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Molecular detection of some growth-hormone genes in chickens

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ABSTRACT

Due to the fact that the chicken growth hormone gene (cGH) is critical for controlling development and metabolism, there could be a link between GH polymorphisms and economic features in chickens. The objective of this study was to detect the single nucleotide polymorphisms (SNPs) in the GH and insulin-like growth factor-binding protein 2 (IGFBP2) genes, and to investigate their associations with body weight (BW). Three hundred individuals (100 birds for each commercial strains Sasso and Ross and one local breed; Baladi) were assayed. And the three primers were amplification with a total of 54 samples from three genotypes (Ross=18, Sasso=18 and Baladi=18). A total of SNPs sequenced was 138,106bp consisting of 13,265bp coding regions and 124,841bp non-coding regions. A total of 197 SNPs were identified, with 34 in coding SNPs regions and 163 in non-coding SNPs regions. The values of variation in coding regions, transitions accounted for 53% (18),26% (9) and 21% (7),while in non-coding regions were 27% (111),33% (132) and 40% (163) in Ross, Sasso and Baladi for three genes. This result is most likely due to the high, medium, and low body weights of Ross, Sasso, and Baladi breed. The higher frequency of A↔G and A↔C and lower frequency of G↔T may be related to higher body weight in the Ross strain. While, the higher frequency of A↔C, C↔T and lower G↔T may be related to lower body weight with Baladi breed. Our study confirms the analyzed three genes affecting meat production in poultry.

Keywords: Single Nucleotide Polymorphisms (SNPs), Growth Hormone gene (GH), Insulin-like Growth Factor Binding protein 2 (IGFBP2), Sasso, Ross strains and Baladi breed.

INTRODUCTION

The chicken genome differs from those of other vertebrates in a number of ways. The development of DNA markers has enabled the creation of effective and reliable genetic linkage maps in livestock species production. For two commercial strains and one local chicken, Sasso, Ross, and Baladi, the results confirmed that microsatellite markers could be used as a molecular tool in fingerprint analysis. The researchers proposed using a large genome scan analysis based on more approved microsatellites to cover the entire chicken genome, which might be useful in future marker-assisted selection systems (Mekky *et al.,* 2021).

Chicken growth hormone (GH) is important in chicken performance because of its critical functions in growth. The pituitary gland produces and hides GH, a polypeptide hormone that affects a wide range of biological activities, including development, egg production, body structure, appetite regulation, ageing, and reproduction (Vasilatos-Younken *et al.,* 1999; Putnova *et al.,* 2001). The IGF-I gene variant in Leghorn hens was investigated to see if there was a link between egg output and body weight. The growth hormone receptor (GHR) is an associate of the type 1 cytokine superfamily with a molecular weight (MW) of 71500 Dalton and codes for a membrane receptor for GH with 638 amino acids (Kazemi *et al.,* 2018). The chicken GHR (cGHR) gene has 10 exons and 9 introns and is found on chromosome Z (Hull *et al.,* 1999). Sequencing of the Yellow Wai Chow GH gene revealed 1 still substitution, 31 inserts, and additional replacements distributed throughout the introns (Ip *et al.,* 2001). An original *MspI* position in the 4 intron of the gene of cGH was recently discovered (Nie *et al.,* 2002).

The influence of a polymorphism in the IGFBPII gene in intron 2 on the growth rate with the Kampung indigenous chickens was investigated, and three populations were identified; CC, CT, TT and CT had a larger genotypic frequency than the other populations, and the allelic effect of the C allele was stronger than the allelic effect of the T allele, with important relationships with development rate at 8-4 and 8-12 WKs of age (P<0.05) (Sidadolog *et al.,* 2013). Another study looked at polymorphisms in the IGF-I gene in chickens, resulting in invariants such as IGFI-SNP1, IGFI-SNP2, IGFI-SNP3, and IGFI-SNP4, with a significant link between IGFI-SNP1 and average body weight and nutrition efficiency at 44, 73, and 107 days of age (P<0.05) (Amills *et al.,* 2003). The bioavailability of insulin-like growth factor (IGF) is regulated by a family of structurally conserved insulin-like growth factor binding proteins (IGFBPs) (Kutsukake *et al.,* 2008). In Hubbard F15 and Cobb E broiler lines, IGF1, IGFBP2, and TGFß3 (transforming growth factor ß3) gene polymorphisms with growth and meat output were identified and examined the correlations between these genes' biological roles and interdependencies (Hosnedlova *et al.,* 2020).

Our goal was to explore the GH1, GH2, and IGFPII genes for the detection of some productive traits loci in three chicken populations selected for body weight. The implications of this motivated us to look for SNP polymorphisms in the GH1, GH2, and IGFPII genes to see if the frequency of such polymorphisms could be altered by body weight.

MATERIAL AND METHODS

Three growth hormone genes; GH1 (Exon1 and Intron1), GH2 (Intron3 and Exon4) and IGFBPII (Exon3 and Intron3) genes were amplification with a total of 54 samples from three genotypes (Ross=18, Sasso= 18 and Baladi= 18) three primers pairs were tested by PCR. Sequence data from a total of 54 samples for three genotypes was obtained for genes as shown in Table (2) and Figures (2, 3 and 4). The genes, Breed, positions, accession numbers, primers sequences, and a number of single nucleotide polymorphisms SNPs see Table (2). They were also chosen as being related to the body weight traits.

Selection of genes and collection of samples:

Three hundred individuals (100 birds for each of two commercial strains Sasso and Ross and one local breed; Baladi) under Sinai environmental conditions, were assayed in the present study. Sampling was done at South Sinai Research Station located at Ras Sudr, Desert Research Center, Matariya, Cairo, Egypt. The target genes of GH1, GH2, and IGFPII were selected according to the reports of (Wardęcka *et al.,* 2005; and Kazemi *et al.,* 2018).

DNA Extraction:

DNA isolation was approved out as previously according to (Ibrahim *et al.,* 2021) and conserved in National Gene Bank, Agricultural Research Center, Egypt.

PCR reactions:

GH1, GH2 and IGFBPII

In a total volume of 50µL, the PCR reaction mixture contained roughly 80ng of genomic DNA, 10pmol of each primer, and 25µL of master mix; Denaturation at 95°C for 5 minutes was followed by 35 cycles at 95°C for 60 seconds, primer annealing at 57°C with GH1, GH2 and 60°C with IGFPII for 30 seconds, PCR product synthesis at 72°C for 60 seconds, and final synthesis at 72°C for 10 minutes.

Sequence analysis :

Gene JET PCR purification kit was used to directly purify the GH1, GH2, and IGFBPII genes, which were then sequenced on an ABI3730XL DNA Analyzer. The sequenced nucleotides were aligned with published data in the NCBI databases (non-redundant nucleotide database) using the BLAST tool (http://ncbi.nlm.nih.gov/BLAST/) and uploaded to the Gene Bank using the Bank It (http://www.ncbi.nlm.nih.gov/BankIt/). And using of FinchTV1.4.0(http://www.geospiza.com/finchtv) programme, the three populations were aligned using CLC Sequence Viewer and MEGA-X.

Statistical analysis:

The significant effects and the variations between three chicken breeds were assessed and implemented using SPSS software. The effects of Growth Hormone GH and Insulin-like Growth Factor Binding Protein 2 (IGFBP2) gens on the studied traits were investigated

RESULTS

Bird's body weight:

Variations between three genotypes: Ross, Sasso strains and Baladi breed in body weight are documented in (Table 1) The Ross had higher body weights (1175.57 vs. 576.14 and 218.79g) compared with the Sasso strain and the Baladi breed. Moreover, the relative weights in a week of 0, 1, 2, 3, 4, 5 and 6 were significantly ($p<0.05$) higher in the Ross than the other genotypes as shown in Table (1) and Figure (1).

Table 1. Differences between Ross, Sasso strains and Baladi breed in body weight.

Genotypes	Weeks							
	0	1	2	3	4	5	6	Average
Baladi	31	55	113	193	283	379	477	218.79
Sasso	40	104	236	459	728	1056	1410	576.14
Ross	53*	164*	452*	1007*	1505*	2151*	2897*	1175.57*

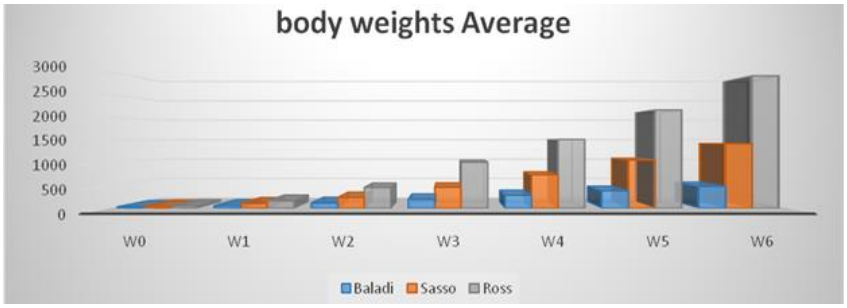


Fig. 1. Body weights average for three genotypes from a week (0) to weeks (6).

The transversions in the three genes

The transversions in total of 440 SNPs were found in all the sequenced DNA fragments from the three genes GH1, GH2, and IGFBPII. Transversions with the GH2 gene were more prevalent in the Baladi breed than in the other breeds. (Table 2) shows that the lowest SNPs are more common than transversions in the Baladi and Sasso breeds in GH1 and IGFBPII, respectively.

Table 2. Characteristics of genes amplified for SNP detection of productive traits

Genes	Breed	Positions	Accession no.	**Forward**	**Revers**	No of SNPs
Gh1	Ross	Exon1,Intron1	MW654249	ATCCCCAGGCAAACATCCTC	CCTCGACATCCAGCTCACAT	13
	Sasso		MW654250			12
	Baladi		MW654251			8
Gh2	Ross	Intron3,Exon4	MW628887	CTAAAGGACCTGGAAGAAGGG	AACTTGTCGTAGGTGGGTCTG	99
	Sasso		MW628888			121
	Baladi		MW628889			150
IGFBPII	Ross	Exon3,Intron3	MW648376	TTTGGTTGAGTCCTAGGCTTG	GGCGTACTACACTGCAGAGG	17
	Sasso		MW648377			8
	Baladi		MW648378			12
Totals						**440**

Figures 2, 3 and 4 showed the graphical alignments of three genes of GH1, GH2 and IGFBPII with three chicken populations.

Fig. 2. Graphical alignment showing the loci of the three genotypes submitted sequence on the GH1 gene.

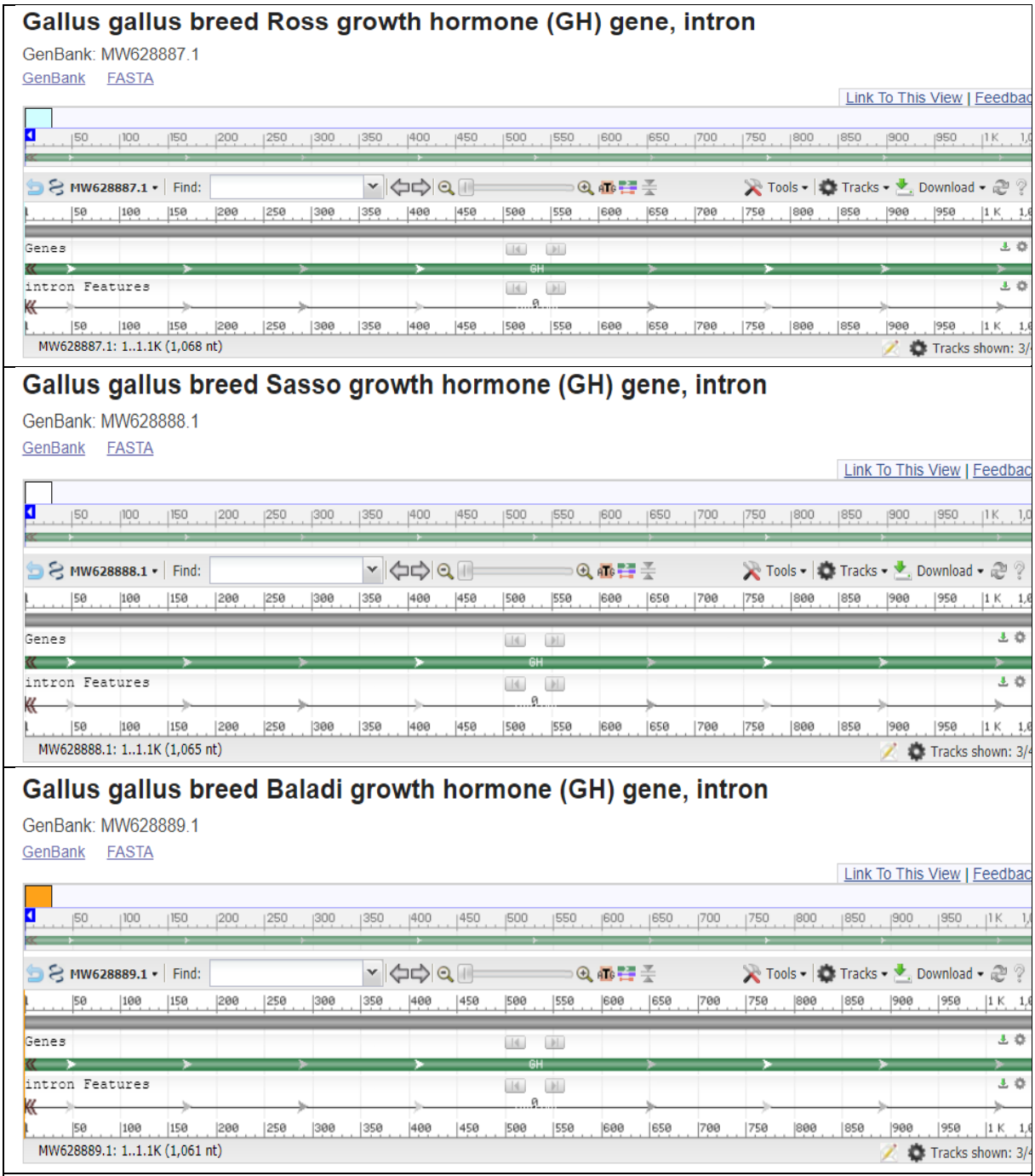


Fig. 3. Graphical alignment showing the loci of the three genotypes submitted sequence on the GH2 gene.

Fig. 4. Graphical alignment showing the loci of the three genotypes submitted sequence on the IGFBPII gene.

Determination of coding sequence (CDs) and non-coding sequence areas were done by the Basic Local Alignment Search Tool (BLAST) system and done with National Center for Biotechnology Information (NCBI) (Figures 5 and 6).

CODING REGIONS:

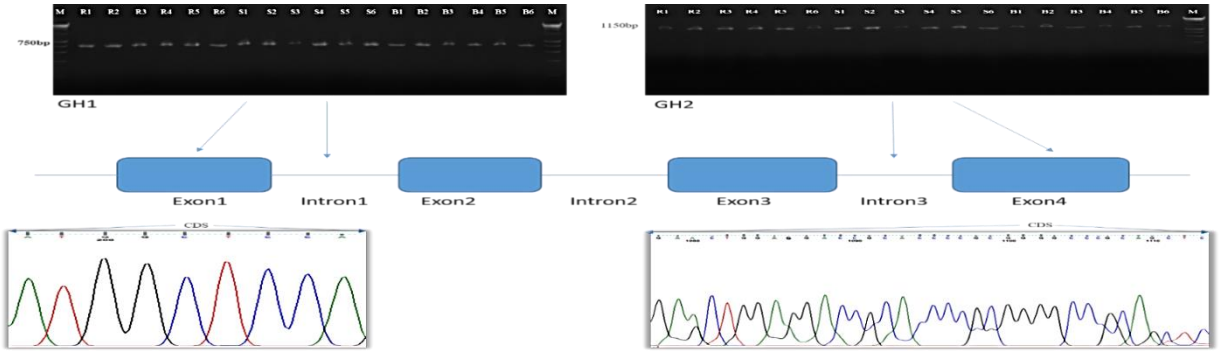


Fig. 5. Profiling of two growth hormone genes GH1 and GH2 of three chicken genetic stock, Electrophoresis analysis of PCR products, structure and SNPs position of GH genes; CDs coding sequence based on sequencing results.

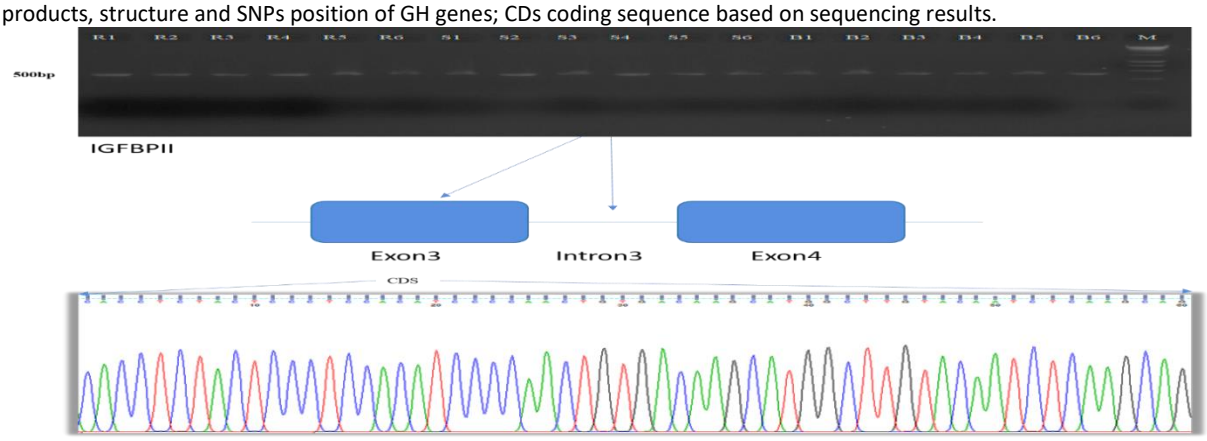


Fig. 6. Profiling of insulin-like growth factor binding protein 2 (IGFBPII) gene of three chicken genetic stock, Electrophoresis analysis of PCR products, structure and SNPs position of IGFBPII gene; CDs coding sequence based on sequencing results.

Regions of coding and non-coding:

The length of the genomic sequence used in this investigation was 138,106bp consisting of 13,265bp coding regions and 124,841bp non-coding regions. A total of 440SNPs made up of 34 coding SNPs and 406 non-coding SNPs were obtained. Overall, the total percentage of variations identified were 9.40 and 90.60% for coding and non-coding regions, respectively in all the genes. The percentage of variation identified was 2.11 and 97.89% for coding and non-coding regions, respectively, with the GH1 gene. Also, the percentage of variations were 9.99 and 90.01% for coding and non-coding regions, respectively, with the GH2 gene. Finally, the percentage of variation identified were 9.40 and 90.60% for coding and non-coding regions with the IGFBPII gene. The highly polymorphic regions of 15 and 145 for GH2 in coding and non-coding areas, respectively with the Ross strain and the Baladi breed. While, the lowest value polymorphism of 1 and 6 with GH1 in coding and non-coding areas with Sasso and Baladi breeds, as shown in Table (3).

Table 3. Nucleotide polymorphism with three breeds of the Ross, Sasso strains and Baladi breed.

Genes	Coding regions					Non-Coding regions				
	Polymorphic sites					Nucleotides diversity				
	L(bp)	Ross	Sasso	Baladi	∑	L(bp)	Ross	Sasso	Baladi	∑
GH1	6-78					275-603				
Total		3	1	2	6		10	11	6	27
∑	91					4,224				4,315
VAR %	**2.11**					**97.89**				
GH2	1077-1123					12-1074				
Total		15	8	5	28		84	113	145	342
∑	13,174					118,597				131,771
VAR %	**9.99**					**90.01**				
IGFBPII						62-268				
Total		0	0	0	0		17	8	12	37
∑	0					2,020				2,020
VAR %	0					100				
T.SNPs.	**13,265**					**124,841**				**138,106**
T.VAR %	**9.40**					**90.60**				
Total		**18**	**9**	**7**	**34**		**111**	**132**	**163**	**406**

T.VAR; the total variations
T.SNPs; the total SNPs:

The values of variations for coding regions, transitions were taken into account 53% (18) of the total, while for non-coding regions it was 27% (111) with the Ross strain for three genes. Also, the values of polymorphism in coding regions and transitions accounted for 26% (9), while in non-coding regions it was 33% (132) in the Sasso strain for three genes. On the other hand, the values of polymorphism in coding regions and transitions accounted for 21% (7), while for non-coding regions it was 40% (163) in the Baladi breed for three genes as shown in Table 3. This result is probably related to the high, medium, and lower body weight of the Ross, the Sasso, and the Baladi breeds, respectively. Whereas, the value weight of the Ross, Sasso strains, and Baladi breeds was 2905, 2241, and 1214g at 6, 9 and 15 weeks of age, as the marketing ages.

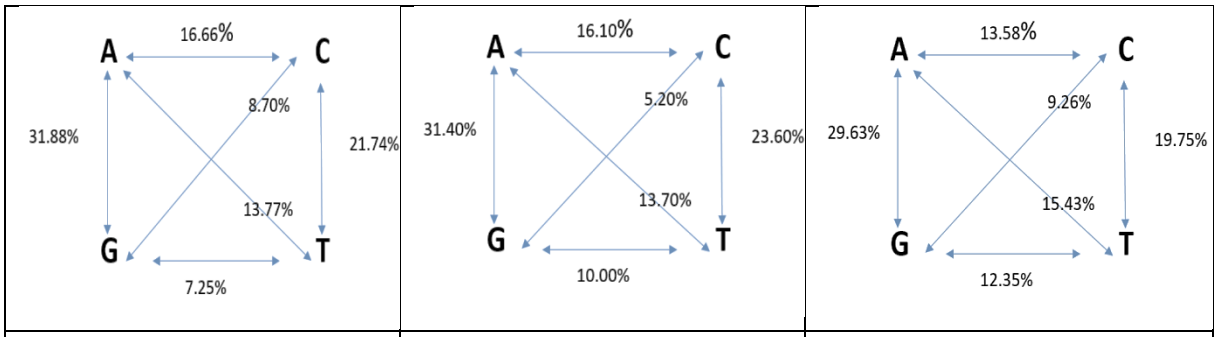
Type of polymorphism:

The transitions C↔T are over-represented with 66.67 of the total substitutions for the IGFBPII gene with the Baladi breed. While, the lowest value of transitions C↔G was 5.45 for the GH2 gene with the Sasso strain. Also, the total number of SNPs ranged from 8 with the Baladi and the Sasso to 142 with the Baladi breed for GH1, IGFBPII, and GH2 genes, respectively. While the lowest frequency of C↔T, C↔G, A↔T, and G↔T SNPs are most likely linked to higher body weight for the Ross strain with the genes of (GH2 and IGFBPII), (GH1 and IGFBPII), (GH1), and (GH2 and IGFBPII) respectively. While the highest frequencies of C↔T, A↔G, C↔A, C↔G, A↔T, and G↔T SNPs are most likely linked to lower body weight for the Baladi breed with the genes of (IGFBPII), (IGFBPII), (GH1), (GH2), (GH2), and (GH2), respectively, as shown in Table (4).

Table 4. SNPs frequency with three breeds of the Ross, Sasso, and Baladi among numbers

SNPs Convert from to	Genes									Total per genotypes		
	Gh1			**Gh2**			**IGFBPII**					
	Ross (%)	Sasso (%)	Baladi (%)	Ross (%)	Sasso (%)	Baladi (%)	Ross (%)	Sasso (%)	Baladi (%)	Ross (%)	Sasso (%)	Baladi (%)
C↔T	46.16 (6)	58.33 (7)	50 (4)	13.90 (15)	16.36 (18)	14.08 (20)	52.95 (9)	62.5 (5)	66.67 (8)	21.74 (30)	23.60 (30)	19.75 (32)
A↔G	23.08 (3)	25 (3)	25 (2)	34.26 (37)	33.64 (37)	30.28 (43)	23.53 (4)	12.5 (1)	25 (3)	31.88 (44)	31.40 (41)	29.63 (48)
C↔A	15.38 (2)	16.67 (2)	25 (2)	17.59 (19)	17.27 (19)	13.38 (19)	11.76 (2)	-	8.33 (1)	16.66 (23)	16.10 (21)	13.58 (22)
C↔G	7.69 (1)	-	-	9.26 (10)	5.45 (6)	10.56 (15)	5.88 (1)	12.5 (1)	-	8.70 (12)	5.20 (7)	9.26 (15)
A↔T	7.69 (1)	-	-	16.70 (18)	16.36 (18)	17.61 (25)	-	-	-	13.77 (19)	13.70 (18)	15.43 (25)
G↔T	-	-	-	8.33 (9)	10.91 (12)	14.08 (20)	5.88 (1)	12.5 (1)	-	7.25 (10)	10.00 (13)	12.35 (20)
Total	**13**	**12**	**8**	**108**	**110**	**142**	**17**	**8**	**12**	**138**	**130**	**162**
Variation	**39.40**	**36.36**	**24.24**	**30.00**	**30.60**	**39.44**	**45.95**	**21.62**	**32.43**	**32.09**	**30.23**	**37.68**

Figure 7. (a, b, and c) showed that the high frequencies of A↔G and A↔C, while the lower frequency of G↔T may be related to higher in body weight with the Ross strain. Also, the higher frequency of A↔C and C↔T, while lower frequency of G↔T that may be related to body weight with the Baladi breed.



a. The Ross strain | b. The Sasso strain | c. The Baladi breed

Fig. 7. The distribution of transitions and among SNPs with three genotypes in chickens.

Figures (8 , 9) showed that the genetic diversity with three genotypes had a higher value of diversity in the Ross strain while the lowest in the Sasso strain with the IGFPII gene than the other genes. Also, the genetic stability with three genotypes has a higher value in the Sasso strain and the lowest in the Ross strain with the IGFPII than the other genes.

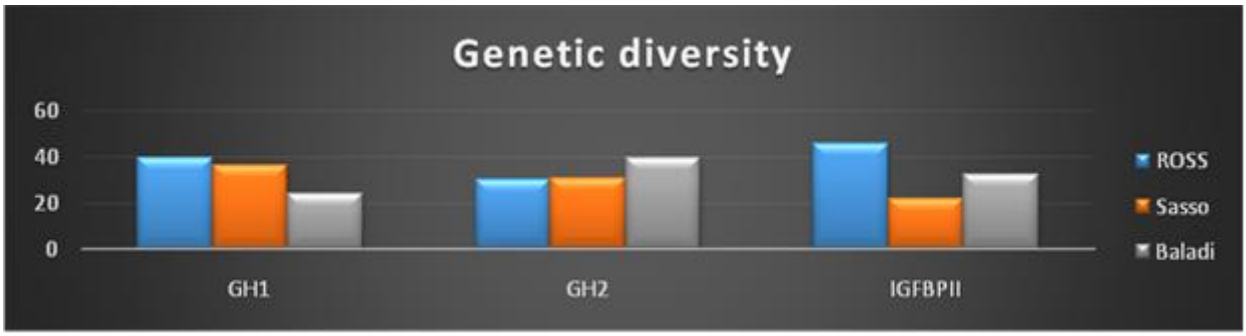


Fig. 8. Genetic diversity with three genotypes

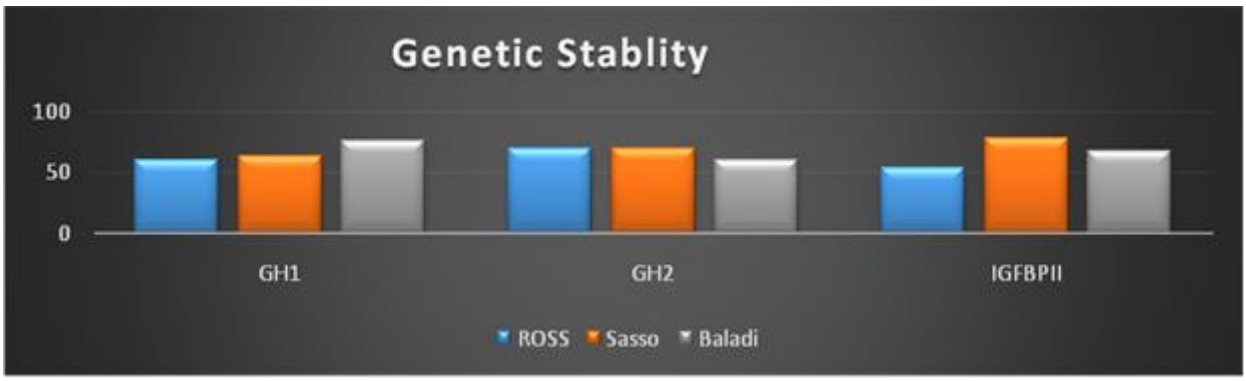


Fig. 9. Genetic stability with three genotypes

DISCUSSION

The sequence variations for the GH1, GH2, and IGFBPII genes in three chicken genotypes were assayed. The findings of the average comparison investigation revealed significant changes among GH genotypes in BW (on one day and puberty), EW (at puberty and thirteen weeks of age), ASM, EN, and AEW at 345–375 days of the era, and PF, as well as IGFBPII populations in the LI trait (Kazemi *et al.,* 2018). The candidate gene approach is becoming significantly less expensive and more effective in detecting candidate QTL genes and quantifiable trait nucleotides with major effects on economically important traits by combining relative data with comparative (including genomes, transcriptomics, proteomics, and metabolisms), biological functions, QTL mapping and fine mapping, signaling pathways, and gene webs (Kuhn *et al.,* 2007). Currently, these investigations suggest that introns in the GH1, GH2 and IGFBPII genes may play an essential function in the control of gene expression. Like GH genes known in other mammals, the chicken cGH gene contains one exon and one intron. There were 46SNPs found, with four in the five non-translate areas, 1 in the three non-translate sections, 5 in exons (2 of nonsynonymous), and the remaining 36 in introns (Nie *et al.,* 2005). The size gene of cGH has been estimated to be around 3.5Kb (Tanaka *et al.,* 1992; Mou *et al.,* 1995; Ip *et al.,* 2001). The outcomes of our study confirm that the analyzed regions of chicken GH are high variations. New polymorphisms in the chicken GH gene and their relationships with axial essential deficits need to be investigated further (Wardecka *et al.,* 2005). In a generation of broiler chickens, the effect of a polymorphism in the GHR gene intron 5 on some economically important traits like egg number, amount of double-yolked eggs, and period of sexual maturity were investigated, and two alleles and haploid genotypes of B+ and B- with frequencies of 0.68 and 0.32 were created, respectively. However, there was no evidence of a link between GHR genotypes and these features (Dunn *et al.,* 2004). The most significant links between polymorphisms in the IGF-I gene and development parameters in Vietnamese native chickens, including body weight at 2, 4, and 6 weeks of age and average daily weight gain from 0 to 6 weeks of age ($p<0.05$) (Anh *et al.,* 2015).

An SNP originating as a C to T mutation in intron 2 of the chicken gene of IGFBPII was found to be substantially linked with BW at hatch, 7, 14, 21, and 28 days ($p<0.05$) (Lei *et al.,* 2005). The IGFBPII gene SNP in intron 2 was discovered to have a substantial connection with growth traits and carcass composition in commercial broiler lines (Li *et al.,* 2006). In Arian broiler lines, the IGFBPII gene had a major effect on the ratio of drumstick mass and the ratio of carcass mass ($p<0.01$) (Aliabad *et al.,* 2010). In Chinese native chickens, significant relationships were identified between the IGFBPII gene and abdominal fat mass and the ratio of abdominal fat characteristics ($p<0.01$) (Leng *et al.,* 2009). In the *Jinghai Yellow* breed, there were significant associations between genotypes and hatch weight and EW at 300 days, demonstrating significant relationships between genotypes and BW and reproductive traits in four Chinese chicken breeds (Zhao *et al.,* 2011). The highest average thigh muscle weight was found in Cobb E line poultry produced by an AC IGF1 population (519.75 g), a BB IGFBP2 genotype (552.00 g), and an AB TGF3 genotype (538.72g). The AA IGF1 population is found in Hubbard F15 strain hens (2585.00 and 35.32 g, respectively). The Hubbard F15 strain chickens had an AA of IGF1 (501.75 g), an AA of IGFBP2 (498.75 g), and an AA genotype

of TGF3 (498.75 g), as well as the lowest average BW and AFW at 42 days (2541.33 g and 31.53 g, respectively), and maybe lacking in the lower breast (494.33 g). A Hubbard F15 strain chicken with an AA of IGF1 (470.68 g) and a BB of IGFBP2 (445.00 g) at the lowermost thigh muscle and a Cobb E-line chicken with a BB of IGFBP2 (445.00g of TGF3 (458.67 g) using skin (Hosnedlova *et al.,* 2020). In addition to three cell obligatory receptors InsR, IGF-1R, and IGF-2R, insulin-like growth factor compulsory proteins (IGFBPs), and the IGFBP protease, the IGF structure contains three ligands (insulin, IGF-1, and IGF-2) (Li, *et al.,* 2017).

CONCLUSION

The higher frequency of A↔G and A↔C while the lower frequency of G↔T may be related to higher body weight with the Ross strain. Also, the higher frequency of A↔C and C↔T while the lower frequency of G↔T may be related to lower body weight with the Baladi breed at the marketing age stage. the values of body weight varied for the Ross, Sasso, and Baladi. Consequently, the polymorphic nature of these candidate genes, as well as their linkages to these qualities, it can be employed as active genetic markers to improve two commercial chicken strains, Ross and Sasso, as well as the local Baladi chicken breed. Finally, it can be concluded that the SNPs affecting meat production in chickens observed in this study suggest that these SNPs can be utilized in future efforts to assess genetic diversity and as markers to assist in the selection of local chickens.

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الكشف الجزيئي عن بعض جينات هرمون النمو في الدجاج

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الملخص

نظرًا لأن جين هرمون نمو الدجاج (GH) مهم للتحكم في التطور والتمثيل الغذائي ، فقد يكون هناك ارتباط بين مظاهر أشكال هرمون النمو والسمات الاقتصادية في الدجاج. ينظم جين IGFBPII مجموعة واسعة من الأنشطة البيولوجية المتعلقة بالنمو والتطور والتمايز. يمكن استخدام الدراسة الحالية في الجهود المستقبلية لتقييم التنوع الجيني وكعلامات للمساعدة في اختيار الدجاج المحلي المتفوق. تم فحص ثلاثمائة فرد (100 طائر لكل من السلالتين التجاريتين ساسو وروس وسلالة محلية واحدة ؛ بلدي) في هذه الدراسة. كان لسلالة الروس وزن جسم أكبر (1175.57 مقابل 576.14 و 218.79 جم) مقارنة بالساسو والسلالة البلدي. في هذه الدراسة كان إجمالي النيوكليتدات الفردية 138.106 تكونت من 13.265 نيوكليتدة في المناطق التي تشفر لبروتين و 124.841 نيوكليتدة بالمناطق الغير مشفرة لبروتين. إجمالي النيوكليتدات 197تتكون من 34اشفرت لبروتين و 163غير مشفرة للبروتين. قيمة التباينات المختلفة في المناطق التي تشفر لبروتين حوالي53٪ (18) و 26٪ (9) و 21٪ (7) بينما في المناطق غير المشفرة للبرتين كانت 27٪ (111) و 33٪ (132) و 40٪ (163) في كلا من الروس والساسو والسلالة البلدي للثلاثة جينات. هذه النتيجة على الأرجح ان تتسبب في أوزان الجسم العالية والمتوسطة والمنخفضة لكلا من سلالة الروس والساسو والبلدي. قد يكون معدل التغير العالي لـ A↔Gو A↔C والتغير المنخفض G↔T مرتبطًا بارتفاع وزن الجسم في سلالة الروس . في حين أن التحول العالي لـ A↔Cو C↔T و G↔T الأقل قد يكون مرتبطًا بانخفاض وزن الجسم مع سلالة البلدي. تؤكد دراستنا أن الجينات الثلاثة التي تم تحليلها تؤثر على إنتاج اللحوم في الدواجن.

الكلمات المفتاحية : المواقع الفردية المتغيرة وجين النمو1 و جين النمو 2 وجين بروتين ربط عامل النمو الشبيه بالأنسولين 2 وسلالة الروس وسلالة الساسو وسلالة البلدي