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Genetic Diversity, Population Structure and their Association with Body Weight in Egyptian Chicken Strains

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ABSTRACT

Genetic characteristics and population structure within and among Egyptian indigenous chicken strains are important for identifying some genetic resources. The present study aimed to use microsatellite markers to determine similarity and genetic distance among different genotypes and their association with growth and production traits in Egyptian indigenous chicken strains. The current study included 800 chickens and 100 genomic DNA samples obtained from four Egyptian local chicken strains of four different areas (Dokki-4, Mandarah, Anshas, and Al-Salam) in Egypt. Their genetic characteristics, population structure, phylogenetic relationships, and their association with body weight were analyzed using seven microsatellite markers. The performance of 200 chicks from each strain was assessed in terms of individual body weight and growth rate. Al-Salam strain had a significantly higher body weight than the other strains up to 12 weeks of age among the four lines of Egyptian local chickens. Additionally, male chickens across all strains indicated significantly higher body weight than females from 2 weeks of age until the end of the experiment. The study revealed a total of 68 alleles from the 7 loci across 4 chicken strains, with an average of 9.71. The average of observed heterozygosity, expected heterozygosity, and polymorphism information content were 0.799, 0.358, and 0.707, respectively. The Mandarah strain had the highest observed allele number of 5.37; however, the lowest observed allele number was 3.12 for the Dokki strain. Analysis of population structure revealed that the four chicken strains should be divided into three clusters based on the highest log-likelihood values (ΔK value, 56.3). The results showed a degree of heterozygosity in the Mandara strain with 66.7% individual memberships, indicating a level of admixture. On the other hand, the Al-Salam strain indicated a high genetic diversity with 99% individual membership. The current study provides valuable insights for future genetic polymorphism studies, the advancement of breeding programs, and strategies for the conservation of the Egyptian local chicken strains.

Keywords: Body weight, Chicken, Genetic diversity, Microsatellite

INTRODUCTION

Poultry production is considered an integral part of agriculture all over the world, with particular emphasis on native poultry due to their meat quality and production potential (Bennett et al., 2018). Chickens play an essential role in providing economically feasible and nutritionally essential human resources (Fontanesi, 2009). A lot of poor rural societies keep native chickens for numerous reasons, such as white meat and egg production (FAO, 2016). In Egypt, native chicken production accounts for 55% of white meat production (Hassanane et al., 2018). Therefore, chicken genetic resources are a necessary part of the biological basis for world diet safety. In recent years, Egyptian local chicken breeds have received insufficient attention on a commercial scale since breeders focus on the use of highly productive commercial broiler chickens (Ramdan et al., 2014a; Nassar et al., 2019). Moreover, native chicken breeds indicate greater disease resistance and environmental adaptability, compared to commercial strains (Padhi, 2016; Rashid et al., 2020). The Egyptian chicken strains exhibit tolerance to heat stress conditions, greater resistance to diseases, and are well-adapted to challenging environments (El-Gendy et al., 2011). Egyptian local chickens have a series of necessary meat

qualities containing superior tenderness and favored tastes that are often preferred by consumers. However, their growth rate and egg production rates are lower than commercial chicken breeds (Nassar et al., 2019). Therefore, crossbreeding among native strains and foreign strains has been used as a strategy to produce some Egyptian strains, such as Dokki-4, El-Salam, Anshas, and Mandarah (Kosba and El-Halim, 2008; Heba et al., 2017).

The importance of preserving chicken biodiversity has received increasing attention in recent years. Although there are a lot of efforts to characterize chicken breeds using morphological measurements and performance traits, molecular methods for characterizing chicken breeds have not been extensively explored (Ramadan et al., 2014b; 2018). Utilizing molecular genetic tools for selection offers a valuable method to enhance the quality of chicken meat and carcasses (Zhou et al., 2006). According to Sahu et al. (2022), ADL0273 has significant effects on some economic traits in chickens. Understanding the genetic characteristics and identifying similarities and differences within and among different breeds is crucial for genetic improvement programs in farm animals (Mirhoseinie et al., 2005). Microsatellite markers are invaluable tools in assessing genetic variation and similarities among strains and species. These markers display a high polymorphism rate and are codominant, making them particularly useful in genetic studies (Yang et al., 2013). The implementation of marker-assisted selection has significantly expedited the breeding process for enhancing chicken growth. Notably, this approach has yielded substantial advancements in genetic improvement while also reducing costs and time requirements (Boichard et al., 2016). Microsatellite markers for studying biodiversity within and among breeds are now identified, with each marker sequence located on loci associated with performance traits (FAO, 2016). Microsatellites have become optimal markers for an assortment of molecular investigations due to their adaptability, operational flexibility, and minor price, compared to other marker methods (Kantartzi, 2013; Zimmerman et al., 2020). Given this, the present study aimed to use microsatellite markers to determine similarity and genetic distance among different genotypes and their association with growth and production traits in Egyptian indigenous chicken strains.

MATERIALS AND METHODS

Ethical approval

The experimental procedure used in this investigation was approved by the Animal Care and Use Committee (Medical Research and Ethics Committee) of the National Research Centre in Egypt, with certificate number 1484052023.

This study was executed at Nobaria Research Station, Animal Production Biotechnology Lab, Central Laboratory Network, National Research Centre, and Department of Cell Biology, Institute of Biotechnology Research, National Research Centre, Giza, Egypt.

Populations and management

Four Egyptian chicken strains (Dokki-4, Mandarah, Anshas, and Al-Salam) were utilized in this study. A total of 200 chicks for each strain were brought from Fayoum Poultry Station, Egypt. Three replicates were used. The one-day-old chicks were wing-banded, intermingled, and brooded (10 chicks/m^2) in floor pens. This process was carried out in a conventional-type house, placed in floor-rearing pens in a conventional-type house until reaching 12 weeks of age.

Incubation and ration

Chicks were incubated at 35°C from hatch to 3 days of age, with a gradual reduction to 32°C by day 7. Subsequently, the temperature was decreased by 2°C per week until reaching 24-25°C by week 5. Humidity was at 45-55% during the experimental period. The chicks were provided with a diet containing 22-23% crude protein (CP) and 2800 Kcal metabolizable energy (ME)/ kg from hatch to 4 weeks. From week 4 to week 12, the diet included 19-20% CP and 3100 Kcal ME/ kg. Water and feed were available *ad libitum*. The lighting period was 24 hours/day. All chickens were vaccinated according to the vaccination program described by Nassar et al. (2019). The chicks were vaccinated against Newcastle disease at day 7 (eye drop, Hitchner, Nobilis®, The Netherlands), day 10 (sub-cutaneous injection with Newcastle inactivated vaccine, Nobilis®, USA), and day 21 (eye drop, La Sota strain, Nobilis®, The Netherlands). Additionally, vaccinations against infectious bursal disease occurred on days 14 and 24 (eye drops) using a Gumboro D-78 strain (Nobilis®, The Netherlands) and against the avian influenza virus using the sub-cutaneous injection of H5N2 (Nobilis®, The Netherlands) inactivated vaccine at the first week of age.

Phenotypic measurements

The parental and progeny chicken populations were weighed at hatch and then biweekly up to 12 weeks of age individually. Biweekly body weight gains and growth

rates were then calculated. The growth rate was calculated using the Formula 1.

(Formula 1)
Growth rate = Body weight gain / Average of both weights at first and end period × 100.

Sampling and DNA processing

At 8 weeks of age, blood samples (3 ml/ chicken) were randomly collected from a total of 100 chickens representing four local Egyptian chicken strains, with 25 from each strain. The blood was collected in tubes containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant and kept at -20°C. Genomic DNA was extracted using 200 µl of each sample according to Thermo Scientific Gene JET Whole Blood DNA Purification Kit (Paisley PA4 9RF, UK). The DNA quality and quantity were determined using UV-spectrophotometer, the UV spectrum at 260 nm and 280 nm (FLUOstar OPTIMA F-Microplate Fluorimeter, Germany) using the Formula 2 described by Sambrook et al. (1989).

(Formula 2)
DNA concentration (µg / µl) = A260 x 400 x 0.05

Microsatellites and polymerase chain reaction program

Seven Microsatellite markers related to chicken performance traits were used based on information available in the gene bank database. Details of microsatellite markers are shown in Table 1. The conditions and program of PCR were described by Ramadan et al. (2018).

The PCR products were electrophoresed at 100 V on a 2% Agarose gel and visualized by staining with ethidium bromide. Sambrook et al. (1989) used the procedure for the allele separation using 8% acrylamide gel.

Table 1. Microsatellite primer codes, sequences, and distribution in chicken chromosomes

Marker	Chromosome number	Forward primer	Reverse primer	References
ADL0158	10	TGG CAT GGT TGA GGA ATA CA	TAG GTG CTG CAC TGG AAA TC	(Das et al., 2015)
ADL0273	Z	GCC ATA CAT GAC AAT AGA GG	TGG TAG ATG CTG AGA GGT GT	(Goto et al., 2016)
ADL0292	5	CCA AAT CAG GCA AAA CTT CT	AAA TGG CCT AAG GAT GAG GA	(Choi et al., 2015)
LEI0079	1	AGGCTCCTGAATGAATGCATC	TCATTATCCTTGTGTGAAACTG	(Podis et al., 2013)
LEI0094	4	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC	(Cho et al., 2020)
MCW0064	8	CTTCAAGAGCCATAGGTGGTCT	TCTCAGCACTACAAAATACACAGG	(Zhou et al., 2006)
MCW0300	27	CAGAGAAACGTGCATGTGGAC	TGTGCACATTTCTCTGCTGAC	(Ambo et al., 2009)

ADL: Avian Disease and Oncology Laboratory, Michigan State University Lansing, LEI: University of Leicester, Leicester, UK, East, MCW: Microsatellite chicken Wageningen, Netherlands

Genotyping

The genotype of chickens was described by Ramadan et al. (2018). Convert version 1.3.1 was used to prepare input files compatible with various genetic software packages, as suggested by Glaubitz (2004). Heterozygosity (H) was estimated using POPGENE 3.2 software package, while PIC was determined using CERVUS 3.0 software. Sysat 7.0 software was employed to draw the dendrogram presentations (Yeh et al., 1999).

Statistical analysis

The Xlstat software, a general linear model XLSTAT 2017, was used for data analysis as a two-way analysis of variance. The main effects were line and sex. The traits analyzed included body weights at hatch, 2, 4, 6, 8, and 12 weeks of age using the following model.

$Yijk= \mu + Li + Sj + LSij + eijk$

Where, Yijk is the k_{th} observation of the j^{th} sex within the i^{th} line, µ denotes the overall mean, Li accounts for the effect of the i^{th} line, Sj determines the effect of the j^{th} sex, Sj refers to the effect of the j^{th} sex, LSij signifies the interaction between the i^{th} line and the j^{th} sex, and Eijk is the random error.

All data are presented as least square means ± standard deviations. Mean values were separated when significance existed, using Duncan (1955). P value less than 0.05 was considered statistically significant.

Genetic structure and the admixture degree are defined using the Baysian algorithm implanted by the STRUCTURE software v.2.3.4 (Pritchard et al., 2000). To infer the ancestral population number, 10 independent runs were achieved for each K value from 2 to 6. For all runs, the admixture models had a burn-in period of 50,000 repeats, followed by 100,000 repeats of the Markov Chain

Monte Carlo algorithm. The Structure Harvester website implementing the Evanno method was used to identify the K value that fits the maximal value of L(k) of the given data. Structure software is a tool that uses a systematic Bayesian clustering approach aiming at defining the cluster number of individuals on their genotypes at multiple loci using Markov Chain Monte Carlo (MCMC) estimation. The MCMC begins by randomly assigning individuals to a pre-determined number of groups. Variant frequencies are estimated in each group, and individuals are re-assigned based on those frequency estimates (Earl and vonHoldt, 2012).

RESULTS AND DISCUSSION

Lest square Means and standard deviation of body weight for hatch, 2, 4, 6, 8, and 12 weeks of age for the four chicken lines (males and females) are shown in Table 2. Al-Salam strain had a significantly higher body weight than the other strains until 12 weeks of age among the four lines of Egyptian local chickens (Table 2, $p \leq 0.05$). Al-Salam chickens were shown to have the highest body weight at hatch, 2 and 12 weeks of age (35.95, 110.80, and 906.60 g, respectively), followed by the Mandarah strain indicating an increase in body weight at 12 weeks of age (879 g), followed by Anshas chickens (826.52 g). On the other hand, Dokki-4 chickens recorded the lowest body weight at 4, 6, 8, 10, and 12 weeks of age (33.45, 89.26, 154.75, 244.45, 458.05, and 806.26 g, $p \leq 0.05$). These findings align with similar results reported in various studies, emphasizing the impact of breed variations on body weight (Ajayi and Ejiofor, 2009; Taha et al., 2012).

The findings indicated significant sexual dimorphism in body weight at all ages studied, except at hatch. The body weights of males were significantly higher than females from 2 to 12 weeks of age ($p \leq 0.05$).

At 12 weeks of age, males had higher body weight (884.82 g) than females (814.73 g), and the differences were statistically significant (Table 2, $p \leq 0.05$). This aligns with findings by Rashed (2012), who reported significant sexual dimorphism in body weights at all ages, excluding hatch time. The body weight of males from 2 to 19 weeks of age was significantly higher than that of females. These results agree with previous reports by Ramadan et al. (2014a; 2019). Compared to females, the data analysis indicated that males had higher weights in both CairoB-2 selected chickens and the control group.

Table 3 shows the genetic variability using seven microsatellite markers in four chicken strains in terms of the observed allele numbers, observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC). Microsatellite markers are used as valuable tools to improve the chickens' performance, biodiversity within and among breeds, and breeding plan programs (FAO, 2016). Association among microsatellite markers and body weight traits were observed with age in the four Egyptian chickens. Previous studies demonstrated similar results, indicating the selected chicken strain had more alleles than the control line (Nassar et al., 2013; Ramadan et al., 2014a,b). Similarly, EL-Gendy and Hela (2014) stated that a genetically improved strain (CE1) had a higher number of alleles (5.72) than the control line (2.35).

According to Table 3, a total of 68 alleles were observed from 7 loci in four chicken strains, with a mean of 9.71. The mean values for Ho, He, and PIC were 0.799, 0.358, and 0.707, respectively. The highest number of alleles was 14 alleles at locus MCW006, followed by 13 alleles at locus LEI0094, 10 alleles at loci ADL0158 and ADL0292, and 8 alleles at loci LEI0079. However, the lowest number of alleles was 5 at locus ADL0273.

The Ho values were generally less than the expected ones in the four Egyptian chicken strains for 7 loci. The highest Ho value was 0.799, whereas the average He was 0.358. The greatest Ho value was 0.838 at locus MCW006 and 0.508 at locus MCW030. The lowest values of both Ho and He were The locus with the maximum Ho was LEI0094 (0.816), followed by ADL0292 (0.734), LEI0079 and MCW0300(both at 0.701), MCW0064 (0.700), ADL0158 (0.683). The average polymorphic information content was 0.707, and the minimal polymorphic information content was 0.614 at locus ADL0273. Comparing the genetic diversity observed in Egyptian chickens in this experiment, similar patterns were found in Korean chickens by Seo et al. (2017), where Ho ranged from 0.709 to 0.882, He ranged from 0.466 to 0.852, and PIC ranged from 0.648 to 0.865. While in 3 breeds reared in Egypt, El-sayed et al. (2021) found that the Ho ranged from 0.1 to 0.85, He ranged from 0.42 to 0.71, and PIC ranged from 0.4 to 0.69. Rashid et al. (2020) detected 171 alleles using 16 microsatellite markers and the average of alleles was reported 10.7, where the mean of Ho was 0.669, He was 0.71, and PIC was 0.749.

The sample size, number of observed alleles, Ho, He, and PIC content for the four Egyptian local chickens using seven microsatellites are shown in Table 4. The Mandarah strain had the highest observed number of alleles (5.37), followed by the Al-Salam strain (3.48 alleles), Anshas strain (3.35 alleles), and Dokki strain (3.12 alleles). The same trend was observed for He. The Mandarah strain had the highest He

(0.809), followed by the Al-Salam strain (0.724), Anshas strain (0.708), and Dokki strain (0.604). The He values were generally higher than Ho values across the four chicken strains and the seven loes. The Mandarah strain had the highest Ho (0.435), followed by the Anshas strain (0.361), Dokki strain (0.285), and Al-Salam strain (0.265).

Table 2. Live body weight (g) least square mean and standard error at different ages of four lines of local chickens

Trait / S.O.V / Strain	Hatch	2	4	6	8	12
Mandarah	33.10^{b}	100.05^{b}	212.85^{b}	295.40^{c}	580.85^{c}	879.00^{b}
Al-Salam	35.95^{a}	110.80^{a}	225.55^{a}	359.02^{a}	599.15^{a}	906.60^{a}
Dokki-4	33.45^{b}	89.26^{b}	154.75^{d}	244.45^{d}	458.05^{d}	806.80^{d}
Anshas	35.34^{a}	110.38^{a}	161.35^{c}	349.20^{b}	590.99^{b}	826.52^{c}
SE	0.38	0.36	0.41	0.41	0.40	0.40
Sex						
Male	34.89^{a}	107.28^{a}	209.94^{a}	341.05^{a}	416.72^{a}	889.52^{a}
Female	34.58^{a}	97.96^{b}	194.75^{b}	283.08^{b}	500.80^{b}	814.73^{b}
Standard error	0.27	0.25	0.3	0.29	0.3	0.28
Strain*Sex						
Mandarah♂	34.30^{b}	102.40^{c}	221.00^{b}	335.90^{c}	592.60^{c}	889.50^{c}
Mandarah♀	34.10b^{c}	97.70^{d}	204.70^{f}	254.90^{f}	569.10^{e}	868.50^{d}
El-Salam ♂	36.10^{a}	119.70^{a}	235.58^{a}	359.02^{b}	639.40^{a}	914.70^{a}
El-Salam ♀	35.60^{ab}	108.90^{b}	215.52^{c}	359.03^{b}	558.90^{f}	898.50^{b}
Dokki-4 ♂	34.10^{bc}	102.30^{c}	169.50^{g}	263.40^{e}	576.70^{d}	864.90^{e}
Dokki-4 ♀	32.80^{c}	76.23^{e}	150.00^{h}	225.50^{g}	339.40^{g}	748.70^{g}
Anshas ♂	34.86^{ab}	111.74^{a}	213.70^{d}	405.90^{a}	622.19^{b}	889.01^{c}
Anshas ♀	35.83^{ab}	109.02^{b}	208.80^{e}	301.90^{d}	559.80^{f}	764.03^{f}
Standard error	0.54	0.51	0.58	0.59	0.6	0.54
Probability						
Strain	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Sex	0.432	0.0001	0.0001	0.0001	0.0001	0.0001
Strain*Sex	0.023	0.0001	0.0001	0.0001	0.0001	0.0001

N: 150 per sex within the line; ^{abcd}: Means, within age and different chicken strains (S.O.V), with different superscripts are significantly different ($p \leq 0.05$).

Table 3. The statistics for a microsatellite profile, observed allele, the observed and expected heterozygosity, polymorphic information content for seven microsatellites in Egyptian local chicken

Locus	NA	H_e	H_o	PIC
ADL0273	5	0.701	0	0.614
ADL0158	10	0.761	0.459	0.683
LEI0094	13	0.837	0.491	0.816
MCW0300	8	0.816	0.508	0.701
ADL0292	10	0.832	0.344	0.734
LEI0079	8	0.806	0.311	0.701
MCW0064	14	0.838	0.393	0.700
Total	68	5.591	2.506	4.949
Mean	9.71	0.799	0.358	0.707
SD	3.09	0.05	0.17	0.056

Na: Observed number of alleles content; Ho: Observed heterozygosity; He: Expected heterozygosity; PIC: Polymorphic information; ADL: Avian Disease and Oncology Laboratory, Michigan State University Lansing, USA; LEI: University of Leicester, Leicester, UK, East; MCW: Microsatellite chicken Wageningen, The Netherlands.

Table 4. The sample size, number of observed alleles, the observed (Ho) and expected (He) heterozygosity, polymorphic information content, mean (SD) for four Egyptian local chickens using seven microsatellites

Population	Sample size	Na	He	Ho	PIC
Mandarah	42	5.37 ± 2.26	0.809 ± 0.07	0.435 ± 0.22	0.722 ± 0.05
Al-Salam	42	3.48 ± 1.06	0.724 ± 0.10	0.265 ± 0.21	0.644 ± 0.07
Dokki	42	3.12 ± 1.66	0.604 ± 0.28	0.285 ± 0.29	0.559 ± 0.24
Anshas	42	3.35 ± 0.79	0.708 ± 0.07	0.361 ± 0.23	0.602 ± 0.09

Na: Observed number of alleles, Ho: Observed heterozygosity, He: Expected heterozygosity, PIC: Polymorphic information content, SD: Standard deviation

Table 5. The genetic differentiation among different chicken strains.

Strains	Dokki-4	Anshas	El-salam	Manarah
Dokki-4	****	0.3944	0.2236	0.0481
Anshas	0.9303	****	0.3579	0.0305
El-salam	1.4977	1.0275	****	0.2929
Mandarah	3.0338	3.4894	1.2279	****

Note: The genetic differentiation among different chicken strains. Nei's genetic identity (above diagonal) and genetic distance (below diagonal). ****: Not evaluated.

Table 6. Correlation matrix (Pearson) between the seven microsatellite markers and different ages of four local Egyptian chickens

Variables	Hatch	2-week	4-week	6-week	8-week	12-week	ADL0273	ADL0158	LEI0094	MCW0300	ADL0292	LEI0079	MCW0064
hatch	1	0.74	0.55	0.67	0.49	0.34	-0.28	-0.22	-0.21	-0.22	-0.28	-0.19	-0.18
2-week	0.74	1	0.81	0.79	0.87	0.64	-0.08	-0.09	-0.02	0.06	-0.11	-0.09	0.06
4-week	0.55	0.81	1	0.77	0.81	0.55	0.26	0.37	0.19	0.40	0.35	0.38	0.36
6-week	0.67	0.79	0.77	1	0.64	0.40	-0.30	-0.21	-0.31	-0.14	-0.24	-0.17	-0.15
8-week	0.49	0.87	0.81	0.64	1	0.76	0.19	0.19	0.22	0.35	0.16	0.10	0.26
12-week	0.34	0.64	0.55	0.40	0.76	1	-0.03	-0.01	-0.08	0.01	0.02	-0.18	0.04
ADL0273	-0.28	-0.08	0.26	-0.30	0.19	-0.03	1	0.92	0.95	0.93	0.95	0.84	0.96
ADL0158	-0.22	-0.09	0.37	-0.21	0.19	-0.01	0.92	1	0.8363	0.87	0.97	0.94	0.81
LEI0094	-0.21	-0.02	0.19	-0.31	0.22	-0.08	0.95	0.84	1	0.86	0.83	0.73	0.90
MCW0300	-0.22	0.06	0.40	-0.14	0.35	0.01	0.93	0.87	0.86	1	0.91	0.86	0.89
ADL0292	-0.28	-0.11	0.35	-0.24	0.16	0.02	0.95	0.97	0.83	0.91	1	0.91	0.90
LEI0079	-0.19	-0.09	0.38	-0.17	0.10	-0.18	0.84	0.94	0.73	0.86	0.91	1	0.73
MCW0064	-0.18	0.06	0.36	-0.15	0.26	0.04	0.96	0.81	0.90	0.89	0.89	0.73	1

ADL: Avian Disease and Oncology Laboratory, Michigan State University Lansing, LEI: University of Leicester, Leicester, UK, East, MCW: Microsatellite chicken Wageningen, The Netherlands

The highest PIC value was 0.722 for the Mandarah strain, followed by 0.644 for the Al-Salam strain, and 0.602 for the Anshas strain. Meanwhile, the lowest PIC value was 0.559 for the Dokki strain. Mandarah strain had the highest values of allele numbers, He, Ho, and PIC (5.37, 0.809, 0.435, and 0.722, respectively). Meanwhile, the Dokki strain had the lowest values of the observed alleles, the He and PIC (3.12, 0.604, and 0.559, respectively). Al-Salam strain had the lowest Ho value (0.265). The findings of the current study on allelic numbers, Ho, and He are in line with values reported by Pirany et al. (2007) and Dorji et al. (2012), who found that the mean of allele numbers was 10.33 and 14.17 in Indian and Bhutanese, respectively. On the other hand, Van Marle-Koster and Nel (2000) indicated a moderately lower mean of allele numbers than the present results, ranging

from 2.3 to 4.3 in five African chicken populations. The estimated means of the allele numbers, Ho, He, and PIC of the four Egyptian strains might be linked to the results in diverse chicken lines (Rajkumar et al., 2007). The variances in genetic polymorphism may be due to diversity in genetic base, breed, line, and strain, likewise using different microsatellite markers.

The genetic differentiation among different chicken populations was analyzed by the molecular procedure based on the identity and distance matrix (Table 5). The closest genetic makeup was observed between Dokki-4 and Anshas, which had an identity score of 0.3944 and a genetic distance of 0.9303. The same trend was observed between the Al-Salam and Mandarah chickens, who had an identity score of 0.0481 and a genetic distance of 1.2279. Phylogenies were constructed using the neighbor-joining procedure and genetic distance DA (Figure 1). The multiple alignments of the concatenated 7 loci aggregated the Dokki-4 and Anshas chickens closely in one cluster, whereas Al-Salam and Mandarah chickens joined in a different close cluster branch. Both were supported by 100% bootstrap confidence.

The genetic structure analysis was performed to identify the uniformity of the four breeds and investigate their admixture and genetic differentiation. The structure analysis showed that the most potential number of derived clusters was K = 3. The delta k value was 56.3, where the highest average of link polt posterior probability (Ln Pr) of K, termed X. Ln Pr (x/k) was shown at K = 3, and then it dropped subsequently (Figure 2). Therefore, it is expected that K = 3 is the most probable number of ancestral stains, contributing to the observed genetic diversity in the four given strains (Figure 3). Based on the individual q values, Dokki and Mandara chickens were assigned to an independent cluster 1, and the average genetic distance between their chickens and other clusters was 2.28, while the average genetic distance between them was 0.10 (Figure 4). Dokki chickens had an individual genotype membership (76.9%) within cluster 1, while the Mandara breed was a heterogeneous breed, where the percentage of its assigned chickens had a low proportion membership (66.7%). This might happen because they have multiple origins or can reflect gene pool dilution due to the inbreeding or crossbreeding of Egyptian chickens. On the other hand, Anshas chickens could be in a second independent cluster 2 with 82% of its membership and the mean genetic distance of 1.87 among its chickens and other clusters. Al-Salam breed chickens were designated to cluster 3 and the mean genetic distance among its chickens and other clusters was 1.29. Al-Salam breed was highly homogenous, showing very high proportions of individual chicken memberships (99%). Al-Salam strain chickens are heritably diverse and retain high genetic diversity. The seven microsatellite markers studied had a significantly positive association with the body weight of different strains at 8 weeks of age (Table 6, $p < 0.05$). Studies indicated that microsatellites markers MCW0010 (Liu et al., 2007; Zhang et al., 2008), MCW0018 (Sewalem et al., 2002; Navarro et al., 2005), LEI0079 (Liu et al., 2007), c3-77696549 and c5-105790 (Uemoto et al., 2009) were associated with chicken body weight at 6 weeks of age. These findings harmonize with the outcomes of Uemoto et al. (2009), who found significant positive correlation coefficients among MS c3-77696549 and c5-105790 and practically all their studied traits (body, carcass, breast, and leg meat weights).

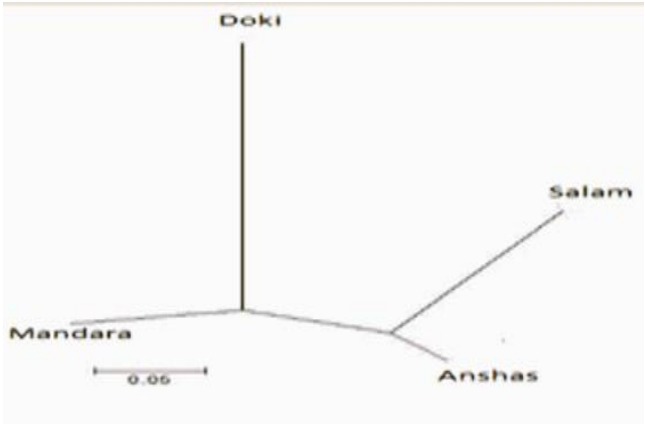

Figure 1. Dendrogram trees between four chicken genotypes using the nearest neighbor hierarchical cluster method

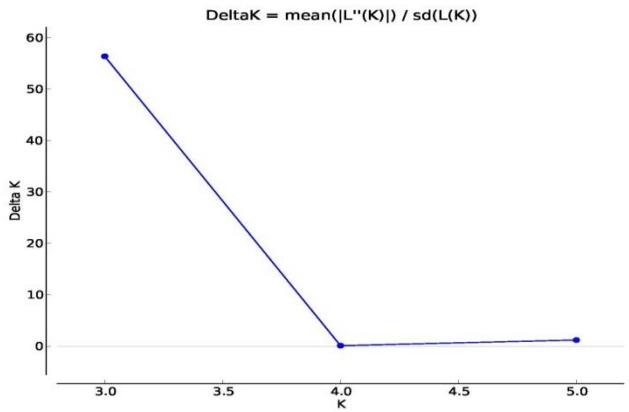

Figure 2. The genetic structure analysis to identify the uniformity and genetic differentiation of the breeds. The modal value of this distribution is the true K (=3), the uppermost level of the structure.

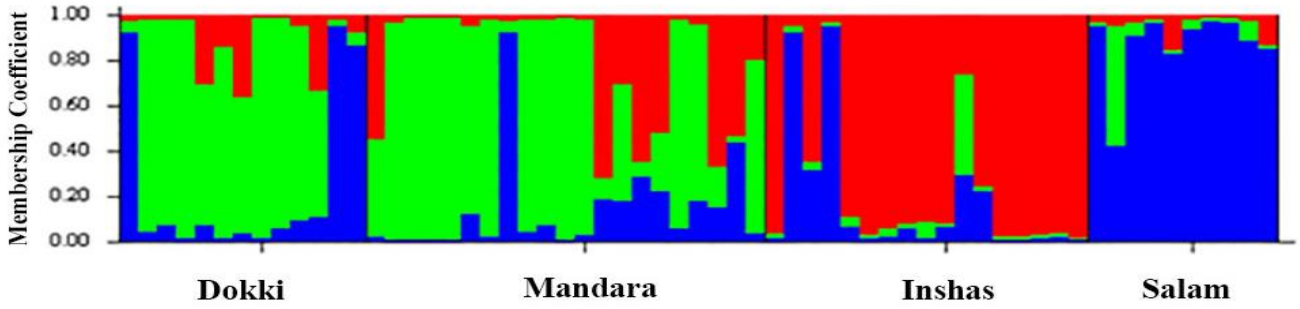

Figure 3. Clustering assignment of the four Egyptian breeds defined by STRUCTURE analyses. Each chicken is represented as a vertical rectangle that is divided into segments whose color and size correspond to the relative proportion of the chicken genome of a particular cluster. The inferred clusters were Dokki (green), Mandara (blue), and Anshas (red).

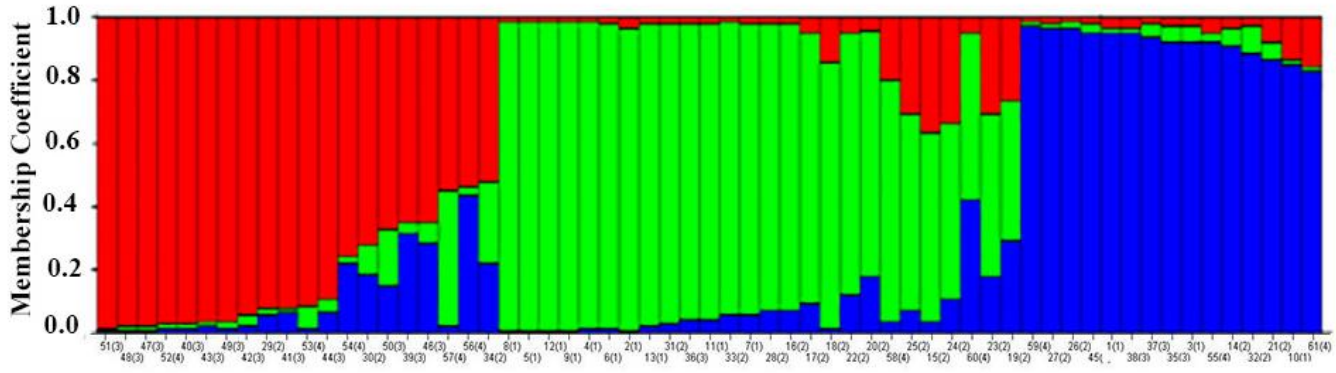

Figure 4. Graphical representation of individual genotype membership coefficients (q) in each of 3 the clusters: Anshas cluster (red), Dokki cluster (green), and Mandara (blue). Anshas and half of Al-Salam's chickens are in a group and Dokki and half of Al-Salam chickens lie in the group.

CONCLUSION

From all results of the current study, it is recommended to use the 7-microsatellite marker to study genetic biodiversity and selection and crossing program for the four local Egyptian chickens. These seven markers showed the highest significant correlation coefficients with body weight at 8 weeks of age. The findings of the present study offer valuable insights for identifying superior local chickens based on genotype characteristics. These insights provide new clues for further studies on breeding programs in local Egyptian chicken strains.

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Author's contribution

This study was done in collaboration with all authors. Karima Fathy Mahrous[2] and Esteftah Mohamed El-Komy conceived the idea, designed the experiments and supervised the research. NIA, HRD, EME and GSR, performed the experiments and co-wrote the paper. LMS performed the experiments. HRD, EME and GSR analyzed the data. KFM critically revised the manuscript. All authors read and approved the final manuscript.

Competing interests

There is no competing interest to declare.

Availability of data and materials

All data generated or analyzed during the current study are included in this published article.

Ethical considerations

This article has been checked by all authors for ethical issues such as plagiarism, publication consent, misconduct,

data fabrication and/or falsification, double publication and/or submission, and redundancy aspects before submission.

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