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## **Antibody Response and DNA Polymorphism Indicators among Local Saudi Chicken Lines and Other Commercial and Exotic Chicken Lines**

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### **ABSTRACT**

This study was conducted to assess antibody response indicators against Newcastle Disease (ND) and Sheep Red Blood Cells (SRBC) in addition to DNA polymorphism using randomly amplified polymorphic DNA technique (RAPD). Five hundred female chicks represented equally Hajar1 Saudi chicken Line, Hajar2 Saudi chicken line, Hisex commercial layers, Ross 308 broiler and Egyptian Fayoumi were used in the present study in 4 replicates per each line for the first five weeks of age. Weekly body weight, weekly ND antibody titer and SRBC antibody titer at 3, 7 and 10 day post injection were assessed. Six RAPD markers have been selected and used for genetic diversity assessment within and among lines. The results indicated comparable values of body weight, ND titer, SRBC titer between Hajar1 and Hajar2 lines. Whereas, there were significant diversity indicators among different lines in the different measuring points. Intra-lines gene diversity started from 0.16 for Hajar2 line and reached 0.26 for Fayoumi line. Hisex and Ross chicken samples showed comparable gene diversity. The dendrogram showed two main clusters, the first cluster included only Ross broilers, while the second cluster included the rest of chicken lines. Higher genetic similarity have been observed between Saudi chicken lines and Hisex than similarity between them and Fayoumi chicken samples. The current results indicated the antibody response diversity among the chicken lines. Intra and inter populations genetic diversities considered the first to be reported using Hajar1 and Hajar2 lines in the context of the global plan of conserving animal genetic resources.

**Key words:** Chicken, antibody response, Hajar1, Hajar2, diversity, RAPD

### **INTRODUCTION**

Recently two lines of Saudi chicken, Hajar1 and Hajar2, have been characterized for their phenotypic characters (Ahmed and Alabbad, 2014). In addition some genetic parameters of both lines have been reported (Alabbad, 2014). There were noticeable diversity in some productive and physiological parameters between the two lines (Ahmed *et al.*, 2014). Just few studies showed comparisons between local Saudi chicken lines and commercial layers or broiler lines in terms of productive traits, physiological performance and some genetic parameters (Alamer and Ahmed, 2012; Ahmed and Alamer, 2011; Khalil *et al.*, 2004; Al-Bisher *et al.*, 1998). While, no reports are available on DNA polymorphism regarding those lines. Conservation and utilization of poultry

genetic resources mainly based on identification and evaluation processes, structural variation among individuals' genome could indicate potential genetic diversity (Soller *et al.*, 2006). Evaluation of genetic distances among chicken using molecular markers may provide useful information as a preliminary evaluation of chicken genetic resources (Weigend and Romanov, 2001) so, the assessment of diversity in antibody response and genetic diversity among those lines and other commercial and regional chicken lines is needed. The Randomly Amplified Polymorphic DNA (RAPD) assays have been used for estimating genetic diversity among different breeds and varieties in poultry (Ghanem *et al.*, 2012; Nikkhoo *et al.*, 2011; Rabie and Abdou, 2010; Ahlawat *et al.*, 2004; Singh and Sharma, 2002). It has also been used in other avian species (Salem *et al.*, 2005). On spite of our prior knowledge that RAPD markers have drawbacks as dominant markers compared to codominant markers but RAPD markers still have advantage of simplicity, speed, minimum cost and unrequired prior knowledge about breeds (Mahmood *et al.*, 2009) which qualify them to be used as first indicators for DNA polymorphism in flocks without prior knowledge about their genetic makeup. Accompany the suspected DNA polymorphism, Saudi chicken lines exposed to hot arid environment which alter their capacities regarding growth, physiological and immune response compared to other chicken lines (Ahmed *et al.*, 2014; Ahmed and Alamer, 2011; Ahmed, 2011), so body weight and immune response of the current lines could be good indicator for physiological parameters differences next to DNA polymorphism. In the current study females of five chicken lines Hajar1 Saudi chicken, Hajar2 Saudi chicken, Hisex commercial layers chicks, Ross 308 broiler chicks and Egyptian Fayoumi chicks were subjected to evaluation for their body weight and antibody response against Newcastle Disease (ND) and Sheep Red Blood Cells (SRBC) within the first five weeks of age. In addition DNA polymorphism within and between lines were detected using Randomly Amplified Polymorphic DNA (RAPD) technique. This research study investigates the suspected diversity between the different chicken lines using RAPD technique as an initial DNA polymorphism indicator in addition to some physiological indicators. We hypothesized that the Hajar1 and Hajar2 lines have highly diverse from Ross 308 chicks and Hisex commercial layers and much closer to Egyptian Fayoumi chicken. While, little similarity of physiological and genetic diversities could exist between Fayoumi and the two Saudi chicken lines.

## **MATERIALS AND METHODS**

**Experimental chicks and management:** Female chicks from five chicken lines were used in the present study. The five lines represented Hajar1 Saudi chicken line, Hajar2 Saudi chicken line, Hisex commercial layers chicks, Ross 308 broiler chicks and Egyptian Fayoumi chicks. One hundred chicks were used in the present study in each line from day old until the 5th week of age. All chicks received an identical vaccination program and same management treatments since one-day-old. Birds were exposed to 23 h day<sup>-1</sup> of light during the experimental period. All chicks were subjected to day old vent sexing, they wing banded and housed randomly in 4 replicates per each line in floor pens at an open house system on wood shaving litter. Feed and water were provided *ad libitum* according to each line feeding requirements according to NRC guidelines for commercial lines and it is also applied for local layer lines.

**Parameters and data collection:** Body weight were recorded for all chicks at day-old, 1, 2, 3, 4 and 5 weeks of age to the nearest gram. Blood samples were collected in a plain tubes to obtain serum samples for all chicks via wing vein at 1, 2, 3, 4 and 5 weeks of age using 25 gauge syringe

while, 28 gauge syringes were used at the younger ages. The serum samples were collected after centrifugation (876×g, 3 min). Serum samples were used to evaluate Newcastle Disease (ND) antibody titer. Titration was performed using microtitre Heamagglutination Inhibition (HI) test as described by OIE (2009). At 7 days of age all birds were injected with 0.5 mL of 15% Sheep Red Blood Cells (SRBC) suspension intramuscular. Blood samples were obtained from each bird at 3, 7 and 10 days post immunization. Sera were collected, centrifuged and stored at -20°C. Titrations were assayed using micro agglutination (Nelson *et al.*, 1995). The research complied with King Faisal University animal care guidelines.

**DNA isolation:** At the 5th week of age blood samples were obtained from wing vein of all birds into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant agent. DNA was extracted from blood samples using QIAamp DNA blood Mini Kits (Qiagen, Valencia, CA, USA, Cat No. 51104) according to the supplier's instructions. All samples were quantified using spectrophotometer. DNA samples were adjusted to 10 ng  $\mu\text{L}^{-1}$  for the following PCR reaction.

**RAPD primers and PCR amplification:** RAPD primers in the present study were selected after screening of 21 decamer primers from operon technologies company-USA to ensure the production of reproducible amplification products and select the highest polymorphic primers. Six primers have been selected for PCR reaction as shown in Table 1. PCR amplifications were performed twice for all samples using thermal cycler reaction of 20  $\mu\text{L}$  volume included 20 ng of genomic DNA, 250 nM dNTPs, 2  $\mu\text{L}$  of Primer, 2 mM  $\text{MgCl}_2$  and 1U Taq polymerase. The reaction mixture was preheated at 94°C for 3 min followed by 40 cycles (94°C for 1 min, 36°C for 1 min and 72°C for 2 min). Then a final extension at 72°C for 10 min was applied. All PCR products were separated by electrophoresis in a 1.5% agarose gel stained with ethidium bromide in 1 X TBE buffer and photographed under UV light. All gel photographs were scored manually for presence or absence of bands.

**Statistical analysis:** Body weight and antibody responses data was subjected to a one way analysis of variance for the effect of line using general linear model. Means were separated using Tukey Honestly Significant Difference (HSD) tests. Data was analyzed using the general linear model procedure of JMP IN 5.1 software (Sall *et al.*, 2005). RAPD data was recorded as binary matrix, the genetic diversity within and among lines were estimated according to (Nei, 1978). Observed number of alleles (Ne), effective number of alleles (Na), Shannon's information index or

Table 1: Operon codes, DNA sequence, total and polymorphic band percentage of each RAPD markers obtained from the five chicken lines

Primer codes	Sequence 5'-3'	Total bands	Percentage of polymorphic bands (%)				
			Pop1*	Pop2	Pop3	Pop4	Pop5
OPP-05	CCC CGG TAAC	10	21.43	64.29	14.29	28.57	42.86
OPN-15	CAG CGA CTGT	12	71.43	64.29	64.29	50.00	71.43
OPA-04	AAT CGG GCTG	9	50.00	42.86	50.00	35.71	57.14
OPP-04	AGT CCT CGCC	12	57.14	57.14	57.14	35.71	57.14
OPA-16	AGC CAG CGAA	14	78.57	50.00	64.29	28.57	71.43
OPAL-03	CCC ACC CTTG	10	64.29	71.43	21.43	42.86	50.00

\*Pop1: Saudi Hajar1, Pop2: Saudi Hajar2, Pop3: Hisex layers, Pop4: Ross 308 broiler and Pop5: Egyptian fayoumi chicken populations, respectively

gene diversity (I) and Expected heterozygosity (He) were calculated using POPGENE software (Yeh *et al.*, 1999). UPGMA dendrogram was constructed according to Nei (1973).

**RESULTS**

Figure 1 demonstrates the body weight of different chick's lines starting from day old until the fifth week of age. The means between Hajar1 and Hajar2 lines were comparable except for the 4th week of age. There were significant differences ( $p < 0.05$ ) in body weight among local Saudi chicken lines and all other lines. As expected, Ross broilers recorded the highest significant ( $p < 0.05$ ) body weight in each measuring point, while Hisex ranked in the second place at all measuring points except for the second week of age. The Egyptian Fayoumi chicken ranked in the third place starting from 3 weeks of age.

Results of ND antibody titer (Fig. 2) indicated significant higher antibody levels of Egyptian Fayoumi chicken than Saudi local chicken lines and both commercial lines at the first week of age.

By the second week of age, Hajar1 and Hajar2 chicken recorded the highest ND antibody titer ( $p < 0.05$ ) followed by Egyptian Fayoumi chicken however, both commercial lines recorded the lowest

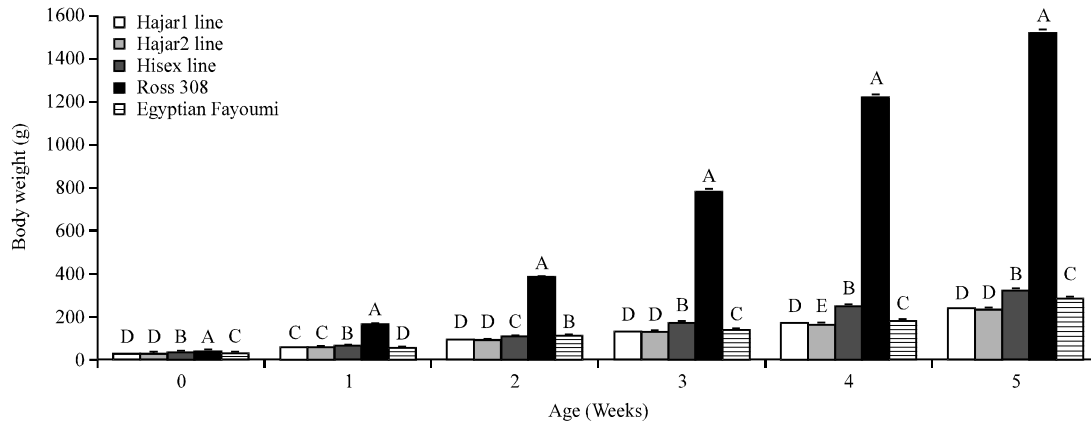


Fig. 1: Body weight of the five chicken lines for the first five weeks of age. Values within a week with different superscript differ significantly ( $p \leq 0.05$ )

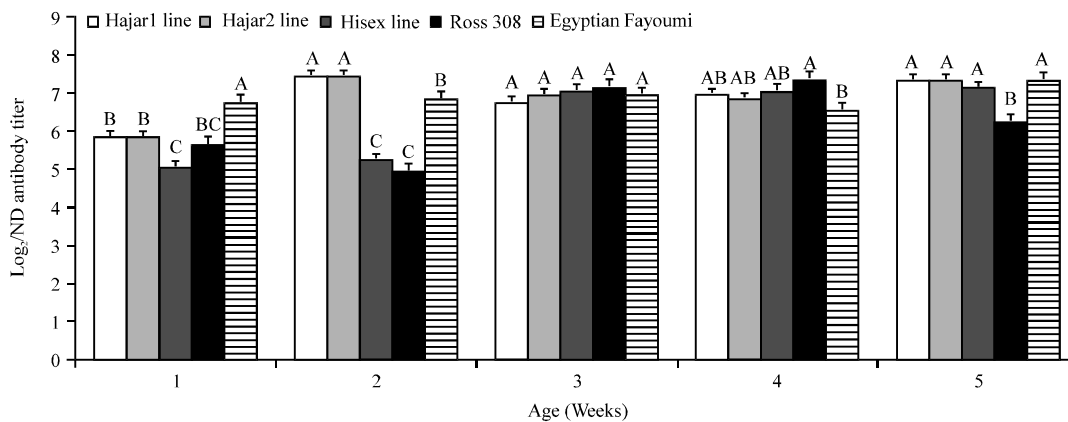


Fig. 2: Newcastle disease antibody titer of the five chicken lines for the first five weeks of age. Values within a week with different superscript differ significantly ( $p \leq 0.05$ ), the titer values are  $\log_2$  of the reciprocal dilution

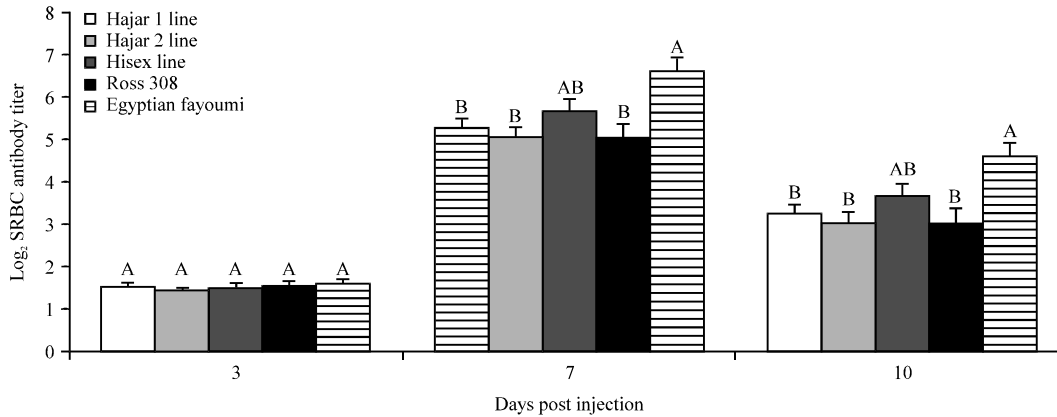


Fig. 3: Antibody titer against sheep red blood cells (SRBC) of the five chicken lines at 3, 7 and 10 days post injection. Values within a week with different superscript differ significantly ( $p < 0.05$ ), the titer values are  $\log_2$  of the reciprocal dilution

Table 2: Overall genetic parameters estimate in five chicken lines using RAPD markers

Chicken line parameters	Saudi Hajar1 line	Saudi Hajar2 line	Hisex layers	Ross 308 broiler	Egyptian fayoumi chicken
Ne*	1.153±0.02	1.122±0.01	1.180±0.02	1.227±0.03	1.261±0.03
Na	1.143±0.10	1.167±0.10	0.905±0.10	0.738±0.10	1.167±0.10
I	0.174±0.02	0.166±0.01	0.189±0.02	0.193±0.03	0.263±0.02
He	0.103±0.01	0.093±0.01	0.118±0.01	0.130±0.02	0.168±0.01
Polymorphic loci (%)	57.14	58.33	45.24	36.90	58.33

Ne: Observed No. of alleles, Na: Effective No. of alleles, I: Shannon's information index or gene diversity, He: Expected heterozygosity

significant antibody levels ( $p < 0.05$ ) at the same week. There were not any significant differences between all lines at the 3rd week of age, no significant differences observed in ND antibody titer among Hajar1, Hajar2 and Hisex birds for the following weeks.

There were not any significant differences ( $p < 0.05$ ) in antibody response against SRBC among all chicken lines at 3 days post exposure (Fig. 3). At 7 and 10 days post exposure Egyptian Fayoumi chicken recorded significantly ( $p < 0.05$ ) higher antibody levels against SRBC than Hajar1, Hajar2 and Ross broilers while, Hisex chicken antibody level did not differ significantly ( $p < 0.05$ ) than all other chicken lines.

After initial testing of 21 decamer primers from operon technologies company-USA, 15 primers successfully amplified the genomic DNA samples from all lines. Out of those primers, six primers were selected to be used for their high polymorphic band ratio as shown in Table 1. The percentage of polymeric bands within each population ranged from 21.4-78.5. The total percentage of polymorphic loci for all primers was recorded 58.3% for Hajar2 and Fayoumi chicken samples (Table 2). The observed number of alleles (Ne), Effective number of alleles (Na), Shannon information index (I) and expected heterozygosity (He) for the five chicken lines are shown in Table 2. The He values ranged from 0.09 for Hajar2 line to 0.16 for Fayoumi line. The results indicated diversity in He among the five chicken lines. The Shannon index values demonstrated intra-lines gene diversity started from 0.16 for Hajar2 line and reached 0.26 for Fayoumi line. Hisex and Ross chicken samples showed comparable gene diversity 0.189 and 0.193, respectively (Table 2). Hajar1 line recorded a slight higher within line genetic diversity (0.174) compared to

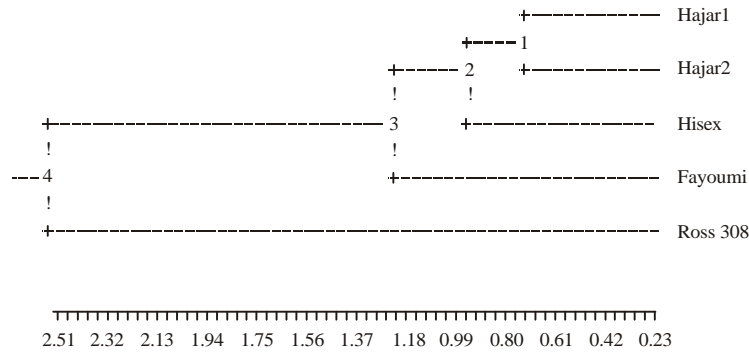


Fig. 4: Dendrogram of genetic relationship among the five chicken line

Table 3: Genetic dissimilarity matrix among the five chicken lines based on RAPD data

Parameters	Saudi Hajar1 line	Saudi Hajar2 line	Hisex layers	Ross 308 broiler	Egyptian fayoumi chicken
Saudi Hajar1 line	0.000				
Saudi Hajar2 line	0.012	0.000			
Hisex layers	0.016	0.017	0.000		
Ross 308 broiler	0.038	0.058	0.053	0.000	
Egyptian Fayoumi chicken	0.023	0.021	0.023	0.053	0

Hajar2 line (0.166). The dissimilarity matrix values among the five chicken lines are presented in Table 3, the values of pairwise comparisons among the five chicken lines ranged from 0.012-0.053. On one hand, the dissimilarity values was relatively higher between Saudi chicken lines and Ross broilers. On the other hand, Saudi chicken lines recoded lower values of dissimilarity with Hisex line. The dissimilarity between Saudi chicken lines and Fayoumi chicken line was higher than the dissimilarity between Saudi chicken lines and Hisex chicken line. Dissimilarity value between Hajar1 and Hajar2 lines recorded the minimum value in the matrix (0.012). The UPGMA dendrogram (Fig. 4) showed two main clusters, the first cluster included only Ross broilers, while the second cluster included the rest of chicken lines. Higher genetic similarity have been observed between Hajar1, Hajar2 and Hisex samples, while lesser similarity were observed between Hajar1, Hajar2 and Fayoumi chicken samples.

## DISCUSSION

The current results pointed out to the immunological and genetic diversity among and within five chicken lines from different origins. The comparable body weight of the two Saudi chicken lines is expected due to previous reports indicated insignificant differences in body weight between the two lines at different ages (Ahmed and Alabbad, 2014). On spite of the expected diversity in body weight among chicken lines due to their different genetic origins, the assessment of body weights have been performed to emphasize the diversity of the present samples of birds.

The ND antibody response are very important indicator because ND is one of the most important diseases of poultry (Alexander, 2003). The presence of antibodies against Newcastle disease virus in backyard and commercial poultry birds in Saudia Arabia has been indicated in a survey study using positive ELISA and HI tests (Alkhalaf, 2009). In addition to the well-known effect of genetic pool of different chicken lines on response to ND vaccines (Chang *et al.*, 2012). Moreover, different

chicken lines reported to produce different levels of natural antibodies, too (De Jong *et al.*, 2013). The present results indicated diversity in antibody response to ND at the first and second weeks of age, this period suspected to be the period of maternal, passive, immunity effect which is more affected by genetic background before vaccination program, Acquired immunity, application (Ahmed, 2011). The following ND antibody titer levels at 3, 4 and 5 weeks of age indicated higher comparable values which could be due to response to the vaccination program. This relationship between genetic makeup and ND immune response have been demonstrated previously (Li *et al.*, 2013).

Response to SRBC provided knowledge about the ability of chick's humoral immune to response without prior exposure possibility. The results indicated variation in humoral immune response to SRBC at 7 and 10 days post exposure, this observed variation most likely due to the different genetic background of the five chicken lines. This suggestion in agreement with Emam *et al.* (2014). They demonstrated the role of genetic line background on antibody response to different antigens. The results indicated the diversity of different phenotypic characters that could be partially affected by genetic background of different chicken lines.

In the current study, indicators of genetic diversity have been achieved to support the required future conservation strategy local lines. RAPD technique was efficient in giving polymorphism indicators in the present study the number of amplified bands by each individual primer ranged from 9-14 in agreement with previous studies (Singh and Sharma, 2002; Ahlawat *et al.*, 2004; El-Gendy *et al.*, 2006). Hajar1 and Hajar2 lines recorded the lowest genetic diversity 0.17 and 0.16 within populations compared to the other commercial and local lines. The low levels of diversity values within those populations suspected to be due to common ancestors, population size and geographical location. However, Hajar1 and Hajar2 exposed to natural selection pressure under the harsh environment which is suspected to favor well-adapted genes and increase within population similarity. This results in agreement with the findings of genetic diversity of 0.17 within Grey local Syrian chicken (Al-Jallad *et al.*, 2012), 0.12 of CB Bangladeshi chicken strain (Mollah *et al.*, 2009) and a genetic diversity value of 0.15 within Brown Nicobari fowl in India (Ahlawat *et al.*, 2004). It is worth mentioning that a higher genetic diversity of 0.58 have been reported within Jordan local chickens (Al-Atiyat, 2010). Also it was 0.20-0.21 in NN and FZ Bangladeshi chicken strain (Mollah *et al.*, 2009). Those results justified due to random mating and wide distribution over large geographical area which is applied to the genetic diversity of 0.26 in Fayoumi chicken in the present study. The intra-population genetic diversity of Hisex and Ross broilers was 0.18 and 0.19 which is potentially due to the selection and breeding processes within the commercial lines.

Hajar1 and Hajar2 lines recorded the minimum genetic distance with each other followed by Hisex then Fayoumi chicken. While Ross broilers located in a different cluster in the phylogenetic tree. According to an extended study of chicken genetic diversity from different continents Granevitze *et al.* (2007) reported that chicken populations could be classified in 7 genetic clusters, Fayoumi chicken listed in the C cluster while, white egg layers listed in D cluster (Granevitze *et al.*, 2009). The present study demonstrated preliminary indicator about the shorter genetic distance between Saudi local lines (Hajar1 and Hajar2) and Hisex commercial layer compared to their distance from Fayoumi chicken. This finding could point out to a possible origin of Local Chicken line more closer to Genetic cluster D than cluster C. Cluster D which is included White egg layers suspected to be most closely related to the Asian Red Jungle Fowl with their colorful feather



(Hillel *et al.*, 2003). The genetic cluster A reported to include broilers (Granevitze *et al.*, 2009) this could explain the high genetic distance between Ross broilers and all other studied lines.

## CONCLUSION

In conclusion, the current research work demonstrated the antibody response diversity among five commercial and local chicken lines in addition, it is highlighted the intra and inter populations' genetic diversity. The genetic diversity indicators pointed out to the lower genetic distance between local Saudi chicken lines. On other hand, Saudi chicken lines have closer genetic distance with commercial layers than Fayoumi chicken. However, Ross broiler chicks located apart in another phylogenetic cluster with the highest genetic distance from all lines. The present results are the first indicators about biodiversity within and among Hajar1 and Hajar2 Saudi chicken lines and other chicken lines. The present study approach is in consistency with the global plan of conserving animal genetic resources worldwide. Further studies are required to assess other physiological and productive performance indicators in addition to genetic polymorphism relationship based on larger number of samples and co-dominant markers seeking support for the potential genetic conservation plan for Hajar1 and Hajar2 lines.

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