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GENETIC DIVERSITY OF HEAT SHOCK PROTEINS AND GROWTH HORMONE RECEPTOR GENES IN TWO EGYPTIAN CHICKEN BREEDS

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ABSTRACT: One hundred individuals as 12 blood samples for each breed of Fayumi and Dandarawi, 100 for all parameters and 12 for genetic parameters with the GH, GHR, IGFBP-II, HSP70 and HSP90 gene were assayed. When compared to the Fayoumi chicken breed, Dandarawi had higher in body weights. Furthermore, the averages of total Cytosine and Guanine (C+G) in the Dandarawi chicken breed were 148.5 nucleotides. These facts would suggest that the Dandarawi allow it to adapt to hot climate environments with the genes had it and is thus selected to increase production under hot environments. The GH1, GH2, GHR, IGFBPII, HSP70, and HSP90 genes had greatly informative markers that have values of PIC >0.50 with two chicken breeds. The T and C were identified as highly variable sites in the GH1 gene at positions 275 and 338 for non-coding regions, respectively. Also, the convert of T to G were found in positions of 26, 95, 130, 167 and 171 for non-coding regions, respectively in GH2 gene. Moreover, the highly variations identified T to C were in positions of non-coding regions, respectively in GHR and IGFBPII gene. Finally, the variations identified C to G and A to C were in positions for coding regions, respectively in HSP90 gene. The highly polymorphic regions of 18 positions were detected for GH2 in non-coding regions with two chicken breeds. The polymorphism with the lowest value was one with HSP70 in the coding area and two chicken breeds. Therefore, the results point to that the genes of HSP90, 70 in the Dandarawi breed might function well under HS than the Fayoumi chicken breed. It was discovered that a single nucleotide polymorphism in HSP70 gene resulted from the amino acid cysteine being used in state of arginine due to the replacement of the T nucleotide with C. The differences between the breeds indicated the variance, which redirect the opportunity of continuing the choice database.

Keywords: SNPs, Genetic diversity, Fayoumi and Dandarawi chicken breeds.

INTRODUCTION

Fayoumi chicken breed:

The origin of this breed is unknown. It is an active and resilient variety of fowl. Many reasons for this scenario have been given; the first is that, through the rule of King Mohamed Ali, it was brought to Egypt from a place called "Biga". The second is that it was brought to Egypt under Napoleon's rule and is descended from the silver Campine breed (Hossari, 1958). While Abdel Warith (1993) claimed that the Fayoumi breed has more although it shares more phenotypic similarities with the Silver Campine than any other population, its genomic makeup might change. Since in the Fayoumi breed, except for sex-linked, whereas was in the silver Campine population, it is autosomal. Additionally, Lamont (1997) discovered that the genetic makeup of the Fayoumi chicken is highly distinct from that of other fowl, according to the Iowa State University website. She attested to the fact that Fayoumi birds had substantially higher virus resistance than other bird species.

Dandarawi chicken breed:

This native chicken breed is located in Dandrah/Assuit in South Egypt. The wings and body have some white, black color in the males, as well as a white hackle and saddle. Grey or reddish-brown color with Females and have a slight crest that faces backwards. They have a single comb whereas females weigh 1.1-1.2 kg, males range from 1.3 to 1.5 kg. Between 6 and 6.5 months, they are fully-grown. They are immune to diseases and tolerant of heat stress up to 40^{o}C **(El-Itriby et al., 1963**). This population and the favorable breed are often compared. Recently hatched chicks have down that ranges in color from yellow to creamy white, with changing gradations of black striping on the back. Mature birds exhibit sexual dimorphism, with females having salmon-colored plumage with white breasts and males having a Ply-black form of white and black. Certain birds' feathers have stippling, which gives them a greyish appearance. White shanks and a beak are present. The female comb is solitary and tiny, whereas the male comb is shaped like a cup. Their beards, crests, and muffs can identify mature birds; the majority of the birds have these features.

The chicken GH gene is roughly 3.5 kb in size and has five exons and four introns, alike to the GH genes found in other animals such as cattle Mou et al (1995), sheep Byrne et al (1987), and goats Kioka et al (1989). The GH gene diversity in different animals have been recognized. For instance, a variation in intron three of the bovine growth hormone gene was discovered to be associated with milk protein contented Lei and others (2007). In fact, guiding components have been found in the GH gene's intron region in a variety of animals Nie et al (2005).

The GH gene, in particular, is regarded as the most promising candidate gene for chicken growth performance and carcass quality features (Anh et al, 2015), requiring more thorough genetic investigations into the growth and production related genes as well as more accurate assessments of their genetic variations. Growth hormone (GH) regulates several metabolic pathways, including growing, regeneration, sexual maturity and immunological reactions, it coordinates these tasks in cells with receptor (Feng et al 1998). The chicken (cGH) gene, which has a length of 4098 base pairs and five exons and four introns, is found on chromosome one (Lechniak et al, 1999). The relationship

between SNPs and some traits were significant associations with chicken as BW and drumstick weight at six WK of age (Ghelghachi et al, 2013). Sexual maturity, EN and BW (Aminafshar et al, 2012), body weight at four, six, eight and ten weeks (wks) of age, as well as that daily weight gain (Anh et al, 2015), several studies have confirmed the significance of GH gene polymorphisms.

The GHR gene that is a member of the type one-cytokine superfamily, produces a 638 amino acid long protein that functions as the membrane receptor for growth hormone (Kazemi et al 2018). The exons ten and introns nine is set on chromosome Z in The chicken GHR gene (Hull et al, 1999). The binding of GH to its receptor causes internal and extracellular metabolic processes to occur (Kazemi et al 2018). This gene and the GH-IGF-I system control follicle development in animal populations that are in their fast-growing stage (Monget et al, 2002). Due to its strong impacts on the production of double-yolk eggs (Li et al, 2008). According to studies on live body weight and subcutaneous fat thickness in chickens (Attarchi et al., 2017 and Ouyang et al., 2008), the GHR gene is one of the most sensible native genes for generative features, egg production, and ESHq.

The IGFBPII gene on chromosome 7 has 275 amino acids, is approximately 38Kbp in size, and has four short exons and three long introns (Lei et al., 2005). In native and commercial breeds, significant correlations between the gene and BW at hatch and days seven, fourteen, twenty-one and twenty-eight have been documented (Lei et al., 2005). Growth rate in chickens at 4-8, 8-12, and 0-4 weeks of age (Sidadolog et al, 2013), and BW at hatch and twelve wks of age (Zhao et al, 2015). Furthermore, some Single Nucleated Polymorphisms have a significant impact on growth traits in native breeds, including an SNP (1196CA) in the 3'-flanking position on abdominal fat mass and ratio (Leng et al, 2009) and an Single Nucleated Polymorphisms (C/T) in intron 2 on BW at 2-12 wks of age, metatarsal, shank, femur length and weight.

For instance, organisms create the heat shock protein HSP70 to aid in their resistance to heat stress. The HSP70 gene functions as a chaperone, whose job it is to appropriately control protein refolding in order to protect cells from heat stress damage (Tkáová & Angeloviová, 2012). The HSP70 gene in gallus gallus has a coding-region length of 1,905 bp, only one exon, and is located on the fifth chromosome (Morimoto et al., 1986). The chicken HSP70 gene's whole sequence is available in gen bank (J02579). The current study looked at the polymorphisms of the GH1, GH2, GHR, IGFBPII, HSP70, and HSP90 loci and their associations with some reproductive and productive traits in the local breeds of Fayumi and Dandarawi. This was done because these candidate genes are crucial for poultry production, it is important to preserve and improve the genetic diversity of native chicken populations, and there has not been a thorough study of the area.

The aim of study investigated genetic diversity of growth hormone, growth hormone receptor and heat shock proteins genes in two Egyptian chicken breeds (Fayoumi and Dandarawi).

MATERIALS AND METHODS

Samples collections and DNA extraction

A total of 100 individuals as 12 blood samples for each two poultry breeds of

Fayumi and Dandarawi (100 for all parameters and 12 for genetic parameters) were assayed in the present study which was collected from the Faculty of Agriculture, Al-Azhar University, Egypt. DNA isolation was carried out as previously described by Ibrahim *et al* (2021) and conserved in the National Gene Bank, Agricultural Research Center, Egypt.

Experimental population

The birds were maintained in batteries, in a single bird cage and individual body weight weights were recorded for each bird separately within genotype at 0, 2, 4, 6, 8, 10, 12, 14, and 16 weeks of age.

Blood samples collections:

Twelve (6, Fayumi and 6, Dandarawi) for gene sequences. One hundred for all parameters and 12 for genetic parameters) chicken samples were used in this study. Chicken blood samples were collected in falcon tubes containing 0.2 ml of EDTA (0.5 M) as an anticoagulant.

PCR reaction

Growth Hormone (GH) and Growth Hormone Receptor (GHR), insulin-like growth factor-binding protein 2 (IGFBP-II), Heat Shock Protein (HSP70) and (HSP90) genes were assayed with two breeds; The PCR reaction mixture contained approximately 50 ng of genomic DNA, 10pmol of each primer, and 25µL of master mix in a total volume of 50µL. The following cycles were applied: denaturation at 95^{o}C for Five min, tracked by 35 cycles at 95^{o}C for 60 s, primer annealing from 57 to 60^{o}C for 30 s, and PCR products synthesis at 72^{o}C for 60 s, and then final synthesis at 72^{o}C for 10 min **(El-**Sayed et al 2022b).

Primers

It was recommended by Ip et al. (2001), Kazemi et al. (2018) and Feng et al. (1997), Li et al. (2006) and Xie et al. (2014) to use a set of primer sequences for the GH1, GH2, GHR, IGFBP-II, HSP70 and HSP90 genes, the sequences as indicated in Table (1).

Sequence analysis and three-dimensional (3D) structure prediction

In this study, investigate these genes including growth hormone (GH) and Growth Hormone Receptor (GHR), insulin-like growth factor-binding protein 2 (IGFBP-II), Heat Shock Protein (HSP70) and (HSP90) using in silico sequence analysis, three-dimensional (3D) structure prediction from the Fayoumi and Dandarawi chicken breeds. The analyses and predicting protein structure were performed using the Phyre2 are free web-based services for protein structure prediction (https://www.ebi.ac.uk/Tools/st/emboss_transeq/).

Statistical analysis

Gene frequencies design and analysis evaluations were implemented using CLC sequence Viewer 6 software. The effects of the Growth Hormone GH and Growth Hormone Receptor GHR gene on the studied traits were assessed.

The PIC was calculated from the formula: PIC = 1- ((freq1)^2+(freq2)^2…….). The average of body weights for two chicken breeds from a week (0) to weeks (16) with means were calculated with the excel sheet.

RESULTS AND DISCUSSIONS

In a previous study, two breeds were used to assess the genetic diversity of GH, GHR, IGFBP-II, HSP70 and HSP90 genes. In Fayoumi chicken breed the accession numbers of OM280325, OM280327 were recorded for GH1, GH2 with intron2 and intron4 respectively. Also, the accession numbers of OM228700, OM280329, OM280331 and OM280333 were recorded for GHR with

exon1 and 2, IGFBPII with intron2, HSP70 and HSP90 respectively. While, in Dandarawi chicken breed OM280326, OM280328, OM228701, OM280330, OM280332 and OM280334 for GH1, GH2, GHR, IGFBPII, HSP70 and HSP90 were recorded respectively as shown in (Figure 1).

body weight

Differences between two chicken breeds; the Dandarawi had higher body weights compared with the Fayoumi chicken breed. Additionally, as demonstrated in (figure 2), the Dandarawi chicken breed had higher relation weightiness in a wks of zero, one, two, three, four, six, eight, ten, twelve, fourteen and sixteen than the Fayoumi chicken breed. El-Sayed et al (2022a) investigated that the Ross had higher in BWs compared with the Sasso and Baladi breed in a wks of zero, one, two, three, four and six were significantly ($p<0.05$) higher in the Ross than the other genotypes. However, significant variations between genotypes were discovered in other variables ($P<0.05$) other than BWs at eight and twelve wks of age attributes by Kazemi et al (2018). Also, no significant relationship between Single Nucleated Polymorphism and these traits was found for BW at day 140 at age at initial laying, quantity of eggs laid, egg specific gravity, and EW at 240 and 450 days of age (Feng et al 1997).

In the Fayoumi chicken breed, the means of counts for adenine, cytosine, guanine, and thiamine were 94.3, 71.8, 76.2 and 84.3 base nucleotides respectively. Additionally, the Fayoumi chicken breed had a mean total of 148 nucleotides for both cytosine and guanine (C+G). Adenine, Cytosine, Guanine and Thiamine mean counts for the Dandarawi chicken breed were 94.3, 71.7, 77.2 and 83.5 base nucleotides respectively. Furthermore, the means of total Cytosine and Guanine (C+G) in the Dandarawi chicken breed were 148.5 nucleotides. These data imply that the Dandarawi population is from Dandra in South Egypt. Similar results were reported by El-sayed et al (2023) who investigated the count of C nucleotides ranged from 68 to 69 of wild rock male pigeons and in the rest of the other genotypes, while the count values of (G) were equal (86) in all genotypes and the lowest value of 154 in (C+G) with wild rock male pigeons, while the highest value in (A+T).

For the non-coding regions of the GH1 gene, the highly variants observed in this analysis were T to C in position of 275 and 338 respectively. Additionally, the GH2 gene non-coding regions at locations of 26, 95, 130, 167 and 171 each changed from T to G. Also, locations of 587, 625 and 205 for the non-coding positions for the GHR and IGFBPII genes respectively, had highly changes found in T to C. Moreover, for the coding region at positions of 6 and 7 respectively with the HSP70 gene. In the coding region of the HSP90 gene, the changes found as C to G and A to C at the positions of 67, 73 and 68 respectively. In two chicken breeds and 18 highly polymorphic sites for GH2 with non-coding regions. In contrast was reported by Lei and colleagues, (2005) for the coding region of HSP70 has the lowest value polymorphism of one with two breeds of chicken an SNP produced by a C to T transition in intron two of the hens IGFBPII gene was investigated to be significantly related with body weight ($P<0.05$) at hatching and at 7, 14, 21 and 28 days of age. Also, the IGFBPII gene Single Nucleated Polymorphisms in intron two, growth traits, and carcass compositions in commercial grill lines

were found to be strongly correlated by Li and others (2006). Researchers found that the IGFBPII gene strongly affected both the ratio of carcass weight and the ratio of drumstick weight in Arian grill lines (*P<0.01*) by Aliabad et al. (2010). In China local chickens however, significant associations among IGFBPII gene polymorphisms and parameters linked to belly fat weight and percentage were observed (*P<0.01*) (Leng et al. 2009). In the same trend was reported by El-Sayed et al. (2022a) for the Ross strain exhibited a higher degree of variety with three genotypes, whereas the Sasso strain had the lowest through the IGFPII gene than the last genes. In addition, with the IGFPII gene, the genetic stability with three populations is greater in the Sasso strain and lowermost in the Ross strain**.**

That genes allow it can acclimate to warm climate environments, therefore when chosen to improvement output under high temperatures in the Dandarawi breed as shown in Table (2), it performs well. On the other hand, the higher frequencies for Adenine were 0.286, 0.410, 0.356 and 0.284, 0.425, 0.325 with GH1, HSP70 and HSP90 for two chicken breeds. In addition, the higher frequencies of Guanine, Thiamine and Cytosine were 0.352, 0.325, 0.298 and 0.374, 0.323, 0.302 with GH2, GHR, and IGFBPII in Fayoumi and Dandarawi chicken breeds respectively. Also, El-Sayed et al (2023) reported that the frequency values varied from 0.567 to 0.572 with short distances female pigeon and short distances male pigeon in (C+G), while in (A+T) varied from 428 to 0.433 with male racing pigeon's short distances and short distances female pigeon respectively.

Finally, Botstein et al. (1980) classified the PIC as highly informative markers with PIC values greater than 0.50, reasonably informative markers with PIC values between 0.25-0.50, and somewhat informative markers with PIC values less than 0.25. Table (3) shows that the GH1, GH2, GHR, IGFBPII, HSP70 and HSP90 genes exhibited highly informative markers with PIC values that extra than 0.50 in the current investigation with two chicken breeds. In GH1 gene the highly variations identified T to C were in positions of 275 and 338 for non-coding region respectively. Also, in GH2 gene the convert of T to G was in positions of 26, 95, 130, 167 and 171 respectively, for non-coding regions. Moreover, the highly variations identified T to C were in positions of 587, 625 and 205 in GHR and IGFBPII gene respectively, for non-coding regions. Also, the highly variation in positions of 6 for coding region in HSP70 gene. Finally, the variations identified C to G and A to C were in positions of 67, 73 and 68, 75 respectively, for coding regions with HSP90 gene. Two chicken breeds and 18 highly polymorphic sites for GH2 in non-coding regions. While, as indicated in Table 4 and Figure 3, the lowest rate biodiversity in HSP70 in the coding region with the two chicken breeds.

Our findings suggested that the Dandarawi breeds with HSP90 and HSP70 genes might function under HS better than those of the Fayoumi chicken breed. Also, the outcome of the amino acid exchange that there is one amino acid was different when compared to the NCBI database using the Blastx tool to investigated SNPs. With the AT nucleotide substitution for a C nucleotide resulted in the amino acid cysteine rather than arginine, causing a Single Nucleated Polymorphism in the chicken population HSP70 gene. Also, the differences between the breeds indicated the

variance, which redirect the probability of continuing the select program.

Yalcin et al. (2001) reported that heat stress has a greatly detrimental influence on the development performance of broilers and egg production of laying hens. In addition, studies on the effects of HS on laying chickens have shown a constant decline in egg weight and eggshell thickness by Emery et al. (1984). In addition to having the lowest SOD activity, one of the most critical components of tissue anti-oxidative methods, the muscle's low reactivity to HSP70, 90 and HSFs may contribute to tissue injury via protein oxidation during heat stresses (Xie et al. 2014). The HSP90 gene influences hormone control to promote heat tolerance when exposed to heat stress (Wu et al., 2015). Additionally, two polymorphic sites were discovered at the start of the coding region: one transition A → G at position +258 and one transversion C → G at location +276 (Mazzi et al. 2019). Finally, El-Sayed et al. (2023) reported that the amino acid E (*glu*) was converted from K (*lys*) with the LDH-A gene, while in of the DR-D4 gene were converted from R (*arg*) and L (*leu*) to S (*ser*) and F (*phe*) only in long-distance male pigeons as opposed to other pigeon breeds.

Prediction of sequence and three-dimensional (3D) structure

The genes for growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factor-binding protein II (IGFBP-II), heat shock protein (HSP70), and heat shock protein (HSP90) from the two chicken breeds were subjected to in silico sequence analysis and three-dimensional (3D) structure prediction, as shown in tables 5, 6, and 7.

CONCLUSION

In could be concluded that, when compared between two breeds the Dandarawi had higher body weights. The GH1, GH2, GHR, IGFBPII, HSP70 and HSP90 genes had highly informative markers that have *PIC* values *>0.50* with two chicken breeds. T and C were identified as highly variable sites with the GH1 gene in two positions for non-coding regions. Also, the convert of T to G was in five positions for non-coding regions with GH2 gene. Moreover, for the GHR and IGFBPII genes, the highly variable sites identified T through C were in three positions for non-coding regions. The polymorphism with the lowest value was one with HSP70 in the coding region and two chicken breeds. According to the findings of this study, the Dandarawi breed has been bred to produce more under warm conditions and has genes that enable it to adapt to warm temperature conditions. As a final point, the association between these traits and genes may be beneficial for molecular marker-assisted selection (MAS) to develop the chicken breeding programs. As a result, our findings indicated that the Dandarawi chicken breed HSP90 and HSP70 genes might function better under HS than the Fayoumi chicken breeds. An SNP in the chicken HSP70 gene was reported to result from the amino acid *cysteine* being used instead of *arginine* due to the replacement of the T nucleotide with C. The differences between breeds indicated the variance which reproduce the probability of continuing the selection program

Gallus gallus breed Fayumi growth hormone (GH) gene, intron 4

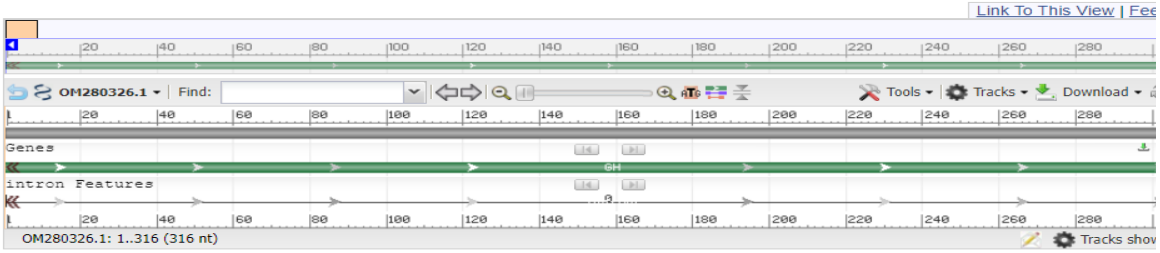
GenBank: OM280325.1

GenBank FASTA

Gallus gallus breed Dandarawi growth hormone (GH) gene, intron 4

GenBank: OM280326.1

GenBank FASTA

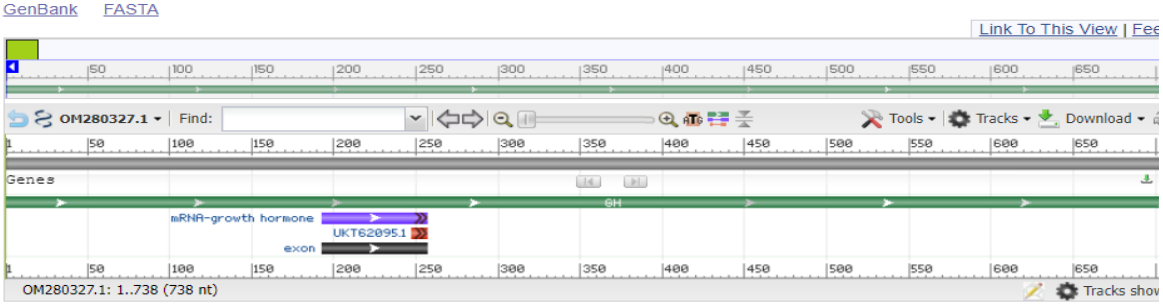


GH, Intron 4

Gallus gallus breed Fayumi growth hormone (GH) gene, partial cds

GenBank: OM280327.1

GenBank FASTA



Gallus gallus breed Dandarawi growth hormone (GH) gene, partial cds

GenBank: OM280328.1

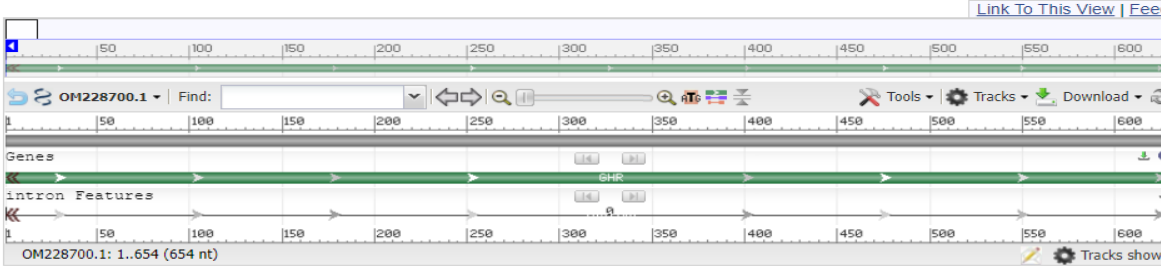
GenBank FASTA

GH, Exon 1

Gallus gallus breed Fayumi growth hormone receptor (GHR) gene, intron 2

GenBank: OM228700.1

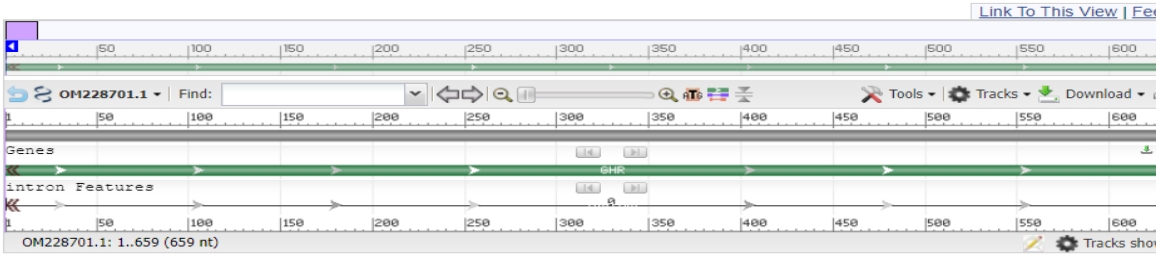
GenBank FASTA



Gallus gallus breed Dandarawi growth hormone receptor (GHR) gene, intron 2

GenBank: OM228701.1

GenBank FASTA

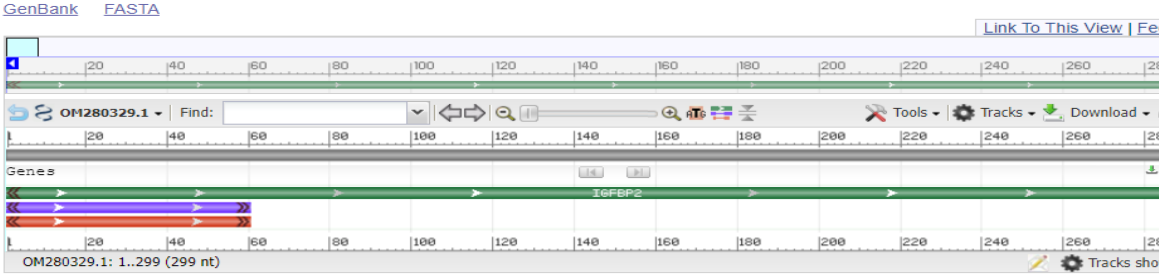


GHR, Intron 2

Gallus gallus breed Fayumi insulin-like growth factor binding protein 2 (IGFBP2) gene, partial cds

GenBank: OM280329.1

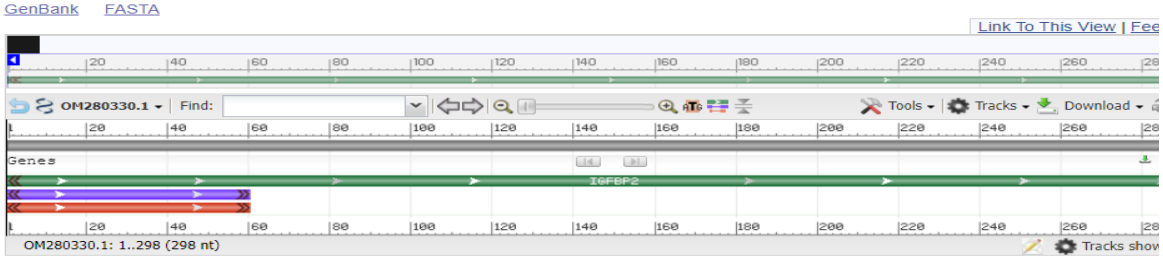
GenBank FASTA



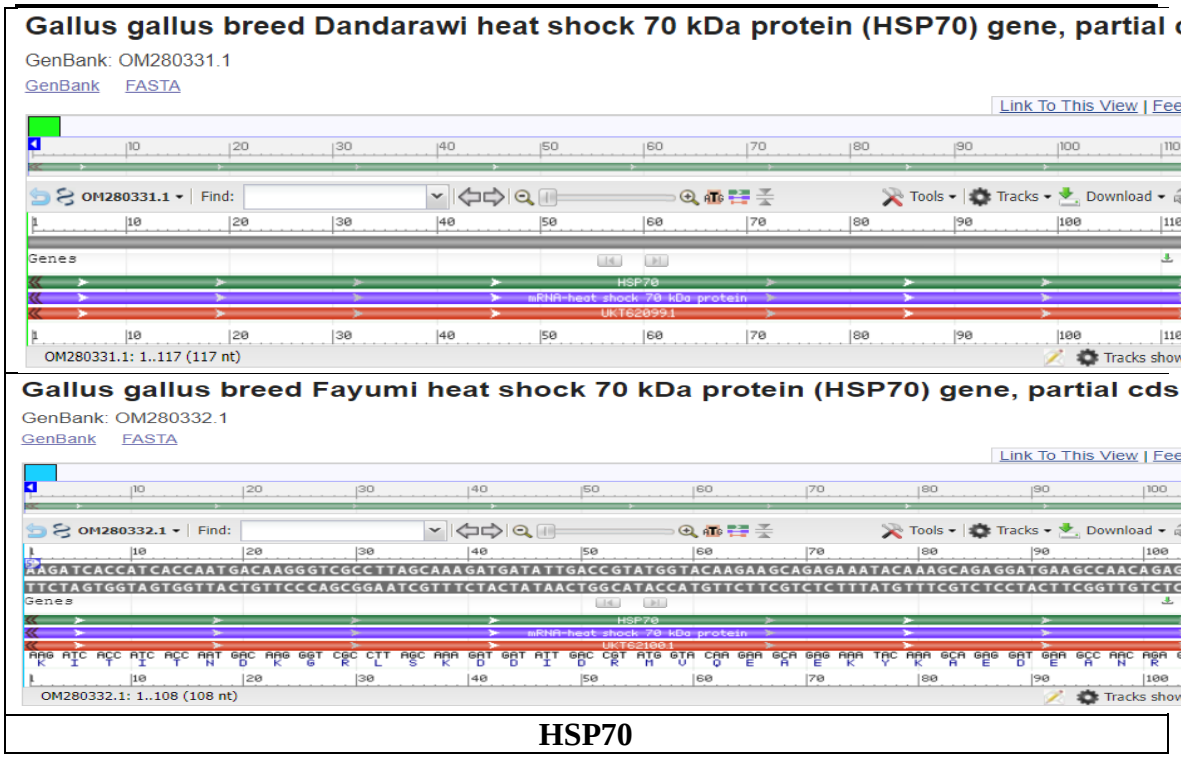
Gallus gallus breed Dandarawi insulin-like growth factor binding protein 2 (IGFB) gene, partial cds

GenBank: OM280330.1

GenBank FASTA



IGFBPII



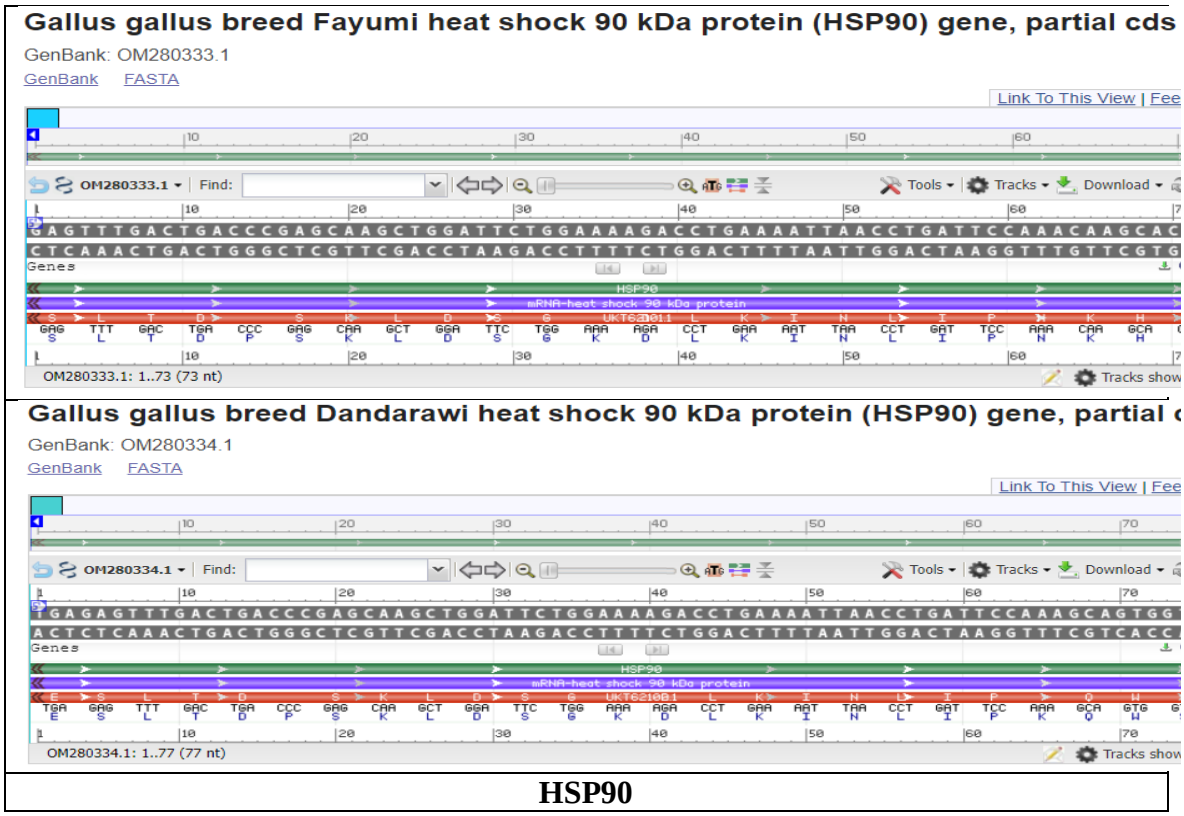


Figure (1): Graphical alignment showing the loci of two chicken breeds submitted sequence on the GH1, GH2, GHR, IGFBPII, HSP70 and HSP90 gene.

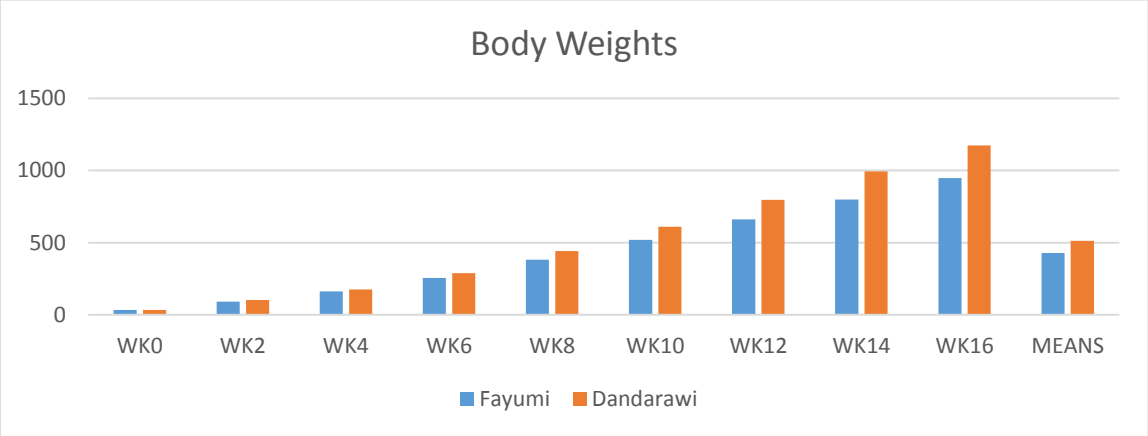


Figure (2): The average of body weights for two chicken breeds from a week (0) to weeks (16) with means.

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Fayumi,GH(Ex1)        TCCAATGCTGCTGCATGCTTTGGGTGATGGGATATGATGGTGGGGTGTGCTGTGGTGGGC
Dandarawi,GH(Ex1)     TCCAATGCTGCTGCATGCTTTGGGTGATGGGATACGATGGTGGGGTGTGCTGTGGTGGGC
                      ************************************************************

Fayumi,GH(Ex1)        TGTACACACGCAGAGCCAGCTCTGAACTAAAATGTGGTAACTTACAGATCAGTGACAAAG
Dandarawi,GH(Ex1)     TGTACACACGCAGAGCCAGCTCTGAACTAAAATGTGGCAACTTACAGATCAGTGACAAAG
                      ************************************************************

Fayumi,GH(Ex1)        GATCTCCTTCCCTACAGTGCAACTTCAAACCATGAGCTGACTCAGGTAACCCTGAGCCTA
Dandarawi,GH(Ex1)     GATCTCCTTCCCTACAGTGCAACTTCAAACCATGAGCTGACTCAGGTAACCCTGAGCCTA
                      ************************************************************

Fayumi,GH(Ex1)        ACCTTGACAGGGGGCAGGAATGAGCTGCAGAATACGAAGACAAAGGCAAACCAGAGTTGT
Dandarawi,GH(Ex1)     ACCTTGACAGGGGGCAGGAATGAGCTGCAGAATACGAAGAACAAGGCAAACCAGAGTTGT
                      ************************************************************

Fayumi,GH(Ex1)        AATGGTGATTGCTATCATACGTGTTGCCAGGGATATTAAAACTCAGTTCCAAGGCTTTAA
Dandarawi,GH(Ex1)     AATGGTGATTGCTATCATATGTGTTGCCAGGGATATTAAAACTCAGTTCCAAGGCTTTAA
                      ************************************************************

Fayumi,GH(Ex1)        AACGGAGATCAGGATGATGTCTAATCAGACTCATAGAATGGCCCGGGTTGAAAAGCACTT
Dandarawi,GH(Ex1)     AACGGAGATCAGGATGATGTCTAATCAGACTCATAGAATGGCCCGGGTTGAAAAGCACTT
                      ************************************************************

Fayumi,GH(Ex1)        CAAAGATCACCTAATTTCAACCCCTTGCACTTGTCCAAGTCCTGCAGGCTCCAGGGCATT
Dandarawi,GH(Ex1)     CAAAGATCACCTAATTTCAACCCCTTGCACTTGTCCAAGTCCTGCAGGCTCCAGGGCATT
                      ************************************************************

Fayumi,GH(Ex1)        CCTCCACTGAAGTTAAACCCTACAGAGAT    689
Dandarawi,GH(Ex1)     CCTCCACTGAAGTTAAACCCTACTGAGAT    689
                      ****************************
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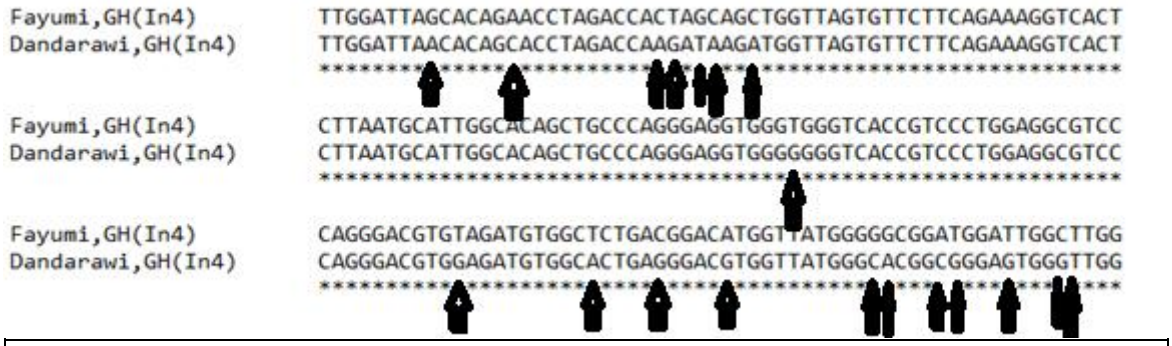
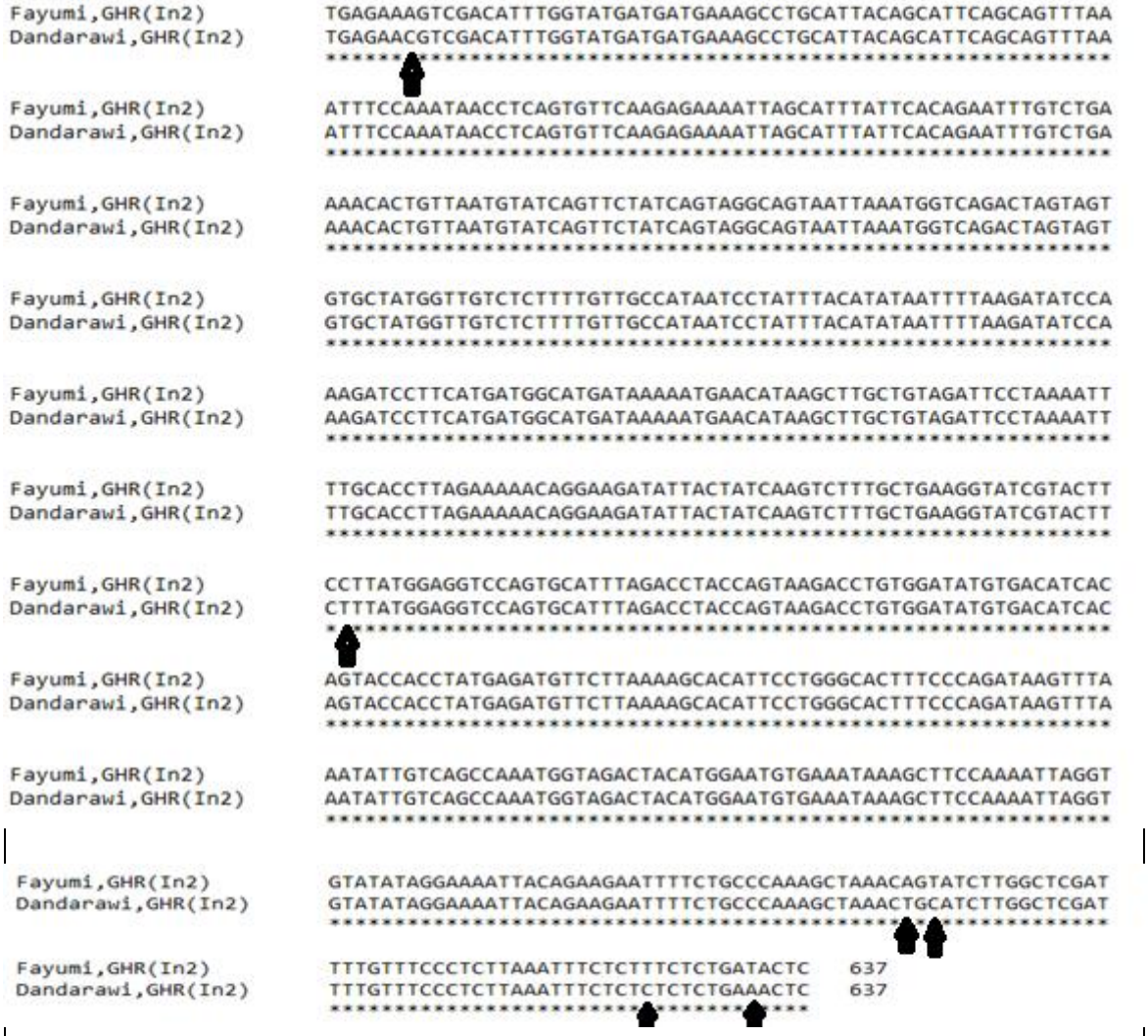
GH1, Exon 1



GH2, intron 4



GHR, intron 2

Figure (3):. The converted positions for GH1, GH2, GHR, IGFBPII, HSP70 and HSP90 gene sequences of two chicken breeds (Fayoumi and Dandarawi).

Table (1): Genes, primer sequence, and other specific information for each gene.

Genes	Primer sequences (5'-3')	TM	Region	Reference
GH1	F: ATCCCCAGGCAAACATCCTC R: CCTCGACATCCAGCTCACAT	59.3	Exon I, intron II	Ip et al.(2001) and Kazemi et al. (2018)
GH2	F:CTAAAGGACCTGGAAGAAGGG R: AACTTGTCGTAGGTGGGTCTG	57	Intron IIII	Kazemi et al. (2018)
GHR	F: GGCTCTCCATGGGTATTAGGA R: GCTGGTGAACCAATCTCGGTT	59.3	Exon I, Intron II	Feng et al.(1997)
IGFBP-II	F: TTTGGTTGAGTCCTAGGCTTG R: GGCGTACTACACTGCAGAGG	60	Intron 2	Li et al.(2006)
HSP70	F:CGTCAGTGCTGTGGACAAGAGTA R: CCTATCTCTGTTGGCTTCATCCT	60	Exon1 (CDS)	Xie et al. (2014)
HSP90	F: GAGTTTGACTGACCCGAGCA R: TCCCTATGCCGGTATCCACA	60	---	Xie et al. (2014)

Table (2): Nucleotides count with means of GH1, GH2, GHR, IGFBPII, HSP70, and HSP90 in two chicken breeds.

Genes	**GH1**	**GH2**	**GHR**	**IGFBPII**	**HSP70**	**HSP90**	**Means**
	Fayoumi						
Nucleotides	**counts**						
A	197	36	206	67	34	26	94.3
C	159	38	112	89	17	16	71.8
G	171	63	112	77	18	16	76.2
T	162	42	207	66	14	15	84.3
C+G	330	101	224	166	35	32	148
	Dandarawi						
	counts						
A	196	39	205	67	34	25	94.3
C	160	36	114	90	15	15	71.7
G	171	67	112	76	17	20	77.2
T	162	37	206	65	14	17	83.5
C/G	331	103	226	166	32	35	148.8

Table (3): In two chicken breeds, there are nucleotide frequencies and polymorphic information content PIC values for GH1, GH2, GHR, IGFBPII, HSP70 and HSP90.

Genes	**GH1**	**GH2**	**GHR**	**IGFBPII**	**HSP70**	**HSP90**
	Fayoumi					
Nucleotides	**Freq.**					
A	0.286	0.201	0.323	0.224	0.410	0.356
C	0.231	0.212	0.176	0.298	0.205	0.219
G	0.248	0.352	0.176	0.258	0.217	0.219
T	0.235	0.235	0.325	0.221	0.169	0.205
PIC	0.75	0.74	0.73	0.75	0.71	0.74
	Dandarawi					
	Freq.					
A	0.284	0.218	0.322	0.225	0.425	0.325
C	0.232	0.201	0.179	0.302	0.288	0.195
G	0.248	0.374	0.176	0.255	0.212	0.260
T	0.235	0.207	0.323	0.218	0.175	0.221
PIC	0.75	0.73	0.73	0.75	0.66	0.74

Table (4):SNPs and their locations in six genes sequence in two breeds of indigenous Egyptian chickens.

Genes	**SNP Position**	**Nucleotide changed from Fayoumi to Danadrawi**	**changes in Position**
GH1	275, 338	T→C	None coding
	461	C→A	
	462	A→C	
	500	C→T	
	684	A→T	
GH2	8, 162	G→A	None coding
	14, 166	A→C	
	25, 29, 32	C→A	
	26, 95, 130, 167, 171	T→G	
	28	G→T	
	140	T→A	
	145, 175	C→G	
	150	A→G	
	161	G→C	
GHR	7	A→C	None coding
	362	C→T	
	585	A→T	
	587, 625	T→C	
	633	T→A	
IGFBPII	205	T→C	None coding
HSP70	6	C→A	CDs
HSP90	67, 73	C→G	CDs
	68, 75	A→C	CDs
	71	C→T	CDs
	72	A→G	CDs
	74	G→T	CDs
	76	T→G	CDs

Table (5): The predicted homology model of Growth Hormone-1 (GH-1) and Growth Hormone-2 (GH-2) in Fayumi and Dandarawi , as displayed by The Phyre2 web portal for protein modeling, prediction and analysis and coloured by rainbow N → C terminus.

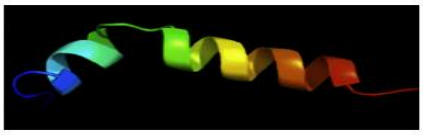
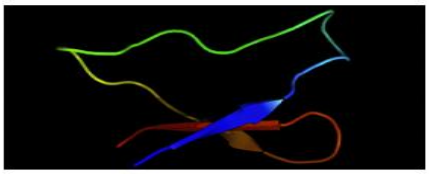
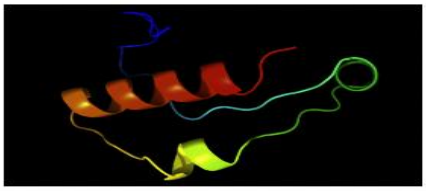
Gene Name	Region	Gene Bank No.	Species used in this study	3D protein modeling
Growth Hormone-1 **(GH-1)**	**intron 4**	OM280325.1	Fayumi	
		OM280326.1	Dandarawi	
Growth Hormone-2 **(GH-2)**	**Intron IIII**	OM280327.1	Fayumi	
		OM280328.1	Dandarawi	

Table (6): The predicted homology model of GHR and IGFBPII in Fayumi and Dandarawi , as displayed by The Phyre2 web portal for protein modeling, prediction and analysis and coloured by rainbow N → C terminus.

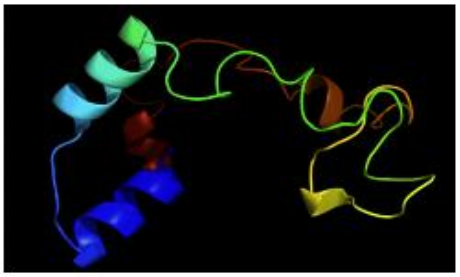
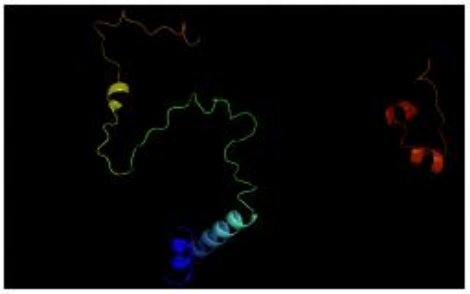
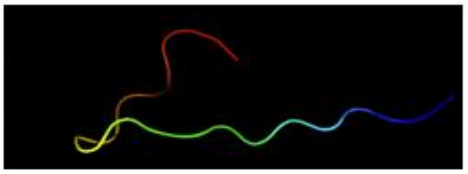
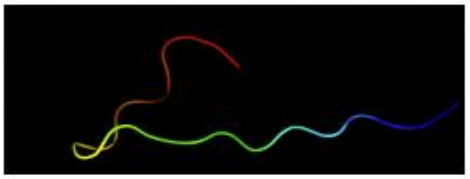
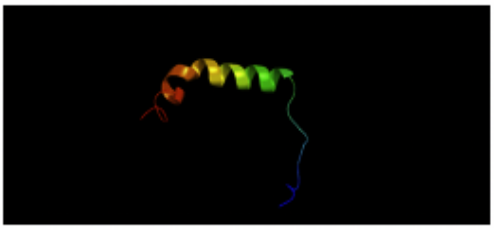
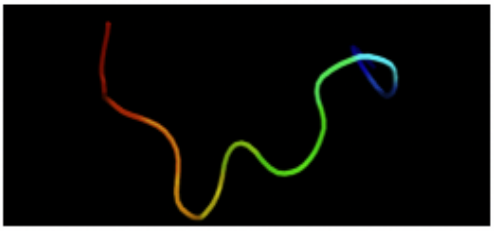
Gene Name	Region	Gene Bank No.	Species used in this study	3D protein modeling
Growth Hormone Receptor (**GHR**)	**Intron 2**	OM228700.1	Fayumi	
		OM228701.1	Dandarawi	
Insulin-like Growth Factor Binding Protein 2 (**IGFBP2**)	**Intron2**	OM280329.1	Fayumi	
		OM280330.1	Dandarawi	

Table.(7): The predicted homology model of HSP70 in Fayumi and Dandarawi , as displayed by The Phyre2 web portal for protein modeling, prediction and analysis and coloured by rainbow N → C terminus.

Gene Name	Region	Gene Bank No.	Species used in this study	3D protein modeling
Heat Shock 70 kDa protein (HSP70)	partial cds	OM280332.1	Fayumi	
		OM280331.1	Dandarawi	

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الملخص العربى

التنوع الجيني لبروتينات الصدمة الحرارية وجينات مستقبلات هرمون النمو في سلالتين من الدجاج المصري

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تم فحص عدد مائة فرد بواقع 12 عينة دم لكل سلالة من الفيومي والدندراوي ، و 100 لجميع المقاييس و 12 للمعلمات الجينية مع كلا من جين GH و GHR و IGFBP-II و HSP70 و HSP90 .بالمقارنة مع سلالة الدجاج الفيومي كانت سلالة الدندراوي أعلى في وزن الجسم. علاوة على ذلك بلغ متوسط مجموع السيتوزين والجوانين (C + G) في سلالة الدجاج الدندراوي 148.5 نيوكليوتيدة. تشير هذه الحقائق إلى أن الدندراوي يمكن ان يتكيف مع البيئات المناخية الحارة مع وجود تلك الجينات فيه وبالتالي يتم اختياره لزيادة الإنتاج في البيئات الحارة. وتحتوي جينات GH1 و GH2 و GHR و IGFBPII و HSP70 و HSP90 على علامات مفيدة للغاية لها قيم PIC> 0.50مع السلالتين. تم تحديد T و C كمواقع شديدة التغير في جين GH1في الموضعين 275 و 338 للمناطق التي لا تنتج بروتين على التوالي. أيضًا تم العثور على تحول من Tإلى G في مواقع 26 و 95 و 130 و 167 و 171 للمناطق غير المنتجة للبروتين على التوالي في جين GH2 .علاوة على ذلك كانت الاختلافات العالية التي تم تحديدها من T إلى C في المناطق التي لا تنتج بروتين على التوالي في جين GHR و IGFBPII .أخيرًا كانت الاختلافات المحددة من C إلى G و A إلى C في مواقع محددة في جين HSP90 تم الكشف عن المناطق متعددة الأشكال من 18 موقعًا لـ GH2 في المناطق غير المشفرة مع السلالتين من الدجاج. كان التعدد المظهري أقل قيمة واحدًا مع HSP70 في السلالتين. بذلك تشير النتائج إلى أن جينات HSP90 و70 في سلالة الدندراوي قد تعمل بشكل جيد تحت الاجهاد الحراري من سلالة الدجاج الفيومي. تم اكتشاف أن تعدد أشكال النوكليوتيدات الفردي في جين HSP70 ناتج عن استخدام الحمض الأميني السيستين في حالة الأرجينين بسبب استبدال النيوكليوتيدات T بـ C .