ABSTRACT

CHEEMA, MANZOOR AHMED. A comparison of the immune performance of commercial growth selected broiler genotypes. (Under the direction of Muquarrab A. Qureshi.)

Two studies were conducted to analyze the interaction of genetics and nutrition on immune responsiveness of broilers. The first study involved four modern day broiler strains: Ross 3F8 (3F8, vent sexable), Ross x Cobb (RC, vent sexable), Ross 308 (308, feather sexable), and Cobb x Cobb (CC, vent sexable) raised on marginal (D1) and high protein (D2) diets. The second study compared male and female broiler chickens from a 2001 hatched Ross 308 broiler strain (ROSS) with male and female of 1957 Athens-Canadian Randombred Control (ACRBC) strain, when raised on diets 'typical' of those used in 1957 (D1) and 2001 (D2). The immunological measurements were the lymphoid organ weights relative to body weights, humoral response to sheep red blood cells (SRBC), T-lymphocyte proliferation response to *in vivo* Phytohemagglutinin-P (PHA-P), and innate macrophage response as measured by elicitation of abdominal exudate cells to Sephadex G-50®, phagocytosis of SRBC and nitrite production. Three additional measurements were included in the first study, namely chemotaxis of peripheral blood lymphocytes to formyl-met-leu-phe, T lymphoblastogenesis in response to Concanavalin-A (Con-A), and non-specific tumoricidal activity by splenic natural killer (NK) cells against ^{51}Cr labeled RP-9 cells.

In the first study, the CC strain had persistent antibody titers against SRBC (P = 0.0182) as well as greater innate immune response as measured by macrophage phagocytic ability for SRBC (P = 0.011). Cell-mediated immune response as measured by T-lymphocyte proliferation response to Con-A and PHA-P (P = 0.0018 and $P \leq 0.04$, respectively) was higher in Ross 308 strain. The high protein diet contributed to a greater T-lymphocyte proliferation whereas the diet effects were variable for monocyte-macrophage functions and humoral response. Interaction between strain and diet was seen in humoral response, and the Ross 308 performed better on D1 while CC performed better on D2.

In the second study, the ACRBC strain, despite being a low weight gain strain, showed significantly higher primary and secondary lymphoid organ weights, relative to body weight ($P \leq 0.04$). Furthermore, the ACRBC strain showed significantly higher antibody titers against SRBC than the ROSS 308 broilers. However, the ROSS 308 strain had significantly greater PHA-P-induced lymphoblastogenic response *in vivo* (P = 0.012). For macrophage functions, the ROSS 308 strain exhibited a higher number of inflammatory exudate cells in response to Sephadex (P = 0.0261) as well as phagocytic potential against SRBC ($P < 0.05$). In addition, the ROSS 308 birds had a greater increase in nitrite production after LPS stimulation compared to the ACRBC strain birds. Diet and sex effects were inconsistent in the second study.

Overall, these studies indicated that genetic differences for immunocompetence exist in commercial broiler strains. These genetic differences tend to indicate that modern broiler strains are less capable of launching a successful humoral response against SRBC

whereas cell mediated and innate immune responses seem to have benefited from artificial selection for increased body weight.

A COMPARISON OF THE IMMUNE PERFORMANCE OF COMMERCIAL GROWTH SELECTED BROILER GENOTYPES

By

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BIOGRAPHY

Manzoor Ahmed Cheema was born August 8, 1975 in Gujranwala, Pakistan. In 1994 he completed higher-secondary school education from Islamabad, Pakistan. In 1999 he received his Doctor of Veterinary Medicine (DVM) degree from College of Veterinary Sciences, Lahore, Pakistan. The following year, he enrolled in the M.S. program in Immunology, with a minor in Biotechnology, at North Carolina State University, in the fall of 2000, under the supervision of Dr. M. A. Qureshi in the Department of Poultry Science.

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INTRODUCTION

One of the concerns of commercial poultry farmers is the protection of their flocks against disease challenge. This objective could be achieved through selecting birds that are resistant against particular pathogens, and/or those displaying better immunocompetence, i.e., the general quality of host's immune system to launch sufficient defense against infections (Knap and Bishop, 2000). Previous studies have linked genetic makeup of poultry to disease resistance and/or susceptibility (Lamont *et al*., 1987; Ruff and Bacon, 1989; Lakshman *et al.*, 1997; Poulsen *et al.*, 1998; Yonash *et al.*, 2001). Furthermore, genetic improvement for growth performance over the years has been seen to negatively influence immune performance of chickens. High growth rate in broiler chickens has also been linked to increased susceptibility for Marek's Disease (Han and Smyth, 1972). Poor immunocompetence as measured by humoral response to SRBC and T-cell proliferation response to Phytohemaggultinin-P, as well as increased lesion score for *Escherichia coli* (Qureshi and Havenstein, 1994; Rao *et al*., 1999) were found in broiler chickens with higher body weight (BW). In another study, broilers with high growth rate had increased mortality rate under commercial conditions of management compared to slow growing broilers (Yunis *et al*., 2000).

Dietary protein and energy content have also been shown to affect immune response. For example, chickens that were raised on diets deficient in proteins and calories showed reduced T-lymphocytes and humoral response against SRBCs (Glick *et*

al., 1981, 1983). Similarly, Praharaj et al. (1995) found that chicken diet with 17% less metabolizable energy (ME) and 20% less crude protein content greatly reduced antibody titers against SRBC in commercial White Leghorns and White Plymouth Rock strains from 6 to 13 d post-inoculation (PI), compared with a high protein diet. Furthermore, chicks that were raised on a low protein displayed higher acute phase immune response, as measured by plasma alpha 1-acid glycoprotein (AGP) and interleukin-1- (IL-1) like activity, in response to *Escherichia coli* lipopolysaccharide, in comparison with those fed a high protein diet (Takahashi *et al*., 1995).

Sex of the chickens also affects the immune performance. Mortality due to Marek's Disease challenge was reported to be higher in male birds of $B^{13}B^{21}$ Cornell Randombred White Leghorn chickens divergently selected for low antibody response to SRBC than in females of the same genetic stock, signifying a sex effect in disease susceptibility (Martin *et al*., 1989). Furthermore, age of the chickens also has an affect on immune performance. Due to immunological immaturity young chickens have a greater incidence of diseases such as infectious bursal disease, avian encephalomyelitis, Marek's Disease, *E. coli* and Salmonella infections (van der Zijpp, 1983).

Two studies were conducted to determine the impact of genetics, dietary protein and energy levels, age and sex of broiler chickens on immunocompetence. The first study utilized four modern day broiler crosses including the Ross 3F8 (3F8, vent sexable), Ross x Cobb (RC, vent sexable), Ross 308 (308, feather sexable) and Cobb x Cobb (CC, vent sexable) when raised on marginal (D1) and high protein (D2) diets. The impact of age on immune performance was quantified by comparing immune performance at one and four

weeks of age. The second study compared a popular commercial broiler strain produced in the calendar year 2001 (Ross 308, feather sexable) with the Athens Canadian Randombred Control (ACRBC) strain that was established in 1957, when raised on diets typical of those used during calendar years 1957 and 2001. The second study was carried out following a previous finding in which 1991 commercial broiler strain and ACRBC strain were compared and a negative impact of high growth performance in broilers on humoral response against SRBC was reported (Qureshi and Havenstein, 1994). In both studies, the immunological endpoints measured were lymphoid organ weights relative to body weights, humoral response to sheep red blood cells (SRBC), T-lymphocyte proliferation response to *in vivo* Phytohemagglutinin-P (PHA-P), and innate macrophage response as measured by elicitation of abdominal exudate cells to Sephadex G-50®, phagocytosis of SRBC and nitrite production. In addition to these measures, the first study included chemotaxis of peripheral blood lymphocytes to formyl-met-leu-phe, T-lymphoblastogenic response to *in vitro* Con-A exposure, and non-specific tumoricidal activity by splenic natural killer cells against ^{51}Cr labeled RP-9 cells.

CHAPTER 1

LITERATURE REVIEW

This review of literature will focus on the non-specific as well as adaptive immune responses, including macrophage, natural killer cell, and B- and T-cell functions in avian species. For this review, previous experiments on the impact of genetic background and the influence of nutritional components on different immune parameters in broiler chicken were also surveyed.

A. Overview of the Immune System

The immune system provides protection against infectious diseases that are caused by various microorganisms including viruses, bacteria, pathogenic fungi and parasites, and it is broadly divided into two categories – namely innate or non-specific immune system and the acquired or specific immune response. Innate immunity is the basic defense mechanism for an animal provided by non-discriminatory barriers of anatomic, physiologic, endocytic and phagocytic, and inflammatory mechanisms. The acquired immunity is a specialized response against infectious agents, and is characterized by specificity, diversity, memory, and self/non-self recognition (Kuby, 1994). The effector cells involved in the innate immune response include granulocytes and macrophages, while the adaptive immune response involves lymphocytes. However, there is not a complete demarcation between these two types of immunities and cells

involved in the innate immune response take part in effector actions of the adaptive immune response (Janeway *et al*., 2001).

A.1. Components of the Immune System

Cells of the immune system arise from stem cells during the first few weeks of embryonic development. Intraembryonic mesoderm develops into progenitors of endothelial cardiac cells, muscle cells, and hematopoietic stem cells. The hematopoietic stem cell is pluripotent and differentiates along one of three pathways, namely as megakaryocytes and platelets, lymphoid stem cells or as myeloid stem cells. Lymphoid progenitor cells differentiate into T-lymphocytes, B-lymphocytes or natural killer (NK) cells; the myeloid progenitor cell generates osteoclasts (for bone formation) or myeloid cells (monocytes, macrophages, dendritic cells, granulocytes) (Melchers and Rolink, 1999).

A.2. B-lymphocytes

B-lymphocytes originate from bone marrow in the mice, humans and other mammals, and from the bursa of Fabricius in birds. Mature B-cells have antibody molecules on their surface that serve as B-cell receptors. Each B-cell receptor (BCR) molecule has an identical binding site for antigens (Melchers and Rolink, 1999). The majority of B-cells express major histocompatibility (MHC) II molecules, and they are classified as antigen presenting cells.

Early events in B-cell activation include antigen contact with specific B-cells, that leads to transmembrane signaling, increased expression of Class-II MHC molecules, exit from the resting state (G_0) to the G_1 phase of the cell cycle, and in case of a strong signal, proliferation (DeFranco, 1995 and 1997). B-cell receptors are also rapidly internalized after antigen binding, leading to antigen uptake, degradation in endosomes or lysosomes, and the antigenic peptide binds in the groove of MHC II molecules after which this complex comes to the cell surface and stimulates specific helper T-cells (Davidson *et al*., 1991).

Activated helper T-cells also produce cell surface molecules. The most important of these is CD40 L that interacts with the B-cell receptors and thus provide important activating signals (Clark *et al*., 1996). Following activation, B-cells may either proliferate and terminally differentiate into antibody (Ab) secreting cells (plasma cells) in the T-cell zone of lymphoid organs or may migrate to the follicular area and initiate formation of a germinal center (Forster *et al*., 1996; Kesloe, 1996). In germinal centers, B-cells activate somatic hypermutation on their immunoglobulin genes and then undergo a stringent selection for high affinity binding to antigen, resulting in production of memory B-cells and plasma cells secreting antibodies of high affinity (Berek *et al*., 1991; MacLennan *et al*., 1997).

In some cases, B-cells are activated to produce antibodies without the involvement of helper T-cells (Bachman *et al*., 1995; Szomolanyi-Tsuda and Welsh, 1996). These T-cell independent antigens are categorized as either type 1 (potent

polyclonal activators of B-cells) or type 2 (trigger vigorous and prolonged signaling by the BCR) based on their properties.

A.3. Immunoglobulins

Immunoglobulins (Ig) play a central role in the humoral immune response. They appear as receptors on the surface of B-cells and initiate processes such as activation, differentiation and even programmed cell death or apoptosis (Frazer and Capra, 1999; Hase *et al*., 2002). As secreted molecules in plasma and other bodily fluids they are able to bind foreign antigens, either neutralizing them directly or indirectly through activation of the complement system or antibody-dependent cell cytolysis by monocytic phagocytes (Mazanec *et al.*, 1992).

The antibody molecule is Y-shaped molecule, consisting of three equal-sized segments loosely connected by a hinge-like junction. The two ends are variable between different antibody molecules and are involved in antigen binding. The variable region provides specificity to the antibody. The third end is conserved and interacts with the effector molecule. Antibody molecules are made of heavy or H chains and light or L chains (Janeway *et al*., 2001). The structure of the heavy chain determines the class or isotype of the immunoglobulins IgM, IgD, IgG, IgA, and IgE (Kuby, 1994). The distinctive functions of these isotypes are determined by the carboxy-terminal of the heavy chain, which contributes the constant region of the antibody. Structurally, each immunoglobulin molecule is composed of four polypeptide subunits, two identical heavy chains and two identical light chains, stabilized by multiple interchain disulfide bonds

(Frazer and Capra, 1999). Out of these, IgG is the most abundant class of antibody found in serum and activates the complement cascade as well as the effector cells such as macrophages, polymorphonuclear cells and lymphocytes through Fcγ receptor (Duncan and Winter, 1988; Edberg *et al*., 1995). IgM is secreted by plasma cells as a pentamer in which five monomers are held together by disulfide bonds linking their carboxy terminal domains and CH3/CH3 domains (Janeway *et al*., 2001). IgM is expressed as membrane bound antibody (Ab) on B-cells and serves as the antigen specific receptor for B-cells. The activation-signal is transmitted by accessory molecules Igα and Igβ (Hombach *et al*., 1990). IgA is the predominant immunoglobulin class formed in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts. IgD, together with IgM, is the major membrane bound immunoglobulin expressed by mature B-cells, and it is thought to function in the antigen activation of a B-cell by an antigen (Frazer and Capra; 1999, Janeway *et al*., 2001).

Macfarlane Burnet (1959) is credited for the 'clonal selection theory' of antibody production. He postulated that antibodies are natural products that appear on the cell surface as receptors with which antigens react selectively. The interaction of an antigen with the surface receptors signals a clonal proliferation of a population of cells that are phenotypically restricted for the specificity of the given antibody. Some daughter cells of the clone then differentiate into antibody forming cells, and others remain as immunologic memory cells able to participate in later booster responses. In addition to germline diversity, Tonegawa (1983) suggested the concept of somatic mutation during which the variable region of the heavy chain (V_H) is generated by the rearrangement of

specific variable (V_H), diversity (D) or joining (J_H) gene segments. The variable region of the light chain (V_L) is produced through rearrangement of specific V_L and J_L gene segments. In addition to germline diversity and somatic mutation, affinity of serum antibodies for antigen increases over a period of weeks after antigen exposure and this is called affinity maturation (Siskind and Benacerraf, 1969).

A.4. T-lymphocytes

The T-lymphocytes derive their name from their site of maturation in the thymus. T-cells recognize antigens along with the MHC molecule as suggested from experiments of Zinkernagel and Doherty (1974). It is now recognized that out of two types of T-cells, helper T-cells recognize antigen carried by MHC II molecules while cytotoxic T-cells recognize antigens on the MHC I molecule.

In humans and mice, 90-95% of T-lymphocytes have αβ T-cell receptors (TCR) and they are responsible for classical helper or cytotoxic T-cell responses (Davis and Chien, 1999). γδ TCR are less numerous than αβ TCR in case of humans and mice (Alam *et al*., 1996). However γδ TCR make up a significant fraction of T-lymphocytes in cows, sheep and chickens. The γδ subset of TCR is suggested to recognize antigen (Ag) directly without the involvement of the MHC molecule (Benoist and Mathis, 1999).

Many of the cytokines produced by T-cells influence B-cell differentiation (Poo *et al*., 1988). T_{H1} cells employ mediators to activate macrophages, to direct activity against parasitic and fungal agents as well as being involved in the delayed type hypersensitivity reaction (Benoist and Mathis, 1999). T_{H2} cells interact with B-cells through CD40/

CD40L pair formation that causes activation and proliferation of B-cells (Foy *et al*., 1996).

Cytotoxic T-lymphocytes release perforins and granzymes and induce apoptosis of target cells bearing foreign peptides associated with MHC I molecules. As the name indictaes, perforins cause pore formation in the membrane of target cells, while granzymes are employed in the protease cascade of apoptotic pathways (Liu *et al*., 1995; Darmon *et al*., 1995).

A.5. Natural Killer Cells

A third class of lymphoid cells lacks the antigen binding receptor of T- and B-cell lineage, and they are called natural killer (NK) cells. Lanier *et al*. (1986) suggested two different lineages of NK-cells, one differentiates in bone marrow and the other, a class of non-MHC restricted cytotoxic T-lymphocytes (CTL), originates from T-cells. NK-cells provide host non-specific resistance against certain kinds of tumors and infectious diseases (Herberman and Ortaldo, 1981). They are part of the innate immune system. In some cases, the NK-cell makes direct membrane contact with a tumor cell in a non-specific, antibody-independent process. Some NK-cells express CD16 that have an affinity for IgG antibodies bound to tumor cells and can subsequently destroy the tumor. This specific process is called antibody-dependent cell-mediated cytotoxicity.

A.6. Macrophages

Granulocyte-monocyte progenitor cells differentiate into promonocytes, leave the bone marrow and enter into the blood stream. They then migrate into tissues and differentiate into specific tissue macrophages. Macrophages are dispersed throughout the body. Some take up residence in particular tissues becoming fixed macrophages, whereas others remain mobile and are called free or wandering macrophages. Free macrophages move by ameboid movement throughout the tissues. Fixed macrophages serve different functions in different tissues. For example, in the liver they are called Kupffer cells; in the connective tissues, histiocytes; in the lung, alveolar macrophages; in the kidney, mesangial cells; and, in the brain, microglial cells (Kuby, 1994; Janeway *et al.*, 2001).

Macrophages are normally in a resting state, but in the course of an immune response, a variety of stimuli activate macrophages. Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokines secreted by activated T_H-cells, by mediators of the inflammatory response, and by bacterial cell-wall products. One of the most potent activators of macrophages is interferon gamma (IFN-γ) secreted by activated T_H-cells (Unanue and Allen, 1987).

Macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called ‘chemotaxis’. In phagocytosis there is adherence of antigen to the macrophage cell membrane that induces membrane protrusions, called pseudopodia, to extend around the attached material. The pseudopodia fuse, enclosing the material within the phagosome. Lysosomes fuse with phagosomes to

form phagolysosome. Lysosomes contain hydrogen peroxide, oxygen free radicals, peroxidase, lysozyme, and various hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called exocytosis (Kuby, 1994).

Reactive nitrogen intermediates are produced when macrophages are activated with bacterial cell-wall lipopolysacharide (LPS) or muramyl dipeptide (MDP), together with a T-cell derived cytokine interferon-gamma (IFN-γ) (Hibbs *et al*., 1972; Weinberg, 1978; Hussain and Qureshi, 1998). As a result of this encounter, macrophages begin to express high levels of nitric oxide synthetase, which oxidizes L-arginine to yield citruline and a reactive radical, nitric oxide (NO) (Hibbs *et al*., 1987). Although NO has potent antimicrobial activity, it can combine with the superoxide anion ($O_2^{\cdot -}$) to yield even more potent antimicrobial substances. Recent evidence suggests that much of the antimicrobial activity of macrophages against bacterial, fungal, helminthic, and protozoal pathogens is due to NO and NO-derived substances (Kuby, 1994; Kun *et al*., 2001).

Another important function of macrophages is antigen processing and presentation. The phagocytosed antigen is degraded within the endocytic processing pathway into peptide that associates with class-II MHC molecules. These peptides and class-II MHC molecules then move to the macrophage membrane. This presentation of antigen is a critical requirement for the activation of T_H-cells, a central event in the development of both humoral and cell-mediated immune response (Unanue and Allen, 1987).

A.7. Primary Lymphoid Organs

Primary lymphoid organs provide the environment where lymphocytes mature.

A.7.a.Thymus

T-cell progenitors formed during hematopoiesis (in bone marrow) enter the thymus as immature thymocytes and mature there to become self-MHC restricted and self- tolerant T-cells. Both the cortex and the medulla of the thymus are interspersed by a network of stromal cells composed of epithelial cells, interdigitating dendritic cells, and macrophages, which interact with and contribute to maturation of thymocytes (van Ewijk, 1991). Stages of thymocyte development include T-cell receptor generation by a series of random gene rearrangements, and positive and negative selection that shapes the mature T-cell receptor repertoire (Kang and Raulet, 1997). During the selection process, the thymocytes, that are capable of recognizing foreign antigen on self-MHC, are selected while self-reactive thymocytes are eliminated. The earliest cell population in the thymus does not express the surface molecules CD4 or CD8 and they are called double negative thymocytes. Expression of a pre-T-cell receptor, pTα:β, on the cell surface is associated with small amounts of CD3 and the loss of CD25, cessation of β-chain gene rearrangement, cell proliferation, and the expression of CD4 and CD8. There are two processes that select for single positive T-cells in thymus: 1) positive selection of thymocyte bearing receptors capable of binding self-MHC:self-peptide complexes, which result in the commitment to self-MHC expressing a foreign antigen. Co-receptor CD8 is selected to recognize antigen presented by MHC I while CD4 is selected to recognize

antigen presented by MHC II. Thymic cortical epithelial cells mediate positive selection (Cosgrove *et al*., 1992). 2) Negative selection eliminates thymocytes bearing high-affinity receptors for self-MHC molecules alone or self-antigen presented by self-MHC. Dendritic cells and macrophages carry out the negative selection (Sprent and Webb, 1995).

An estimated 95-99% of developing thymocytes undergo programmed cell death without ever maturing. The neonatal animals whose thymus has been removed, show reduced numbers of T-lymphocytes and a failure to initiate cell-mediated immune response (Kuby, 1994).

A.7.b. Bone Marrow

Both B- and T-lymphocytes originate in the bone marrow, but only B-lymphocytes mature there. T-lymphocytes migrate to the thymus for the maturation process. In adults, the majority of Ig-secreting cells are localized in the bone marrow. Shortly after immunization, most antibody-forming cells occur in peripheral lymphoid tissues, but later on, especially during secondary type responses, most antibody-forming cells are localized in the bone marrow (Benner *et al.*, 1981).

A.8. Secondary Lymphoid Organs

The spleen and other mucosal lymphoid tissues provide sites where mature lymphocytes can trap and interact with antigens.

A.8.a. Spleen

The spleen is an adaptation of the animal for filtering blood and trapping blood-borne antigens, so that the animal can respond to systemic infections. Splenic compartments are of two types – the red pulp and the white pulp, which are separated by a diffuse marginal zone. The red pulp consists of a network of sinusoids populated with macrophages and numerous erythrocytes. It is in this site where old and defective erythrocytes are destroyed and removed. The white pulp surrounds the arteries, forming a periarteriolar lymphoid sheath (PALS) populated mainly by T-lymphocytes. The marginal zones in the periphery of PALS are rich in B-cells organized in primary lymphoid follicles (Kraal, 1992). Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers where rapidly dividing B-cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes. The effects of splenectomy are dependent upon the age of the individual. At a young age, spelenectomy leads to an increased incidence of bacterial sepsis, whereas in adults its effects are less severe (Kuby, 1994; Balazc *et al*., 2002).

A.8.b. Mucosal Associated Lymphoid Tissue

In mammals, the mucosal-associated lymphoid tissues (MALT) include the tonsils, appendix, Peyer's patches, and the loosely clustered lymphoid cells in the lamina propria of the intestinal villi. The number of antibody producing plasma cells in the MALT is considerably larger than in spleen, lymph nodes, and bone marrow combined

(Kuby, 1994). The mucosal membranes in the respiratory, digestive, and urogenital tracts contain specialized cells called M-cells that deliver small samples of foreign antigen from the lumen of the organ to a deep pocket within the mucosa that is filled with clusters of B-cells, T-cells, and macrophages. M-cells express class-II MHC molecules to present antigens to the T_H-cells (Kuby, 1994).

B. Avian Immune System

Similar to the mammals, the avian species also have an adaptive immune system, which is further differentiated into humoral and cell-mediated immune branches. In fact, the B-lymphocytes are the most important players in humoral immune response and they derived their name from the bursa of Fabricius in birds. Glick (1956) established the bursa of Fabricius' role in humoral immunity after observing a failure in antibody formation in bursectomized birds.

B.1. Humoral Immune Response

The bursa of Fabricius is the site for B-cell development and re-arrangement of germ-line Ig genes allowing normal antibody responses in birds (Glick, 1956). Mammalian immunoglobulin homologs for IgM, IgG and IgA have been characterized in birds (Benedict and Berestecky, 1987).

In contrast to mammalian immunoglobulin genes, avian B-cells have only a single functional V and J gene segment for the heavy and light chain loci that reduce combinatorial diversity of Ig genes in chickens (Reynaud *et al*., 1985). However, the

chicken Ig light chain has a segment of 25 genes, and the heavy chain has 80-100 genes that are termed 'pseudogenes', since they lack a leader exon, promoter region, and a functional signal sequence (heptamer-spacer-nonamer) at the 3' end (Reynaud *et al*., 1994). To produce the desired diversity in antibody molecules to account for the multitude of antigens, chickens utilize a DNA recombination process, a non reciprocal transfer of nucleotide sequence blocks from the V region pseudogenes to functionally rearranged V_H and V_L genes (McCormack *et al*., 1991).

Like in mammals, avian humoral immune response may be thymic dependent or thymic independent. The thymic dependent pathway requires the interaction of three cell types: the T-cells, the B-cells and an antigen-presenting cell (APC) such as the macrophage. The T-cell is activated following its recognizing of an antigen displayed by the MHC II molecule on an APC. It then activates a B-cell, either directly (cognate interaction) or indirectly, by releasing a series of activating factors that belong to the interleukin family. The B-cells then differentiate into antibody producing plasma cells. In the thymus independent pathway, B-cells either make direct contact with soluble antigens through the Ig receptors or they recognize the antigen as it is displayed by the macrophage. Upon first exposure to an antigen, birds produce a primary humoral immune response that is characterized by high levels of IgM. A secondary immune response is produced after subsequent exposure with the same antigen and IgG prevails during that response. Both the primary and the secondary responses have four phases. These include a latent phase, the period shortly after initial antigen exposure in which no antibodies are detected in the blood serum; immuno-progressive phase, an exponential production phase

of antibodies; antibody peak phase, when the antibodies reach maximum level in serum; and regression phase, when concentration of antibodies in serum rapidly declines (Glick, 1991).

B.2. Cell-Mediated Immune Response

The T-cell receptor (TCR) complex is made up of a TCR chain for antigen recognition (TCRα and TCRβ, or TCRγ and TCRδ) and an associated signal-transducing CD3 complex. In addition to TCR1 and TCR2 in mammals, avian species have an additional subtype of T-cells, TCR3 (Göbel *et al*., 1996). The α and β T-cells are reactive to monoclonal antibodies (mAb) against TCR2 and TCR3, whereas the γ and δ T-cells are reactive to TCR3 mAb (Sowder *et al*., 1988). Chicken T-cells utilize the same strategy of random gene rearrangements of DNA fragments encoding the variable region (V, D, and J) as in mammals, and the random arrangement is coupled with the joining of independent α, β, or γ, δ, chains which gives rise to 10^{15}-10^{18} different combinations in variable region of the TCR (Davis and Bjorkman, 1988). Most peripheral αβ T-cells express either CD4 or CD8, whereas the majority of splenic and all blood γδ T-cells are CD4 and CD8 negative. About 5-15% of splenic γδ T-cells are CD8+ (Sowder *et al*., 1988).

B.3. Macrophage Functions

As in mammals, macrophages in birds belong to a mononuclear phagocytic system, and are considered the first line of defense against invading organisms. Being mobile scavenger cells, macrophages participate in the innate immune responses. When the monocytes migrate to various tissues, they differentiate into tissue macrophages that are also APC and participate in the acquired immune response. Because chickens lack resident abdominal macrophages, intra-abdominal injection of Sephadex G-50 can elicit macrophage recruitment at the site of injection (Trembicki *et al*., 1984; Qureshi *et al*., 1986; Harmon *et al*., 1992). Macrophages develop their phagocytic potential at embryonic day 12 for liver macrophages and at day 16 for splenic macrophages (Jeurissen *et al*., 1989). Phagocytosis of opsonized antigens is increased over unopsonized antigens. Avian Sephadex-elicited macrophages are activated by signals from *E. coli* LPS or lymphokines (Qureshi *et al*., 2000a). Blood monocytes display chemotaxis towards the antigen, whereas macrophages, once differentiated in the tissues, depend on invading microorganisms brought to them. The invading microorganisms are trapped in phagosomes which fuse with lysosomes. Lysosomal enzymes eventually digest the microorganisms (Qureshi and Dietert, 1995). However, there are certain avian bacteria such as *Staphylococcus aureus* that are resistant to phagocytosis by macrophage. Other bacteria (such as *Pasteurella multocida*) possess a capsule that helps them to escape internalization by macrophages (Harmon *et al*., 1992; Pruimboom *et al*., 1996). Similarly, avian viruses show different responses when they interact with macrophages. Some viruses, such as the herpesvirus of turkeys (HVT-FC16), infectious bronchitis virus

(IBV), and reticuloendotheliosis virus (REV), are unable to infect the macrophages. Other viruses such as the adenovirus, infectious laryngeotracheitis (ILT) virus, reovirus, infectious bursal disease virus (IBDV), myelocytomatosis virus, and Newcastle Disease virus (NDV) infect macrophages and cause cytopathogenic effects (reviewed by Qureshi *et al*., 2000a). Chicken anemia virus has been shown to decrease the phagocytic potential of macrophages as well as their cytokine production (McConnell *et al*., 1993). Newcastle disease virus infects macrophages and eventually causes apoptosis and necrosis (Lam, 1996). Avian influenza virus of waterfowl origin can activate the phagocytic potential of the macrophages but depresses humoral and cell-mediated immune functions (Laudert *et al*., 1993). Suppressor macrophages have been proposed to play a role in the inhibition of T-cell tumors growth in Marek's Disease (MD) infection (von Bulow and Klasen, 1983). Furthermore, macrophages can destroy parasites like *Toxoplasma cruzi* and *Leishamania mexicana*. However, *Plasmodium gallinaceum* and *Toxoplasma gondii* can infect and multiply within macrophages (Meirelles and DeSouza, 1985; Ramirez *et al*., 1991; Ramirez *et al*., 1995). For fungal diseases caused by *Candida tropicalis* and *Aspergillus fumigatus*, avian macrophages inhibit the infection (Harmon and Glisson, 1989). In addition, macrophages isolated from cases of Poult Enteritis and Mortality Syndrome (PEMS) in turkeys showed increased production of pro-inflammatory cytokines which might explain intestinal inflammation and digestive problems found in this condition (Heggen *et al*., 2000). Among the dietary ingredients, supplements of zinc methionine and vitamin E enhance phagocytic potential of macrophages (Kidd *et al*., 1994a; Kidd *et al*., 1994b; Gore and Qureshi, 1997).

B.4. Natural Killer Cells

In chickens, the NK-cell sub-populations have been found in the spleen, the gut intraepithelial lymphocytes (IEL), the bursa of Fabricius and the thymus (Lillehoj and Chai, 1988). The cytotoxic activity of NK-cells depends upon the sub population type, target cells involved and the genetic background of the chickens. Myers and Schat (1990) demonstrated a difference in the cytotoxic potential of NK-cells derived from spleen and IEL, whereas both cell types infected lymphoblastoid cell line RP9, only the IEL were cytotoxic for rota-virus in infected chick kidney cells. The discovery of NK-cell receptor genes on MHC B complex led to speculation that NK-cells are responsible for the chicken's MHC-determined susceptibility to MD (Kaufman *et al*., 1999).

B.5. Intestinal Immunity

The avian intestinal tract forms a major barrier between external and internal environments. The bursa of Fabricius not only acts as a primary lymphoid organ but also is a part of the gut-associated lymphoid tissue (GALT). The bursa duct connects the bursa lumen with the intestinal lumen, and allows screening for antigens (Schat and Myers, 1991). The cecal tonsils (CT) are made of a central crypt, diffuse lymphoid tissue, and germinal center, and are located at the base of the proximal ends of the ceca. B-cells are located in the sub-epithelial region of the CT, but T-cells are present in a deeper zone (Payne and Powell, 1984). There are many plasma cells in the CT that produce IgM, IgG, and IgA (Jeurissen *et al*., 1989). Macrophages are found throughout the CT but are most prevalent directly under the epithelium. The CT have the ability to acquire particulate

matter from the intestinal lumen, and are thought to function in antigen sampling (Sorvari *et al*., 1977). Peyers patches have a similar distribution of B-lymphocytes, T-lymphocytes and a diffuse macrophage population. However, they also have a lower number of plasma cells producing IgM, IgG and IgA that are detected in them (Befus *et al*., 1980; Jeurissen *et al*., 1989). M-cells in the endothelium of the GALT actively acquire antigens from the intestinal mucosa via pinocytosis. In chickens, these antigens are engulfed by macrophages within the germinal centers of the Peyers patches and CT, and by cells of follicle-associated bursa epithelium. Macrophages of the GALT have been shown to launch the humoral immune response to intestinal antigens (Befus *et al*., 1980; Burns, 1982; Ekino *et al*., 1985).

C. Historical Overview

C.1. Animal Breeding

Biological evolution or natural selection is a slow process that selects for animals better adapted for survival in a specific environment. Human intervention in terms of livestock breeding accelerates the process of biological selection of animals tailored for human needs. Animal breeding has a long history going back thousands of years. In fact Darwin (1859) in his first chapter of 'The Origin of Species' titled 'Variation under Domestication' recognized this phenomenon of selection as a minor evolutionary event. The aim of a breeder is to create a population with high economic production efficiency, i.e., high production combined with relatively low feed intake (Luiting, 1990). This is

achieved by altering gene frequencies and distributions by employing various mating systems. As in other livestock animals, application of population genetic theory, and emerging biological techniques have led to advances in poultry breeding and incremental improvements in the birds' growth and reproductive potential. Over the years there has been either a genetic selection for the improvement of existing characteristics governed by certain genetic traits or they have been selected for other characteristics governed by another set of alleles, that have allowed selection for different line of poultry stocks, such as broiler and layer breeders, from a common dual purpose ancestor (Siegel, 1999).

Havenstein *et al*. (1994) showed that over a period of 35 years, a modern commercial broiler strain hatched in calendar year 1991 gained 3.9 times more body weight (BW) at 56 days of age when compared to a randombred control line developed in 1957. Between the years 1976 and 1991, the marketing age for broilers to achieve a given body weight (BW) had been reduced by about 1 day/year. While new technologies in poultry processing, better nutrition, and management conditions have contributed to this end, their data indicated that 85-90% of the gain in broiler BW has been brought about by genetic selection for increased BW (Sherwood, 1977; Havenstein *et al*., 1994). Similar results were found in studies on turkeys and broiler chickens that showed significant improvement of live weight at slaughter, feed conversion and growth rate over the years between 1960 and 1996 (Rauw *et al*., 1998). In a recent study by Havenstein *et al.* (2003, North Carolina State University, NC, Raleigh, personal communication), the growth rate of the Ross 308 broiler strain hatched in calendar year 2001 had increased by 2.5% per year at 42 to 84 days of age when compared with the performance of the then popular

Arbor Acres feather sexable broiler strain hatched in 1991. In the same study, improvement in commercial broilers as well as diet over the period of ten years significantly increased carcass yield, total breast meat and the percentage of whole body fat and abdominal fat pad.

C.2. Association between Genetics and Immunity

Previous literature indicated that immune parameters are genetically linked. Congenic White Leghorn chickens that had a common genetic background but different *B* haplotypes showed varied resistance and susceptibility to coccidiosis. The haplotypes $15I_5$-*15* showed more resistance to *Eimeria tenella* and lesser intestinal lesion scores to *Eimeria acervulina* when compared to 15.6-*2* and 15.7-*2* haplotypes (Ruff and Bacon, 1989).

Recently, Yonash *et al.* (2001) identified three DNA markers associated with immune responses in a population of meat type chickens divergently selected for high or low antibody response to *Escherichia coli* (*E. coli*). ADL0146 was associated with antibody response to SRBC and Newcastle Disease virus (NDV), ADL0290 was linked to Ab against NDV, and ADL0298 was associated with Ab *to E. coli* and survival.

When two different chicken lines were infected with a mixture of infectious bronchitis virus and a pool of pathogenic strains of *E. coli*, a remarkable difference in mortality was seen (Bumstead *et al*., 1989). Brown Leghorns showed 3% mortality while White Leghorns showed 87% mortality. These differences in mortality were shown to be inherited from a dominant autosomal gene and not linked to maternal effects. As to the

exact genetic association with disease resistance, Lamont *et al*. (1987) demonstrated major histocomaptibility *B* complex to be responsible for resistance against fowl cholera after exposure to *Pasteurella multocida*. Similar results were provided by Lakshman *et al*. (1997) who showed that some MHC class II bands were associated with production traits and with MD resistance.

Natural selection over the centuries has enabled individuals to allocate resources according to demands for growth, reproduction, maintenance and well being (Siegel, 1999). Lerner (1954) described genetic homeostasis as a state of self-regulation of populations that is based on natural selection and favors intermediate rather than extreme phenotypes. The process of artificial selection for a particular trait can disturb genetic homeostasis. Luiting *et al*. (1995, 1997) described residual feed intake (RFI) as the total amount of food resources available for functions other than body maintenance, growth, and reproduction, i.e. 'reaction to pathogens', 'reaction to stress', etc., in different environments and for different metabolic stages of life. Artificial selection for a particular trait may deplete all resources including RFI with no buffer to respond to stresses and challenges. This diversion of resources from essential functions may lead to the development of pathology associated with increased susceptibility to disease, impaired reproduction, or inefficient metabolism and thus impaired animal welfare (Moberg, 1985; Newman, 1994). In fact, any stimulus that challenges homeostasis will act as a stressor, and changes in normal biological functions are sometimes apparent as animals attempt to respond to a stress (Siegel, 1995).

A negative relationship between high growth performance and disease resistance has been established in previous studies. Han and Smyth (1972) reported that chickens selected for high growth rate were more susceptible to experimental infection with Marek's Disease virus as compared with birds selected for low growth rate. White Leghorn chickens selected for their low response for the delayed type hypersensitivity wattle reaction (DWR) to Calmette-Guerin bacillus (BCG) antigen gained more weight than birds selected for higher DWR (Afraz *et al*., 1993). However, the high responding birds showed a significantly higher incidence, as well as mortality from Marek's disease, suggesting that DWR is not related to cell mediated immune response against MD virus. White Leghorn chickens appear to have a greater genetic potential to give rise to progenitor cells for macrophage production compared to broiler strain (Nicolas-Bolnet *et al*., 1995).

Similar findings were seen in other turkey flocks. In a natural outbreak of fowl cholera and erysipelas at the Ohio Agricultural Research and Development Center, Saif *et al*. (1984) found that resistance to these diseases varied with the genetic background. The line developed for high egg production had higher mortality rate from fowl cholera than the randombred control line from which it was developed. On the other hand the line selected for high growth rate showed the highest mortality to erysipelas when compared with the egg line and its control line. Bayyari *et al*. (1997) showed that the cell-mediated immune response, as detected by the toe web response to PHA-P, lymphocyte count and relative spleen weights, was poorer in turkeys selected for fast growth rate compared to their parent randombred control line. The turkey strain selected for higher egg production

showed lower relative spleen and bursa of Fabricius weights. Additionally, hemolytic complement activity was greater in the *in vitro* mitogenic response to PHA-P, compared to its parent randombred control line. These results suggest that selection for faster growth rate is accompanied by changes in cell-mediated immune response that may potentially affect overall immune response. Nestor *et al*. (1996b) found similar results in that a turkey line selected for increased growth rate exhibited decreased resistance to *Pastuerella multocida* and NDV in comparison to the control from which it was derived. Mortality to NDV was significantly higher in the turkey line selected for high body weight at 16 weeks of age compared with a line selected for high egg production and two randombred controls (Tsai *et al.,* 1991). Sharaf *et al.* (1998) showed that a turkey line selected for high egg production had poor antibody response compared with its progenitor randombred control line when vaccinated with Newcastle Disease virus and *Pastuerella multocida* administered singly or in combination.

In addition to immunological deficiencies, commercial broilers have shown a remarkably high incidence of tibial dyschondroplasia, 47.5%, compared with a randombred control line where it was 1.2% (Sharaf *et al.,* 1998).

C.3. Association between Nutrition and Immunity

Diet of a bird plays an important role in the immune response of birds. The effect of nutrition involves both content of feed as well as the feeding regimen. Poultry diets include nutrients from five major groups: carbohydrates, proteins (amino acids), fats, minerals and vitamins (Latshaw, 1991). Nutritional requirements critical for the immune

system development at one week following hatch include linoleic acid, vitamin A, iron, selenium, and several B vitamins (Klasing, 1998). These micronutrients are needed in diets of both newly hatched chicks' as well as in hens' diet for maternal transfer to chicks. An excess of micro-nutrients can have negative repercussions on the immunity as seen in a positive interaction of vitamin A with malabsorption syndrome (Veltmann *et al.,* 1985). Components of the immune system including leukocytes and immunoglobulins that are produced every day comprise less than 1% of total increase in body weight of a chicken, which led Klasing (1998) to suggest that the amount of substrate resources needed by the immune system is very low relative to needs for growth or egg production. One response against foreign pathogens is to starve them of nutrition, for instance avidin secreted by macrophages binds to biotin and removes it from the site of infection, or transferrin produced from liver that binds iron and sequesters it in the liver (Korpela, 1984; Tufft and Nockels, 1991).

Dietary constituents can exert direct immunoregulatory influence as reflected by n-3 fatty acids that increase the antibody response to antigens, while at the same time decreasing the cell mediated response to a mitogen (Fritsche *et al.,* 1991; Krover and Klasing, 1997). Low protein concentrations in broiler diets induced higher cytokine and acute phase proteins in response to the *E. coli* endotoxin LPS when compared with chickens fed a high protein diet (Takahashi *et al.,* 1995). Amongst micronutrients; vitamin E depresses the production of prostaglandins and modulates the release of cytokines from stimulated leukocytes (Romach *et al.,* 1993). Vitamin A is required to maintain epithelial tissue, and to maintain an effective cellular immune response by

acting through the retinoic acid receptor (Latshaw, 1991; Halevy *et al.,* 1994). An adequate amount of nutrient is needed to reduce pathology. Vitamin E and selenium act as antioxidants, and their deficiency affects cell membrane integrity leading to increased pathogenecity to *Salmonella minnesota* lipopolysaccharides (Sword *et al.,* 1991). Higher levels of vitamin E have immunostimulatory activity (Latshaw, 1991). Vitamin C reduces lesions by *Mycoplasma gallisepticum*, Newcastle Disease virus and by *Eschirichia coli* infection (Gross, 1995).

The physical and chemical make up of a diet affects the absorption of pathogens in the intestinal tract. Factors such as the amount of fiber, the viscosity of the fiber, and dietary fats that are refractory to digestion affect microbial populations and capacity of microorganisms to attach to enterocytes (Klasing, 1998). Diets that are high in non-starch polysaccharides, such as those found in barley and rye, increase the capacity of anaerobic enterococci such as Clostridial species, to attach to enterocytes and cause infection (Wagner and Thomas, 1978; Untawale and McGinnis, 1979; Hofshagen and Kaldhusdal, 1992; Kaldhusdal and Hofshagen, 1992).

Apart from the content of the diet, the feeding regimen can also affect the immunocompetence of birds. Praharaj *et al.* (1996) showed that the birds fed on alternate days displayed fewer lesions and reduced mortality to *E. coli* challenge in comparison with full fed birds. They also showed lower frequency of marble spleen disease virus than those on an *ad libitum* feeding regimen. However, longer periods without feed elevate coticosterone levels and eventually impair both antibody and cell-mediated immune responses. This is mediated possibly via cytokines (Latshaw, 1991).

D. Parameters of Immune Response

D.1. Antibody Response to Sheep Red Blood Cells (SRBCs)

Antibody response against SRBCs is carried out by B-lymphocytes, and it requires activation of B-lymphocytes into plasma cells after their interaction with helper T-cells and antigen presenting cells.

D.1.a. Genetic Influence

A negative correlation exists between high production rate and the antibody response to sheep red blood cells in chickens. In a study comparing 1991 and 1957 broiler strains fed diets typical of those years, a randombred control strain from 1957 performed significantly better in antibody response (total, IgM and IgG) against sheep red blood cells (SRBC) compared with the 1991 commercial broiler strain (Qureshi and Havenstein, 1994). Body weights from these strains were measured at 21, 42, 56, 70, and 84 days of age. The 1991 strain on the 1991 diet showed an increase in body weight by 3.7, 4.2, 3.9, 3.5, and 3.2 times respectively, compared to 1957 strain raised on 1957 diet (Havenstein *et al.,* 1994). Similar results were seen in other studies that showed genotypes with higher body weight give poor antibody response to SRBC than lower body weight line of broilers (Miller *et al.,* 1992; Rao *et al.,* 1999). While comparing two congenic lines of White Leghorn chickens heterozygous at the MHC *B* haplotype,

Dunnington *et al*. (1996) found that the B^{21} haplotype possessed higher antibody titers and lower body weights (BW) in most instances while B^{13} haplotype had lower antibody titers and higher BW.

D.1.b. Nutritional Influence

The effect of nutrition on antibody response to SRBC is variable. Tsiagbe *et al.* (1987) found a dose related increase in total and IgG antibodies against SRBC when the broiler chicken diet was supplemented with methionine. However, Rao *et al.* (1999) found no significant differences in humoral response to SRBC among the chicks fed high, medium, and low protein diets. Similarly, dietary protein and energy content had no significant influence on broiler chick responses to SRBC (Praharaj *et al.,* 1997).

D.2. Blastogenic Response to Phytohemagglutinin-P (PHA-P)

When lectin PHA-P is injected intradermally into animals, the response primarily involves stimulation of T-cell division with minimal effects on B-cells (Tizard, 1995). Therefore, lymphoproliferation in response to PHA-P is considered a good *in vivo* measure of T-lymphocyte function (Qureshi *et al.,* 1997). Other studies also base PHA-P mediated response to the active recruitment of basophils as well as CD4 positive lymphocytes. Thereby, this reaction is sometimes termed as cutaneous basophilic hypersensitivty (CBH) response (Stadecker *et al.,* 1977). CD4+ T-cells are responsible for induction of delayed type hypersensitivity (DTH) reaction in response to protein antigens (Benoist and Mathis, 1999).

D.2.a. Genetic Influence

Corrier (1990) found significant genetic differences in DTH reaction as measured by cutaneous basophilic hypersensitivty response when the toe web of broiler chicks was injected with PHA-P in comparison to injection in layer chickens. Chicken genotypes selected for high body weight showed the lowest DTH response as measured by wattle thickness at 12 hours after PHA-P injection, but genotypes with the low body weight gave the highest response 12-24 hours after PHA-P injection (Rao *et al.,* 1999). When a cross between PHA-P high responder and low responder lines was tested for mitogenic response to PHA-P, an intermediate result was seen, suggesting that at least two alleles control PHA-P response in birds (Morrow and Abplanalp, 1981).

D.2.b. Nutritional Influence

Many nutrients have been shown to depress the cutaneous basophilic hypersensitivity response to chemoattractants. High dietary vitamin E levels of 300 IU/kg significantly decreased the toe web swelling in response to PHA-P injection in comparison with birds fed 10 IU/kg of vitamin E (Boa-Amponsem *et al.,* 2000). On the other hand a positive correlation between vitamin A and interdigital mitogenic response to PHA-P was observed. Broiler chickens fed low levels of vitamin A (400 IU/kg) showed a low response to PHA-P, while the highest level of dietary vitamin A (15000 IU/kg) produced a high mitogenic response (Lessard *et al.,* 1997). Tsiagbe *et al.* (1987)

found methionine supplementation in broiler diets to increase the *in vivo* response of T-cells to PHA-P injected in the wing web.

D.3. Natural Killer (NK) Cell Activity

NK-cells have been suggested to provide non-specific natural immunity to host against certain kinds of tumors and infectious diseases (Herberman and Ortaldo, 1981).

D.3.a. Genetic Influence

Lillehoj and Chai (1988) demonstrated genetic differences in NK-cell cytotoxicity when examining two strains of chickens FP (B^{15} B^{21}) and SC (B^{2} B^{2}). Gut intraepithelial lymphocytes with NK-cell activity in SC chickens showed significantly higher cytotoxicity in ^{51}Cr labeled avian LSCC-RP9 (avian leukosis tumor cell line) after 4 hours of incubation, than NK-cells from gut intraepithelium of the FP line. No significant line differences were observed in cytotoxicity of NK-cells derived from the spleen, thymus and bursa. A gene in the chicken MHC B locus' B-F/B-L region has been identified that is transcribed in NK-cell lines and is closely related to lectin-like natural killer (NK) receptors (Kaufman *et al.,* 1999).

D.3.b. Nutritional Influence

Dietary supplements also have an impact on NK-cell activity. A mixture of vitamins (A, D, E, and B) and electrolytes added to the drinking water decreased tumoricidal activity of NK-cells against RP9-cells when compared with birds provided

with unsupplemented drinking water, B complex vitamins (riboflavin, pantothenic acid, niacin, B_{12}, pyridoxine, biotin, folic acid) plus electrolytes in drinking water, or vitamins A, D, E, and B in drinking water (Ferket and Qureshi, 1992).

D.4. Macrophage Activity

Macrophages are members of mononuclear phagocytic system and play an important role in the innate immune response. The effector functions of macrophages measured in following studies included induction of cytotoxic activity in response to a particular biological signal, phagocytosis of an antigen, and release of nitric oxide in response to stimulation with *Escherichia coli* (*E. coli*) lipopolysaccharides (LPS).

D.4.a. Genetic Influence

Qureshi and Miller (1991) found that commercial broiler chickens varied in their ability to elicit macrophages by intra-abdominal injection of Sephadex-G50. Internalization and killing of opsonized and unopsonized SRBC as well as opsonized *E. coli*. They also varied in the cytolytic ability of the stimulated macrophage supernatants against LSCC-RP9 tumor cell line after they were activated with LPS. Puzzi *et al.* (1990) showed genetic differences within White Leghorn chicken lines that were congenic for the MHC *B* complex $15I_5$-*B* macrophage function. Macrophages from B^2 and B^{13} lines showed higher phagocytosis of SRBC in comparison to B^5 and B^{21} lines. Results were the same for *in ovo* macrophage activation by Sepahdex G-50 or LPS as well as for *in vitro* LPS activation. Similar results were seen in superoxide anion activity being higher in the

B^2 and B^{13} lines. Qureshi *et al.* (1989) suggested a reciprocal relationship between the MHC (*B* complex) dosage on monocyte macrophage function. Mononuclear leukocytes from tetrasomics ($B^{15}B^{15}B^{15}B^{15}$) showed enhanced chemotaxis to f-met-leu-phe (FMLP) and *Enterobacter cloacae* culture supernatant in comparison with the chemotaxis levels in disomics and trisomics. However, peritoneal macrophages from both tetrasomic and trisomic chickens exhibited reduced phagocytic activity for unopsonized SRBC than disomic chickens.

Hussain and Qureshi (1997) reported that macrophages from Cornell K strain chickens ($B^{15}B^{15}$) exhibit higher level of iNOS expression and nitric oxide production in response to stimulation with *E. coli* lipopolysaccharides, than the macrophages from GB1 ($B^{13}B^{13}$) and GB2 (B^6B^6) chickens.

D.5. Lymphoblastogenic Response to Con-A

Concanavalin A is a T-lymphocyte mitogen which induces lymphoproliferation in T-cells upon co-culture (Bayyari *et al.,* 1997; Li *et al.,* 1999; Qureshi *et al.,* 2000b). Therefore, *in vitro* exposure of lymphocytes to Con-A and resultant lymphoproliferation is considered to mimic the antigen-induced T-lymphocyte expansion *in vivo*.

D.5.a. Genetic Influence

Purified peripheral blood mononuclear cells and whole blood lymphocyes from turkeys selected for high body weight showed poor lymphoblastogenic response to Con-A when compared to the response in a randombred control line (Li *et al.,* 1999). A single

autosomal gene was believed to control mitogenic response of chicken peripheral blood leukocytes in CB ($B^{12}B^{12}$) and WA ($B^{9}B^{9}$) chicken lines (Miggiano *et al.,* 1976). Morrow and Abplanalp (1981), however, showed that variation in Con-A stimulation is controlled by at least two major genes, and one of them may be linked to the major histocompatibilty complex. Furthermore, the genes controlling the PHA-P and Con-A stimulation in chickens were shown to be independent in a comparison of three inbred lines of chicks. Lassila *et al.* (1979) observed a variable response to PHA-P and Con-A in White Leghorn chickens congenic for the MHC *B* haplotype. Line P (B^{2} B^{2}) showed higher lymphoproliferation than V chickens (B^{15} B^{15}) and hybrids (B^{2} B^{15}). The Con-A response was the same in all of the lines, implying that the mitogenic response to Con-A and PHA-P is influenced by different loci.

D.6. Chemotaxis of Mononulear Leukocytes

Mononuclear leukocytes take part in wound healing and host immunity, by their ability to respond to chemotactic factors released at the site of inflammation (Qureshi *et al.*, 1988). This response is considered to be part of innate defense mechanism of the host.

D.6.a. Genetic Influence

The mononuclear leukocytes' chemotactic ability for f-met-leu-phe and *Enterobacter cloace* culture supernatant was shown to be influenced by the MHC *B* complex (Qureshi *et al.,* 1989). The tetrasomic line ($B^{15}B^{15}B^{15}B^{15}$) performed better than

trisomic and disomic lines under these conditions. In another study involving *B* congenic White Leghorns, a difference was seen in the chemotaxis for blood mononuclear leukocytes to f-met-leu-phe. However, a converse relationship existed between macrophage function and chemotaxis (Puzzi *et al.,* 1990).

D.7. Lymphoid Organ Weights

Primary and secondary lymphoid organs provide the site for maturation of lymphocytes, and for the interaction between lymphocytes and antigens.

D.7.a. Genetic and Nutritional Influence

An interaction between diet and genotype was seen for relative weight of bursa of Fabricius; but the genotype with the lowest body weight showed the highest bursa weight when raised at 43 days of age on high crude protein diet (Rao *et al.,* 1999). Konashi *et al.* (2000) reported that the relative weights of the bursa of Fabricius and thymus were affected by dietary amino acid deficiencies, with branched chain amino acids deficiencies causing the most reduction in weight of these organs. Kwah *et al.* (1999) showed that dietary arginine deficiency significantly reduced spleen and thymus relative weights.

E. Current Study

There were two primary objectives of the present study:

1) To determine the relative immune performance of several modern commercial broiler strains when raised on high and low protein diets.

2) To determine what changes in the immune performance have accrued over the years, by comparing a modern broiler strain produced in calendar year 2001 with a Randombred control strain established in 1957 when raised on rations typical of those years.

For this study both humoral and cell-mediated immune functions were investigated. Comparisons included the potential of macrophages to phagocytise SRBC, and to determine their relative levels of nitrite production; the ability of natural killer cells to kill the RP9 tumor B-cell line; to compare T-cells' lymphoproliferation in response to mitogens PHA-P (*in vivo*) and Con-A (*in vitro*); and to compare the T-cell dependent humoral response of B-cells after challenging the birds with 3% SRBC intravenously. Lymphoid organ weights relative to total body weight were measured during the maturation of the bird's immune system.

CHAPTER 2

A comparison of the immune profile of commercial broiler strains when raised on marginal and high protein diets

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ABSTRACT A study was conducted to compare the immunocompetence of four commercial broiler strains: Ross 3F8 (3F8, vent sexable), Ross x Cobb (RC, vent sexable), Ross 308 (308, feather sexable), and Cobb x Cobb (CC, vent sexable). All strains were fed two different dietary regimens, marginal protein diet (D1) which had 20.1 and 18.1% crude protein, and high protein diet (D2) containing 21.9 and 20% crude protein, for the starter and finisher diets, respectively. Immunological endpoints quantified included the humoral response to sheep red blood cells (SRBC), the T-cell proliferation response to the mitogens Concanavalin-A (*in vitro* Con-A response) and Phytohemagglutinin-P (*in vivo* PHA-P response), the innate immune response from comparing the tumoricidal potential of natural killer (NK) cells, the elicitation of monocyte-macrophages by Sephadex G-50®, the phagocytosis of SRBC, and the chemotactic response to formyl-met-leu-phe- (FMLP). Body weights (BW) and lymphoid organ weights relative to BW were also measured. Strain CC showed comparatively higher and more persistent level of antibody titers against SRBC ($P = 0.0182$) as well as better macrophage phagocytic ability for SRBC ($P = 0.0118$) than the other strains. The Ross 308 strain had significantly higher cell-mediated immune response, as measured by T-lymphocyte proliferation response to PHA-P ($P = 0.0081$) and Con-A ($P = 0.04$) as well as chemotaxis response, to FMLP ($P = 0.0001$) than the other strains. The diet effect was variable for monocyte-macrophage functions; however, birds on the high protein diet showed greater cell-mediated response than those on the low protein diets as measured by Con-A and PHA-P responses. An interaction was seen between strains and diets in antibody response with the Ross 308 showing higher titers on D1 while the CC had

higher response on D2. These results indicate that genetic differences exist between various commercial broiler chicken lines for cell-mediated, humoral and innate immune responses. Furthermore, dietary protein levels influence the expression level of a broiler chicken's immunocompetence.

Key words: Broilers, genetic, diet, immunocompetence.

INTRODUCTION

Commercial poultry breeding has among its objectives the improvement of production potential and disease resistance. Over the years, most of the selection emphasis has been on the improvement of growth and feed conversion, and these changes have been shown to be negatively associated with immunological performance of poultry, as reported by Han and Smyth (1972), Qureshi and Havenstein (1994), Rao *et al.* (1999), and Yunis *et al.* (2000). These and other studies (Qureshi and Miller, 1991) have, therefore, established that commercial broiler lines differ in several baseline immune function parameters. While genetic make up of the birds has been clearly shown to have a significant impact on disease resistance and/or susceptibility (Lakshman *et al.,* 1997; Ruff and Bacon, 1984; Lamont *et al.,* 1987), limited information is available (Yonash *et al.,* 2001) which relates a particular gene, gene locus or a marker with a particular immune response parameter in commercial broiler lines. Nevertheless, commercial broiler producers are acutely aware that the breeder lines must be "co-selected" for both performance and immunocompetence parameters.

Nutrition has also been shown to affect the immune response. For example, chickens that were raised on diets that were 2/3 deficient in amino acids compared to the basal dietary levels had reduced numbers of lymphocytes in the thymus. Chicken diets reduced by 2/3 in calories, amino acids or both compared with basal dietary levels showed a decline in humoral immune response to sheep red blood cells (SRBC) (Glick *et al.,* 1981, Glick *et al.,* 1983). Takahashi *et al.* (1995) observed an acute phase response to *Escherichia coli* (*E. coli*) lipopolysaccharide (LPS) in chicks raised on low protein diets versus those fed on high protein diets. Physical and chemical make up of the diet also affects the absorption of pathogens through the intestinal tract. Factors such as the amount of fiber, the viscosity of the fiber, and fats that are refractory to digestion affect microbial number and the capacity of the microorganisms to attach to enterocytes (Klasing, 1998).

Lastly, the age of the bird has impact on immune performance. Because of immunological immaturity, young chicks may suffer from diseases like infectious bursal disease, avian encephalomyelitis, Marek's Disease, *E. coli* and Salmonella infections. Also, the measurement of early immune competence can be complicated by the presence of maternal antibodies (van der Zijpp, 1983).

With these considerations in mind, the present study was conducted to evaluate immune performance of four different commercial broiler strains raised on low and high protein diets. Age-related immune performance was quantified at one-week and four-weeks of age. The parameters measured included: body weight, relative lymphoid organ weights, humoral response to SRBC, cell-mediated immune response to *in vitro*

Concanavalin-A and *in vivo* Phytoheamagglutinin-P stimulation, monocyte macrophage potential as measured by chemotaxis of blood monocytes to FMLP, *in vivo* elicitation of macrophages in response to Sephadex G-50, phagocytic potential of macrophages for SRBC, nitrite production in response to LPS stimulation, and non-specific tumoricidal activity by splenic natural killer (NK) cells.

MATERIALS AND METHODS

Chickens and Diets

Three commercial broiler lines and two dietary regimens (i.e., high and marginal protein diets) were utilized in these studies. The experiments were conducted on two separate hatches and are thus called as Trial 1 and Trial 2. Strains utilized in Trial one were Ross 3F8 (3F8, vent sexable), Ross 308 (308, feather sexable), and Cobb x Cobb (CC, vent sexable). In Trial 2, Ross x Cobb (RC, vent sexable) was used instead of Ross 3F8, whereas remainder of the strains were the same as in Trial 1, namely Ross 308 and Cobb x Cobb. Fertile eggs were received from the sponsoring commercial broiler company on two separate occasions. These eggs were incubated and hatched, with greater than 95% hatchability, at the North Carolina State University Department of Poultry Science, Raleigh, NC. The chicks were feather and vent-sexed at day of hatch and the males were wing-banded and used in these trials. Birds were arranged in six treatment groups in a 3 x 2 factorial arrangement; i.e., three strains (Ross 3F8, Ross 308, and Cobb x Cobb for Trial 1; Ross x Cobb, Ross 308, and Cobb x Cobb for Trial 2), and two dietary regimens (D1 – marginal protein diet, D2 – high protein diet). Chicks were

housed in wire-cage Alternative Design batteries, with 64 total cages, each containing 10 birds. The diets (both starter and grower rations) were mixed and delivered by the sponsors on an as needed basis to the research facility. The marginal protein starter diet had 3075 Kcal ME/kg and 20.10% crude protein (CP) while the marginal protein finisher diet had 3152 Kcal ME/kg and 18.10% CP. The high protein starter and finisher diets contained 3086 Kcal ME/kg, 21.90% CP and 3130 Kcal ME/kg, 20% CP, respectively (Table 13). In both trials starter lasted from days 1-14, while the finisher was fed from day 15 until the end of the experiment at 28 days of age. The room temperature was maintained at 92-95 F for the first week and then reduced to 82-85 F for the remaining period of the trial. Feed and water were supplied *ad libitum*.

Experimental Endpoints

Body Weights and Relative Lymphoid Organ Weights

At 2 weeks of age in Trial 1 and at 3 weeks of age in Trial 2, the thymus (all lobes on the left side of the neck), spleen, bursa of Fabricius and cecal tonsils were removed from 8 birds per group. The chick and its organs were weighed, and the organ weights were expressed as the percentage of the bird's body weight.

Antibody response

Sheep red blood cells (SRBC) were used as T-dependent antigens to quantify the antibody response. In both trials, 10 birds per group were injected intravenously at 7 days

of age with SRBC (3% suspension in PBS[1]; 1 mL per chick), followed by a booster injection of SRBC suspension given at 14 days after the first injection. Blood samples were collected at 4, 7, 10, and 14 days post first injection, and again at 5 and 10 days post boost.

Serum from each sample was collected, heat inactivated at 56C for 30 minutes, and was then analyzed for total anti-SRBC antibodies as previously described (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994).

Lymphoproliferative response to PHA-P

The lymphoproliferative response to *in vivo* Phytohemagglutinin[2] (PHA-P) stimulation was assessed as described previously by Corrier (1990). The T-cell mitogen, PHA-P, was injected intra-dermally (100 μg/100 μL/bird) into the toe web of the left foot of 10 birds/group at 2 and 4 weeks of age, and an equal volume of PBS was injected in the toe web of the right foot of the same bird. The thicknesses of the toe webs were then measured at 24 and 48 hours post injection in Trial 1 and additionally at 72 hours post injection in Trial 2, using a micrometer. Swelling response was measured by subtracting the pre-injection measurements from the post-injection measurements of the toe web thickness.

[1] Fisher Scientific, Pittsburgh, PA 15275.

Lymphoproliferative response to Concanavalin-A

The blastogenic responses of chicken leukocytes to Concanavalin-A[2] (Con-A) was measured by using peripheral blood lymphocytes as described by Qureshi *et al.* (2000b). In Trials 1 and 2, 10 birds per group were bled at one and four weeks of age. Peripheral blood lymphocytes were separated by Ficoll[3] density gradient, washed and the cell count was brought to 2 x 10^6 cells/mL in RPMI-1640 growth medium[2]. Then, in a flat bottomed 96 well plate, 100 μL of cell suspension was added with an equal quantity of Con-A at a concentration of 25 μg/100 μL. Control wells contained 100 μL of RPMI-1640 growth medium instead of cell suspension. Plates were incubated for 24 hours at 41C with 5% CO_2, after which 50μL of 1 mg/mL stock solution of MTT[2] (3-[5,5-dimethylthiazol-2-yl]-2,5-[diphenyltetrazolium]) was added to each well, and the plates were incubated again for 4 hours. Afterwards, all liquid from the wells was removed and 150 μL of acid-isopropanol (50 mL of 0.015 M phosphate buffer saline (PBS) pH 7.2, and 100 mL of acid-isopropanol [0.04 N HCl in isopropanol]) was added to each well. The solution was then pipetted repeatedly to dissolve the blue formazan crystals. The optical density (OD) of the solution was then measured at 540 nm by using an enzyme linked immunosorbent assay plate reader[4]. The lymphoproliferative index was calculated as OD of Stimulated – OD of Unstimulated cells/OD of Unstimulated cells.

[2] Sigma Chemical Co., St. Louis, MO 63178.
[3] Atlanta Biologicals, Norcross, GA 30093.

[4] Bio-Tek Instruments, Burlington, VT 05402

Macrophage Function Assessment

Macrophage functions were assessed in each trial as previously described (Qureshi and Miller, 1991). Ten birds from each treatment group were injected with a 3% suspension of Sephadex G-50®[2] at a concentration of 1 mL/100 g of body weight at 9 and 29 days of age. Approximately 42 hours after injection, birds were euthanized, and the abdominal cavity was flushed with sterile heparin (0.5 U/mL) - saline (0.75%) solution. Abdominal exudate cells (AEC) were then collected in siliconised glass tubes and centrifuged at 285 x g for 10 minutes to obtain an AEC pellet. Cell pellets from individual birds were resuspended in 4 mL of RPMI-1640 growth medium supplemented with 5% heat-inactivated fetal calf serum[3] and antibiotics (100 U/mL penicillin[3] and 50 μg/mL streptomycin[3]). The total number of nonerythroid AEC were counted on a hemocytometer.

In order to quantify the phagocytic potential, a 1% SRBC suspension in RPMI[1] growth medium was used as a particulate antigen. AECs from 10 birds were pooled to generate five samples (2 birds per sample). Macrophage monolayers were established by adding 1 x 10^6 AECs/mL from each sample into Petri dishes[5] containing four glass coverslips[6]. After one hour of incubation, coverslips were washed to remove any non-adherent cells. These macrophage monolayers were co-incubated with a 1.0 mL suspension of unopsonized SRBC which was added to each Petri dish. After 60 minutes incubation at 41C in a humidified atmosphere and 5% CO_2, the coverslips were washed

[5] Corning, Corning, NY 14831.

to remove non-internalized SRBCs, fixed and stained with Leukostat®[7] and mounted on microscopic slides. A total of 200 macrophages were scored microscopically for phagocytosis as well as for numbers of SRBC per phagocytic macrophage.

Nitrite Production

The production of nitrite (a stable end product of nitric oxide) by macrophages in response to LPS was assessed as previously described by Green *et al.* (1982). Macrophages from 8 birds pooled as two birds per sample were cultured in 24-well plates ($1x10^6$ cells per well per pooled sample) and exposed to lipopolysaccharides[2] from *E. coli* (1 μg/well) for 24 hours. The culture supernatants were collected and the concentration of nitrite was determined as described by Green *et al.* (1982). The standard curve for the nitrite assay was generated using various dilutions of 10mM stock solution of sodium nitrite in RPMI-1640 CM. The nitrite levels in culture supernatants were calculated by comparing the optical density (OD) readings against the nitrite standard curve.

Chemotactic response

At 1 and 4 weeks of age in each trial, the chemotactic potential of leukocytes was assessed using the Boyden blind well chamber as previously described (Lohr and Snyderman, 1981; Qureshi *et al.,* 1988). Blood samples were drawn from ten birds per group. Peripheral blood lymphocytes were separated from whole blood, washed and the

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[6] Thomas Scientific, Swedesboro, NJ 08085.
[7] Fisher Scientific, Orangeburg N.Y. 10962.

cell count was adjusted to $2x10^6$ cells/mL as described earlier. Samples from three birds per group were pooled while one bird's sample was discarded. The chemoattractant f-met-leu-phe[2] (FMLP) was dissolved in RPMI-1640 growth medium at 10^{-5} M concentration, and was added in the lower wells of the blind well chambers, while RPMI-1640 growth medium was added into the control wells. Polycarbonate membrane filters[7] (5 μm pore size) were placed over the lower wells. The upper well insert was screwed into place and filled with 0.2 mL of leukocyte suspension. The chemotactic chambers were incubated for 1 hr at 41C in humidified 5% CO_2 in air. Following this, the cell suspension in the upper chamber was removed with a Pasteur pipette, the filters were fixed in methanol and stained with Mayer's hematoxylin[2]. The filters were air-dried, mounted on glass slides and the chemotaxis was quantified by counting the mononuclear cells in 10 randomly selected 100 x oil immersion microscopic fields in the areas where the cells had migrated. The chemotactic response was expressed as the mean of mononuclear cells per 100 x oil field for triplicate filters.

Natural Killer (NK) Cell Activity

NK-cell activity was quantified at 1 and 4 weeks of age by using a previously described technique (Qureshi and Miller, 1991). A B-lymphocyte tumor cell line, LSCC-RP9, was used as target cells, which were passaged using RPMI-1640 growth medium supplemented with 20 % fetal bovine serum (CM, complete medium) for 24 hours in advance of the assay. They were then Ficoll-purified to remove dead cells and the viable

cell concentration was then adjusted to 2 x 10^7 cells/mL. One mL of RP9-cells was incubated with 300 μCi of ^{51}Cr (as sodium chromate) for radio-labeling. For the effector cells, spleens from 5 birds per group were removed, homogenized and the resulting single cell suspension was adjusted to 2.5 x 10^7 cells/mL. One hundred micro liters of target cells and an equal quantity of the splenocyte suspension were co-cultured in a 96 well plate (1.25 effector cells:1 target cell). The radioactivity in supernatants and pellet lysates from each well was separately determined in a gamma counter[8] after a 4-hour co-culture. Results were expressed as percentage specific ^{51}Cr release, as calculated by the following formula: Percentage specific ^{51}Cr release = experimental release – spontaneous release/total releasable count – spontaneous release x 100.

Statistical Analysis

Data were analyzed using the General Linear Models® procedure of SAS (SAS Institute, 1990). Means were separated for significance by Duncan's multiple range test at a significance level of $P < 0.05$.

[8] Packard Multi-Prias 2, Packard Instrument, Downers Grove, IL 60517.

RESULTS AND DISCUSSION

Body Weights and Relative Lymphoid Organ Weights

Body weights (BW) of the chicks in the two trials are given in Table 1. Body weights were taken at 2 weeks of age in Trial 1 and at 3 weeks of age in Trial 2. In Trial 1, all three strains, i.e., 3F8, 308 and CC had comparable body weights, and no significant strain or diet effects were observed. Also, no interaction between strains and diets was found (Table 1). However in Trial 2, at 3 weeks of age, CC and RC (an additional strain not used in Trial 1), had significantly higher BW than the 308 chickens, but were not significantly different from each other (Table 1). Neither a diet effect nor any interactions were observed in Trial 2. These observations suggest that there is an age-dependent difference in growth performance between lines used in this study.

The weights of the lymphoid organs, i.e., of the bursa, spleen, and thymus as a percentage of BW are provided in Table 2. The bursal, splenic and thymic weights were not significantly different for the different strains or diets when measured at 2 or 3 wk of age. An interaction between diet and strain was seen for thymic weight in Trial 1, with the 3F8 birds raised on D1 having significantly higher splenic weights as a percentage of total body weights, compared to when they were raised on D2 (Table 2). There is

evidence in the literature suggesting improved lymphoid organ growth in low weight strains when the dietary crude protein level is increased from 18% to 23% (Rao *et al.,* 1999). Our findings, however, yielded no such differences when comparing various strains or diets.

Antibody Response

The total antibody responses of the various strains against SRBC for Trials 1 and 2 are summarized in Tables 3 and 4, respectively. In Trial 1 (Table 3), strains 308 and CC had significantly higher antibody response as compared with strain 3F8 at 4 days post primary injection ($P = 0.03$). At 7 days, strain CC had greater titers than 3F8 ($P = 0.04$), while 308 was not significantly different from the other two strains. At 14 days post primary injection CC had significantly higher antibody response than 308 and 3F8 ($P = 0.03$). Both the 308 and CC strains showed significantly higher titers than 3F8 at 5 days post boost ($P = 0.001$), at 10 days post boost CC showed persistence in titers (4.29 ± 0.35) and was significantly higher than 308 ($P = 0.018$) while 3F8 was not significantly different from either of the other two strains.

In Trial 2 (Table 4), no significant differences in antibody response were observed at day 4, 7, 10, or 14 of primary response for different strains or diets. Furthermore, there was no significant interaction between diets or strains. On day 5-post boost, no significant differences were seen in antibody response of the different strains or diets. However, an interaction between diets and strains was seen with CC showing significantly greater humoral response on Diet 2 than on Diet 1 ($P = 0.033$). On day 10

post boost, CC and RC had significantly higher persisting antibody titers than 308 (P = 0.0001). No significant difference in the performance of the strains on the different diets was seen, but an interaction between diets and strains was seen, with 308 exhibiting higher titers on Diet 1 than on Diet 2.

Taken together, data from both trials indicate that the CC strain of broilers can be classified as high responders for anti-SRBC antibody response. While strain 308 was also a high antibody producer (Table 3, Trial 1), its antibody level did not persist as long as for strain CC in either Trial 1 or Trial 2. Unfortunately we cannot draw any conclusion about the relationship of antibody response with regard to growth rate since antibody response was quantified at an age (2 and 3 wk) when these chickens were not at their maximum genetic growth potential. Earlier studies designed to correlate growth with immune performance suggest a negative correlation between body weight and humoral response against SRBC (Qureshi and Havenstein, 1994; Rao *et al.,* 1999). Recently, Yonash *et al.* (2001) identified three DNA markers (ADL0146, ADL0290 and ADL0298) associated with antibody responses against SRBC, *E. coli* and Newcastle Disease virus (NDV). It is quite possible that these marker(s) would segregate differentially in the chicken lines used in these studies. Studies with white leghorn chickens have also shown a correlation between the major histocompatibility (*B*-complex) haplotype and antibody response. For example, Dunnington *et al.* (1996) showed that the B^{21} haplotype was associated with a high antibody response and low body weight while the B^{13} haplotype had lower antibody titers and higher body weights. The two diets used in these studies did not show any difference during the primary phase of antibody response in either trials.

However, the CC birds, which had the highest antibody response, did well during the secondary response when fed the high protein (D2) diet. These differences led to the observed strain x diet interaction found on day 5-post boost in both trials (Table 3, P = 0.0217, and Table 4, P = 0.0338). On the contrary, the strain 308 birds, which showed reduced antibody persistence, performed better on the low protein diet (D1) than on the high protein diet (D2), at 5 and 10 days post boost. Therefore, a case can be made that the CC birds respond higher on the D2 diet whereas strain 308 may show higher response on the low protein D1 diet for anti-SRBC antibody production. Other studies (Rao *et al.,* 1999), however, have shown that variation in dietary crude protein do not lead to significant differences in antibody titers in broiler strains.

Lymphoproliferative response to PHA-P

The lymphoblastogenic responses to PHA-P, as measured in Trials 1 and 2, are provided in Tables 5 and 6, respectively. In both trials, the response was measured at 2 and 4 wk of age using different birds at each time frame. In Trial 1, at 2 wk of age (Table 5), strain 3F8 showed significantly greater swelling of the toe web, at 24 hours post PHA-P injection, when raised on diet D2 compared to when they were raised on diet D1 (P = 0.03). Birds on Diet 2 showed significantly greater swelling than D1 at 48 hours post PHA-P injection, at 2 weeks of age (P = 0.01). These findings were not replicated at 4 weeks of age. In Trial 2 (Table 6), strain 308 had significantly higher swelling response than CC at 24, 48 and 72 hours post PHA-P injection at 2 wk of age ($P \leq 0.04$). At 4 weeks of age no significant differences were seen between the strains. When lectin PHA-

P is injected intra-dermally into animals, the response primarily involved stimulation of T-cell division with minimal effects on B-cells (Tizard, 1995). Therefore, lymphoproliferation in response to PHA-P is considered a good *in vivo* measure of T-lymphocyte function (Qureshi *et al.,* 1997). Other studies also base PHA-P mediated response to the active recruitment of basophils as well as CD4 positive lymphocytes. Thereby, this reaction is sometimes termed as cutaneous basophilic hypersensitivty (CBH) response (Stadecker *et al.,* 1977). Based on our data, none of the 3 strains used in Trial 1 (Table 5) emerged as a clear high or low responder either at 2 or 4 wk of age. Within the 3F8 strain, the birds showed higher swelling response when raised on D2 than on D1 (low protein diet) ($P = 0.0341$). However, diet effects disappeared when the birds reached 4 wk of age. This was evident in both Trials 1 and 2. Since strain 308 had higher lymphoproliferative response at 2 wk of age in Trial 2 (Table 6), it appears that strain 308 is comparatively higher performer for PHA-P mediated lymphoproliferative response. The differences between strains observed in our study for PHA-P response as well as for dietary effects, are not surprising since several previous studies have shown such differences among broiler, white leghorns and turkey strains (Tsiagbe *et al.,* 1987; Corrier 1990; Bayyari *et al.,* 1997; Rao *et al.,* 1999). Furthermore, it has been suggested that the PHA-P response in chickens is under polygenic control (Morrow and Abplanalp, 1981). This may represent interesting options for breeders since "intermediate" responders may arise when high and low responder genotypes are crossed (Morrow and Abplanalp, 1981). In our studies, strain RC may represent an intermediate genotype for PHA-P response as observed at 2 wk of age (Table 6).

Lymphoblastogenesis Response to Concanavalin A

Concanavalin-A is a T-lymphocyte mitogen that induces lymphoproliferation in T-cells upon co-culture (Bayyari *et al.,* 1997; Li *et al.,* 1999; Qureshi *et al.,* 2000b). Therefore, *in vitro* exposure of lymphocytes to Con-A and resultant lymphoproliferation is considered to mimic the antigen-induced T-lymphocyte expansion *in vivo*. Furthermore, this endpoint has been shown to be under the control of single autosomal gene in highly inbred chickens (Miggiano *et al.,* 1976). In another study, Con-A stimulation in chickens was reported to be controlled by at least two major genes, one of which is linked to the MHC (Morrow and Abplanalp, 1981).

In the current study, we compared the Con-A-mediated lymphoproliferation between different broiler strains at 1 and 4 wk of age in both Trials 1 and 2 (Table 7). In Trial 1, Con-A-mediated lymphoproliferation was poor due to unexplained reasons (data not shown). However, at 4 weeks of age, peripheral blood leukocytes (PBLs) from strain 308 had a significantly higher stimulation index ($P = 0.0018$) than either the 3F8 or CC strains (Table 7). Diet had no significant effect on the Con-A response. Also, no interaction was observed between diets and strains. In Trial 2, strain 308 had a significantly higher stimulation index than strain RC ($P = 0.0281$), while CC was not significantly different from either of the other strains at 1 wk of age. Birds on the D2 diet exhibited significantly greater lymphoproliferative response than birds on the diet D1 ($P = 0.0081$). The interaction between diet and strain was not significant. At 4 weeks of age, the CC birds were significantly higher responders than the RC birds, while strain 308

birds were intermediate. Diets did not cause any significant differences in performance, or any significant interaction between strains and diets. Taken together, the data from the two trials indicates that the 308 birds consistently have higher responsiveness for Con-A mediated lymphoproliferation than birds from the other strains.

Mononuclear Phagocytic System Function Assessment

Abdominal Exudate Cells recruitment

Chickens lack resident cell populations belonging to the mononuclear phagocytic system (MPS, i.e., monocytes, macrophages) in their abdominal cavity (Glick *et al.,* 1964; Sabet *et al.,* 1977; Qureshi *et al.,* 1986, 2000a). Elicitation of such cells into the abdominal cavity in response to an inflammatory stimulus results in a phenomenon called *in vivo* chemotaxis. Sephadex-elicited abdominal exudate cells (AEC) were quantified as one measure of MPS function in these trials, and the data were summarized in Tables 8 and 9. In Trial 1, AEC numbers were significantly higher at one week of age in strain 308 than in either the 3F8 or CC strains (P = 0.0182). Chickens raised on D1 yielded a significantly higher number of AEC than those raised on the high protein diet D2 (P = 0.0384). At 4 wk of age, however, the strain 308 birds were lower responders than the 3F8 and CC strain birds suggesting an age related response to *in vivo* AEC elicitation (P = 0.0453). The marginal protein diet, D1, repeated its performance at 4 wk with P = 0.0131. No interaction between diet and strain was seen in this case. In Trial 2 (Table 9), strain RC failed to elicit a significant number of AEC at 4 wk of age post Sephadex G-50 injection, whereas strains 308 and CC were high responders.

Phagocytosis

At one week of age in Trial 1, macrophages from 3F8 and CC strains had a significantly higher percentage of phagocytic macrophages than the macrophages from strain 308 birds ($P = 0.0009$). An interaction between diets and strains was observed with strain 3F8 exhibiting significantly higher phagocytic response on D2 than on diet D1 ($P = 0.0112$). The average number of SRBC per phagocytic macrophage was significantly higher at one week of age in macrophages from strains CC than from strains 3F8 and 308 ($P = 0.0454$). The diets did not contribute to these differences. An interaction between strains and diets was seen, however, and strain 3F8 had significantly greater phagocytic response against SRBC when raised on D2 than on D1 ($P = 0.0089$).

At the 4th week of age in Trial 1, macrophages from strain CC had significantly higher phagocytic uptake than macrophages from either the 3F8 or 308 strains ($P = 0.0118$). This response was higher on D2 than on D1, and there was no interaction between diets and strains. Strains 308 and CC were high responders based on average number of SRBC/phagocytic macrophages at 4 weeks of age.

In Trial 2, macrophages from strain CC had the highest phagocytic potential at 1 wk of age on D2, resulting in a significant strain x diet interaction ($P= 0.0232$). At 4 wk the same strain had the highest phagocytosis level on D1 ($P = 0.0009$). Over-all, the CC strain exhibited a higher level of phagocytosis, either alone or in interaction with diet, than the other strains. Strain 3F8 was intermediate in its phagocytic function. Diet effects

were rather sporadic; the trends leaning towards greater phagocytic potential with diet D2 at the younger age.

Macrophage phagoctyic potential has been shown to be associated with the *B* complex haplotype in chickens (Qureshi *et al.,* 1986). Macrophages from White Leghorn congenic lines B^2 and B^{13} showed higher phagocytosis of SRBC than lines carrying B^5 and B^{21} (Puzzi *et al.,* 1990). Furthermore, alterations in MHC expression or gene dosage may affect phaogocytic function (Qureshi *et al.,* 1989)**.** Genetic differences in macrophage phagocytic, bacterial and tumor cell killing potential were also observed in commercial broiler lines with undefined MHC haplotypes (Qureshi and Miller, 1991). It should be pointed out that the differences in macrophage functions observed in our study represent non-specific phagocytosis since SRBC were not opsonized with either antibodies or complement.

Nitrite Production

Inducible nitric oxide synthase (iNOS) activity of macrophages in response to LPS stimulation was quantified by measuring nitrite levels in Trials 1 and 2 (Table 10). In both trials (except 19.42 μM value in Trial 1, wk 1 in 308, D1 group) the nitrite values ranged from 2.35 to 9.9 with an approximate SEM value of 1.24. This range as well as analysis conducted to determine the effects of diet or diet x strain interactions indicated that there were no major differences among the various treatment groups in nitrite production. It would be interesting to quantify CD14 (the LPS binding molecule) and TLR4 (the LPS signaling molecule) expression on macrophages from these genetic

groups. Previous studies have shown that various strains of chickens differ in the constitutive as well as inducible expression of CD14 and TLR4 (Dil and Qureshi, 2002a,b).

Chemotactic Response

The chemotactic response of blood mononuclear cells to FMLP as assessed in Trials 1 and 2 is reported in Table 11. The chemotactic assay which was done at one week of age during Trial 1 showed no significant difference in chemotactic activity of the monocytes from the different strain or diet groups. There was also no significant interaction between diets and strains. At 4 weeks of age, strain 308 showed significantly higher chemotactic activity than either the 3F8 or CC strains (P = 0.0001). The birds placed on D2 exhibited greater chemotactic response than those provided D1 (P = 0.0124). An interaction between diet and strain was also seen with strain 308 showing significantly higher chemotactic response on D2 than the rest of the strain x diet groups (P = 0.0548).

During Trial 2, chemotactic activity of strain 308 at one week of age was significantly higher than for strains RC and CC, while strain RC was better than strain CC (P = 0.0001). The chemotactic response also exceeded on D2 than on D1 (P = 0.0172). However, no interaction between diets and strains was observed. Strain 308 was again a high responder at 4 weeks of age in comparison to the RC and CC strains (P = 0.001). No significant difference in the chemotactic activity between diets, nor any interaction between diets and strains, was observed. Overall, in both trials, strain 308 had

significantly greater chemotactic response than the other strains, also the birds raised on the high protein diet, D2, gave higher *in vitro* chemotactic response to FMLP than those raised on D1.

Genetic control of chemotactic response has been previously reported by Qureshi *et al.* (1988) and Puzzi *et al.* (1990) when comparing $15I_5$ *B*-congenic White Leghorn chickens. Both studies reported B^5 and B^{21} as being a higher responder than B^2 and B^{13}. In another study, Qureshi *et al.* (1989) reported an inverse relationship between phagocytic and chemotactic responses, suggesting that a chicken line higher in macrophage phagocytic function may not perform as well in responding to inflammatory signals. In the studies reported herein, chicks from the 308 strain were high responders for chemotactic ability, whereas the phagocytic response was relatively weaker. Other evidence of MHC (*B* complex) affects on chemotactic response comes from *B*-complex gene dosage model where monocytes from tetrasomic ($B^{15}B^{15}B^{15}B^{15}$) chicks showed enhanced chemotaxis to FMLP and *Enterobacter cloacae* culture supernatant compared to disomics and trisomics (Qureshi *et al.,* 1989).

Natural Killer Cell Activity

Data on the natural killer (NK) cell activity in Trials 1 and 2 are summarized in Table 12. In Trial 1, NK-cells from the CC strain showed significantly higher NK activity at one week of age than NK-cells from either the 3F8 or 308 strains. At 4 wk of age, D2 fed birds had significantly higher NK activity than D1 fed birds ($P = 0.0007$). The strain x diet interaction was not significant.

In Trial 2, no significant difference in specific chromium release was observed at one wk of age for the different strains or diets. Also, no significant interaction was observed. At 4 wk of age, strain effects were not significant, but there was a diet effect because birds on D1 had significantly greater NK activity than the birds on D2 (P = 0.0189). There was no interaction between strains and diets.

Lillehoj and Chai (1988) demonstrated genetic differences in NK-cell cytotoxicity when examining two strains of chickens FP (B^{15} B^{21}) and SC (B^{2} B^{2}). Gut intraepithelial lymphocytes with NK-cell activity in SC chicken showed significantly higher cytotoxicity in ^{51}Cr labeled avian LSCC-RP9 (avian leukosis tumor cell line), following 4 hr of incubation, than the NK-cells isolated from the gut intraepithelium of FP line. No significant line differences were observed in cytotoxicity of NK-cells derived from the spleen, thymus or bursa. In the present study, NK-cells derived from the spleen did not show consistent differences. A gene in the B-F/B-L region of the chicken MHC has been identified that is transcribed in NK-cell lines and is closely related to lectin-like natural killer (NK) receptors (Kaufman *et al.,* 1999). The importance of NK-cells in the protective immunity of chickens is still not well understood.

Taken together, the study reported herein suggests that genetic differences do exist amongst commercial chicken lines for various base-line immune function parameters. It was not possible to address and/or correlate the immune response endpoints with regard to growth performance, since the body weights of birds at market age were not available. Nevertheless, out of the four strains tested using a broad immune response panel, two strains, namely strains 308 and CC performed significantly higher in

their immune response than the strains 3F8 and RC. Among the two high responding strains, CC can be classified as high responder for the humoral (adaptive) endpoint. The CC strain also had greater macrophage phagocytic function perhaps thereby contributing to higher humoral immunity from its improved antigen uptake, processing and presentation. On the contrary, strain 308 exceeded in cell-mediated immune responses, since it exhibited greater T-lymphocyte proliferation as well as chemotactic responses. The diet effect was not consistent for monocyte-macrophage functions. However, birds raised on the high protein diet showed effective T-cell proliferation in response to mitogens Con-A and PHA-P. Interaction between strain and diet was seen in antibody response with strain 308 giving higher antibody titers against SRBC on D1 and strain CC showing higher response on D2. These observations therefore imply that breeders may be able to co-select their strains for performance as well as immune parameter(s) suited to the type of disease challenges encountered in specific production conditions.

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Table 1. Body weights of commercial broiler chicken strains when raised on marginal and high protein diets[2]

		Trial 1		Trial 2
Strain[1]	Diet[2]	Body weight (g) (Trial 1)	Strain[1]	Body weight (g) (Trial 2)
		2 wk		3 wk
3F8	D1	267.12	RC	680.93
3F8	D2	289.87	RC	639.57
308	D1	294.00	308	581.45
308	D2	288.50	308	582.46
CC	D1	290.12	CC	644.32
CC	D2	283.50	CC	611.18
Pooled SEM[3]		11.61		20.11
Strain Averages				
3F8	x	278.50	RC	660.25[a]
308	x	291.25	308	581.95[b]
CC	x	286.81	CC	627.75[a]
Sources of variation			Probability	
Strain		0.5421		0.0015
Diet		0.7106		0.1432
Strain x Diet		0.3668		0.5406

Data represents mean body weights of 8 birds/strain/diet that were taken at 2 or 3 wk of age in trials 1 and 2, respectively.

[a,b] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

1. 3F8 = Ross 3F8 (vent sexable)
   RC = Ross x Cobb (vent sexable),
   308 = Ross 308 (feather sexable)
   CC = Cobb x Cobb (vent sexable).

2. D1 = Marginal protein starter diet with 3075 Kcal ME/kg and 20.10% crude protein (CP) while marginal protein finisher diet had 3152 Kcal ME/kg and 18.10% CP.
   D2 = High protein diet starter and finisher containing 3086 Kcal ME/kg, 21.90% CP and 3130 Kcal ME/kg, 20% CP, respectively.

3. SEM = Standard Error of Mean.

Table 2. Primary and secondary lymphoid organ weights of commercial broiler chicken strains when raised on marginal and high protein diets[2]

		Trial 1				Trail 2		
Strain[1]	Diet[2]	Bursa (%)	Spleen (%)	Thymus (%)	Strain[1]	Bursa (%)	Spleen (%)	Thymus (%)
		2 wk	2 wk	2 wk		3 wk	3 wk	3 wk
3F8	D1	0.28	0.08	0.25[a]	RC	0.23	0.09	0.20
3F8	D2	0.22	0.08	0.17[b]	RC	0.22	0.09	0.19
308	D1	0.24	0.09	0.24[a]	308	0.25	0.09	0.21
308	D2	0.23	0.08	0.19[ab]	308	0.22	0.09	0.19
CC	D1	0.22	0.09	0.20[ab]	CC	0.23	0.10	0.21
CC	D2	0.26	0.10	0.22[ab]	CC	0.23	0.11	0.21
Pooled SEM[3]		0.02	0.007	0.02		0.02	0.009	0.02
Sources of variation		Probability						
Strain		0.7885	0.4688	0.9666		0.8906	0.2718	0.7864
Diet		0.7345	0.8145	0.0756		0.4830	0.8748	0.5308
Strain x Diet		0.1704	0.3459	0.0550		0.7951	0.8392	0.8347

Thymus (all lobes on left side of neck), bursa of Fabricius and spleens were removed from 8 birds/strain/diet and weighed to the nearest gram, at 2 and 3 wk of age. The values (%) were computed as a percentage of lymphoid organ's weight to total body weight, from the individual bird.

[a,b] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = Standard Error of Mean.

Table 3. Anti-sheep red blood cells antibody response of commercial broiler chicken strains when raised on marginal and high protein diets[1] (Trial 1)

Strain[1]	Diet[2]	D PPI[3]				D PSI[3]	
		4	7	10	14	5	10
3F8	D1	0.20	0.55	0.40	0.70	6.80 [bc]	3.30
3F8	D2	0.22	0.50	0.60	0.30	6.12 [c]	3.50
308	D1	1.10	1.20	0.80	0.30	8.40 [a]	3.40
308	D2	1.22	1.00	0.11	0.20	6.90 [bc]	2.40
CC	D1	0.90	1.80	1.00	1.00	7.70 [ab]	4.22
CC	D2	0.88	1.50	1.33	1.66	8.70 [a]	4.37
SEM[3] range		.35 - .37	.43 - .45	.42 - .44	.41 – .43	.44 - .49	.48 - .51
Strain Averages							
3F8	x	0.21[b]	0.52[b]	0.50	0.50[b]	6.46[b]	3.40[ab]
308	x	1.16[a]	1.10[ab]	0.45	0.25[b]	7.65[a]	2.90[b]
CC	x	0.89[a]	1.65[a]	1.16	1.33[a]	8.20[a]	4.29[a]
Sources of variation		Probability					
Strain		0.0327	0.0446	0.1948	0.0323	0.0014	0.0182
Diet		0.8811	0.6042	0.8835	0.8706	0.2934	0.5843
Strain x Diet		0.9818	0.9613	0.4461	0.4280	0.0217	0.3565

A 3% suspension of SRBC was injected @ 1 mL/bird (10 birds per strain per diet). Serum samples were collected at days 4,7,10, and 14 post first injection and then at days 5 and 10 after second injection. Antibody titers were quantified by plate agglutination assay.

[a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = standard error of the mean, D PPI = days post primary injection, D PSI = days post secondary injection.

Table 4. Antibody response against sheep red blood cells of different strains of chickens when raised on marginal and high protein diets[2] (Trial 2)

Strain[1]	Diet[2]	D PPI[3]				D PSI[3]	
		4	7	10	14	5	10
RC	D1	0.10	1.00	0.44	0.66	5.66^{ab}	2.33^{ab}
RC	D2	0.70	1.00	0.60	0.40	4.10^{b}	2.88^{a}
308	D1	0.70	1.60	0.50	0.00	6.10^{a}	1.88^{b}
308	D2	0.00	0.33	0.10	0.30	5.20^{ab}	0.33^{c}
CC	D1	1.00	0.70	0.10	0.40	4.00^{b}	2.60^{ab}
CC	D2	0.77	0.60	0.50	0.30	5.90^{a}	2.70^{ab}
SEM[3] range		.32-.33	.36-.38	.23-.24	.22-.23	.64-.67	.34-.36
Strain Averages							
RC	x	0.40	1.00	0.52	0.53	4.88	2.61^{a}
308	x	0.35	0.96	0.30	0.15	5.65	1.11^{b}
CC	x	0.88	0.65	0.30	0.35	4.95	2.65^{a}
Sources of variation		Probability					
Strain		0.2002	0.5778	0.5679	0.2542	0.4689	0.0001
Diet		0.6865	0.1366	0.7905	0.9053	0.7415	0.3121
Strain x Diet		0.1332	0.1773	0.2300	0.4474	0.0338	0.0142

A 3% suspension of SRBC was injected @ 1 mL/bird (10 birds per strain per diet) at 7 days of age. Serum samples were collected at days 4,7,10, and 14 post first injection (dpi) and then at days 5 and 10 post boost (dpi). Antibody titers were quantified by plate agglutination assay.

[a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = standard error of the mean, D PPI = days post primary injection, D PSI = days post secondary injection.

Table 5. Lymphoblastogenic response against PHA-P by different strains of chickens when raised on marginal and high protein diets[2] (Trial 1)

Strain[1]	Diet[2]	24 hr increase (mm)	48 hr increase (mm)	24 hr increase (mm)	48 hr increase (mm)
		2 wk	2 wk	4 wk	4 wk
3F8	D1	0.83[bc]	0.49	1.07	0.75
3F8	D2	1.04[a]	0.70	0.94	0.57
308	D1	0.99[abc]	0.53	0.74	0.46
308	D2	1.00[ab]	0.72	0.93	0.60
CC	D1	0.98[abc]	0.56	0.97	0.66
CC	D2	0.79[c]	0.53	0.99	0.67
Pooled SEM[3]		0.07	0.05	0.10	0.09
Sources of variation		Probability			
Strain		0.3447	0.4172	0.1818	0.2684
Diet		0.8323	0.0124	0.7470	0.9112
Strain x Diet		0.0341	0.0766	0.3095	0.2669

PHA-P was injected @ 100µg/bird in the toe web of the right foot of 10 birds/strain/diet at 2 and 4 weeks of age. Swelling was measured by a constant tension micrometer at 24 and 48 hours. The increase in swelling response was computed by subtracting the pre-injection value from the post-injection value at a given time point.

[a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = standard error of the mean.

Table 6. Lymphoblastogenic response against PHA-P by different strains of chickens when raised on marginal and high protein diets[2] (Trial 2)

Strain[1]	Diet[2]	24 h increase (mm)	48 h increase (mm)	72 h increase (mm)	24 h increase (mm)	48 h increase (mm)	72 h increase (mm)
		2 wk	2 wk	2 wk	4 wk	4 wk	4 wk
RC	D1	0.56	0.38	0.31	0.68	0.74	0.81
RC	D2	0.50	0.42	0.33	0.74	0.86	0.93
308	D1	0.61	0.45	0.42	0.72	0.75	0.71
308	D2	0.56	0.52	0.39	0.78	0.90	0.95
CC	D1	0.43	0.39	0.35	0.76	0.86	0.93
CC	D2	0.41	0.30	0.20	0.58	0.76	0.81
Pooled SEM[3]		0.06	0.05	0.04	0.07	0.09	0.10
Strain Averages							
RC	x	0.53[ab]	0.40[ab]	0.32[ab]	0.71	0.80	0.87
308	x	0.59[a]	0.48[a]	0.40[a]	0.75	0.83	0.83
CC	x	0.42[b]	0.35[b]	0.27[b]	0.67	0.81	0.87
Sources of variation		Probability					
Strain		0.0344	0.0430	0.0176	0.5715	0.9591	0.9097
Diet		0.4228	0.8850	0.1369	0.7344	0.4846	0.3478
Strain x Diet		0.9373	0.3015	0.1533	0.2279	0.3730	0.2162

PHA-P was injected @ 100µg/bird in the toe web of the right foot of 10 birds/strain/diet at 2 and 4 weeks of age. Swelling was measured by a constant tension micrometer at 24 and 48 hours. The increase in swelling response was computed by subtracting the pre-injection value from the post-injection value at a given time point.

[a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = standard error of the mean.

Table 7. Lymphoblastogenic response of leukocytes against Concanavalin A from different genetic strains of chickens when raised on marginal and high protein diets[2]

		Trial 1		Trial 2	
Strain[1]	Diet[2]	Stimulation index	Strain[1]	Stimulation index	Stimulation index
		4 wk		1 wk	4 wk
3F8	D1	0.25	RC	0.09	0.35
3F8	D2	0.14	RC	0.13	0.26
308	D1	0.51	308	0.25	0.36
308	D2	0.33	308	0.34	0.41
CC	D1	0.19	CC	0.06	0.36
CC	D2	0.20	CC	0.38	0.53
Pooled SEM[3]		0.07		0.06	0.05
Strain Averages					
3F8	x	0.19[b]	RC	0.11[a]	0.31[b]
308	x	0.42[a]	308	0.30[a]	0.39[ab]
CC	x	0.19[b]	CC	0.20[ab]	0.44[a]
Sources of variation		Probability			
Strain		0.0018		0.0281	0.0509
Diet		0.1231		0.0081	0.3231
Strain x Diet		0.4016		0.0818	0.0669

Peripheral blood leukocytes were separated out of whole blood using a Ficoll density gradient (10 birds/strain/diet), concentration was adjusted to $2x10^6$ cells per mL, and the cells were then incubated with 25 μg/100μL of Con-A mitogen. After 24 hours of incubation, lymphoblastogenesis was quantified using MTT assay. Lymphoproliferative index was calculated as = stimulated – unstimulated/unstimulated.

[a,b] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = Standard Error of Mean.

Table 8. Macrophage function of different strains of chickens on marginal and high protein diets[2] (Trial 1 – 1 and 4 wk)

		1 wk			4 wk		
Strain[1]	Diet[2]	# AEC (10^6)	Percentage Phagocytosis	# SRBC/ macrophage	# AEC (10^6)	Percentage Phagocytosis	# SRBC/ macrophage
3F8	D1	5.55	24.30^{c}	1.75^{c}	18.57	55.51	1.92
3F8	D2	4.60	32.99^{ab}	2.08^{ab}	15.42	64.35	1.91
308	D1	11.83	26.51^{bc}	1.94^{bc}	11.85	57.94	2.14
308	D2	9.11	21.04^{c}	1.85^{bc}	5.71	65.34	2.10
CC	D1	10.16	32.06^{ab}	2.23^{a}	26.85	60.47	1.95
CC	D2	4.30	34.36^{a}	2.00^{ab}	10.28	76.27	2.03
SEM[3] Range		1.76-1.86	2.17-2.66	0.08-0.10	4.04	2.82-3.09	0.06-0.07
Strain Averages							
3F8	x	5.07^{b}	28.66^{a}	1.91^{b}	17.00^{a}	59.93^{b}	1.91^{b}
308	x	10.47^{a}	23.77^{b}	1.89^{b}	8.78^{b}	61.64^{b}	2.12^{a}
CC	x	7.23^{b}	33.21^{a}	2.12^{a}	18.57^{a}	68.37^{a}	1.99^{ab}
Sources of variation		Probability					
Strain		0.0182	0.0009	0.0454	0.0453	0.0118	0.0141
Diet		0.0384	0.3571	0.9264	0.0131	0.0001	0.9107
Strain x Diet		0.3985	0.0112	0.0089	0.2329	0.3126	0.6700

A 3% Sephadex G-50 suspension was injected @ 1mL/100g BW into the abdominal cavities of 7 birds/strain/diet at 1 week of age. After 40 hours abdominal exudate cells harvested and the cell count was adjusted to $1x10^6$/mL. For phagocytic activity, macrophages were fed 1% SRBC, incubated for 1 hr, fixed, stained, and scored for % phagocytic macrophages as well as average # of SRBC/phagocytic macrophages.

[a-c] Mean ± SE within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2]. For strain and diet designations, see footnote of Table 1.

[3.] SEM = Standard Error of Mean.

Table 9. Macrophage function of different genetic strains of chickens on marginal and high protein diets[2] (Trial 2 – 1 and 4 wk)

		1 wk			4 wk		
Strain[1]	Diet[2]	# AEC (10^6)	Percentage Phagocytosis	# SRBC/ macrophage	# AEC (10^6)	Percentage Phagocytosis	# SRBC/ macrophage
RC	D1	14.81	41.72[b]	1.89	9.12	43.06[b]	1.79
RC	D2	13.40	40.40[b]	2.09	17.50	44.93[ab]	1.85
308	D1	15.93	38.60[b]	1.92	21.38	46.41[ab]	1.84
308	D2	10.87	40.63[b]	2.21	40.16	31.66[c]	1.86
CC	D1	15.46	38.17[b]	1.86	23.75	50.98[a]	1.82
CC	D2	10.21	55.25[a]	2.24	26.62	34.85[c]	1.67
SEM[3] Range		2.89	2.90-3.86	0.08-0.10	6.47-6.86	2.30-2.69	0.06-0.07
Strain Averages							
RC	x	14.10	41.06	1.99	13.31[b]	44.00	1.82
308	x	13.40	36.92	2.07	30.77[a]	39.04	1.85
CC	x	12.84	46.71	2.05	25.18[ab]	42.91	1.74
Sources of variation		Probability					
Strain		0.9090	0.1080	0.7287	0.0379	0.1049	0.2864
Diet		0.1060	0.0399	0.0009	0.0755	0.0001	0.6766
Strain x Diet		0.7570	0.0232	0.7175	0.4832	0.0009	0.3107

A 3% Sephadex G-50 suspension was injected @ 1mL/100g BW into the abdominal cavities of 7 birds/strain/diet at 1 week of age. After 40 hours the abdominal exudate cells were harvested, the cell count was adjusted to $1x10^6$/mL. For phagocytic activity macrophages were fed 1% SRBC, incubated for 1 hr, fixed, stained, and scored for % phagocytic macrophages as well as average # of SRBC/phagocytic macrophages.

[a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2]. For strain and diet designations, see footnote of Table 1.

[3.] SEM = Standard Error of Mean.

Table 10. LPS – mediated nitrite production response by macrophages from different strains of chickens when raised on marginal and high protein diets[2] (Trials 1 and 2)

		Trial 1			Trial 2	
Strain[1]	Diet[2]	Nitrite (μM)	Nitrite (μM)	Strain[1]	Nitrite (μM)	Nitrite (μM)
		1 wk	4 wk		1 wk	4 wk
3F8	D1	8.86	4.11[ab]	RC	5.11	5.76
3F8	D2	6.73	4.33[ab]	RC	6.86	6.30
308	D1	19.42	2.35[b]	308	5.44	6.12
308	D2	7.81	4.59[ab]	308	7.89	8.33
CC	D1	9.90	6.25[a]	CC	4.62	7.58
CC	D2	7.89	2.63[b]	CC	6.60	7.46
SEM[3] Range		2.10 – 2.42	0.96		1.07	0.75
Strain Averages						
3F8	x	7.79[b]	4.22	RC	5.98	6.03
308	x	13.62[a]	3.47	308	6.66	7.23
CC	x	8.89[b]	4.44	CC	5.61	7.52
Sources of variation		Probability				
Strain		0.0319	0.5773		0.6109	0.1234
Diet		0.0072	0.6250		0.0218	0.1593
Strain x Diet		0.0626	0.0108		0.9456	0.2923

Macrophage cultures (from 10 birds/strain/diet) were exposed to lipopolysaccharides from *E. coli* (1μg/1x10^6 macrophages) for 24 hours, at 1 and 4 weeks of age. The culture supernatant was tested for nitrite levels by treating it with Griess reagent method.

[a,b] Mean ± SE within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = Standard Error of Mean.

Table 11. Chemotactic response of peripheral blood leukocytes (monocytes) from different strains of chickens when raised on marginal and high protein diets[2] (Trials 1 and 2)

		Trial 1			Trial 2	
Strain[1]	Diet[2]	Chemotaxis 1 wk (#/ 100 x microscopic field)	Chemotaxis 4 wk (#/ 100 x microscopic field)	Strain[1]	Chemotaxis 1 wk (#/ 100 x microscopic field)	Chemotaxis 4 wk (#/ 100 x microscopic field)
3F8	D1	20.93	13.45^{c}	RC	24.70	22.46
3F8	D2	19.30	14.03^{c}	RC	32.40	23.36
308	D1	22.43	23.30^{b}	308	35.86	29.53
308	D2	25.30	35.73^{a}	308	39.23	26.20
CC	D1	18.43	13.03^{c}	CC	20.10	21.93
CC	D2	19.26	16.46^{c}	CC	24.10	19.70
SEM[3] range		4.74	2.16 – 2.64		2.10 – 2.58	1.53
Strain Averages						
3F8	x	19.76	13.74^{b}	RC	28.55^{b}	22.91^{b}
308	x	22.86	29.51^{a}	308	37.55^{a}	27.86^{a}
CC	x	18.85	14.75^{b}	CC	22.10^{c}	20.81^{b}
Sources of variation				Probability		
Strain		0.5473	0.0001		0.0001	0.0019
Diet		0.8159	0.0124		0.0172	0.2393
Strain x Diet		0.9233	0.0548		0.5643	0.3901

Peripheral blood leukocytes were separated from whole blood by Ficoll density gradient (9 birds/strain/diet and pool of 3 birds was used as one sample) at 1 and 4 weeks of age. Chemotactic response was quantitated against f-met-leu-phe (10^{-5} molar) using a blind well chamber assay. The cell number was $2x10^{5}$ per sample.

[a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = Standard Error of Mean.

Table 12. Splenic natural killer cell activity of different strains of chickens when raised on marginal and high protein diets[2] (Trials 1 and 2)

		Trial 1			Trial 2	
Strain[1]	Diet[2]	Specific Chromium Release (%)	Specific Chromium Release (%)	Strain[1]	Specific Chromium Release (%)	Specific Chromium Release (%)
		1 wk	4 wk		1 wk	4 wk
3F8	D1	1.12[bc]	1.43	RC	3.09	15.60
3F8	D2	1.72[bc]	3.24	RC	2.51	14.02
308	D1	-3.27[c]	-0.37	308	2.39	17.23
308	D2	2.91[b]	3.34	308	3.07	11.21
CC	D1	-0.37[d]	-0.28	CC	3.20	16.81
CC	D2	10.83[a]	2.40	CC	3.25	10.84
SEM[3] Range		0.96 – 1.05	0.95		0.38 – 0.40	1.82 – 3.16
Strain Averages						
3F8	x	1.42[b]	2.34	RC	2.80	14.81
308	x	-0.18[b]	1.48	308	2.73	14.22
CC	x	5.22[a]	1.06	CC	3.22	13.82
Sources of variation		Probability				
Strain		0.0001	0.3978		0.4102	0.8995
Diet		0.0001	0.0007		0.8690	0.0189
Strain x Diet		0.0001	0.6091		0.2891	0.4796

Spleens from 5 birds/strain/diet were homogenized and differentially centrifuged to get splenic leukocytes (used as a source of natural killer cells) at 1 and 4 weeks of age. RP9 cells were labeled with ^{51}Cr. The splenic and ^{51}Cr labeled RP9 cells were plated at the ratio of 50:1 and were then incubated for 4 hours at 39 C. The amounts of chromium released was quantitated by gamma counter, specific chromium release (%) was calculated as:

$$\frac{\text{Exp release (supernatant) – spontaneous release (from RP9) x 100}}{\text{Total (supernatant + pellet) – spontaneous (from RP9)}}$$

[a-d] Mean ± SE within a column with no common superscript differ significantly ($P \leq 0.05$).

[1,2.] For strain and diet designations, see footnote of Table 1.

[3.] SEM = Standard Error of Mean.

Table 13. Analysis of Diets used.

Ingredient	D1[1] Starter 1-14 d	D1[1] Grower 15-28 d	D2[2] Starter 1-14 d	D2[2] Grower 15-28 d
Kcal ME/kg	3075	3152	3086	3130
Crude protein %	20.10	18.10	21.90	20.00
Lysine %	1.15	1.01	1.32	1.14
Met + Cys %	0.88	0.78	0.98	0.86
Threonine %	0.77	0.69	0.84	0.76
Calcium %	0.89	0.80	0.96	0.85
Avail. Phosphorus %	0.43	0.38	0.47	0.42
Sodium %	0.21	0.21	0.21	0.21

1. Marginal protein diet.
2. High protein diet.

CHAPTER 3

A comparison of the immune performance of a 2001 commercial broiler with a 1957 Randombred broiler strain when fed "typical" 1957 and 2001 broiler diets

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ABSTRACT: A study was conducted to compare the immunocompetence of the 2001 Ross 308 (ROSS) feather-sexable broiler strain and the 1957 Athens Canadian Randombred Control (ACRBC) strain when raised on diets "typical" of those being used in 1957 and 2001. This study was a follow up on a previous study quantifying the immunological differences that exist between a 1991 commercial strain and the 1957 ACRBC broiler strain when raised on diets typical of calendar years 1991 and 1957 (Qureshi and Havenstein, 1994). Immunological endpoints analyzed included the total, IgM, and IgG humoral response against sheep red blood cells (SRBC), *in vivo* lymphoproliferation against a T-cell mitogen, Phytohemagglutinin-P (PHA-P), and inflammatory and phagocytic responses of the macrophages. Body weights (BW) and relative lymphoid organ weights were also measured. The ROSS strain on modern diet had significantly higher BW at 24 days of age ($P = 0.0001$), while the ACRBC had higher relative (i.e. as a percent of BW) lymphoid organ weights ($P \leq 0.04$). The ACRBC strain showed significantly higher humoral responses against SRBC than the ROSS 2001 birds ($P < 0.05$). However, the ROSS had significantly greater PHA-P-induced lymphoblastogenic response *in vivo* ($P < 0.01$). Elicitation of inflammatory exudate cells was significantly higher in the ROSS birds than in the ACRBCs ($P = 0.0261$). While the percentage of macrophages that phagocytized SRBC was comparable between the two strains, the number of RBCs phagocytized by individual macrophages was higher ($P < 0.05$) in the ROSS than in the ACRBC chickens. Furthermore, the change in nitrite production following LPS stimulation in ROSS birds was significantly higher than in the ACRBC. Diet and sex effects were inconsistent amongst all parameters tested. In

conclusion, the current study suggests that the impact of genetic selection for BW results in a decrease in the adaptive arm of the immune response, i.e., antibody production. In contrast, it appears that the cell-mediated and inflammatory responsiveness aspects of the immune response are improved by selecting birds for high growth rate.

Key words: Broilers, genetic, diet, immunocompetence.

INTRODUCTION

Over the past 40-50 years, the poultry industry has witnessed a dramatic increase in the production potential of commercial poultry. This has been facilitated through application of population genetic theory, and emerging biological techniques. The genetic improvements in carcass weight and growth rate are well-documented in studies by Marks (1979), Chambers *et al.* (1981), Sherwood (1977), and Havenstein *et al.* (1994). However, selection for high production potential in poultry is not without negative consequences. Whereas the process of natural selection enables an individual to allocate resources according to demands for growth, reproduction, maintenance and well being, artificial selection for production potential can disturb the genetic homeostasis, leading to deficient resources for well being of the individual (Lerner, 1954; Beilharz, 1998).

While genetic differences have been shown to influence the disease outcome in chickens (Bumstead *et al.,* 1989; Afraz *et al.,* 1993; Lakshman *et al.,* 1997), a negative consequence of high growth rate, in the form of increased susceptibility to disease, such

as for Marek's disease, have also been reported (Han and Smyth, 1972). Under commercial broiler management conditions, fast growing broilers exhibited high mortality from commonly encountered infectious or metabolic diseases when compared with the slower growing groups of birds (Yunis *et al.,* 2000). Diet effects on immunocompetence are also well defined. For example, deficiencies in amino acids, calories, or both, in chicken diets compared with basal dietary levels, resulted in a decline in thymic T-cells, as well as humoral immune response to SRBC (Glick *et al.,* 1981; Glick *et al.,* 1983). Takahashi *et al.* (1995) observed higher acute phase immune response, as measured by plasma alpha 1-acid glycoprotein (AGP) and interleukin-1-(IL-1) like activity, in response to *E. coli* lipopolysaccharide (LPS) in chicks raised on low protein diet in comparison with those fed on a high protein diet. Amongst micro-nutrients, vitamin E and A have been found to modulate cellular immune response against infections (Sword *et al.,* 1991; Latshaw, 1991; Halevy *et al.,* 1994). An influence of the animal's sex on immune parameters has also been reported. For example, a significant negative correlation between phagocytic activity and T cell-mediated response was found in female birds of a White Leghorn chicken line, which was absent in male birds of the same genotype (Cheng and Lamont, 1988).

In the current study, a modern 2001 commercial broiler strain (Ross 308, feather sexable) was compared for immune performance against an Athens Canadian Randombred Control (ACRBC) strain. The ACRBC was developed in 1957 from several early white-feathered broiler strains and is used as a baseline control relative to the modern day broiler (Havenstein *et al.,* 1994). In addition to the genetic strains, two sexes

and two diets typical of those fed during calendar years 2001 and 1957 were also used to determine their impact on the immune response. This study was carried out to see what changes have occurred in broilers since 1991 when a similar study was conducted (Havenstein *et al.,* 1994; Qureshi and Havenstein, 1994). Their study also extended our previous findings which showed that while the 1991 commercial broiler strain gained 3.9 times more weight compared with the 1957 ACRBC at 56 days of age, it also exhibited a lower humoral immune response (Qureshi and Havenstein, 1994). Therefore, the present study is a follow up for assessing the genetic and dietary improvements, which may have possibly affected immunocompetence in broilers over the period of ten years from 1991 to 2001. Parameters examined were: body weight and relative lymphoid organ weights, humoral immune response to sheep red blood cells (SRBC), *in vivo* elicitation of macrophages, quantification of phagocytic function and nitrite production, and cell-mediated immune responses to *in vitro* Concanavilin-A (Con-A) and *in vivo* Phytohemagglutinin-P (PHA-P) stimulation.

MATERIALS AND METHODS

Chickens and Diets

The present study compares immune performance of male and female birds from the 2001 Ross 308 feather-sexable strain (ROSS) and from the 1957 Athens-Canadian Randombred Control (ACRBC) strain when they were raised on rations typical of those

used during calendar years 2001 and 1957. Fertile eggs were received on two separate occasions for two trials. The ACRBC strain eggs were kindly provided by the University of Georgia, Athens, GA, while eggs for the Ross 308 feather sexable strain were kindly provided by Allen's Hatchery, Liberty, NC. All eggs were incubated and hatched at the North Carolina State University Poultry Educational Unit, Raleigh, NC. On day 1, the chicks were feather and vent sexed, and both males and females were neck banded. The study was carried out in two trials, with Trial 1 lasting for 22 days and Trial 2 lasting for 24 days. Treatments were arranged in a 2 x 2 x 2 factorial arrangement; i.e. two strains (2001 ROSS and 1957 ACRBC), two sexes and two dietary regimens. The groups were randomly assigned into 4 blocks of 8-litter floor pens/block (32 pens total with 21 birds per pen). The 2nd trial utilized the same factorial arrangement. The treatments were assigned into 4 rooms of 16-litter floor pens/room (64 pens in total with two pens per group in one room). The 1957 starter diet contained 2895 Kcal ME/kg and 21.3% crude protein and was fed for entire period in Trials 1 and 2, since the grower phase for the 1957 diet in Trial 1 started at 36 days of age and was beyond the span of present immune function part of the study. The 2001 starter diet contained 3205 Kcal ME/kg and 23% crude protein and was fed from hatch to 21 d, and the grower phase of 2001 diet was fed from 22-24 days and it contained 3150 Kcal ME/kg and 20.5% crude protein. Complete analysis of the two diets used in the present study is presented in Table 8.

Room temperature was maintained at 92-95 F for the first week and was then reduced to 82-85 F for the remaining period of the trial. Feed and water were supplied *ad libitum*.

Experimental Endpoints

Body Weights and Relative Lymphoid Organ Weights

Thymus (all lobes on the left side of the neck), spleen, bursa of Fabricius and cecal tonsils were removed at 24 days of age from 8 birds per group in Trial 2. These organs and the corresponding chicks were weighed and the organ weights were expressed as a percentage of the bird's body weight (BW).

Antibody response

Sheep red blood cells (SRBC) were used as a T-dependent antigens to quantify the antibody response. In Trial 1, eight birds per group were injected intravenously with SRBC (3% suspension in PBS[1], 1 mL per chick) at 7 days of age followed by a booster injection of SRBC suspension at 10 days following the first injection. Blood samples were collected at 5 and 10 days after the first injection, and again at 5 and 10 days post boost. In Trial 2, 10 birds per group were injected intravenously at 7 days of age with SRBC. A booster injection was then given at 11 days after the first injection. Blood samples were collected at 4, 6, and 11 days after the first injection and at 4 and 7 days post boost.

The serum from each sample was collected, heat inactivated at 56 C for 30 minutes, and then analyzed for total, mercaptoethanol[2]- (ME) sensitive (IgM), and ME-resistant (IgG) anti-SRBC antibodies as previously described (Delhanty and Solomon,

[1] Fisher Scientific, Pittsburgh, PA 15275.

1966; Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994). Briefly, 50 μL of serum was added in equal amount of PBS in the first column of a 96 well plate[4] and was incubated for 30 minutes at 37 C. A serial dilution was then made (1:2) and 50 μL of 2% SRBC suspension was added to each well. Total antibody titers were then read after 30 minutes of incubation at 37 C. For ME-sensitive (IgM) response, 50 μL of 0.01 M mercaptoethanol in PBS was used instead of PBS, followed by the aforementioned procedure. The difference between the total and the IgG response was considered to be equal to the IgM antibody level.

Lymphoproliferative response to PHA-P

The lymphoproliferative response to Phytohemagglutinin-P[2] (PHAP), an indicator of T-cell induced delayed type hypersensitivity reaction, was assessed as described previously by Corrier (1990). In Trials 1 and 2, T-cell mitogen, PHA-P was injected intra-dermally (100 μg/100 μL/bird) into the toe web of the left foot of 8 birds/group at 19 days of age, while an equal volume of PBS was injected into the toe web of the right foot. The thicknesses of the toe webs were then measured after 24 and 48 hours using a micrometer. The swelling response was measured by subtracting the pre-injection measurement from the post-injection measurement of the toe web.

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[2] Sigma Chemical Co., St. Louis, MO 63178

Macrophage Function Assessment

Macrophage functions were assessed in Trial 2 only using methods previously described by Qureshi and Miller (1991). Ten birds from each treatment group were injected with a 3% suspension of Sephadex G-50®[2] at a concentration of 1 mL/100 g of body weight at two weeks of age. Approximately 42 hours after injection, the birds were euthanized, and the abdominal cavity was flushed with a sterile heparin (0.5 U/mL) and saline (0.75%) solution. Abdominal exudate cells (AEC) were collected in siliconised glass tubes and centrifuged at 285 x g for 10 minutes to produce an abdominal exudate cell (AEC) pellet. The AEC pellet from each individual bird was resuspended in 4 mL of RPMI-1640 growth medium supplemented with 5% heat-inactivated fetal calf serum and antibiotics[3] (100 U/mL penicillin[3] and 50 μg/mL streptomycin[3]). Total nonerythroid AEC were then counted on a hemocytometer.

In order to quantify the phagocytic potential, a 1% SRBC suspension in RPMI[1] growth medium was used as a particulate antigen. The AECs from 10 birds were then pooled to generate five samples (2 birds per sample). Macrophage monolayers were established by adding 1 x 10^6 AECs/mL from each sample into Petri dishes[4] containing four coverslips[5]. After one hour of incubation, the coverslips were washed to remove any non-adherent cells. These macrophage monolayers were co-incubated with a 1.0 mL suspension of unopsonized SRBC which was added to each Petri dish. After 60 minutes incubation at 41C in a humidified atmosphere and 5% CO_2, the coverslips were washed

[3] Atlanta Biologicals, Norcross, GA 30093.
[4] Corning, Corning, NY 14831.
[5] Thomas Scientific, Swedesboro, NJ 08085.

to remove non-internalized SRBCs. They were then fixed and stained with Leukostat®[6] and mounted on microscopic slides. A total of 200 macrophages from each of the four coverslips per bird were scored microscopically for phagocytosis as well as for numbers of SRBC per phagocytic macrophage.

Nitrite Production

Production of nitrite (a stable end product of nitric oxide) by macrophages in response to LPS[2] stimulation was assessed as previously described by Green *et al.* (1982). Macrophages from 8 birds pooled as two birds per samples were cultured in 24-well plates ($1x10^6$ cells per well per pooled sample) and exposed to LPS from *E. coli* (1 μg/ well) for 24 hours. The culture supernatants were collected and the concentration of nitrite was determined as described by Green *et al.* (1982). The standard curve for the nitrite assay was generated using various dilutions of 10mM stock solution of sodium nitrite in RPMI 1640 growth medium. The nitrite levels in culture supernatant fractions were calculated by comparing the optical density (OD) readings against the nitrite standard curve.

[6] Fisher Scientific, Orangeburg, NY 10962.

Statistical Analysis

Data were analyzed by using General Linear Models® procedure of SAS (SAS Institute, 1990). Means were separated for significance by Duncan's multiple range test at significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Body Weights and Relative Lymphoid Organ Weights

Body and lymphoid organ weights were recorded only in Trial 2 and are provided in Table 1. ROSS chickens (both males and females) raised on 2001 and 1957 diets had significantly higher body weights than the ACRBCs raised on both diets (2001 or 1957) at 22 days of age ($P = 0.0001$). Furthermore, the birds on the 2001 diet had significantly higher growth than those on the 1957 diet ($P = 0.0001$), and a significant interaction between strains and diets was observed with the ROSS chickens showing greater growth on 2001 diet ($P = 0.0003$). Similar differences in BW were reported in a companion study by Havenstein *et al.* (2003, North Carolina State University, Raleigh, NC, personal communication) who showed that the BW of the ROSS on the 2001 diets were 4.22, 4.96, 4.88, 4.30 and 3.86 times heavier than those of the ACRBC on the 1957 diet at 21, 42, 56, 70 and 84 days of age, respectively. Similarly, the ROSS on the 2001 diet was 3.81, 4.62, 4.45, 3.92, and 3.43 times larger than the ACRBC on the 2001 diets at the same ages. The ROSS on the 1957 diet was 3.47, 3.94, 3.69, 3.44, and 3.13 times heavier than the ACRBC on the 1957 diet.

While ROSS birds had greater BW, the ACRBC showed significantly higher relative weights for thymus, bursa, spleen and cecal tonsils ($P < 0.05$), Table 1. Furthermore, significant interactions were observed indicating that female birds of the ACRBC strain and the 2001 diet had higher relative thymus weights ($P = 0.0380$) and spleen weights ($P < 0.05$). The ACRBC strain on the 2001 diet showed higher relative cecal tonsil weights ($P = 0.0209$). In the present study, the 1957 starter diet had 21.3% CP and was provided for the entire length of the experiment, compared with 23.0% CP in the 2001 starter diet that was fed until day 21 and 20.5% CP in 2001 grower diet that was fed from 22-24 days. In a previous study by Rao *et al.* (1999), a low weight gain strain showed significantly improved relative bursal weight when dietary crude protein level was increased from 18% to 23%. In our study, thymus and spleen, and perhaps even cecal tonsils of 1957 birds when grown on 2001 diet (higher protein levels till 21 days of age) showed significant increases in their relative weights, similar to the observation made by Rao *et al.* (1999). Therefore, this study clearly establishes that the modern day commercial broiler strain selected for enhanced growth exhibits reduced relative growth of both primary and secondary lymphoid organs. This may support the "resource allocation theory" (Rauw *et al.,* 1998) which suggests that artificial selection for a particular trait, such as BW, in animals leads to a change in the allocation of resources to the different functions of the animal, that may affect its ability to maintain its immunocompetence and health.

Antibody response

Antibody response data against SRBC as measured by total, IgM and IgG levels in Trials 1 and 2 are given in Tables 2, 3 and 4. Total antibody titers were determined at 5 and 10 d post primary injection (PPI) in Trial 1, and at 4, 7, and 11 d PPI in Trial 2 (Table 2). For the primary antibody response in Trial 1, the ACRBC strain exhibited higher titers which were significantly greater than those from the ROSS strain (P = 0.0001) at 10 d PPI. However, in Trial 2 the response of both strains to SRBC at every d PPI was lower than in Trial 1 and was not significantly different between strains (Table 2). The secondary antibody response was measured at 5 and 10 d post second injection (PSI) in Trial 1, and at 4 and 7 d PSI in Trial 2 (Table 2). In this case, the ACRBC birds exhibited significantly higher total antibody titers at 5 and 10 d PSI in Trial 1. In Trial 2, however, while antibody titers peaked in both strains at 4 d PSI, no statistical difference was observed between the two strains at 4 or 7 d PSI (Table 2). A significant effect of the diets on antibody response was observed only in Trial 1 at 5 d PSI in which birds raised on 1957 diet allowed development of higher titers than 2001 diet. A significant interaction between diet and strain was observed at 10 d PPI in Trial 1 in which the ACRBC strain on the 1957 diet had higher antibody response than all the remaining combinations.

Immunoglobulin M (IgM) titers were determined during the primary response at 5 and 10 d PPI in Trial 1 and at 4, 7, and 11 d PPI in Trial 2 (Table 3). The IgM titers for the secondary response were quantified at 5 and 10 d PSI and at 4 and 7 d PSI in Trials 1 and 2, respectively (Table 3). In Trial 1, the ACRBC strain had significantly higher IgM

titers than the ROSS strain at d 10 PPI ($P = 0.0001$) and d 5 PSI ($P = 0.0125$). As seen previously in Table 2, at both 10 d PPI and at 5 d PSI, the ACRBC was a high responder for total anti-SRBC antibodies. The data, therefore, indicates that the significant increase observed in the total antibodies for the ACRBC was in fact due to higher IgM levels. At the same time points, birds raised on the 1957 diet exceeded in their titers than birds raised on the 2001 diet ($P \leq 0.0065$). An interaction between diets and strains was observed at d 10 PPI, with the ACRBC having greater IgM response on the 1957 diet ($P = 0.0119$). In Trial 2, no strain differences were seen in IgM levels at 4 d PPI, but at 7 d PPI the ACRBC birds approached having a higher antibody level ($P = 0.0880$) than the ROSS birds (Table 3). During the secondary response, the ROSS birds had significantly higher IgM levels at 4 and 7 d PSI ($P = 0.0001$).

Immunoglobulin G (IgG) titers were determined on the same days as the IgM levels (Table 4). Significant differences were seen between the two strains. During the PSI period, in Trial 1, a strain effect was observed at 10 d PSI ($P = 0.0062$) with the ACRBC strain having higher IgG levels than the ROSS strain (Table 4). Females also gave higher titers than males at d 10 PSI ($P = 0.0235$). In Trial 2, the IgG response was significantly higher for the ACRBC than for the ROSS at 4 and 7 d PSI ($P \leq 0.0004$). Significant diet x sex interactions for IgG levels were observed at both 4 and 7 days post boost ($P < 0.01$), with the females having higher levels than the males on the two diets.

Taken together, the antibody data from Trial 1 clearly showed that ACRBC chickens had significantly higher total, IgM and IgG responses than the modern day birds (Tables 2 - 4). While the differences in total and IgM levels could not be reproduced in

Trial 2, the ACRBC was clearly the high responder of the two strains following the booster injection when higher IgG levels are expected. This was the case when the ACRBC was found to have significantly higher IgG titers over the modern day broiler (Table 4). These observations are similar to the ones made 10 years earlier while comparing ACRBC with the 1991 commercial broiler in which ACRBC was found to be a high responder in antibody production than the 1991 broiler strain (Qureshi and Havenstein, 1994). Birds raised on 1957 diet appeared to have produced slightly higher levels of antibodies, either alone or in interaction with ACRBC strain, but these diet effects were sporadic and inconsistent. Additional studies in chickens (Miller *et al.,* 1992; Boa-Amponsem *et al.,* 1999; Rao *et al.,* 1999) and turkeys (Nestor *et al.,* 1996a) have supported our current and previously reported conclusions that the antibody response or disease resistance potential of fast growing strains of poultry is relatively weaker than in the slow growing types from which they are selected. These studies, therefore, indicate that such changes in antibody responsiveness may be a correlated response to the selection for increased BW. While most studies have not linked a particular gene or gene locus with such genetic changes, Yonash *et al.* (2001) identified a DNA marker ADL0146 that was associated with antibody response to SRBC and Newcastle disease virus (NDV) in a population of meat type chickens divergently selected for high or low antibody response to *E. coli*. Furthermore, Dunnington *et al.* (1996) found that the B^{21} haplotype was associated with higher antibody titer and lower BW in most instances, while B^{13} was associated with lower antibody titers and higher BW.

Lymphoproliferative response to PHA-P

Phytohemagglutinin-P, a T-cell mitogen, induces proliferation in T-lymphocytes. Injection of PHA-P at a selected site in chickens can be considered as an inducer of a localized *in vivo* T-lymphoproliferative response. This response was measured at 24 and 48 h post PHA-P injection into the toe web, and is reported in Table 5. In both trials, the ROSS strain exhibited significantly higher swelling response than the ACRBC at both 24 and 48 h post PHA-P injection ($P \leq 0.01$ in Trial 1; $P \leq 0.001$ in Trial 2). Other than a dietary effect that was observed at 24 h after PHA-P injection in Trial 1, where the birds on the 2001 diet had higher lymphoproliferative response than those on the 1957 diet, no other significant dietary effects were observed. In addition, no consistent strain x diet x sex interactions were observed (Table 5).

The lymphoblastogenic response to PHA-P has been reported to be polygenic. When a cross between PHA-P high responder and low responder lines was tested for mitogenic response to PHA-P, an intermediate result was seen suggesting that more than one gene controls the PHA-P response in birds (Morrow and Abplanalp, 1981). Corrier (1990) showed that broiler chicks exhibited significantly greater swelling of the toe web when injected with PHA-P than layer-type chickens. Our study was carried out with the objective to determine a possible genetic difference between two broiler strains with high and low growth performance. Our data indicates that the modern high weight gain broilers (ROSS) showed greater PHA-P mediated response than the low-weight selected ACRBC strain. Since PHA-P induces T-cell division with minimal effects on B-cells (Tizard, 1995) and is considered a good *in vivo* measure of T-lymphocyte function

(Qureshi *et al.,* 1997), our results indicate that the T-lymphocytes from the ROSS birds have higher lymphoproliferative potential. This suggests that genetic selection for growth has positively affected the cell-mediated arm of the immune response. In contrast to our results, Bayyari *et al.* (1997) reported that turkeys selected for increased 16 wk BW had lower toe web response to PHA-P than their randombred parent line. While this may suggest a possible difference between chickens and turkeys, interestingly Bayyari *et al.* (1997) further reported that upon *in vitro* PHA-P stimulation lymphocytes from high BW turkey lines exhibited significantly greater lymphoproliferation than the lymphocytes from the smaller parent line.

Macrophage Function Assessment

Macrophage functions were examined only in Trial 2 (Tables 6 and 7). Abdominal exudate cells (AECs) were quantified as a measure of the ability of the two genetic strains to respond to an inflammatory signal such as Sephadex. The AEC numbers were significantly higher in the ROSS strain 48 h post 3% Sephadex injection than in the ACRBC strain (P = 0.0261). Chicks from both strains had greater AEC numbers on the 1957 diet as compared with the 2001 diet (P = 0.0049).

When the phagocytic potential of Sephadex elicited glass adherent macrophages against SRBCs was quantitated, both ROSS and ACRBC strains exhibited comparable percentage of phagocytic macrophages (Table 6). However the relative phagocytic activity of individual macrophage in terms of numbers of internalized SRBCs was significantly higher in the 2001 ROSS strain than in the 1957 ACRBC strain (P =

0.0122). For both phagocytic endpoints, the birds raised on the 2001 diet were significantly high responders than those on the 1957 diet ($P \leq 0.0044$). A strain x diet x sex interaction was observed for the percentage of phagocytic macrophages ($P = 0.003$) indicating that different levels of phagocytosis were dependent upon all three factors. Overall, the ROSS strain had significantly higher levels of AEC and phagocytic potential for SRBC than the ACRBC strain. The 2001 diet also produced higher levels of phagocytosis than the 1957 diet.

Another function related to macrophages is the constitutive and inducible production of nitric oxide (NO) (Dil and Qureshi, 2002a, b). The NO activity was measured in the form of nitrite in the culture supernatants of the macrophages from both strains of chickens after stimulation with or without LPS *in vitro* (Table 7).

Constitutive nitrite production as measured in the absence of LPS stimulation was significantly higher in the ACRBC strain than in the ROSS birds ($P = 0.017$). This was not the case for inducible nitrite production after LPS stimulation. No significant differences in nitrite levels were observed between the two strains after LPS stimulation (Table 7). Strain x sex as well as strain x diet x sex interactions were observed for these traits, however, indicating the response was related to all three factors. Significantly lower nitrite production in the absence of LPS suggests poorer constitutive expression of nitric oxide synthase in the ROSS strain than in the ACRBC strain. Inducible expression of nitrite was comparable in the two strains.

The findings of our study suggest that the macrophages from modern-day broilers are more adept in the recognition and response to an inflammatory signal than the

macrophages from the randombred ACRBC chickens. That is, the ROSS birds had significantly higher AEC numbers in response to Sephadex stimulation (Table 6) as well as almost 1.5 times higher nitrite production in response to stimulation with LPS (Table 7). It is, however, interesting to note that while no difference in the overall phagocytic percentage of macrophages for SRBC was observed between the two strains, the macrophages from the ROSS chickens had a significantly higher level of phagocytic activity (Table 6). This seems to be an improvement from 10 years ago when the then 1991 strain in comparison to the then 1957 ACRBC strain had comparable macrophage functional activities (Qureshi and Havenstein, 1994). It is well known that chicken macrophage phagocytic activity is modulated by the genetic make up of the birds (Qureshi *et al.,* 1989; Puzzi *et al.,* 1990). Additionally, nitric oxide synthase activity differs amongst chicken genetic lines (Hussain and Qureshi, 1997, 1998). Commercial broiler chicken lines also show significant variability in several macrophage functions such as phagocytosis, bacterial uptake and killing, and cytokine (such as tumor necrosis factor) production (Qureshi and Miller, 1991). The observed improvement in macrophage phagocytic and possibly in inducible nitric oxide synthase (iNOS) activity is suggestive of positive correlated genetic change brought about by selection for performance traits such as growth.

In conclusion, it is clear from the current study that the impact of genetic selection for BW is in a negative direction on the adaptive arm of the immune response, i.e., antibody production. It seems that the cell-mediated and inflammatory responsiveness aspects of the immune system are improved in birds selected for rapid growth rate. Diet

and sex effects are inconsistent between growth selected and randombred strains for all parameters tested.

Both arms of the adaptive immune response, i.e., the humoral and cell-mediated immune responses are interactive yet distinct in their effector functions. For example, antibody response may be more effective in controlling bacterial infections where as cell-mediated immune response would be desirable in eliminating virus infected cells.

The dichotomous changes that have taken place in the immune performance of chicken lines selected for high growth rate suggest that it may be important for the breeders to adopt a breeding program which takes into account the genetic correlations that exist between these important biological functions. There is a need for a high humoral immune response against SRBC in birds, for protecting against diseases such as *E. coli* infections (Rao *et al.,* 1999), as well as, a need for higher cell-mediated immune response as it relates to viral infections (Fredricksen and Gilmour, 1983; Schat and Xing, 2000). Furthermore, birds with higher macrophage phagocytic potential and nitrite production could protect against bacterial, viral and parasitic infections (Qureshi *et al.,* 2000a). Since it is highly unlikely that the genetic correlations between growth rate and the adaptive and cell-mediated immune responses approach 1 or –1, it should be possible for breeding program to incorporate all three of these important traits for improvement through artificial selection. It appears that breeders of meat-type chickens should, at a minimum, be including some measure of humoral immune response and its genetic correlation with body weight into their selection programs. It is probably not necessary

to include cell-mediated immune response measures, since they will be improved automatically due to their positive correlation with body weight.

Table 1. Body and lymphoid organ weights of a 2001 modern commercial broiler and the 1957 Athens-Canadian randombred strain (Trial 2)

Strain[1]	Diet[2]	Sex	Body weight (g)	Thymus (%)	Bursa (%)	Spleen (%)	Cecal Tonsils (%)
2001	2001	Male	767.37	0.22 [b]	0.26	0.13	0.024
2001	2001	Female	774.00	0.28 [b]	0.26	0.11	0.029
2001	1957	Male	647.62	0.21 [b]	0.31	0.14	0.042
2001	1957	Female	609.87	0.25 [b]	0.30	0.10	0.038
1957	2001	Male	222.25	0.22 [b]	0.40	0.15	0.048
1957	2001	Female	206.25	0.47 [a]	0.50	0.26	0.053
1957	1957	Male	199.37	0.25 [b]	0.47	0.14	0.054
1957	1957	Female	177.12	0.24 [b]	0.45	0.15	0.044
SEM[3] Range			20.56 – 23.74	0.03	0.04	0.02	0.004
Strain Averages							
2001	x	x	699.71[a]	0.24[b]	0.28[b]	0.12[b]	0.03[b]
1957	x	x	201.25[b]	0.30[a]	0.46[a]	0.18[a]	0.04[a]
Sources if variation					Probability		
Strain			0.0001	0.0466	0.0001	0.0002	0.0001
Diet			0.0001	0.0335	0.3431	0.0839	0.0599
Sex			0.2476	0.0040	0.5374	0.3400	0.7724
Strain x Diet			0.0003	0.2018	0.5304	0.0545	0.0209
Strain x Sex			0.9049	0.2173	0.4482	0.0034	0.6209
Diet x Sex			0.3975	0.0195	0.3188	0.0406	0.0540
Strain x Diet x Sex			0.5234	0.0380	0.3508	0.1948	0.5927

Mean body weights and relative lymphoid organ weights of 8 birds per group were taken at 24 days of age.
[a,b] Means within a column with no common superscript differ significantly ($P \leq 0.05$).
[1.] 2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.
[2.] 2001 = Broiler diet typical of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet typical of those being fed in 1957.
[3.] SEM = Standard Error of Mean.

Table 2. Total anti-sheep red blood cells antibody titer of a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain

			Trial 1				Trial 2				
Strain[1]	Diet[2]	Sex	D PPI[3]		D PSI[3]		D PPI[3]			D PSI[3]	
			5	10	5	10	4	7	11	4	7
2001	2001	Male	1.12	0.62	3.12	1.00	0.30	0.20	0.50[ab]	4.60	3.30
2001	2001	Female	2.75	0.75	4.00	1.62	1.10	0.50	0.10[a]	4.60	3.00
2001	1957	Male	0.12	0.12	4.12	1.50	0.40	0.60	0.30[ab]	4.40	3.70
2001	1957	Female	3.37	0.87	4.25	1.62	0.70	0.75	0.70[a]	6.00	4.40
1957	2001	Male	2.75	1.62	4.00	1.75	0.90	0.66	0.40[ab]	5.40	3.70
1957	2001	Female	0.75	1.12	4.00	2.25	0.40	0.40	0.60[ab]	4.40	3.50
1957	1957	Male	3.00	2.11	4.87	2.44	0.40	1.20	0.70[a]	4.60	3.40
1957	1957	Female	3.00	3.14	5.00	2.85	0.22	0.60	0.44[ab]	4.90	4.10
	SEM[3] Range		0.64	.38 –.43	.38 –.41	.32 –.36	.23 –.25	.23 –.30	.20 –.21	0.35	0.41
Strain Averages											
2001	x	x	1.84	0.59[b]	3.87[b]	1.43[b]	0.62	0.51	0.40	4.90	3.60
1957	x	x	2.37	2.00[a]	4.46[a]	2.32[a]	0.48	0.71	0.53	4.82	3.67
Source of variation			Probability								
Strain			0.2487	0.0001	0.0362	0.0005	0.4000	0.3090	0.3612	0.7667	0.7994
Diet			0.2487	0.0728	0.0066	0.0669	0.1563	0.0870	0.3612	0.3745	0.0784
Sex			0.1204	0.2321	0.3136	0.0901	0.5380	0.6027	0.9256	0.3745	0.4466
Strain x Diet			0.1204	0.0165	0.5744	0.4092	0.5380	0.9170	0.6675	0.1408	0.2063
Strain x Sex			0.0004	0.7692	0.4324	0.8666	0.0112	0.1030	0.9256	0.0253	0.9325
Diet x Sex			0.0517	0.0693	0.5744	0.5448	0.7952	0.5461	0.5628	0.0052	0.1106
Strain x Diet x Sex			0.8378	0.4394	0.4324	0.6702	0.2322	0.8187	0.0376	0.7667	0.9325

One week old birds were given a sheep red blood cell injection (day 0) followed by a second injection on days 10 and 11 in Trials 1 and 2, respectively. Blood serum samples from 8 birds per strain/sex/diet were analysed for the presence of total anti-SRBC antibodies. The data represent mean ± standard errors of $\log_2$ of the reciprocal of the last dilution exhibiting agglutination.

[a,b] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1] 2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

[2] 2001 = Broiler diet typical of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet typical of those being fed in 1957.

[3] SEM = standard error of the mean, D PPI = days post primary injection, D PSI = days post secondary injection.

Table 3. IgM (mercaptoethanol-sensitive) anti-sheep red blood cells antibody titer of a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain

			Trial 1				Trial 2				
Strain[1]	Diet[2]	Sex	D PPI[3]		D PSI[3]		D PPI[3]			D PSI[3]	
			5	10	5	10	4	7	11	4	7
2001	2001	Male	1.00	0.25	2.00	0.75	0.20	- 0.00	0.50	2.50	1.10
2001	2001	Female	2.75	0.62	2.87	0.75	1.00	0.40	0.10	2.70	1.40
2001	1957	Male	0.12	0.12	3.37	1.00	0.30	0.30	0.30	2.50	1.90
2001	1957	Female	3.00	0.87	3.25	1.12	0.70	0.62	0.70	2.60	1.50
1957	2001	Male	2.62	1.25	3.00	1.12	0.80	0.66	0.30	1.40	0.20
1957	2001	Female	0.62	1.00	3.12	1.12	0.40	0.40	0.40	1.00	0.60
1957	1957	Male	3.00	2.11	4.50	1.22	0.40	1.20	0.40	1.20	0.60
1957	1957	Female	2.00	3.14	3.85	1.00	0.22	0.30	0.22	0.70	0.40
SEM[3] Range			0.58	.36 –.41	.40 –.43	.25 –.29	.24 –.25	.24 –.27	.19 –.20	0.39	0.29
Strain Averages											
2001	x	x	1.71	0.46[b]	2.87[b]	0.90	0.55	0.33	0.40	2.57[a]	1.47[a]
1957	x	x	2.06	1.87[a]	3.62[a]	1.11	0.45	0.64	0.33	1.07[b]	0.45[b]
Source of variation			Probability								
Strain			0.4107	0.0001	0.0125	0.2767	0.5861	0.0880	0.6252	0.0001	0.0001
Diet			0.5005	0.0065	0.0011	0.4420	0.2639	0.1860	0.5710	0.5917	0.1875
Sex			0.3315	0.0905	0.8413	0.9001	0.3707	0.5402	0.8911	0.5917	0.9041
Strain x Diet			0.1578	0.0119	0.6778	0.4010	0.5861	0.8987	0.4015	0.7205	0.3999
Strain x Sex			0.0001	0.7577	0.2768	0.6543	0.0121	0.0103	0.8911	0.2848	0.7177
Diet x Sex			0.2055	0.1402	0.1313	0.9001	0.7976	0.3269	0.3594	0.8580	0.1202
Strain x Diet x Sex			0.9402	0.4163	0.8413	0.6543	0.3707	0.4391	0.0610	1.0000	0.9041

One week old birds were given a first sheep red blood cell injection (day 0) followed by a second injection on days 10 and 11 in Trials 1 and 2, respectively. Blood serum samples from 8 birds per strain/sex/diet were analysed for the presence of total anti-SRBC antibodies. The data represent mean ± standard errors of $\log_2$ of the reciprocal of the last dilution exhibiting agglutination.

[a,b] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1.] 2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

[2.] 2001 = Broiler diet typical of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet typical of those being fed in 1957.

[3.] SEM = standard error of the mean, D PPI = days post primary injection, D PSI = days post secondary injection.

Table 4. IgG (mercaptoethanol-resistant) anti-sheep red blood cells antibody titer of a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain

			Trial 1				Trial 2				
Strain[1]	Diet[2]	Sex	D PPI[3]		D PSI[3]		D PPI[3]		D PSI[3]		
			5	10	5	10	4	7	11	4	7
2001	2001	Male	0.12	0.37	1.12	0.25	0.10	0.20	0.00	2.10	2.20
2001	2001	Female	0.00	0.12	1.12	0.87	0.10	0.10	-0.00	1.90	1.60
2001	1957	Male	0.00	0.00	0.75	0.50	0.10	0.30	-0.00	1.90	1.80
2001	1957	Female	0.37	0.00	1.00	0.50	-0.00	0.12	0.00	3.40	2.90
1957	2001	Male	0.12	0.37	1.00	0.37	0.10	-0.00	0.10	4.00	3.50
1957	2001	Female	0.12	0.12	0.87	1.12	-0.00	-0.00	0.20	3.40	2.90
1957	1957	Male	0.00	-0.00	0.37	1.22	-0.00	-0.00	0.30	3.40	2.80
1957	1957	Female	1.00	-0.00	1.14	1.85	0.00	0.30	0.22	4.20	3.70
	SEM[3] Range		0.23	.12 –.13	.18 –.19	.28 –.32	0.07	0.10	0.09	0.46	0.41
Strain Average											
2001	x	x	0.12	0.12	1.00	0.53[b]	0.07	0.18	-0.00[b]	2.32[b]	2.12[b]
1957	x	x	0.31	0.12	0.84	1.14[a]	0.02	0.05	0.20[a]	3.75[a]	3.22[a]
Source of variation			Probability								
Strain			0.2617	1.0000	0.2519	0.0062	0.3274	0.0774	0.0028	0.0001	0.0004
Diet			0.1362	0.0085	0.1078	0.0975	0.3274	0.2709	0.4048	0.2545	0.4001
Sex			0.0640	0.1782	0.0943	0.0235	0.3274	0.7986	0.9335	0.2545	0.5004
Strain x Diet			0.4529	1.0000	0.7863	0.0532	1.0000	0.7986	0.4048	0.4024	0.5004
Strain x Sex			0.2617	1.0000	0.4569	0.3823	1.0000	0.1093	0.9335	0.4024	0.8660
Diet x Sex			0.0272	0.1782	0.0336	0.3948	1.0000	0.6708	0.5048	0.0203	0.0084
Strain x Diet x Sex			0.4529	1.0000	0.2254	0.5570	0.3274	0.3509	0.5048	0.8190	0.8660

One week old birds were given a sheep red blood cell injection (day 0) followed by a second injection on days 10 and 11 in Trials 1 and 2, respectively. Blood serum samples from 8 birds per strain/sex/diet were analysed for the presence of total anti-SRBC antibodies. The data represent mean ± standard errors of $\log_2$ of the reciprocal of the last dilution exhibiting agglutination.

[a,b] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1.] 2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

[2.] 2001 = Broiler diet typical of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet typical of those being fed in 1957.

[3] SEM = standard error of the mean, D PPI = days post primary injection, D PSI = days post secondary injection.

Table 5. Lymphoblastogenic response against PHA-P by a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain – 24 and 48 hours increase.

			Trial 1		Trial 2	
Strain[1]	Diet[2]	Sex	24 hours increase (mm)	48 hours increase (mm)	24 hours increase (mm)	48 hours increase (mm)
2001	2001	Male	0.58	0.33	0.93[a]	0.47
2001	2001	Female	0.79	0.42	0.62[cd]	0.41
2001	1957	Male	0.48	0.33	0.71[bc]	0.47
2001	1957	Female	0.57	0.37	0.85[ab]	0.57
1957	2001	Male	0.45	0.23	0.39[e]	0.20
1957	2001	Female	0.55	0.26	0.40[e]	0.22
1957	1957	Male	0.31	0.21	0.45[de]	0.20
1957	1957	Female	0.41	0.21	0.44[de]	0.20
SEM[3] Range			0.09	0.06	0.07	0.04 – 0.05
Strain Averages						
2001	x	x	0.60[a]	0.36[a]	0.78[a]	0.48[a]
1957	x	x	0.43[b]	0.23[b]	0.42[b]	0.20[b]
Source of variation			Probability			
Strain			0.0129	0.0043	0.0010	0.0001
Diet			0.0298	0.5010	0.5796	0.2948
Sex			0.0721	0.4268	0.4430	0.6832
Strain x Diet			0.8401	0.8648	0.6674	0.2189
Strain x Sex			0.6801	0.5632	0.4170	0.8476
Diet x Sex			0.6469	0.7182	0.0433	0.3428
Strain x Diet x Sex			0.6734	0.9728	0.0232	0.1664

At 19 days of age, PHA-P was injected @ 100 μg/100 μL/bird in the toe web of right foot of 8 birds per strain/sex/diet. Swelling was measured by a constant tension micrometer at 24 and 48 hours post injection. The increase in swelling was computed by subtracting the pre-injection value from the post-injection value at a given time point.

[a,b] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1.] 2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

[2.] 2001 = Broiler diet typical of those being fed in calendar year 2001 with crumbled. starter and pelleted grower, 1957 = broiler starter diet typical of those being fed in 1957.

[3.] SEM = standard error of the mean.

Table 6. Macrophage response of a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain (Trial 2)

Strain[1]	Diet[2]	Sex	# AEC	Percentage Phagocytosis	# SRBC/Macrophage
2001	2001	Male	4.91	43.97 [a]	2.40
2001	2001	Female	8.02	29.89 [bc]	2.09
2001	1957	Male	9.34	23.27 [£]	1.90
2001	1957	Female	12.66	33.29 [b]	1.91
1957	2001	Male	5.46	34.26 [b]	2.14
1957	2001	Fe-ale	4.46	30.56 [bc]	1.74
1957	1957	Male	6.85	31.25 [b]	1.71
1957	1957	Female	7.59	28.21 [bc]	1.90
SEM[3] Range			1.55 – 1.79	2.58 – 2.98	0.10 – 0.11
Strain Ave²ages					
2001	x	x	8.73[a]	32.60	2.07[a]
1957	x	x	6.09[b]	31.07	1.87[b]
Sources of var©ation				Probability	
Strain			0.0261	0.4330	0.0122
Diet			0.0049	0.0044	0.0038
Sex			0.1866	0.1685	0.1063
Strain x Diet			0.3288	0.1284	0.1930
Strain x Sex			0.1529	0.7326	0.7953
Diet x Sex			0.6752	0.0019	0.0056
Strain x Diet x Sex			0.7430	0.0033	0.4156

3% Sephadex suspension was injected @ 1mL/ 100 g bw into the abdominal cavities of 5 birds/strain/diet/sex at two weeks of age. After 40 hours birds were harvested to get abdominal exudate, cell count was adjusted to 1 x 10^6/mL. For phagocytic activity macrophages were fed 1% SRBC, incubated for 1 hr, fixed, stained and scored for % phagocytic macrophages as well as average # of SRBC/ phagocytic macrophages.

[a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1.] 2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

[2.] 2001 = Broiler diet typical of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet typical of those being fed in 1957.

[3.] SEM = standard error of the mean.

Table 7. LPS – mediated nitrite production response by macrophages from a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain (Trial 2)

Strain[1]	Diet[2]	Sex	Nitrite (μM) with LPS	Nitrite (μM) without LPS
2001	2001	Male	14.04[abc]	4.90 [cd]
2001	2001	Female	15.33[ab]	5.21 [cd]
2001	1957	Male	20.54[a]	9.78 [ab]
2001	1957	Female	8.59[c]	3.49 [d]
1957	2001	Male	15.49[a]	7.08 [bc]
1957	2001	Female	14.73[abc]	12.61 [a]
1957	1957	Male	8.69[bc]	4.78 [cd]
1957	1957	Female	15.61[a]	7.83 [bc]
Pooled SEM[3]			2.47	1.29
Strain Averages				
2001	x	x	14.62	5.84[b]
1957	x	x	13.63	8.07[a]
Sources of variation			Probability	
Strain			0.5714	0.0170
Diet			0.3800	0.2890
Sex			0.5208	0.4784
Strain x Diet			0.4194	0.0064
Strain x Sex			0.0183	0.0001
Diet x Sex			0.4288	0.0150
Strain x Diet x Sex			0.0036	0.2641

Macrophage cultures (from 5 birds/ strain/ sex) were exposed to lipopolysaccharides from *E. coli* (1 μg/ 1x 10^6 macrophages) for 24 hours. The culture supernatant was tested for nitrite levels by treating it with Griess reagent method.

[a-d]Means within a column with no superscript differ significantly ($P \leq 0.05$).

[1.] 2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

[2.] 2001 = Broiler diet typical of those being fed in calendar year 2001 with crumbled starter and palleted grower, 1957 = broiler starter diet typical of those being fed in 1957.

[3.] SEM = standard error of the mean.

Table 8. Predicted Analysis of diets used.

Ingredient	1957 Starter Diet 1-24d	2001 Starter Diet 1-21d	2001 Grower Diet 22-24d
Kcal ME/kg	2895	3205	3150
Crude protein %	21.3	23.0	20.5
Lysine %	1.18	1.25	1.10
Met + Cys %	0.75	0.91	0.87
Threonine %	0.82	0.87	0.76
Calcium %	1.35	0.90	0.55
NPP %	0.61	0.42	0.23
Sodium %	0.18	0.22	0.22

SUMMARY AND CONCLUSIONS

The results of the present research provide a broad overview of different variables affecting immunocompetence of commercial broiler chickens including genetics, dietary protein and energy level, and the age and sex of the birds. Immune parameters analyzed included the innate immune response measures of the macrophage-monocyte phagocytic and chemotactic potential, tumoricidal activity of NK-cells, as well as the acquired immune response measured as antibody production following a SRBC injection. The cell-mediated response was measured as T-cell proliferation following injection with the mitogens PHA-P and Con-A.

Taken together, the two studies reported showed genetic differences in commercial broiler lines for various base-line immune function parameters. A dichotomy was observed in the both studies between the humoral and cell-mediated immune responses. In the first study, strain CC was the high responder for antibody response against SRBCs, while strain 308 showed a higher level of T-lymphocyte proliferation to the mitogens PHA-P and Con-A. Parameters quantifying innate immune response indicated that strain CC had higher macrophage phagocytic potential, and that strain 308 performed better with regard to its chemotactic response to f-met-leu-phe. Diet affects showed that high protein rations led to a higher T-cell proliferation in response to mitogens Con-A and PHA-P in Trial 1. Furthermore, an interaction between strains and diets was observed, indicating that the antibody response of strain 308 was higher on D1 while strain CC produced a higher antibody level on D2.

Although, it was not possible to address the correlation between immune response and growth performance in the first study, one of the aims of the second study was to investigate the impact that the long-term selection for high growth performance by broiler breeders has had on immune performance of modern broilers. In that study, it was evident that genetic selection for high BW has a negative affect on the adaptive arm of the immune response, i.e., antibody production in relation to the levels seen in the ACRBC. In contrast, it seems that the cell-mediated and inflammatory responsiveness aspects of the immune response was higher in the modern day growth selected broiler, i.e., in the 2001 ROSS, than it was in the 1957 ACRBC.

Both arms of the immune response, the humoral and cell-mediated, are interactive yet distinct in their effector functions. The dichotomous immune performance of the modern broiler line observed in this study suggests that it may be important for the breeders to adopt a breeding program that is geared towards the simultaneous improvement of both arms of the immune system. This could be done by taking into account the genetic correlation between body weight and the two arms of the immune system. In fact, this is supported by findings of Parmentier *et al.* (1995) who reported that chickens selected for enhanced humoral response against SRBCs also showed higher antibody titers to vaccination from *E. coli*, Newcastle Disease virus, infectious bronchitis virus, and infectious bursal disease virus. Furthermore, previous research indicates that better cell-mediated immune response would be desirable in eliminating virally-infected disease targets (Fredricksen and Gilmour, 1983; Schat and Xing, 2000). The macrophages play an extremely important role in microbicidal activities, tumor cell

killing and regulating immune responses through their release of biologically active cytokines and oxidative radicals (Qureshi *et al.,* 2000a; Dil and Qureshi, 2002a,b).

The present study implies that breeders should co-select their strains for performance as well as immune parameter(s) suited to the type of disease challenges encountered. Genetic improvement for better immunocompetence may not only be beneficial in reducing mortality, but also from the point of view of reducing risk to the consumer and the environment; i.e., it will reduce dependence on medication and vaccination and may reduce the excretion of pathogens. As pointed out earlier, immunocompetence is the general ability of the bird to launch immune response of sufficient specificity and magnitude and is broader in its effect than resistance against a particular antigen. While selection of birds against a particular antigen may ensure protection against that disease, it may not necessarily provide protection against multiple pathogens. Selection for broader immunocompetence probably be preferable. Furthermore, challenge testing may be undesirable from the perspective of biosecurity and animal welfare. Methods using indirect selection for better immunocompetence, that do not require disease exposure, may also be preferable in the development of such a breeding program.

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