

## **IMMUNOLOGICAL VARIATIONS AMONG COMMERCIAL BROILER STRAINS**

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### **SUMMARY**

The humoral antibody titer against Newcastle Disease Virus (NDV), and sheep red blood cells (SRBC), cell mediated immune response and relative lymphoid organs (bursa and spleen) weights were determined in six commercial broiler strains, (Hubbard, Arbor Acres, Cobb, Avian, Lohman and ISA).

The results of antibody titer against NDV changed with age, at one day-old, the maternal antibody titer of Arbor Acres and Lohman chicks were significantly higher than those of Hubbard, ISA and Avian chicks. This ranking changed thereafter. At two weeks of age, Hubbard chicks had the highest titer, while at three weeks of age ISA chicks were the highest. However, no significant differences were found in antibody titer against SRBC or cutaneous basophil hypersensitivity among strains.

Also, the relative bursa and spleen weights varied among strains. The relative spleen weight of Arbor Acres was significantly the highest at four weeks of age compared to those of the other strains, (except that of Lohman strain). Although the relative bursa weight of both Cobb and Avian chicks were significantly higher at two weeks of age, but they were lower at six weeks of age than those of Arbor Acres.

In conclusion, the results indicate that, it is important to measure the antibody titer against NDV in order to know when to vaccinate the broiler chicks.

**Keywords:** Broiler strain, immune response, lymphoid organs

### **INTRODUCTION**

Since late 1940s, tremendous change has taken place in growth rate, age at slaughter, and feed conversion of commercial broilers. The majority of that change has been brought about by the efforts of commercial broiler breeding organization (Sherwood, 1977; Havenstein *et al.*, 1994a & b). The productive

performance which varied in different commercial broiler strain has been documented by Stino *et al.* (1977), Souza *et al.* (1996) and Abdou *et al.* (1998).

Genetic differences in immune responsiveness and resistance to certain diseases of chickens have been shown to be influenced by the Major Histocompatibility Complex (MHC), immunoglobulin allotypes and genes not associated with either MHC or allotypes (Van der Zijpp, 1983; Ruff and Bacon, 1984; Puzzi *et al.*, 1990 and Lamont, 1991). The genetic makeup of commercial broilers has been dramatically changed by selection for superior growth rate and may be coincidentally accompanied by decreased resistance to disease or reduce immunological response (Han and Smyth, 1972; Saif *et al.*, 1984; Sacco *et al.*, 1991, 1994a and b; Tsai *et al.*, 1992; Qureshi and Havenstein, 1994; and Bayyari *et al.*, 1997). The reciprocal situation, selection for improved immunological response, has also been shown to result in decreased body weight (Siegel and Gross, 1980; Siegel *et al.*, 1982; Van der Zijpp, 1983; Okada *et al.*, 1988; Martin *et al.*, 1990; Afrazet *et al.*, 1994; Bayyari *et al.*, 1997).

Immune response and disease resistance in young broiler chicks are crucial for the survival and productivity of broiler chicks. They reach market weight at a continuously earlier age, ranging mostly between five to seven weeks (Yonash *et al.*, 1996).

The purpose of this study was to compare the immune function of six commercial broiler strains. The immune system functions were assessed in terms of antibody titer against Newcastle disease virus and Sheep Red Blood Cells, cutaneous basophil hypersensitivity, and relative weight of bursa and spleen.

## MATERIALS AND METHODS

Three thousand hatching eggs from each of six commercial broiler strains (Hubbard, Arbor Acres, Cobb, Lohman, ISA Vidate, and Avian) were obtained from different commercial broiler breeder companies.

The isolation test that was carried out in Animal and Poultry Health Institute on 30 eggs from each strain proved that eggs were free from *Mycoplasma gallisepticum* and *synoviae*. The eggs were incubated in the hatchery of Cairo Poultry Company. After hatching, all chicks were wing-banded, placed intermingled in traditional commercial farm (open sided house) located in Kafr-El-Amar village - Qalyubia.

During the first four weeks of age, the chicks were fed a commercial starter ration (3000-3100 K cal ME/KG and 22% crude protein) and then fed a commercial finisher ration (3300 -3400 Kcal ME/KG and 20% crude protein). During the entire experiment, feed and water were provided *ad libitum* and illumination was provided continuously (24 hrs / day).

### Immunization and Titration

Live Newcastle disease vaccine (NDV) was administered at five and 20 days of age using B1 and lasota strain vaccine, respectively, whereas live infection bursal disease vaccine (IBD) was administered at 14 and 22 days of age. Drinking water was administered with live vaccines. At ten days of age 0.5 ml bivalent inactivated vaccine (NDV and IBD) was injected subcutaneously in the back of the neck.

Blood samples were collected randomly from ten chicks of each strain at one day of age (to detect maternal antibody titer), and at two, three, four, five and six weeks of age. Antibody titer against NDV was detected by hemagglutination inhibition (HI) test as described by Beard (1980).

At six weeks of age, ten chicks from each strain were injected intravenously in the brachial vein with 0.2 ml of 10% suspension of packed sheep red blood cells (SRBC). Sera were collected on the seventh day postimmunization and antibody titer against SRBC were determined using the microtiter procedure described by Van der Zijpp and Leenstra (1980). Titers were expressed as the log<sub>2</sub> of the reciprocal of highest dilution giving complete agglutination.

### Cutaneous Basophil Hypersensitivity (CBH) Responses

Ten male chicks from each strain were randomly selected at 6-weeks of age. *Cutaneous Basophil Hypersensitivity* response was determined by injecting 0.1 ml of phytohemagglutinin-p (PHA-P) (100 µg/ml) subcutaneously into a defined area on the right wattle, whereas saline (0.1ml) was injected in the left wattle and served as a control. The thickness of both wattles at 24 hr after injection were measured in mm using a micrometer. The CBH response was calculated as a relative response. [thickness of right wattle(PHA-P response) ÷ thickness of left wattle(saline response)].

### Relative Bursa of Fabricius and Spleen Weight

At two, four, and six weeks of age, ten chicks from each strain were sacrificed, and bursa of fabricius and spleen were removed and weighed. Their relative weights were expressed as percentage of body weight (milligram of organ /100 grams of body weight).

### Statistical Analyses

The data were analyzed using the SAS (1988) general linear model procedure, with one-way ANOVA model using the strain as main effect. Where appropriate, means were separated using Duncan's Multiple Range test.

## RESULTS AND DISCUSSION

**Antibody titer:** The maternal antibodies are called passive acquired immunity and believed to provide temporary protection during the critical period before and after hatching when the immune system is still immature (Seto,1981).

Maternal antibody titers against NDV were significantly ( $P < .05$ ) higher in both Arbor Acres and Lohman chicks than those of Hubbard, ISA, and Avian chicks (Table 1). The variation in maternal antibody titers among strains may be attributed to immunological status of parent stocks, but not to the genetic makeup. Whether, about 50% of antibody level present five days before ovulation in the breeder hens is usually transferred to the newly hatched chicks (Eidson *et al.*,1980). The maternal antibodies that circulate in the blood stream of newly hatched chicks are composed only from immunoglobulin G. It is transferred from the blood of the hen into the ovarian follicles and are incorporated into the yolk during oogenesis and released into the circulation when the yolk is digested by the embryo. It could be detected as early as the 11<sup>th</sup> day of incubation (Solomon, 1971). Both immunoglobulin M and A are transferred to the eggs through the albumen. During embryogenesis, the developing embryo swallows the albumen and the two antibodies are deposited and act locally in the intestinal and respiratory tracts of the chick (Gordon and Jordan, 1982). It is very important to measure the maternal antibody titer in the newly hatched chicks, and to know their half life in order to predict when the first vaccination should occur.

Table 1. Mean ( $\pm$  SEM) antibody titer against NDV of different commercial broiler strains at various ages

Strain	Age(wk)					
	0*	2	3	4	5	6
Arbor	6.4 $\pm$ .31 <sup>a</sup>	3.4 $\pm$ .21 <sup>b</sup>	4.4 $\pm$ .38 <sup>c</sup>	6.5 $\pm$ .27 <sup>a</sup>	4.9 $\pm$ .24 <sup>c</sup>	6.2 $\pm$ .29 <sup>a</sup>
Acres						
Lohman	5.8 $\pm$ .31 <sup>a</sup>	3.2 $\pm$ .20 <sup>b</sup>	4.8 $\pm$ .38 <sup>abc</sup>	6.7 $\pm$ .27 <sup>a</sup>	5.2 $\pm$ .24 <sup>bc</sup>	6.1 $\pm$ .29 <sup>a</sup>
Cobb	5.5 $\pm$ .3 <sup>ab</sup>	3.5 $\pm$ .20 <sup>b</sup>	4.5 $\pm$ .38 <sup>bc</sup>	6.2 $\pm$ .27 <sup>a</sup>	5.9 $\pm$ .24 <sup>a</sup>	6.5 $\pm$ .29 <sup>a</sup>
Hubbard	4.7 $\pm$ .3 <sup>ac</sup>	4.6 $\pm$ .20 <sup>a</sup>	4.1 $\pm$ .38 <sup>c</sup>	5.9 $\pm$ .27 <sup>a</sup>	4.5 $\pm$ .24 <sup>c</sup>	5.8 $\pm$ .29 <sup>a</sup>
ISA	4.0 $\pm$ .3 <sup>dc</sup>	3.3 $\pm$ .20 <sup>b</sup>	5.6 $\pm$ .38 <sup>a</sup>	6.7 $\pm$ .27 <sup>a</sup>	5.9 $\pm$ .24 <sup>a</sup>	6.2 $\pm$ .29 <sup>a</sup>
Avian	3.5 $\pm$ .31 <sup>d</sup>	3.6 $\pm$ .22 <sup>b</sup>	5.3 $\pm$ .38 <sup>ab</sup>	6.3 $\pm$ .27 <sup>a</sup>	5.7 $\pm$ .24 <sup>ab</sup>	5.8 $\pm$ .29 <sup>a</sup>

- Value in the same column having different superscripts are significantly different ( $P < 0.05$ ).

\* Maternal antibody titer.

The immunity induced by immunization or infection is called active acquired immunity (Gordon and Jordan,1982). The antibody titers against NDV that represent acquired immunity are shown in Table (1). The ranking of strains varied with age. At two weeks of age, Hubbard chicks produced significantly

( $P < 0.05$ ) higher antibody titers than any of the other strains. ISA vidette chicks produced significantly higher antibody titer than Arbor Acres, Cobb and Hubbard at three weeks of age but not than Lohman and Avian. While at five wks of age both ISA and Cobb chicks produced significantly higher antibody titer than Arbor Acres, Lohman and Hubbard but not than Avian. On the other hand, the superiority of antibody titer of Hubbard chicks that was observed at two weeks of age reversed to the poorest level at 3, 4 and 5 weeks of age. At four and six weeks of age there were no significant differences between the different broiler strains.

The antibody titers against SRBC are illustrated in Figure (1). No significant differences ( $P < 0.05$ ) were observed in antibody titer against SRBC among the different strains, although ISA vidette produced the highest level. However, both Arbor Acres and Avian chicks produced the lowest level.

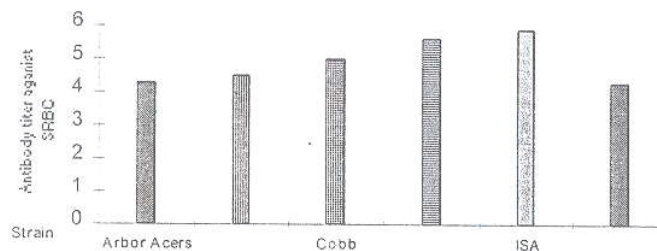


Figure 1. Mean antibody titers against SRBC of six wk. old commercial broiler strains.

The significant variation on antibody titer against NDV but not against SRBC among strains were partially consistent with the result of Van der Zijpp (1983), who reported significant differences in antibody titer of SRBC between cockerels of three genetic origins (White Plymouth Rock, White leghorn, and Warren).

The present study is consistent with the results of Biozzi *et al.* (1970), Peleg *et al.* (1976) and Inooka *et al.* (1984). They found that antibody producing ability (to disease antigens such as NDV or to nondisease antigens such as SRBC) was influenced by genetic factor. The higher antibody titer level, that was produced by ISA vidette chicks against both NDV and SRBC, supports the result of Biozzi *et al.* (1980). They demonstrated that antibody producing ability to SRBC was controlled by multiple genes. It could be correlated with other immune abilities such as antibody producing ability to several antigens or resistance to several diseases. These facts suggest that the genetic regulation for antibody production are involved with those of general antibody production and resistance, namely common genetic control system, exist among mechanisms in disease resistance and antibody production (Inooka *et al.*, 1984).

### Cutaneous Basophil Hpersensitivity (CBH)

CBH is elicited in chicken by an intradermal injection of PHA-P. It is a simple and useful method for assessing *in vivo* cell mediated immunocompetence. It has been used widely in previous investigation (Stadecker *et al.*, 1977; Backman and Mashaly, 1987; and Brake *et al.*, 1988). Evaluation of the effect of genetic origin on CBH is illustrated in Fig. (2). No significant differences in the Wattle swelling test were found between strains, however Lohman chicks had higher Wattle swelling than the other strains. These results are partially not in agreement with those of Van der Zijpp (1983), who detected significant differences in CBH of three genetically different strains (white Plymouth Rock, White Leghorn, and a medium heavy breed cross (Warren).

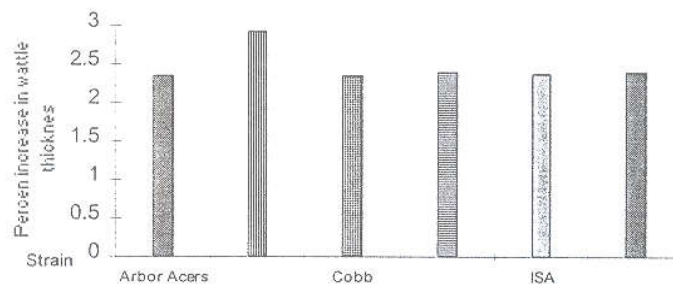


Figure 2. Percent increase in wattle thickness following PHA injection in different commercial broiler strains .

This contradiction may be due to strain differences. 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 chickens for various T-cell functions, suggests that many of these functions were highly heritable but linked to MHC nor to Ig allotypic loci (Miggiono *et al.*, 1976; Pink and Miggiono, 1977; and Lasilla *et al.*, 1979). The similarity in wattle test among strains indicated that the activities of immunocompetence responsible for CBH are similar in all strains .

### Relative Bursa and Spleen Weight :

Bursa of Fabricius is a primary lymphoid organ in birds. It is found at the junction of the hind gut and cloaca. It serves as a site for B cell maturation (Glick *et al.*, 1956). In the present study generally, the bursa reaches its maximum relative weight at two weeks of age, then regresses (Table 2). This observation agrees with Glick (1994), who showed that the bursa has three

phases for growth pattern : 1) the most rapid growth during the first three weeks after hatch, 2) a plateau period, and 3) regression .

Table 2 . Relative bursa of Fabricious weight ( ± SEM ) of different commercial broiler strains at 2 , 4 and 6 weeks of age

Strain	Age (wk)		
	2	4	6
Arbor Acres	173.0 ± 1.6 <sup>b</sup>	161.9 ± 2.2 <sup>a</sup>	116.6 ± 15.2 <sup>a</sup>
Lohman	221.9 ± 1.6 <sup>ab</sup>	195.8 ± 2.2 <sup>a</sup>	88.5 ± 15.2 <sup>ab</sup>
Cobb	243.5 ± 1.6 <sup>a</sup>	163.7 ± 2.2 <sup>a</sup>	66.0 ± 15.2 <sup>b</sup>
Hubbard	203.9 ± 1.6 <sup>ab</sup>	149.6 ± 2.2 <sup>a</sup>	83.1 ± 15.2 <sup>ab</sup>
ISA	217.1 ± 1.6 <sup>ab</sup>	187.3 ± 2.2 <sup>a</sup>	105.3 ± 15.2 <sup>ab</sup>
Avian	237.2 ± 1.6 <sup>a</sup>	139.8 ± 2.2 <sup>a</sup>	85.8 ± 15.2 <sup>ab</sup>

- Value in the same column having different superscripts are significantly different (P<0.05).

The rank of relative bursa weight also varied among strains with age, i.e. at two weeks of age both Cobb and Avian chicks had significantly ( P<.05 ) higher relative weight than those of Arbor Acres chicks . On the other hand, contrary was observed at six weeks of age, Whether, Arbor Acres chicks had significantly (P<.05) higher relative bursa weight than those of Cobb chicks. This may indicate that the bursa of both cobb and Avian chicks reaches maturation earlier than those of Arbor Acres chicks.

The spleen is a secondary lymphoid organ responsible for filters blood borne antigens and is a major site for mounting immune responses. Generally, the maximum relative weights of spleen were detected at six weeks of age (except those of Arbor Acres chicks, that maximum relative weight observed at five weeks of age). No significant differences were observed in relative spleen weight among strains at all ages studied except at four weeks of age, Arbor Acres chicks had significantly higher relative spleen weight (Table3) .

Table 3 . Relative spleen weight ( ± SE ) of different commercial broiler strains at 2 ,4 and 6 weeks of age

Strain	Age (wk)		
	2	4	6
Arbor Acres	74.0 ± 7.4 <sup>a</sup>	208.2 ± 14 <sup>a</sup>	165.4 ± 18.1 <sup>a</sup>
Lohman	87.0 ± 7.4 <sup>a</sup>	177.0 ± 14 <sup>ab</sup>	194.5 ± 18.1 <sup>a</sup>
Cobb	81.6 ± 7.4 <sup>a</sup>	154.7 ± 14 <sup>b</sup>	168.8 ± 18.1 <sup>a</sup>
Hubbard	77.3 ± 7.4 <sup>a</sup>	158.2 ± 14 <sup>b</sup>	202.5 ± 18.1 <sup>a</sup>
ISA	87.9 ± 7.4 <sup>a</sup>	152.6 ± 14 <sup>b</sup>	178.4 ± 18.1 <sup>a</sup>
Avian	82.3 ± 7.4 <sup>a</sup>	142.2 ± 14 <sup>b</sup>	176.5 ± 18.1 <sup>a</sup>

- Value in the same column having different superscripts are significantly different (P<0.05).

In conclusion, the results reported here would imply that the immune status varied between different commercial broiler strains. Thus it is important to measure the antibody titer against NDV in broiler chicks.

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### إختلاف سلالات بدارى التسمين التجارية فى بعض المقاييس المناعية

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تم قياس مستوى الاجسام المناعية المتكونة ضد مرض النيوكاسل وكرات الدم الحمراء للغنم كما تم تقدير المناعة الخلوية والوزن النسبي للاعضاء الليمفاوية (البرسا - الطحال) وذلك في ستة أنواع بدارى تسمين تجارية وهى : الهابرد - الاربوراكز - كب - إفيان - لوهمان والايزا .

أوضحت نتائج مستوى الاجسام المناعية ضد مرض النيوكاسل أن هناك تبايناً بين الأنواع حسب العمر فعلى سبيل المثال فإن المناعة الامية والتي قيست فى اليوم الأول من العمر كانت أعلى معنوياً فى كتاكيت الاربوراكز واللوهمان عنه فى كتاكيت الهابرد - الايزا والإفيان ، بينما تغير الوضع بعد ذلك حيث انه على عمر اسبوعان احتلت كتاكيت الهابرد المرتبة الاولى بينما على عمر ٣ اسابيع فإن كتاكيت الايزا حازت هذه المكانة .

من ناحية اخرى لم تلاحظ اى اختلافات معنوية فى مستوى الاجسام المناعية ضد كرات الدم الحمراء للغنم أو فى اختبار فرط الحساسية (والذى يعتبر مؤشر للمناعة الخلوية) بين هذه الأنواع .

نلاحظ أيضاً أن الوزن النسبى للبرسا والطحال تباين بين الأنواع تبعاً للعمر . فعلى سبيل المثال احتل الوزن النسبى لطحال كتاكيت الاربوراكز المرتبة الاولى عند عمر ٤ اسابيع وكان اعلى معنوياً عن مثيله فى الأنواع الاخرى (باستثناء كتاكيت اللوهمان) .

كما أن الوزن النسبى للبرسا كان أعلى معنوياً على عمر اسبوعين فى كتاكيت الكب والإفيان عنه فى كتاكيت الاربوراكز ولكن تبدل الوضع عند عمر ستة أسابيع .