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CHARACTERIZATION OF NEWCASTLE DISEASE ANTIBODY RESPONSE AND SOME RELATED PERFORMANCE INDICATORS OF TWO LOCAL SAUDI CHICKEN LINES AND TWO CROSS LINES DURING THE REARING PERIOD

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ABSTRACT

The present study was conducted to assess antibody response against Newcastle disease (ND) vaccination of one hundred and sixty female chicks represented equally two local Saudi chicken lines Hajar 1 and Hajar 2, In addition to crossbreed HiH1, and HiH2 lines during the rearing period from four to 16 wk of age. Body weight (BW), hemoglobin concentration (Hb) and hematocrit percent (Ht) were evaluated as accompany performance indicators. The chicks were housed in a cage housing system and fed commercial rations. Birds were received identical vaccination program. Body weights were recorded for all birds individually at 4,8,12, and 16 weeks of age. Blood samples were collected in both heparinized and plain tubes at 4, 8, 12, and 16 weeks of age. Circular micro-capillary tube reader was used to evaluate Ht values. Haemoglobin concentrations were determined through cyanomet hemoglobin method. ND antibody titers were assayed using Enzyme-Linked Immunosorbent Assay (ELISA) kit. The two crossbreed lines showed superior body weight, especially starting from the 8th week of age while HiH2 and Hajar2 lines showed higher ND titers than Hajar1 and HiH1 lines. Hb values were significantly higher for Hajar1 and Hajar2 lines. Ht did not show differences among the chicken lines at any measuring point. The diverse genetic background of the birds is a key role in shaping the cross line birds' immune capacity against ND. The superior body weight of the cross lines is not necessarily an indicator of their immune response tendency.

Key words: Immune response, ELISA, Saudi chicken lines, crossing, ND.

INTRODUCTION

A recent study established and characterized a base population of new Saudi chicken lines named Hajar1 and Hajar2 (Ahmed and Alabbad 2014). The two lines demonstrated phenotypic and genetic diversity (Alabbad, 2014; Ahmed and Rezk, 2015). In addition, they have shown high levels of maternal immunity transfer to the newly hatched chicks (Ahmed 2011). Their unique response to one of the Orthomyxovirida virus group, Newcastle disease, vaccine under stressful conditions compare to commercial lines was reported (Alamer and Ahmed 2012). Newcastle disease virus (NDV) causes a high percentage of mortality rate in susceptible chickens; those losses are seen most often in domestic fowls (Ibu *et al*., 2009). Genetic improvement processes are promising to improve antibody response against ND in chickens (Liu *et al*., 2014). Breeding companies tended to consider growth and immune response as selection tools for commercial breeding (Cheema *et al*., 2007). Crossing of endangered local breeds and established breeds can result in economic benefits from the enhanced productivity of the cross line, and concurrently the conservation of native animal diversity (Shrestha, 2005). The only way to control outbreaks of the ND in local chickens is use of effective vaccines (Paulillio *et al*., 2009). Al-Zubeedy (2009) stated that humoral immunity is the essential protection against the NDV in chickens. Therefore, the long lasting antibody level to NDV in flocks of chickens could be done through vaccination programs. Several ELISA kits are available commercially for antibody detection against NDV and the main advantage of the ELISA is the accuracy, in addition to reproducibility, with high sensitivity and specificity (Alexander, 2003). In spite of the suspected tolerance of the local chicken to the harsh environment, this heat tolerance could correlate with less growth rate (Lamont *et al*., 2014) or less productivity and reduced immune competence (Rajkumar *et al*., 2011). Currently there are not any information about the immune response of the two cross line chicken HiH1 and HiH2 during the rearing period. Additionally, there is a shortage of information about the immune profile of the two local Saudi chicken lines Hajar1 and Hajar2 during this period. The present experiment tried to participate in drawing the complete picture about the four chicken lines during the rearing period through characterization of Newcastle disease antibody response using ELISA technique and assessment of some other related indicators. We hypothesized that enhanced body weight could be shown by the cross lines while local

chicken lines Hajar1 and Hajar2 will produce higher antibody levels against ND during the rearing period, with comparable values of the related traits among the four lines.

MATERIALS AND METHODS

Birds and general management: The present study was carried out in the poultry research unit, King Faisal University Experiment Station. Saudi local chicken lines Hajar1 and Hajar2 (Ahmed and Alabbad, 2014) and the cross lines HiH1, and HiH2 were used as the experimental birds in the present study (Hakami, 2015). One hundred and sixty female chicks represented all chicken lines equally. The chicks were obtained at day old from each line. At hatching, all chicks were sexed using vent sexing methodology; the experimental female chicks were obtained. Then, the chicks were wing banded with a unique serial for each line.

The chicks were housed in a cage housing system for brooding and rearing period in a closed house with two-tier cage system with the tunnel ventilation system. Each cage contained Pan Feeders and nipple drinking system with central heating and lighting system. The cage system provided floor space of 320 cm/bird. Chicks of each line were divided into three replicates.

Birds were subjected to 33ºC during the first 3 days of age, then the temperature declined gradually to reach 26ºC by the end of the third week of age. All experimental chicks were fed a commercial starter layer ration (20% crude protein and 2800 kcal ME/kg) through the brooding period. By the 6th week of age until the end of the experimental period, they fed a commercial growing ration (14% crude protein and 2700 kcal ME/kg). A photoperiod of 24 h of light was provided for the first two weeks of age thereafter, a fixed lighting system was applied. All birds were received identical vaccination program as set by the poultry unit at King Faisal university research station (Table 1).

Parameters and data collection: Body weights of all birds were recorded for all birds individually starting from the 4th week of age with 4 weeks intervals until 16 weeks of age. Blood samples were collected from the wing veins of all birds in both heparinized and plain tubes at 4, 8, 12, and 16 weeks of age. Heparinized samples were kept refrigerated for one hour. Hematocrite percent was determined directly one hour after blood collection by centrifugation of blood samples in heparinized capillary tubes, The hematocrite percent determined by circular micro-capillary tube reader. Simultaneously, Haemoglobin concentrations were determined through cyanomethemoglobin method using a commercial kit (Ref. No10751, Human Diagnostics mbH, Germany). Serum samples were separated, then, the Newcastle disease (ND) antibody titers were assayed using a commercial kit for Enzyme-Linked Immunosorbent Assay (ELISA) (X-ovo Flockscreen ND virus antibody kit, Cat NO. V125, X-OvO limited, Dunfermline, UK).

Birds in the current study were subjected to a high quality of care, according to the mandatory animal care guideline of the Deanship of scientific research at King Faisal University

Statistical Analysis: Typically, the data were subjected to a normality test, and then the data subjected to a one-way analysis of variance (ANOVA) for the effect of chicken line. Data was analyzed using the general linear model procedure. JMP IN software version 5.1 (Sall and Lehman, 2005) SAS Institute Inc., USA was used for statistical analysis. Statistical significance was considered as $P \leq 0.05$.

RESULTS

Figure 1 demonstrates the body weights of the different chicken lines from 4 to 16 weeks of age. At the 4th week of age, all chicken lines recorded almost similar body weight. Starting from the 8th week of age, body weights were affected by the genetic crossing where, HiH2 line recorded the highest body weight. Whereas, H2 recorded the lowest body weight. H1 birds were heavier than H2 birds in the 8th week of age ($P < 0.05$). At the 12th week of age both cross lines recorded apparent higher body weight ($P < 0.05$) compared to the local lines. The difference between cross lines was insignificant as same as the difference between the local lines. The chicken of the cross line HiH2 recorded an advantage in body weight over all chicken lines at the 16th week of age ($P < 0.05$), while there were not significant differences in body weight neither between HiH1 and H2 nor H1 and H2.

Comparable Hematocrit values were recorded among all chicken lines at 4, 8, 12, and 16 weeks of age (Figure 2). Hemoglobin concentration did not demonstrate significant differences ($P < 0.05$) neither between H1 and H2 birds, nor HIH1 and HiH2 birds at any measuring point (Figure 3). Local chicken lines recorded the highest significant ($P < 0.05$) hemoglobin concentration compared to both cross lines at 4, 8, and 12 weeks of age. In spite of numerical differences, H1 and HiH2 chicken recorded comparable hemoglobin concentrations at 16 weeks of age.

Figure 4 demonstrates Newcastle disease antibody titer of the four chicken lines based on ELISA titration. At the early age of the present experiment comparable antibody titer values were recorded at 4 weeks of age among all chicken lines. Although the antibody level of HiH1 birds was comparable to both local chicken lines at 8 weeks of age, but it was significantly ($P < 0.05$) lower than the HiH2 birds' antibody titer. Birds of the HiH2 line showed a consistent

trend of low antibody titer at the 12th week of age where, HiH1 birds recorded significant lower (P <0.05) antibody titer compared to H2 birds. There were no significant differences among H1, H2, and HiH2 birds' antibody titer at 12 weeks of age. The antibody response trend became stronger by the 16th week of age where, H2 chicken line and their cross line HiH2 recorded the highest significant (P <0.05) antibody titer against ND compared to H1 and HiH1 cross line

Table 1. Vaccination program of the experimental chicken lines during the first 16 weeks of age

Birds' age	Type of vaccine	Method of vaccination
5 days	HB1+IB (single dose)	Eye drops
14 days	IBD (1.5 dose)	Eye drops
22 days	Lasota (1.5 dose)	Eye drops
26 days	Lasota (1.5 dose)	Drinking water
42 days	Lasota +IB (1.5 dose)	Drinking water
50 days	Fowl box (single dose)	Wing web stab
70 days	ND oil emulsion (single dose)	Intramuscular injection

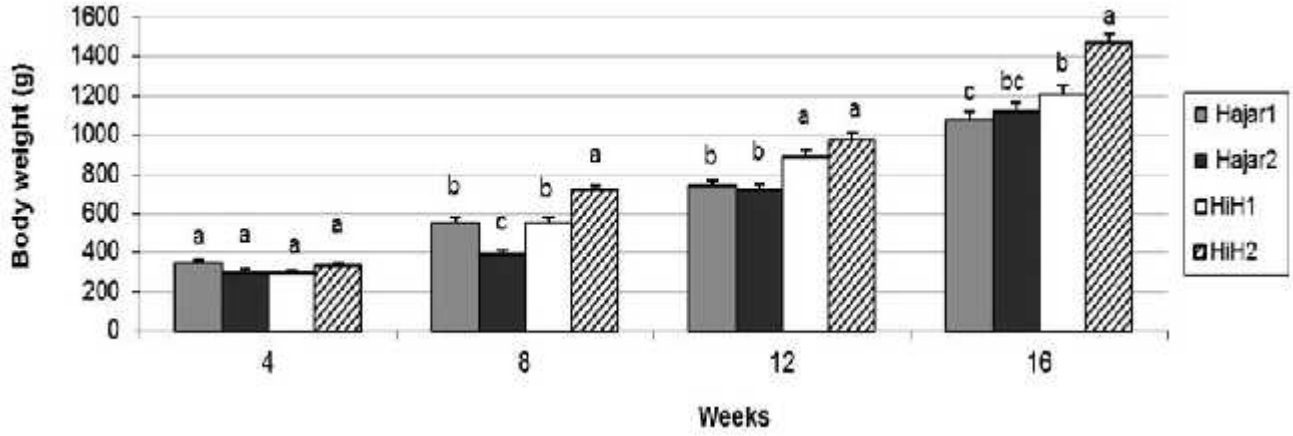

Figure (1). Body weight of the experimental chicken lines during the period from 4-16 weeks of age
* HiHi and HiH2 are Hisex sires× Hajar1 dams and Hisex sires× Hajar2 dams crosses, respectively.
a,b,c columns within a week with different superscript differ significantly (P ≤0.05)

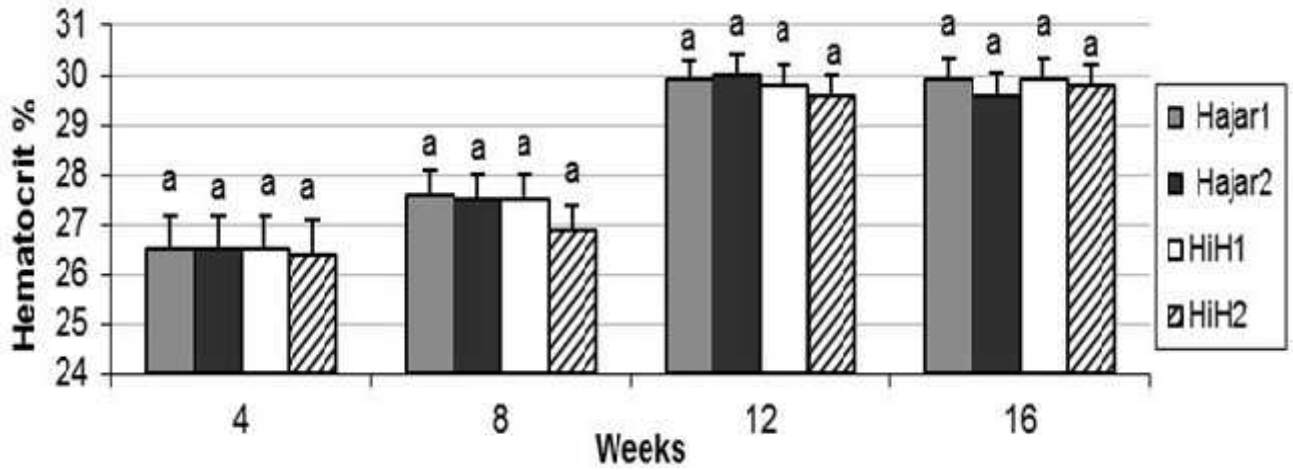

Figure (2). Hematocrit percent of the experimental chicken lines during the period from 4-16 weeks of age
* HiHi and HiH2 are Hisex sires× Hajar1 dams and Hisex sires× Hajar2 dams crosses, respectively.

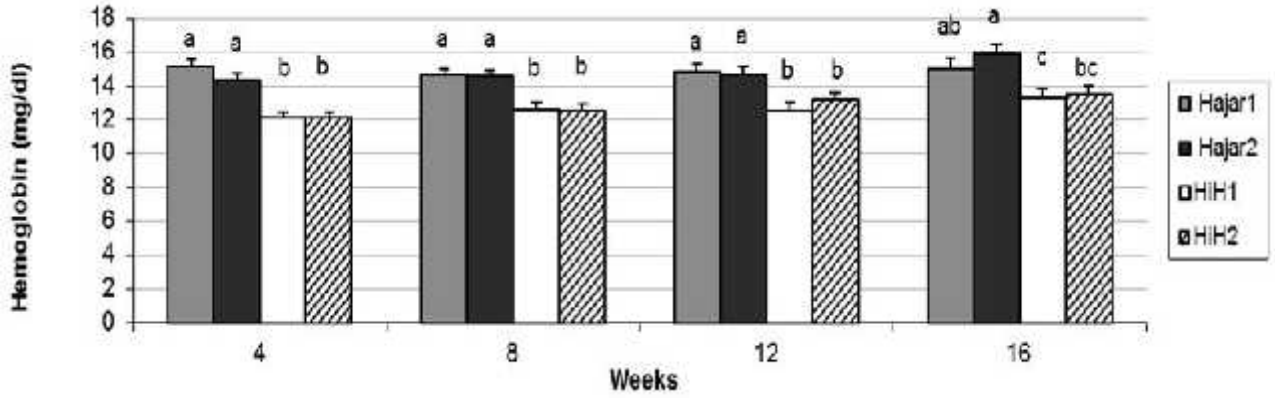

Figure (3). Hemoglobin concentration of the experimental chicken lines during the period from 4-16 weeks of age

* HiHi and HiH2 are Hisex sires× Hajar1 dams and Hisex sires× Hajar2 dams crosses, respectively.

a,b,c columns within a week with different superscript differ significantly (P ≤0.05)

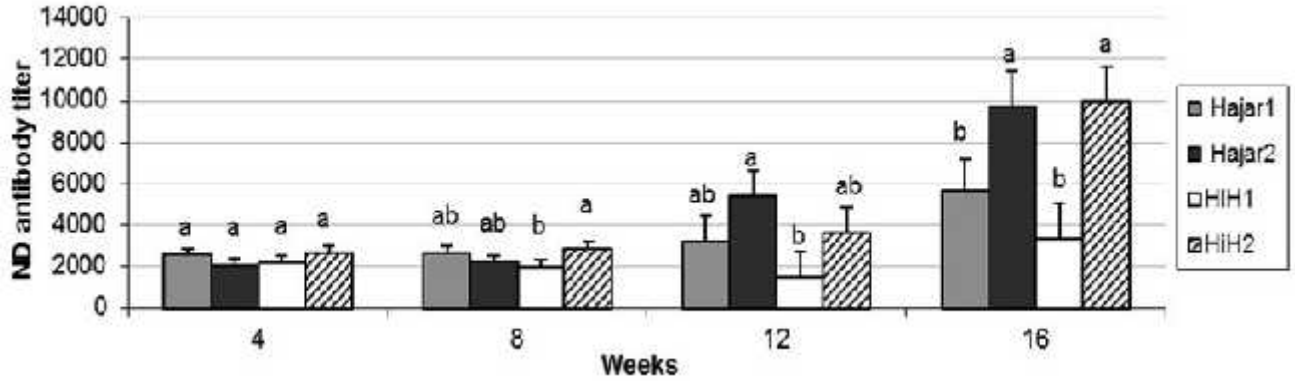

Figure (4). Serum Newcastle disease (ND) antibody titer of the experimental chicken lines during the period from 4-16 weeks of age

* HiHi and HiH2 are Hisex sires× Hajar1 dams and Hisex sires× Hajar2 dams crosses, respectively.

a,b columns within a week with different superscript differ significantly (P ≤0.05)

DISCUSSION

The current results tried to investigate the immunological performance against ND of chicken cross lines, HiH1 and HiH2, against the two Saudi chicken lines Hajar1 and Hajar2 during the rearing period. This approach is important since the cost of immune suppression or low response could be a limiting factor against sustaining the cross lines. Local chicken lines were subjected to natural selection that affect multi locus quantitative traits and associated with preserves adaptive genetic variation and fitness of individuals in their local environment, this natural selection effect have been reported earlier by Giovambattista *et al.*, (2001). According to crossbreeding process, the performances of local chickens considerably improved as shown in cross line's body weight in the present study. This improvement due to crossbreeding has been reported previously (Yang and Jiang, 2005). Despite the positive effect of crossbreeding on body weight in the present study, but the HiH1 cross line demonstrates low antibody response against ND. This could be due to increased metabolic resources availability for growth on the cost of the immune system, this negative relationship between immune response and other production traits have been reported previously (Dorshorst *et al.*, 2011). The relatively high immune response of the HiH2 cross line was not expected. The high immune response of HiH2 suspected to be affected by the H2 local line genetic pool, which demonstrated a high immune response against ND too. We suspect that the genetic makeup of H2 chickens was responsible for this attitude and the capacity of Hajar2 to re-allocate resources and use them efficiently differ from H1 chicken line capacity. The portion of allocated resources to a specific demand affected by the genetic makeup of the animal (Siegel and Honaker, 2009). The genetic and physiological differences between H1 and H2 lines have been highlighted previously (Hakami, 2015; Ahmed and Alabbad, 2014; Bougrein, 2013, Ahmed, 2010). The detailed differences in the genetic pool between the two local chicken lines need

more investigations to interpret the birds performance indicators. Hematocrit values of the four chicken lines demonstrated comparable values over the experimental period; this could be due to comfort management system during the rearing period. In addition, a previous study (Ahmed *et al*., 2014) mentioned that differences in Hematocrit percent among local Saudi chicken and cross lines are shown up after 12 weeks of age, which could explain the lack of genetic effect on the hematocrit percent in the earlier ages in the present study. The effect of birds' age on hematocrit values have been reported previously (Khawaja *et al*., 2012). Hemoglobin indicates the amount of oxygen transportation in addition to the process of carrying the carbon dioxide in his way out (Etim *et al*., 2014). Hemoglobin concentration change in relation to many factors, including genetic background of the bird (Peter *et al*., 2011). The relatively high values of hemoglobin concentration of the local chicken lines compared to cross lines in the present study could be explained as a result of their adaptation and tolerance during long time in a harsh environment. Under long time of hot-arid environment birds tried to cope with that through physiological changes in red blood cells in terms of number and efficiency, the changes in red blood cells count directly impact hemoglobin concentration as reported earlier (Hauptmanova *et al*.,2006). The high hemoglobin values suspected to be due to the unique genetic background of the current chicken lines, which is consistent with the previous findings (Elagib and Ahmed, 2011).

The present results indicated that although the superiority of the crossbreed line HiH2 and H2 local chicken line in ND antibody titer during the rearing period, but the local chicken lines H1 and the cross line HiH1 maintain an acceptable limit of response in most of the measuring points. Hemoglobin concentration affected mostly by the genetic origin of the birds. The differences in genetic background of the birds play an important role in shaping the cross line birds' immune capacity against ND. The enhanced body weight of cross lines not necessarily an indicator for their immune competence.

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Conflict of interest: The authors declare that they have no conflict of interest

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