DIFFERENCES IN IMMUNE RESPONS BETWEEN BROILER STRAINS AND THEIR CROSSES.

By

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Abstract: *Humoral antibody titers against sheep red blood cells (SRBC), cell mediated immune response and spleen weights and relative spleen weights were determined in two broiler strains. Arbor Acres (AA), PureLine (PL), and the crosses between Arbor Acres males and a synthetic Egyptian broiler female line B-2 females (AA•B-2) and PureLine males • B-2 females (PL•B-2).*

The result of antibody titers against SRBC varied between strains and crosses. Arbor Acres and PL broilers had significantly lower level of antibody responses to SRBC three days post primary immunization (3 PPI) than the crosses. There were no significant differences between the AA and PL broiler strains or between the AA• B-2 and PL•B-2 crosses. Six days PPI AA•B-2 chicks had significantly lower level of antibody titers against SRBC than the AA and PL•B-2 birds. On the other hand, nine days PPI no significant differences were observed between all strains and crosses. No significant differences in the delayed type hypersensitivity response (DTH) were found between the broiler strains or the crosses.

Also, the spleen weights varied significantly among strains and crosses. The relative spleen weights of the AA•B-2 and PL•B-2 broilers were significantly higher than the PL at six weeks of age.

Key Words: In Immune Respons, Broiler, Crosses.

INRODUCTION

One of the major problems that encounter the poultry industry is infectious disease. It is responsible for major economic losses and can have devastating effects particularly in intensive production. Adaptive immunity to pathogens is critically dependent on T cell responses, particularly CD4 T helper (Th) cell responses, in mammalian and avian species (Arstila *et al.*, 1994). Upon activation, mammalian CD4 T cells make several cytokines that fall into two groups, referred to as Th1 and Th2. A given mammalian T cell is likely to make either Th1 or Th2 cytokines (Mosmann *et al.*, 1986; Cherwinski *et al.*, 1987). The Th1 cytokines include tumor necrosis factor-β and interferon-γ (IFNγ). The IFNγ induces inflammation and activates macrophages to kill resident intracellular pathogens, such as mycobacteria or salmonellae (Kagaya *et al.*, 1989). The Th2 cytokines include interleukin (IL)-4, - 5, and -10, which enhance the maturation response of B cells so that efficient antibodies are made to deal with extracellular pathogens, such as streptococci (Lebman and Coffman, 1988). Whether an immune response is dominated by antibody production or inflammation can make a remarkable difference in the severity of a

given infectious disease (Kim *et al.*, 1985; Stevens *et al.*, 1988; Heinzel *et al.*, 1991).

The sheep red blood cells (SRBC) is a complex, multideterminant natural antigen provoking a T-B cell dependent antibody response (Van der Zijpp *et al,.* 1983). In chickens, the delayed type hypersensitivity response (DTH) represents the Th1 immune response *in vivo*. By measuring local swelling upon antigen injection into the wattle, wing-web, or toe web of immunized birds, the DTH response can be generally assessed (York and Fahey, 1990) or specifically (Corrier, 1990). Chicken lines with low DTH responses appear to show better resistance to Marek's disease (Afraz *et al.*, 1994).

The aim of the present work was to compare the humoral; cell mediated immune response against both SRBC and BSA antigens, and relative weights of spleens of two broiler strains and their crosses with a local female broiler line (B-2).

MATERIAL AND METHODS

The present experiment was carried out at the Poultry Research Center and the Laboratory of Biotechnological Methodology for Poultry Improvement, Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

This experiment was conducted to determine the effect of crossing on immune response of two broiler strains and their crosses with B-2 as following:

1- Arbor Acers as a commercial broiler strain, (AA).

2- PureLine grandparent males × PureLine grandparent females as a male broiler strain (PL), (PureLine Genetics LLC, Preston, CT, USA).

Their crosses with the local female broiler line (B-2)

3- PureLine grandparent males • B-2 line females as a local broiler cross (PL•B-2).

4- Arbor Acers parent males • B-2 line females as a second local broiler cross (AA•B-2).

All chicks of the PL, PL•B-2, and AA•B-2 lines were produced by artificial insemination. The PL•B-2 chicks were produced by using 15 PureLine grandparent males with 150 B-2 line females from the fourth selected generation (F. K. R. Stino, Department of Animal Production, Faculty of Agriculture, Cairo University, unpublished data). The AA•B-2 chicks were produced by using 15 Arbor Acers commercial broiler parent males with 150 B-2 line females from the fourth selected generation. The three PL, PL•B-2, and AA•B-2 chicks were hatched in the same incubator on the same day. The AA birds were bought as commercial day old broilers.

General Management

One day old chicks from each strain were wing-banded and weighed to the nearest gram. Chicks from each strain were distributed randomly into four replicate individual deep litter (with wood shavings, 10 birds/m^2) floor pens. Hanging tube feeder and plastic water drinkers were used. All chicks were reared under the same conditions, exposed to continuous light for the first 3 days then they were exposed to 23 hours of light daily. All chicks were fed a commercial starter broiler ration (23 % crude protein and 3050 kcal ME/kg) during the first 2 weeks of age. From 15 days to the end of the experiment, they were fed a commercial broiler grower ration (21 % crude protein and 3150 kcal ME/kg). Both feed and water were provided *ad libitum*.

Immunization and titration against Sheep red blood cells

This experiment was conducted to monitor the early events during the

initiation of the primary humeral immune responses following the injection of SRBC antigen.

The SRBC suspension was prepared by pooling 100 ml of blood from the jugular vein of three to five native Egyptian sheep using a heparinized syringe. The blood was then placed in dried clean centrifuge tubes. An equal amount of phosphate buffer saline (PBS) was added and the suspension was centrifuged at 3000 RPM for 10 minutes. The supernatant was poured off and about 20-30 volumes of PBS were added to the packed cells. The cells were then gently resuspended, re-centrifuged and the supernatant was again poured off. Ten ml of the packed cells were added to 90 ml of PBS to prepare 10% SRBC suspension for immunization. Two ml of packed cells were added to 98 ml of PBS to prepare the 2% SRBC suspension for titration.

At 28 days of age, 48 birds from each strain (12 bird from each replicate) were randomly chosen to detect primary antibody titers against SRBC. All chosen birds, of all strains, were injected i.m. with 0.5 ml of 10% SRBC suspension to induce a T cell dependent antibody response. Approximately 2 ml of blood samples were collected from the wing vein of each bird at 31, 34 and 37 days of age (days 3, 6 and 9 post injection) in nonheparinized tubs, and the blood was allowed to clot. These samples were centrifuged at 3000 RPM for 10 minutes and the serum was separated. Antibodies against SRBC were measured using the micro titer procedure of Van der Zijpp and Leenstra (1980). Titers were expressed as the log 2 of the reciprocal of the highest dilution giving complete agglutination.

Delayed type hypersensitivity (DTH) response to bovine serum albumin (BSA)

This experiment was conducted to investigate the development of the delayed type hypersensitivity (DTH) response. At 35 days of age, 48 males from each strain (12 from each replicate) were randomly chosen. All chosen birds, from all strains, were injected with 4 mg BSA, in 1 ml saline, sub cutaneously (s.c.), in the back of the neck. After 7 days, the right wattle thickness was measured by a paper thickness micrometer before injection (control). After that, the same birds, of each strain, were injected with 1 mg BSA, in 0.1 ml saline into the flat surface of the right wattle. At 12, 24, and 48 hours post injection, the thickness of the right wattles were measured again to calculate the difference in the thickness between before and after BSA injection.

The percentage increase in wattle thickness was calculated according to the following equation:

$$\text{Relative response \%} = \frac{\text{Increased wattle thickness (post injection – pre injection)}}{\text{initial wattle thickness (pre injection)}} \times 100$$

Spleen Weight and Relative Spleen Weight

At six weeks of age, thirty two chicks from each strain (8 birds from each replicate) were slaughtered after 8 hours of fasting. Spleens were removed and weighted. Their relative weights were expressed as percentage of live fasted body weight.

Statistical analysis

Data were analyzed as a one-way analysis of variance using the SAS software, general linear model (SAS institute, 2004). All data are reported as means ± standard errors. Mean values were separated using Duncan's multiple range test (Duncan's, 1955) if significant differences were present. Significant level was set at 5%.

The Statistical model used in the analysis was :

$$Y_{ij} = \mu + s_i + e_{ij}$$

Where:

Y_{ij} = the observation on the j^{th} bird in the i^{th} strain.

μ = the overall mean.

s_i = the effect of the i^{th} strain.

e_{ij} = the random error term.

RESULTS

Antibody titers

The results demonstrated that there was a significant effect of strain on antibody response to SRBC (Table 1). After three days post immunization the AA and PL birds had significantly lower level of antibodies against SRBC than the AA*B-2 and PL*B-2 birds. There were no significant differences between PL and AA birds or between AA*B-2 and PL*B-2 birds at day 3 post primary immunization (PPI). At day 6 PPI, AA*B-2 birds had significantly lower level of antibodies against SRBC as compared to AA or PL*B-2 birds which showed the highest levels. However, PL birds were intermediate. On the other hand, at day 9 there were no significant differences observed between any of the strains or crosses.

Table 1. Primary antibody titers against SRBC at different days post immunization.

Strains or crosses	Days post immunization		
	3	6	9
PL	2b*	8ab	6a
AA	2b	9 a	6a
AA*B-2	4 a	7 b	7a
PL*B-2	4 a	9a	5a
SEM (n=12)	0.395	0.436	0.527
Probability	**	**	NS
	0.0151	0.0204	0.3841

*Values followed by different superscripts, within day post immunization, are significantly different (P<0.05)

** Significant, NS = not significant

Delayed type hypersensitivity (DTH)

Delayed type hypersensitivity (DTH) is a simple and useful method for assessing in vivo cell mediated immunocompetence. The relative response to BSA, as wattle test in the different groups is presented in Table 2. There were no significant differences (P>0.05) between all the strains and/or crosses at all the times measured. However, AA birds had higher numerical relative response than the other groups at 12 hours post injection, while PL*B-2 birds had lower numerical relative response than the other groups at the same time. At 24 and 48 hours post injection higher numerical relative response was observed for the PL birds, whereas lower response was observed for the PL*B-2 birds.

Table 2. Relative increase in wattle thickness (%) as delayed type hypersensitivity after injection with BSA.

Strains or crosses	Time post injection (hours)		
	12	24	48
PL	99	126	114
AA	145	110	80
AA•B-2	90	119	82
PL•B-2	85	99	65
SEM (n=12)	20	20	21
Probability	NS*	NS	NS
	0.1627	0.7951	0.4227

* No significant differences were observed

Spleen Weight and Relative Spleen Weight

The results indicated that there were significant differences ($P<0.05$) observed between broiler strains and crosses in both spleen weights and relative spleen weights at 6 weeks of age (Table 3). The AA strain had significantly the highest spleen weights as compared to all other groups. However, no significant differences were observed in spleen weights between the PL, AA•B-2 or PL•B-2 groups.

The current results indicated that the PL had significantly lower relative spleen weights than both the AA•B-2 and PL•B-2 groups. No significant differences were observed in relative spleen weights among the AA, AA•B-2 or PL•B-2 groups (Table 3).

Table3. Spleen Weights and Relative Spleen Weights of different broiler strains and crosses at 6 weeks of age.

Strains or crosses	Spleen weight	Spleen percentage
PL	2.7 ^{b*}	0.13 ^{b}
AA	3.5 ^{a}	0.16 ^{ab}
AA•B-2	2.5 ^{b}	0.19^{a}
PL•B-2	2.5 ^{b}	0.19 ^{a}
SEM (n=32)	0.1951	0.0119
Probability	**	**
	0.0021	0.0031

* Values in the same column having different superscripts are significantly different ($P<0.05$)

** Significant

DISCUSSION

SRBC antibody titers

Phenotypic variations in immune response and disease resistance among the different broiler strains and crosses were apparent. The AA and PL strains had the poorest antibody response to SRBC at 3 days post primary immunization (dPPI). The other two broiler crosses had higher anamonstic (secondry) titer responses to this agent. Previous reports have demonstrated that chickens selected for high antibody response to SRBC also had enhanced immune responses to other T-dependent antigens (BSA & *E. coli*) and to vaccination with Newcastle disease virus or other avian pathogens (Gross *et al.*, 1980; Parmentier *et al.*, 1994, 1996). Conversely, meat-type chickens selected for high

antibody response to *E. coli* also responded well to SRBC, BSA, and Newcastle disease virus (Heller *et al.*, 1992).

Variation in antibody response to SRBC among different chicken lines or populations has been previously reported (Van der Zijpp, 1983; Martin *et al.*, 1989; Loudovaris *et al.*, 1990). In one study, total antibody levels to SRBC, after primary immunization, were influenced by the genetic line. The differences in antibody levels were due to the rate of production and persistence of the antibodies, in particular IgG (Martin *et al.*, 1989). It is speculated that the differences in antibody response to SRBC among different broiler lines probably reflect differences in the rate of production or persistence of the IgM and IgG isotypes. Genetic control of antibody response to SRBC in chickens is largely due to genes within the Major Histocompatibility Complex (MHC) locus (Dunnington *et al.*, 1989; Loudovaris *et al.*, 1990).

This phenomenon may indicate that crossing, the native Egyptian breed, White Baladi, which is part of the B-2 genotype, with PL and AA possess an active innate immunity (anatomic, physiological, and phagocytic barriers). These barriers interfere with the entry of diseases to reach the target cells and proliferate within them. This may result in reducing virus numbers, that can react and stimulate acquired immune response (humoral and cell mediated). On the other hand, immunization with SRBC using i.m. injection as route of administration makes this antigen by pass most innate barriers. Thus it reaches directly the secondary lymphoid organs, and reacts with the immunecompetant cells. This interpretation may indicate also that the White Baladi breed may possess a genetic make up that qualify their immune system to react against some antigens like SRBC with high efficiency.

In general, the current experiment showed that primary antibody titers against SRBC antigens increased and peaked significantly after 6 dPPI and then it declined gradually. Similar observations were reported by Benjamini and Leskowitz (1991) and Kuby (2000). They stated that the first contact of an individual with an antigen generates a primary antibody response. This response is characterized by a latent (lag) period that lasts 3-4 days followed by an exponential increase to a maximum titer which is attained by 5-7 days, then antibody levels eventually plateaue and thereafter decline.

Genetic resistance to disease is more critical in broiler chickens due to the shortened time to market and the time required to develop protective immunity with vaccination. The current report demonstrates that there are phenotypic variations in antibody response to SRBC among the broiler strains and crosses. Although the data imply that the differences may be due to genetic variation among the strains, the role of maternal effects must also be considered. However, based on the moderate heritabilities for immune response in chickens (Van der Zijpp *et al.*, 1983; Kaiser *et al.*, 1997, 1998), it is anticipated that the phenotypic differences in these broiler strains are only partially genetic in nature. Broiler crosses AA•B-2 and PL•B-2 were the most phenotypically distinct, based on antibody response to SRBC and they had the highest immune response 3 days post immunization.

Delayed type hypersensitivity (DTH)

The current results indicated that no significant differences in the wattle swelling test were found between strains and/or crosses. However, the AA strain had higher wattle swelling than the other strain and crosses. Previous studies indicated that other tests of T-cell mediated immune response of chickens were significantly different among birds of different genetic lineages (Fredericksen and Gilmour, 1983; Lamont and Smyth, 1984; and Cheng and Lamont, 1988). Successful divergent

selection of chicken for various T-cell functions, suggested that many of these functions were highly heritable but linked to MHC or to Ig allotypic loci (Miggiono *et al.*, 1976; and Lasilla *et al.*, 1979). The similarity in wattle test among strains and crosses indicated that the activities of immunocompetance responsible for BSA are similar in all strains and crosses.

Spleen Weight and Relative Spleen Weight

The spleen is a secondary lymphoid organ that filters blood borne antigens and is a major site for increased immune responses.

Silversids *et al.*, (1997) stated that spleen weights are a general indicator of stress in chicken. They also stated that spleen weight was significantly different between different crosses at day 42 of age. Our results indicated that there were significant differences between 6-week spleen weight of some broiler strains and crosses. The AA broilers had the highest spleen weight (3.5gm).

Cheema *et al.*, (2003) reported that the modern day commercial broiler strains, selected for enhanced broiler performance, exhibits reduced relative growth of lymphoid organs including the spleen which was used as indicator for immune response of broilers. Thus, it seams that the decrease in relative spleen weights in the PL strain had a negative affect on its immune response.

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الملخص العربي

الإختلافات في الإستجابة المناعية بين أنواع من كتاكيت اللحم وخلطانها

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**قسم الإنتاج الحيواني – كلية الزراعة – جامعة القاهرة

تم قياس مستوى الأجسام المناعية المتكونة ضد كرات الدم الحمراء للغنم كما تم تقدير المناعة الخلوية وذلك في سلالتين من بدارى التسمين وخلطانها. خليط ناتج من خلط ذكور الأربورإيكرز مع إناث B-2 (وهي امهات انتاج لحم مصرية محلية داخل فيها جدود سلالة الأربورإيكرز والدجاج البلدي الأبيض المصري تم انتاجها بالانتخاب لأعلى وزن عند عمر ٦ أسابيع لفترة اربعة اجيال)، وخليط ناتج من خلط ذكور البيورلاين مع إناث B-2، سلالة الأربورإيكرز وسلالة البيور لاين وهما سلالتين تجاريتين لإنتاج اللحم .

أوضحت نتائج مستوى الأجسام المناعية ضد كرات الدم الحمراء للغنم أن هناك تباينا معنويا بين الأنواع حسب العمر فكانت سلالتي الأربرإيكرز والبيورلاين الأقل معنويا في الإستجابة المناعية في اليوم الثالث بعد الحقن عن الانواع الاخرى. من ناحية اخرى لم يلاحظ اختلافات معنوية في إختبار فرط الحساسية (والذي يعتبر مؤشرا جيدا للمناعة الخلوية) بين هذه الانواع. لوحظ ايضا أن نسبة وزن الطحال بالنسبة للوزن الحي كان الأقل معنويا في البيورلاين عن مثيله في الأنواع الأخرى عند عمر ٦ أسابيع.