GENETICS, HUMORAL IMMUNORESPONSIVENESS, AND DISEASE RESISTANCE IN CHICKENS

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(ABSTRACT)

Lines of White Leghorn chickens selected > 20 generations for (HA) and against (LA) antibody response to SRBC injected i.v. from 41 to 51 days of age, are now known to have diverged in primary antibody response to SRBC. Experiments described in this dissertation were designed to further evaluate the immune competence of these lines as influenced by age, diet, and a disease agent. A crossing experiment was also conducted to further describe the mode of inheritance of such competence.

Humoral immunocompetence was evaluated by primary, memory, and maternal antibody responses to SRBC. Primary antibody response, measured 5, 10, and 20 days after inoculation with SRBC was greater in HA than LA chicks inoculated at 14, 21, and 28 days of age. In chicks injected at 7 days of age, a higher frequency of responders was observed for HA than LA chicks suggesting an earlier onset of immunocompetence in the high than low antibody line.

Immunological memory antibody responses (secondary and tertiary) was studied in parallel experiments on two groups of chicks hatched at a 14-day interval. Chicks in both hatches were from the same matings of parental Lines HA and LA. Memory responses were evident in chicks at 14 days of age. Antibody responses to a second and third inoculation with SRBC were similar for both lines suggesting that genetic factors that influence primary and memory responses are not the same. The responses of LA chicks to repeat inoculations with SRBC were anamnestic whereas those of HA chicks initially inoculated at 28 days of age were not anamnestic. This study did not establish any major influence of nutrient density on either primary or memory immune responses even though the higher

nutrient density diet improved growth performance.

Assays in chicks indicated that maternal antibodies were transferred earlier into eggs laid by HA hens than in those of LA hens (7 to 9 days vs 10 to 12 days after inoculation) regardless of dosage administered. Response patterns whether assessed in terms of frequency of detection or magnitude of response showed divergence between the lines.

Chicks of parental, reciprocal F_1, F_2, and backcrosses of mating combinations of Lines HA and LA were injected with SRBC at 36 days of age. Contrasts between parental lines for antibody titers measured 5 and 12 days later showed higher antibody titers in HA than LA chicks. Sex-linked effects were evident because reciprocal contrasts for F_1 crosses, individual heterosis, and maternal heterosis were sex dependent.

Response to marble spleen disease virus (MSDV) measured 6 days after inoculation of chicks from parental, reciprocal F1, F2, and backcross matings of the lines, indicated that the mode of inheritance of spleen weight differed after infection. In the infected chicks, parental contrasts for absolute and relative spleen weights showed greater resistance to MSDV in LA than HA chicks. No other genetic effect was consistently important after infection.

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INTRODUCTION

The impact of disease on the poultry industry has both economic and genetic dimensions. Economically, losses that are directly accounted for include mortality, medication, veterinary services, downgrades, depopulation, lower production, and poorer feed efficiency. Losses, often associated with subclinical infections, such as reduced egg quality, fertility, hatchability, vaccination stress, and morbidity are routinely ignored in economic analysis because they are more difficult to quantify.

Calnek and Witter (1991) estimated annual costs to the global poultry industry from Marek's disease at $943 million. For coccidiosis, producers in the U.S.A. alone incur as much as $400 million annually (Schwartz, 1994). A loss seldom considered in viewing the impact of diseases is that they can distort genetic parameters used for designing breeding programs for the long term improvement of stocks. For instance, phenotypic variation of most traits may be inflated in diseased populations and thereby affect selection differentials as well as expected genetic progress. Furthermore, depopulation necessitated by outbreaks of certain diseases can deny the industry of valuable genes (Hutt, 1958; Warner *et al*., 1987).

Non-genetic measures such as vaccination, antimicrobial, and eradication programs have greatly reduced the devastating effects of most diseases, but have neither eliminated the agents nor achieved complete control of several others. Drug resistance (McDougald, 1990) and the resurgence of more virulent strains of infectious agents reduce the effectiveness of these non-genetic measures in the short term, thus indicating a need for other approaches. In the long-term therefore, genetic selection for stocks of chickens resistant against major infectious agents would complement efforts at non-genetic manipulations in a more permanent way. A genetic approach has the added benefit of producing products devoid of drug residues for health-conscious consumers.

Classification of disease resistance

Numerous diseases that afflict domestic animals occur when environmental insults meet genetic predisposition (Warner *et al.*, 1987). Genetic systems that exist to avert diseases from occurring include non-host resistance, resistance to infection, and resistance to disease development.

Non-host resistance or complete immunity implies complete lack of interaction between pathogen and host such as occurs between plant and animal viral diseases. ***Resistance to infection*** or true resistance prevents infection. Normally, host-pathogen interactions occur before an infection is established. Under this category of resistance, the outcome of such an interaction is expressed as incompatibility and depends upon the genotypes of the host and the pathogen (Dixon *et al.*, 1991). Receptor mechanisms which have been found to be important under this category (Crittenden, 1974; Plant and Glynn, 1976), are mostly inherited in simple Mendelian fashion (Gavora, 1990). ***Resistance to disease development*** refers to the ability of the host to prevent the pathogen from causing disease after an infection. This system is the commonest and the least pathogen-specific. Most diseases for which genetic resistance is being sought currently come under this class and are therefore given prominence in this proposal.

The interaction between a pathogen and its host is dynamic, with the infection and the degree of resistance dependent upon: (1) mechanisms employed to invade and multiply in the host by the pathogen and (2) types of defensive mechanisms the host can use to defend itself (Bains, 1979). Genetic resistance to disease development is thus complex and involves several systems of the body with the immune system being a very important component (Warner *et al.*, 1987).

Disease resistance and immune response

Interest in finding a relationship between disease resistance and immune response arose when it was seen that

selection for resistance against most diseases tended to be specific and had little effect on general disease resistance (Carson, 1951; Gavora and Spencer, 1983). Yet from a practical point-of-view, the ability of a bird to withstand challenge by multiple pathogens determines cost of production and ultimately profitability. Large responses to selection for immune traits show that these are manipulable genetically (Siegel and Gross, 1980; van der Zijpp and Nieuwland, 1986). Correlated responses to selection for immune traits cover a wider range of diseases than would be achieved by selection for individual diseases (Gross *et al*., 1980; Martin *et al*., 1986; Dunnington *et al*., 1992a,b). A further advantage in working with immune traits is that commercial breeding populations need not be exposed to infectious agents.

Striking differences have been reported among populations selected for similar immune responses. In lines selected for antibody response to sheep red blood cells, responses to concanavalin A (con A) mitogen was higher in the high (HA) than low (LA) antibody line (Scott *et al*., 1991). However, in lines of chickens selected against this same antigen at Wageningen, response to con A mitogen was reversed (Kreukniet *et al*., 1994b). Differences have also been reported in response to Marek's disease virus (Pinard *et al*., 1993). Such reports suggest the need for genetic analysis to study immune response genes and their mode of inheritance in the various populations. Understanding the mode of inheritance of these genes will be required in designing breeding programs to exploit the traits. According to Bumstead and Miller (1987), the initial steps in studying genes responsible for resistance consist of identifying resistant and susceptible populations followed by crosses between them.

LITERATURE REVIEW

An important step in studying genetic disease resistance would be to identify populations in which these resistance genes occur and understand the natural mechanisms maintaining them in these populations. The purpose of this section of the proposal is to review findings of other investigators regarding genetic variation in disease resistance, immunocompetence, and the relationship between them.

GENETIC VARIATION IN DISEASE RESISTANCE

Infectious agents to be covered in this proposal include protozoan parasites, bacteria, and viruses of economic importance to poultry production.

Protozoan parasites

One of the most economically important protozoan diseases of poultry is coccidiosis, caused by *Eimeria spp* in intensively-reared chickens which are in daily contact with their droppings (Schwartz, 1994). The disease is characterized by enteritis and diarrhea. The seven most pathogenic species are *E. tenella*, *E. necatrix, E. brunetti, E. maxima, E. acervulina, E. mitis and E.praecox* (Ovington *et al*., 1995). These parasites have both extracellular and intracellular, as well as asexual and sexual life cycles. The number of asexual stages depends upon the *Eimeria* species and the host's immune responses (Ovington *et al*., 1995).

Long (1968) and Bumstead and Millard (1987) exposed 3-week old chicks of different breeds and inbred lines of chickens to several *Eimeria* species and measured disease resistance by changes in body weight, mortality and oocyst output. The influence of host strain on the response criteria was clearly demonstrated. Pinard *et al.* (1998) found the Egyptian Fayoumi to be the most resistant of the five outbred lines tested based upon mortality, lesion scores and growth reduction. Other traits in

which variation among populations in resistance to coccidiosis has been expressed include packed cell volume (PCV), and lesion scores (Mathis *et al*., 1984; Bumstead and Millard, 1987; Martin *et al*., 1986; Lillehoj and Ruff, 1987). In general, the various measures of response to *Eimeria* did not correlate with one another. Further, host resistance generally depended upon the *Eimeria* species involved (Long, 1968; Bumstead and Millard, 1992).

Jeffers (1978) presented data which supported the importance of host strain x coccidial species interactions in the expression of host susceptibility. According to Mathis *et al.* (1984) PCV was a better measure of disease resistance to *E.tenella* infection than to *E. acervulina* because, compared to the latter, the former causes extensive hemorrhage which substantially depresses PCV. The relationship between body weight and lesion scores varies among *Eimeria* species. Conway *et al*. (1990) observed that lesion scores did not fully reflect the degree of disease severity in induced infection. Their results corroborated those of Long *et al*. (1980), in that weight changes in susceptible broiler birds infected with *E.tenella* did not correspond with those of immunized birds having similar lesion scores.

The genetic basis of the variation in resistance to coccidiosis among populations was demonstrated by differences among sire families (Mathis *et al*., 1984) as well as responses to genetic selection. Rosenberg *et al*.(1954) obtained a divergence of 35% in survival rate following one generation of selection in response to *E. tenella* infection. After 12 generations of selection for response to *E. tenella*, mortality for the control, resistant, relaxed resistant, and susceptible lines were 41%, 21%, 29%, and 35% respectively (Johnson and Edgar, 1982). High dosages of oocysts spread the infection to tissues other than ceca, the usual target of *E. tenella*, with the tissues varying with the host strain. It was concluded that genes controlling tissue specificity and response to acute cecal coccidiosis might

be identical or complimentary.

Variation has also been reported in the relationship between primary and secondary responses to *Eimeria* species (Lillehoj, 1988). Certain strains highly susceptible to primary infection were the more resistant to challenge infection whilst some were resistant to both suggesting different genetic mechanisms controlling non-specific, specific, and immunological memory (Lillehoj, 1988; Lillehoj *et al*., 1989; Lillehoj and Trout, 1993).

Causes of variation in resistance to coccidiosis

Major genes

Gavora (1990) has provided evidence of correlations between criteria to measure resistance to *Eimeria* species and haplotypes at the major histocompatibility complex (MHC). In general, B^{5} and B^{15} haplotypes provided more protection against *E. tenella* and *E. acervulina* than B^{2}, B^{12}, or B^{13}. According to Lillehoj *et al*. (1989), considerable differences in resistance were observed between strains sharing common MHC alleles (e.g. B^{13}) but having different genetic backgrounds; results corroborated by Ruff and Bacon (1989) and Dunnington *et al*. (1992a). The involvement of non-MHC linked loci in determining resistance to *Eimeria* species have also been reported (Johnson and Edgar, 1984; Martin *et al*., 1986).

Immune responses

Eimeria parasites exhibit both extracellular and intracellular stages of life cycles in their infection of the host. The role of the humoral component of the immune system appears to be important during the early phase of the infection and circulating antibodies specific for coccidial parasites are detectable within one week of inoculation with oocysts (Lillehoj, 1991). The level and duration of antibody response depends on host factors such as age and genetics of the host, and the species of *Eimeria* (Lillehoj, 1988). However, bursectomy or transfer of protection by using *Eimeria* immune antibodies show that antibodies are not involved in host resistance to coccidial

challenge infection (Rose, 1974; Giambrone *et al*., 1981; Lillehoj, 1987).

On the other hand, there is extensive evidence which shows that cell-mediated immune responses are essential in limiting oocyst production in primary and subsequent infections (Wakelin and Rose, 1990; Lillehoj and Trout, 1993; Ovington *et al*., 1995). A significant negative correlation between oocyst production and lymphocyte proliferation was found to be important in protection against coccidiosis caused by *E. maxima* (Talebi and Mulcahy, 1995). Previous research had indicated that Cyclosporin A, which is known to block the activation of T-cells, abrogated host protective immunity to *Eimeria* parasites (Lillehoj, 1987).

Generally, antigens of the various *Eimeria* species vary in immunogenecity, with *E. maxima* and *E. brunetti* being the more immunogenic whilst *E. necatrix* and *E. tenella* are the less immunogenic (Ovington *et al*., 1995). The antigenic determinants of the different species are not cross-reactive (Wallach *et al*., 1990). Thus Bumstead and Millard, (1992) noted that chickens from lines which produced the largest numbers of oocysts following infection with *E. tenella* produced the fewest number when infected with *E maxima, E. mitis*, or *E. praecox*. Within *Eimeria* species, immunogenecity also varies between stages of development, the asexual stage being more immunogenic than the sexual stage (Rose and Hesketh, 1976). Even though coccidial merozoite antigens that induced T-cell proliferation were associated with resistance to infection (Jenkins *et al*., 1988), merozoite fractions that induced lymphocyte proliferation were different from those that induced interferon (IFN-γ) production, indicating that different antigens favor different cellular responses (Martin *et al*, 1995). Earlier studies (Prowse and Pallister, 1989) have shown that interferons (IFN) play a role in resistance to primary infection which may be population dependent (Ovington *et al*., 1995).

The sub-populations of T-cells may also cause variation between susceptible and resistant strains. In chickens infected

with *E. tenella*, there were more $CD4^+$ intestinal intra-epithelial lymphocytes (I-IEL) than $CD8^+$ (Lillehoj and Bacon, 1991; Lillehoj, 1994). In general, it appears that chickens which developed more severe lesions and produced more oocysts had less $CD8^+$ T-lymphocytes than chickens that had less severe lesions and fewer oocysts (Lillehoj, 1994).

Mode of inheritance

Champion (1954) concluded, after a comparison of the survival rate of parental lines (resistant and susceptible), F_1, F_2, and their backcrosses exposed to *E. tenella*, that sex-linkage, maternal effects or cytoplasmic inheritance did not influence susceptibility to *E. tenella*. His lines which had been selected for only two generations did not show significant evidence of dominance. In a diallel crossing experiment involving nine lines, heterosis of up to 34% was observed in the survival rate of some individual crosses exposed to *E. tenella* (Jeffers *et al*., 1970). Differences in reciprocal crosses were attributed to maternal effects. The importance of maternal effects in resistance to *Eimeria* spp was confirmed by Buvanendran and Kulasegaram (1972) who tested male progeny of 4 strains crossed reciprocally. According to Smith *et al*. (1994), maternal antibodies transmitted early immunity against *E. maxima* which lasted for 2 to 3 weeks.

In two separate experiments in which three different inbred lines were crossed, female crossline chicken had significantly greater survival rates than male chicks (Jeffers *et al*., 1970) suggesting involvement of the W-chromosome of the dam lines in genetic resistance to *E. tenella*. Working with MHC genes, Clare *et al*. (1985) have shown that resistance to cecal coccidiosis was dominant in B^2B^5 heterozygotes. The mode of inheritance of resistance to *Eimeria* parasites is thus population dependent.

Bacterial parasites

Colibacillosis is a non-contagious infectious disease of

immature poultry and occasionally older fowl caused by coliform (intestinal) bacteria especially pathogenic strains of *Escherichia coli* (Schwartz, 1994). These bacteria are opportunistic in that the disease develops as a secondary infection following severe stress, immunosuppression, and respiratory illness. The disease is characterized by septicemia, diarrhea, airsaculitis, granulomas, and poor productivity.

Variation among breeds and lines of chickens in resistance to *E. coli* challenge as measured by changes in body weight and lesion scores have been reported (Boa-Amponsem *et al*., 1991; Praharaj *et al*., 1996). Soller *et al*. (1981) reported differences among 35 sire families of a commercial poultry strain in immune response to *E. coli* vaccination. A heritability based upon the paternal half-sib correlations of .25 has resulted in attention directed at the immune system in dealing with resistance to this bacteria. Evidence for the variation in the rate of maturation of the immune response to *E. coli* has been reported (Heller *et al*., 1981; Peleg *et al*., 1985).

Substantial responses of meat lines to divergent selection for response to *E. coli* vaccination have been reported. In one case the two lines differed by 50% in antibody titers after 4 cycles of selection (Pitcovski *et al*., 1987) whilst Leitner *et al*. (1992) reported a ratio of response of 2.2 between the high and low selected lines. Heller *et al*. 1990 analyzed the immunological bases of the difference between the divergently selected lines reported by Leitner *et al*. (1992). Chickens from the S_3 and S_4 generations of the high (HC) and low (LC) selected lines and the control (CT) line were tested for antibody response to antigens of *E. coli*, SRBC, Newcastle disease virus (NDV) and bovine serum (BSA), as well as T-cell mitogens. Line HC exhibited better responses in all the compartments (humoral, phagocytic, and cell-mediated) of the immune system which prompted the suggestion that the rate of maturation of the entire system was under similar genetic influence.

Subsequently, the nature of genetic influence on the

variation between these two lines was investigated. Findings of Uni *et al*. (1993) associated the differences between the lines with major genes at the MHC locus. The difference in haplotype frequencies, however, became significant only after pooling data over replicated selected lines. An analysis of diallel matings between the lines showed a lack of heterosis but significant reciprocal effects (Leitner *et al*., 1994). Maternal rather than sex-linked effects were invoked to explain reciprocal differences because the sexes responded similarly to *E. coli* vaccination. Heritabilities calculated in the S_9 generation were .44 and .42 in HC and LC lines, respectively (Yonash *et al*., 1996), with estimates from the dam component always higher than those from the sire component.

Viral diseases

Marek's disease (MD) is often used as a model to study immune responses against viral disease-causing agents and tumors in chickens (Schat, 1991). MD is one of the most researched of all neoplastic diseases in chickens due to its economic importance and its similarity with Burkitt Lymphoma in humans.

Variation among breeds and lines of chicken in the incidence of MD has been reported by several workers (Biggs *et al*., 1968; Crittenden *et al*., 1972; Grunder *et al*., 1972; Gavora, 1990; Spencer *et al*., 1992; Pinard *et al*., 1993). This variation has been shown to have a genetic basis. Biggs *et al*. (1968) found that among 47 sire families incidence of MD ranged from 0 to 23%, 1 to 22%, and 41 to 100%. Estimates of heritability of resistance to MD ranged from .06 to .67 (Gavora 1990; Pinard *et al*., 1993). Although selection for resistance to MD has resulted in large responses (Hutt, 1958; Cole, 1968) indicating multifactorial mode of inheritance, resistant lines often succumb to more virulent strains of MD virus (Grunder *et al*., 1972). Gavora and Spencer (1983) proposed a combination of selection and vaccination as the best protection against MD.

Factors which cause stocks to differ in susceptibility to MD include major genes, background genomic effects, and immune

responses. Haplotypes of the major histocompatibility complex (MHC) which have been associated with levels of susceptibility to MD include B^2, B^3, B^6, B^{13}, B^{15}, B^{17}, B^{18}, B^{19} and B^{21} (Briles *et al*., 1980; Sander, 1992) with B^{21} considered the most effective allele as a heterozygote (Gavora, 1990; Hartmann *et al*., 1990; Bacon and Witter, 1994). Lines known to differ in MD were both fixed for B^2 (Pazderka *et al*., 1975) whilst B^{13} exhibited greater resistance than B^{21} in different populations (Calnek *et al*., 1989; Martin *et al*., 1989a). Non-MHC linked loci also influence MD (Pevzner *et al*., 1981; Fredericksen *et al*., 1982) and in some cases accounted for a third of the total variation in MD incidence (Fredericksen *et al*., 1982).

Immune responses and MD resistance

Susceptibility of chickens to MD was unaltered by bursectomy, and passive antibody administered seven days after infection was not protective (Witter, 1976). Resistance in old immunocompetent chickens was, however, reduced by surgical thymectomy (Sharma *et al*., 1973) demonstrating the importance of cell- mediated immune (CMI) responses in MD resistance (Schat, 1991). Also, maternal influences on MD vary among breeds (Ball *et al*., 1971; Crittenden *et al*., 1972) and appear to be important in the initial stages of infection (Witter, 1976).

Gene action in MD incidence can be additive (Schmittle and Eidson, 1968), heterotic (Biggs *et al*., 1968; Crittenden *et al*., 1972) and dominant (Hartmann *et al*., 1990) with maternal effects more important than sex-linked effects (Witter, 1976; von Krosigk *et al*., 1972). In general, gene action appears to be modulated by environment, pathogen and population characteristics.

GENETIC VARIATION IN IMMUNOLOGICAL TRAITS

Immune-mediated protection from disease is primarily based upon an interaction among macrophages (inflammatory leukocytes), T and B lymphocytes (Lamont and Dietert, 1990). These sources of protection represent three major functional arms of the immune system namely, phagocytic, cell-mediated, and humoral immunity,

respectively. An important feature of the response is the necessity of cell communication and cooperation, which is achieved by either cell-to-cell contact or by the interaction of secreted factors (cytokines) with receptors on cell surfaces (Male and Roitt, 1993).

The avian immune system is characterized by: the presence of primary lymphoid organs, the bursa of fabricius and the thymus; secondary or peripheral tissue (e.g., the spleen), and the lack of organized lymph nodes (Firth, 1977). Because most immune responses involve multiple immune cell interactions, genetic influences are possible at several stages in the process. Biozzi *et al*. (1979) claimed that the three immune functions were under separate genetic control in mice.

In a review on chickens, van der Zijpp (1983) provided evidence for breed and stock variation in the levels of serum immunoglobulin, responses to various antigens (e.g., *Diptheria toxoid*) and mitogens (PHA, Con A), and delayed-type hypersensitivity. Differences among stocks have also been reported in the rates at which immunocompetence is developed (Rees and Nordskog, 1981). Heritability estimates for various immunoglobulins ranged from .75 to 1.18 (van der Zijpp, 1983). Although substantial responses to selection for bursa weight have been reported, according to a recent review by Kreukniet (1995) the selection did not consistently influence antibody response to most antigens. Several attempts to obtain correlated responses for immunocompetence through selection for T-cell and humoral responses have been reviewed (Martin, 1989; Kreukniet, 1995). Most of these selection experiments were short-term and evidence of divergence of lines in response to infectious and non-infectious agents were inconsistent or lacking.

Two sets of populations which have undergone long-term selection for antibody response to SRBC and which have been the subject of several investigations are located in Virginia, USA and Wageningen in the Netherlands. The remainder of this review will be devoted to lessons learnt from these populations

regarding the relevance of selecting for immunocompetence on disease resistance.

Direct responses

Virginia lines. Two lines derived from the same base population of White Leghorn chicken were divergently selected for high (HA) and low (LA) antibody response to SRBC antigen. Each chicken was injected , through the brachial vein with .1mL of .25% suspension of SRBC at the age of 41-51 days (Siegel and Gross, 1980; Martin *et al.*, 1990). The trait selected for was total antibody production five days after an injection with SRBC.

Divergence between the lines was immediate, being 1.24, 2.10, and 2.96 in S_1, S_2 and S_3 generations, respectively (Siegel and Gross, 1980). Realized heritabilities of .17 to .44 for the HA and .19 to .26 for LA in the S_3 (Siegel and Gross, 1980) and the S_{14} generations (Martin *et al.*, 1990) were indicative of substantial additive genetic variation for the selected trait which is probably a trait with an intermediate optimum for fitness. Divergence between the lines attenuated after three generations such that it was the same for generations S_8 and S_3 (Siegel *et al.*, 1982) and the response to selection again commenced (Martin *et al.*, 1990).

Wageningen lines. This bidirectional selection experiment was initiated from a base population of ISA Warren cross chicks. Selection was for high (HA) or low (LA) total antibody production measured five days after immunization intramuscularly with 1mL of 25% SRBC at 37 days of age (van der Zijpp and Nieuwland, 1986; Pinard *et al.*, 1992). A random-bred control population, (C) was included. Divergence in agglutination titers in response to SRBC antigen was significant from generation three, being higher for line HA (5.6) than for line LA (3.9). Differences between the lines continued to increase, and by generation nine antibody titers were respectively 10.6, 6.2 and 1.9 for the lines HA, C, and LA, respectively (Pinard *et al.*, 1992). According to Pinard

et al. (1992), the heritability estimate using data on all the lines over 9 generations was .31. The estimate of heritability was higher for line LA (.36) than line HA (.29) and line C (.22). Realized heritability was also higher in line LA (.25) than in the HA line (.21). In all the lines antibody titers were higher for females than males (Pinard *et al*., 1992).

Correlated responses

The principal interest in selecting for antibody production is to improve general immunocompetence and obtain resistance to wide ranging infectious agents, without any concomitant deleterious effects on commercially important production traits.

Immune characteristics

Virginia lines. Mean persistence of antibody titers 21 days post inoculation was higher in line HA than LA (Siegel and Gross, 1980), indicating a probable divergence in memory mechanisms. SRBC antigen administered to 14-day old (Ubosi *et al*., 1985a) or 370- day old chickens (Siegel *et al*., 1982) elicited similar differences in antibody production as was observed between the lines at the selection age. The kinetics of the antibody response differed between the selected lines. Also, dosage of SRBC antigen influenced the differences between lines in their antibody response to primary immunization (Ubosi *et al*., 1985a). In line HA, secondary responses consisted mainly of IgG antibodies whilst in line LA, both primary and secondary titers were largely IgM antibodies (Martin *et al*., 1989). Anamnestic response was apparent in only the LA line (Ubosi *et al*., 1985a; Martin *et al*., 1989b).

Based upon *in vitro* responses of peripheral lymphocytes (PBL) to con A and PHA-M mitogens, T-cell activity was higher in line HA than line LA (Scott *et al*., 1991). Total and IgG antibody titers of HA chicks in response to *Brucella abortus* were higher than for LA chicks (Scott *et al*., 1994; Dunnington *et al*., 1992b). Thus, both B and T-cell functions appear to have been

diverged by the direct selection for antibody to SRBC. The effect of this selection on macrophage function as well as antigen presentation have not been investigated in these lines.

Wageningen lines. Both total and IgG antibody titers post secondary inoculation followed the same divergence as was observed in primary responses to SRBC antigen (Pinard *et al.*, 1992). The secondary total antibody titer was lower than the primary ones in the HA chicks, whereas a recall response was observed only in line LA chicks (van der Zijpp and Nieuwland, 1986; Pinard *et al.*, 1992). Other studies comparing different routes of immunization revealed that secondary total antibody titers were higher than the primary only in the group immunized intraperitoneally (Kreukniet *et al.*, 1992). In an earlier study, a divergence in cell-mediated immunity was discounted whilst phagocytic activity based upon carbon clearance rate appeared to be higher in the HA than LA chickens (van der Zijpp and Nieuwland, 1986). An in-depth study of these lines in latter generations, however, revealed that T-cell activity was consistently higher in line LA than in line HA (Kreukniet *et al.*, 1994a) and that phagocytic activity was similar in both lines (Kreukniet *et al.*, 1994b).

Infectious immunogens

Virginia lines. A series of experiments were conducted to characterize lines LA and HA in respect of their resistance to infectious disease agents (Gross *et al.*, 1980). Air sac lesions in response to *M. gallisepticum* challenge were 56% and 100% of the HA and LA lines, respectively, in one trial and 3% and 50% of the HA and LA, respectively, in a second. Chicks from the HA line had greater weight gains after challenge with *E. necatrix* and *E. tenella* than those of line LA (Gross *et al.*, 1980; Martin *et al.*, 1986). None of the chicks of line HA died compared to 84% mortality in line LA when challenged with Newcastle disease virus (Gross *et al.*, 1980). Seven days after challenge with splenomeglia virus, 7% of HA and 52% of LA chicks had spleens

that weighed more than 1.7g (Gross *et al*., 1980). Also, line HA chicks exhibited greater resistance to fowl mites than chicks of line LA (Gross *et al*., 1980).

Several studies have however shown greater resistance to *E. coli* (Gross *et al*., 1980; Dunnington *et al*., 1991) and *Staphylococcus aureus* (Gross *et al*., 1980) by line LA than HA chicks. Martin *et al*. (1989a), using sublines of HA and LA with $B^{21}B^{21}$, $B^{13}B^{21}$, $B^{13}B^{13}$ genotypes, found a line by MHC genotype interaction for MD resistance with a heterozygous advantage for the MHC. Such results suggest that mechanisms for MD resistance may be different from that of other viral agents (e.g., NDV, splenomeglia virus) whose symptoms are more acute. Lifetime mortality due to non-specific causes was higher in line LA than HA chickens (Siegel *et al*., 1982).

Wageningen lines: Unlike the Virginia selection lines, these antibody lines have not been challenged with many disease agents *per se*. After exposing the lines to *Salmonella* antigen, van der Zijpp and Nieuwland (1986) concluded that the difference in antibody producing capacity between the lines was not reflected in the response to this antigen. Chicks from line LA were more susceptible to challenges with MD virus and died earlier than the chicks from line HA and the control line (Pinard *et al*., 1993). The results suggested that the LA line had been adversely affected by the selection since the HA and the control lines hardly differed in resistance to MDV (Pinard *et al*., 1993).

Production traits.

Virginia Lines. Body weights of pullets from line LA were significantly greater than for those of line HA at 4 and 24 wk of age in both the S_8 (Siegel *et al*., 1982) and S_{14} (Martin *et al*., 1990) generations. LA pullets commenced egg production at younger ages (Siegel *et al*., 1982; Martin *et al*., 1990) and at lower body weights (Martin *et al*., 1990) than HA pullets. Line LA had greater hen-day egg production to the fixed age of 300 days than their HA counterparts in generations S_8 and S_{14} (Siegel

et al., 1982; Martin *et al*., 1990). However, when percentage hen-day production was also measured later in life (364 to 385 days of age), the difference between the lines no longer existed (Siegel *et al*., 1982). The production of defective eggs was greater in LA than in HA pullets in generation S_7 (Siegel *et al*., 1982) but not in generation S_{14} (Martin *et al*., 1990) whilst the incidence of double-yolk eggs for line LA exceeded that for line HA in generation S_{14} (Martin *et al*., 1990).

Wageningen Lines. Selection for antibody production to SRBC has resulted in divergence in body weight as the LA line chicks are heavier than the HA line chicks (Kreukniet *et al*., 1994a), a result consistent with that of the Virginia lines (Martin *et al*., 1990). In an extensive review, Kreukniet (1995) indicated that the energy utilization was better in line HA than LA both before and after immunization with SRBC.

Mode of inheritance of immune traits

Considerable responses to selection and the medium realized heritability estimates reported in the literature indicate substantial polygenic additive gene action in 5-day antibody titers to SRBC antigen (Siegel and Gross, 1980; Martin *et al*., 1990; Pinard *et al*., 1992). Yet, the effects of major genes on this trait have been demonstrated. Response to SRBC antigen was dependent on particular haplotype combinations present at the MHC as well as the background genome in which they were expressed (Dunnington *et al*., 1989). Higher antibody responses were reported in $B^{13}B^{21}$ individuals from line HA and in $B^{21}B^{21}$ individuals from line LA. In the Wageningen antibody lines, B genotypes explained only 3.5% of the total variation in the antibody response estimated in the F_2 population whilst 31% was due to the rest of the genotype (Pinard and van der Zijpp, 1993).

The influence of nonadditive gene action on antibody titers was apparent by generation S_3 of the Virginia lines (Siegel and Gross, 1980). Heterosis of 9% and 10% were reported by Siegel *et al*. (1982) for generations S_3 and S_6, respectively. Working with

the S_9 generation of the Wageningen lines, Pinard *et al*. (1993) suggested that the lack of heterosis in their lines was due to the absence of 121 B-haplotype in the LA line. Biozzi *et al*. (1979) interpreted heterosis in antibody response to SRBC in lines of mice to be due to partial dominance of the character, high responder. The chicken lines of Virginia are now in their 22nd generation of selection and would be suitable to study the importance of nonadditive gene action in immune responses to SRBC antigen.

Other modes of inheritance of antibody response to SRBC have been suggested. The difference in magnitude between heritability estimates based upon dam and sire components of variance indicated parental effects may vary (van der Zijpp and Leenstra (1980). Differences in the response of reciprocal crosses between lines of chicken selected for high or low antibody titers to SRBC antigen have been reported (Pinard and van der Zijpp, 1993; Gross *et al*., 1988). These results suggest that sex-linked or maternal effects contribute to variation in antibody response to SRBC antigen. Genetic analysis of these lines can provide information that would be useful in designing breeding programs to exploit immune traits.

OBJECTIVES

1. To investigate the ontogeny of immune responses in lines selected for high and low antibody response to SRBC antigen.
2. To study the mode of inheritance of responses to pathogenic and non-pathogenic immunogens of lines selected for high or low antibody production to SRBC antigen.

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CHAPTER I

Diet and Humoral Immunoresponsiveness of Lines of Chickens Divergently Selected for Antibody Response to Sheep Red Blood Cells.

ABSTRACT

Growth and humoral immune response was studied in lines of White Leghorn chickens selected for high (HA) or low (LA) 5-day antibody titers to an i.v. inoculation with 0.1ml of a 0.25% suspension of SRBC antigen(s). Chicks were fed either a high (E) or low (A) nutrient density diet from hatch onwards. Chicks from each line-diet subclass were inoculated i.v. with 0.1ml of either 0.25% or 2.50% suspension of SRBC at either 7, 14, 21, or 28 days of age. Antibody titers were measured 5, 10, and 20 days after inoculation.

LA chicks were heavier than HA chicks at 7 days of age and thereafter. Chicks fed diet E were heavier than those fed diet A. Feed efficiency was influenced by diet (E > A) at 21 and 28 days of age and line (LA > HA) at 28 days of age. In all but one case, antibody titers to SRBC were higher in HA than LA chicks. Also, the frequency of non-responders of chicks inoculated with SRBC at 7 days of age was higher for LA than HA chicks. The higher dosage elicited greater 5-day antibody responses in LA but not HA chicks. Dietary effects on SRBC antibody were generally unimportant except for occasional interactions with dosage and line.

Key Words: body weight, SRBC, antibody titers, lines, diet, dosage.

Abbreviations: SRBC = sheep red blood cells; BW = body weight; HA = high antibody response line; LA = low antibody response line; H = high SRBC dosage; S = selection dosage of SRBC; E = high nutrient density diet; A = low nutrient density diet; doa = day of age.

INTRODUCTION

Selection experiments have provided evidence for classifying immunocompetence in chickens as a polygenic trait (11, 15, 18, 20) and are therefore influenced by both genetic and non-genetic factors. Of the non-genetic factors affecting immune responses, nutrition is economically important because of its influence on production costs (13). The main inference from nutritional studies suggests that diets and feeding regimens which promote fast growth rate also result in reduced immunocompetence (1, 3, 4, 12, 14). Yet, commercial poultry diets are formulated to meet the nutritional requirements for specialised meat and egg producing stocks (9, 13) with little, if any, attention to immune competence.

Of concern to poultry producers is that lines selected for improved immune responses exhibit a negative association with characteristics such as juvenile growth rate and egg production (7, 10, 16). Also, recent evidence (20) suggests that selection for immune response influences maturation of the immune system. Most studies on nutrition and immunocompetence of chickens utilised stocks which had not undergone direct artificial selection for immune traits (3). Thus, the question to be addressed is whether nutritional manipulation can alleviate the poorer production performance of immunocompetent chickens without dissipating such built-in competence.

The purpose of this experiment was to ascertain the effect of diet and SRBC dosage on the immune responses of lines of chickens divergently selected for antibody responses to SRBC.

MATERIALS AND METHODS

Stocks, Diets, and SRBC dosages.

Chicks used in this experiment were obtained from matings of White Leghorn lines selected for high (HA) or low (LA) antibody production in response to 0.1ml of a 0.25% suspension of SRBC administered i.v. between 41 to 51 days of age (7, 15). Two diets which differed in nutrient density were fed in mash form. The diet on which the genetic lines were developed was the lower nutrient density diet (A), and contained 20% crude protein and 2685 kcal ME/kg. The higher nutrient density diet (E) contained 24% crude protein and 3146 kcal ME/kg. Two SRBC dosages were compared. They were 0.1ml of either 0.25% suspension of SRBC (the dosage used in the selection experiment) or a higher dosage of 2.50% suspension of SRBC. Lower and higher SRBC dosages were designated as S and H, respectively.

Procedures: Eggs obtained from S_{22} generation age-contemporary parents of each line were incubated in the same machine. On day 22 of incubation, 128 chicks from each line were removed from the hatcher, randomized into 16 groups, wingbanded, weighed, and vaccinated for Marek's disease. They were housed with sexes intermingled in groups of 8 per pen in an electric battery cage brooding unit. Assignment of the 16 pens of chicks of each genetic line to each diet was at random. Feed and water were provided *ad libitum* and lighting was continuous.

Blood was obtained at hatch from 10 chicks as well as 10 hens (parents of the chicks) selected at random from each line and the plasma tested for SRBC antibody using the microtiter procedure (19). At 7, 14, 21, and 28 days of age (doa), chicks from two pens per line-diet subclass were injected in the brachial vein with 0.1ml of either 0.25% or 2.50% suspension of SRBC. Five, 10, and 20 days after inoculation, blood was obtained from the brachial vein for antibody titer measurement using the microtiter procedure (19). Titers were expressed as $\log_2$ of the reciprocal of the highest dilution giving visible agglutinin.

Chicks were individually weighed at 7, 14, 21, and 28 doa. Throughout the experiment feed consumption was recorded on pen basis. At 24 doa, chicks from each pen (except those immunized at 7 doa) were divided into 2 equal groups and moved to developer batteries with wire floors.

Statistical Analysis: Individual body weights were transformed to common logs and analyzed

within each age by ANOVA with line, diet, sex, SRBC dosage, and interactions among them as main sources of variation in a fixed effects model. Feed efficiency per pen (BW/weight of feed consumed) was transformed to arc sin square roots prior to ANOVA in which line, diet, SRBC dosage, and interactions between them were the main variables. Antibody titers of responders to SRBC given at 7, 14, 21, and 28 doa and measured at 5, 10, and 20 days after inoculation were analyzed with the same statistical model as for BW but without sex. The frequency of responders to SRBC at 7 doa was analyzed by the Chi-square statistic.

RESULTS

Body weight and feed efficiency at various ages

Body weights at different ages were influenced by line, diet, and sex, whereas effects due to dosage of SRBC and interactions among main effects were absent. Growth patterns of males, and females for line-diet subclasses were similar, hence only those for females are presented (Figure 1). LA chicks were heavier than HA chicks at all ages within each diet. Also, chicks fed diet E, were heavier than those fed diet A from 7 days of age onwards. At 28 doa HA chicks fed diet E were heavier than LA chicks fed diet A. Although these trends were the same for males and females, sexual dimorphism for BW was not significant until 21 doa.

Although feed efficiency was similar for both lines through 21 doa (Table 1), by 28 doa, LA chicks were more efficient than HA chicks. By 21 doa and thereafter, feed efficiency of chicks fed diet E was superior to that of chicks fed diet A. Dosage of SRBC had no effect on feed efficiency.

Non-responders to SRBC inoculation

Although antibody responses were observed in chicks inoculated with SRBC at 7, 14, 21, and 28 doa, the frequency of non-responders was highest for the group inoculated at 7 doa with both line and dosage effects being evident. For this group of chicks the frequency of non-responders was higher for line LA than HA for plasma tested 5 days (25% vs 14%), 10 days (30% vs 10%) and 20 days (35% vs 15%) after inoculation. Non-responders were observed more frequently in chicks given the 0.25% than 2.50% SRBC dosage for plasma tested 5 days (29% vs 11%) and 10 days (25% vs 5%), but not 20 days (30% vs 20%) after inoculation. Diet had no effect on the frequency of non-responders to SRBC inoculation.

Beyond the inoculation age of 7 days, non-responders were observed only in LA chicks. The non-responders in LA chicks inoculated at 14, 21, and 28 doa ranged from 5 to 13%, 2 to 16%, and 23 to 30% for antibody titers measured 5, 10, and 20 days after inoculation, respectively.

Antibody response to SRBC : 5 days after inoculation

Antibody titers for responders 5 days after inoculation with SRBC are presented in Table 2. For chicks inoculated at 7 doa there was no difference between lines for antibody titers. Chicks given the higher dosage of SRBC and those fed the lower nutrient density diet had higher titers than their counterparts given the lower dosage and fed the higher nutrient density diet. For chicks inoculated at 14 doa there were line (HA>LA), and dosage (H>S), but not diet effects. The line effects persisted for those inoculated at 21 doa. Antibody titers of LA chicks remained low regardless of inoculation age while those for HA chicks increased considerably with age of inoculation.

For the 21 day inoculation age, the dosage by diet interaction was significant, while at 28 days both dosage by diet and dosage by line interactions were significant. The dosage by diet interaction at 21 doa was because chicks inoculated with the lower dosage exhibited no dietary effect (E = 8.8 ± 1.4; A = 9.0 ± 1.3) while with the higher dosage chicks fed diet E had a higher antibody titers than those fed diet A (10.1 ± 1.1 vs 7.8 ± 1.4). This dosage of SRBC by diet interaction persisted in chicks inoculated at 28 doa (Table 3). The dosage by line interaction at 28 doa was because there was no dosage effect on HA chicks, whereas the higher dosage elicited higher antibody response in LA chicks.

Antibody responses to SRBC: 10 days after inoculation

Antibody titers of chicks 10 days after inoculation with SRBC, are shown in Tables 4 and 5. Lines followed the same trends as were observed for antibody response 5 days after inoculation in that titers were higher for HA than LA chicks in all cases except for those chicks inoculated at 7 doa. Dosage effects disappeared, except for LA chicks inoculated at 14 and 28 doa where antibody levels were higher for the H than S SRBC dosage resulting in the line by dosage interaction (Table 5). There was no dietary influence on antibody levels 10 days after inoculation for chicks inoculated at 7, 14, and 21 doa. At 28 doa there was a line by diet interaction because titers were higher for LA but not HA chicks fed diet E than A (Table 5).

Antibody responses to SRBC: 20 days after inoculation

Antibody responses 20 days after inoculation of chicks with SRBC are presented in Table 6. There were no differences between lines or dosages for chicks inoculated at 7 doa, however, titers were higher for those fed diet E than A. The diet effect was not present for chicks inoculated at 14, 21, and 28 doa. HA chicks generally maintained higher antibody levels than LA chicks and, at subsequent inoculation ages, titers were higher for H than S chicks, results consistent with those observed 5 and 10 days after inoculation. There were, however, significant line by dosage interactions for chicks inoculated at 14 and 28 doa. For chicks inoculated at 14 doa, a line difference was observed with the lower SRBC dosage, (HA = 5.5 ± 0.6; LA = 2.0 ± 0.1), but not with the higher dosage (HA = 4.7 ± 0.5; LA = 4.3 ± 0.4). For chicks inoculated at 28 doa, however, while there was a line difference at both dosages, the dosage effect was present for the HA line (H = 4.6 ± 0.3; S = 3.5 ± 0.2), but not for the LA line (H = 2.1 ± 0.1; S = 2.0 ± 0.1).

DISCUSSION

Reports for lines selected for humoral immune response (3, 7, 10) or juvenile BW (6, 8) indicate an antagonistic relationship between these traits. Yet, simultaneous improvement of the two traits is desirable in commercial poultry production. Results of the present work suggest that growth rate of immunocompetent lines may be improved by feeding diets of higher nutrient densities, without concomitant losses in antibody titers. This relationship seems inconsistent with much of the literature which suggests that nutritional manipulations that increase growth also tend to reduce immunocompetence (1, 3, 4, 12, 14). The inconsistency may be that in growth-selected stocks the tendency to overconsume food may have a negative influence on resources available for immune responses. The inconsistency may also be due to differences in the mechanism for antibody production in specialized antibody lines such as those used in this research and non-specialized lines utilized in the others. Parmentier *et al*. (10) explained that the responses of their high anti-SRBC antibody line was partly antigen-specific, whereas that of their low line might be due to antigen-unspecific mechanisms which result in lower and delayed antibody responses. Further evidence for different mechanisms in high antibody producing lines is the lack of SRBC dosage effects on antibody levels observed in this experiment and in other

recent work with these lines (2). These observations suggest a lower threshold due to the selection for high affinity lymphocytes specific for SRBC antigens.

For chicks inocualted with SRBC at 7 doa, the relation between diets depended on when antibody titers were measured. Five days after inoculation titers were higher for chicks fed diet A than E. There was no difference between diets 10 days after inoculation, while 20 days after inoculation titers were higher for those fed diet E than A. Perhaps more noteworthy was the result that for antibody production 5 days after inoculation, the higher nutrient density diet enhanced responses to the higher antigen concentrations. This inference corroborates an earlier report (5) showing that a calorie-protein deficient diet reduced primary immune responses to a 5.0% but not a 0.5% SRBC antigen concentration, despite the lack of dietary differences in immmunocompetent cells.

A higher proportion of responders was observed in the high than low antibody line for the selected trait, 5-day antibody titers as well as for persistence of the response 10 and 20 days after inoculation. These results in young chicks are consistent with reports on these (17) and other lines (20) and indicate that maturation of the immune system can be modified by artificial selection.

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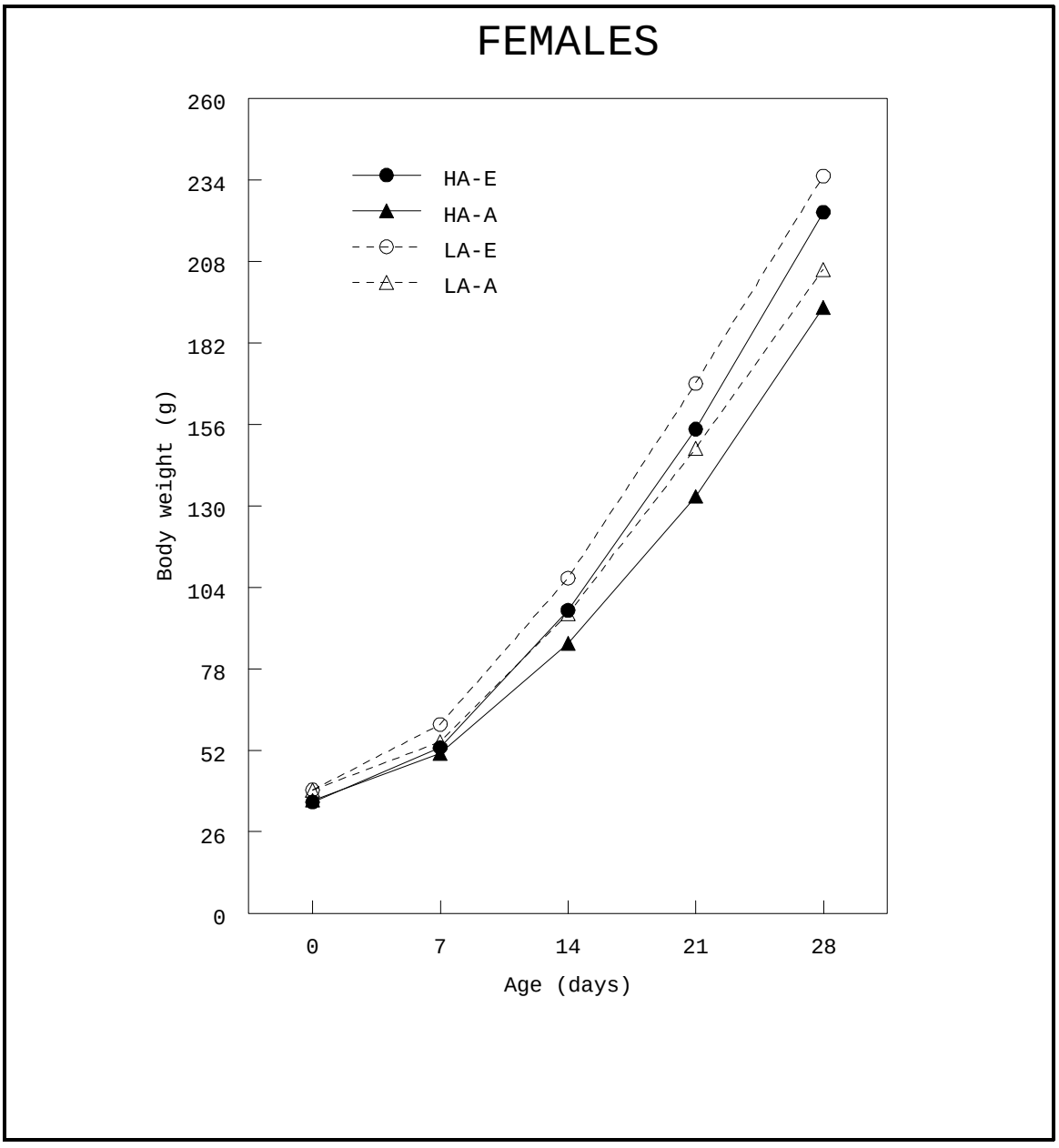

Fig. I.1. Body weights of females at hatch, 7, 14, 21, and 28 days of age by line and diet. Lines HA and LA were selected for high and low antibody response to SRBC, respectively. Diets E and A were high and low nutrient densities, respectively.

Table I.1. Mean feed efficiencies to 7, 14, 21, and 28 days of age by line, SRBC dosage and diet

	Period (days)			
Variable	0 to 7	0 to 14	0 to 21	0 to 28
Line[1]				
HA	1.53	0.72	0.57	0.52 *
LA	1.50	0.73	0.60	0.55
Dosage[2]				
H	1.53	0.72	0.58	0.53
S	1.49	0.73	0.58	0.53
Diet[3]				
E	1.53	0.76	0.62 *	0.57 **
A	1.50	0.70	0.54	0.49
SEM	.03	.04	.02	.01

[1] Lines HA and LA have been selected for high and low antibody response to SRBC, respectively.

[2] Dosages of SRBC used were H (2.50%) and S (0.25%).

[3] Diets E and A were high and low nutrient densities, respectively.

* $P \leq 0.05$, ** $P \leq 0.01$.

Table I.2. Mean ± SEM antibody titers of responders 5 days after inoculation with SRBC by age at inoculation, line, SRBC dosage and diet

Age	Line[1]		Dosage[2]		Diet[3]	
(days)	HA	LA	H	S	E	A
7	4.6 ± 0.5	3.8 ± 0.4	4.9 ± 0.5 *	3.3 ± 0.2	3.8 ± 0.3 †	5.0 ± 0.6
14	9.5 ± 0.4 **	3.5 ± 0.4	7.1 ± 0.7 *	5.9 ± 0.7	6.6 ± 0.7	6.5 ± 0.7
21	12.4 ± 0.3 **	3.7 ± 0.4	[4]			
28	[4]					

[1] Lines HA and LA have been selected for high and low antibody response to SRBC, respectively.

[2] Dosages of SRBC used were H (2.50%) and S (0.25%).

[3] Diets E and A were high and low nutrient densities, respectively.

[4] [redacted] significant interactions involving main effects.

Differences between lines, dosages, or diets within an age were significant at † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

Table I.3. Mean ± SEM for significant interactions of dosage with line and with diet for antibody titers 5 days after inoculation with SRBC at 28 days of age

	Line[1]		Diet[2]	
Dosage[3]	HA	LA	E	A
H	13.0 ± 0 **	6.6 ± 0.8	11.2 ± 0.7 *	9.3 ± 1.3
	NS	*	*	NS
S	12.9 ± 0.1 **	3.5 ± 0.3	8.9 ± 1.3 NS	9.2 ± 1.4

[1] Lines HA and LA have been selected for high and low antibody response to SRBC, respectively.

[2] Diets E and A were high and low nutrient densities, respectively.

[3] Dosages of SRBC used were H (2.50%) and S (0.25%).

* $P \leq 0.05$; ** $P \leq 0.01$; NS $P > 0.10$.

Table I. 4. Mean ± SEM antibody titers of responders 10 days after inoculation with SRBC by age at inoculation, line, SRBC dosage, and diet

Age	Line[1]		Dosage[2]		Diet[3]	
(days)	HA	LA	H	S	E	A
7	4.0 ± 0.4	3.3 ± 0.3	3.8 ± 0.3	4.0 ± 1.2	3.3 ± 0.4	4.3 ± 0.4
14	[4]				7.4 ± 0.7	7.3 ± 0.7
21	11.6 ± 0.3	**3.4 ± 0.4	7.9 ± 0.9	8.4 ± 0.8	8.4 ± 0.8	7.9 ± 1.0
28	[4]					

[1] Lines HA and LA have been selected for high and low antibody response to SRBC, respectively.

[2] Dosages of SRBC used were H (2.50%) and S (0.25%).

[3] Diets E and A were high and low nutrient densities, respectively.

[4] significant interactions involving main effects.

Difference between lines at age 21 was significant at $**P \leq 0.01$.

Table I.5. Mean ± SEM for significant interactions of line with dosage and diet for antibody titers 10 days after inoculation with SRBC at 14 and 28 day of age

	14 doa			28 doa					
	Dosage[2]			Diet[3]			Dosage[2]		
Line[1]	H		S	E		A	H		S
HA	9.4 ± 0.7	NS	9.3 ± 0.6	11.4 ± 0.4	NS	11.9 ± 0.4	11.6 ± 0.4	NS	11.8 ± 0.3
	**		**	**		**	**		**
LA	5.7 ± 0.6	**	2.6 ± 0.4	3.7 ± 0.4	*	2.5 ± 0.2	3.4 ± 0.4	*	2.5 ± 0.2

[1] Line HA and LA have been selected for high and low antibody response to SRBC, respectively.

[2] Dosages of SRBC used were H (2.50%) and S (0.25%).

[3] Diets E and A were high and low nutrient densities, respectively.

* $P \leq 0.05$, ** $P \leq 0.01$, NS $P > 0.10$

Table I.6. Mean ± SEM antibody titers of responders 20 days after inoculation with SRBC by age at inoculation, line, SRBC dosage and diet

Age	Line[1]		Dosage[2]		Diet[3]	
(days)	HA	LA	H	S	E	A
7	2.9 ± 0.5	2.5 ± 0.5	3.2 ± 0.7	2.3 ± 0.3	3.7 ± 0.9 †	2.2 ± 0.2
14	[4]				4.7 ± 0.4	4.7 ± 0.4
21	3.8 ± 0.3 **	2.4 ± 0.2	4.0 ± 0.4 **	2.7 ± 0.2	3.3 ± 0.3	3.6 ± 0.5
28	[4]				3.6 ± 0.3	3.5 ± 0.3

[1] Lines HA and LA have been selected for high and low antibody response to SRBC, respectively.

[2] Dosages of SRBC used were H (2.50%) and S (0.25%).

[3] Diets E and A were high and low nutrient densities, respectively.

[4] [ ] significant interactions involving main effects.

Difference between line, dosage, or diet were significant at † $P \leq 0.10$;** $P \leq 0.01$.

Chapter II

Diet and immunological memory of lines of White Leghorn chickens divergently selected for antibody response to sheep red blood cells

ABSTRACT Antibody responses to a first, second, and third injection with SRBC, and growth were studied in lines of White Leghorn chickens selected for high (HA) or low (LA) 5-d antibody titers to an i.v. inoculation with 0.1mL of a 0.25% suspension of SRBC. The experiment involved parallel studies on 2 groups of chicks hatched from the same matings of parental lines HA and LA at a 14-d interval. Chicks of each age-line subclass were fed either a high (E) or low (A) nutrient density diet from hatch onwards. When chicks of Hatches 1 and 2 were 28 and 14 d of age (doa) respectively, they were injected with 0.1mL of 0.25% suspension of SRBC, and antibody titers measured 3 and 6 d later. A second and a third injection of the same concentration of SRBC was given to chicks of each age-line-diet subclass at 10-d intervals and antibody titers measured 3 and 6 d after each injection in different chicks randomly sampled from each age-line-diet subclass.

After the first injection, antibody (primary) responses of HA chicks were higher than those of LA chicks regardless of age and diet. This difference (HA > LA) observed for the primary response was seldom evident in the responses to the second (secondary) and third (tertiary) injections. Antibody responses of LA chicks after the second and third injections were anamnestic. For HA chicks given the first injection at 28 doa, neither the secondary nor tertiary responses suggested anamnestic capacities, while there was apparent memory exhibited by the secondary and tertiary responses of HA chicks initially injected at 14 doa.

LA chicks were significantly heavier than HA chicks at all ages. Even though the higher nutrient density diet (E) increased BW of chicks of both lines, its effect on memory responses was sporadic. The results of this experiment show that, even though divergent selection has been successful in the primary responses, correlated responses in immunological memory were not always

observed, suggesting that the two types of responses might be under different genetic control.

(*Key Words*: sheep red blood cells, diet, body weight, antibody responses, immunological memory)

INTRODUCTION

Vaccination, an important tool in disease control, depends upon immunological memory to be effective. Recall antibody responses differ from primary antibody responses in several respects. They occur more rapidly, persist longer, attain higher titers, are of higher affinity, and isotype class switching takes place (Roitt, 1994; Ahmed and Gray, 1996). Immunological memory would seem to be influenced by genetic selection. Parmentier *et al*. (1996) reported that a line of chickens selected for high humoral response to SRBC antigen responded better to vaccination with viral antigens than a line selected in the opposite direction.

Ubosi *et al*. (1985) studied the kinetics of primary and secondary immunizations of a pair of chicken lines divergently selected for antibody response to SRBC. They reported that antibody titers peaked about 3 days after secondary immunization (PSI) rather than the 5-d peak following primary inoculation. An anamnestic response to the secondary inoculation was, however, observed only in the low antibody response line (Ubosi *et al*., 1985). Responses of these lines to secondary inoculations with SRBC were also studied by Martin *et al*. (1989) who found memory response only in the low response line. According to Pinard *et al*. (1992) the lower secondary response in their high antibody line might adversely affect the effectiveness of vaccination. None of the above studies measured responses to further inoculations or the effect of nutrition which may influence immune responses in chickens (Cook, 1991). Yet in commercial poultry production, it is common to give sequential vaccinations of a particular antigen especially to breeding flocks (Bains, 1979), and diets vary. Also, the effects of such factors as age on immunological memory in chickens needs elucidation. This paper reports on an investigation into the effects of nutrient density on memory responses of lines of chicken divergently selected for antibody response to SRBC.

MATERIALS AND METHODS

Stocks, Diets, and Husbandry

The chicks used in this experiment were progeny from White Leghorn lines that had undergone 23 generations of selection for high (HA) or low (LA) antibody response to 0.1mL of a 0.25% suspension of SRBC given intravenously between 41 and 51 days of age (Martin *et al.*, 1990). Two diets which differed in nutrient density were fed in mash form throughout the experiment. The lower density diet (A), which was also the diet under which the lines had been selected, contained 20% crude protein and 2685 kcal ME/kg. The higher nutrient density diet (E) contained 24% crude protein and 3146 kcal ME/kg.

Two groups of chicks (192 total) were hatched at a 14-d interval from the same matings of Lines HA and LA. At hatch, 96 chicks per line were wingbanded, vaccinated for Marek's disease, randomized into groups of 48 and placed in floor pens covered with litter. For each hatch, half of the chicks from each line was randomly assigned to each diet. Feed and water were provided *ad libitum* and lighting was continuous. These husbandry practices were consistent with those under which the lines were selected. Chicks were individually weighed at 14, 28, and 38 doa and sex determined on Day 38.

When chicks from Hatches 1 and 2 were 28 and 14 d of age (doa) respectively, they were injected with 0.1mL of 0.25% suspension of SRBC. Three and 6 d later, 8 of the injected chicks from each line-diet-age subclass were randomly chosen, bled, and their plasma tested for total SRBC antibody (Wegmann and Smithies, 1966). Antibody titers were expressed as the $\log_2$ of the reciprocal of the last dilution in which agglutination was macroscopically observed. Ten d after the first (primary) injection, chicks not bled for post primary antibody response (PPI) were injected again with 0.1mL of 0.25% SRBC suspension. Three and 6 d later blood was obtained from 16 different, but

randomly chosen, chicks from each line-diet-age subclass of those given the second as well as those given only the first inoculation. Plasma was tested for SRBC antibody as before.

Ten d after the second injection, the 16 remaining chicks from each line-diet-age subclass that had not been bled, were given a third injection of SRBC. Eight of these chicks, together with 8 each of those which received first and second injections respectively, were randomly selected and bled 3 and 6 d later. Throughout the experiment, no chick was bled on consecutive bleeding days.

Statistical Analysis

Individual BWs were transformed to common logs and subjected to ANOVA (SAS, 1985) with line, diet, sex, age at initial injection, type of response, and interactions among them as the main sources of variation. A preliminary analysis of antibody titers using the model for BW, showed a lack of sex effects and interactions involving sex. Because of significant interactions involving age at initial injection, subsequent analyses were carried out within age at first injection (14 or 28 d) with line, diet, type of response, and interactions among them as the main sources of variation. When chicks were bled after repeated injections, multiple types of responses resulted (i.e., primary, secondary, and tertiary). Comparisons between lines were biased by the extreme differences between them for primary antibody responses to SRBC. Therefore a particular type of response at a given bleeding time was analyzed by ANOVA within age with line, diet, and the interaction between them as the main sources of variation. Tests of significance were at $P < 0.05$.

RESULTS

Body Weight at Various Ages

The number of injections of SRBC a chick received, and the group in which it was hatched, had no influence on the BW at 14, 28, or 38 doa. Thus, hatches were pooled for data summarized in Table 1. Only 5 of 78 possible interactions were significant and

they were considered unimportant for further analysis. At all ages Line LA males and females were heavier than those of Line HA, chicks fed Diet E were heavier than those fed Diet A, and males were heavier than females.

Antibody Responses to Primary Inoculation

Antibody titers measured 3 days after the first injection with SRBC (3PPI) were the lowest throughout the study (Table 2). For both age groups HA chicks produced higher titers than LA chicks, with no difference between diets. Six days after the initial injection (6PPI) antibody titers increased several fold over those for 3PPI. The increase was noted especially in HA chicks of both ages. Line HA chicks maintained higher titers (6PPI) than those of Line LA regardless of diet or age of initial injection. Moreover, the older HA chicks had a greater 6-d antibody response (6PPI) than the younger HA chicks (12.6 ± 0.4 *vs* 7.9 ± 0.6), while in LA chicks 6PPI titers were not influenced by age at initial injection. Also, the 6-d titers for HA chicks were the highest primary response observed in the experiment. The highest primary responses for LA chicks, however, occurred after 6-d PPI (Table 2).

Antibody Responses to Secondary Inoculation

In all comparisons, 3-day secondary antibody responses (3PSI) were similar to or higher than 3-(3PPI) and 6-d (6PPI) primary responses (Table 2). The increase in the case of LA chicks, was three- to four-fold for the chicks given the initial injection at 14 doa. For those LA chicks given the initial injection at 28 doa, however, the increase was less.

Primary responses of chicks not given the second injection (13PPI) were declining in HA but not LA chicks. For chicks injected initially at 14 doa, the line by type of response interaction was significant because in HA chicks, 3PSI titers were similar to the declining primary titers (13PPI), whereas in the LA chicks, titer 3PSI far exceeded those 13PPI. For chicks injected initially at 28 doa, differences due to line (HA > LA)

were significant. Types of response differed when chicks were fed Diet E (3PSI > 13PPI) but were similar when fed Diet A.

When types of responses were compared in chicks initially injected at 14 doa, there was a line difference (HA > LA) for 13PPI but not for 3 PSI. However, for chicks injected initially at 28 doa, line differences (HA > LA) were observed for both 13PPI (9.8 ± 0.8 *vs* 2.9 ± 0.4) and 3PSI (11.9 ± 0.6 *vs* 5.5 ± 1.2). For the 13PPI titers of these chicks, those fed the lower density diet (the one in which the lines had been selected) generally sustained higher antibody titers (7.1 ± 0.7 *vs* 5.6 ± 0.6) than those fed the higher density diet.

Six days after the second injection, antibody responses (16PPI, 6PSI) of chicks initially injected at 14 doa reflected line by diet and line by type of response interactions (Table 3). The line by diet interaction occurred because although there was no line difference for chicks fed Diet A, titers were higher for HA than LA chicks when fed Diet E. Conversely, titers were higher for HA chicks fed Diet E than A whereas no diet differences were observed for LA chicks. The line by type of response interaction was due to a line difference (HA > LA) in titers of chicks given only the first injection (16PPI), but not for those given the second injection.

For chicks injected initially at 28 doa, the magnitude of the residual primary inoculation (16PPI) compared with 6PSI depended upon diet. The interactions occurred because chicks fed Diet A had higher primary titers than those fed Diet E (6.6 ± 0.7 *vs* 4.6 ± 0.6), whereas no dietary differences (A = 10.9 ± 0.5 *vs* E = 11.5 ± 0.4) were found for the secondary responses.

Antibody Responses to the Third Injection

The pattern of response after the third injection showed consistent increases in titers from 3 to 6 d after injection (Table 2). Comparisons among 3-d tertiary antibody responses (3PTI), 13-d secondary titers (13PSI), and 23-d primary titers (23PPI) for chicks bled on the same day, revealed a line by diet

by type of response interaction for chicks initially injected at 14 doa. Subsequent analyses were undertaken within line-diet-type of response subclasses. A consistent pattern emerged for all cases in that 3PTI titers were higher than 13PSI which in turn exceeded titers of chicks injected only once (23PPI). In contrast, for the older chicks (initially injected at 28 doa), rankings of the type of response depended upon the line-diet subclass (Table 2). Except for LA chicks fed Diet A where titers 13 PSI were lower than those 3PTI, the titers 13PSI and 3PTI were similar and both higher than 23PPI. A more detailed analysis of individual types of response revealed that for 23PPI titers of both age groups, Diet E was associated with higher titers than Diet A. Line effects (HA > LA) influenced 23PPI and 13PSI but not 3PTI titers in the 28-day injected group, and only 23PPI titers in chicks initially injected at 14 doa.

In the younger chicks injected initially at 14 doa, rankings of the type of response were: 6-d post tertiary inoculation response (6PTI) being the highest, followed by 16-d secondary response (16PSI), with 26-d primary titer (26PPI) the least. When lines and diets were compared for each of the three antibody responses, it was evident that HA chicks had higher residual primary responses (26PPI) than LA chicks (2.9 ± 0.3 *vs* 2.1 ± 0.2) whereas line differences were lacking for the 16-d secondary responses (6.0 ± 0.5 *vs* 4.7 ± 0.8) and the 6-d tertiary responses (11.2 ± 0.5 *vs* 10.7 ± 1.0).

For chicks injected initially at 28 doa and bled 6-d after the third injection, the tertiary antibody titers (6PTI) were higher than the other responses (16PSI and 26PPI) measured that day. Chicks of Line HA fed Diet E and LA chicks fed Diet A had similar secondary (16PSI) and primary (26PPI) responses. In contrast, for HA chicks fed Diet A and LA chicks fed Diet E, the 16PSI titers surpassed 26PPI titers (Table 2). When analyzed separately, the differences between lines (HA > LA) influenced 26PPI titers of both age groups, as well as the 6PTI titers of

the 28-d injected group. There was no difference between the secondary residual (16PSI) and the tertiary (6PTI) titers of chicks initially injected at 14 doa.

DISCUSSION

The lines involved in this experiment have been developed through selection for high or low primary antibody responses 5 d after a single injection with SRBC administered intravenously between the ages of 41 and 51 days (Siegel and Gross, 1980). Chicks from the line selected for low antibody response to SRBC were heavier than those selected for high antibody response to SRBC, a result consistent with previous data for these lines (Martin *et al.*, 1990; Boa-Amponsem *et al.*, 1998) and for similar lines developed in the Netherlands (Kreukniet *et al.*, 1994; Parmentier *et al.*, 1996, 1998). Also males were heavier than females and chicks fed higher nutrient density diets had higher BW than those fed lower nutrient diets, a well documented phenomena for chickens. Several reports on these lines indicate that the high responder line reaches maximum titers at between 5 and 7 d after an initial injection with SRBC (Siegel and Gross, 1980; Ubosi *et al*., 1985; Martin *et al*., 1989). The present report is consistent with these findings regarding peak antibody response of the HA line and further indicates that this peak period was not influenced by diet or age of chicks when initially injected.

The benefits of repeated inoculations with the same antigen appeared consistent throughout the experiment when the booster responses were compared with non-booster titers, as have been previously reported for these lines (Ubosi *et al*., 1985; Martin *et al*., 1989). The question arises, however, as to whether the response to repeated inoculations resulted from immunological memory mechanisms or was simply an enhanced primary response due to further maturation of the immune system with age. Accelerated responses associated with re-exposure to antigen are due principally to increases in the frequency of antigen-specific

immune cells which are of higher affinity than those involved in primary responses (Roitte, 1994; Ahmed and Gray, 1996).

The results of the present work suggest that both 3 and 6-d secondary responses in LA chicks exhibited clear, definite immunological memory regardless of age of initial injection with SRBC antigens (Figure 1). The responses were rapid and achieved higher titers than the primary titers. In the case of the HA chicks initially injected at 28 doa, neither the secondary nor the tertiary responses suggested an anamnestic response. The secondary responses of chicks of the HA line injected initially with SRBC at 14 doa, however, appeared to be anamnestic. It is noteworthy that these chicks were given the second injection at 24 doa. The similarity of the secondary titers of these chicks with the primary titers of chicks of the same line injected initially at 28 doa, suggests that antibody responses in this line are probably due to primary mechanisms influenced by age. The memory responses of these lines as discussed above so far agree with those of Ubosi *et al*. (1985) and Martin *et al*. (1989), as well as lines reported by Pinard *et al*. (1992). They also suggest that a high initial response may preclude resources available for anamnestic responses from immunological memory.

Tertiary responses observed in our experiment did not exceed secondary responses in most cases, which is contrary to expectations (Roitt, 1994; Coligan *et al*., 1994). This inconsistency may have been due to the presence of high levels of residual secondary antibody at the time of the third injection which neutralized a proportion of the SRBC antigen. Although the higher nutrient density diet improved BW of both lines, its effect on memory responses appears sporadic. The results of this experiment show that even though divergent selection has been successful in the primary responses, no correlated responses in immunological memory have been achieved suggesting that the two types of responses are under different genetic control.

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TABLE II.1. Means and SEM for body weights (g) of chicks by sex, line, diet, and age

Sex	Line[1] or Diet[2]		Age (d) 14	28	38
♂♂	Line	HA	96 ± 2 **	197 ± 6 **	306 ± 9 **
		LA	106 ± 2	222 ± 5	334 ± 6
	Diet	A	98 ± 2 **	199 ± 5 **	300 ± 6 **
		E	104 ± 2	220 ± 6	340 ± 9
♀♀	Line	HA	88 ± 3 **	165 ± 5 **	245 ± 7 **
		LA	101 ± 2	189 ± 5	281 ± 6
	Diet	A	91 ± 2 **	166 ± 4 **	248 ± 6 **
		E	98 ± 3	188 ± 6	278 ± 7

** Within an age, differences between lines and between diets are significant ($P \leq 0.01$).

[1] Lines HA and LA were selected for high and low response to SRBC, respectively.

[2] Diets A and E were of lower and higher nutrient density, respectively.

TABLE II.2. Means and SEM for SRBC antibody titers of chicks by age at first injection, line, diet, and type of response

Age (d) at first injection	Line[1]	Diet[2]	Type of response	Days after SRBC injection: PPI 3 / PSI – / PTI –	6 / – / –	13 / 3 / –	16 / 6 / –	23 / 13 / 3	26 / 16 / 6
14	HA	A	PPI	1.5 ± 0.3 a	7.5 ± 0.2 a	7.0 ± 0.8	4.5 ± 0.5 x	2.9 ± 0.2 x	2.8 ± 0.3x
			PSI	–	–	8.5 ± 1.1	10.0 ± 0.7 y	6.1 ± 0.7 y	5.9 ± 0.4y
			PTI	–	–	–	–	8.1 ± 0.9 z	11.0 ± 0.5z
		E	PPI	1.6 ± 0.2 a	8.3 ± 0.9 a	6.8 ± 0.9	6.5 ± 0.3 x	5.3 ± 0.6 x	2.9 ± 0.3x
			PSI	–	–	9.0 ± 1.1	12.3 ± 0.5 y	7.1 ± 0.5 y	6.1 ± 0.6y
			PTI	–	–	–	–	9.6 ± 0.7 z	11.4 ± 0.5z
	LA	A	PPI	1.0 ± 0.6 b	2.8 ± 0.4 b	3.1 ± 0.4 x	2.8 ± 0.5 x	2.1 ± 0.3 x	2.1 ± 0.2x
			PSI	–	–	11.1 ± 0.6 y	10.5 ± 0.5 y	5.8 ± 0.6 y	4.0 ± 0.5y
			PTI	–	–	–	–	7.8 ± 0.8 z	9.4 ± 0.9z
		E	PPI	1.0 ± 0.6 b	2.3 ± 0.3 b	2.6 ± 0.6 x	2.3 ± 0.6 x	1.3 ± 0.2 x	2.1 ± 0.2x
			PSI	–	–	8.8 ± 1.2 y	9.6 ± 1.2 y	5.7 ± 0.6 y	5.4 ± 1.0y
			PTI	–	–	–	–	10.3 ± 0.6 z	11.9 ± 1.0z
28	HA	A	PPI	2.4 ± 0.3 a	13.0 ± 0.0 a	10.8 ± 1.0	9.0 ± 0.7 x	3.0 ± 0.4 x	4.4 ± 0.6x
			PSI	–	–	12.0 ± 0.4	11.9 ± 0.6 y	8.0 ± 0.5 y	6.5 ± 0.7y
			PTI	–	–	–	–	8.5 ± 0.8 y	11.8 ± 0.5z
		E	PPI	3.0 ± 0.5 a	12.1 ± 0.7 a	8.8 ± 0.8 x	6.0 ± 0.6 x	4.8 ± 0.6 x	4.0 ± 0.5x
			PSI	–	–	11.6 ± 0.8 y	12.5 ± 0.3 y	7.1 ± 0.8 y	3.6 ± 0.3x
			PTI	–	–	–	–	8.8 ± 0.8 y	11.8 ± 0.5y
	LA	A	PPI	1.5 ± 0.3 b	2.4 ± 0.3 b	3.4 ± 0.4	4.3 ± 0.4 x	1.4 ± 0.3 x	2.1 ± 0.2x
			PSI	–	–	5.9 ± 1.8	10.1 ± 0.7 y	4.1 ± 0.6 y	3.9 ± 0.5x
			PTI	–	–	–	–	7.6 ± 0.8 z	9.1 ± 1.2y
		E	PPI	1.1 ± 0.3 b	2.1 ± 0.4 b	2.4 ± 0.3 x	3.3 ± 0.7 x	2.1 ± 0.4 x	2.0 ± 0.3x
			PSI	–	–	5.0 ± 0.6 y	10.5 ± 0.6 y	5.7 ± 0.9 y	4.9 ± 0.6y
			PTI	–	–	–	–	7.1 ± 0.8 y	11.1 ± 0.7z

a,b Means in a column within an age group with no common letter differ significantly ($P \leq 0.05$).
x,y,z Means in a column within an age-line-diet subclass with no common letter among type of response differ significantly ($P \leq 0.05$).
[1] Lines HA and LA were selected for high and low response to SRBC, respectively.
[2] Diets A and E were of lower and higher nutrient densities, respectively.
[3] Responses PPI, PSI, and PTI represent after chicks given 1, 2 or 3 injections of SRBC. Subsequent injections were given 10 and 20 d after the first injection.

TABLE II.3. Means and SEM for line by diet and line by type of response of SRBC titers

Line[1]	Diet[2] A	Diet[2] E	Type of response[3] 16 PPI	Type of response[3] 6PSI
HA	7.3 ±0.8 *	9.4 ± 0.8	5.5 ±0.4 **	11.1 ±0.5
	NS	**	**	NS
LA	6.6 ±1.1 NS	5.9 ± 1.1	2.5 ±0.4 **	10.1 ±0.6

* $P \leq 0.05$; ** $P \leq 0.01$; NS when $P \leq 0.05$.

[1] Lines HA and LA were selected for high and low response to SRBC, respectively.

[2] Diets A and E were of lower and higher nutrient density, respectively.

[3] Chicks were 14 doa when given the first inoculation and 24 doa when given the second inoculation of SRBC. PPI and PSI represent SRBC antibody titers measured 16 and 6 days after receiving the first and second inoculation of SRBC.

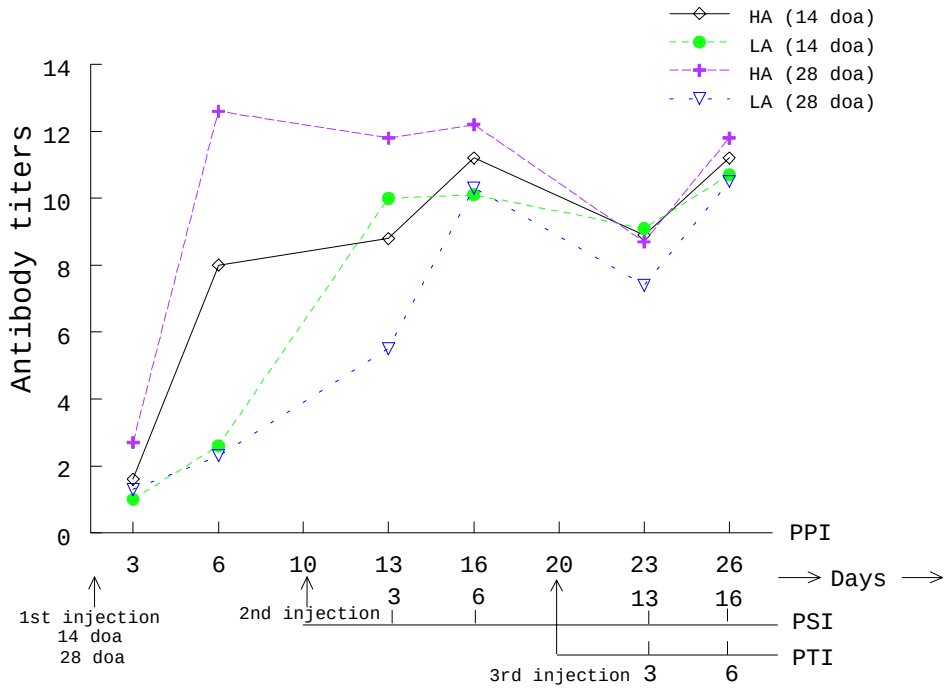

Figure II.1. Antibody responses of HA and LA chicks injected initially at 14 or 28 doa and subsequently injected 10 and 20 days later. Responses were measured at 3 and 6 d post primary (PPI), post secondary (PSI), and post tertiary (PTI) injections.

CHAPTER III

Antibody transmitting ability of hens from lines of chickens differing in response to SRBC antigen

ABSTRACT 1. Hens from White Leghorn lines selected for high (HA) or low (LA) antibody response to sheep red blood cells (SRBC) were inoculated with 0.1mL of either 0.25% or 2.50% SRBC suspension. Eggs laid over the next 15 days were grouped into 5, 3-day collection periods and incubated. Maternal antibody titres were determined in chicks at hatch and 7 days after hatch. In a subsequent experiment, hens of the two lines were inoculated with 0.1mL of 2.50% suspension of SRBC, and eggs laid on days 10 through 13 after inoculation were incubated. Maternal antibody titres were determined in 15 and 18-day embryos as well as in chicks at 0, 5, 10, 15, 20, and 25 days after hatch.

2. Dosage of SRBC had no effect on the antibody titres in line HA, however, the higher dosage elicited greater antibody titres than the lower dosage in line LA.

3. Maternal antibodies were detected earlier in chicks of line HA (eggs laid on days 7 → 9) than those of line LA (eggs laid on days 10 → 12) regardless of dosage administered to the hens.

4. In both lines, antibodies specific to SRBC were observed on day 15 of incubation, with the frequency of responders greatest at hatch. The high frequency of HA responders was maintained for 15 days after hatch, whereas there was an immediate decline with age in LA responders.

5. It was concluded that lines HA and LA have diverged in the pattern of maternal antibody levels as a result of the divergent selection for antibody response to SRBC.

INTRODUCTION

Although control of some diseases of chickens is attainable through vaccination, chicks aged 21 days or younger may respond poorly to vaccination because their immune system has not matured (Schwartz, 1994). Protection of chicks through passive immunization was reported for *E. coli* (Heller *et al.,* 1990), with the level of humoral immune response of offspring more a maternal than a paternal effect (Leitner *et al*., 1994). Also, the presence or absence of maternal antibody can contribute to a maternal effect for viability of progeny (Nordskog and Pevzner, 1977), especially during the early period after hatch. Pinard and van der Zijpp (1993) obtained results which suggested that maternal or sex-linked effects were important in antibody titres to SRBC in lines of chickens selected for immune response to SRBC antigen. Differences in the response of reciprocal crosses between lines of chickens selected for high or low antibody titres to SRBC antigen indicated maternal effects for resistance to avian adenovirus group II (Gross *et al*., 1988). The experiments reported here were designed to determine whether lines selected divergently for response to SRBC antigen differed in their ability to transmit SRBC antibodies to their progeny and whether this ability was influenced by dosage of antigen.

MATERIALS AND METHODS

Stocks and reproduction

Age contemporary chickens from lines of White Leghorns divergently selected for high (HA) or low (LA) antibody production 5 days after inoculation intravenously with 0.1mL of a 0.25% suspension of sheep red blood cell (SRBC) antigen (Siegel and Gross, 1980; Martin *et al*., 1990) were used in the 2 experiments. Hens maintained in single-bird cages with feed and water provided *ad libitum* were artificially inseminated weekly

with pooled semen from males of their respective lines. Prior to each experiment, parents tested negative for SRBC antibody.

Experiment 1

Thirty hens, 72 weeks of age from generation S_{22} of lines HA and LA were assigned to 2 groups of 15 hens each. One group was inoculated in the brachial vein with 0.1mL of 0.25% SRBC suspension and the other with 0.1mL of 2.50% SRBC suspension. Egg collection commenced 24 h after inoculation. Eggs collected on 3 consecutive days constituted a group, with 5 such groups at the end of the 15-day collection period. Eggs were stored in a climatically controlled room and turned twice daily prior to incubation. The day before the last egg collection, hens were bled through the brachial vein for SRBC antibody determination by the microtitre procedure of Wegmann and Smithies (1966). Blood was collected into tubes containing 2 drops of EDTA as anticoagulant, refrigerated, and plasma tested for antibody within 24 h of collection.

At hatch, blood was obtained from 6 chicks of each line - dosage - period of collection subclass and determination of total SRBC and 2-mercaptoethanol (2-MER) antibodies made by the microtitre methods of Wegmann and Smithies (1966) and Delhanty and Solomon (1966) respectively. The remaining chicks were wingbanded and placed in electric battery brooders with wire floors (20 pens) by subclass groups. A mash diet (200g protein/kg; 11.2 MJ AME/kg) and water were available at all times. Lighting was continuous.

Seven days after hatch, the remaining chicks were bled and plasma tested for total SRBC and 2-MER antibodies as previously described. Antibody titres were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination.

Antibody titres of the hens were subjected to ANOVA with line, SRBC dosage, and the interaction between them as main variables. Total and 2-MER antibody titres of the progeny were analysed by ANOVA within each bleed day. Main effects were line, SRBC

dosage, period of egg collection, and interactions among them. When interactions were significant, separate analyses were conducted within each main effect. Significance was at $P \leq 0.05$.

Experiment 2

At 31 weeks of age 60 S_{23} generation HA and LA hens were inoculated in the brachial vein with 0.1mL of 2.50% SRBC suspension, which was the dosage which elicited greater antibody levels in experiment 1. Eggs laid 10 through 13 days after inoculation were incubated. Chicks were removed from the hatcher on day 22 of incubation and reared in electric battery brooders with wire floors as in experiment 1.

Blood for the determination of SRBC and 2-MER antibody titres was obtained from 10 embryos or chicks from each line at 15 and 18 days of incubation, and at 0, 5, 10, 15, 20, and 25 days after hatching. Assay procedures were the same as those described for experiment 1.

The frequency of progeny from the 2 lines that had detectable SRBC antibodies (responders) was tested within ages (*i.e.*, bleed days) by Fisher's exact test. Antibody titres of responders were analysed by ANOVA with line, age, and the interaction between them as main variables. When interactions were significant, separate analyses were conducted within each main effect. Significance was at $P \leq 0.05$.

RESULTS

Experiment 1

Antibody titres of hens

There was a significant interaction between lines and SRBC dosages for antibody titres of hens. Analysis within main effects showed that the interaction was because there was no difference between dosages in antibody response for line HA, whereas for line LA the higher dosage elicited greater response than the lower dosage (Fig. 1).

Antibody titres of progeny

Because the line-dosage-collection period interactions were significant for both SRBC and 2-MER titres, analyses were

conducted within collection periods. Regardless of dosage, no antibodies specific to SRBC were detected in chicks hatched from eggs collected on days 1 → 6 (Table 1). From eggs produced on days 7 → 9 (period 3) SRBC antibodies were detected in HA but not LA chicks. In both lines chicks from eggs laid on days 10 → 12 (period 4) by hens which received the higher SRBC antigen dosage had at hatch higher antibody titres (4.1±0.5) than those from hens given the lower dosage (2.5±0.4) There were dosage-line interactions for period 3 for chicks at hatch and days 10 → 12 (period 4) when chicks were 7 days of age (Table 2). The interactions occurred because while there was a dosage effect on antibody titres in HA progeny, there was no dosage effect in LA progeny.

Experiment 2

The pattern of antibody transfer from hens to progeny varied between lines (Fig. 2). In general, antibodies were detected in embryos on day 15 of incubation with the frequency of responders greater in HA than LA chicks. Frequency of responders increased with time of incubation in both lines. Response patterns showed a divergence after hatch, with the decline in LA chicks such that by 25 days of age only 20% had detectable antibody. In contrast, all HA chicks maintained a detectable response through 15 days after hatch, with 70% of HA chicks still testing positive 25 days after hatch (Fig. 2).

When analysis included only those chicks exhibiting detectable antibody titres, means for line LA did not change over the entire period of the experiment (Fig. 3). For line HA, however, antibody titres changed over time. Comparisons between lines at each age showed higher titres for line HA than LA in 18-day embryos and in chicks through 10 days after hatch. Standard errors of titres at these ages ranged from 0.23 to 0.28 except for day of hatch when it was 0.41. The results for 2-MER antibodies were similar to those for total SRBC antibodies. This consistency demonstrated that maternal antibodies were of the IgG type.

DISCUSSION

The divergence in antibody response to SRBC antigen between hens of lines HA and LA used to produce the chicks reported on here, has earlier been documented as a correlated response to selection for this trait in juveniles (Siegel *et al.*, 1982). The higher antibody titres of HA hens within dosages, however, has not been reported even though such differences at the selection age exist for these populations (Ubosi *et al*., 1985), and similarly selected chicken lines (Kreukniet and van der Zijpp, 1990). Such a difference in response to dosage suggests a difference in the proportion of high affinity lymphocytes specific for SRBC antigen.

Previous studies with reciprocal crosses (Gross *et al*., 1988; Pinard and van der Zijpp, 1993) suggested that maternal effects may have diverged from selection for high or low antibody response to SRBC antigen in young chickens. To our knowledge, however, the data presented in this report are the first to specifically demonstrate this effect. Working with *E. coli*, Heller *et al*. (1990) observed a positive correlation between a hen's antibody titre and the level of maternal antibody in her chicks at hatching. Such an inference cannot be generalized in our experiment because level of maternal antibody in chicks was influenced by the days after inoculation of hens, genetic line, and dosage of SRBC antigen.

Our results suggest that, in selecting for high and low antibody response to SRBC, divergence also occurred in the time required for maternal antibody to be transferred into the egg, level of antibody in the 18-day embryo, duration of peak maternal antibody in the progeny, and the rate of decline in antibody response in progeny. The implication of these results is that differences exist among genetic stocks in response to passive immunization.

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TABLE III.1

Mean ± SEM of SRBC antibody titres at hatch and 7 days after hatch of progeny from HA and LA hens[1] inoculated with SRBC. Experiment 1

Collection Period	Collection[2] Days	At hatch HA	At hatch LA	7 days HA	7 days LA
1	1 → 3	NR[3]	NR	NR	NR
2	4 → 6	NR	NR	NR	NR
3[4]	7 → 9	4.0 ± 0.3	NR	2.4 ± 0.3	NR
4	10 → 12	4.7 ± 0.3 **	2.1 ± 0.3	3.8 ± 0.3	**1.3 ± 0.1
5	13 → 15	3.3 ± 0.2 **	2.4 ± 0.3	2.2 ± 0.2	**1.3 ± 0.2

** A highly significant ($P \leq 0.01$) difference between pairs of means.

[1] HA and LA: lines selected for high or low antibody response to SRBC, respectively.

[2] Eggs from days after hens were inoculated with SRBC.

[3] No progeny exhibited SRBC antibody.

[4] Data not analyzed because of no variation in line LA (*i.e.*, zero response).

TABLE III.2

Mean ± SEM of SRBC antibody titres at hatch and 7 days after hatch of progeny for egg collection periods where there were line[1] by SRBC antigen dosage interactions. Experiment 1

SRBC dose	At hatch[2]		7 days[3]	
	HA	LA	HA	LA
0.25%	3.0 ±0.4 ** *	NR[4]	3.0 ±0.4 ** *	1.2 ±0.1
2.50%	4.5 ±0.3 **	NR	4.4 ±0.2 **	1.6 ±0.2

*,** significant ($P \leq 0.05$) and highly significant ($P \leq 0.01$) difference between pairs of means, respectively.

[1] HA and LA: lines selected for high or low antibody response to SRBC, respectively.

[2] Eggs from days 7 → 9 after hens were inoculated with SRBC.

[3] Eggs from days 10 → 12 after hens were inoculated with SRBC.

[4] No progeny exhibited SRBC antibody.

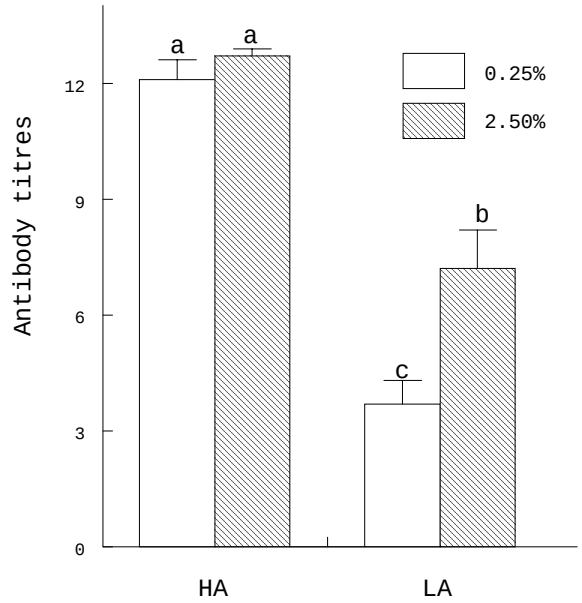

FIG. III.1. Means and SEM of antibody titres of HA and LA hens inoculated with 0.1mL of 0.25% or 2.50% SRBC. Means with no common letters differ at $P \leq 0.05$. Experiment 1.

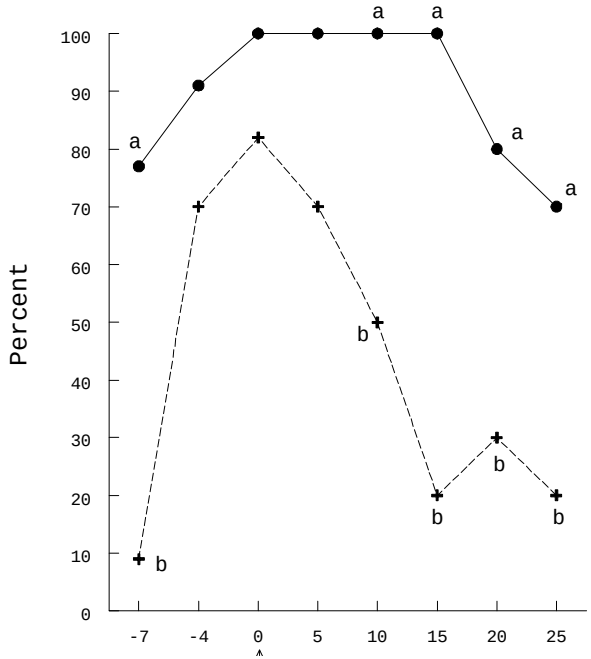

FIG. III.2. Percent of responders to SRBC antigen of embryos and chicks (↑ is day of hatch) of lines HA and LA. The -7 and -4 days = 15 and 18 days of incubation. a,b within a day values with no common letter differ significantly ($P \leq 0.05$). Experiment 2.

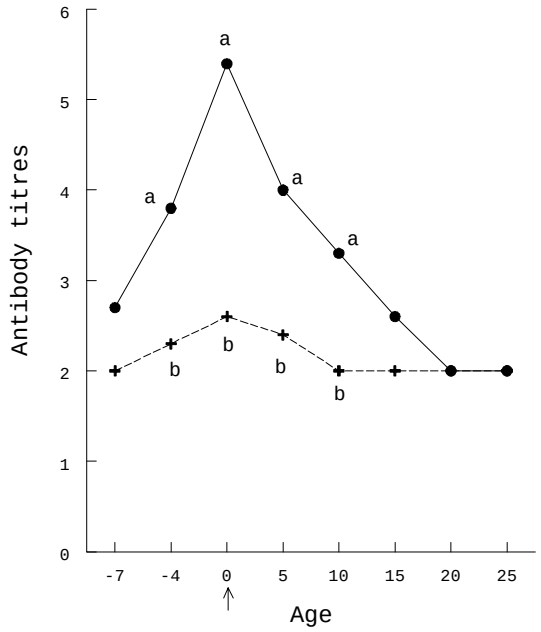

FIG. III.3. Antibody titres of embryos and chicks (↑ is day of hatch) of responders for lines HA and LA. The -7 and -4 days = 15 and 18 days of incubation. a,b within a day values with no common letter differ significantly ($P \leq 0.05$). Experiment 2.

Chapter IV

Genetic Architecture of Antibody Responses of Chickens to Sheep Red Blood Cells

Introduction

Genetic resistance to disease development is complex and involves several systems of the body with the immune system being an important component (Warner et al. 1987; Male and Roitt 1993). Selection for resistance to particular diseases tends to be specific and has little effect on general disease resistance required in modern livestock and poultry production (Carson 1951; Gavora 1990). Correlated responses to selection for antibody titers to foreign proteins provides resistance to a wider range of diseases than would be achieved by selection for individual disease resistance, however, the enhanced resistance can not be generalized to all diseases. In a two-way selection experiment for high or low antibody response to sheep red blood cell (SRBC) antigens, the high line exhibited stronger antibody to Newcastle disease, was more resistant to *Mycoplasma gallisepticum*, *Eimeria necatrix*, a splenomeglia virus, and feather mites, but was more susceptible to *Escherichia coli* and *Staphylococcus aureus* infection than the low line (Gross et al. 1980). Subsequent studies with these lines showed greater resistance by the high than low line to *Eimeria tenella* (Martin et al. 1986) and that the high line exhibited greater immunoresponsiveness to *Brucella abortus* than the low line (Dunnington et al. 1992).

There is need to understand more about relationships between general immune response and disease resistance. Working with immune responses has an added benefit in that commercial breeding populations need not be exposed to infectious agents. Efficient utilization of genetic variation in immunoresponsiveness in livestock and poultry breeding where crossing of populations predominates requires an understanding of the modes of inheritance of immune traits. Such information on poultry is rather limited. Considerable responses to selection and moderate realized heritability estimates (Siegel and Gross 1980; Martin et al. 1990; Pinard et al. 1992) suggest substantial additive genetic variation for antibody response to SRBC antigens. In addition,

the influence of major genes of the MHC on immunoresponsiveness has been reported (e.g., GÜNTHER et al. 1974; DUNNINGTON et al. 1989), although its importance relative to the rest of the genome appeared minor (PINARD and VAN DER ZIJPP 1993). Heterosis appeared important in some studies (SIEGEL et al. 1982), but was minimal in others (PINARD and VAN DER ZIJPP 1993).

Through long-term divergent selection, lines of chickens have been developed that differ markedly in antibody response to SRBC. This paper reports on the genetic architecture of response to SRBC antigen based on various crosses of these lines of chickens.

Materials and methods

The experiment involved two lines of White Leghorn chicken derived from the same base population but selected divergently for high (HH) and low (LL) antibody response 5 days after an injection into the brachial vein with 0.1mL of 0.25% suspension of SRBC antigen between the ages of 41 and 51 days (SIEGEL and GROSS 1980; MARTIN et al. 1990). Matings between age-contemporary chickens from the S_{22} generation of these lines were made to produce the parental lines and reciprocal F_1 generation crosses. Chicks were housed in deep litter pens based on mating combination. At 126 days of age, 15 cockerels and 60 pullets of these 4 populations i.e., HH, HL, LH, LL (first letter denotes sire line) were randomly selected and housed in individual cages in an environmentally controlled room.

At 36 weeks of age complete diallel matings were made among the dams and sires of the 4 populations to produce 16 progeny types consisting of parentals, reciprocal F_1, F_2, and backcrosses. At hatch, 100 straight-run chicks of each progeny type were wingbanded, vaccinated for Marek's disease and placed in floor pens covered with litter. Chicks were fed *ad libitum* a mash diet containing 20% crude protein and 2685 kcal ME/kg. Lighting and water were available continuously.

At 36 days of age, 10 chicks of each sex-progeny type subclass were inoculated through the brachial vein with 0.1mL of

0.25% suspension of SRBC antigen. Five and 12 days later, a sample of 0.5 mL of blood was obtained from the brachial vein of each chick and transferred to tubes containing 2 drops of the anticoagulant ethylene diamine tetraacetate (EDTA). Blood was refrigerated for 24 hours to allow red blood cells to settle. Antibody determination from plasma followed the microtiter hemagglutination procedure of WEGMANN and SMITHIES (1966). Titers were expressed as $\log_2$ of the reciprocal of the last dilution in which agglutination was macroscopically observed.

Antibody titers 5 and 12 days after inoculation with SRBC were subjected to an analysis of variance with progeny type, sex and the interaction between them as the main variables. The relationship between antibody titers and the proportion of line HH genes in the progeny type was examined graphically and also with a polynomial regression model which fitted linear, quadratic and cubic effects sequentially (FREUND and LITTELL 1981).

Genetic analysis was undertaken within sexes and with sexes pooled in order to study sex-linked and maternal effects on antibody production. The models of DICKERSON (1969) and NOTTER (1987) provided useful definitions for several crossbreeding parameters such as recombination and maternal heterosis, however they ignored sex-linkage. EISEN et al. (1966) and BARBATO (1991) gave the framework for separating maternal and sex-linked effects pertinent for male homogametic species such as poultry. Contrasts were conducted based upon those models (EISEN et al. 1966; DICKERSON, 1969; NOTTER, 1987; BARBATO, 1991) to ascertain differences due to parental, reciprocal, heterosis (individual and maternal), and individual recombination effects (see Table 2 for formulae). Individual heterosis was assessed for each reciprocal F_1 cross as well as their mean. Maternal heterosis was estimated under the assumption of the absence of paternal heterosis (HHF_1-F_1HH; LLF_1-F_1LL) or presence of paternal heterosis ($4\overline{BC}-2\overline{F}_2-\overline{F}_1-P)/2$. In estimating recombination effects,

maternal heterosis was ignored { $2(\overline{F}_2 - \overline{F}_1)$ } or allowed

{ $4(\overline{F}_2 - \overline{BC})$ }. Scaling tests (MATHER and JINKS 1982) were used to

test the adequacy of an additive-dominance model for 5- and 12-day antibody titers.

Significance was declared at $P < 0.05$ and < 0.01. When significant, main effects with multiple means were compared by Duncan's multiple range test.

Results and discussion

Antibody response to SRBC differed among progeny of the various mating combinations for titers both 5 and 12 days after inoculation (Tables 1 and 2). In general, neither sex nor its interaction with progeny type was important. Progeny of the high antibody parental line (HH), had higher 5- and 12-day antibody titers than progeny of the low antibody parental line. These differences included all the transmissible genetic effects. Even though the two reciprocal F_1 crosses were similar in antibody response when pooled over sexes, an inspection of the data revealed a sex difference between them at both ages, that is antibody titers of female progeny were biased towards that of their male parental progeny types, whereas the difference between the male progeny was not significant (Table 2). If these patterns were due to maternal effects (nuclear or cytoplasmic), both sexes would be affected similarly. Since females receive the sex chromosome Z from only their sires, genes on this chromosome appear to influence antibody response to SRBC antigen.

Heterosis, recombination, and sex-linkage

Heterosis was expressed in the female progeny of the HHLL F_1 cross for 5-day antibody titers and in the male progeny of the reciprocal F_1 cross (LLHH) for both 5- and 12-day titers. When reciprocals were pooled, the significant mean heterosis of 22% for antibody titers at 5 days was due mainly to heterosis in the

male progeny. Thus, individual heterosis arising out of heterozygosity of the progeny was highly influenced by sex-linked effects.

At 5 and 12 days after immunization, maternal heterosis (29 and 40%, respectively) was expressed in the male progeny of backcross matings involving the high antibody parental line and 13 and 23%, respectively, for the low antibody parental line. For backcross progeny involving female progeny maternal heterosis for 5- and 12-day antibody titers were 14 and 7%, respectively, in the high background and 16 and 18%, respectively, in the low background. There seems, thus, to be confounding of sex-linkage and heterozygosity *per se* as a cause for the maternal heterosis. The contribution of sex-linked genes appears clear because all females of a "HHF_1" backcross receive a Z chromosome from the high parental line, and the male progeny of "HHHL" backcrosses are homozygous for the high parental line Z chromosome (Table 1). Therefore the HHF_1 progeny should be expected to exhibit higher antibody titers than the reciprocal backcrosses (i.e., F_1HH) if the Z chromosome is assumed to influence antibody titers. The data appear consistent with this reasoning especially for 5-day antibody titers. Furthermore, in the absence of additive maternal effects, it is doubtful whether maternal heterosis can arise.

Recombination effects were not important in 5- and 12-day antibody titers as evident from F_2 and F_1 population means as well as the results on contrasts conducted (Tables 1 and 2). The scaling tests provided further confirmation on the lack of recombination effects because the additive-dominance model failed due to sex-linkage.

Relationships between mean antibody titers (pooled over sexes) and progeny types (i.e., proportion of H in the genotype) are summarized in Fig 1. For 5-day titers both linear and quadratic components were significant, while only the linear component was significant for 12-day titers. The equations were $Y=2.7346 + 0.1588x - 0.0007x^2$ for 5-day titers and Y=3.4588 +

0.1027x for 12-day titers.

General comments

Substantial responses to artificial selection with heritability estimates ranging from 0.26 (SIEGEL and GROSS 1980) to 0.49 (PINARD et al. 1992) place 5-day antibody titers to primary inoculation with SRBC among traits influenced by additive gene action. The importance of other modes of inheritance of this trait in chickens was suggested by a positive heterotic effect of 9% after 3 generations of selection (SIEGEL and GROSS 1980). However, by the S_9 generation, antibody titers of parental line crosses were lower than their mid-parent average (UBOSI et al. 1985) when data were pooled over ages. Heterosis was, however, expressed in the HL of the F_1 cross at 35 days of age. PINARD and VAN DER ZIJPP (1993) inferred from a comparison of reciprocal F_1 crosses of their selected lines for SRBC antibody that maternal or sex-linked effects contributed to 5-day antibody response to primary immunization with SRBC.

The literature (SIEGEL and GROSS 1980; UBOSI et al. 1985; PINARD and VAN DER ZIJPP 1993) lends support to the results of the present work regarding reciprocal effects on antibody titers to SRBC, as well as the higher titers of F_1 progeny with HH sires than F_1 progeny with LL sires. However unlike the present work, sex-linked and maternal effects were confounded.

Results from this experiment are also consistent with the hypothesis of homogametic heterosis proposed by STONAKER (1963) to explain the contribution of sex chromosomes to heterosis. He proposed that greater hybridization effects can be achieved in the sex chromosomes than is attainable in the autosomes with the same degree of inbreeding. Because in our experiment maternal effects were not important and because maternal heterosis was not expressed in 5-day antibody titers, the use of crossbred dams in the improvement of this trait is not indicated.

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Summary

Two lines of White Leghorn chickens selected divergently for high (HH) or low (LL) antibody response 5 days after an injection with 0.1mL of 0.25% suspension of sheep red blood cell (SRBC) antigen were used to produce parental, reciprocal F_1, F_2 and backcross progeny. At 36 days of age males and females of the various progeny types were injected with SRBC suspension and antibody titers measured at 5 and 12 days later.

Progeny of the high antibody line had higher titers at both 5 and 12 days after inoculation with SRBC than those of the low line. Reciprocal effects for SRBC titers were important only for female progeny suggesting sex-linked effects of the Z chromosome. Titers for F_1 progeny were intermediate and different from the parental lines at both 5 and 12 days after inoculation. Antibody titers 5 days after inoculation exhibited heterosis which emanated from the homogametic sex. Although maternal effects generally had no influence on antibody titers, maternal heterosis in the selected trait was due to sex-linkage. Recombination effects were negligible for both traits.

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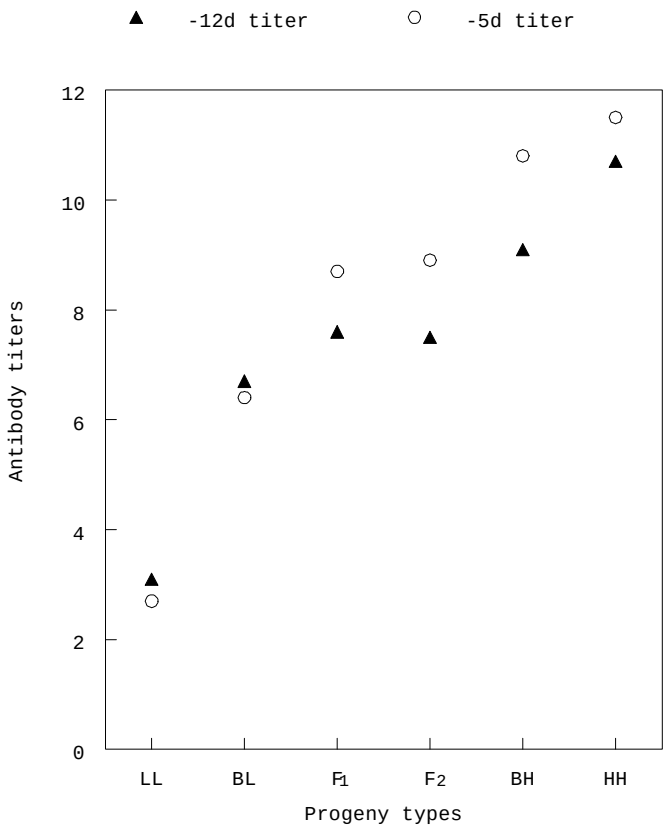

FIG.IV.1. Mean antibody titers 5 and 12 days after inoculation with SRBC by progeny types

Table IV.1. **Mean antibody titers (log_2) 5 and 12 days after inoculation with SRBC of chicks from all mating combinations of lines HA[1] and LA[1]**

Progeny[2]	5 days ♂♂	5 days ♀♀	5 days Pooled	12 days ♂♂	12 days ♀♀	12 days Pooled
Parental lines and F_1						
HHHH	11.9	11.1	11.5 a	10.4	11.0	10.7 a
HHLL	7.5	10.4	8.9 bc	6.4	9.0	7.7 de
LLHH	9.3	7.3	8.3 bc	8.1	6.6	7.4 de
LLLL	1.6	3.5	2.6 g	2.2	3.8	3.0 g
Backcross to HH parental line						
HHHL	12.2	11.1	11.7 a	10.4	9.2	9.8 ab
HHLH	11.9	11.9	11.9 a	10.6	10.0	10.3 ab
HLHH	9.8	9.9	9.8 b	7.3	8.2	7.7 de
LHHH	8.9	10.2	9.6 b	7.7	9.7	8.7 bc
Backcross to LL parental line						
LLHL	7.2	7.3	7.3 de	7.3	7.3	7.3 de
LLLH	6.4	6.1	6.3 ef	7.9	6.8	7.4 de
HLLL	5.4	5.2	5.3 f	6.0	5.2	5.6 f
LHLL	6.6	6.4	6.5 ef	6.4	6.8	6.6 ef
F_2						
HLHL	9.2	8.7	8.9 bc	7.7	6.3	7.0def
HLLH	8.0	9.3	8.7 bc	6.6	7.1	6.9 ef
LHHL	8.6	7.7	8.2 bcd	8.7	7.0	7.9 de
LHLH	9.3	10.4	9.9 b	8.1	8.5	8.3 cd

Overall SEM was 0.27 and 0.23 for 5 and 12 day antibody titers, respectively.

[1] HA = HH and LA = LL lines, respectively.

[2] The first two letters designate the sire and the second two letters the dam population for the mating combination.

a-g any two means in a pooled column followed by the same letter are not different at $P \leq 0.05$.

Table IV.2. **Significance of genetic effects on antibody titers 5 and 12 days after inoculation with SRBC of chicks according to contrasts involving various mating combinations of lines HA[1] and LA[1]**

Contrast	5 days ♂♂	5 days ♀♀	5 days Pooled	12 days ♂♂	12 days ♀♀	12 days pooled
Parental lines (P)						
HH-LL	**	**	**	**	**	**
Reciprocal effects						
HL-LH	NS	*	NS	NS	*	NS
Heterosis						
HL-(HH+LL)/2	NS	*	*	NS	NS	NS
LH-(HH+LL)/2	*	NS	NS	*	NS	NS
(HL+LH)-(HH+LL)	*	NS	*	NS	NS	NS
Maternal heterosis[2]						
HHF_1-F_1HH	**	NS	**	**	NS	**
LLF_1-F_1LL	NS	NS	NS	NS	NS	*
$(4\overline{BC}-2\overline{F}_2-\overline{F}_1-P)/2$	NS	NS	NS	NS	NS	*
Recombination						
$2(\overline{F}_2-\overline{F}_1)$	NS	NS	NS	NS	NS	NS
$4(\overline{F}_2-\overline{BC})$	NS	NS	NS	NS	NS	NS
Scaling[3]						
$A=2\overline{BC}_{HH}-HH-(HL+LH)/2$	NS	NS	NS	NS	NS	NS
$B=2\overline{BC}_{LL}-LL-(HL+LH)/2$	*	NS	NS	**	NS	*
$C=4\overline{F}_2-2\overline{F}_1-HH-LL$	*	NS	*	NS	NS	NS

[1]HA=H and LA=L lines, respectively.
[2]HHF_1-F_1HH = (HHHL+HHLH)-(HLHH+LHHH).
LLF_1-F_1LL = (LLHL+LLLH)-(HLLL+LHLL).
[3] BC_{HH} =(HHHL+HHLH+HLHH+LHHH)/4.
=(LLHL+LLLH+HLLL+LHLL)/4.
* = $P \leq 0.05$; ** = $P \leq 0.01$; NS = $P > 0.05$.

Chapter V

Mode of inheritance of unselected traits in Lines of chickens selected for high or low antibody response to sheep red blood cells: Resistance to marble spleen disease virus.

ABSTRACT Two lines of White Leghorns that had undergone long-term selection for high (HH) or low (LL) antibody response to sheep red blood cell antigen(s) formed the nuclear lines for this experiment. Matings were made in various combinations to produce in a single hatch from age contemporary breeders the parental lines, reciprocal F_1 and F_2 crosses, and backcrosses for 16 progeny types. At 50 days of age chicks were inoculated with either a 1% or 10% suspension of spleen extract from chickens infected with marble spleen disease virus (MSDV). A third group served as uninjected controls. Response to MSDV was evaluated by spleen weight 6 days after inoculation. Spleen weights relative to body weights of control chicks were heavier for the HH than LL line with evidence from the crosses of sex-linkage and negative heterosis. Line LL chicks were more resistant to MSDV than Line HH chicks with F_1 crosses intermediate to and different from either parental line with no evidence of heterosis.
(*Key words:* disease resistance, heterosis, SRBC, spleen, Marble spleen disease)

INTRODUCTION

Genetic selection for resistance of chickens against major infectious agents has been recognized for a long time as an adjunct to nongenetic means of disease control. A fundamental problem in the study of the genetics of disease resistance, however, has been the exposure of breeding populations to the infectious agent which may result in mortality and morbidity. Also, it would be desirable to select for general rather than specific disease resistance (Gavora, 1990) as commercial stocks of poultry are required to perform under diverse production environments.

The need for a marker with easily measurable phenotypic expression, that would identify potentially resistant genotypes led to the selection for antibody responses in several species including guinea pigs, rats, Japanese quail, swine (see review by Warner *et al.*, 1987), mice (Biozzi *et al.*, 1979), chickens (Siegel and Gross, 1980; Pinard *et al.*, 1992) and goats (Eide *et al.*, 1991). Bi-directional selection for antibody response to sheep red blood cell (SRBC) antigens has resulted in the alteration of components of the immune system, such as antibody response (Biozzi *et al.*, 1979; Siegel and Gross, 1980; Pinard *et al.*, 1992), macrophage activity (Biozzi *et al.*, 1979), B-cell population (Kreukniet, 1995), T-cell population (Scott *et al.*, 1991; Kreukniet *et al.*, 1994), as well as the CD4 and CD8 sub-populations of T lymphocytes (Kreukniet, 1995).

Correlated responses in disease resistance to such bi-directional selection were reported by Gross *et al.* (1980) who found that the high antibody line to SRBC exhibited stronger antibody response to Newcastle disease, was more resistant to *Mycoplasma gallisepticum*, *Eimeria necatrix*, and a splenomeglia virus, but was more susceptible to *Escherichia coli* and *Staphylococcus aureus* than the low line. Such correlated responses could be due to altered functions of the immune system, linkage with disease resistance genes, or cross-reactivity of the antigenic determinants of SRBC and the infectious organisms. In order to exploit such correlated responses to indirectly improve disease resistance traits, it is important to understand the genetic mechanisms which control resistance to these diseases.

Marble spleen disease of chickens, caused by avian adenovirus group II organism, and characterized by splenomegaly and no other lesions, was identified by Domermuth *et al.*

(1979). Splenic lesions consist of reticuloendothelial hyperplasia and lymphocytic degeneration (Veit *et al.*, 1981). Experiments involving high and low SRBC antibody lines and reciprocal crosses between them showed that the resistance or susceptibility of a cross to marble spleen disease virus (MSDV) depended upon the sire and dam lines of the crosses and that genotype by environment interactions affected spleen size (Gross *et al.*, 1988). Further investigations have been conducted with chicks of these same lines and various crosses between them to study the genetic architecture of responses to MSDV. This paper reports on the results of that study.

MATERIALS AND METHODS

Stocks and Husbandry

Two lines of White Leghorn chickens derived from the same base population, but selected divergently for high (HH) or low (LL) antibody response 5 days after an injection into the brachial vein with 0.1mL of 0.25% suspension of SRBC antigen(s) between the ages of 41 and 51 days (Siegel and Gross, 1980; Martin *et al.*, 1990) were the nuclear lines for the experiment. Matings between age-contemporary chickens from the S_{22} generation of these lines were made to produce the parental lines and reciprocal F_1 crosses. Chicks were housed in deep litter pens based on mating combinations. At 126 days of age, 15 cockerels and 60 pullets of these 4 populations i.e., HH, HL, LH, LL (first letter denotes sire line) were randomly selected and housed in individual cages in an environmentally controlled room.

At 36 weeks of age, matings were made among the sires and dams of the 4 populations to produce 16 progeny types consisting of parentals, reciprocal F_1, F_2, and backcrosses. At hatch, 100 unsexed chicks of each progeny type were wingbanded, vaccinated for Marek's disease, and placed in floor pens covered with woodshavings as litter. Chicks were provided *ad libitum* a mash diet containing 20% crude protein and 2685 kcal ME/kg. Lighting and water were also available continuously.

At 50 days of age, chicks were weighed and randomly subdivided within each sex-progeny type subclass into 3 groups of 10 each (n=960). Two of the groups were injected via the brachial vein with 0.1mL of either 1% or 10% suspension of spleen extract from chickens infected with MSDV. The third group served as the uninjected controls. Six days later, which

is the time of maximal response (Domermuth *et al.*, 1979), chicks were weighed, killed by cervical dislocation and their spleens removed and weighed (0.01g)

Statistical and Genetical Analyses

Body weights at 56 days of age, and absolute and relative spleen weights (g/kg BW) were subjected to ANOVA with progeny type, MSDV dosage, sex, and interactions among them as the main variables. Body weights were transformed to logarithms and relative spleen weights to arc sine square roots before ANOVA. Significance was declared at $P \leq 0.05$ and ≤ 0.01. Duncan's multiple range test was used to compare multiple means when ANOVA showed significant main effects.

Genetic analysis was undertaken within sexes so that maternal and sex-linked effects could be separated. The expected means of the progeny types in terms of nuclear genetic effects transmitted directly or through the maternal environment, individual and maternal heterosis, and recombination effects have been given by Dickerson (1969) and Notter (1987) who also provided information on paternal heterosis. Both authors dealt with mammalian species and ignored sex-linkage. Eisen *et al.* (1966), and Barbato and Vasilatos-Younken (1991) discussed models for separating maternal and sex-linked effects pertinent to male homogametic species such as poultry. Contrasts among the least square means of the progeny types were conducted based upon those models to ascertain differences due to parental, reciprocal, heterosis (individual and maternal), and individual recombination effects (see Table 2 for formulae). Individual heterosis was assessed for each reciprocal F_1 cross as well as their mean. Maternal heterosis was estimated under the assumption of the absence of paternal heterosis (HHF_1 - F_1HH; LLF_1 - F_1LL) or the presence of paternal heterosis $(4\overline{BC} - 2\overline{F}_2 - \overline{F}_1 - P)/2$. In estimating recombination effects, maternal heterosis was ignored $\{\ 2(\overline{F}_2 - \overline{F}_1)\ \}$ or allowed $\{\ 4(\overline{F}_2 - \overline{BC})\ \}$.

Scaling tests A, B, and C (Mather and Jinks, 1982) were used to test the adequacy of an

additive-dominance model for absolute and relative spleen weights. For these tests

$A = 2\overline{BC}_{HH} - HH - [(HL + LH)/2]$, $B = 2\overline{BC}_{LL} - LL - [(HL + LH)/2]$, and

$C = 4\overline{F}_2 - 2\overline{F}_1 - HH - LL$ where, $\overline{BC}_{HH}$ = (HHHL + HHLH + HLHH + LHHH)/4 and $\overline{BC}_{LL}$ =(LLHL + LLLH + HLLL + LHLL)/4. Relationships between traits and the proportion of line HH genes in the various progeny types were examined with a polynomial regression model which fitted linear and quadratic effects sequentially (Freund and Littell, 1981).

RESULTS

Relative spleen weights were similar for the 1% and 10% MSDV dosages in all progeny types. For absolute spleen weights these dosages were also similar in 15 of 16 progeny types. Therefore, the 1% and 10% MSDV dosages were combined for subsequent analyses.

As measured by absolute spleen weight, 6 out of 16 progeny types exhibited resistance to MSDV (no difference between control and inoculated), while for relative spleen weight, LLLL, LHLL, and HLHL progeny were resistant to MSDV (Table 1). Body weights of control and inoculated chicks were different in only 2 of 16 progeny types (Table 1) and were therefore not subjected to further analysis. Sexes responded similarly to the MSDV inoculation as dosage by sex interactions were not important for any of the traits. Spleen weights were heavier on an absolute weight basis (1.61 $\pm$ 0.03 *vs* 1.45 $\pm$ 0.02 g) and smaller on a relative basis (2.6 $\pm$ .04 *vs* 2.9 $\pm$ .04 x 10^{-3}) for males than females.

Inheritance of Traits before Inoculation

Among chicks not inoculated with MSDV (controls), neither the parental lines nor reciprocal F_1 crosses differed for absolute spleen weights (Table 2). Although the mean heterosis was not significant for this trait, negative heterosis was expressed in male progeny of the HHLL cross and positive heterosis in the female progeny of the LLHH cross suggesting that sex-related factors influence spleen size. Further indications that sex-related factors may affect absolute

spleen weights were that recombination effects $(\overline{F}_2 - \overline{F}_1)$ and maternal heterosis were expressed only in the males (Table 2) while the linear regression was not significant (Table 3).

Unlike absolute spleen weights, both parental and reciprocal contrasts were important for relative spleen weights of the control chicks (Table 2). Males and females of Line HH had larger relative spleens than those of Line LL. The contrasts suggest that the sex chromosome Z, from Line LL reduced relative spleen weights. The reciprocal contrast for this trait was significant only in females. The male progeny of the HHLL cross exhibited negative heterosis which was reflected in the mean heterosis and indicated homogametic heterosis effects. Maternal heterosis occurred only in the male progeny of the backcross to the low antibody parental line whereas the recombination effects $(\overline{F}_2 - \overline{F}_1)$, biased by maternal heterosis, was important only in males. The regression of relative spleen weights of the controls on parental Line HH indicated a stronger linear relationship in females than in males (Table 3) .

Inheritance of Traits after Inoculation

Absolute and relative spleen weights after inoculation with MSDV differed significantly between the selected lines with Line LL being more resistant than Line HH. The F_1 crosses, while not different from each other, were intermediate to and significantly different from either parental line (see Table 1 for means). This trend persisted for both sexes for absolute and relative spleen weights as confirmed by the significant parental contrasts and the lack of reciprocal effects for these traits (Table 2). In female chicks inoculated with MSDV the only other significant contrast, apart from the parental effects mentioned earlier was recombination, which increased absolute and relative spleen weights (Table 2). For male chicks inoculated, no other effect was significant for absolute spleen and only maternal heterosis was expressed for relative spleen weights in addition to the parental effect (Table 2). Mean heterosis was essentially zero for relative spleen weights after inoculation (Table 2). The linear regression of the traits on percent Line HH genome was significant for both sexes (Table 3; Figures 1, 2).

Out of 24 scaling tests conducted, only 3 were significant. These were scales B (0.0053 $\pm$

0.0019) and C (0.0077 ± 0.0033) for relative spleen weights and scale B (0.36 ± 0.16) for absolute spleen weights of inoculated male chicks.

DISCUSSION

The size of the spleen of avian species may be influenced by genotype (Ubosi *et al.*, 1985), stressors (Gross *et al.*, 1988), and season (John, 1994). In the absence of a well-developed lymph node system in the chicken (John, 1994), the spleen is the major organ involved in immune responses to some antigens (White *et al.*, 1975). This reasoning is consistent with splenomegaly having been shown to be an important characteristic of response to MSDV infection in chickens (Domermuth *et al.*, 1979).

Although small relative spleen weights appear to be associated with resistance to MSDV, this may be coincidental as the low antibody line may have resisted infection by MSDV with a superior MSDV specific immune response. Absolute spleen weights of control chicks gave little indication of potential resistance to MSDV. Our results showing that the mode of inheritance of normal spleen differed after infection were consistent with those of Praharaj *et al.* (1995) who challenged a wide range of stocks of chickens with MSDV and reported increases in relative spleen weights and differences among stocks for degree of response.

Relative spleen weight was influenced mainly by additive genetic effects as well as sex-linkage before infection. Homogametic heterosis is an indicator of sex-linkage (Stonaker, 1963). On the other hand, the most consistent effect in both absolute and relative spleen weights after inoculation was parental, suggesting that additively transmitted line effects greatly influence resistance to MSDV. Resistance to MSDV was influenced markedly by the proportion of low antibody line genome in the progeny. This result was similar to that reported by Gross *et al.* (1988) who worked with the S_{12} generation chicks of these lines. The lack of heterosis for resistance to MSDV in lines so diverged in their response to SRBC antigen is not necessarily contrary to expectation (Falconer and Mackay, 1996), because chickens are not routinely exposed to MSDV. As stated in the introduction to this paper, methods are needed in commercial poultry breeding to select for general, rather than specific, disease resistance. The greater resistance of the low than high antibody line to MSDV observed in this experiment suggests that high antibody producing lines may not be superior in

all cases and that general disease resistance may be enhanced by an intermediate optimum.

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TABLE V.1. Mean absolute (g) and relative spleen weights, and body weights (g) for control and MSDV inoculated chicks from all mating combinations of lines HH[1] and LL[1]

	Absolute spleen wt			Relative spleen wt ($x10^{-3}$)			56-day body wt		
Progeny[2]	Control		Inoculated	Control		Inoculated	Control		Inoculated
Parental lines and F_1									
HHHH	1.4	*	1.9	2.7	*	3.7	512		526
HHLL	1.1	*	1.6	1.8	*	2.7	597		583
LLHH	1.4		1.5	2.3	*	2.7	590		581
LLLL	1.1		1.0	1.8		1.7	595		562
Backcross to HH parental line									
HHHL	1.5		1.8	2.7	*	3.3	559		532
HHLH	1.2	*	1.7	2.3	*	3.2	562		539
HLHH	1.5		1.8	2.5	*	3.2	580	*	552
LHHH	1.2	*	1.6	2.3	*	2.9	565		522
Backcross to LL parental line									
LLHL	1.0	*	1.4	1.8	*	2.3	574		600
LLLH	0.9	*	1.3	1.7	*	2.2	576		596
HLLL	1.1	*	1.5	1.9	*	2.6	587		570
LHLL	1.4		1.3	2.4		2.3	580		565
F_2									
HLHL	1.6		1.8	2.9		3.1	563		565
HLLH	1.2	*	1.6	2.0	*	2.8	588	*	560
LHHL	1.1	*	1.5	2.0	*	2.7	572		550
LHLH	1.2	*	1.6	2.2	*	2.8	558		557
Pooled SEM	0.04		0.07	0.06		0.09	5		4

[1] HH and LL were selected for high and low antibody response to SRBC, respectively.

[2] The first two letters designate the sire and the second two letters the dam population for the mating combination.

* Difference between adjacent means significant at $P \leq 0.05$.

TABLE V.2. Genetic effects (in %) on absolute and relative spleen weights of chicks, for control MSDV inoculated chicks according to contrasts involving various mating combinations of lines HH[1] and LL[1]

Contrast	Control (not inoculated)				Inoculated			
	Absolute		Relative		Absolute		Relative	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Parental lines (P)								
HH-LL	26	31	37 *	59 *	117**	80**	119**	117**
Reciprocal effects								
HL-LH	-19	-23	-16	-27 *	8	-5	-4	-3
Heterosis								
HL-(HH+LL)/2	-27 *	3	-30 *	-14	17	7	2	-2
LH-(HH+LL)/2	-10	34*	-17	18	10	7	-2	2
(HL+LH)-(HH+LL)	-19	18	-24 *	2	14	7	0	0
Maternal heterosis[2]								
HHF_1-F_1HH	3	-4	4	0	0	6	5	5
LLF_1-F_1LL	-31 *	-11	-31 *	-9	-7	-4	-10 *	-8
$(4\overline{BC}-2\overline{F}_2-\overline{F}_1-P)/2$	16	-33	11	-28	11	-44	11	-34
Recombination								
$2(\overline{F}_2-\overline{F}_1)$	43 *	-12	27 *	5	5	5	12	5
$4(\overline{F}_2-\overline{BC})$	2	22	20	17	13	32*	4	23 *

[1] HH and LL were selected for high and low antibody response to SRBC, respectively.
[2] HHF_1-F_1HH = (HHHL+HHLH)-(HLHH+LHHH).
LLF_1-F_1LL = (LLHL+LLLH)-(HLLL+LHLL).
* $P \leq 0.05$; ** $P \leq 0.01$.

TABLE V.3. Regressions[1] of mean absolute and relative spleen weights on percent HH genetic background by sex of progeny for control (C) and MSDV inoculated (I) chicks

Spleen wt		b_1			$b_2(10^{-5})$			R^2
Absolute								
♂♂	C	-0.001	±	0.005	4.86	±	4.5	0.72
	I	0.016	±	0.003**	-5.91	±	3.3	0.97
♀♀	C	0.009	±	0.002*	-5.78	±	2.2	0.89
	I	0.010	±	0.002**	-1.91	±	1.9	0.98
Relative								
♂♂	C	-0.002	±	0.009	10.10	±	8.3	0.78
	I	0.021	±	0.005**	-2.92	±	4.4	0.98
♀♀	C	0.012	±	0.002**	-2.06	±	1.9	0.99
	I	0.019	±	0.004**	1.35	±	3.8	0.99

[1] b_1 is linear, b_2 is quadratic.

* $P \leq 0.05$; ** $P \leq 0.01$.

GENERAL SYNTHESIS

The impact of disease on the poultry industry has both economic and genetic dimensions. Costs of production increase as a result of losses due to clinical and subclinical infections. Disease can distort genetic parameters used for designing breeding programs, whereas mortality and depopulation necessitated by outbreaks of certain diseases deny the industry of valuable genes. Non-genetic measures commonly used to control diseases, such as medication and biosecurity have not achieved complete control of most diseases. Genetic selection for disease resistance should be embarked upon as an adjunct to the non-genetic measures to control diseases. Because of the risks involved in exposing breeding populations to specific pathogens, and the need to select for general rather than specific disease resistance, immunological traits may be considered as potential markers for the identification of resistant genotypes.

The lines involved in this dissertation have been subjected to long-term selection (> 20 generations) for (HA) and against (LA) antibody response to 0.1mL of 0.25% suspension of sheep red blood cells (SRBC) injected i.v. between 41 and 51 days of age. The lines are now known to have diverged in primary antibody response to SRBC at the selection age. Experiments described in this dissertation were designed to further evaluate the immune competence of these lines as influenced by diet, age, and a disease agent. Also conducted was a crossing experiment to describe further the mode of inheritance of such competence.

Humoral responsiveness considered in this dissertation included primary (Chapter I), memory (Chapter II), and maternal (Chapter III) antibody responses to SRBC. Primary antibody response was measured in chicks of lines HA and LA at the ages of 7, 14, 21, and 28 days (Chapter I). Chicks of Line HA mounted a higher antibody response measured 5 and 10 days after inoculation with SRBC than those of Line LA. Even though the magnitude of the total primary antibody titers did not vary between lines when

chicks were injected at 7 days of age, the frequency of responders was higher for HA than LA chicks. This suggests that the onset and/or threshold of immune competence occurs earlier in the high than the low antibody line. An early development of the immune system is desirable in animal production because it affects the mortality and health status of the young animal as well as its ability to respond to vaccines. Even though growth of HA chicks was improved by feeding a higher nutrient density diet, their antibody production was not affected. This result suggests the possibility of alleviating the poorer growth performance of immunocompetent chickens by nutritional manipulation without dissipating the built-in competence. Whether this inference can be extended to include growth-selected stocks which tend to overconsume food and direct proportionately more resources to growth, requires further study.

Immunological memory for antibody responses of Lines HA and LA was studied in parallel experiments on two groups of chicks hatched at a 14-day interval from the same matings of parental Lines HA and LA (Chapter II). The inoculation regimen started when chicks in groups 1 and 2 were 28 and 14 days of age, respectively. Memory responses were evident in the chicks at 14 days of age which suggests that both primary and memory responses (Chapters I, II) mature simultaneously. Despite the large line difference (HA > LA) in primary responses of these chicks to SRBC as a result of the selection imposed, responses to a second and a third inoculation with SRBC appeared to be similar. This finding suggests that genetic factors that influence primary and memory responses are not the same. It appears that the responses of LA chicks to repeat inoculations with SRBC were anamnestic, because they were rapid and achieved higher titers than the primary responses. Because the secondary and tertiary responses of HA chicks initially injected at 28 days of age did not exhibit anamnestic characteristics, the memory expressed by HA chicks given SRBC initially at 14 days of age needs additional study. Future experimentation should establish whether (1) memory

response in HA chicks is confounded with age and (2) HA chicks are capable of mounting higher antibody responses (both primary and secondary) to SRBC than the present levels.
Nutrient density had no major influence on immunological memory even though feeding the higher density diet enhanced growth performance. Thus this study did not establish any consistent evidence of the importance of diet on immune responses (Chapters I, II) indicating that the nutrient levels of both diets involved in these experiments were adequate for the fullest expression of immune responsiveness. There was, however, an indication (Chapter I) that at higher antigen concentrations, a higher density diet would enhance humoral immune competence.

Passive antibodies are important in protecting immunologically immature or compromised individuals against specific antigens. Two experiments were conducted to study the pattern of transmission and persistence of maternal SRBC antibody in HA and LA chicks. Antibody titers were determined in the hens injected with 0.1mL of either 0.25% or 2.5% SRBC and in 15 and 18-day embryos as well as 0, 5, 10, 15, 20, and 25 days after hatch. Dosage of SRBC had no effect on the antibody titers in HA hens whereas the higher dosage elicited greater antibody response in LA hens suggesting that the mechanism for high antibody titers in Line HA probably involves high affinity lymphocytes specific for SRBC. Assays in the chicks indicated that maternal antibodies were transferred into HA eggs earlier (7 - 9 days after inoculation) than in those of LA (10 - 12 days after inoculation), regardless of dosage administered to the hens. Response patterns, whether assessed in terms of frequency of detection or level of antibodies showed divergence between the lines. Large divergence in frequency of responses was observed in 15-day embryos (HA = 78% vs LA = 8%) and chicks at 25 days of age (HA = 70% vs LA = 20%). Also, the level of antibody was higher in HA than in LA chicks throughout the period from the 18th day of incubation until 10 days after hatch.

These studies indicate that genetics may play a role in the

phenomenon of age resistance, and that chicks of high antibody lines may be better protected at younger ages through maternal antibodies (Chapter III) and early onset of humoral immunocompetence (Chapters I, II).

In order to study the modes of inheritance of antibody response to SRBC (Chapter IV) and resistance to marble spleen disease virus (Chapter V), 16 mating combinations of Lines HA and LA were made from parents of the S_{22} generation. Chicks of parental, reciprocal F_1, F_2, and backcrosses of these matings were injected with SRBC at 36 days of age, and antibody titers measured at 5, and 12 days later (Chapter IV). Contrasts between parental lines were highly significant and indicated Line HA progeny had higher 5 and 12-day antibody titers than those of Line LA. Reciprocal contrasts for the F_1 crosses for both traits (5 and 12-day titers) were important only in female progeny suggesting sex-linked effects of the Z chromosome. Two other sources of evidence reinforced the sex-linkage hypothesis. Individual heterosis for 5-day antibody response was significant in males and also when data were pooled over sexes. These data are consistent with the phenomenon of homogametic heterosis caused by sex chromosomes. Maternal heterosis as measured by backcrosses to the high antibody line (HHF_1 - F_1HH) was consistent with the hypothesis of sex-linked effects of Z chromosome on antibody titers in the high line. On the basis of these data therefore, parental line effects and sex-linked effects were major factors influencing antibody titers of these lines. Subsequent experimentation however, is needed to confirm and indicate whether this is a single sex-linked major gene or a number of segregating alleles at loci affecting antibody response.

Male and female chicks (50 days of age) from the 16 crosses of Lines HA and LA representing parental lines, reciprocal F_1, F_2 and backcrosses were injected i.v. with either 1% or 10% spleen extract from chickens infected with marble spleen disease virus (MSDV). A third group served as controls. Response to MSDV was

evaluated 6 days after inoculation (Chapter V). The mode of inheritance of normal spleen weight in these lines differed after infection. Although neither parental lines nor reciprocal F_1 crosses differed for absolute spleen weights and heterosis was negligible, recombination effects and maternal heterosis, as well as the non-linear relationship between absolute spleen weight and genotype (% HA genome) suggested sex-related factors. After infection with MSDV, parental contrasts for both absolute and relative spleen weights were highly significant showing greater resistance to MSDV in LA than HA chicks. Neither reciprocal nor individual heterosis effects were evident.

Such results indicate that the high antibody line could not extend the superiority in humoral immunity to resistance to MSDV. Thus, although divergent selection may have produced divergence in immune response mechanisms to different antigens, the question remains as to whether general disease resistance is achievable or whether stocks should rather be selected to match particular production environments.

VITA

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