# PHENOTYPIC CHARACTERIZATION AND GENETIC DIVERSITY OF INDIGENOUS CHICKENS OF JORDAN IN COMPARISON WITH NATIVE AND COMMERCIAL BREEDS FOR CONSERVATION AND BREEDING PURPOSES

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⊠Supporting Information

**ABSTRACT**: Indigenous chickens of Jordan are facing extinction and need genetic conservation because they were unable to commercially compete in the poultry industry because of low genetic ability compared to commercial layers. The study aimed to investigate phenotypic and genetic diversity of village chickens in Jordan using discriminant analyses procedures to provide a basis for sustainable genetic conservation and utilization program to overcome any possible extinction. The sampled chicken population of 578 one-year old chickens (125 males and 433 females) was phenotypically characterized for 15 biometric and plumage traits from major cities of the three regions; Middle, North, and South. The traits variations within and between breeds were detected statistically by stepwise discriminant and canonical-discriminant of uni- and multivariate analyses. The results showed the sampled population as village chickens in Jordan is comprised of indigenous (Baladi) breed (85%) and few exotic and commercial breeds. The breeds were distinct and differentiated based on phenotypic traits indicating high genetic variability. The major phenotypic traits that showed significant power to differentiate breeds were comb type, body weight, comb size, earlobe color, wattle size, face color and breast size in males and comb type and size, body weight, face and breast size, leg color and wattle size in females. Recent and past crossings, admixture or migration from exotic and commercial breeds were noted. Moreover, low levels of phylogeographic structure were observed across the studied breeds. In conclusion, there is a need to conserve the indigenous breed *in situ* and *in vivo* for its adaptive gene pool in the coming days of persisted climate change and disease threats.

**Keywords**: Breed conservation, Distance, Genetic diversity, Morphology, Native chickens.

## INTRODUCTION

The sustainability of biodiversity and genetic resources is considered the first step towards food security in each country. Thus, many countries nowadays apply the convention on biological diversity (CBD) as main regulation and law to govern species biodiversity (Chandra and Idrisova, 2011). Regarding domestic animals and livestock, the CBD of each country develop a strategic action plan for their genetic diversity conservation and genetic resources utilization. The genetic resources are representing ability of genetic makeup or livestock breed to produce and reproduce in specific conditions. Nowadays, commercial breeds have been genetically improved for high production in intensive system. On the other hand, indigenous or native (Baladi –Arabic name–) breeds are surviving better in low-input and village production systems. In fact, farmers and villagers are replacing indigenous breeds with commercial strains and/or their crossbreds. This practice is considered main threat of indigenous chickens' biodiversity in developing countries resulting in losing genetic resources as well as chicken extinction. The Food and Agriculture Organization of the United Nations (FAO, 2004) recommended, in such a situation of extinction, to apply appropriate management and conservation strategies. In general, livestock extinction is globally concerned for conserving genetic resources of future need and utilization (FAO, 2008). Nevertheless, utilization of biodiversity is the main aim of countries' national plan which include collecting and disseminating information and applying practical practices to conserve biodiversity. Particularly, the practical application of genetic conservation is contested by farmer breeding practices, global warming, exotic breeds/trains, and social-economic issues (Hoffmann, 2022).

Jordan, since the 1970s, has developed commercial poultry farms by importing high producing exotic layers and

broilers to meet the increasing demand of eggs and meat. Major developments, in the poultry industry, have occurred in 2000s in which large numbers of high-producing strains of layers are released into market and reared in villages and suburban areas without governmental regulation prevent unplanned crossbreeding with the indigenous chickens. Indigenous chickens (*Gallus gallus*) of Jordan are domesticated chicken reared in villages and backyards with low input requirements and conditions. Their phenotypes are features for numerous variations in body shape and size, feather and leg color, comb type and size, slow growth rates and small egg size (Abdelqader et al. 2007; Al-Atiyat, 2009). The latter two features were the reasons why some farmers crossed them with exotic breeds to benefit from heterosis effects or hybrid vigor towards more egg and meat production (Ahmed et al., 2020). Jordanian farmers are in general practicing crossbreeding of exotic commercial layers and ornamental breeds and ancient breeds (Al-Atiyat, 2009). It is worthy to mention, the most common threat to the indigenous chicken's diversity of the word is crossbreeding (Leroy et al, 2016). Consequently, avoiding crossbreeding practices by farmers is important step to conserve indigenous chicken genetic resources and their genetic variations. Furthermore, conserving genetic variation is priority for both current and future utilization indigenous chicken that most tolerable breed of persisted global warming in Jordan. Additionally, Jordan indigenous chicken has better interest for avoiding extinction afterward the last decade outbreak of avian flu (poultry influenza) which associated with extensive culling to control the epidemic (Dunn et al., 2019).

Worthwhile, phenotypic description of indigenous chicken is characterizing and documenting all genes that contribute to phenotypic and plumage traits. This is considered genetic characterizations which is a prerequisite to sustainable conservation plan (FAO, 2013). So that the plan is comprised of evaluation phenotypic traits, breeding history, genetic diversity level within and between populations which is measured by statistical analysis such as multivariate discriminant procedure (González Ariza et al., 2021). The objective of the study was to assess the genetic diversity of Jordan indigenous chickens for detecting the conservation possibility and future perspective under current climate situation.

## MATERIALS AND METHODS

### Chicken population

First, this study is interested in the indigenous or Baladi chickens which were found in rural and urban areas of Jordan. Sampled population was village chickens which found in these areas, and it was comprised of various breeds; indigenous, ancient exotic and commercial breeds. We sampled mainly the indigenous individuals and some predefined exotic and commercial individuals as a reference groups. The sampled indigenous chicken population were classified and named after each sampled governorate from three major regions of Jordan: North, Middle and South regions. The other sampled breeds were predefined exotic breeds of Cochins, Fayoumi, Lamborghini and Pakistani. In addition to commercial breeds of Hy-Line White and ISA Brown chickens. The Cochin chicken is a breed of large feather-legged chickens come from China (Larkina et al., 2021). Fayoumi chicken breed is robust breed in a harsh environment and native to Egypt (Dessie et al., 2011). Lamborghini chicken is all black in appearance known as Ayam Cemani chicken comes from Java (Indonesia) and nicknamed the Lamborghini of all chicken breeds because of their price, rarity, and prestige (Dharmayanthi et al., 2017). Pakistani chicken breed is thought to have evolved from cockfighting chickens and being native to Pakistan and thus called Pakistani in Jordan (Abdelqader et al., 2007). The Hy-Line White chickens and ISA Brown chicken are modern strains of commercial layers producing eggs. ISA Browns are brown-feathered and brown egg layers, and Hy-lines chickens are white-feathered and white egg layers.

### Ethical Regulations and considerations

In this research, handling chickens was practiced with the permission of the appropriate regulations and guidelines of the Ethics Committee of Mutah University (No.: AGR/1/15/2018).

### Data recording and statistical analysis

The plumage and biometric data were documented following pictorial guidelines of chickens phenotypic characterization (FAO, 2008). The traits were body weight and color, comb color, type and comb size, beak color, face color and size, wattle color and size, ear lob, eye and leg color, and breast color and size. The survey was executed to ensure random sampling. The chickens of one-year old were randomly selected, weighted and phenotypic traits were recorded. The total population size was 558 individuals: 125 males and 433 females.

The statistical analyses were based on the SAS program version 9.2 (SAS, 2010). The first analysis was phenotypic clustering model (PCM). Second, simple discriminant analysis was performed to calculate probability of an individual chicken into predefined group. Then stepwise discriminant was also used to define traits of better discriminating power. Last, canonical -discriminant analysis of uni- and multi-variate analysis- was performed to generate canonical variables (CAN). The CANs were counted for pairing each breed with other breeds into one genetic group and cluster of potential population or breed. Finally, the Mahalanobis distances were estimated of the covariance matrix (Winaya et al., 2023). Consequently, the SAS TREE procedure was operated to build a dendrogram using the statistical method of unweighted pair's group. Finally, the PROC CLUSTER procedure was accomplished utilizing distances data to form the clusters.

## RESULTS

### Phenotypic traits and discriminant power

Table 1 shows the phenotypic traits and sampling location details. The results showed the sampled population as village chickens in Jordan is comprised of indigenous (Baladi) breed (85%) and many exotic and commercial breeds; Cochins (2.5%), Fayoumi (1.5%), Hy-Line (3.5%) , ISA-brown (1%), Lamborghini (2%), Pakistani (4%). The contribution of samples per governorate varied from the highest in Krak (21.5%) to lowest by Tafilh governorate (5.5%).

### Diversity and differentiation analyses

The Biometric and plumage variable showed a wide range of differentiation ability (Table 2). Five traits only (body weight, wattle size, earlobe color, face color, comb size) were significantly able to differentiate individuals of the breeds in males. They were body weight, wattle size, earlobe color, face color and comb size which abled to significantly separated the breeds' males. In females, the variables were comb size, body weight, face size, comb type, leg color, breast size, and wattle size that significantly discriminated them between pairwise females' breeds on average. Body weight of males was the most powerful discriminant variable, while comb size of was the most powerful discriminant variable.

The results of discriminant procedure showed Pakistani male chickens were highly differentiated ($P<0.0001$) from others (Table 3). The indigenous male breed was also significantly differentiated from Pakistani breed with the longest distance value. On the other side, the Mahalanobis distances of the female breeds were significantly differentiated ($P<0.001$). The longest distance was between Baladi female breed and the Hi-Line female breed. The lowest nonsignificant distance was between Baladi and Fayoumi breeds. In general, the longest significant distance was noted between Baladi and each of commercial chickens (Hy-Line White -White Leghorns- and ISA Brown laying hens -Rhode Island Red chicken-) breeds, reflecting the long genetic distance between them (Table 3).

Table 4 shows Eigenvalue, variance proportion, and canonical correlation variables. Function Canonical value number one (CAN1) is qualified to the differences among the males of the breed. The high percentage of Eigenvalue variation (63.6% and 75.2% for male and female, respectively) and thus total variation in the grouping of discriminant function 1 in this study is related to the differences among males and females the breeds in the studied traits and evidence of high genetic variation (73.2% for male and 70.9% for female). In details, the variation proportion of males (70%) of function 1 was higher than other functions. Additionally, CAN1 is higher than the other functions' values. Likewise results of variation proportion and the canonical correlation of function were noted.

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**Table 1** - Frequency of Jordan indigenous chicken breeds, their sampling location of both males and females

| Breed | No. | Percent (%) | No. | Percent (%) | Total No. | Overall Percent (%) |
|---|---|---|---|---|---|---|
| | Females | | Males | | Females and Males | |
| Baladi | 364 | 84 | 108 | 86 | 472 | 85 |
| Cochins | 4 | 1 | 5 | 4 | 9 | 2.5 |
| Fayoumi | 14 | 3 | - | 0 | 14 | 1.5 |
| Hy-Line | 30 | 7 | - | 0 | 30 | 3.5 |
| ISA brown | 7 | 2 | - | 0 | 7 | 1 |
| Lamborghini | 5 | 1 | 4 | 3 | 9 | 2 |
| Pakistani | 9 | 2 | 8 | 6 | 17 | 4 |
| Total | 433 | 100 | 125 | 100 | 558 | 100 |
| Governorate | | | | | | |
| Ajlun | 30 | 7 | 11 | 9 | 41 | 8 |
| Aman | 20 | 5 | 5 | 3 | 25 | 4 |
| Aqba | 35 | 8 | 17 | 14 | 52 | 11 |
| Blqa | 45 | 10 | 10 | 8 | 55 | 9 |
| Jrsh | 17 | 4 | 5 | 4 | 22 | 4 |
| Krak | 90 | 21 | 27 | 22 | 117 | 21.5 |
| Maan | 79 | 18 | 18 | 15 | 97 | 16.5 |
| Mdba | 59 | 14 | 15 | 12 | 74 | 13 |
| Mfrq | 35 | 8 | 10 | 8 | 45 | 8 |
| Tfilh | 23 | 5 | 7 | 6 | 30 | 5.5 |
| Total | 433 | 100 | 125 | 100 | 558 | 100 |

## Table 2 - Summary of stepwise selection of traits

| Entered | Partial R-Square | F Value | Pr > F | Wilks' Lambda | Pr < Lambda | Average Squared Canonical Correlation | Pr > ASCC |
|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | |
| Body weight | 0.1302 | 6.04 | 0.0001 | 0.8698 | 0.0007 | 0.0434 | 0.0007 |
| Wattle size | 0.0907 | 3.99 | 0.0095 | 0.7909 | <.0001 | 0.0707 | 0.0001 |
| Earlobe color | 0.0657 | 2.79 | 0.0437 | 0.739 | <.0001 | 0.0899 | <.0001 |
| Face color | 0.0723 | 3.07 | 0.0307 | 0.6855 | <.0001 | 0.1085 | <.0001 |
| Comb size | 0.0634 | 2.64 | 0.0529 | 0.6421 | <.0001 | 0.1293 | <.0001 |
| **Female** | | | | | | | |
| Comb size | 0.3567 | 39.37 | <.0001 | 0.6433 | <.0001 | 0.0595 | <.0001 |
| Body weight | 0.1029 | 8.12 | <.0001 | 0.5771 | <.0001 | 0.0746 | <.0001 |
| Face size | 0.0737 | 5.62 | <.0001 | 0.5346 | <.0001 | 0.0853 | <.0001 |
| Comb type | 0.0736 | 5.6 | <.0001 | 0.4952 | <.0001 | 0.096 | <.0001 |
| Leg color | 0.0486 | 3.6 | 0.0017 | 0.4711 | <.0001 | 0.1032 | <.0001 |
| Breast size | 0.036 | 2.62 | 0.0168 | 0.4542 | <.0001 | 0.1087 | <.0001 |
| Wattle size | 0.0233 | 1.67 | 0.1268 | 0.4436 | <.0001 | 0.112 | <.0001 |

## Table 3 - Mahalanobis distance and Prob > Mahalanobis distance between Males and Females of the chicken breeds

| Male breed | | | | | | Cochins | Lamborghini | Pakistani |
|---|---|---|---|---|---|---|---|---|
| Baladi | | | | | | 2.83[NS] | 4.93[NS] | 9.51*** |
| Cochins | | | | | | | 5.286[NS] | 13.92*** |
| Lamborghini | | | | | | | | 19.16*** |

| Female breed | Baladi | Cochins | Fayoumi | Hy-Line | ISA brown | Lamborghini | Pakistani |
|---|---|---|---|---|---|---|---|
| Baladi | | 2.13 [NS] | 1.53 [NS] | 9.52*** | 9.70*** | 1.33[NS] | 7.94*** |
| Cochins | | | 1.59 [NS] | 14.52*** | 13.39** | 1.41 [NS] | 8.38[NS] |
| Fayoumi | | | | 14.92*** | 10.47*** | 1.67 [NS] | 8.85*** |
| Hy-Line | | | | | 26.45*** | 13.16*** | 26.86*** |
| ISA brown | | | | | | 9.78* | 16.37*** |
| Lamborghini | | | | | | | 10.73** |

\* :P<0.05, \*\*: P<0.01 \*\*\*: P<0.001 , NS: not significant.

## Table 4 - Eigenvalue, variation proportion, and canonical correlation of each function for males and females

| Canonical | Eigenvalue | Variation proportion | Canonical correlation | Pr > F |
|---|---|---|---|---|
| **Males** | | | | |
| 1 | 0.636 | 0.732 | 0.624 | 0.001 |
| 2 | 0.146 | 0.168 | 0.357 | 0.620 |
| 3 | 0.087 | 0.100 | 0.283 | 0.729 |
| **Females** | | | | |
| 1 | 0.752 | 0.709 | 0.655 | <.0001 |
| 2 | 0.154 | 0.145 | 0.365 | <.0001 |
| 3 | 0.105 | 0.099 | 0.308 | 0.144 |
| 4 | 0.032 | 0.030 | 0.177 | 0.978 |
| 5 | 0.015 | 0.015 | 0.123 | 0.998 |
| 6 | 0.003 | 0.003 | 0.054 | 1.000 |

The genetic contribution of chicken individuals' breed into the overall genetic pools of breeds is shown in Table 5. It is noted that 60.19% of Baladi males shared pure genetic pool of Baladi, and 22.22% of its genetic pool hared with Cochins. Fewer proportions were noted between Baladi males and males of Lamborghini and Pakistani breeds. However, good proportion of males' assignment (40%) as an error rate indicated that misassignment of crossbreds or non-Baladi males as Baladi breed. On the other hand, almost exotic breeds were assigned with high proportion to their own breed (80, 75 and 75% for Cochins, Lamborghini and Pakistani breeds, respectively). The results also showed that most females Baladi (27.75%) were assigned to unique genetic pool of Baladi chicken

followed by Lamborging and Fayoumi with fair proportion; 19.78 and 16.48%, respectively. However, near 72% of Baladi females were misclassified as pure Baladi. For instance, 21.43% of Fayoumi and 3.33 % of Hy-Line females were assigned as Baladi females. On the other hand, 50% of Cochine and Fayoumi breeds were predefined Baladi chicken were sharing 50% of their genetic pools as phenotype with Baladi females. The results are indicated by error rate (Table 5). The results might indicate a shared genetic pool between Baladi breeds and the exotic studied breeds or crossbreds.

Based on previous results of CAN's function 1 in which CAN1 exhibited the major variations for males and females. The variations in the CAN1 for both males and females was related to the following phenotypic variables, the body weight and color, comb color, peak color and face color. Thus, they allowed for a clear distinction between male breeds (Table 6). For more details in females, the Can values showed high correlated coefficients between combinations of the plumage traits of chicken breeds indicating that comb type is most discriminant variable.

Furthermore, the results showed significant coefficient values for CAN1 of male breed in which Pakistani breed had higher value for CAN1. Similarly, it was noted higher value of female Pakistani in CAN1 (Table 6). Better illustration of the results is seen as plotted in Figure 1 in which male and female breeds differentiated along CAN1 axis and Can2 axis. Thus, the canonical variables were successful in differentiating breeds' sexes from each other. In details, individuals of the breeds distributed in either separated group or intermixed group indicating how phenotypically and genetically close. In other words, the results illustrate how individuals of both sexes from breeds were related based on individual principal component analysis. For example, the first genetic group of the male plumage in Figure 1 is represented by black circle grouped where most samples are Baladi males except few individuals of Colachins, Lamborghini and Pakinstani. Both exotic breeds of Lamborghini and Pakinstani chickens are grouped in separated circle each. Pakistani males formed in one separated and distinct group (green circle). On the other hand, this principal component analysis shows a clustering of females as presented individuals of different breeds plotted with others providing evidence of clear separation, intermixing, or crossbred in Baladi breed. However, major samples of Baladi are not crossbred individuals and thus they were clustered together from all sampled regions of Jordan. In fact, more obvious results were noticed for the female individuals indicating that the individuals were grouped from same breed representing their closeness in sharing sample variables (Figure 1). However, some Baladi individuals

**Table 5** - Membership proportion (%) between studied chicken breeds

| | Male | | | | | | |
|---|---|---|---|---|---|---|---|
| Breed | Baladi | Cochins | Lamborghini | Pakistani | | | |
| Baladi | 60.19 | 22.22 | 10.19 | 7.41 | | | |
| Cochins | 20 | 80 | 0 | 0 | | | |
| Lamborghini | 25 | 0 | 75 | 0 | | | |
| Pakistani | 25 | 0 | 0 | 75 | | | |
| Priors | 0.25 | 0.25 | 0.25 | 0.25 | | | |
| Rate | 0.40 | 0.2 | 0.25 | 0.25 | | | |

| | Female | | | | | | |
|---|---|---|---|---|---|---|---|
| Breed | Baladi | Cochins | Fayoumi | Hy-Line | ISA brown | Lamborghini | Pakistani |
| Baladi | 27.75 | 10.71 | 16.48 | 10.71 | 5.49 | 19.78 | 9.07 |
| Cochins | 0 | 50 | 0 | 0 | 0 | 50 | 0 |
| Fayoumi | 21.43 | 7.14 | 50 | 0 | 0 | 14.29 | 7.14 |
| Hy-Line | 3.33 | 0 | 0 | 96.67 | 0 | 0 | 0 |
| ISA brown | 0 | 0 | 0 | 0 | 85.71 | 14.29 | 0 |
| Lamborghini | 0 | 20 | 0 | 0 | 0 | 0 | 80 |
| Pakistani | 0 | 0 | 0 | 0 | 0 | 0 | 77.78 |
| Priors | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| Rate | 0.72 | 0.50 | 0.50 | 0.03 | 0.14 | 0.20 | 0.22 |

of both sexes did not reflect this relationship; they were clustered with other breeds or located in a place far away.

To highlight how the Baladi can be utilized for its purity, genetic resources, and conservation, separated clusters were reconstructed for dendrogram tree of different branching level for males and females (Table 7). For males, the first branch included Baladi chickens breed formed in a separate cluster or genetic group or gene pool with Cochins (Figure 2). The second branch formed from males of Lamborghini breed. The last branch had Pakistani males far away from other branches or breeds' genetic pools. The dendrogram of females (Figure 3) shows a cluster of Baladi in separated branch. The second cluster was a sub-group that includes Cochins and Lamborghini and Fayoumi chickens. This group of sub-clustered with Cochins and Fayoumi breeds forming the first major cluster as one group / gene pool close to Baladi chickens. The rest breeds were far separated from those breeds in a third cluster of branches. Pakistani breed was grouped with the ISA brown instead Baladi indicating an intermediate position Hy-Lin and ISA brown. The female dendrogram branching was like the males' except that three breeds (Fayoumi, Hy-Lin and ISA brown) were missed in males' dendrogram.

**Table 6** - Total-sample standardized canonical coefficients, and total variations explained by each canonical variable (CAN).

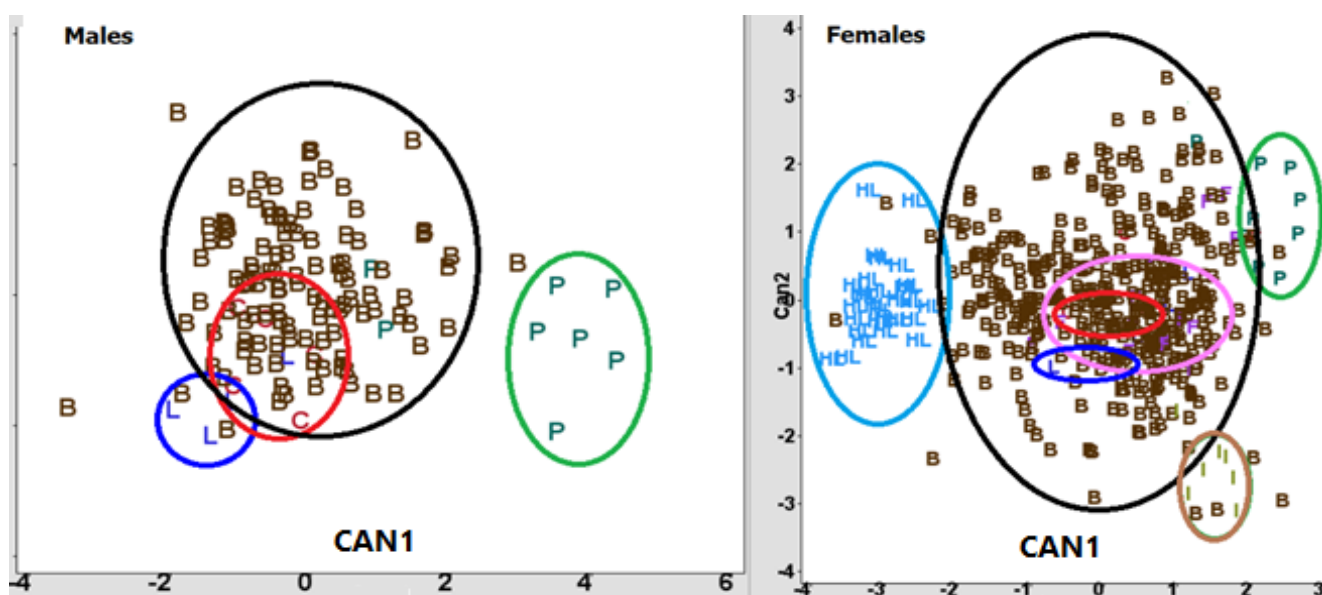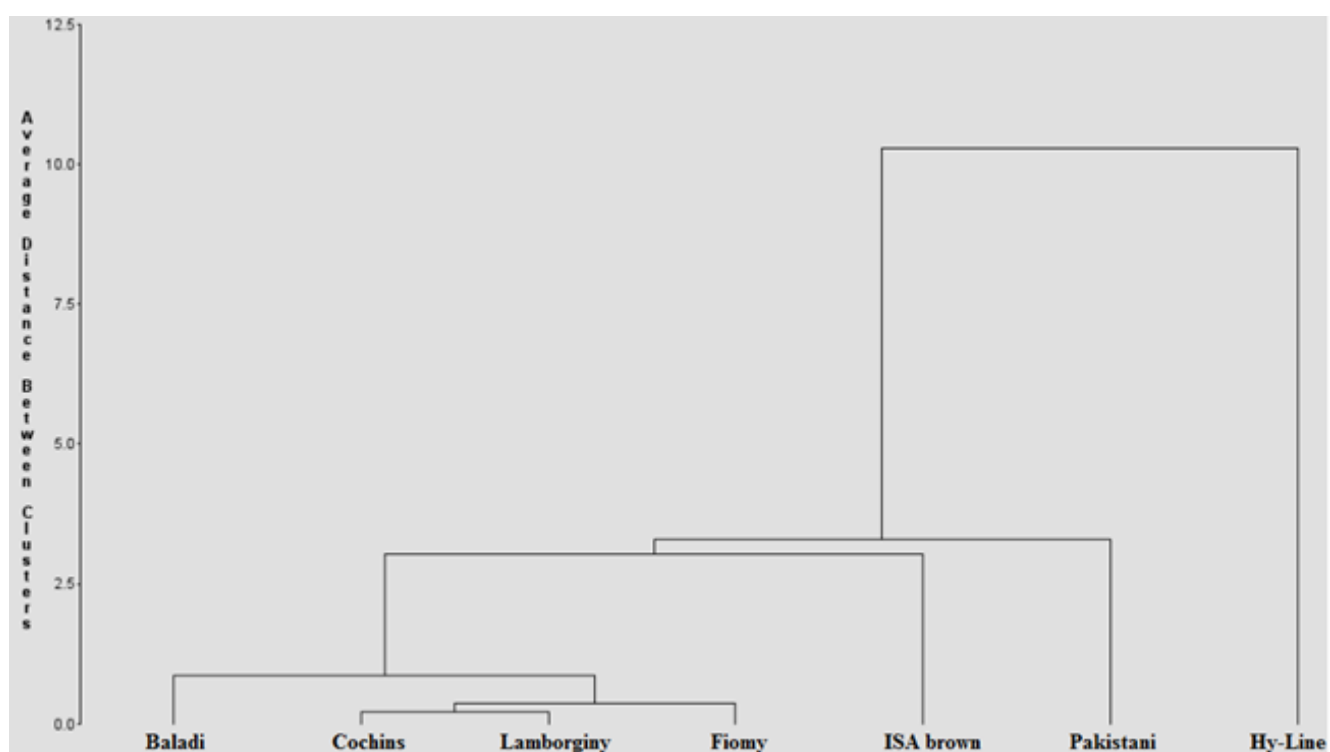| Variable | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | CAN1 | CAN2 | CAN3 | CAN4 | CAN1 | CAN2 | CAN3 | CAN4 |
| Body weight | 0.00108 | 0.00006 | -0.00037 | -0.00035 | 0.00062 | -0.00168 | 0.00192 | 0.00061 |
| Body color | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00001 | 0.00000 | -0.00001 |
| Comb color | 0.00036 | 0.00095 | -0.00043 | -0.00017 | 0.00000 | 0.00004 | -0.00006 | 0.00002 |
| Comb type | -0.14447 | 0.33245 | -0.08804 | 0.17721 | -0.15034 | 0.28590 | -0.04539 | 0.16189 |
| Comb size | -0.00475 | 0.04714 | -0.02647 | -0.00885 | -0.08415 | -0.00398 | 0.01888 | -0.01554 |
| Peak color | 0.00038 | 0.00065 | -0.00126 | -0.00064 | 0.00004 | 0.00002 | 0.00007 | 0.00000 |
| Face color | 0.00240 | -0.00010 | -0.00047 | 0.00012 | -0.00004 | -0.00007 | -0.00011 | 0.00009 |
| Face size | 0.00051 | 0.00930 | 0.05111 | 0.03171 | 0.02153 | 0.07010 | 0.00581 | -0.00871 |
| Wattle color | 0.00070 | -0.00005 | -0.00089 | 0.00181 | -0.00003 | 0.00070 | 0.00063 | 0.00037 |
| Wattle size | -0.04848 | -0.04855 | -0.02255 | 0.06530 | 0.02708 | 0.00945 | -0.06279 | 0.04872 |
| Earlobe color | 0.00054 | 0.00041 | 0.00077 | -0.00025 | 0.00000 | 0.00000 | -0.00002 | 0.00001 |
| Eye color | -0.00157 | 0.00090 | 0.00017 | 0.00021 | -0.00035 | 0.00016 | -0.00007 | 0.00070 |
| Breast color | 0.00008 | -0.00002 | 0.00000 | 0.00008 | 0.00000 | 0.00001 | -0.00001 | 0.00007 |
| Breast size | 0.02873 | -0.00047 | 0.00107 | -0.00331 | 0.01378 | 0.02955 | 0.00217 | -0.09914 |
| Leg color | -0.00003 | 0.00032 | 0.00047 | 0.00181 | 0.00096 | 0.00185 | 0.00295 | -0.00017 |



**Figure 1-** Discriminating both sexes of the breeds based on canonical variables. (Breeds: Baladi = B; Cochins = C; Fayoumi = F; Hy-Line = HL; ISA brown= I; Lamborghini = L; Pakistani = P).

**Table 7 -** Canonical variables (CAN) values of each male and female breeds

| Male breed | CAN1 | CAN2 | CAN3 | Female breed | CAN1 | CAN2 | CAN3 | CAN4 |
|---|---|---|---|---|---|---|---|---|
| Baladi | -0.144 | 0.1301 | -0.018 | Baladi | 0.1257 | 0.0096 | -0.042 | 0.0663 |
| Cochins | -0.564 | -0.945 | 1.204 | Cochins | 0.5958 | 0.4087 | -0.64 | -0.755 |
| Lamborghini | -1.21 | -1.605 | -0.901 | Fayoumi | 0.7465 | -0.033 | -0.69 | -0.719 |
| Pakistani | 2.9001 | -0.363 | -0.054 | Hy-Line | -2.933 | 0.0096 | 0.292 | -0.173 |
| | | | | ISA brown | 1.4743 | -2.467 | 1.2305 | -0.29 |
| | | | | Lamborghini | 0.4605 | -0.328 | -0.657 | -0.179 |
| | | | | Pakistani | 1.8625 | 1.5506 | 1.5019 | -0.328 |
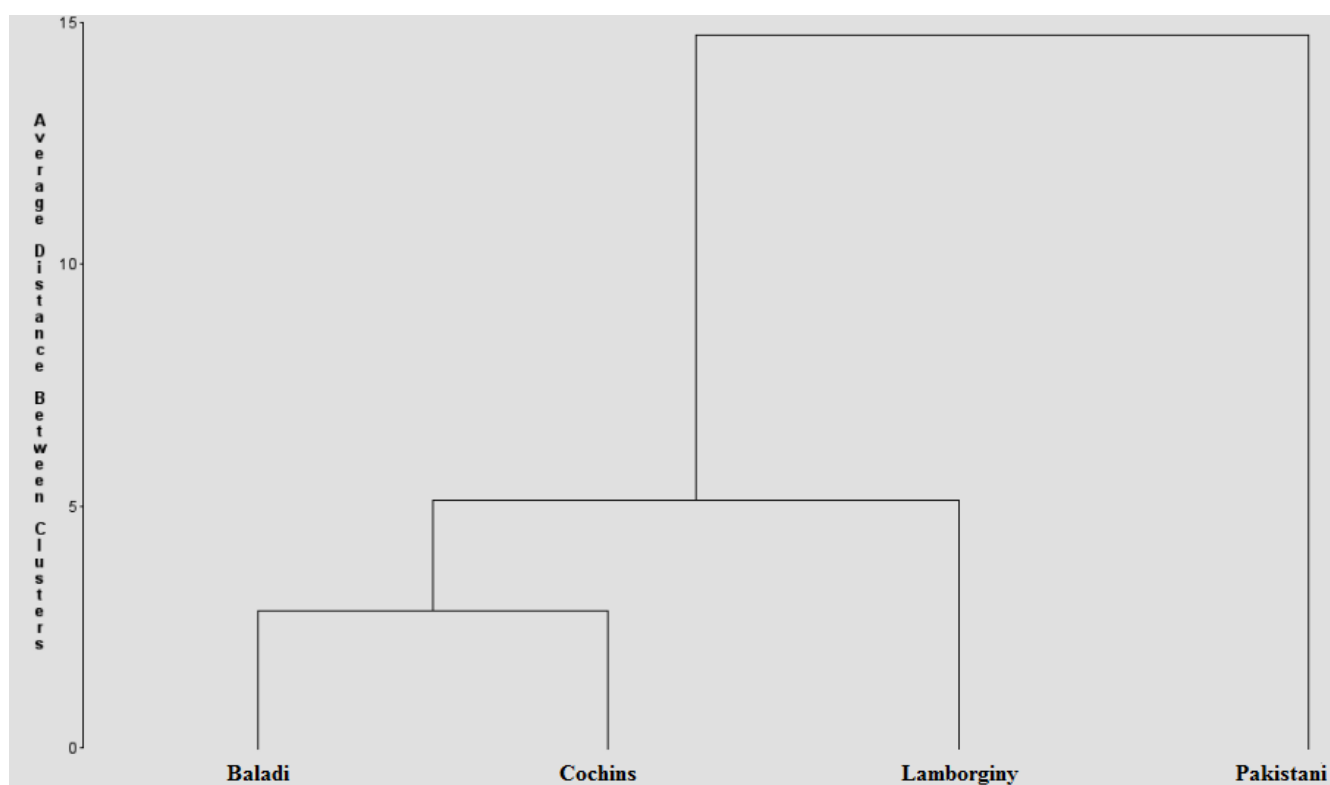


**Figure 2 -** The constructed dendrogram of males



**Figure 3 -** The constructed dendrogram of females

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## DISCUSSION

The indigenous chickens of Jordan are well adapted to the local climatic conditions of dominant heat stress and drought, scarce feed, with better resistance to diseases and stresses. Their phenotypic traits were developed because of natural and artificial selection throughout past ages. Considering their domestication and dispersal from literature, they were developed in different parts of the world from different old breeds and formed the present look like chicken phenotype. In fact, the phenotype and plumage traits of the chicken are like old and indigenous chickens of worldwide indigenous chickens. Furthermore, they were reared along with breeds come from countries as close as Egyptian (Fayoumi breed) and as far as Pakistan (Pakistani breed) (Abdelqader et al., 2007).

The results showed great variation in plumage and morphometric traits which might be a result of mainly selection and geographical proximate. Similar findings were reported for worldwide indigenous breeds of many countries (Al-Atiyat, 2009; Daikwo et al., 2011; Al-Atiyat et al., 2017). For example, in Jordan the average live weight, of Baladi chickens of Al-Karak Governorate, were 1201 and 1681 grams for females and males of one-year old (Al-Atiyat et al., 2023). In Algeria, the body weight of males was about 1.4 kg and that of females was about 1.1 kg (Dahloum et al., 2016). In Ethiopia, the body weight of males was 1.630 kg and females 1.370 kg for village chickens. In Saudi Arabia, the body weight of males was 1.50 kg and females 1.3 kg (Abudabos et al., 2017). It was found that the chickens of different geographical regions were distinct based on phenotype traits. The Jordan chicken clearly stated that breeds within the region was due to the geographical proximity along with long natural selection. The discriminant traits that separated the chickens from other studied breeds were comb type, body weight, comb size, earlobe color, wattle size, face color and breast size in males and plumage variables that significantly discriminated between pairwise breeds' comparisons were comb type and size, body weight, face and breast size, leg color and wattle size in females. Similar plumage variables were reported by Halima et al. (2007), Dana et al. (2010) and Adekoya et al. (2013). Furthermore, the canonical discriminant analysis explained the total co-variation between plumage traits of the chicken breeds. In particular, the multiple correspondence analyses showed the variation was accounted for by the CAN2 and CAN3, and thus the Canonical discriminant analysis was proved for successful identifying variation of phenotypic traits between breeds. It agrees with similar studies of worldwide chicken breeds (Rosário et al., 2008; González Ariza et al., 2022; Muluneh et al., 2023). Finally, based on phenotypic traits, the Mahalanobis distances show an expected differentiation for males and females breeds.

The large distances were observed for clearly distinct breeds; mainly the Pakistani, Hy-line and Issa Brown from other ones. It is worth mentioning that Baladi chicken was closer to the ancient exotic breeds and commercial breeds found in Jordan and reared long with for long time ago. Nevertheless, the result might explain why Baladi individuals were located within groups of other breeds. This demonstrates that the significant (P<0.0001) studied phenotype traits of the Baladi were in similar with others and able to discriminate them away or close. It was found that most males of exotic breeds were correctly assigned (100%) into each of own breed. However, the results showed less proportion (75-80%) for exotic females. The results prove that full description of phenotypic traits of Jordan chickens help with finding guideline of distinct traits for breed assignment and predefinition procedure for genetic conservation and breeding programs. In agreement, Larkina et al. (2021) proposed a phenotypic clustering model for breeding programs for local, commercial, and fancy breeds.

The history of Jordan chicken domestication can be inferred the uncovered origin and development. There is diversity across regions; Central (Amman, Madaba, Zarqa) areas show more diversity than the two populations from the South of the country and North. There is evidence of crossbreds or exotic breeds of chickens which were sometimes hard to assign as Baladi or otherwise. It is common that village chicken population consisted of the indigenous, crosses and exotic chickens. In general, indigenous chickens are better for their adaptability, hatchability and have reproduction performance than exotic and crossbreed chickens (Khan, 2008; Dzungwe et al., 2022). However, there is a need for practical research to improve the implementation of long-term crossbreeding programs in developing countries (Leroy et al., 2016) including Jordan. Summing up, clearly identified breeds and genetic diversity may be attributed to route of dispersion and arrival of domestic chicken in the country. It is worth remembering that the Central (Amman, Madaba, Zarqa) area of the country includes the capital Amman where there is a major sale yard market of chicken. Amman and Maddaba might receive chicken from different geographic areas across time. In addition, most commercial farms of chickens – mainly layers– are found near both regions.

## CONCLUSION

The phenotypic and plumage variations between breeds were detected within Baladi breed. The breeds had distinct differentiation reflecting the existence of high genetic variability between studied breeds based on phenotypic traits. The traits showed significant ability to differentiate breeds were body weight, comb type and comb size, face

color and breast size and wattle size in males and females. In addition to earlobe color for males and leg color females each only. Past and recent crossing and migration of the exotic and commercial breeds was notified. However, canonical discriminant analysis was capable to assess genetic differentiation of Baladi Jordan chicken breeds. On the other hand, this study presented further support for the origin of Jordan chickens as well as for the importance conserving. They still have a unique genetic pool with shared genes with other studied breeds which can be clearly reported by low phylogeographic structure across the studied breeds. In conclusion, there is always a need to conserve the breed in situ and in vivo for better knowing of its origin and utilization of the genetic resources for better tolerance of climate stresses and diseases. It is also recommended further studies based on the D-loop chicken mitochondrial DNA for determining purity and origin, and SNP chip studies for detecting adaptive genes and selection signatures.

## DECLARATIONS

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### Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

### Authors' contribution
R.M. Al-Atiyat contributed to research concept, data collection and analyses and the write up of the manuscript. M. AL-Rawashdeh, Kh. Abu-Alruz, and M. Alasasfa contributed to technical and logistic support. N. Salameh, F. Nawiseh, S. Al-Khamaiseh and MJ Tabbaa contributed to experimental design, data collection and execution. RMA, SKH and MJT contributed to data analysis and writing final drafted manuscript. All authors have read and approved the final manuscript.

### Consent to publish
The authors agree to the publication of this manuscript.

### Competing interests
The authors declare no competing interest.

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