

A Comparison of the Immune Response of a 2001 Commercial Broiler with a 1957 Randombred Broiler Strain When Fed Representative 1957 and 2001 Broiler Diets¹

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ABSTRACT Immunocompetence of the 2001 Ross 308 broiler strain and the 1957 Athens Canadian Randombred Control (ACRBC) strain was compared when they were given diets representative of those that were being used in 1957 and 2001. Antibody response against SRBC, *in vivo* lymphoproliferation against Phytohemagglutinin-P (PHA-P), and inflammatory and phagocytic responses of the macrophages were measured. The Ross 308 strain on the 2001 diet had higher BW at 24 d of age ($P = 0.0001$), whereas the ACRBC had greater lymphoid organ weights (except thymus) relative to BW ($P \leq 0.003$). The ACRBC strain showed greater antibody responses against SRBC than the 2001 Ross 308 birds for much of the trial ($P \leq 0.0362$). However, the Ross 308 broilers had greater PHA-P-induced toe-web swelling response ($P \leq 0.0129$). In-

flammatory exudate cell numbers were higher in the Ross 308 broilers than in the ACRBC birds ($P = 0.0261$). The percentage of macrophages that phagocytized SRBC was comparable between the two strains, but the number of SRBC phagocytized by individual macrophages was higher ($P = 0.0122$) in the Ross 308 broiler than in the ACRBC chickens. Nitrite production by macrophages following lipopolysaccharide stimulation was comparable between the two strains. Interactions of diet, strain, and sex were inconsistent among all parameters tested. In conclusion, the current study suggested that genetic selection for improved broiler performance has resulted in a decrease in the adaptive arm of the immune response but an increase in the cell-mediated and inflammatory responses.

(*Key words:* broiler, diet, genetic change, immunocompetence, sex)

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INTRODUCTION

Over the past 40 to 50 yr, the poultry industry has witnessed a dramatic increase in the production potential of commercial poultry. This increase has been facilitated through application of population genetic theory and emerging biological techniques. 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). Selection for high production potential in poultry, however, 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 genetic homeostasis, leading to deficient resources for the well-being of the individual (Lerner, 1954; Beilharz, 1998).

Although genetic differences have been shown to influence the disease outcome in chickens (Bumstead et al., 1989; Afraz et al., 1994; Lakshmanan et al., 1997), a negative consequence of high growth rate in the form of increased susceptibility to disease, such as for Marek's disease, has 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 slower-growing groups of birds (Yunis et al., 2000). Several studies have shown specific dietary effects on immune response. 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, 1983). Takahashi et al. (1995) observed greater acute-phase immune response, as measured by

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Abbreviation Key: ACRBC = Athens Canadian Randombred Control; AEC = abdominal exudate cells; LPS = lipopolysaccharide; MES = mercaptoethanol-sensitive; PHA-P = phytohemagglutinin-P; PPI = postprimary injection; PSI = postsecondary injection.

plasma α 1-acid glycoprotein and interleukin-1-like activity, in response to *Escherichia coli* lipopolysaccharide (LPS) in chicks raised on a low-protein diet in comparison with those fed a high-protein diet. Among the micronutrients, vitamins E and A have been found to modulate cellular immune response against infections (Latshaw 1991; Sword et al., 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 from 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 with the Athens Canadian Randombred Control (ACRBC) strain for immune performance. 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 (for history see Hess, 1962; Gowe and Fairfull, 1990). In addition to the genetic strains, two sexes and two diets representative of those fed during calendar years 2001 and 1957 were also used to determine their impact on the immune response. The present study was carried out to see if there is evidence of any change in these relationships in broilers since 1991 when a similar study was conducted (Havenstein et al., 1994; Qureshi and Havenstein, 1994). The study by Qureshi and Havenstein (1994) showed that while the 1991 commercial broiler strain gained 3.9 times more weight than the 1957 ACRBC at 56 d of age, it also exhibited a lower humoral immune response. The current study examines growth rate and immune function parameters in a 2001 broiler strain as they are affected by strain, diet, and sex. Parameters examined included: BW and relative lymphoid organ weights, humoral immune response to SRBC, in vivo elicitation of macrophages, quantification of phagocytic function and nitrite production, and cell-mediated immune responses to 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 and from the 1957 ACRBC strain when they were raised on diets that were representative 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 Sam Aggrey,³ and eggs for the Ross 308 feather sexable strain were kindly provided by Allen's Hatchery.⁴ All eggs were

incubated and hatched at the North Carolina State University Poultry Educational Unit, Raleigh, NC. On d 1, the chicks were feather and vent sexed, and both males and females were neck tagged. The study was carried out in two trials, with trial 1 lasting for 26 d and trial 2 lasting for 24 d. Treatments were arranged in a $2 \times 2 \times 2$ factorial arrangement, i.e., two strains (2001 Ross 308 and 1957 ACRBC), two sexes, and two dietary regimens [the same facility used for the Qureshi and Havenstein (1994) study]. The groups were randomly assigned into four blocks of eight-litter floor pens/block (32 pens total with 21 birds per pen). The second trial utilized the same factorial arrangement. The treatments were assigned into four rooms of 16-litter floor pens/room (64 pens in total with two pens per group in one room). The 1957 starter diet contained 2,895 kcal ME/kg and 21.3% CP and was fed as mash for entire period in trials 1 and 2, because the grower phase for the 1957 diet in trial 1 started at 43 d of age and was beyond the span of present immune function part of the study. The 2001 starter diet contained 3,205 kcal ME/kg and 23% CP and was fed from hatch to 21 d, and the grower phase of the 2001 diets was fed from 22 to 24 d and it contained 3,150 kcal ME/kg and 20.5% CP. Other management and dietary details are provided in Havenstein et al., 2003.

Room temperature was maintained at 33.33 to 35°C for the first week; temperature was then reduced to 27.77 to 29.44°C for the remaining period of the trial. Feed and water were supplied ad libitum.

Experimental Endpoints

BW 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 d of age from eight birds per group in trial 2. These organs and the corresponding chicks were weighed, and the organ weights were expressed as a percentage of BW.

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

The serum from each sample was collected, heat inactivated at 56°C for 30 min and then analyzed for total, mercaptoethanol⁶-sensitive (MES) IgM and mercaptoethanol-resistant IgG anti-SRBC antibodies as previously described (Delhanty and Solomon, 1966; Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994). Briefly, 50 μ L of serum was added in an equal amount of PBS in the first column of a 96-well v-shaped bottom plate,⁷ and the solution was incubated for 30 min at 37°C. A serial

³University of Georgia, Athens, GA.

⁴Liberty, NC.

⁵Fisher Scientific, Pittsburgh, PA.

⁶Sigma Chemical Co., St. Louis, MO.

⁷Corning, Corning, NY.

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 min of incubation at 37°C. The well immediately preceding a well with a distinct SRBC button was considered as the endpoint titer for agglutination. For MES (IgM) response, 50 μL of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, 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 PHA-P,⁶ 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 intradermally (100 $\mu\text{g}/100 \mu\text{L}$ per bird) into the toe web of the left foot and an equal volume of PBS was injected into the toe web of the right foot of 8 birds/group at 19 d of age. The thickness of the two toe webs was then measured with a micrometer after 24 and 48 h. The swelling response was measured by subtracting the preinjection measurement from the postinjection measurement of the PHA-P-injected toe web. Because no change in toe web thickness occurred due to PBS injection, these data did not need to be reported or used in further statistical comparisons.

Macrophage Function Assessment. Macrophage functions were assessed in trial 2 only using methods previously described by Qureshi and Miller (1991). A total of 80 birds (10 from each treatment group) were injected with a 3% suspension of Sephadex G-50⁶ at a concentration of 1 mL/100 g of BW at 2 wk of age. Approximately 42 h 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 $285 \times g$ for 10 min to obtain an 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 antibiotics⁸ (100 U/mL penicillin⁸ and 50 $\mu\text{g}/\text{mL}$ streptomycin⁸). Total nonerythroid AEC were then counted on a hemocytometer.

In order to quantify the phagocytic potential, a 1% SRBC suspension in RPMI⁷ growth medium was used as a particulate antigen. The AEC from 10 birds were then pooled to generate five samples (2 birds/sample). Macrophage monolayers were established by adding 1×10^6 AEC/mL from each sample into Petri dishes⁷ containing four coverslips.⁹ After 1 h 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 that was added to each Petri dish. After 60 min incubation at 41°C in a

humidified atmosphere and 5% CO₂, the coverslips were washed to remove non-internalized SRBC. They were then fixed and stained with Leukostat¹⁰ 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. The production of nitrite (a stable end product of NO) by macrophages in response to LPS⁶ stimulation was assessed as previously described by Green et al. (1982). Macrophages from 64 birds (8 from each treatment group) were assessed for nitrite production at 2 wk of age. Samples from two birds were pooled and cultured in 24-well plates (1×10^6 cells per well per pooled sample) and exposed to LPS from *E. coli* (1 $\mu\text{g}/\text{well}$) for 24 h. 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 readings against the nitrite standard curve.

Statistical Analysis

Data were analyzed using the general linear models procedure of SAS software (SAS Institute, 1996). Strain, diet, sex, and the two-way and three-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

BW and Relative Lymphoid Organ Weights

Body and lymphoid organ weights were recorded only in trial 2 and are provided in Table 1. The Ross 308 broilers (both males and females) raised on the 2001 and 1957 diets had higher BW than the ACRBC raised on the same diets at 24 d of age ($P = 0.0001$). Furthermore, the birds on the 2001 diet were heavier than those on the 1957 diet ($P = 0.0001$). However, a significant interaction between strains and diets was observed with the Ross 308 chickens showing greater relative growth on the 2001 or 1957 diet ($P = 0.0007$) than ACRBC raised on the 1957 or 2001 diet. Similar differences in BW are reported in a companion study by Havenstein et al. (2003) that showed that the BW of the Ross 308 broilers on the 2001 diets were 4.22, 4.96, 4.88, 4.30, and 3.86 times higher than those of the ACRBC on the 1957 diet at 21, 42, 56, 70, and 84 d of age, respectively. Similarly, the Ross 308 broiler 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 308 broiler 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.

Although Ross 308 birds had greater BW, the ACRBC had greater ($P \leq 0.0003$) relative weights for bursa, spleen,

⁸Atlanta Biologicals, Norcross, GA.

⁹Thomas Scientific, Swedesboro, NJ.

¹⁰Fisher Scientific, Orangeburg, NY.

TABLE 1. Body and lymphoid organ weights¹ of a 2001 modern commercial broiler and the 1957 Athens-Canadian randombred strain when fed 1957 and 2001 diet (trial 2)

Strain ²	Diet ³	Sex	Body weight (g)	Thymus (%)	Bursa of Fabricius (%)	Spleen (%)	Cecal tonsils (%)
2001	2001	Male	767.37	0.22	0.26	0.13	0.024
2001	1957	Male	647.62	0.21	0.31	0.14	0.042
1957	2001	Male	222.25	0.22	0.40	0.15	0.048
1957	1957	Male	199.37	0.25	0.47	0.14	0.054
2001	2001	Female	747.37	0.30	0.27	0.11	0.033
2001	1957	Female	609.87	0.25	0.30	0.10	0.038
1957	2001	Female	206.25	0.47	0.50	0.26	0.053
1957	1957	Female	177.12	0.24	0.45	0.15	0.044
Pooled SEM			20.15	0.03	0.04	0.02	0.004
Strain averages							
2001	x	x	693.06 ^a	0.24	0.29 ^b	0.12 ^b	0.03 ^b
1957	x	x	201.25 ^b	0.30	0.46 ^a	0.18 ^a	0.04 ^a
Source of variation			Probability				
Strain			0.0001	0.0736	0.0001	0.0003	0.0001
Diet			0.0001	0.0237	0.4099	0.0825	0.1105
Sex			0.0993	0.0028	0.4792	0.3264	0.9912
Strain × diet			0.0007	0.2798	0.6103	0.0674	0.0421
Strain × sex			0.7349	0.2990	0.5235	0.0048	0.4268
Diet × sex			0.6762	0.0136	0.2807	0.0406	0.0269
Strain × diet × sex			0.8411	0.0611	0.4183	0.2239	0.8137

^{a,b}Means within a column and classification with no common superscript differ significantly ($P \leq 0.05$).

¹Mean body weights and relative lymphoid organ weights of eight birds per group were taken at 24 d of age.

²2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

³2001 = Broiler diet representative of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet representative of those being fed in 1957.

and cecal tonsils (Table 1). Whereas thymic weights between the two strains only approached significance ($P = 0.0736$), significant interactions were observed indicating that female birds provided the 2001 diet had significantly greater relative thymus weight than females given the 1957 diet, or for males provided either of the two diets ($P = 0.0136$). Females on the 2001 diet also had greater relative spleen weights than the females on 1957 diet and the males on the 2001 diet ($P = 0.0406$). The relative spleen weights of the females on the 2001 diet were not significantly different from relative spleen weights of male birds on 1957 diet. Female birds of ACRBC strain had greater relative spleen weights than females of the Ross 308 strain, and males of both strains ($P = 0.0048$). The ACRBC strain on the 2001 and 1957 diets showed greater relative cecal tonsil weights than the Ross 308 strain on both diets ($P = 0.0421$). The males on the 1957 diet had greater ($P = 0.0269$) cecal tonsil weights than males on the 2001 diet, but they were not significantly different from females on either diet.

In a previous study by Rao et al. (1999), a strain exhibiting low BW gain showed significantly improved relative bursal weight when dietary CP level was increased from 18% to 23%. In the current study, the cecal tonsils of ACRBC birds when grown on 2001 or 1957 diet showed significant increases in their relative weights when compared to Ross 308 broilers grown on the same diets. The data from the current study suggests that the modern day commercial broiler strain selected for enhanced broiler performance 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 increased 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, respectively. Total antibody titers were measured at 5 and 10 d postprimary 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 did not exhibit significantly different antibody response compared to Ross 308 strain at 5 d PPI. A significant diet-by-sex interaction for total antibodies was present at 5 d PPI in which females on the 1957 diet had higher titers than females on the 2001 diet or for the males provided either of the two diets ($P = 0.0517$). A strain × sex interaction was also present at 5 d PPI where the ACRBC males had higher antibody levels than the Ross 308 males ($P = 0.0004$). A significant diet-by-strain interaction for total antibodies was observed at 10 d PPI due to the ACRBC strain on the 1957 diet having higher antibody response levels than the other sex diet combinations ($P = 0.0165$). Furthermore, at 10 d PPI, the ACRBC strain had higher total anti-SRBC titers than the Ross 308 strain ($P = 0.0001$). At 4 d PPI in trial 2, a significant strain × sex effect was present in which Ross 308 females had

TABLE 2. Total anti-SRBC antibody titer of a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain when fed 2001 and 1957 diets¹

Strain ²	Diet ³	Sex	Trial 1				Trial 2				
			Days PPI ⁴		Days PSI ⁵		Days PPI			Days PSI	
			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	1957	Male	0.12	0.12	4.12	1.50	0.40	0.60	0.30 ^{ab}	4.40	3.70
1957	2001	Male	2.75	1.62	4.00	1.65	0.90	0.66	0.40 ^{ab}	5.40	3.70
1957	1957	Male	3.00	2.11	4.87	2.44	0.40	1.20	0.70 ^a	4.60	3.40
2001	2001	Female	2.75	0.75	4.00	1.62	1.10	0.50	0.10 ^a	4.60	3.00
2001	1957	Female	3.37	0.87	4.25	1.62	0.70	0.75	0.70 ^a	6.00	4.40
1957	2001	Female	0.75	1.12	4.00	2.25	0.40	0.40	0.60 ^{ab}	4.40	3.50
1957	1957	Female	3.00	3.14	5.00	2.85	0.22	0.60	0.44 ^{ab}	4.90	4.10
Pooled SEM			0.64	0.41	0.38	0.34	0.23	0.27	0.20	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	2.29 ^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 × diet			0.1204	0.0165	0.5744	0.4092	0.5380	0.9170	0.6675	0.1408	0.2063
Strain × sex			0.0004	0.7692	0.4324	0.8666	0.0112	0.1030	0.9256	0.0253	0.9325
Diet × sex			0.0517	0.0693	0.5744	0.5448	0.7952	0.5461	0.5628	0.0052	0.1106
Strain × diet × sex			0.8378	0.4394	0.4324	0.6702	0.2322	0.8187	0.0376	0.7667	0.9325

^{a,b}Means within a column and classification with no common superscript differ significantly ($P \leq 0.05$).

¹Six-day-old birds were given an injection of SRBC (d 0) followed by a second injection on d 10 and 11 in trials 1 and 2, respectively. Blood serum samples from eight birds per strain/sex per diet were analyzed for the presence of total anti-SRBC antibodies. The data represent mean ± standard errors of log₂ of the reciprocal of the last dilution exhibiting agglutination.

²2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

³2001 = Broiler diet representative of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet representative of those being fed in 1957.

⁴PPI = days postprimary injection.

⁵PSI = days postsecondary injection.

higher titers than ACRBC females ($P = 0.0112$). A strain × diet × sex interaction was present at 11 d PPI in trial 2, but the differences were not consistent for different groups.

The secondary antibody response was measured at 5 and 10 d postsecondary injection (PSI) in trial 1 and at 4 and 7 d PSI in trial 2. In this case, the ACRBC birds exhibited significantly higher total antibody titers at 5 and 10 PSI in trial 1. In trial 2, none of the strain, diet, or sex differences were significant at 4 or 7 d PSI. The only significant effects observed in trial 2 were for the strain × sex and the sex × diet interactions at 4 d PSI. The sex × diet interaction was caused by the females on the 2001 diet having lower antibody titers than the females on the 1957 diet ($P = 0.0052$). The significant strain × sex interaction at 4 d PSI in trial 1 was the result of a much larger difference in the response of the ACRBC females than for Ross 308 males ($P = 0.0253$).

Immunoglobulin M titers were measured 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. In trial 1, a strain × sex interaction ($P = 0.0001$) was present at 5 d PPI, due to the Ross 308 females having higher IgM titers than the Ross males, and the ACRBC females having lower titers than the ACRBC males ($P = 0.0001$). At 10 d PPI significant strain, diet, and strain × diet effects

were observed for the levels of IgM antibodies. The ACRBC had higher IgM levels than the Ross 308 broilers ($P = 0.0001$), and birds on the 1957 diets had higher levels than those on the 2001 diets ($P = 0.0065$), resulting in the strain × diet interaction ($P = 0.0119$). As seen previously in Table 2, at both 10 d PPI and at 5 d PSI in trial 1, the ACRBC was a high responder for total anti-SRBC antibodies. The data in Table 3, therefore, suggest that the significant increase observed in total antibodies for the ACRBC in trial 1 was in fact due to higher IgM levels. At 5 d PSI, birds raised on the 1957 diet had greater titers than birds raised on the 2001 diet ($P = 0.0011$). In trial 2, no strain differences were seen in IgM levels at 4 d PPI or 11 d PPI, but the ACRBC birds approached having a significantly higher antibody level ($P = 0.0880$) at 7 d PPI than the Ross 308 birds. Significant strain × sex interaction was seen at 4 d PPI, when the females of Ross 308 strain had higher IgM titers than did the females of ACRBC and males of Ross 308 strain ($P = 0.0121$). This interaction continued to be present at 7 d PPI ($P = 0.0103$), but not at 11 d PPI. During the secondary response, the Ross 308 birds had higher IgM levels than the ACRBC at 4 and 7 d PSI ($P = 0.0001$).

Immunoglobulin G titers were measured on the same days as the IgM levels (Table 4). A significant diet × sex interaction was present at 5 d PPI where the females on 1957 diet had significantly higher IgG titers than females on 2001 diet and males on 1957 diet, while they were not

TABLE 3. IgM (mercaptoethanol-sensitive) anti-SRBC antibody titer of a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain when fed 2001 and 1957 diets¹

Strain ²	Diet ³	Sex	Trial 1				Trial 2				
			Days PPI ⁴		Days PSI ⁵		Days PPI			Days PSI	
			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	1957	Male	0.12	0.12	3.37	1.00	0.30	0.30	0.30	2.50	1.90
1957	2001	Male	2.62	1.25	3.00	1.12	0.80	0.66	0.30	1.40	0.20
1957	1957	Male	3.00	2.11	4.50	1.22	0.40	1.20	0.40	1.20	0.60
2001	2001	Female	2.75	0.62	2.87	0.75	1.00	0.40	0.10	2.70	1.40
2001	1957	Female	3.00	0.87	3.25	1.12	0.70	0.63	0.70	2.60	1.50
1957	2001	Female	0.62	1.00	3.12	1.12	0.40	0.40	0.40	1.00	0.60
1957	1957	Female	2.00	3.14	3.85	1.00	0.22	0.30	0.22	0.70	0.40
Pooled SEM			0.58	.38	.40	.27	.24	.24	.19	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 × diet			0.1578	0.0119	0.6778	0.4010	0.5861	0.8987	0.4015	0.7205	0.3999
Strain × sex			0.0001	0.7577	0.2768	0.6543	0.0121	0.0103	0.8911	0.2848	0.7177
Diet × sex			0.2055	0.1402	0.1313	0.9001	0.7976	0.3269	0.3594	0.8580	0.1202
Strain × diet × sex			0.9402	0.4163	0.8413	0.6543	0.3707	0.4391	0.0610	1.0000	0.9041

^{a,b}Means within a column and classification with no common superscript differ significantly ($P \leq 0.05$).

¹Six-day-old birds were given a first injection of SRBC (d 0) followed by a second injection on d 10 and 11 in trials 1 and 2, respectively. Blood serum samples from eight birds per strain/sex per diet were analyzed for the presence of total anti-SRBC antibodies. The data represent mean \pm standard errors of log₂ of the reciprocal of the last dilution exhibiting agglutination.

²2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

³2001 = Broiler diet representative of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet representative of those being fed in 1957.

⁴PPI = days postprimary injection.

⁵PSI = days postsecondary injection.

significantly different from males on 2001 diet. A diet effect was seen at 10 d PPI where birds on the 2001 diet had higher titers than those on the 1957 diet ($P = 0.0085$). During the PSI period, in trial 1, a diet × sex interaction for IgG was present at 5 d PSI where females on the 1957 diet had higher IgG response than males on the 1957 diet ($P = 0.0336$). A strain effect was observed at 10 d PSI ($P = 0.0062$) with the ACRBC strain having higher IgG levels than the Ross 308 strain. At the same time point females gave higher titers than males ($P = 0.0235$). A significant strain × diet effect on IgG was seen at 10 d PSI where the ACRBC raised on the 1957 diet had higher antibody response than the ACRBC on the 2001 diet, and the Ross 308 on both diets ($P = 0.0532$). In trial 2, the IgG response was higher for the ACRBC than for the Ross 308 broilers at 11 d PPI ($P = 0.0028$), and at 4 and 7 d PSI ($P \leq 0.0004$). Significant diet × sex interaction for IgG levels was observed at 4 d post boost, where females on the 1957 diet and males on 2001 diet had higher titers than males on the 1957 diet and females on the 2001 diet ($P = 0.0203$). A diet × sex interaction was also present at 7 d PSI where females on the 1957 diet had higher titers than males on the 1957 diet and females on the 2001 diet, but they were not significantly different than males on the 2001 diet ($P = 0.0084$).

Taken together, the antibody data from trial 1 clearly show that ACRBC chickens have significantly higher total, IgM, and IgG responses than the modern strain of

broilers used (Tables 2 to 4). While the differences in total and IgM levels could not be reproduced in trial 2, the ACRBC was clearly the higher responder of the two strains following the booster injection when higher IgG levels are expected and were observed (Table 4). These observations are similar to the ones made 10 yr earlier when the ACRBC was compared with the 1991 commercial Arbor Acres broiler. In that study, the ACRBC was found to have a higher antibody response than the 1991 Arbor Acres broiler strain (Qureshi and Havenstein, 1994). Birds raised on the 1957 diet in the current study appeared to produce slightly higher levels of antibodies, but that effect was sporadic and inconsistent. Additional studies in chickens (Miller et al., 1992; Boa-Amponsem et al., 1999; Rao et al., 1999) have supported the current and previously reported conclusions that the antibody response and disease resistance potential of fast growing strains of poultry is relatively weaker than in the slow growing types from which they are selected. In turkeys, growth-selected F line (Nestor et al., 1996a) has been shown to have higher mortality than the randombred control (RBC2) line when experimentally challenged with *Pasteurella multocida* (Sacco et al., 1991; Nestor et al., 1996b), and Newcastle disease virus (Tsai et al., 1992). Interestingly, F line poulters exhibited higher anti-SRBC antibody response than their randombred control counterparts as well as had significantly higher CD4+ helper-T lymphocyte populations (Li et al., 2000). These authors

TABLE 4. IgG (mercaptoethanol-resistant) anti-SRBC antibody titer of a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain when fed 2001 and 1957 diets¹

Strain ²	Diet ³	Sex	Trial 1				Trial 2				
			Days PPI ⁴		Days PSI ⁵		Days PPI			Days PSI	
			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	1957	Male	0.00	0.00	0.75	0.50	0.10	0.30	0.00	1.90	1.80
1957	2001	Male	0.13	0.37	1.00	0.53	0.10	0.00	0.10	4.00	3.50
1957	1957	Male	0.00	0.00	0.37	1.22	0.00	0.00	0.30	3.40	2.80
2001	2001	Female	0.00	0.13	1.13	0.87	0.10	0.10	0.00	1.90	1.60
2001	1957	Female	0.37	0.00	1.00	0.50	0.00	0.12	0.00	3.40	2.90
1957	2001	Female	0.13	0.12	0.88	1.13	0.00	0.00	0.20	3.40	2.90
1957	1957	Female	1.00	0.00	1.15	1.85	0.00	0.30	0.22	4.20	3.70
Pooled SEM			0.23	.12	.18	.30	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	1.18 ^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 × diet			0.4529	1.0000	0.7863	0.0532	1.0000	0.7986	0.4048	0.4024	0.5004
Strain × sex			0.2617	1.0000	0.4569	0.3823	1.0000	0.1093	0.9335	0.4024	0.8660
Diet × sex			0.0272	0.1782	0.0336	0.3948	1.0000	0.6708	0.5048	0.0203	0.0084
Strain × diet × sex			0.4529	1.0000	0.2254	0.5570	0.3274	0.3509	0.5048	0.8190	0.8660

^{a,b}Means within a column and classification with no common superscript differ significantly ($P \leq 0.05$).

¹Six-day-old birds were given an injection of SRBC (d 0) followed by a second injection on d 10 and 11 in trials 1 and 2, respectively. Blood serum samples from eight birds per strain/sex per diet were analyzed for the presence of total anti-SRBC antibodies. The data represent mean ± standard errors of log₂ of the reciprocal of the last dilution exhibiting agglutination.

²2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

³2001 = Broiler diet representative of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet representative of those being fed in 1957.

⁴PPI = days postprimary injection.

⁵PSI = days postsecondary injection.

suggested that the BW selection may have resulted in changes in T lymphocyte subpopulations and, therefore, may have affected disease resistance. The current study, therefore, indicate that such changes in antibody responsiveness may be a correlated response to the selection for increased broiler performance including 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 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 B²¹ haplotype was associated with higher antibody titer and lower BW in most instances while B¹³ 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 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 308 strain exhibited 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 ($P = 0.0298$), where the birds given the 2001 diet had higher lymphoproliferative response than those on the 1957 diet, no other significant dietary effects were observed. In addition, no significant interactions were observed.

The lymphoblastogenic response to PHA-P is presumed to be polygenic (Morrow and Abplanalp, 1981). Corrier (1990) showed that broiler and layer-type chicks differ in their responses to PHA-P, i.e., broiler chicks are higher responders than layer-type chickens. The data from the current study indicate that Ross 308 broilers have a greater PHA-P mediated response than does the ACRBC strain. Since PHA-P induces T-cell division with minimal effects on B-cells (Tizard, 1995), it is considered a good in vivo measure of T-lymphocyte function (Qureshi et al., 1997). Thus, the data from the current study indicated that the T-lymphocytes from the Ross 308 birds have higher lymphoproliferative potential than the ACRBC. This suggests that genetic selection for growth has positively affected the cell-mediated arm of the immune response. In contrast to the current 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 the high

TABLE 5. Lymphoblastogenic response against phytohemagglutinin-P (PHA-P) by a 2001 modern commercial broiler and the 1957 Athens-Canadian Randombred strain, 24 and 48 h increases when fed 2001 and 1957 diets¹

Strain ²	Diet ³	Sex	Trial 1		Trial 2	
			24 h increase	48 h increase	24 h increase	48 h increase
2001	2001	Male	0.58	0.33	0.93 ^a	0.47
2001	1957	Male	0.48	0.33	0.71 ^{bc}	0.47
1957	2001	Male	0.45	0.23	0.39 ^e	0.20
1957	1957	Male	0.31	0.21	0.45 ^{de}	0.20
2001	2001	Female	0.79	0.42	0.62 ^{cd}	0.41
2001	1957	Female	0.57	0.37	0.85 ^{ab}	0.57
1957	2001	Female	0.55	0.26	0.40 ^e	0.22
1957	1957	Female	0.41	0.21	0.44 ^{de}	0.20
Pooled SEM			0.09	0.06	0.07	0.04
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 × diet			0.8401	0.8648	0.6674	0.2189
Strain × sex			0.6801	0.5632	0.4170	0.8476
Diet × sex			0.6469	0.7182	0.0433	0.3428
Strain × diet × sex			0.6734	0.9728	0.0232	0.1664

^{a-e}Means within a column and classification with no common superscript differ significantly ($P \leq 0.05$).

¹At 19 d of age, PHA-P was injected at 100 $\mu\text{g}/100 \mu\text{L}$ per bird in the toe web of right foot of eight birds per strain/sex per diet. Swelling was measured by a constant tension micrometer at 24 and 48 h postinjection. The increase in swelling was computed by subtracting the preinjection value from the postinjection value at a given time.

²2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Randombred control.

³2001 = Broiler diet representative of those being fed in calendar year 2001 with crumbled, starter and pelleted grower, 1957 = broiler starter diet representative of those being fed in 1957.

BW turkey line 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 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 higher in the Ross 308 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 SRBC was quantitated, both strains exhibited comparable percentage of phagocytic macrophages (Table 6). However, the relative phagocytic activity of the individual macrophages, in terms of numbers of internalized SRBC, was higher in the 2001 Ross 308 strain than in the 1957 ACRBC strain ($P = 0.0122$). For both of these phagocytic endpoints, the birds raised on the 2001 diet were higher responders than were those on the 1957 diet ($P \leq 0.0044$). A strain × diet × sex interaction was observed for the percentage of phagocytic macrophages ($P = 0.003$), indicating the difference between the sexes on the two diets were significantly different for the two strains.

Another function related to macrophages is the constitutive and inducible production of 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 higher in the ACRBC strain than in the Ross 308 birds ($P = 0.017$). Strain × diet ($P = 0.0064$), strain × sex ($P = 0.0001$), and diet × sex ($P = 0.0150$) interactions were also observed. On the contrary, no significant differences in nitrite levels were observed between the strains, diets or sexes after LPS stimulation. Strain × sex as well as strain × diet × sex interactions were observed for inducible NO production, however, the response was not consistent among different groups. Significantly lower nitrite production in the absence of LPS suggests poorer constitutive expression of NO synthase in the Ross 308 strain than in the ACRBC strain.

The findings of the current study suggest that macrophages from modern-day broilers appear to be more adept in eliciting responses against antigens, as well as in the engulfment of antigens than are macrophages from ACRBC chickens. That is, the Ross 308 birds had significantly higher AEC numbers in response to Sephadex stimulation (Table 6) whereas nitrite production in response to LPS stimulation was comparable between the two strains. It is, however, interesting to note that while no difference in the overall phagocytic percentage of macro-

TABLE 6. Macrophage response of a 2001 modern commercial broiler and the 1957 Athens-Canadian Rando-bred strain when fed 2001 and 1957 diets (trial 2)¹

Strain ²	Diet ³	Sex	AEC Number	Phagocytosis (%)	SRBC/macrophage number
2001	2001	Male	4.91	43.97 ^a	2.40
2001	1957	Male	9.34	23.27 ^c	1.90
1957	2001	Male	5.46	34.26 ^b	2.14
1957	1957	Male	6.85	31.25 ^b	1.71
2001	2001	Female	8.02	29.89 ^{bc}	2.09
2001	1957	Female	12.66	33.29 ^b	1.91
1957	2001	Female	4.46	30.56 ^{bc}	1.74
1957	1957	Female	7.59	28.21 ^{bc}	1.90
Pooled SEM			1.62	2.74	0.11
Strain averages					
2001	x	x	8.73 ^a	32.60	2.07 ^a
1957	x	x	6.09 ^b	31.07	1.87 ^b
Source of variation			Probability		
Strain			0.0261	0.4330	0.0122
Diet			0.0049	0.0044	0.0038
Sex			0.1866	0.1685	0.1063
Strain × diet			0.3288	0.1284	0.1930
Strain × sex			0.1529	0.7326	0.7953
Diet × sex			0.6752	0.0019	0.0056
Strain × diet × sex			0.7430	0.0033	0.4156

^{a-c}Means within a column and classification with no common superscript differ significantly ($P \leq 0.05$).

¹Three percent Sephadex suspension was injected at 1mL/100 g BW into the abdominal cavities of five birds/strain per diet per sex at 2 wk of age. After 40 h birds were harvested to get abdominal exudate, cell count was adjusted to 1×10^6 /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.

²2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Rando-bred control.

³2001 = Broiler diet representative of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet representative of those being fed in 1957.

TABLE 7. Lipopolysaccharide (LPS)-mediated nitrite production response by macrophages from a 2001 modern commercial broiler and the 1957 Athens-Canadian Rando-bred strain when fed 2001 and 1957 diets (trial 2)¹

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

^{a-d}Means within a column and classification with no superscript differ significantly ($P \leq 0.05$).

¹Macrophage cultures (from 5 birds/strain per sex) were exposed to LPS from *Escherichia coli* (1μ g/ 1×10^6 macrophages) for 24 h at 2 wk of age. The culture supernatant was tested for nitrite levels by treating it with Griess reagent method.

²2001 = Ross 308 feather-sexable; 1957 = Athens-Canadian Rando-bred control.

³2001 = Broiler diet representative of those being fed in calendar year 2001 with crumbled starter and pelleted grower, 1957 = broiler starter diet representative of those being fed in 1957.

phages for SRBC was observed between the two strains, the macrophages from the Ross 308 chickens had a significantly higher level of phagocytic activity (Table 6). In a previous study the 1991 Arbor Acres broiler had comparable macrophage phagocytic activity when compared with the ACRBC strain (Qureshi and Havenstein, 1994). In the current study, although the Ross 308 broiler (instead of Arbor Acres broiler) was used as a representative of the modern-day broiler bird, the increased macrophage phagocytic activity in Ross 308 broilers is suggestive of an improvement in macrophage function in modern day broiler as compared to randombred control birds which was the same in both 1991 (Qureshi and Havenstein, 1994) and the current study. 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, NO synthase activity differs among 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 activity is suggestive of a positive correlated genetic change brought about by selection for performance traits such as growth.

In conclusion, it appears from the current study that genetic selection for improved broiler performance has had a negative impact on the adaptive arm of the immune response (antibody production). At the same time, it appears that cell-mediated and inflammatory responsiveness of the immune system has been improved in strains that have been selected for rapid growth rate. Diet and sex effects were inconsistent between growth selected and randombred strains for all immune function parameters tested.

Both arms of the adaptive immune response (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 whereas cell-mediated immune response would be desirable in eliminating virus-infected cells. The apparent dichotomous changes that have taken place in the immune performance of chicken lines selected for increased 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 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., 2000). 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 commercial

breeders to incorporate all three of these important traits in their breeding programs (Falconer, 1989; Kean et al., 1994). It appears that breeders of meat-type chickens should, at a minimum, consider including some measure of humoral immune response and its genetic correlation with BW into their selection programs.

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