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Immunocompetence Profile of Saudi Native Chickens Compared to Exotic Breeds under High Environmental Temperature

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Abstract: A study was conducted to evaluate the immunocompetence profile of Saudi native chicken compared to not commercial (Leghorn) and commercial (Lohmann) breeds under high environmental temperature. A total of 600 1-d-old chicks representing three studied breeds (200 each) were used. All birds were kept under similar husbandry, environmental and hygienic conditions. Measurements of cell-mediated and humoral immune responses were determined for the different breeds. The current results revealed that the Saudi native and Leghorn chickens had significantly lower body weight compared to the Lohmann breed. There was no significant difference among breeds for antibody levels against Newcastle disease virus vaccine (NDV). However, Saudi native chickens recorded slightly higher level compared with the other two breeds at earlier tested time. Saudi native chickens generally expressed significantly ($P<0.05$) higher cell-mediated immune response *in vivo* than commercial egg-type chickens, suggesting that raising chickens under high environmental temperatures has a more negative effect on cellular immune response of commercial chickens rather than native ones. In terms of relative weight of lymphoid organs, there were no significant differences among breeds for relative weight of either spleen or bursa of Fabricius. Nevertheless, significantly ($P<0.0001$) lower percentage of thymus was found in Leghorn breed compared to native and the Lohmann breeds. Concerning globulin level, it could be noticed that the Saudi native chickens recorded significantly highest level ($P<0.001$) compared to the other breeds. Regarding to humoral immunity, the results indicated that there was no significant difference among breeds for total antibody concentrations against sheep red blood cells (SRBC). It may be concluded that Saudi native chickens are less affected by hot temperature for cellular immunity than commercial chickens.

Key words: Innate immunity, cell mediated immunity, Saudi native chicken, NDV antibody, hot climate

INTRODUCTION

Native chickens play an important role in the household food supply and socio-cultural community in many developing countries. Currently, the world poultry production is facing frequent outbreaks of precarious infectious diseases like avian influenza and Salmonella which cause serious health problems and economic loss. High yield commercial chickens may lose the genetic resistance to various diseases through the long-term of selection programs. Lower disease resistance coupled with hot environment in open and semi-closed chicken houses resulted in rapid transmission of infectious diseases. A global great attention of conservation of native chicken genetic resources had been given. Generation after generation, the indigenous chicken likely adapted to harsh and extreme local environmental conditions, which enhanced their immune resistance. However, several studies showed that local chicken breeds had a considerably better immunity and higher resistance to diseases compared to exotic breeds, particularly under harsh environmental

conditions (Fathi *et al.*, 2007; 2012; El-Safty, 2012). Oluyemi *et al.* (1979) reported that the Egyptian Fayoumi breed was resistant to avian leukosis complex.

Genetic selection for disease resistance to major infectious agents in chicken has been recognized for a long time as an adjunct to non-genetic means of disease control (Boa-Amponsem *et al.*, 1997; El-Safty *et al.*, 2006; Chatterjee *et al.*, 2007). Moreover, as a consequence of natural selection, indigenous breeds have been shown to be more resistant to disease (Minga *et al.*, 2004). The Aseel, an ancient chicken breed from India, has a reputation for being well adapted to tropical conditions, and has been found to be the most immunocompetent in tests comparing it to a number of other breeds (Singh *et al.*, 2004). Superiority in immunological parameters such as phagocytic activity and H/L ratio was found in Libyan native chickens compared with broiler chicks (El-Safty, 2012). A report of Permin and Ranvig (2001) showed that high-yielding Lohmann Brown hybrids had lower worm burden and worm-egg excretion than the traditional breed Danish

Landrace following infection with the helminth *Ascaridia galli*, which indicates that some sort of genetic resistance exists. A total score index for immunocompetence traits of CARI-Nirbheek chickens, a cross of CARI Red and Aseel breeds, was considerably better than that of the high-yielding breeds such as White Leghorn and broiler-type birds (Singh *et al.*, 2004). Emam *et al.* (2014) compared Iranian indigenous chicken populations and commercial laying hen strains for immune response profiles. They suggested that the variation observed in immune responses between these strains of chickens is most likely due to differences in the genetic background between each strain of chicken rather than by commercial selection programs for high production. Romanov *et al.* (1996) stated that local breeds have genes that are capable to increase their adaptation to the hazard environmental variation including prevailing diseases. Baelmans *et al.* (2004) showed different levels of classic and alternative complement in eco-breeds, which also different from commercial layers (Parmentier *et al.*, 2004).

Genetic improvement toward high-yielding chickens would adversely affect heat tolerance and immunity of the birds. It has been established that the heat stressed chickens had a lower resistance to diseases. Mashaly *et al.* (2004) indicated that heat stress not only adversely affects production performance but also inhibits immune function. Regnier *et al.* (1980) suggested that heat-induced immunosuppression may depend on breed of the birds. Heat stress was also reported to cause a reduction in antibody production in young chickens (Zulkifli *et al.*, 2000). White blood cell counts and antibody production were significantly inhibited in heat stressed hens (Mashaly *et al.*, 2004). Little is known of natural disease resistance and abilities of the immune system of Saudi native chicken compared with commercial strains under high environmental temperature. In this context, the present study aimed at comparing the immunocompetence profile of native chickens and either standard or commercial breeds raised under high environmental temperature in the Al-Qassim province, Saudi Arabia.

MATERIALS AND METHODS

Flock management: A total of 600 healthy 1-d-old chicks (200 White Lohmann, 200 White Leghorn and 200 Saudi native) were used in the current study. All birds were placed in floor pens bedded with wood shaving litter. The birds were weighed to the nearest gram every four weeks during the growing period. At 16 weeks of age, the females were transferred to individual wire cages until the end of the experiment. All chicks were kept under identical environmental, health and nutritional conditions in open houses without temperature-controlled facilities. Feed and water for all birds were

provided *ad libitum*. The average of high ambient temperature during the growing and laying periods was $34 \pm 1.5^{\circ}\text{C}$.

Antibody titer against Newcastle disease virus (NDV):

Chicks were orally vaccinated with Newcastle disease "La-Sota" vaccine (Intervet International B.V., Boxmeer, Holland) via drinking water on d 12 and 28. Fifteen chicks from each breed were randomly selected and blood was collected from the wing vein. The sera were stored at -20°C until the end of the experiment. Antibody titers against Newcastle disease virus (NDV) was determined by the Haemagglutination Inhibition (HI) test as described by Alexander *et al.* (1983) on d 28, 35 and 42. The data were expressed as the logarithm base 2.

Relative lymphoid organs: At six weeks of age, 15 chicks were randomly chosen and weighed for determination of relative weight of lymphoid organs. The birds were slaughtered and the bursa of Fabricius, spleen, thymus (all lobes from left side of the neck) were removed and weighed to the nearest milligram. The relative weights of lymphoid organs were compared in the different breeds.

Assay for cell-mediated immunity: Cell-mediated immunity *in vivo* was evaluated by injection of the mitogen phytohemagglutinin-P (PHA-P) into the wattle. Fifteen laying hens from each different breed aged 36 weeks were randomly assigned. Each hen was intradermally injected in the right wattle with 100 μg phytohemagglutinin-P (Sigma Chemical Co., St. Louis, MO 63178) in 0.1 ml of sterile saline. The initial wattle thickness was measured with a constant tension caliper before injection and at 24, 48 and 72 hr after PHA-P injection. The resultant swelling response in the wattle was calculated as the difference between its thickness before and after injection.

Total protein, albumin and globulin: After completion of PHA-P assay, a 2.0 ml blood sample was withdrawn from the jugular vein. Plasma total protein and albumen were determined using spectrophotometer with commercial kits (Stanbio Laboratory, 1261 North Main St. Boerne, Texas 78006, USA). The globulin was calculated as the difference between the total protein and albumen.

Antibody response against sheep red blood cells: At 46 weeks of age, 20 birds per breed were injected intramuscularly with SRBC (7% suspension in PBS, 1 ml/bird). Blood samples were collected at 7, 14 and 21 days after the injection. Serum was stored at -20°C until tested. Serum was heat inactivated at 56°C for 30 min and then analyzed for total immunoglobulins (Ig), mercaptoethanol-sensitive (MES) IgM, and

mercaptoethanol-resistant IgG antibodies as previously described (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994; Fathi *et al.*, 2003). Titers were expressed as the log₂ of the reciprocal of the highest dilution giving complete agglutination. The difference between the total and the IgG response was considered to be the IgM antibody level.

Statistical analysis: Data were subjected to a one-way ANOVA using JMP Ver. 11 (SAS Institute, 2013) with dietary treatment as a fixed effect. The results are presented as mean and the pooled SEM. The significance of difference among the groups was assessed using Duncan's new multiple range test. Significance was set as $P < 0.05$.

RESULTS AND DISCUSSION

Body weight: The results of body weight for each breed are presented in Table 1. Lohmann chickens recorded significantly ($P < 0.05$) heavier body weight compared to either Saudi native or Leghorn birds at all studied ages during the growing period. Body weight of Leghorn and native birds was almost the same. This result was also found by Demeke (2003), who stated that there was no difference between White Leghorn and local chickens raised under scavenging condition in mean daily body weight gain during the first two months of age.

Newcastle antibody titer: The antibody titers as detected by haemagglutination for all breeds following vaccination with NDV vaccine are illustrated in Fig. 1.

As shown in Fig. 1, Saudi chickens had the higher level of the antibody titer response against Newcastle disease vaccine compared to the other two breeds at 28 d., but this difference was not statistically significant. At later age, the difference among breeds was diminished. It is obvious that Saudi native chickens had the higher level of specific antibodies compared to Leghorn breed at earlier ages. That is meant that Native birds are capable to defeat Newcastle disease at early time of infection. Taha *et al.* (2012), stated significant differences between breeds for antibody titer responses to Newcastle disease virus. Moreover, higher antibody titers were found in Fayoumi lines. Additionally, Okoye *et al.* (1999) reported that the Nigerian rural chickens were more resistant to Infectious bursal disease than the exotic layer pullets. Also, Okoye and Aba-Adulugba (1998) reported that local cockerels were more resistant to *Salmonella pullorum* infection than White Rock cockerels.

PHA-P Response: Fig. 2 shows the lymphoproliferative response (swelling) due to intradermal injection of PHA-P in the wattle for the different studied breeds. Saudi native chickens recorded the highest significantly ($P < 0.05$) response compared to the other two breeds,

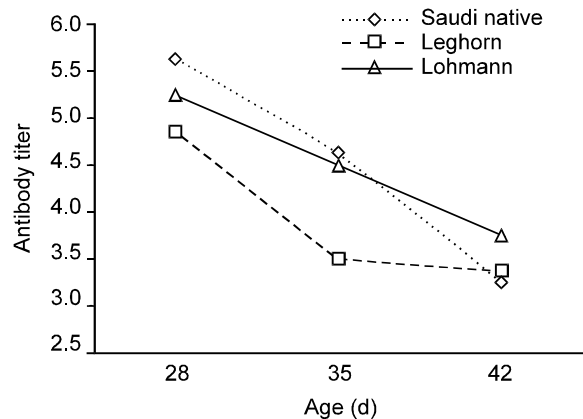


Fig. 1: Specific antibody against Newcastle Disease Virus- NDV- after 1, 2 and 3 weeks for Saudi native, Leghorn and Lohmann chickens

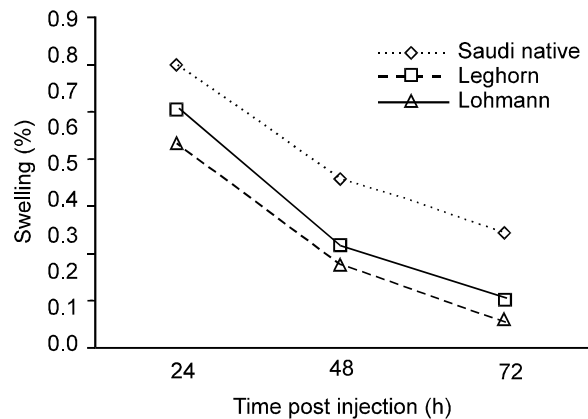


Fig. 2: The wattle swelling response after intradermal injection of phytohaemagglutinin-P in chicks expressing different breeds. Each value is the mean expressed as the difference from the pre-injection value

particularly after 72 hours of injection. The difference between Leghorn and Lohmann chickens was not statistically significant at all tested moments post PHA-P injection.

Intensive selection for production traits impaired the capability of poultry to generate protective immune response and disease resistance (Adriaansen-Tennekes *et al.*, 2009). Likewise, Tirawattanawanich *et al.* (2011) confirmed that the Thai indigenous breed had higher immune responses than those of commercial line chickens under tropical environmental conditions. The deleterious effect of high ambient temperature on immune response could be more pronounced on commercial chickens than that of native and standard breeds. This could be a consequence of the heat stress causing immunosuppression for birds frequently selected to high-yielding egg production performance. Environmental heat stress can alter the susceptibility of

Table 1: Means \pm SE of body weight (g) for Saudi native, Leghorn and Lohmann birds during the grow-up period

Age	Strain			Prob.
	Saudi native	Leghorn	Lohmann	
4 weeks	139.8 ^b \pm 1.72	155.9 ^b \pm 2.66	212.4 ^a \pm 2.75	0.0001
8 weeks	383.5 ^b \pm 5.70	387.7 ^b \pm 6.29	481.7 ^a \pm 4.79	0.0001
12 weeks	629.2 ^b \pm 7.96	655.0 ^b \pm 8.73	834.0 ^a \pm 6.55	0.0001
16 weeks	785.7 ^b \pm 8.87	800.1 ^b \pm 10.14	1034.1 ^a \pm 6.89	0.0001

^a and ^b Means within the same row with different letters are significantly differed

Table 2: Means \pm SE of weight and percentage of lymphoid organs (spleen, bursa and thymus) at 6 weeks of age

Lymphoid organ	Strain			Prob.
	Saudi native	Leghorn	Lohmann	
Spleen, g	0.56 ^b \pm 0.04	0.69 ^{ab} \pm 0.08	0.84 ^a \pm 0.07	0.01
Spleen %	0.17 \pm 0.01	0.21 \pm 0.03	0.20 \pm 0.01	NS
Bursa of Fabricius, g	0.44 ^{ab} \pm 0.03	0.37 ^b \pm 0.05	0.56 ^a \pm 0.05	0.01
Bursa %	0.13 \pm 0.01	0.11 \pm 0.02	0.14 \pm 0.02	NS
Thymus, g	1.47 ^a \pm 0.14	0.95 ^b \pm 0.08	1.67 ^a \pm 0.14	0.0005
Thymus %	0.44 ^a \pm 0.03	0.28 ^b \pm 0.02	0.40 ^a \pm 0.02	0.0001

^a, ^b and ^{ab} Means within rows with different superscript are significantly ($P < 0.05$) different from each other. NS. Non-Significant.

Table 3: Means \pm SE of serum total protein, albumin and globulin amounts at 36 weeks of age

Item	Strain			Prob.
	Saudi native	Leghorn	Lohmann	
Total Protein, (g/dl)	7.93 ^a \pm 0.52	5.77 ^b \pm 0.37	8.07 ^a \pm 0.46	0.01
Albumin Level, (g/dl)	3.82 ^a \pm 0.40	2.56 ^b \pm 0.27	4.26 ^a \pm 0.47	0.01
Globulin Level, (g/dl)	4.11 ^a \pm 0.63	3.21 ^b \pm 0.31	3.81 ^b \pm 0.64	0.001

^a, ^b and ^c Means within rows with different superscript are significantly ($P < 0.05$) different from each other.

poultry to infective agents and it is therefore important to learn how stress affects the immune system (Hangalapura *et al.*, 2003; Osei-Amponsah *et al.*, 2013). Local breeds raised in natural environments have the potential to offer more flavourable products for the increasingly health-conscious consumer (Moula *et al.*, 2009).

Relative weight of lymphoid organs: Relative weights of lymphoid organs are listed in Table 2. The results indicated that there were no significant differences among breeds for both relative weight of spleen and bursa of Fabricius. Concerning relative weight of thymus, Leghorn birds recorded significantly ($P < 0.0001$) lowest percentage compared to Saudi native and Lohmann birds. However, the bursa and thymus are the important lymphoid organs involved in the development and differentiation of B or T lymphocytes (Eerola *et al.*, 1987; Toivanen *et al.*, 1987).

Blood parameters: Serum globulin levels may reflect antibody production. It partially reflects the potential of a bird to resist pathogen infection. The levels of total protein, albumin and globulin at 36 weeks of age are listed in Table 3. Leghorn chickens had significantly ($P < 0.01$) lowest level of total protein compared to Saudi native and Lohmann chickens. The same trend was observed for albumin level. In terms of globulin level, it

could be noticed that the Saudi native chickens recorded the highest significantly ($P < 0.001$) level compared to either standard or commercial breeds.

Antibody response against sheep red blood cells: Non-infectious antigens can be used to measure the response of the immune system as an estimate of the predicted immune activity level that would occur in an actual challenge situation (Lamont *et al.*, 2003). The use of sheep red blood cells as an antigen to compare specific humoral immunity in chicken breeds is well documented (Fathi *et al.*, 2003; Osei-Amponsah *et al.*, 2013).

The results presented in Table 4 indicated that there was no significant difference among breeds for total antibody titer against SRBC at 7, 14 and 21 d post-primary injection. The same was true for IgG and IgM levels binding SRBC. Local Ghanaian ecotypes and Sasso T-44 chicken were superior to broilers in terms of their ability to respond to SRBC antigens (Osei-Amponsah *et al.*, 2013). Ghanaian native chickens showed the highest immune response to inoculation with SRBC antigens compared with commercial broilers. This could be attributed to the fact that the Sasso T-44 is a scavenging chicken genotype in France and therefore could have similar immunological abilities as local Ghanaian chickens. Local chickens raised in extensive scavenging systems are likely to face more

Table 4: Means \pm SE of Total antibody production (Ab), immunoglobulin G (IgG) and immunoglobulin M (IgM) titer in Saudi native, Leghorn and Lohmann strains at 46 weeks of age

Date of measure	Strain			Prob.
	Saudi native	Leghorn	Lohmann	
	Total antibody level			
Day 7 Post Injection	4.57 \pm 0.53	4.50 \pm 0.42	5.00 \pm 0.31	NS
Day 14 Post Injection	3.72 \pm 0.36	3.38 \pm 0.26	3.86 \pm 0.26	NS
Day 21 Post Injection	3.00 \pm 0.31	2.76 \pm 0.16	3.00 \pm 0.31	NS
	Immunoglobulin G (IgG)			
Day 7 Post Injection	2.43 \pm 0.20	2.75 \pm 0.37	3.29 \pm 0.29	NS
Day 14 Post Injection	2.29 \pm 0.18	2.25 \pm 0.16	2.57 \pm 0.20	NS
Day 21 Post Injection	1.86 \pm 0.14	1.88 \pm 0.23	2.00 \pm 0.22	NS
	Immunoglobulin M (IgM)			
Day 7 Post Injection	2.14 \pm 0.55	1.75 \pm 0.25	1.71 \pm 0.29	NS
Day 14 Post Injection	1.43 \pm 0.20	1.13 \pm 0.23	1.29 \pm 0.29	NS
Day 21 Post Injection	1.14 \pm 0.26	0.88 \pm 0.13	1.00 \pm 0.31	NS

NS. Non-Significant.

health challenges and hence are likely to be more immune competent. For instance, Egyptian indigenous naked neck and normally feathered chickens have been reported to have higher immune response than commercial chicken lines (El-Safty *et al.*, 2006). Rajkumar *et al.* (2011) also reported higher antibody response in naked neck breeds of chicken. In conclusion, although Saudi native chickens have a lighter body weight, they are high-responder for cell mediated immunity compared to commercial chickens under high environmental temperature.

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