77

BARD

FINAL REPORT

PROJECT NO. I-938-85

**Genetic Relationships Between the Immune Response,
Resistance to Infectious Agents and Production Per-
formance of Chickens**

**E.D. Heller, P.B. Siegel, A. Cahaner, W.B. Gross,
B.A. Peleg**

1990

Standard BARD Cover Page for Scientific Reports

Date: JULY 25th 1990

BARD
P.O. BOX 6
Bet Dagan, ISRAEL

19-08-1990

BARD project No. I-938-85 c

Title

GENETIC RELATIONSHIP BETWEEN THE IMMUNE RESPONSE, RESISTANCE TO INFECTIOUS
AGENTS AND PRODUCTION PERFORMANCE OF CHICKENS.

Investigators' Names (Principal listed first)

E.D. HELLER, A. Cohen

P.B. SIEGEL, W.B. Gross

B.A. Peley

Investigators' Institutions

HEBREW UNIVERSITY OF JERUSALEM
FACULTY OF AGRICULTURE

VIRGINIA POLYTECHNIC INSTITUTE
AND STATE UNIVERSITY. COLLEGE
OF AGRICULTURE AND LIFE SCIENCE.

Kimron Veterinary Institute
Bet Dagan

Project's starting date: SEPT. 1986

Type of Report: 1st Annual 2nd Annual Final XXXXXX

D. Heller
Signature
Principal Investigator

Y. Haas.
Signature
Institution's Authorizing Official

BARD PROJECT IS-938-85.

GENETIC RELATIONSHIP BETWEEN THE IMMUNE RESPONSE, RESISTANCE TO
INFECTIOUS AGENTS AND PRODUCTION PERFORMANCE OF CHICKS.

THIRD ANNUAL AND FINAL REPORT

E.D. Heller and P.B. Siegel

B. Table of Contents.

	Page
Table of Contents	2
Abstract.	3
Objective of the Original Research Proposal.	4
Report on Work Conducted in Rehovot.	5
Summary of Previous Years	5
The Selected Lines After 2 Cycles	6
The Selected Lines After 3 Cycles	8
MHC Assessment of the Selected Lines	13
Conclusion Remarks	19
Tables and Figures	20
Final Report on Work Conducted in Blacksburg.	41
Cooperation.	47
Research Achievements.	47
List of Publications.	

C. ABSTRACT.

The objectives of this project were to select chickens for increased immune responses of two independent populations, a broiler (Israel) and a Leghorn (USA) populations and to study the relationship between genetic constitution and environment on the immune response.

After 3 cycles of selection 6 broiler lines were established. Two replicate lines of high responders at 10 days of age to E.coli antigen (HC1 and HC2), two replicate lines of low responders (LC1 and LC2) and two lines of unselected controls (CT1 and CT2). A significant difference in antibody response to E.coli was found between LC and HC lines. Selection differentials and responses to selection were calculated and the realized heritability over the 3 generations was found to be .34 and .21 in the LC and HC lines respectively. When S3 chicks were vaccinated with ND vaccine, HC1 and HC2 chicks responded significantly higher than the control or LC1 and LC2 lines. we concluded that selection for high and low antibody response to E.coli, resulted in a correlated antibody response to ND vaccine. When these chicks were vaccinated with SRBC, HC1 and HC2 chicks exhibited significantly higher antibody response than the control, LC1 or LC2 chicks. Reproductive traits were recorded and none was influenced by the selection, only hatchability of HC eggs was higher than LC eggs. In vivo carbon clearance revealed that HC chicks cleared significantly faster than LC chicks. T cells in vitro proliferation due to specific antigen or mitogens revealed that HC chicks T cells responded higher to the E.coli antigen, PHA or Con-A. The B-cell mitogen PWM brought us to about the same conclusions.

RFLP analysis of Chickens MHC using PvuII or BglII endonucleases and ClassII or ClassIV probes revealed that some of the band are represented only in one of the lines. This finding will enable a fast and reliable instrument for selection. The results of the Virginia group demonstrated a delicate balance among maintenance, immunoresponsiveness, growth and reproduction. The balance, which depend upon differential allocation of a finite pool of resources, is influenced not only by previous and current environment, but also the genotype. Differences among genetic stocks in resistance to infectious agents may be masked or accentuated by environmental manipulation.

D. Objectives of the original research proposal.

1. To compare the effects of two ongoing selection programs for immune response in poultry: (i) Two way directional selection for early age and late age of development of immune maturity as measured by the response to the specific disease antigens, Newcastle disease virus (NDV) vaccine and E.coli vaccine (Israel), and (ii) Two way directional selection for high and low response to foreign complex antigen, sheep erythrocytes (Virginia), with respect to:

- a. Rate of development and overall level of the immunological response in chicks.
- b. effects on competence at the cellular level (macrophages, T-cells)
- c. Kinetics of the response to immunization
- d. Correlated effects of the selection on response to other antigens and disease agents, and on growth, reproduction livability and behavioral traits of the chickens.

2. To study the effects of crossing the selected lines to one another, on rate of development and overall level of the immune response, on response to other antigens and disease agents, and on growth, reproduction, livability and behavioral traits.

3. To study the relationship between genetic constitution (as affected by selection lines and their crosses) and environment (as affected by social stress and handling and by early water deprivation and low brooder house temperatures) on the immune response and on the outcome of disease challenge.

Body of the Report: Work conducted in Rehovot, Israel

Summary of previous years: base populations and generation S₁

In generation S₁, there was no difference between the selected lines in antibody response to E. coli or to NDV vaccinations, apparently due to technical problems. Hence S₁ generation was regarded as base population, and the six selected lines were initiated again: for low or high response to early vaccination with NDV (LN and HN, respectively), for low or high response to early vaccination with E. coli (LC and HC, respectively), and for low or high average response ("Index") to early vaccination with both antigens (LI and HI, respectively). In S₂, the re-established S₁, only the selection on immune response to E. coli was effective. No response was obtained to the selection on NDV antibodies, apparently because young chicks carried maternal antibodies. Therefore, LC and LI were actually two independent replicates of selection for low titer of E. coli antibodies, and therefore designated as LC₁ and LC₂. Similarly, HC and HI lines were designated as HC₁ and HC₂ replicates of selection for high E. coli titer of antibodies. Also, while this generation could be regarded as S₂ by the project's time-scale, it actually has gone through only one effective selection cycle, and therefore referred S₁ throughout this report (it should not be confused with S₁ generation mentioned in the previous annual report, which actually served as base population). To simplify the result of the ELISA titer, each serum Ln antibody titer calculated from the linear regression subtracted 6.25 (optical density of a serum which was equals to that of the negative serum P/N=1). Means over hatches of E. coli titer and body weight of males and females of each line, and their deviations from the entire S₁ generation are presented in Table 1. Hatch effects within this generation, as well as within the later ones, were removed by multiplicative correction factor. The mean of all 7 lines in S₁ was taken as the mean of the base population. It was preferred over the control line because of the latter's limited size. A significant, symmetric response to the divergent selection was observed in S₁ (Table 1).

The procedure of the selection on E. coli antibodies among S₁ males and females within LC₁, LC₂, HC₁ and HC₂ lines was described in the 2nd annual report. Means of the selected parents within each line, and the

intended and actual selection differentials (S) are given in Table 2. The actual S 's were weighted by the number of S_2 offspring per parent, in order to correspond to line means in the offspring generation. On the average, actual S 's were quite similar to the intended ones, indicating the lack of natural selection for or against *E. coli* antibodies. Parents from the LN, HN and CT lines were randomly taken to reproduce these lines.

The selected lines after 2 cycles (generation S_2)

Experimental procedures

At the age of about 8 months of age, the selected S_1 males and females were mated (by A.I.) within each of the 7 lines. Females' age and body weight on the day of first egg, as well as total egg production until 300 days of age, were recorded as in previous year. Egg collection, incubation and hatching procedures were also as in the previous year. Hatchability was recorded in all hatches, and day-old chicks were weighed in one hatch, mainly to represent egg weight.

A total of 762 S_2 chicks (393 males and 369 females) were reproduced in two consecutive hatches, ten days apart. All of them were vaccinated with *E. coli* at ten days of age, as in previous year.

Because of the high NDV antibody titer of the S_1 hens, a third hatch of the LN, HN and CT lines was raised up to 24 days of age, when maternal antibodies disappear. At that age each chick was subcutaneously vaccinated with inactivated NDV in Aluminum Hydroxide (0.5ml/chick). Ten days later, the chicks were bled for NDV antibody determination, and then challenged with 1000 EID₅₀/chick. Mortality was recorded for 12 days and the survivors were evaluated for pathogenic symptoms. There was no difference in the mortality between the two lines and the control line, indicating that these chicks can be considered as base population for selection on response to NDV of chicks free of maternal NDV antibodies. Hence, divergent lines for NDV response were initiated again by re-assigning 1st and 2nd hatch males and females from the pooled two lines to "new" LN and HN lines, according to the mean NDV antibody titer of their full sibs at the third hatch.

Line means

Mean antibody titer and mean body weight at 6 weeks of age, by line and sex, and their deviations from the entire S_2 mean, are presented

in Table 3. They were corrected for hatch effect as in previous generation. Year effects were similarly removed, bringing all generation means to the value of 2.41, and adjusting each individual record accordingly. This correction facilitate comparisons between line means in different generations, and a reliable calculation of realized heritabilities from selection differentials in one generation, and line means (i.e., response to selection) in the next generation.

Highly significant difference was found between the replicated low-coli (LC) and high-coli (HC) lines (Table 3). Small, insignificant difference in E. coli antibodies was found between LN and HN lines. The control line had almost the lowest titer average, for an unclear reason. However, assuming that the mean of all seven lines can be taken as an approximation of the base population mean, the response to the selection for high antibodies titer appears larger than that of selection for low titer (.77 versus -.48, Table 3). Mean body weights of the LC lines were larger than those of the HC lines.

Selection differentials

Based on individual antibody titer of each S2 chick, and of his full and half sibs, 20 males and 30 females were selected within each line to produce the next generation of LC1, HC1, LC2, and HC2. Equal number of chicks were randomly chosen from each dam-family in the control line for its reproduction. Mean E. coli antibody titer of the selected S2 parents within each line, and the intended and actual selection differentials (S) are given in Table 4. The actual S's were weighted by the number of S3 offspring per parent. As in previous generation (Table 2); actual S's were very similar to the intended ones (Table 4), proving the lack of natural selection for or against E. coli antibodies. Absolute values of S of the LC parents were smaller than those of the HC parents.

The sib-selection on NDV antibodies titer, which was applied to establish new LN and HN lines (see previous section for details), caused an indirect differentials in E. coli antibodies. Males and females selected for low NDV had also lower E. coli titers, while those selected for high NDV had higher E. coli titers (Table 4).

Weighted (by number of offspring) mean body weights of the selected S2 parents within each lines, and the actual indirect selection differentials in body weight, are given in Table 4. Mean body weight of the LC parents in S1 was larger than that of the HC parents by 4.4%

(1175g vs. 1125g, Table 2). This apparent random difference increased to about 6% difference (1552g vs. 1462g, Table 4) in mean body weight of LC and HC lines in S2, respectively. Therefore in S2, some selection against body weight was practiced in the LC lines, with an average indirect S of -106g and no interference with the selection for low antibody titer (Table 4).

The selected lines after 3 cycles (generation S3)

Experimental procedures

At the age of about 8 months of age, the selected S2 males and females were mated (by A.I.) within each of the 6 selected lines and the control line. Due to the unexplained low E. coli antibodies titers of the control line (CT) in S2 generation, males and females from another population which was derived from the same base population, were used to establish a new control line (CT2). These chickens hatched and were raised together with the other seven lines. Females' age and body weight on the day of first egg, as well as total egg production until 300 days of age, were recorded as in previous year. Egg collection, incubation and hatching procedures were similar to those in the previous year.

A total of 689 S3 chicks (324 males, 365 females) were reproduced in two consecutive hatches, ten days apart. All of them were vaccinated with E. coli at 10 days of age, as in first and second hatch of S2 generation, and with NDV at 20 days of age, as in the third hatch of previous generation. Also first hatch vaccinated on the age of 23 days with 5% of sheep erythrocytes (SRBC) injected intermuscular. Each chick was bled twice: at 20 days of age - to determined E. coli antibodies titer 10 days post-vaccination, at 30 days of age - to determined NDV antibodies 10 days post-vaccination and to determined SRBC antibody 7 days post-vaccination.

Line means

Mean antibody titer and mean body weight at 6 weeks of age, by line and sex, and their deviations from the entire S3 generation mean, are presented in Table 5. Highly significant difference was found between the replicated low-coli (LC) and high-coli (HC) lines. It had increased substantially, from 1.10 in S1 [0.53-(-0.57), Table 1] and 1.25 in S2 [0.77-(-0.48), Table 3], to 1.84 in S3 [0.90-(-0.94), Table 5]. Small

but significant difference in E. coli antibodies was found between LC and HC lines. The mean of the new control line (CT2) was equal to the mean of the pooled S_3 generation, justifying the consideration of the later as a reliable representative of the base population. The response to the selection for high or low E. coli antibodies titer appears to be symmetrical in this generation (-.94 and .90, Table 5). Mean body weight of all eight lines were very similar (Table 5). The difference in body weight between LC and HC lines in S_2 generation disappeared in S_3 , apparently due to the negative selection in the LC lines (Table 4). It indicates the lack of genetic correlation between antibody titer and body weight.

Selection differentials

Due to the termination of the BARD funding, only LC and HC lines will be maintained. Based on individual antibody titer of each S_2 chick, and of his full and half sibs, 15 males and 30 females were selected within each line to produce the next generation of LC1, HC1, LC2, and HC2. The means of these selected S_3 birds, and the selection differentials of males and females in each line, are given in Table 6.

As a result of the asymmetrical distribution of individual titers within lines (Fig. 1), absolute values of selection differentials (S) of the LC parents were much smaller than those of the HC parents (-0.33 vs. 0.76, Table 6). Indirect differentials in body weight were negligible (Table 6), as in previous generation.

Realized heritability

Realized heritability (h^2_r) was calculated separately from each selection cycle, and for the three cycles combined, in each of the four lines (LC1, HC1, LC2, and HC2). Estimates were also averaged over replicated lines in each selection direction (LC or HC, Table 7). Heritability was calculated separately for each selection line in order to accommodate for asymmetric response to selection, where the means of the entire generations were used to represent the base population.

Heritability could not be calculated in the second cycle for line LC2 and line HC1, because each of them responded in the "wrong way", i.e., the opposite of its selection direction (Table 7). However, such unclear results in a single generation could be random.

In both first and third cycles, realized heritability was larger in the LC lines than in the HC lines, with this difference increasing from

the S_1 (.37 vs. .24) to S_3 (.65 vs. .12). The higher h^2_r in the LC lines was not due to larger response (Tables 1, 3 and 5), but due to smaller selection differentials. It indicates that the extreme individual values in the HC lines (Fig. 1), which made the selection differentials in these lines larger than in LC lines, are mainly of non-genetic origin. When selection differentials and responses to selection, were accumulated over three cycles and two replicated lines, the estimates of realized heritability were .34 and .21 in the LC and HC lines, respectively (Fig 2).

Correlation responses with NDV and SRBC antibody production

The results of the antibody response of S_3 generation to Newcastle disease virus vaccination is given in Fig 3. As it can be seen from this Fig., HC1 and HC2 chickens responded significantly higher than the control and from the LC1 and LC2 lines. LC1 and LC2 chickens responded significantly lower to the vaccination than the control or HC1 and HC2 lines. It can be concluded that the selection for high or low antibody response to E.coli resulted with a correlated antibody response to NDV. The results of the antibody response of S_3 generation to sheep red blood cells immunization is given in Fig 4. As it can be seen from this Fig. HC1 and HC2 chickens exhibited significantly higher response to SRBC than the control, LC1 or LC2 chickens. LC1 and LC2 line of chickens responded significantly lower than HC1 and HC2 chicken lines but non-significantly lower than the control line.

Reproductive performance

Six reproductive traits were recorded for each of the S_1 and S_2 females which were used to reproduce generations S_2 and S_3 :

- 1 - Body weight at first egg
- 2 - Age at first egg
- 3 - Total egg number up to 300 days of age
- 4 - Laying rate (%)
- 5 - Hatchability of S_2 chicks
- 6 - Mean body weight of 3rd hatch chicks

LC hens of S_1 generation started to lay 13 days earlier, but their laying rate was somewhat lower than that of HC hens, hence total egg production up to 300 days of age was similar in the LC and HC lines (Table 8). Percent hatchability of HC eggs was higher than that of LC

eggs. Combining egg production and hatchability, the estimated overall reproductive ability was similar in the LC and HC lines (35 and 38 chicks per hen, respectively). Day old chicks from the LC lines were heavier than those from the HC lines, resulting from heavier LC eggs. The LC chicks in S2 were also heavier at 4 weeks of age (Table 3). However, body weight of hens at sexual maturity (day of first egg) was almost identical in the LC and HC lines.

In the next generation, LC hens of S2 generation started to lay 8 days earlier, and their laying rate was slightly lower than that of HC hens, hence total egg production up to 300 days of age was similar in the LC and HC lines (Table 9). Percent hatchability of HC eggs was much higher than that of LC eggs. Combining egg production and hatchability, the estimated overall reproductive ability of the HC lines was higher than that of the LC lines (42 and 34 chicks per hen, respectively).

Of all reproductive traits, the largest and most significant difference between the LC and HC lines was observed in hatchability. This difference also increased with additional selection cycle, indicating a real correlated response to the selection on early immune response to E. coli vaccination. Besides the practical importance of the advantage in hatchability of the HC lines, it indicates the possible role of the immune system in hatchability.

Challenge test

Similar percentage of mortality and morbidity was observed among chicks from the low-response (LC), high-response (HC) and control (CT) lines after challenge with E. coli at 20 days of age (Table 10). However, following vaccination 10 days earlier, percentage of mortality and morbidity of chicks from the LC lines was higher than that of HC chicks (39% vs. 23%) although significance level was only 0.1 (Table 10). The vaccinated HC chicks had significantly higher antibody titer, in agreement with their better resistance to E. coli challenge.

Carbon Clearance Assay.

The assay (Cheng and Lamont, 1988) was used to evaluate in vivo phagocytic activity of broilers at age 2 and 3 weeks with the following modifications. The supernatant fraction (3.000Xg/10 min) of India ink (Pel:Ken 518.21A 862 Hanover WG) was injected into the brachial vein of birds at volume of 3ml/kg BW. Tow, 4, 6 and 15 min post injection a 200 μ l sample of blood was collected from the right external

jugular and transferred into 1800 μ l of 3.8% sodium citrate. After centrifugation (50xg for 5 min) the relative amounts of carbon remaining in the supernatant of each sample were estimated by spectrophotometer at 675 nm, using the samples collected before carbon injection as zero.

Results

The carbon clearance activity of the HC (1+2), LC (1+2) and CT lines was evaluated in two separate experiments. Twenty broilers (10 males and 10 females) of the CT line were assayed at the age of three weeks. As can be seen in Fig. 4A, females cleared significantly faster the carbon from the blood than the males. In experiment two, 20 chicks of line HC and 20 chicks of line LC (10 males and 10 females in each line) were assayed at the age of four weeks. As can be seen in Fig. 4A, there was no difference between males and females in HC lines while in LC lines females cleared carbon faster but not significantly so Fig 4D. However, males and females together in HC lines cleared significantly faster than males and females together in LC lines Fig 4C.

In-vitro proliferation of chicks T-lymphocytes to *E. coli*

To assay the specific T-lymphocytes response to the *E. coli* antigen, chicks of the HC and LC lines (4 males and 4 females of each line) were challenge with 1×10^6 CFU of O2:K1 *E. coli* bacteria. Forty-eight, 96, and 120 hr' post-challenge (PC) peripheral blood leukocytes were collected and their proliferative response to the antigen was measured (Leitner *et al* 1989). The result (Table 11) shoeing a higher proliferation as expressed as count per minute, in the HC lines in all three time of the assay but only significantly ($P < 0.05$) at 48 hr' PC.

In vitro proliferation of polyclonal T and B lymphocytes by mitogens.

In order to assay the general activity of the chicken lymphocytes, lymphocytes of chickens of the HC and LC lines (9 males and 9 females of each line) were collected and were stimulated by Concavalin-A (Con-A) and Phytohemagglutinin (PHA) for T cells stimulation and Pockweed mitogen (PWM) for B cells stimulation (Lee L., 1984). The assay was performed according to Lee 1974. For obtaining optimal lymphocytes response. Erythrocytes (1×10^6 /well) were added.

The assay was performed on S1 and S3 generations and calculated for

deviation of the selected lines means from the entire generation means in S1 and S3 comparatively. The results (Fig. 5, Fig. 6 and Fig. 7) showed that in S1 generation the differences between the lines were found to be non-significant. Comparing the mitogenic response of PBL of S3 chicks revealed that LC responded non-significantly lower than the control group but HC chicks' PBL responded significantly higher to all three mitogens than the LC or control groups.

These results indicates that along with the selection to E.coli response, differences in lymphocytes response to mitogens emerged and were correlated to the antibody responses.

Interferon production

Chickens from the selected lines (S3 generation) were examined for their T lymphocytes ability to produce interferon. The assay was performed according to Skellick et al. (1986) and Rubinstain et al. (1981). Stimulation for interferon production was performed by addition of Forbol Meristat Acetat (0.2 ng/well) and PHA (1 µg/well) to peripheral blood lymphocytes (1×10^6) for 24 h'. Supernatants were collected and added to monolayer of chick embryo fibroblasts. After 20 hours of incubation Vesicular Stomatitis Virus (VSV) was added (4×10^2 TCID₅₀/well) and 24 hours later the cytopathic effect was estimated by addition of neutral red. Uptake of the dye was measured using ELISA reader (O.D. 540nm). Results are given as percent of protection of the interferon.

The result of this experiment are given in table 13 and indicate that there is no significant differences in interferon production of PBL of the different lines although HC lymphocytes gave slightly more protection than CT lymphocytes while LC lymphocytes showed the lowest protection.

Association of Restriction Fragment Length Polymorphisms Of Chicken MHC Class II Genes With Early Immune Response To E.coli.

Summary.

Restriction fragment length polymorphism analyses of chicken Major Histocompatibility Complex (MHC) were performed on meat-type chickens that have been divergently selected for immune response to immunization with Escherichia coli at 10 days of age for three generations. Two lines have been selected for high early antibody titer (HC) and low

late (LC) antibody titer along with an unselected control line (C).

Southern blotting and hybridization procedures were performed on the genomic DNA, isolated from erythrocytes, using PvuII and BglII endonuclease and MHC class II and class IV probs. RFLP analysis revealed polymorphism at the chicken class II region with both enzymes. After digestion with the restriction endonuclease PvuII ten restriction fragments were presented. While 2 were common to all chickens (P1 and P3), the other 8 showed differential presence. Out of 36 chickens that were examined, 11 PvuII RFLP patterns were identified. No significant antibody titers differences were found between the different patterns. Eight restriction fragments were identified using BglII endonuclease. Five fragments were common to all chickens and the other 3 showed diversity. Screening 36 chickens, 6 different BglII RFLP patterns have been identified. Patterns BE and BF were detected only in line E and were characterized by high antibody titer. Pattern BC was detected only in the line L and associated with low antibody titer ($P < 0.01$).

The results suggest that there may be an association between chicken MHC class II and E.coli immune response. This finding may will enable a rapid selection of meat-type chickens for enhanced E.coli resistance.

Introduction.

In chickens, the MHC genes and their proteins have been divided into three classes, class I, II, and IV which are designated as B-F, B-L and B-G, respectively. Several studies confirm that genes in the chicken MHC influence resistance to disease (Bacon L.D. 1987). Most of these studies were done on Leghorn chickens, using monoclonal antibodies that have been produced to antigens of each of the three subregions of the chicken MHC (Crone et al, 1985; Miller et al 1982; Pink et al, 1985). The B-L antigens (Guillemot et al. 1986) are involved in regulation of immune cell cooperation (Vainio et al., 1984). Isolation of the MHC class II(B-L) β chain sequences (Bourlet et al 1988) provided a tool to characterize the MHC at the molecular level. Soller and Backman (1986) described the potential of RFLP application to poultry breeding. The MHC, with its established role in immune response and disease resistance should show RFLP with direct effect on immunological traits and, therefore, be of value in breeding programs.

In this study we selected meat-type chickens for immunological trait of early and late E.coli antibody response. By RFLP analysis, using PvuII and BglII endonuclease and MHC class II probe, we examined the

association between RFLP of MHC class II and E.coli antibody levels in these meat-type selected lines.

Materials and Methods.

Animals

A commercial grandparent line was used as the based population for the selection. Eighty females were randomly mated with 20 males to produce offspring for the selected and control lines. Two lines were divergently selected for early (HC) or late (LC) immune response to vaccination with E.coli. The "best" (lowest in line L and highest in line E) 15 males and 30 females were selected in each generation from about 60 birds per sex within each line. Similar number of males and females were taken at random to maintain a control line (C). Seven males and about 30 females per line were mated to produce the next generation. The same vaccination, titer determination and selection procedures were used in two cycles, producing generations S_1 and S_2 .

Antibody titers

The antibody titer was determined by ELISA (Leitner et al.1989). 10 day post-immunization of chicks immunized at age of 10 days (E.coli 078;K80 2×10^9 CFU/ml inactivated by sonication) which was injected subcutaneously (0.5ml/chick).

DNA isolation and gene detection

Isolation of genomic DNA was from peripheral blood. Digestion by PvuII and BglII endonuclease, blotting, and hybridization with DNA probe were performed as described by Hillel et al.(1989). The class II probe was provide by Dr. Pitcovsky, Israel (Xu et al.1988).

Analysis of RFLP patterns

Molecular size of the restriction fragments were determined by comparison to size markers of HindIII digested by lambda DNA (Flanagan et al. 1988). The fragments were coded P1-P10 for PvuII and B1-B8 for BglII. Identical patterns of restriction fragments were coded as PA-PF for PvuII and BA-BH for Bgl II.

Results.

Basic structure of B-L broiler chicken RFLP patterns

1. PvuII restriction endonuclease: RFLP analysis, after digestion with the restriction endonuclease PvuII, revealed polymorphism at the chicken class II region. Ten restriction fragments were presented. While 2 fragments were common to all chickens (P1 and P3), the other 8

showed differential presence (Table I). Out of 36 chickens that were examined, 11 PvuII RFLP patterns were identified. No significant antibody titers differences were found between the different patterns.

2. Bgl II restriction endonuclease: Eight restriction fragments were identified using BglII endonuclease. Five fragments were common to all the chickens and the other 3 showed diversity (Table II). Screening 36 chickens, 6 different BglII RFLP patterns have been identified. Patterns distribution in the selected lines HC and LC are presented in Table III. Patterns designated BE and BF were detected only in line HC and were characterized by high antibody titers. Pattern BC was detected only in line L and was associated with low antibody titer. (Significance: $P < 0.01$) Fragments analysis within each B and P fragment group, multiple regression analysis were carried out and selection was performed using stepwise procedure.

Two fragments (B5,P9) were found to be positively associated with E.coli antibody levels (Table IV).

Table I. Restriction fragments, their size and frequency in selected lines (HC ,LC and C) using restriction endonuclease Pvu II

Number	Fragment size (Kilobase)	Frequency (%)		
		line HC	line LC	line C
P1	11.5	100	100	100
P2	7.8	66.6	70.2	68.8
P3	4.7	100	100	100
P4	4.2	0	5	3
P5	3.2	0	5	3
P6	2.8	44.4	35.3	39.5
P7	2.3	77.5	82.1	78.6
P8	1.7	100	82.1	89.2
P9	1.5	100	76.4	85.6
P10	1.2	77.4	70.6	68.5

Table II. Restriction fragments, their size and frequency in the selected lines (HC, LC and C) using restriction endonuclease Bgl II

Number	Fragment size (Kilobase)	Frequency (%)		
		HC line	LC line	C line
B1	14.0	100	100	100
B2	11.2	100	100	100
B3	8.1	100	100	100
B4	6.8	100	100	100
B5	6.0	20	0	8
B6	4.3	36	20	30
B7	3.0	45.5	46.6	60.3
B8	2.7	100	100	100

Relationship between DNA restriction fragments and selection trait
Pattern analysis:

Table III. RFLP patterns, using BglIII enzyme, their mean antibody levels $\pm$ S.E. and distribution in lines HC, LC, and C.

Pattern	E. coli abtibody level	statistical grouping*	Frequency (%)		
			line HC	line LC	line C
BA	9.36 $\pm$ 1.3	bc	25	31.5	12.5
BB	9.22 $\pm$ 1.2	bc	37.5	43.7	50
BC	7.81 $\pm$ 0.3	b	0	18.6	8.2
BD	7.52 $\pm$ 0.8	b	0	6.2	10
BE	10.42 $\pm$ 0.3	c	12.5	0	12.5
BF	10.28 $\pm$ 0.4	c	25	0	6.8

*Different letters designated statistical difference ($P < 0.01$).

Table IV. Effect of DNA restriction fragments on *E.coli* antibody levels.

Restriction fragment	P9	B5
Fragment effect	1.08*	1.6**
Standart error	0.63	0.7

**P<0.05, *P<0.01

Discussion

This study used an MHC class II chicken DNA probe to demonstrate variation among meat-type chickens selected for early and late *E.coli* homural immune response. Digestion of DNA with PvuII and BglII generated RFLP patterns. Two of the BglII patterns were associated with high antibody titer to *E.coli* and were presented only in the E line. One BglII pattern was associated with late antibody response and was presented only in the L line. PvuII digestion reveled greater polymorphism. The small number of individuals in each line examined (N=36) prevented us from finding clear PvuII patterns that are significantly associated with either high or low antibody response to *E.coli*. Looking at individual bands it was obvious that two restriction fragments were associated with increased antibody titer. Those results indicated that this method, though with some limitations, can be applied in genetic selection plans. Certain DNA restriction fragments or certain pattern for the chicken MHC class II genes appear to be associated with the selection trait. It may be possible, therefore to screen animals early in life for presence or absence of a particular pattern or restriction fragment associated with class II B-L genes, and than select on that basis to improve *E.coli* resistance.

Conclusion remarks.

Both groups dedicated their efforts in study the relationship between general immune responses, resistance to infectious agents and production traits and their genetic control. The Rehovot group studied this relationships in commercial meat-type chickens.

The findings strongly indicated that genetic selection for early immune responsiveness can be accomplished without affecting production traits. The original idea was that the selection should be performed using two antigens, a bacterial and a viral. The results of the project's first year indicated that E.coli antigen, as prepared by us was sufficient to allow selection with a broad effect.

Our selected line for high antibody response to E.coli showed higher resistance to pathogenic E.coli bacteria when the challenge was performed after immunization. This line responded with higher antibody titers to Newcastle disease vaccine and Sheep erythrocytes, demonstrated higher phagocytic activity (in vivo carbon clearance) and its lymphocytes responded higher to T and B cell mitogens. Experiments examining the selected lines resistance to challenge with pathogenic Newcastle disease virus and Infectious Bursal Disease virus failed to show conclusive results and needed further calibration. The chicken line selected for low antibody response to E.coli at 10 days of age was found inferior in its response in the other tests.

A preliminary study of the lines' Major Histocompatibility Complex using RFLP (see attached) exhibited the possibility of using this method as additional tool for selection for high immune responses and resistance to diseases.

Table 1. Means (over hatches) of *E. coli* antibody titer¹⁾ and body weight²⁾ of males (M) and females (F) of each line, and their deviations (Dev.) from the entire S1 generation mean. Analysis of variance and significance tests included only lines LC1, LC2, HC1 and HC2.

Line	Sex	Number	<i>E. coli</i> titer		Body weight	
			Mean	Dev.	Mean	Dev.
Low coli (LC1)	M	51	1.74	-0.62	1217	8
	F	50	1.87	-0.58	1007	-28
	M+F	101	1.81	-0.60	1112	-10
Low coli (LC2)	M	39	1.87	-0.49	1249	40
	F	56	1.85	-0.60	1038	3
	M+F	95	1.86	-0.55	1144	22
LC average			1.84	-0.57	1128	6
High coli (HC1)	M	55	2.87	0.51	1185	-24
	F	64	3.17	0.72	1035	0
	M+F	119	3.02	0.61	1110	-12
High coli (HC2)	M	72	2.80	0.44	1183	-26
	F	53	2.92	0.47	1022	-13
	M+F	125	2.86	0.45	1103	-19
HC average			2.94	0.53	1107	-16
S.E.M.	M	--	0.164		24.37	
	F	--	0.024		19.00	
P(F): LC vs. HC	M	--	0.0001		0.0264	
	F	--	0.0001		0.6660	
<hr/>						
Low NDV (LN)	M	60	2.47	0.11	1192	-17
	F	51	2.43	-0.02	1017	-18
	M+F	111	2.45	0.04	1105	-18
High NDV (HN)	M	60	2.16	-0.20	1247	38
	F	38	2.45	0.00	1049	14
	M+F	98	2.31	-0.10	1148	26
Control (CT)	M	54	2.31	-0.05	1207	-2
	F	46	2.30	-0.15	1085	50
	M+F	100	2.31	-0.10	1146	24
Entire S1 generation	M	391	2.36	--	1209	--
	F	358	2.45	--	1035	--
	M+F	749	2.41	--	1122	--

- 1 - Ln titer at 20 days following vaccination at 10 days of age
2 - Body weight in grams at 5 weeks of age

Table 2 Means and selection differentials (S^1) of the S1 males and females selected for reproduction within lines at 6 weeks (intended S) and of those who produced S2 offspring, weighted by their number (actual S).

Line	Sex	E. coli titer				Body weight		Number of parents
		Intended		Actual		Actual		
		Mean	S	Mean	S	Mean	S	
Low coli (LC1)	M	0.94	-0.81	1.32	-0.42	1382	165	7
	F	1.14	-0.73	1.21	-0.66	1033	32	24
	M+F	1.04	-0.68	1.27	-0.54	1208	99	31
Low coli (LC2)	M	1.37	-0.50	1.18	-0.69	1264	8	6
	F	1.52	-0.33	1.27	-0.58	1018	-21	22
	M+F	1.45	-0.42	1.23	-0.64	1141	-7	28
LC average		1.25	-0.55	1.25	-0.59	1175	46	
High coli (HC1)	M	3.84	0.97	3.89	1.02	1211	29	7
	F	4.14	0.97	3.92	0.75	1010	-28	25
	M+F	3.99	0.97	3.91	0.89	1111	1	32
High coli (HC2)	M	3.69	0.89	4.52	1.72	1263	84	7
	F	3.40	0.48	3.40	0.48	1013	-6	22
	M+F	3.55	0.69	3.96	1.10	1138	39	29
HC average		3.77	0.83	3.94	0.96	1125	20	

Low NDV (LN)	M	2.32	-0.15	2.70	0.23	1229	39	7
	F	2.34	-0.09	2.07	-0.36	1004	-6	24
	M+F	2.33	-0.13	2.39	-0.07	1117	17	31
High NDV (HN)	M	2.02	-0.14	2.87	0.71	1326	78	7
	F	2.33	-0.12	2.22	-0.23	1006	-28	26
	M+F	2.18	-0.13	2.54	0.24	1166	25	33
Control (CT)	M	2.00	-0.31	2.02	-0.29	1243	35	7
	F	2.32	0.02	2.16	-0.14	1091	5	27
	M+F	2.16	-0.15	2.09	-0.22	1167	20	34

1) S = selection differential: deviation of selected parents' mean from the line's mean.

Table 3. Means (over hatches) of *E. coli* antibody titer¹⁾ and body weight²⁾ of males (M) and females (F) of each line, and their deviations (Dev.) from the entire S2 generation mean. Analysis of variance and significance tests included only lines LC1, LC2, HC1 and HC2.

Line	Sex	Number	<u>E. coli titer</u>		<u>Body weight</u>	
			Mean	Dev.	Mean	Dev.
Low coli (LC1)	M	50	1.63	-0.61	1649	- 2
	F	48	1.75	-0.83	1407	30
	M+F	98	1.69	-0.72	1528	14
Low coli (LC2)	M	43	1.89	-0.35	1722	71
	F	25	2.45	-0.13	1429	52
	M+F	68	2.17	-0.24	1576	62
LC average			1.93	-0.48	1552	38
High coli (HC1)	M	52	2.82	0.58	1597	-54
	F	65	3.10	0.52	1330	-47
	M+F	117	2.96	0.55	1464	-50
High coli (HC2)	M	59	3.05	0.81	1575	-76
	F	56	3.75	1.17	1343	-34
	M+F	115	3.40	0.99	1459	-55
HC average			3.18	0.77	1462	-52
S.E.M.	M	--	0.177		34.40	
	F	--	0.178		30.44	
P(F): LC vs. HC	M	--	0.0001		0.0190	
	F	--	0.0001		0.4822	

Low NDV (LN)	M	67	2.42	0.18	1682	31
	F	52	2.67	0.09	1415	38
	M+F	119	2.55	0.14	1549	35
High NDV (HN)	M	60	1.89	-0.35	1668	17
	F	59	2.05	-0.53	1402	25
	M+F	119	1.97	-0.44	1535	21
Control (CT)	M	62	1.86	-0.38	1670	19
	F	64	2.14	-0.44	1360	-17
	M+F	126	2.00	-0.41	1515	1
Entire S2 generation	M	393	2.24	--	1651	--
	F	369	2.58	--	1377	--
	M+F	762	2.41	--	1514	--

1 - Ln titer at 20 days following vaccination at 10 days of age

2 - Body weight in grams at 6 weeks of age

Table 4 Means and selection differentials (S^1) of the S2 males and females selected for reproduction within lines at 6 weeks (intended S) and of those who produced S3 offspring, weighted by their number (actual S).

Line	Sex	E. coli titer				Body weight		Number of parents
		Intended		Actual		Actual		
		Mean	S	Mean	S	Mean	S	
Low coli (LC1)	M	0.98	-0.65	0.74	-0.89	1591	-59	6
	F	1.22	-0.53	1.40	-0.35	1322	-85	12
	M+F	1.10	-0.59	1.07	-0.62	1457	-72	18
Low coli (LC2)	M	0.95	-0.94	1.07	-0.82	1658	-64	7
	F	1.46	-9.99	1.55	-0.90	1214	-215	11
	M+F	1.21	-0.96	1.31	-0.86	1436	-140	18
LC average		1.16	-0.78	1.19	-0.74	1447	-106	
High coli (HC1)	M	3.91	1.09	4.04	1.22	1637	40	7
	F	4.25	1.15	4.21	1.11	1293	-37	11
	M+F	4.08	1.12	4.13	1.17	1465	2	18
High coli (HC2)	M	4.26	1.21	4.56	1.51	1618	43	7
	F	4.50	0.75	4.05	0.30	1350	7	15
	M+F	4.38	0.98	4.31	0.91	1484	25	22
HC average		4.23	1.05	4.22	1.04	1475	14	

Low NDV (LN)	M	1.59	-0.83	1.93	-0.53	1780	98	7
	F	2.27	-0.40	2.27	-0.42	1392	-23	22
	M+F	1.93	-0.62	2.10	-0.48	1586	38	29
High NDV (HN)	M	2.17	0.28	2.33	0.44	1609	-59	7
	F	2.80	0.75	2.82	0.77	1440	38	22
	M+F	2.49	0.52	2.58	0.61	1525	-11	29
Control (CT)	M	1.91	0.05	1.91	0.05	1700	30	7
	F	2.38	0.24	2.55	0.42	1349	-11	26
	M+F	2.15	0.15	2.23	0.24	1525	10	33

1) S - selection differential: deviation of selected parents' mean from the line's mean.

Table 5. Means (over hatches) of *E. coli* antibody titer¹⁾ and body weight²⁾ of males (M) and females (F) of each line, and their deviations (Dev.) from the entire S3 generation mean. Analysis of variance and significance tests included only lines LC1, LC2, HC1 and HC2.

Line	Sex	Number	<i>E. coli</i> titer		Body weight	
			Mean	Dev.	Mean	Dev.
Low coli (LC1)	M	34	1.32	-1.11	1528	-40
	F	36	1.17	-1.21	1263	-47
	M+F	70	1.25	-1.16	1396	-43
Low coli (LC2)	M	32	1.74	-0.69	1595	27
	F	39	1.62	-0.76	1376	66
	M+F	71	1.68	-0.73	1486	47
LC average			1.46	-0.94	1441	2
High coli (HC1)	M	34	3.48	1.05	1604	36
	F	43	2.83	0.45	1262	-48
	M+F	77	3.16	0.75	1433	-6
High coli (HC2)	M	38	3.09	0.66	1552	-16
	F	37	3.83	1.45	1315	5
	M+F	75	3.46	1.05	1434	-5
HC average			3.31	0.90	1434	-5
S.E.M.	M	--	0.220		39.21	
	F	--	0.262		31.61	
P(F): LC vs. HC	M	--	0.0001		0.6727	
	F	--	0.0001		0.3323	
Low NDV (LN)	M	56	2.35	-0.08	1557	-11
	F	61	2.16	-0.22	1345	35
	M+F	117	2.26	-0.15	1451	12
High NDV (HN)	M	37	3.20	0.77	1608	40
	F	53	2.82	0.44	1310	0
	M+F	90	3.01	0.60	1459	20
Control-old (CT1)	M	56	2.00	-0.43	1577	9
	F	51	2.23	-0.15	1302	-8
	M+F	107	2.12	-0.29	1440	1
Control-new (CT2)	M	37	2.42	-0.01	1525	-43
	F	45	2.36	-0.02	1305	-5
	M+F	82	2.39	-0.02	1415	-24
Entire S3 generation	M	324	2.43	--	1568	--
	F	365	2.38	--	1310	--
	M+F	689	2.41	--	1439	--

1 - Ln titer at 20 days following vaccination at 10 days of age

2 - Body weight in grams at 6 weeks of age

Table 6 Means and intended selection differentials (S^1) of the
S3 males and females selected for reproduction within lines

Line	Sex	<u>E. coli titer</u>		<u>Body weight</u>		Number selected
		Mean	S	Mean	S	
Low coli (LC1)	M	0.95	-0.37	1550	-18	21
	F	1.00	-0.17	1266	-44	32
	M+F	0.98	-0.27	1408	-31	53
Low coli (LC2)	M	1.24	-0.50	1620	52	21
	F	1.33	-0.29	1386	76	31
	M+F	1.29	-0.39	1503	64	52
LC average		1.13	-0.33	1455	15	
High coli (HC1)	M	4.41	0.93	1561	- 7	17
	F	3.45	0.62	1271	-39	27
	M+F	3.93	0.77	1416	-23	44
High coli (HC2)	M	4.05	0.96	1498	-70	20
	F	4.37	0.54	1322	12	29
	M+F	4.21	0.75	1410	-29	49
HC average		4.07	0.76	1413	-20	

1) S - selection differential: deviation of selected parents' mean
from the line's mean.

Table 7 Realized heritability (h^2_r) of E. coli antibody titer, calculated from averaged sire and dam effects in each of the three selection cycles in two Low coli and two High coli lines

Line	Calculated h^2_r (= R^1 / S^2)			
	S0-S1	S1-S2	S2-S3	S0-S3
LC1	$\frac{-0.54}{-1.40} = 0.39$	$\frac{-0.13}{-0.54} = 0.24$	$\frac{-0.44}{-0.61} = 0.72$	$\frac{-1.11}{-2.55} = 0.44$
LC2	$\frac{-0.50}{-1.46} = 0.34$	$\frac{0.30}{-0.60} = 0.00^3$	$\frac{-0.49}{-0.86} = 0.57$	$\frac{-0.69}{-2.92} = 0.24$
LC Average	0.37	0.12	0.65	0.34
HC1	$\frac{0.45}{2.09} = 0.22$	$\frac{-0.07}{0.89} = 0.00^3$	$\frac{0.20}{1.16} = 0.17$	$\frac{0.58}{4.14} = 0.14$
HC2	$\frac{0.50}{1.95} = 0.26$	$\frac{0.53}{1.10} = 0.48$	$\frac{0.06}{0.89} = 0.07$	$\frac{1.09}{3.94} = 0.28$
HC Average	0.24	0.24	0.12	0.21

1) R - response to selection: deviation of selected line progeny mean from entire population mean.

2) S - selection differential: deviation of selected parents mean from entire population mean.

3) Heritability could not be calculated - response was in the "wrong way", i.e., opposite to the selection direction.

Table 8 Reproductive performances of hens selected in S₁ generation; ANOVA and significance tests included only lines LC1, LC2, HC1 and HC2

Line	Number of hens	BWE1 (g)	AE1 (d)	LR (%)	TEG (eggs)	PHC (%)	MCWT (g)
LC1	26	3759	199	62	62	63	45.7
LC2	19	3786	208	57	54	59	47.4
LC Average	45	3773	204b	60	58	61b	46.6a
HC1	27	3678	209	65	61	72	43.8
HC2	26	3863	224	62	49	68	45.9
HC Average	53	3771	217a	64	55	70a	44.9b
S.E.M.		76.7	4.6	4.4	3.2	5.8	.76
P(F): LC vs. HC		.976	.004	.415	.215	.021	.030

LN	28	3758	221	56	43	72	45.8
HN	29	3739	218	59	47	68	48.4
CT	30	3725	212	67	60	76	47.9
All lines	185	3756	213	61	54	69	46.4

- 1 - BWE1 = Body weight at first egg
- 2 - AE1 = Age at first egg
- 3 - LR = Laying rate (%)
- 4 - TEG = Total egg number up to 300 days of age
- 5 - PHC = Hatchability of S₂ chicks
- 6 - MCWT = Mean body weight of 3rd hatch chicks

Table 9 Reproductive performances of hens selected in S₂ generation; ANOVA and significance tests included only lines LC1, LC2, HC1 and²HC2

Line	Number of hens	BWE1 (g)	AE1 (d)	LR (%)	TEG (eggs)	PHC (%)
LC1	14	3624	207	67	62	63
LC2	14	3490	208	57	52	57
LC Average	28	3557a	208	62	57	60a
HC1	12	3647	206	67	62	76
HC2	21	3923	225	60	45	81
HC Average	33	3785b	216	64	54	79b
S.E.M.		84.1	4.6	4.1	3.5	6.1
P(F): LC vs. HC		.031	.152	.507	.739	.026

LN	26	3509	221	64	52	77
HN	27	3869	227	58	42	64
CT1	26	3688	223	73	56	76
CT2	25	3487	207	71	65	67
All lines	161	3663	217	65	54	70

- 1 - BWE1 = Body weight at first egg
- 2 - AE1 = Age at first egg
- 3 - LR = Laying rate (%)
- 4 - TEG = Total egg number up to 300 days of age
- 5 - PHC = Hatchability of S₂ chicks

Table 10 Mortality and morbidity of 20 days old chicks from the selected and control lines, challenged with 1×10^7 CFU of E. coli, with or without vaccination 10 days earlier

	LC lines (1+2)	HC lines (1+2)	Control line
<u>Non-vaccinated</u>			
Mortality (dead/total)	6/41	10/44	5/20
Morbidity (P+P /total)	22/41	17/44	6/20
Mortality + morbidity (%)	68	61	55
<u>Vaccinated</u>			
Ln antibody titer	1.39±.07	1.77±.04	--
Mortality (dead/total)	8/41	3/44	--
Morbidity (P+P /total)	8/41	7/44	--
Mortality + morbidity (%)	39	23	--

* - P+P = chicks showing Pericarditis and/or Perihepatitis
7 days after challenge

Table 11. In-vitro proliferation of S3 chick T-lymphocytes
as a response to E.coli antigen.

Line	Time post vaccination		
	48h.	96h.	120h.
HC1+HC2	13.6±6.3* ^a	50.5±5.5	77.0±8.6
LC1+LC2	2.2±0.8*	32.9±6.9	55.5±1.3

An asterisk signifies difference ($P_3 < 0.05$) between the lines.
a= The results are given in CPMX10³

Table 12. In-vitro response of S1 and S3 chicks peripheral blood lymphocytes to mitogenic stimulation.

		PHA	The Mitogen ConA	PWM
S ¹	LC1	128.6±51.6 ^a	167.3±61.6	22.4±16.2
	HC1	159.8±70.1	212.2±62.0	30.6±21.0
S ³	LC1	168.8±55.2*	179.0±83.7*	29.1±11.5*
	HC1	287.0±65.1*	274.8±78.7*	48.5±18.0
	CT	183.0±65.7	168.0±65.6	40.7±14.0

S1 n=22

S2 n=16

a=The results are given as CPMX10³ Group Mean±SE.

An asterisk signifies differences (p<0.01) between the lines.

Table 13. Interferon production of S3 chicks peripheral blood lymphocytes due to PHA stimulation.

<u>S3 Chicks line</u>	<u>% protection</u>
HC	41.8±15.8
LC	38.9±16.9
Control	40.0±10.8

n=44

Fig.1

Means and distributions of individual antibody titer to *E. coli* of the control and selected lines in S3 generation

Fig. 2

Mean antibody titer of S0,S1,S2 & S3
and estimates of realized heritabilities

Fig. 3

Means and distributions of individual antibody titer to NDV of the control and selected lines in S3 generation

Fig. 4

Means and distributions of individual antibody titer to SRBC of the control and selected lines in S3 generation

Fig. 5

Fig. 6

DEVIATIONS OF SELECTED LINES MEANS
FROM THE ENTIRE GENERATIONS MEANS
S 0, S 1 AND S 3 COMPARATIVELY

LYMPHOCYTE RESPONSE TO PHA MITOGEN.

18 CHICKENS FROM EACH LINE

Fig. 7

DEVIATIONS OF SELECTED LINES MEANS
FROM THE ENTIRE GENERATIONS MEANS
S 0, S 1 AND S 3 COMPARATIVELY

LYMPHOCYTE RESPONSE TO CON-A MITOGEN.

18 CHICKENS FROM EACH LINE

Fig. 8

DEVIATIONS OF SELECTED LINES MEANS
FROM THE ENTIRE GENERATIONS MEANS
S 0, S 1 AND S 3 COMPARATIVELY

LYMPHOCYTE RESPOSE TO PWM

18 CHICKENS FROM EACH LINE

GENETIC RELATIONSHIPS BETWEEN THE IMMUNE RESPONSE, RESISTANCE TO INFECTIOUS AGENTS AND PRODUCTION PERFORMANCE OF CHICKENS

BARD FINAL REPORT

Virginia, 1990

Genetic variation for production traits and immune responses is well documented in chickens. Lacking is information on the relationships of general immune response, blood antigen loci, resistance to infectious agents and production traits. Results of this project which involved both commercial and experimental populations of chickens, demonstrated a delicate balance among maintenance, immunoresponsiveness, growth and reproduction. The balance, which depends upon differential allocation of a finite pool of resources, is influenced not only by previous and current environments, but also by genotype. Results of experiments conducted under this project demonstrated that differences among genetic stocks in resistance to infectious agents may be masked or accentuated by environmental manipulations. In addition to such genotype by environment interactions, we obtained evidence that effects of differing haplotypes of the major histocompatibility complex (MHC) may vary according to the background genome i.e., major gene complex by background genome interaction.

Selection of high and low antibody response to sheep red blood cells (SRBC)

Individual phenotypic selection for high (HA) and low (LA) antibody response 5 days after an intravenous injection of 0.1 ml of .25% SRBC antigen has resulted in populations of White Leghorn chickens distinctly different for this trait. Not only are total antibody titers to SRBC greater in line HA than LA, but the lines differ in kinetics of response. Antibody response in line HA is rapid, peaks at moderate levels, and persists. In contrast, the peak titer is lower and persistency is less in line LA. When secondary injections are given, titers are greater at 3 than at 5 days post injection and greater for line HA than LA.

IgG and IgM as measured by 2-mercaptoethanol resistant (MER) and susceptible antibodies to SRBC, respectively, have been determined in these lines. Whereas levels of MER peak and then decline in line HA, they persist only at low levels in line LA. Proportions of MER antibody are considerably lower in line LA than in line HA, both on an absolute basis and relative to total antibody. After a secondary injection, the

pattern for MER and for MES in line LA is similar to that for total antibody, as is MER in line HA. For MES, the pattern reverses in line HA.

Differences between lines HA and LA exist for production traits. Line LA chickens weigh more as juveniles and less as adults, mature at young ages, lay more eggs, and have lower heterophil to lymphocyte ratios than those from line HA. Also, there are large differences between these lines for erythrocyte antigen systems including the MHC. The correlated responses of unselected traits to selection for SRBC are not independent of resource allocations within the individual. Moreover, the phenotypic expression also reflects modifications in responses to individuals to external factors. Experiments were conducted to evaluate influences of these factors on responses to infectious agents.

Disease challenges in lines HA and LA

A series of experiments was conducted to examine relationships between selection for antibody response to SRBC and resistance to specific infectious agents. Previous experiments showed that responses among genetic stocks were influenced by the specific environments in which the chickens were maintained and by the severity of the challenge. Accordingly, different environmental settings were included in most trials. Examples of results are that the severity of E. coli infection is reduced more by adding ascorbic acid to the feed of line LA than HA chickens. Line LA chickens are more susceptible to E. tenella than line HA chickens under good husbandry. Under mild stressors the pattern remains the same, however, the difference between lines is less. Line HA is more susceptible than line LA to Str. fecalis, and treatment with corticosterone is more helpful in combating infection from this pathogen in line HA than LA. Responses of these lines to LaSota strain of Newcastle Disease is influenced by social stress. Among line HA chickens, response to challenge is similar for socialized and ignored chickens. In contrast, in line LA resistance to Newcastle is greater for socialized than ignored chickens. Trials involving Mycobacterium avium and avian adenovirus II also show that stressors influence the magnitude of differences between these lines.

Results of these experiments demonstrate that resistance to specific pathogens can be modified by selection for high or low antibody response to SRBC. Also, the magnitude of differences in response among lines can be modified by previous and current husbandry. Resistance advantage among lines differed depending

on the infectious agent, suggesting that an intermediate may be more advantageous than very high or very low titers. These results suggest that breeders may be more successful in combating disease organisms by selecting their populations for intermediate rather than high or low immunoresponsiveness.

To address this thesis we conducted trials where SRBC antibody was concurrently measured in commercial meat stocks, lines selected for high (HW) or low (LW) juvenile body weight, and lines HA and LA. SRBC titers for line HW and the commercial meat stocks were similar to those for line LA and lower than those for line HA. For example, mean SRBC titers 5 days after intravenous inoculation were 1.8, 1.3, 2.9, 3.2 and 7.0 for line LA, line HW, commercial A, commercial B and line HA, respectively.

To evaluate further the concept that, in meat-type poultry, resources are preferentially allocated into growth at the expense of immunoresponsiveness, early and late feather male broiler chicks were reared under ad libitum and alternate-day feeding for 28 days. At that age, half of the alternate-day fed broilers were released to ad libitum feeding. When 47 days of age, broilers were inoculated with E. coli. Mortalities to 72 hours post inoculation were 7, 20 and 50% for alternate, ad libitum, and released groups, respectively. These data, and those from companion experiments, provide further evidence of preferential allocation of resources.

To evaluate effects of selection for body weight on general mortality, comparisons included lines HW, LW and subpopulations of these lines where selection had been relaxed (HR and LR). Mortality data were obtained to 188 weeks of age. Characteristics of the first 40% of individuals in each line to die were compared to values for the surviving 60%. There was no evidence of mortality from infectious pathogen(s) during this experiment. Line LW chickens had the highest mortality, particularly at young ages. Juvenile body weights (at 4 and 8 weeks of age) were greater and egg production was higher in LW survivors than non-survivors. Although body weights and age at first egg in lines HR and LR generally regressed towards pre-selection values after cessation of selection, few physiological traits (body weights, antibody responses, egg production) were different between survivors and non-survivors within each line.

Antibody response to SRBC and thyroid hormones in various populations of chickens

Several experiments were conducted to define the role of thyroid hormones on immunoresponsiveness. Males from lines HA and LA were fed ad libitum either a diet containing 0 (control) or .1% (TF) thiouracil, from hatching through 64 days of age. Chickens were injected intravenously with .1 ml of .25% SRBC at 37 days of age and a booster of the same dosage was given to half of these chickens 23 days later. At 42 days of age, plasma thyroxine (T_4) concentrations were similar for both lines, however, line by diet interactions were present for plasma tri-iodothyronine (T_3) and ratios of T_3/T_4 . When fed the control diet, line HA chickens had higher T_3 concentrations and T_3/T_4 ratios than line LA. This pattern was reversed for chickens fed thiouracil. Primary and secondary antibody titers to SRBC were higher for line HA than LA, but were similar for TF and control chickens. In line HA, persistence of elevated titers as measured by percent change from 5 to 27 days post-primary inoculation was greater in TF than control chickens.

In a companion experiment normal and dwarf chickens from lines HW and LW were used to determine effects of dosage of antigen, line, sex-linked dwarfism, and plasma thyroid hormone concentrations on antibody titers to SRBC. The greatest separation of lines and of dwarf-normal genotypes occurred with an intermediate dose (0.1 ml of 0.25%) of SRBC. Five days after administration of SRBC at this dose antibody responses were greater for line LW than HW and in dwarf than normal chickens. The dwarf-normal but not the line relationship was evident for the booster challenge as well. Separation of lines and dwarf-normal genotypes for plasma thyroid hormone concentrations were greater at 71 than 36 days of age. Also the effect of the dwarfing allele on thyroid hormones was dependent on the background genome. There was no relationship between T_3 and T_4 concentrations and antibody responses to SRBC. These data do not support the suggestions of a relationship between antibodies to SRBC and plasma T_3 and T_4 .

Relationship between background genome and haplotypes of the MHC on antibody response to SRBC and disease resistance

To evaluate the influence of MHC genotypes in diverse backgrounds on production traits and immunoresponsiveness, sublines of lines HA and LA were developed by repeated backcrossing. Once the sublines were developed, progeny with MHC genotypes $B^{13}B^{13}$, $B^{13}B^{21}$ and $B^{21}B^{21}$ were produced in each

subline. Although antibody response to SRBC was consistently higher in background genome HA than LA, there was an interaction between background genome and MHC haplotypes. The interaction resulted from high antibody responses by $B^{13}B^{21}$ chickens of line HA and $B^{21}B^{21}$ chickens of line LA. Thus, response to SRBC was dependent on particular haplotype combinations present at the MHC as well as the background genome.

Much of the final year of the project has been devoted to measuring production traits and disease resistance of chickens with $B^{13}B^{13}$, $B^{13}B^{21}$ and $B^{21}B^{21}$ MHC genotypes in these sublines of lines HA and LA. Additional data for production traits are still being obtained because further confirmation is needed of a cross-over interaction of background genome by MHC haplotype for the important economic trait, hen-day egg production. Means of this trait for $B^{13}B^{13}$ hens were 72 and 78% in HA and LA backgrounds, respectively. For $B^{21}B^{21}$ hens respective values were 79 and 71%.

Challenges to infectious agents did not follow a specific pattern. There was no evidence of a MHC haplotype effect nor significant background genome by MHC haplotype for challenges involving E. tenella, Newcastle disease, tuberculosis or Mycoplasma gallisepticum. A natural exposure to Marek's disease revealed an interaction of background genome by MHC haplotype and an advantage of heterozygotes ($B^{13}B^{21}$; $B^{21}B^{13}$) to the homozygotes. For E. coli resistance, regardless of background genome, $B^{13}B^{13}$ chickens were consistently more resistant than $B^{21}B^{21}$ chickens with heterozygotes being intermediate.

The conclusions from this phase of the research are that the effects of major genes and gene complexes may be influenced by the background genome. The implications of this relationship are important as molecular genetic procedures become applicable in poultry breeding programs.

Publications

Siegel, P. B., M. N. Katanbaf, N. B. Anthony, D. E. Jones, A. Martin, W. B. Gross and E. A. Dunnington, 1987. Responses of chickens to Streptococcus faecalis: Genotype-housing interaction. Avian Diseases 31:804-808.

Katanbaf, M. N., P. B. Siegel and W. B. Gross, 1987. Prior experience and response of chickens to streptococcal infection. Poultry Sci. 66:2053-2055.

- Collins, J. W. and P. B. Siegel, 1987. Human handling, flock size, and responses to and E. coli challenge in young chickens. *Applied Animal Behaviour Sci.* 19:183-188.
- Martin, A., F. M. A. McNabb and P. B. Siegel, 1988. Thiouracil and antibody titers of chickens from lines divergently selected for antibody response to sheep erythrocytes. *Develop. Comp. Immunology* 12:611-619.
- Dunnington, E. A., A. Martin, R. W. Briles, W. E. Briles, W. B. Gross and P. B. Siegel, 1989. Antibody response to sheep erythrocytes for White Leghorn chickens differing in haplotypes of the major histocompatibility complex (B). *Animal Genetics* 20:213-216.
- Gross, W. B., C. H. Domermuth and P. B. Siegel, 1988. Genetic and environmental effects on the response of chickens to avian adenovirus group II infection. *Avian Pathology* 17:767-774.
- Katanbaf, M. N., D. E. Jones, E. A. Dunnington, W. B. Gross and P. B. Siegel, 1988. Anatomical and physiological responses of early and late feathering broiler chicks to various feeding regimes. *Archiv. Geflugelk.* 52:199-126.
- Gross, W. B. and C. H. Domermuth, 1988. Factors influencing the severity of avian adenovirus group II and Escherichia coli infections in chickens. *Avian Diseases* 32:793-797.
- Martin, A., F. M. A. McNabb and P. B. Siegel, 1988. Thyroid hormones and antibody response to sheep erythrocytes of dwarf and normal chickens selected for juvenile body weight. *Genetique, Selection & Evolution* 20:499-510.
- Dunnington, E. A., A. Martin, C. O. Ubosi and P. B. Siegel, 1989. Liveability in female chickens selected for growth - Associations with body weight, egg production and antibody traits. *J. Genetics & Animal Breeding* 106:152-158.
- Martin, A., W. B. Gross and P. B. Siegel, 1989. IgG and IgM responses in high and low antibody-selected lines of chickens. *J. Heredity* 80:249-252.
- Martin, A., E. A. Dunnington, W. E. Briles, R. W. Briles and P. B. Siegel, 1989. Marek's disease and major histocompatibility complex haplotypes in chickens selected for high or low antibody response. *Animal Genetics* 20:407-414.
- Martin, A., E. A. Dunnington, W. B. Gross, W. E. Briles, R. W. Briles and P. B. Siegel, 1990. Production traits and alloantigen systems in lines of chickens selected for high or low antibody response to sheep erythrocytes. *Poultry Sci.* 69(in press).

F. Cooperation.

During the 3 years of this project a permanent channel of communication between the partners, Bitnet, was used. This allowed an immediate flow of information between Blacksburg and Rehovot and vice-versa.

Prof. Siegel visited Israel and Dr. Heller Visited Virginia. These visits were used for exchange of techniques, evaluation of results and future planning.

G. The Research Achievements with Respect to the Original Research Proposal and the Benefit to Agriculture

Conclusion remarks.

Both groups dedicated their efforts in study the relationship between general immune responses, resistance to infectious agents and production traits and their genetic control. The Rehovot group studied this relationships in commercial meat-type chickens.

The findings strongly indicated that genetic selection for early immune responsiveness can be accomplished without affecting production traits.

The original idea was that the selection should be performed using two antigens, a bacterial and a viral. The results of the project's first year indicated that E.coli antigen, as prepared by us, was sufficient to allow selection with a broad effect.

After 3 generations of selection highly significant differences between the replicated low-coli responders (LC1 and LC2) and the high-coli responders (HC1 and HC2). The realized heritability over 3 generations was found to be 0.34 and 0.21 in the LC and HC lines respectively. Mean body weight of all eight lines were very similar. Six reproductive traits that were recorded. Of all these traits only hatchability was found to be of significant difference between the lines developed (line HC showed higher hatchability than LC). Besides to the practical importance of the advantageous hatchability of the HC line, it indicates the possible role of the immune system in hatchability.

Our selected line for high antibody response at early age to E.coli showed higher resistance to pathogenic E.coli bacteria when the challenge was performed after immunization. This line responded with higher antibody titers to Newcastle disease vaccine and Sheep erythrocytes, demonstrated higher phagocytic activity (in vivo carbon clearance) and its lymphocytes responded higher to T and B cell mitogens. Experiments examining the selected lines resistance to challenge with pathogenic Newcastle disease virus and Infectious Bursal Disease virus failed to show conclusive results and needed further calibration. The chicken line selected for low antibody response to E.coli at 10 days of age was found inferior in its response in the other tests.

A preliminary study of the lines' Major Histocompatibility Complex using RFLP (see attached) exhibited the possibility of using this method as additional tool for selection for high immune responses and resistance to diseases.

The Blacksburg group performed selection based on high (HA) and low (LA) response to SRBC of \White Leghorn chickens. The lines developed differed in their antibody titers and in the kinetics of the response. Differences between the lines exist for production traits. Line LA chickens weigh more as juveniles and less as adults, mature at young ages, lay more eggs and have lower heterophil to lymphocyte ratios than those from line HA. There are differences between these lines for erythrocyte B antigen systems including the MHC. The results of experiment involving disease challenges in lines HA and LA demonstrated that resistance to specific pathogens can be modified by selection for high and low antibody response to SRBC. The results suggest that breeders may be more successful in combating disease by selection their populations for intermediate rather than high or low immunoresponsiveness.

Several experiments were conducted to define the role of thyroid hormones on immuneresponsiveness. There was no relationship between T_3 and T_4 concentrations and antibody responses to SRBC.

The results of this project which involved both commercial and experimental populations of chickens, demonstrated a delicate balance among maintenance, immunoresponsiveness, growth and reproduction. The balance, which depends upon differential allocation of a finite pool

of resources, is influenced not only by previous and current environments, but also by genotype. Evidence was obtained that effects of differing haplotypes of the MHC may vary according to the background genome.

These results can be of great benefit to chicken breeders. The addition of a simple procedure, measuring antibody titer to E.coli at 10 days of chicks life or to SRBC, may give a good indication of the chicks immune capacity, a trait of high importance. This can decrease mortality and increase resistance and livability of the flocks.