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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, Ross x Cobb, Ross 308 and Cobb x Cobb (CC)] that were fed either a marginal protein diet (D1) or a high protein diet (D2) for the starter and finisher diets, respectively. Strain CC showed comparatively higher and more persistent antibody titers against sheep red blood cells (SRBC) ($P \leq 0.0182$) as well as higher macrophage phagocytic function for SRBC uptake ($P = 0.0118$) than the other strains. The Ross 308 strain had significantly greater cell mediated immune response, as measured by T-lymphocyte proliferation response to phytohemagglutinin-P (PHA-P), $P \leq 0.04$) and Concanavalin-A (Con-A) ($P \leq 0.0281$), as well as for the chemotaxis response to formyl-met-leu-phe ($P \leq 0.0019$) than the other strains. The diet effect was variable for monocyte-macrophage functions, but birds on the high protein diets showed higher cell-mediated response than the birds on the low protein diets when measured by Con-A and PHA-P responses. An interaction between strains and diets was seen for antibody response with the Ross 308 showing higher titers on D1 while the CC had greater antibody response when raised on D2. These results suggest that genetic differences exist between various commercial broiler chicken lines for cell mediated, humoral and innate immune responses. Furthermore, dietary protein levels appear to influence the immune response levels of broiler chickens but the response obtained varies by strain. The results of these studies imply that immunocompetence is genetically controlled, and, therefore some measures of immunocompetence could be considered as a selection criterion while selecting for performance traits.

Key words: Broilers, genetic changes, diet, immunocompetence

Introduction

Commercial poultry breeding has amongst its objectives, the improvement of production potential and disease resistance. Over the years there has been much emphasis on growth improvement that is negatively associated with some aspects of immunological performance of poultry as reported by Hans and Smyth (1972); Qureshi and Havenstein (1994); Rao *et al.* (1999); Yunis *et al.* (2000); Cheema *et al.* (2003). These and other studies (Qureshi and Miller, 1991) have 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) that relates to the effects of particular genes, gene loci or genetic markers with particular immune response parameters in commercial broiler lines. Nevertheless, commercial broiler producers are aware that breeder lines should probably be "co-selected" for both performance and immunocompetence parameters.

Nutrition has also been shown to affect immune response. For example, chickens that were raised on diets with only one third the amino acids contained in the basal diet had reduced numbers of lymphocytes in the thymus (Glick *et al.,* 1983). Chicken diets with one third the calories, amino acids or both compared with a basal diet sustained only a minimal humoral immune response to sheep red blood cells (SRBC) (Glick *et al.,* 1981). Takahashi *et al.* (1995) observed an acute phase response to *Escherichia coli* lipopolysaccharide (LPS) in chicks raised on low protein diets compared with 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, viscosity of fiber and fats that are refractory to digestion also affect microbial number and the capacity of the microorganisms to attack enterocytes (Klasing, 1998). The age of the bird has an impact on immune performance. Because chicks are hatched with immature immune systems, they may suffer from both viral diseases and bacterial diseases. The measurement of early immune competence can be complicated, however, by the presence of maternal antibodies (van der Zijpp, 1983).

With these considerations in mind, the objective of present study was to evaluate immune performance of four commercial broiler strains when raised on low and high protein dietary regimens. Age-related immune performance was quantified at 1 and 4 wk of age. The parameters measured included: body weight (BW), relative lymphoid organ weights, humoral response to

SRBC, cell mediated immune response to *in vitro* concanavalin-A (Con-A) and *in vivo* phytohaemagglutinin-P (PHA-P) stimulation, monocyte-macrophage-potential as measured by chemotaxis of blood monocytes to formyl-met-leu-phe (FMLP), *in vivo* elicitation of macrophages in response to Sephadex® G-50, phagocytic potential of macrophages for SRBC and nitrite production by macrophages in response to LPS stimulation.

Materials and Methods

Chickens and Diets: Three commercial broiler lines and two dietary regimens (*i.e.*, marginal and high protein diets) were utilized in this study. The experiment was conducted in two trials, with two separate hatches. Strains utilized in Trial 1 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 along with the Ross 308 and the CC. Fertile eggs were obtained from Aviagen™ (Huntsville, Alabama 35805) and hatched in the NC State University Poultry Education Unit, Raleigh, NC. Hatchability was greater than 95% for both hatches. The chicks were feather and vent-sexed at day of hatch and the males were wing-banded for each hatch. 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 in Trial 1; Ross x Cobb, Ross 308 and Cobb x Cobb in Trial 2) and two dietary regimens (D1 – marginal protein regimen, D2 - high protein regimen). Chicks were housed in wire-cage Alternative Design™ batteries (Alternative Design Manufacturing and Supply Inc., Siloam Springs, AR 72761) with 64 cages, each containing 10 birds. The diets (both starter and grower rations) were acquired from commercial sources on need basis. 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. In both trials, the starter was provided for *ad libitum* consumption from d 1-14, while the finisher was provided from d 15 until the end of the experiment at 32 d of age. The room temperature was maintained at 33.33-35 °C for the first wk and then reduced to 27.7-29.44 °C for the remaining period of the trial. Water was supplied *ad libitum* throughout both trials.

Experimental Endpoints

Body Weights and Relative Lymphoid Organ Weights: 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 at 2 wk of age in Trial 1 and at 3 wk of age in Trial 2. Each chick and its organs were weighed and the organ weights were expressed as a percentage of the bird's BW.

Antibody Response: Sheep red blood cells were used as a T-dependent antigen to quantify the antibody response. In both trials, 10 birds per group were injected intravenously with SRBC (3% suspension in PBS; 1 mL per chick) at 7 d of age followed by a booster injection given at 14 d after the first injection (21 d of age). Blood samples were collected at 4, 7, 10, 14 d post first injection and again at 5 and 10 d post boost. Serum from each sample was collected, heat inactivated at 56 °C 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 PHA-P (Sigma-Aldrich, St. Louis, MO 63178) stimulation was assessed as described previously by Corrier (1990). The T-cell mitogen, PHA-P was injected (100 µg/100 µL/bird) intradermally into the toe web of the left foot of 10 birds/group at 2 and 4 wk of age. The swelling response was measured at 24 and 48 h post injection, by subtracting the pre-injection measurement from the post-injection measurement of the toe web thickness.

Lymphoproliferative Response to Con-A: The blastogenic response of chicken leukocytes to Con-A (Sigma-Aldrich, St. Louis, MO 63178) was measured by using peripheral blood lymphocytes as described by Qureshi *et al.* (2000b). In Trial 1 and 2, 10 birds per group were bled at 1 and 4 wk of age. Proliferation of peripheral blood lymphocytes in response to Con-A was measured by the MTT (3-[5,5-dimethylthiazol-2-yl]-2,5-[diphenyltetrazolium]; Sigma-Aldrich, St. Louis, MO 63178) assay and the optical density (OD) of the solution was measured at 540 nm by using an enzyme linked immunosorbent assay plate reader (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 used to collect Sephadex-elicited abdominal exudate macrophages at 9 and 29 d of age. The endpoints quantitated were the number of inflammatory abdominal exudate cells (AEC), percentage phagocytic macrophages as well as the number of SRBC internalized 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); Dil and Qureshi (2002a,b). The standard

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

Strain[2]	Diet[3]	Trial 1 Body weight (g) 2 wk	Strain	Trial 2 Body weight (g) 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		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

[1]Data represents mean body weights of 8 birds/strain/diet that were taken at 2 and 3 wk of age in Trial 1 and 2, respectively. [a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$). [2]3F8 = Ross 3F8 (vent sexable). RC = Ross x Cobb (vent sexable), 308 = Ross 308 (feather sexable), CC = Cobb x Cobb (vent sexable). [3]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.

Table 2: Primary and secondary lymphoid organ weights[1] of commercial broiler chicken strains when raised on marginal and high protein diets

		Trial 1				Trial 2		
Strain[2]	Diet[3]	Bursa (%) 2 wk	Spleen (%) 2 wk	Thymus (%) 2 wk	Strain[1] 3 wk	Bursa (%) 3wk	Spleen(%) 3 wk	Thymus (%) 3wk
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		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

[1]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 ($P \leq 0.05$). [2, 3]For strain and diet designations, see footnote of Table 1.

curve for the nitrite assay was generated using various dilutions of 10mM stock solution of sodium nitrite in RPMI 1640 growth medium (Sigma-Aldrich, St. Louis, MO 63178). The nitrite levels in culture supernatants were calculated by comparing the OD readings against the nitrite standard curve.

Chemotactic response: At 1 and 4 wk of age in each trial, the chemotactic potential of leukocytes was assessed using the Boyden blind well chamber as previously described (Qureshi *et al.*, 1988; Lohr and Snyderman, 1981). Blood samples were drawn from ten birds per group. The chemotactic response was

expressed as the mean of mononuclear cells per 100 x oil field of a research microscope from triplicate polycarbonate membrane filters (Fisher Scientific, Orangeburg, NY 10962).

Statistical Analysis: Data were analyzed using the General Linear Models® procedure of SAS (SAS Institute, 1996). Strain, diet and two-way interactions were included in all analyses. 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 weights of the chicks in the two trials are given in Table 1. Body weights were taken at 2 wk of age in Trial 1 and 3 wk of age in Trial 2. In Trial 1, all three strains, i.e., 3F8, 308 and CC had comparable BW, no significant strain effect or diet effects were observed. Also, no interaction between strains and diets was found (Table 1). However in Trial 2, 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 diet effect nor any interaction was observed in Trial 2. These observations suggest that there is an age-dependent difference in growth performance between the lines used in this study.

The weights of the lymphoid organs, i.e., bursa of Fabricius, spleen and thymus, as a percentage of BW are provided in Table 2. The bursa, spleen and thymus 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 thymus weight in Trial 1 and 3F8 birds had significantly higher thymus weight as a percentage of total BW when they were raised on D1 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 we compared various strains or diets.

Antibody Response: The total antibody responses of the various strains against SRBC for Trial 1 and 2 are summarized in Table 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 d post primary injection ($P = 0.03$). At 7 d, CC had greater titers than the 3F8 ($P = 0.04$), while 308 was not significantly different from the other two strains. At 14 d post primary injection, CC birds displayed significantly higher antibody response than the 308 and 3F8 birds ($P = 0.03$). Both the 308 and CC strains showed significantly higher titers than 3F8 at 5 d post boost ($P = 0.001$), at 10 d post boost CC showed persistence in titers (4.29 ± 0.35) and had significantly higher titer than strain 308 ($P = 0.018$), while strain 3F8 was not significantly different from either of the other two strains. An interaction was seen between diets and strains at 5 d post boost and 308 birds had higher antibody titer when raised on D1 than when raised on D2 ($P = 0.0217$).

In Trial 2 (Table 4), no significant differences in antibody response were observed at 4, 7, 10, or 14 d of primary response for the different strains or diets. Furthermore, there was no significant interactions between diets or strains. On 5 d post boost, no significant difference was seen in antibody response of the different strains or diets. However, an interaction between diets and strains was seen with CC broilers showing significantly greater humoral response on D2 than on D1 ($P = 0.033$). On 10 d post boost, CC and RC birds 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 significantly higher antibody titers on D1 than on D2 ($P = 0.0142$).

Taken together, data from both trials indicate that the CC strain of broilers are high responders for anti-SRBC antibody response. However, strain 308 was also a high antibody producer (Table 3, Trial 1), but its antibody level did not persist as long as for strain CC in either Trial 1 or Trial 2. This study was not designed to relate antibody response with growth. Earlier studies designed to look at that relationship suggest a negative correlation between selection for increased growth rate and humoral response against SRBC (Qureshi and Havenstein, 1994; Rao *et al.,* 1999; Cheema *et al.,* 2003). Recently, Yonash *et al.* (2001) identified three DNA markers (ADL0146, ADL0290 and ADL0298) associated with antibody responses against SRBC, *Escherichia coli* and Newcastle Disease virus. 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 shown a clear 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 a low BW while the B^{13} haplotype had lower antibody titers and higher BW. 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 on 5 d post boost in

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

Strain[2]	Diet[3]	D PPI[4]				D PSI[5]	
		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
Pooled SEM		0.35	0.43	0.42	0.41	0.44	0.47
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	0.35651

[1]A 3% suspension of SRBC was injected @ 1 mL/bird (10 birds per strain per diet). Serum samples were collected at d 4, 7,10 and 14 post first injection and then at d 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 ($P \leq 0.05$). [2,3]For strain and diet designations, see footnote of Table 1. [4,5]D PPI = days post primary injection, D PSI = days post secondary injection.

Table 4: Antibody response[1] against sheep red blood cells of different strains of chickens when raised on marginal and high protein diets (Trial 2)

Strain[2]	Diet[3]	D PPI[4]				D PSI[5]	
		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}
Pooled SEM		0.32	0.36	0.23	0.22	0.67	0.36
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

[1]A 3% suspension of SRBC was injected @ 1 mL/bird (10 birds per strain per diet). Serum samples were collected at d 4, 7,10 and 14 post first injection and then at d 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 ($P \leq 0.05$). [2, 3] For strain and diet designations, see footnote of Table 1. [4,5]D PPI = days post primary injection, D PSI = days post secondary injection.

both trials (Table 3, P = 0.0217; Table 4, P = 0.0338). On the contrary, the strain 308 birds, which showed reduced antibody persistence, had higher antibody levels on the low protein diet (D1) than on the high protein diet (D2), at 5 d post boost in Trial 1 and 10 d post boost in Trial 2. Therefore, a case can be made that the CC birds respond higher on the D2 whereas strain 308 may show higher response on D1 for anti-SRBC antibody production. Other studies (Rao *et al.,* 1999), however, have shown that variation in dietary CP does 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 Trial 1 and 2, are provided in Table 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 h post PHA-P injection when raised on D2 compared to when they were raised on D1 (P = 0.03). Birds on D2 showed significantly greater swelling than when they were raised on D1 at 48 hours post PHA-P injection at 2 wk of age (P = 0.01). These findings were not replicated at 4 wk of age. In Trial 2 (Table 6), strain 308 had significantly higher swelling response than CC at 24, 48 and 72 h post PHA-P injection at 2 wk of age ($P \leq 0.04$). At 4 wk of age no significant differences were seen between the strains. 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. Therefore, this reaction is sometimes termed as cutaneous basophilic hypersensitivity 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 (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 the broilers, White Leghorn type chickens and turkey strains (Corrier 1990; Bayyari *et al.,* 1997; Rao *et al.,* 1999; Tsiagbe *et al.,* 1987). 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 Con-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 Trial 1 and 2 (Table 7). In Trial 1, Con-A mediated lymphoproliferation was poor due to unexplained reasons (data not shown). However, at 4 wk of age, peripheral blood leukocytes 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 two strains. Birds on D2 exhibited a significantly greater lymphoproliferative response than birds on D1 (P = 0.0081). The interaction between diets and strains was not significant. At 4 wk of age, the CC birds were significantly higher responders than the RC strain birds, while strain 308 birds were intermediate. There were no significant differences in performance between diets, nor any significant interactions between strains and diets. Taken together, the data from the two trials indicate that 308 birds consistently have higher response for Con-A mediated lymphoproliferation than birds from the other strains.

Mononuclear Phagocytic System Function Assessment

Abdominal Exudate Cell Recruitment: Chickens lack resident cell populations belonging to the mononuclear phagocytic system (i.e., monocyte, 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

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

Strain[2]	Diet[3]	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		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

[1]PHA-P was injected @ 100μg/bird in the toe web of the right foot of 10 birds/strain/diet at 2 and 4 wk of age. Swelling was measured by a constant tension micrometer at 24 and 48 h 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-c]Means within a column with no common superscript differ significantly ($P \leq 0.05$). [2,3]For strain and diet designations, see footnote of Table 1.

Table 6: Lymphoblastogenic response against PHA-P[1] by different strains of chickens when raised on marginal and high protein diets (Trial 2)

Strain[2]	Diet[3]	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		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

[1]PHA-P was injected @ 100μg/bird in the toe web of the right foot of 10 birds/strain/diet at 2 and 4 wk of age. Swelling was measured by a constant tension micrometer at 24 and 48 h 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-c]Means within a column with no common superscript differ significantly ($P \leq 0.05$). [2,3]For strain and diet designations, see footnote of Table 1.

inflammatory stimulus results in a phenomenon called *in vivo* chemotaxis. Sephadex-elicited AEC were quantified as one measure of mononuclear phagocytic system function in these trials and the data were summarized in Table 8 and 9. In Trial 1, AEC numbers were significantly higher at 1 wk 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

Table 7: Lymphoblastogenic response of leukocytes against Concanavalin A[1] from different genetic strains of chickens when raised on marginal and high protein diets

Strain[2]	Diet[3]	Trial 1	Strain	Trial 2	
		Stimulation index		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		0.07		0.06	0.05
Strain Averages					
3F8	x	0.19[b]	RC	0.11[b]	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

[1]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 h 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 ($P \leq 0.05$). [2,3]For strain and diet designations, see footnote of Table 1.

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

Strain[2]	Diet[3]	1 wk			4 wk		
		# 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
Pooled SEM		1.82	2.41	0.09	4.04	2.89	0.06
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

[1]A 3% Sephadex G-50 suspension was injected @ 1mL/100g BW into the abdominal cavities of 7 birds/strain/diet at 1 wk of age. After 40 h birds were harvested to get abdominal exudate cells and the cell count was adjusted to $1x10^6$/mL. For phagocytic activity, macrophages were fed 1% SRBC, incubated for 1 h, fixed, stained and scored for percentage phagocytic macrophages as well as average number of SRBC/phagocytic macrophages. [a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$). [2,3]For strain and diet designations, see footnote of Table 1.

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

		1 wk			4 wk		
Strain[2]	Diet[3]	# 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
Pooled SEM		2.89	3.45	0.09	6.86	2.52	0.06
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

[1]A 3% Sephadex G-50 suspension was injected @ 1mL/100g BW into the abdominal cavities of 7 birds/strain/diet at 1 wk of age. After 40 h birds were harvested to get abdominal exudate cells, the cell count was adjusted to 1x10^6/mL. For phagocytic activity macrophages were fed 1% SRBC, incubated for 1 h, fixed, stained and scored for percentage phagocytic macrophages as well as average number of SRBC/phagocytic macrophages. [a-c]Means within a column with no common superscript differ significantly ($P \leq 0.05$). [2, 3]For strain and diet designations, see footnote of Table 1.

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

		Trial 1			Trial 2	
Strain[2]	Diet[3]	Nitrite (µM)	Nitrite (µM)	Strain	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
Pooled SEM		2.36	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

[1]Macrophage cultures (from 10 birds/strain/diet) were exposed to lipopolysaccharides from *E. coli* (1µg/1x10^6 macrophages) for 24 h, at 1 and 4 wk of age. The culture supernatant was tested for nitrite levels by treating it with Griess reagent. [a,b]Mean within a column with no common superscript differ significantly ($P \leq 0.05$). [2,3] For strain and

diet designations, see footnote of Table 1.
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 birds suggesting an age related response to *in vivo* AEC elicitation (P = 0.0453). The marginal protein diet, D1, again produced higher AEC numbers at 4 wk with P = 0.0131. No interaction between diets and strains 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 wk of age in Trial 1, macrophages from 3F8 and CC strains had significantly higher percentage of phagocytic macrophages than the macrophages from strain 308 (P = 0.0009). An interaction between diets and strains was observed with strain 3F8 exhibiting significantly higher phagocytic response on D2 than on D1 (P = 0.0112). The average number of SRBC per phagocytic macrophage was significantly higher at 1 wk of age in macrophages from strain 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 4 wk 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 better 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 macrophage at 4 wk 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 with the trend directed toward higher phagocytic response at the younger age when birds were raised on D2.
Macrophage phagocytic 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 phagocytic 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 activity of macrophages in response to LPS stimulation was quantified by measuring nitrite levels in Trial 1 and 2 (Table 10). In both trials, the nitrite values ranged from 2.35 to 9.9 µM, barring 19.42 µM during Trial 1, wk 1 in 308, D1 group. 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 Toll-like receptor-4 (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 Toll-like receptor-4 (Dil and Qureshi, 2002a,b).

Chemotactic Response: The chemotactic response of blood mononuclear cells to FMLP as assessed in Trial 1 and 2 is reported in Table 11. The chemotactic assay which was done at 1 wk of age in Trial 1 showed no significant difference in chemotactic activity of the monocytes from the different strain/diet groups. There was also no significant interaction between diet and strain. At 4 wk 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 chemotaxis 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/diet groups (P = 0.0548).
During Trial 2, chemotactic activity of strain 308 was significantly higher than for strains 3F8 and CC at 1 wk of age, while strain RC was better than strain 308 (P = 0.0001). The chemotactic response was also higher 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 wk of age compared to the RC and CC strains (P = 0.001). No significant difference in the chemotactic activity between diets, nor any interaction between diet and strain was observed. Overall in both trials, strain 308 performed significantly better than the other strains. The birds raised on the high protein diet, D2, also had 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); Puzzi *et al.* (1990) when comparing $15I_5$ *B*-congenic White Leghorn chickens. Both studies reported B^5 and B^{21} as being

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

Strain[2]	Diet[3]	Trial 1		Trial 2		
		Chemotaxis 1 wk (#/100 x microscopic field)	Chemotaxis 4 wk (#/100 x microscopic field)	Strain	Chemotaxis 1 wk (#/100 x microscopic field)	Chemotaxis 4 wk (#/100 x icroscopic field) m
3F8	D1	20.23	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
Pooled SEM		4.74	2.24		2.18	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

[1]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 wk 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 ($P \leq 0.05$). [2, 3]For strain and diet designations, see footnote of Table 1.

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) effects on chemotactic response comes from a B-complex gene dosage model where monocytes from tetrasomic ($B^{15}B^{15}B^{15}B^{15}$) chicks showed enhanced chemotaxis as compared to disomics and trisomics when treated against FMLP and *Enterobacter cloacae* culture supernatant (Qureshi *et al.*, 1989).

Taken together, the study reported herein suggests that differences exist among 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 these birds were not grown to their maximum genetic growth potential. Nevertheless, from the four strains that were tested against a broad immune response panel, two strains, namely 308 and CC performed significantly higher in their immune response than the strains 3F8 and RC. Amongst the two higher 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 immune response from its improved antigen uptake, processing and presentation. On the contrary, strain 308 had better 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 diets showed effective T-cell proliferation in response to mitogens Con-A and PHA-P. An 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).

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References

Bayyari, G. R., W. E. Huff, N. C. Rath, J. M. Balog, L. A. Newberry, J. D. Villines, J. K. Skeeles, N. B. Anthony and K. E. Nestor, 1997. Effect of genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poult. Sci., 76: 289-296.

Cheema, M. A., M. A. Qureshi and G. B. Havenstein, 2003. A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. Poult. Sci. (in press).

Corrier, D. E., 1990. Comparison of phytohemagglutinin-induced cutaneous hypersensitivity reactions in the interdigital skin of broiler and layer chicks. Avian Dis., 34: 369-373.

Dil, N. and M. A. Qureshi, 2002a. Differential expression of inducible nitric oxide synthase is associated with differential Toll-like receptor-4 expression in chicken macrophages from different genetic backgrounds. Vet. Immunol. Immunopathol., 84: 191-207.

Dil, N. and M. A. Qureshi, 2002b. Involvement of lipopolysaccharide related receptor and nuclear factor kappa B in differential expression of inducible nitric oxide synthase in chicken macrophages from different genetic backgrounds. Vet. Immunol. Immunopathol., 88: 149-161.

Dunnington, E. A., W. E. Briles, R. W. Briles and P. B. Siegel, 1996. Immunoresponsiveness in chickens: association of antibody production and the B system of the major histocompatibility complex. Poult. Sci., 75: 1156-60.

Glick, B., E. J. Day and D. Thompson, 1981. Calorie-protein deficiencies and immune response of the chicken. I. Humoral immunity. Poult. Sci., 60: 2494-2500.

Glick, B., K. Sato and F. Cohenour, 1964. Comparison of the phagocytic ability of normal and bursetomized birds. J. Reticuloendothel. Soc., 1: 442-449.

Glick, B., R. L. Taylor Jr., D. E. Martin, M. Watabe, E. J. Day and D. Thompson, 1983. Calorie-protein deficiencies and immune response of the chicken. II. Cell mediated immunity. Poult. Sci., 62: 1889-1893.

Green, L. C., D. A. Wagner, J. Glogowski, P. L. Skipper, J. S. Wishnok and S. R. Tannebaum, 1982. Analysis of nitrate, nitrite and [15N] nitrite in biological fluids. Anal. Biochem.,126: 131-138.

Han, P. F.-S. and J. R. Smyth, Jr., 1972. The influence of growth rate on the development of Marek's disease in chickens. Poult. Sci., 51: 975-985.

Klasing, K. C., 1998. Nutritional modulation of resistance to infectious diseases. Poult. Sci., 77: 1119-25.

Lakshman, N., J. S. Gavora and S. J. Lamont, 1997. Major histocompatibility complex class II DNA polymorphisms in chicken strains selected for Marek's disease resistance and egg production or for egg production alone. Poult. Sci., 76: 1517-1523.

Lamont, S. J., C. Bolin and N. Cheville, 1987. Genetic resistance to fowl cholera is linked to the major histocompatibility complex. Immunogenetics, 25: 284-289.

Li, Z., K. E. Nestor, Y. M. Saif, W. L. Bacon and J. W. Anderson, 1999. Effect of selection for increased body weight on mitogenic responses in turkeys. Poult. Sci., 78: 1532-1555.

Lohr, K. M. and R. Snyderman, 1981. In vitro methods for the study of macrophage chemotaxis. In: H. B. Herscowitz, H.T. Holden, J.A. Bellant abd A. Ghaffar (Ed.), Manual of Macrophage, Collection, Characterization and Function. Marcel Dekker, New York, N.Y.

Miggiano, V., M. North, A. Buder and J. R. L. Pink, 1976. Genetic control of the response of chicken leukocytes to a T-cell mitogen. Nature, 263: 61-63.

Morrow, P. R. and H. Abplanalp, 1981. Genetic control of T-lymphocyte mitogenesis in chickens. Immunogenetics, 13: 189-200.

Puzzi, J. V., L. D. Bacon and R. D. Dietert, 1990. B-congenic chickens differ in macrophage inflammatory responses. Vet. Immunol. Immunolpathol., 26: 13-30.

Qureshi, M. A., S. E. Bloom and R. R. Dietert, 1989. Effects of increased major histocompatibility complex dosage on chicken monocyte-macrophage function. Proc. Soc. Exp. Biol. Med., 190: 195-202.

Qureshi, M. A., R. R. Dietert and L. D. Bacon, 1986. Genetic variation in the recruitment and activation of chicken peritoneal macrophages. Proc. Soc. Exp. Biol. Med., 181: 560-568.

Qureshi, M. A., R. R. Dietert and L. D. Bacon, 1988. Chemotactic activity of chicken blood mononuclear leukocytes fron $15I_5$-B-Congenic lines to bacterially-derived chemoattractants. Vet. Immunol. Immunolpathol., 19: 351-360.

Qureshi, M. A., F. W. Edens and G. B. Havenstein, 1997. Immune system dysfunction during exposure to poult enteritis and mortality syndrome agents. Poult. Sci., 76: 564-569.

Qureshi, M. A., C. L. Heggen and I. Hussain, 2000a. Avian macrophage: effector functions in health and disease. Dev. Comp. Immunol., 24: 103-119.

Qureshi, M. A. and G. B. Havenstein, 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed 'typical' 1957 and 1991 boiler diets. Poult. Sci., 73: 1805-1812.

Qureshi, M. A. and L. Miller, 1991. Comparison of macrophage function in several commercial broiler genetic lines. Poult. Sci., 70: 2094-2101.

Qureshi, M. A., M. Yu and Y. M. Saif, 2000b. A novel "small round virus" inducing poult enteritis and mortality syndrome and associated immune alterations. Avian Dis., 44: 275-283.

Rao, S. V., N. K. Praharaj, M. R. Reddy and B. Sridevi, 1999. Immune competence, resistance to *Eschirichia coli* and growth in male broiler parent chicks fed different levels of crude protein. Vet. Res. Commun., 23: 323-326.

Ruff, M. D. and L. D. Bacon, 1984. Coccidiosis in 15.5-congenic chicks. Poult. Sci., 70: 530-538.

Sabet, T., W. C. Hsia, M. Stnisz. A. El-Domeri and P. VanAlten, 1977. A simple method for obtaining peritoneal macrophages from chickens. J. Immunol. Methods, 14: 103-110.

SAS Institute, 1996. SAS® User's Guide: Statistics Version 6 Edition. SAS Institute, Inc. Cary, NC.

Stadecker, M. J., M. Lukic, A. Dvorak and S. Leskowitz, 1977. The cutaneous basophil response to phytohemagglutinin in chickens. J. Immunol., 118: 1564-1568.

Takahashi, K., S. Yodogawa, Y. Akiba and K. Tamura, 1995. Effect of dietary protein concentration on responses to *Escherichia coli* endotoxin in broiler chickens. Br. J. Nutr., 74: 173-182.

Tizard, I. R., 1995. Immunology: An Introduction. Saunders College Publishing. Philadelphia, New York, London.

Tsiagbe, V. K., M. E. Cook, A. E. Harper and M. L. Sunde, 1987. Enhanced immune responses in broiler chicks fed methionine-supplemented diets. Poult. Sci., 66: 1147-1154.

van der Zijpp, A. J., 1983. Breeding for immune responsiveness and disease resistance. World's Poult. Sci. J., 39: 118-131.

Yamamoto, Y. and B. Glick, 1982. A comparison of the immune response between two lines of chickens selected for differences in the weight of the bursa of Fabricius. Poult. Sci., 61: 2129-2132.

Yonash, N., H. H. Cheng, J. Hillel and A. Cahaner, 2001. DNA micro satellites linked to quantitative trait loci affecting antibody response and survival rate in meat-type chickens. Poult. Sci., 80: 22-28.

Yunis, R., A. Ben-David, E. D. Heller and A. Cahaner, 2000. Immunocompetence and viability under commercial conditions of broiler group differing in growth rate and in antibody response to *Escherichia coli* vaccine. Poult. Sci., 79: 810-816.