Archives of Razi Institute, Vol. 78, No. 2 (2023) 633-642

DOI: 10.22092/ARI.2022.359477.2429

Original Article

Documentation of Genetic Diversity by Insulin-Like Growth Factor1 Receptor (Exon2) Gene for Fallow Deer (*Dama dama*) in Iraq

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Received 23 July 2022; Accepted 13 August 2022
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Abstract

Insulin-Like Growth Factor1 Receptor (Exon2) (IGF1R) gene plays a vital role in physiological impacts, such as growth, development, reproduction, and metabolism. A significant difference was noted between the IGR1R (exon 2) gene and the body weight of *Dama dama*. In addition, the heterozygosity pattern (AB) was significantly higher than the other pattern (AA). There are three single nucleotide polymorphisms (SNPs; 144G>C, 147A>G, and 210A>C) within the IGF-1R (exon 2) locus. The statistical analyses indicated the presence of three different haplotypes (GAA, CAA, and GGC). The analysis of relative frequencies indicated that the most frequent haplotype in the studied *Dama dama* population was Hap3 (GGC) (43.4782%) out of the three observed haplotypes. The results of SSCP-PCR revealed the variability of the target gene between the genotype frequencies in Fallow deer (*Dama dama*) with a high level of significance ($P \leq 0.01$) with two patterns (AA and AB) and an absence of BB pattern. The allele frequency of AA record a high level (71.74%) than the other genotype (AB) (28.26%), with a high-frequency level of the A allele (0.86) than the B allele (0.14). In current findings, SSCP genotyped in the *Dama dama* DNA observed an estimated 72% monomorphic loci and 28% polymorphic loci approximately. Hardy Weinberg equilibrium test (HW) was applied to the SSCP-PCR data matrix, and the statistical test was based on a chi-square ($\chi 2$) test. Chi-square was (55.928%) with a highly significant level ($P \leq 0.01$) recorded in the present study. As related to AA and AB genotypes mean, a significant difference ($P \leq 0.05$) was noted between IGF1R (exon 2) gene with a body weight of *Dama dama*, as well as the heterozygosity pattern (AB), was significantly ($P \leq 0.05$) higher than the other pattern (AA) (30.34±3.01kg versus 24.85±1.94kg), respectively. A significant impact ($P \leq 0.05$) between IGF1R (exon2) polymorphism and heart girth was founded to be related to the AB pattern (heterozygous) (76.92 ± 3.20 cm), whereas the lower value was related to the AA pattern (71.33 ± 2.49 cm). No significant differences in effects were shown in relation to body length and height at the shoulder. The present study is also interested in genetic characterization by calculating (Ne) as a tool for genetic diversity. Therefore, the number of alleles detected (Na) indicates that two alleles only were unique in the population of the study, with (1.3204) being the number of efficient alleles (Ne). Moreover, Shannon's Information index was recorded at 0.4073. The observed homozygosity (O.Hom.) and heterozygosity (HO) were (0.7174 and 0.2826), respectively. The values of expected homozygosity (E.Hom.) and heterozygosity (HE) were 0.7547 and 0.2453, respectively. The genetic diversity of Nei was 0.2427. The results showed an unexpected influx of IGF1R diversity measured by Fis and recorded the value (-0.1646). In this sense, the results of the current study may be considered an approximation to the total genetic diversity of the population of Dama dama in Iraq, but the information obtained is relevant to proposing the strategies of conservation for the genetic diversity observed.

Keywords: Fallow deer (*Dama dama*), IGF1R (exon2) gene, SNPs, SSCP-PCR, Shannon's index, Gene flow

1. Introduction

Deer are hoofed mammals forming the family of Cervidae. Male deer of all species (except the Chinese water deer) and female reindeer grow and shed new antlers yearly. Most often, Deer are social and live in groups, the male is considered a dominant individual in the herd, and the herds are separated by sex (1).

Additionally, this species is under threat seriously in its Turkish native range, and the subspecies Dama dama mesopotamica is listed in Appendix I under the Convention on International Trade in Endangered species; therefore, the import and export of this subspecies are subject to regulation under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (2).

Fallow deer is one of the deer species. The male is a buck; the female is a doe and the young a fawn. Adult bucks are (60-100) kg in weight, (85-95) cm in shoulder height, and typically (140-160) cm long; does are (130-150) cm long, (30-50) kg in weight, and (75-85) cm in shoulder height. The giant bucks may measure 190 cm long and weigh 150 kg (3). Fawns are born in spring at around 30cm and weigh around 4.5kg. Their lifespan is about 12-16 years.

There are several growth factors, such as insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), and transforming growth factors (TGFs), have been isolated from different tissues of several species; these substances are active in stimulating the cells and tissues' growth, as well as wound healing are called growth factors. In the liver, 75% of growth factors are produced by growth hormones besides insulin stimulation; their impacts are expressed on specific receptors and modified via specific binding proteins (4).

There is a significant association identified between polymorphism of genes with production and growth performance in diverse farm animals, including; cattle (5) and sheep (6, 7), among which Insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 1 receptor (IGF-1R) are candidate genes that impact on growth and production in livestock (6). IGF-1 exerts its biological impacts by binding to IGF1R on target tissues (8). Insulin-like growth factor 1 receptor (IGF1R) is a glycoprotein membrane that mediates most of the biological functions of IGF1 and IGF2 genes, which have significant effects on performance and are related to age, sex, horns, parity, meat quality, and carcass in livestock (6, 9). IGF-1R is a tetrameric glycoprotein-tyrosine kinase receptor consisting of two α subunits extracellular and two β subunits intracellular, assisting downstream signals (10). The IGF-1 ligand binding to the receptor on the surface of cells leads to the activation of two major pathways, the PI3-kinase and MAP-kinase, to the regulation of IGF1 response on target tissue (11). On the other hand, IGF-IR can be regulated by microRNAs (miRNA) let-7a and let-7f; it may represent novel regulatory factors of IGF1R expression in antler cell proliferation of sika deer (12).

2. Materials and Methods

The experimental period was performed from May 2021 to April 2022 at Wahj Al-DNA Company for Qualification and Training / Baghdad.

2.1. Herd Information

Accurately, the herd information was noted, including the number of births per female, animal ages, and morphometric measurements. Fallow deer were reared in sheds housing under the natural environmental condition in the middle of Iraq. In the present study, fifty animals of Fallow deer of both sexes and different ages were used. The samples were collected from the middle and south of Baghdad City.

2.2. Blood Samples Collection

A specialized veterinary team from the Faculty of Veterinary Medicine, Al-Qasim Green University, took blood samples from the jugular vein. 3 ml of each blood sample was set in an EDTA tube. After blood collecting in the tubes, it was kept in a cooled, thermally insulated box (4ºC) and then transported to the lab with minimum disturbance and shaking. In the laboratory, all the tubes were kept in a deep freezer (-20 ºC) for molecular analysis.

Directly after taking blood samples from Fallow deer, the morphometric measurements were made on deer using metric tape (± 1 cm). The dimensions include body length, height at the withers, heart girth, and body weight by a digital scale for 300 Kg placed on flat ground.

2.3. Genomic DNA Isolation

Total gDNA was isolated from the whole blood and collected in EDTA anticoagulant tubes with minimum shaking; it can be used for molecular studies and applied by the Easy Pure Blood Genomic DNA kit (Quick-DNA™ Blood MiniPrep Catalog Nos. D3024 & D3025). By using the Nanodrop instrument, the concentration of DNA samples was estimated. 1µl of the extracted DNA was placed in the lens of the Nanodrop instrument in order to detect the concentration in ng /µL at 260 nm wavelength, while the ratio of absorbance at 260 nm and 280 nm was used to assess the purity of DNA.

Agarose gel electrophoresis was adopted after DNA extraction according to the protocol of Sambrook (13) for confirmation of the integrity and presence of DNA. It consists of agarose, 1 X TBE buffer, DNA marker (100bp), and red safe nucleic acid staining solution (10 mg/ml).

2.4. PCR Conditions

PCR is done in three steps (Denaturation, annealing, and extension). MaximePCR Pre Mix Kit (i-

Taq) is the product mixed with every component: i-Taq DNA Polymerase, Dntpmixture, reaction buffer, in one tube for 1 rxn PCR. The components of PCR; are 5U/µl (i-Taq DNA polymerase), 2.5 mM (dNTPs), 1X (reaction buffer (10X), and 1X (gel loading buffer) as well as template DNA and primer set (Table 1).

Table 1. The specific primer of IGF1R (Exon 2) gene for SSCP by the method of Yang, Zhang (14)

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'-TGTGCTTTCCTTGTAACTGAAGCCT- 3'	56.04	44	380 bp
Reverse	5'-GCCCCACGGGTGATATTCC - 3'	55.41	63	

The mixture of the specific interaction for the candidate gene under study was 25µl; 5µl (Taq PCR Pre Mix), 10 picomols/µl (1µl) (forward and reverse primer), 1.5µl gDNA, and 16.5µl (distill water). PCR conditions for the IGF1R (Exon 2) gene are shown in table 2.

Table 2. The optimum PCR conditions of IGF1R (Exon 2) Gene

Phase	Tm (°C)	Time	No. of cycle
Initial Denaturation	94	5 min.	1 cycle
Denaturation	94	45sec.	45 cycle
Annealing	62	45sec.	
1st Extension	72	45sec.	
2nd Extension	72	10min.	1 cycle

2.5. SSCP Conditions

SSCP technique is carried out using Bio-Rad Protein II xi Cell vertical gel electrophoresis unit (Bio-Rad laboratories) for amplified gene fragments. SSCP experiment was based on Orita, Iwahana (15) 's protocol initially described earlier with some modifications. Briefly, about 4 µl of PCR product and 12 µl of a formamide dye is prepared in a PCR tube and denatured at 95 °C for 10 minutes in the Biometra PCR machine, then kept in an ice-chilled box and kept in -20 °C deep freeze for 10 minutes immediately. The amplicons that are denatured were loaded into the bis/acrylamide solution, involving (24 ml) of 50% acrylamide-bisacrylamide, (5 ml) of 1X TBE buffer, (10 ml) of glycerol, and (61 ml) of autoclaved HPLC water. The gel of SSCP was subjected to silver staining to visualize SSCP band patterns (16).

2.6. DNA Sequencing

PCR resolved amplicons were commercially sequenced according to the instruction manuals of the sequencing company. PCR products for different patterns of the D-Loop gene were purified and sequenced by Macrogen Incorporation Geumchen (Seoul, South Korea) to identify single nucleotide polymorphisms (SNPs) in samples under study.

2.7. Statistical Analysis

The data were processed by one-way classification (Analysis of Variance, ANOVA) using Completely

Randomized Design (CRD). The results obtained in all measurements were presented in the tables as mean values ± standard error of the mean (SE).

To detect the impact of different factors and polymorphism in the present study, the Statistical Analysis System (Stat Soft Inc., Tulsa, OK, USA) - SAS (17) Program and General Linear Model - GLM procedure were used.

Duncan (18) multiple range test 1955 (Analysis of Variation-ANOVA) and Last Square Means (LSM) were used to comparing the significant difference between the mean values at $p < 0.05$.

The genetic diversity, including allele frequency, chi-square test (χ^2), and Hardy-Weinberg Equilibrium (HWE), were used to compare the genotype percentage distributions in Fallow deer (*Dama dama*).

$Y_{ijklmn} = \mu + G1_i + A_j + S_k + P_m + e_{ijkmn}$

Where; Y_{ijklmn} is body weight and body dimensions (body length, height at shoulder, and heart girth), μ is the overall mean of traits, and G_i is the effect of IGF1R(exon2) gene genotypes (AA, AB, and BB), A_j is the effect of age (least than 1, 1-3, more than 3 years), S_k is the effect of sex (female and male), P_m is the effect of parity (0, 1, 2, 3) and e_{ijklmn} is the random error that is distributed naturally in an average equal zero and variation equal $\sigma^2 e$. The P value of 5% was defined as statistical significance.

According to Hill and Mackay (19), allele frequency for genes under study was calculated.

Shannon's Information index (I) (20), Nei (21) 's expected heterozygosity, and gene flow estimated (Nm) via PopGene (Yeh et al., 1999) were calculated for the D-Loop gene to estimate genetic variation between populations of Fallow deer (*Dama dama*).

Nm = Gene flow was estimated from Fst, = 0.25(1 – Fst)/Fst.

3. Results and Discussion

3.1. Genomic DNA Extraction

Genomic DNA was extracted from 46 fresh blood samples of Fallow deer by Easy Pure Blood Genomic DNA kit (Quick-DNA™ Blood MiniPrep Catalog Nos. D3024 & D3025) prepared by Zymo/USA through Wahj Al-DNA Company for qualification and training/ Baghdad.

DNA purity was investigated by Quawell Nanodrop / Hong Kong with wavelength 260/280 nanomol, 1.75 to 2.08 nanomol.

All samples of genomic DNA were loaded by gel electrophoresis within (0.5%) agarose for a half-hour using nucleic acid staining solution (red safe), then DNA bands are shown clearly in figure 1.

3.2. The Amplification of IGF1R (Exon 2) Gene

The Polymerase Chain Reaction (PCR) technique amplified for genomic DNA to study the impact of the Insulin-like Growth Factor 1 Receptor (IGF1R) exon 2 gene in Fallow deer production. The PCR product of the target gene was tested by 2% agarose gel electrophoresis with 1X TBE buffer. The bands of candidate genes appeared at 380 bp using pairs of primer prepared by Integrated DNA Techno (IDT) company/Canada (Figure 2).

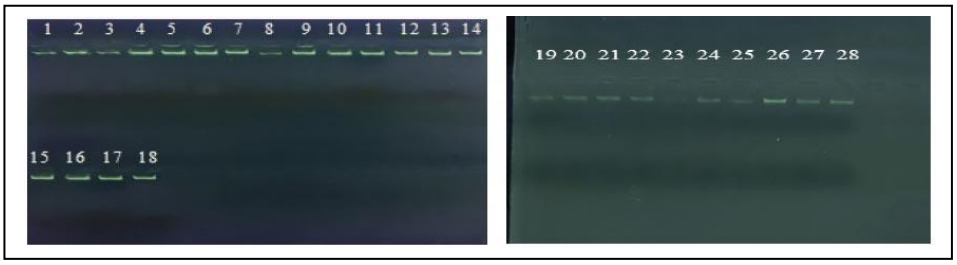

Figure 1. The bands of gDNA were on 0.5% agarose gel at 60 volts for 30 min with adding 3 µl red safe. Lane 1-28 genomic DNA extracted from blood samples used in the present study

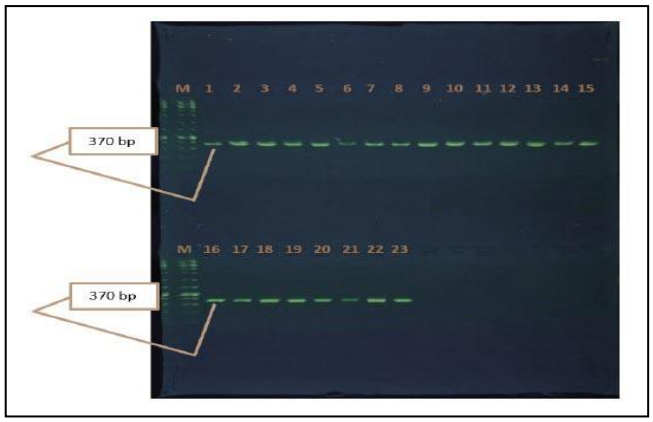

Figure 2. IGF1R (Exon 2) products separated at 380 bp on 2% agarose gel electrophoresis at 5 volt/cm^2 for 60 minutes. M:100 bp ladder

3.3. Sequence alignment with World GenBank

Matching of IGF1R (exon 2) primer (380bp) using National Center of Biotechnology Information (NCBI), (HM988958.1) (22), depending on reference study (14). The amplicons that are successfully amplified of the IGF1R (exon2) gene (380 bp). The current study was shown from different samples through Bio Edit Sequence Alignment Editor Software. All the SNP variations that were suggested were deposited and confirmed within the NCBI database with Accession number: MZ787951.1 (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for IGF1R (exon2) gene in Iraqi Fallow deer (*Dama dama*), the 1st time in the world.

However, the sequencing alignment of *the Dama dama* IGF1R (Exon2) gene was aligned with *Cervus Nippon* (Sika deer) within Genbank sequence ID: (JN009669.1) that registered by Colitti (23), appeared identical 100% in some of the samples whereas, other samples appeared variation when they recorded approximately 99%. Table 3 explains the sequence alignment of the target gene of Iraqi Fallow deer (*Dama dama)* compared with (JN009669.1), as well as the type and ratio of substitution. The identities of three variations in nucleotides were recorded; 144G>C, 147A>G, and 210A>C.

Table 3. Sequence alignment of IGF1R (exon2) gene of Iraqi Fallow deer (*Dama dama*)

Type of substitution	SNPs	Sequence ID with compare	Source	Identities
Transversion	C144G	JN009669.1	*Cervus Nippon*	99%
Transition	G147A	JN009669.1	*Cervus Nippon*	99%
Transversion	C210A			
No variation	---	JN009669.1	*Cervus Nippon*	100%

3.4. Accession Number and Registration of IGF1R (exon 2) Gene within NCBI

The length of the IGF1R (exon2) gene is (1-380) bp. There are three nucleic acid variants observed [C144G (G>C), G147A (A>G), and C210A (A>C)]. These differences were recorded in the currently nucleic acid sequences in the samples under study and were not found in the corresponding reference sequences. Three nucleic acid variations of the IGF1R gene in other locations were reported by other hands (A214G, C246T, and C65T) in Sika deer (14). To confirm the variations of the target gene, the sequencing chromatograms of the investigated samples were verified and documented according to their positions in the PCR amplicons (Figure 3).

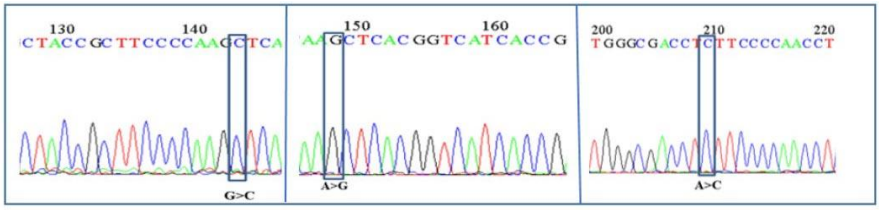

Figure 3. Fallow deer *(Dama dama)* DNA sequences of IGF1R (exon 2) gene chromatogram. The variations that were observed were 144 G>C, 147 A>G, and 210 A>C

The current DnaSP statistical analyses indicated the presence of three different haplotypes (GAA, CAA, and GGC). These haplotypes exhibited various distributions with a variable of nucleic acid diversities. The analysis of relative frequencies indicated that the most frequent haplotype in the studied *Dama dama* population was Hap3 (GGC) (43.4782%) out of the other observed three haplotypes shown in table 4.

Table 4. Genetic analysis of IGF1R (exon2) haplotypes in Fallow deer population

Hap no.	Hap Description	Location	Hap. Frequency
Hap1	GAA	---	0.217391
Hap2	CAA	144	0.347826
Hap3	GGC	147 and 210	0.434782

3.5. Results of SSCP-PCR for IGF1R (Exon2) Gene in Fallow Deer (*Dama dama*)

This study examined IGF1R (Exon2) gene polymorphism among Iraqi Fallow deer and tested for their association with many parameters. The genotypes and allele frequency distributions for target gene polymorphism are shown (Table 5).

Table 5. Distribution and allele frequency with Chi-Square of IGF1R (Exon2) gene polymorphism in Fallow deer (*Dama dama*) in Iraq

IGF1R gene Polymorphism	Number	Percentage (%)
AA	33	71.74
AB	13	28.26
BB	0	0.00
Total	46	100%
Chi-Square (χ^2)	---	55.928 **
Allele	**Frequency**	
A	0.86	
B	0.14	

** ($P \leq 0.01$)

The results revealed the variability of IGF1R (exon 2) gene between the genotype frequencies in Fallow deer (*Dama dama*) with a high level of significance ($P \leq 0.01$) with two patterns (AA and AB) and absence of BB pattern for target gene. The allele frequency of AA record a high level (71.74%) than the other genotype (AB) (28.26%), with a high-frequency level of the A allele (0.86) than the B allele (0.14); this variation is perhaps related with the size of populations under study.

In current findings, SSCP genotyped in the *Dama dama* DNA observed an estimated 72% monomorphic loci and 28% polymorphic loci; the polymorphic may produce from the low number of alleles (Na = 2) and, consequently, this may be affected by the founder effect. The presence of polymorphic loci in this work is also reported in other studies of white-tailed deer populations (24). It is essential to consider that the monomorphic detected probably also be affected by reducing No. Sampled of individuals and the loci number that were analyzed (25).

Hardy Weinberg equilibrium test (HW) was applied to the SSCP-PCR data matrix to determine the loci found or not in equilibrium; the statistical test was based on a chi-square (χ^2) test. Chi-square was (55.928%) with a highly significant level ($P \leq 0.01$) recorded in the present study (Table 5). Dakheel and al-Anbari (26), as related to IGF1R (intron 2) gene polymorphism, suggested that the chi-square value was high significant ($P \leq 0.01$) (75.12%) through his studies on Fallow deer (*Dama dama*).

3.6. Relationship between IGF1R (Exon2) Gene Polymorphism with a Body Weight of Fallow Deer (Dama dama)

As related to AA and AB genotypes mean, there is a significant difference ($P \leq 0.05$) was noted between the IGF1R (exon 2) gene and the body weight of *Dama dama* (Table 6). In general, the heterozygosity pattern (AB) was significantly ($P \leq 0.05$) higher than the other pattern (AA) (30.34±3.01kg versus 24.85±1.94kg), respectively. Whatever these results are in agreement with Dakheel and al-Anbari (26) when they study about Fallow deer and suggested the relation between IGF1R (Intron 2) gene polymorphism with body weight, and he reported the heterozygosity pattern (32.67±8.77 kg) was higher than the homozygosity (25.21 ±1.86 kg and 18.90±4.67 kg) with high significant difference ($P \leq 0.01$). On the other hand, some studies confirmed

the same results as the present finding, like Luo, Qin (27) when studWang, Ouyang (28) on a pig.

Table 6. Relationship between IGF1R (Exon 2) gene polymorphism and body weight of Fallow deer (*Dama dama*)

Polymorphism	No	Mean ± SE (kg)
AA	33	24.85 ± 1.94 b
AB	13	30.34 ± 3.01 a
Level of Sig.	---	*

* ($P \leq 0.05$)

3.7. Relationship between IGF1R (Exon2) Gene Polymorphism with Body Dimensions of Fallow Deer (*Dama dama*)

As observed in table 7, a significant impact ($P \leq 0.05$) in IGF1R (exon2) polymorphism with heart girth was founded to be related to the (heterozygous) AB pattern (76.92 ± 3.20 cm), whereas the lower heart girth was showed in AA pattern (71.33 ± 2.49 cm). However, the current results agree with Dakheel and al-Anbari (26), when their study about Fallow deer reported the relation between IGF1R (Intron 2) gene polymorphism with heart girth, and he suggested the heterozygous pattern (78.33±6.25cm) was higher than the homozygous (70.84±2.51 and 70.80±6.02 cm) with significant difference ($P \leq 0.05$).

Regarding body length and height at the shoulder, no significant differences in effects were shown (Table 7).

Table 7. Relationship between IGF1R gene polymorphism and body dimensions

Polymorphism of IGF1R	Mean ± SE (cm)		
	Body length	Height at shoulder	Heart girth
AA	72.03±2.27	63.54±2.84	71.33±2.49 b
AB	74.69±3.58	61.46±4.36	76.92±3.20 a
Level of Sig.	NS	NS	*

* ($P \leq 0.05$), NS: Non-Significant

3.8. Calculation of Genetic Diversity between the Populations

The genetic diversity will be at risk with unplanned crossing; in consequence, the studies of genetic characterization can conserve existing genetic diversity by providing supporting information for that (24). In this regard, the present study was attractive to that by calculating (Ne) as a tool for genetic diversity. Therefore, the number of alleles (Na) showed that only two alleles were unique in the targeted population. The number of efficient alleles (Ne) was 1.3204, and they are alleles with the capacity to transmit to the 2nd generation as reported by Castillo-Rodríguez, Serna-Lagunes (24). Moreover, Shannon's Information index was recorded at 0.4073 (Table 8).

Table 8. Shannon's Information index for IGF1R (exon2) of Fallow deer (*Dama dama*) in Iraq

Gene	Sample No.	Na	Ne	I
Exon 2	92	2.00	1.3204	0.4073
Mean	92	2.00	1.3204	0.4073

na = the Observed number of alleles.
ne = Effective number of alleles (29)
I = Shannon's Information index (20)

In terms of heterozygosity, genetic diversity results indicate a relatively low level in the Fallow deer (*Dama dama*) populations in Iraq. The observed homozygosity ($O._{Hom.}$) and heterozygosity (H_O) were (0.7174 and 0.2826), respectively. The values of expected homozygosity ($E._{Hom.}$) and heterozygosity (H_E) were 0.7547 and 0.2453, respectively. The genetic diversity of Nei was 0.2427 (Table 9).

This study found that (H_O) was near to (H_E) beside slight variation. These variations between the indicators are due to the allele frequency variations between samples and perhaps due to individual genetic differences in the study population or the mixture of the independent genes (24).

These findings agree with Ambriz-Morales, De La Rosa-Reyna (30), when they studied O.v.sinaloae (*Dama Virginiana Zimmermann*) subspecies of deer in south Mexico, reported that the heterozygosity was convergent between H_O and H_E (0.64 and 0.61 respectively). On the other hand, Kollars, Beck (31) showed low (Ho) values (0.19 - 0.22) when they study on white-tailed deer (*O. virginianus*) in the USA.

Table 9. Diversity indices for IGF1R (Exon2) gene of *Dama dama* in Iraq

Gene	Sample No.	O.Hom. *	E. Hom.**	HO*	HE*	Nei (H_t)***	Ave. Het.
Exon 2	92	0.7174	0.7547	0.2826	0.2453	0.2427	0.2427
Mean	92	0.7174	0.7547	0.2826	0.2453	0.2427	0.2427

*Observed homozygosity and heterozygosity
**Expected homozygosity and heterozygosity were computed (32)
***Nei (21) 's expected heterozygosity

The assessment of the genetic population status of *Dama dama* has been useful for implementing risk prevention measures of a genetic nature, like, multiple paternity, social dominance, and mating system (33). This information was essential for genetic conservation variability in which molecular markers are the appropriate tools for the basic genetic diversity characterization studies.

Via a low level of gene flow, the genetic drift decline within genetic diversity (34). The results showed an unexpected influx of IGF1R diversity measured by Fis and recorded the value (- 0.1646). However, the present value of Fis agreed with Latch, Gee (34) when they study on white-tailed deer in many specific loci in the USA and suggested the values of Fis for some locus (−0.053, −0.002, −0.013, −0.059, −0.051 and −0.027) (Table 10).

Table 10. Gene flow and F-Statistics estimation of IGF1R (exon 2) gene

Gene	Sample No.	Fis.*	Fit.	Fast.	Nm.
Exon 2	92	-0.1646	-0.1646	0.00	0.00
Mean	92	-0.1646	-0.1646	0.00	0.00

* Wright's inbreeding coefficient

In this sense, the results of the current study may be considered an approximation to the total genetic diversity of the population of *Dama dama* in Iraq, but the information obtained is relevant to proposing the strategies of conservation for the genetic diversity observed.

It is important to consider the genetic characterization for the selection with maintained genetic variability, without the risk of an inbreeding system and drift losses.

Authors' Contribution

Study concept and design: I. A. F. and B. Q. H. A.
Acquisition of data: I. A. F. and B. Q. H. A.
Analysis and interpretation of data: I. A. F. and B. Q. H. A.
Drafting of the manuscript: I. A. F. and B. Q. H. A.
Critical revision of the manuscript for important intellectual content: I. A. F. and B. Q. H. A.
Statistical analysis: I. A. F. and B. Q. H. A.
Administrative, technical, and material support: I. A. F. and B. Q. H. A.

Ethics

The animal study was approved by the ethics committee of the Al-Qasim Green University, 51013, Babylon, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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