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Effects of Genetic Variations in the FGFBP2 Gene on Growth Indicators and Production Efficiency in Broiler Chickens

A. H. Essa [1]* S. S. Hussein [2] A. Q. Aswad [3]
M. A. Shareef [4]

[1] College of Law, University of Anbar, Ramadi, 31001, Iraq.
[2] Department of Animal Production, College of Agriculture, University of Anbar, Ramadi, 31001, Iraq.
[3] College of Political Science, University of Anbar, Ramadi, 31001, Iraq.
[4] Department of Accommodation Affairs, Headquarters, University of Anbar, Ramadi, 31001, Iraq.

***Correspondence to:** A. H. Essa, College of Law, University of Anbar, Ramadi, 31001, Iraq.
Email: aymaneng14@uoanbar.edu.iq

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Abstract

A 100 Ross 308-type broiler birds were raised in the College of Agriculture, University of Anbar fields for 42 days, and 60 were selected at the end of the feeding period. Blood samples were taken from the birds by slicing the jugular vein, and the slaughtering process was accomplished. The blood samples were transferred to the molecular genetics laboratories in Baghdad to obtain DNA to determine the phenotype of the FGFBP2 gene. The gene was identified in experimental birds based on the bands resulting from DNA relocation. The genetic phenotypes were determined using PCR-RFLP technology and the Alu 1 restriction enzyme based on the size of the fragments obtained from the FGFBP2 gene. They comprised CC, CD, and DD with allelic frequencies of 26.67, 51.66, and 21.67, respectively and with C and D allelic frequencies of 0.53 and 0.4y, respectively. Birds carrying the DD genotype were superior in breast weight traits of the FGFBP2 gene at 17.484±0.640 compared to CC and CD genotypes at 15.608±0.530 and 15.929±0.503, respectively. A study of the FGFBP2 gene sequences showed 65%

substitution mutations in the nitrogenous base sequence of the broiler samples. The study aimed to identify the relationship between the genotypes and the allelic frequencies of the FGFBP2 gene with carcass traits (carcass weight, covering percentage, and weights of the breast, thigh, and thigh pieces) of broiler chickens and the possibility of genetic improvement to obtain the most economically viable characteristics.

Keywords: FGFBP2, Sequencing, RFLP, PCR.

تأثير التغايرات الوراثية لجين FGFBP2 على مؤشرات النمو والكفاءة الإنتاجية في دجاج اللحم

ايمن هاشم عيسى [1]* صفاء صلاح حسين [2] عبدالله قاسم اسود [3]

مهند عامر شريف [4]

[1] كلية القانون، جامعة الانبار، الرمادي، 31001، العراق.

[2] قسم الإنتاج الحيواني، كلية الزراعة، جامعة الانبار، الرمادي 31001 العراق.

[3] كلية العلوم السياسية، جامعة الانبار، الرمادي، 31001، العراق.

[4] قسم شؤون الأقسام الداخلية، رئاسة الجامعة، جامعة الانبار، 31001، العراق.

***المراسلة الى:** ايمن هاشم عيسى، كلية القانون، جامعة الانبار، الرمادي، 31001، العراق.

البريد الالكتروني: aymaneng14@uoanbar.edu.iq

الخلاصة

تم تربية 100 دجاجة من نوع روس 308 في ظروف بيئية محكمة في حقول كلية الزراعة/ جامعة الانبار لمدة 42 يوم، تم ترقيم الطيور في اليوم الاول من التجربة واخذ بيانات كل طير اسبوعيا وبعد 42 يوم اخذت عينات الدم عن طريق قطع الوريد الوداجي وتم التقطيع للذبائح وقياس اوزان القطعيات، نقلت عينات الدم إلى مختبرات متخصصة في مجال الوراثة الجزيئية لأجل عزل الحامض النووي DNA من عينات الدم لغرض دراسة جين FGFBP2 وتحديد المظاهر الوراثية Phenotype لجين FGFBP2، وباستعمال تقنية PCR – RFLP وانزيم القطع Alu 1 تم تحديد المظاهر الوراثية اعتمادا على حجم القطع التي تم الحصول عليها لجين FGFBP2 وارسلت عينات لدراسة التتابع للقواعد النيتروجينية والحصول على مواقع التغايرات، تم ترميز المظاهر الوراثية التي ظهرت كما يأتي (CD، DD، CC)، وبترددات أليلية (26.67، 51.66، 21.67) وبتكرار جيني حيث كان الأليل C هو الأكثر تكرارًا، بينما كان الأليل D الأقل تكرارًا، أظهرت نتائج الدراسة أن المظهر الوراثي DD كان الأكثر تفوقًا في وزن الصدر، حيث سجل 718.15 ± 17.25 غرامًا، مقارنة بـ 633.66 ± 21.99 و 645.84 ± 20.84 غرامًا في المظاهر الوراثية CC وCD على التوالي. وهذا يشير

إلى أن جين FGFBP2 له تأثير إيجابي على نمو عضلات الصدر، وهو ما يبرهن على تأثيره على تحسين إنتاجية العضلات وزيادة كتلة الصدر في الدواجن. كما أظهرت نتائج تحليل التسلسل وجود طفرة استبدال في القواعد النيتروجينية في جين FGFBP2، خاصة في الإكسون الثاني. تم اكتشاف أربع تغايرات (SNP) في القواعد النيتروجينية التي قد تؤثر على هيكل ووظيفة البروتين الناتج عن الجين. وقد تؤدي هذه التغايرات إلى تأثيرات على استقرار البروتين وتركيبه، مما قد يسهم في تحسين نمو العضلات. وهذه الطفرات هي استباليه بنسبة 65% في تسلسل القواعد النيتروجينية لعينات الدجاج اللاحم. تهدف هذه الدراسة إلى أن التحليل الجيني لجين FGFBP2 يمكن أن يساعد في تحسين خصائص الإنتاج في الدواجن، خاصة من خلال اختيار المظاهر الوراثية التي تؤدي إلى زيادة فعالية النمو والتطور العضلي وسمات الذبيحة في دجاج اللحم.

كلمات مفتاحية: FGFBP2، تسلسل النيوكليوتيدات، البلمرة، انزيمات القطع، المظاهر الوراثية.

Introduction

The increasing demand for animal products due to the growing global population has raised the production of poultry meat by encouraging breeders to enhance growth rates and muscle mass through the use of genetic improvement programs (11, 14 and 15). At present, broiler chickens are marketed at the same age as in the past, while achieving nearly twice the body mass compared to what was typical in earlier years (14, 21 and 22).

Genetic improvement programs, combined with enhanced healthcare, nutrition, and management practices in broiler chicken production, have led to significant improvements in feed conversion efficiency and accelerated body growth (1 and 19). Broiler chickens are selected based on several traits, including growth, which depends mainly on recent weight. At the end of the rearing period, carcass meat yield is a key trait of economic interest, the most important being the breast cut, due to its economic value, as its percentage reaches 50% of the meat (1 and 11). The improvement in the result of the breast meat cut is through the relationship between its weight ratio to that of the body weight of the carcass. Thus, this relationship will increase the benefit from the food consumed by the bird (22 and 23).

The identification of genetic markers linked to carcass traits are associated with genes influencing them and allows for the distinction between genetically different birds (10). The development of molecular genetics and genetic mapping has led to studies to identify genetic regions on chromosomes that affect economically important carcass traits (5).

Through the study of quantitative characteristics, many traits affecting muscle growth and development have been identified, which are based on chromosomes containing genes affecting economically important traits (12 and 14). A study by (3) in the area of chromosomes identified 4 quantitative trait loci (QTL) between the thigh and drumstick, and they indicated that this region was specific to the growth factor genes affecting muscle growth in broiler chickens. The fibroblast growth factor binding protein 2 (FGFBP2) gene was selected for the study of QTL, which is

considered one of the genes affecting and associated with economically important traits of muscle growth.

The FGFBP2 gene encodes a fibroblast growth-factor binding protein, which binds to FGFs, particularly FGF1 and FGF2, that are involved in regulating muscle cell growth and differentiation (4 and 7). Previous studies have shown that FGFBP2 can transport FGF2 from the extracellular medium to the interior, enhancing the biological activities of FGF1 (2, 8 and 18). A study by (9) showed that the FGFBP2 gene contributes to muscle mass growth after hatching, and has an important role in the development of embryonic muscle growth in broiler embryos.

This study investigated the association between single nucleotide polymorphisms (SNPs) in the FGFBP2 gene and production traits in broiler chickens, particularly muscle growth rate and breast muscle yield, by determining the relationship between genetic traits and muscle growth rate, with the gene's effect on increasing breast weight and improving muscle growth efficiency. It also explored the relationship between SNPs and their effect on muscle growth characteristics and carcass traits. Thus, genetic improvement programs can be applied to enhance muscle growth traits and increase production efficiency in the poultry industry by studying the effect of the FGFBP2 gene, thereby selecting birds with strong genetic traits for genetic improvement purposes.

Materials and Methods

Animal care: The broiler chicks were raised under standard management and rearing programs at the College of Agriculture, University of Anbar, with appropriate vaccination schedules applied during the rearing stage.

Animal samples: The study was conducted on 100 (Ross 308) broiler birds from the poultry fields of the college from 9/20/2023 to 11/2/2023. The birds were raised in cages, each containing 10 birds ranging in age from 1 day to 42 days. Each bird was numbered, and weights were taken on the first day of their life and at the end of each week to determine the efficiency of food conversion and the rate of weight gain for each bird. At the end of the experiment, the birds were slaughtered, blood samples were taken, and the studied pieces were weighed.

Cutting process and weighing the pieces: After slaughtering and taking samples of types of blood, the carcass was cut into main pieces comprising the thigh, the drumstick, and the breast. The secondary pieces were the wings, the back, and the neck. They were weighed according (22) using the following equation:

Weight of the carcass piece to body weight = (weight of the carcass piece)/(live body weight) × 100

DNA extraction: Blood samples of 5 ml were randomly collected from each of the 60 after slicing the jugular vein in the neck and completing the slaughtering process. The samples were placed in test tubes containing the anticoagulant EDTA, stored in a refrigerated box, and then transferred to a freezer at -18 °C. Blood analysis was conducted in a laboratory to isolate the genetic material using the Promega Kit and to identify the genotype of the FGFBP2 gene. It also investigated its association with productive traits to identify the genetic features and their effect on productive

performance. DNA extraction samples were loaded into Gel electrophoresis at a concentration of 2% agarose and using ethidium bromide dye,

Primer preparation: Macrogen was prepared in the primer in a lyophilized form. The primer was dissolved by adding nuclease-free water to obtain a product with a final 100 pmol/µl concentration.

Table 1: Sequence of the nitrogenous bases of the FGFBP2 gene prepared by Macrogen.

Primer	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)
FGFBP2-F	GGATGAAGAGATGAAAGCGAGA	58	715
FGFBP2-R	AAACCCCCAGAAGCCACA		

Amplification of the FGFBP2 gene using PCR technology: Polymerase chain reaction (PCR) was carried out using a thermal cycler, with specific forward and reverse primers for each target gene, and previously isolated genomic DNA samples. The reaction conditions were set according to the protocol described in the Materials and Methods section. The PCR products were loaded onto a 2% agarose gel by pipetting 7 µL of each amplified sample along with 4 µL of a molecular size marker (100–1500 bp) as a reference. Electrophoresis was performed initially at 100 volts for 10 minutes to facilitate sample entry into the wells, followed by 80 volts for 90 minutes to achieve optimal separation. The results were documented using a gel documentation system marker lane (M) representing known fragments and fragment size (100-1500) bp. Numbers 1 to 27 represent Ross 308 broilers, as illustrated in Figure 1.

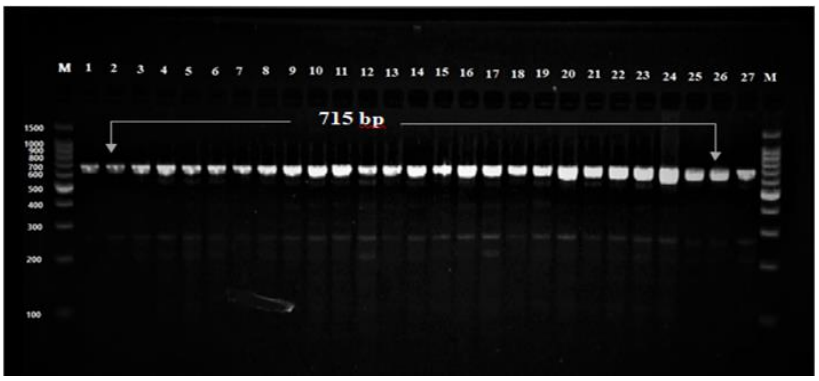

Figure 1: PCR product for the FGFBP2 gene after it was placed in an agarose gel, where ethidium bromide dye was added and exposed to UV.

Genotyping of FGFBP2 gene variants using the Alu I restriction enzyme through the PCR-RFLP technique: The genetic structures of Ross 308 broilers for the FGFBP2 gene were determined using the RFLP technique and the Alu 1 restriction enzyme. The digested PCR products were electrophoresed by injecting 8 µL in each sample and using 4 µL of ladder (100-1500) in agarose gel at a concentration of 2.5%. The device's voltage was set to 100 volts for 10 mins and then to 80 volts for an hour and a half. The result of the transfer was photographed to ensure the success of the extraction process and obtain the required size of the pieces (Fig. 2)

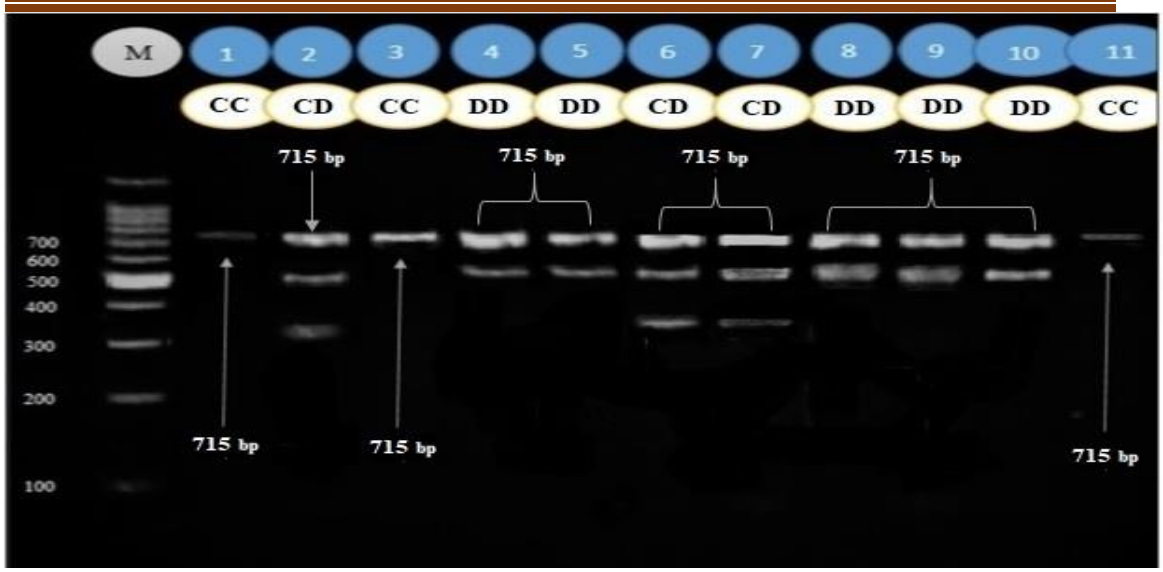

Figure 2: Result of digestion of the FGFBP2 gene using the restriction enzyme Alu 1 after exposure to acerose gel dyed with the ethidium bromide dye of the ultraviolet radiator M column is a cut-off of the volume (100 - 1500) basal pair. Columns 1, 3, and 11 show homozygous dominant patterns, denoted by CC; columns 2, 6, and 7 represent heterozygous samples, denoted by CD; and columns 4, 5, 8, 9, and 10 represent homozygous recessive samples, denoted by DD.

Statistical analysis: The statistical analysis of the data was conducted using the statistical program (20). The completely randomized design (CRD) was used to study the effect of the genetic features of the FGFBP2 gene on the productive performance of broiler chickens. Significant differences between the averages were compared using the (6) multinomial test at a significance level of $P \leq 0.05$.

Results and Discussion

Phenotypes and allelic frequency of the FGFBP2 gene: Table 2 illustrates the percentage distribution of the dominant (CC), heterozygous CD), and recessive (DD) genotypes in the birds at 26.67%, 51.66%, and 21.67%, respectively, showing the clear predominance of the CD phenotype. This predominance indicates the effect of the FGFBP2 gene on growth in birds with the heterozygous phenotype, highlighting the need to consider average gene frequencies when selection is based on the abundance of FGFBP2 gene phenotypes. Priority should be given to birds with the CD phenotype, followed by those with CC and DD phenotypes. The DD phenotype may be associated with reduced growth performance due to the limited influence of the FGFBP2 gene in birds carrying it. Accordingly, genetic improvement in birds with the CD genotype can be achieved through the direct selection method for such individuals.

Table 2: Genotype distribution of the FGFBP2 gene in the Ross 308 broiler chicken samples.

Phenotype	Number	Avg (%)
CC	16	**26.67**
CD	31	**51.66**
DD	13	**21.67**
Total	60	**100.00**
Chi-square value ($\chi 2$)	-----	**NS 0.366**
Significance level	**NS**	

These results closely match those by (9) who showed the AB genetic phenotype being superior to the AA and BB genotypes, whose percentages were 0.30 and 0.20, respectively. It is clear that the population is in a state of equilibrium according to the Hardy-Weinberg equilibrium (HWE) and that the effects of random mating between population members through mutations, migrations, chance, and selection, can affect populations genetically. Table 3 shows the allelic frequencies of the FGFBP2 gene obtained through the presence or disappearance of the cut sites for many different alleles. The allele with one cut site at the 715 bp position is called the C allele, while in the D allele the cut appears at the 710bp base pair position, depending on the abundance of genotypes. The frequencies of the C and D alleles were 0.53 and 0.47, respectively. The results were analyzed using the SAS program (18).

Table 3: Allelic frequencies of the FGFBP2 gene.

Allele	Frequency (%)
C	**0.53**
D	**0.47**
Total	**100**

The relationship of the FGFBP2 gene genotypes with carcass weight: Table 4 presents the breast percentage and weights of various carcass cuts in the broiler chickens covering total carcass weight, dressing percentage, and the individual weights of the breast, thigh, and drumstick. A significant effect of genotype on breast weight was observed ($P < 0.05$). Broilers carrying the DD genotype recorded the highest breast weight, with an average of 718.15±17.25g compared to 633.66±21.99g and 645.84±20.84g for the CC and CD genotypes respectively. This indicates a clear genetic advantage of the DD genotype for enhancing breast muscle development in broiler chickens. The reason for this significant superiority is attributed to the effect of the FGFBP2 gene on the growth rate of the breast of birds carrying the DD genetic feature. About the remaining birds carrying the CC and CD genetic traits through the influence of the FGFBP2 gene region on the quantitative trait loci affecting the muscle growth rate of the breast cut of individuals carrying the DD genetic trait, based on the relationship between the ratio of breast cut weight to carcass weight. This demonstrated the effect of the FGFBP2 gene on the muscle growth of the breast cut through carcass weight observed in birds carrying the DD genetic trait. Consequently, this relationship will increase benefits for the breast meat yield if genetic improvement is carried out by selecting these genetic traits, and no significant differences appear for the other genotypes traits (DD, CD, CC).

The results of this study shows a specific effect limited to breast muscle of the FGFBP2 gene on carcass traits, with no significant differences observed in most cuts, except for the breast weight cut, which showed a significant improvement. This variation in effect may be related to the specific role that FGFBP2 plays in muscle growth, as it has been previously associated with promoting muscle tissue development in some studies, which may explain the significant superiority observed in breast weight in this study. These results align with (9), who studied the effect of the FGFBP2 gene on carcass cut weights and found no significant differences between most cuts, except for breast weight, which showed a significant increase. This strengthens the validity of the results of this study and suggests a specific impact of this gene on certain areas of the carcass.

This study's findings also agree with (8), who investigated the relationship between meat quality and muscle characteristics in broiler chicken breeds. The study found that the FGFBP2 gene did show a significant effect on the weight of most cuts, including the thigh and breast, further supporting the findings from this research. This indicates that the effect of the FGFBP2 gene may be more specialized in certain regions of the body, such as the breast, compared to other muscles. In contrast, this study's results do not align with (17), who compared different broiler strains and focused on the genetic structures of these strains and their impact on quantitative trait loci (QTLs). It identified clear genetic influences on various carcass traits, showing significant differences between the strains, which contradicts the results of our study, where no significant differences were observed between strains for the traits studied.

This discrepancy could be attributed to several factors, including differences in the strains used in the study, as these strains may respond differently to genetic traits under specific breeding conditions. Furthermore, the genetic analysis methods used in may have been more precise or comprehensive than those employed in this study, which may explain why their methods were able to detect more subtle genetic effects. Environmental factors and breeding practices can also have a substantial impact on the results. Variables such as diet, temperature, or stocking density can affect gene expression and modify its effects on carcass traits. Thus, these differences between studies could be explained by the genetic diversity of the strains studied as well as environmental influences that may alter the effect of the FGFBP2 gene on inherited traits (16).

Based on these results, FGFBP2 appears to be a promising gene for improving specific quality traits, such as breast weight, in genetic selection programs. This opens up new possibilities for improving broiler chicken breeds through targeting these genes in future breeding programs. If this gene can positively influence certain traits like breast weight, it could be utilized to improve poultry productivity by enhancing desired traits in commercial production. In this context, genetic modification strategies using techniques like CRISPR or others could be a promising option for developing breeds with balanced characteristics, including fast growth and high meat quality.

Table 4: Relationship between FGFBP2 gene genotypes and some studied economic traits.

Genotype	Mean ± standard error (g)				
	Drumstick weight/g	Thigh weight/g	Breast weight/g	Purity ratio	Carcass weight/g
CC	90.46±0.261	78.34±0.307	633.66±21.99b	72.93±1.09	1880.4±54.56
CD	89.44±0.157	76.38±0.169	645.84±20.84b	72.47±0.56	1877.6±53.29
DD	95.45±0.224	78.65± 0.326	718.15±17.25a	72.34±0.66	1855.2±46.97
Sig level	NS	NS	*	NS	NS

Averages with different letters within one column differ significantly from each other.
*(P<0.05), NS: not significant.

Sequence analysis study of the FGFBP2 gene: Forty samples of the PCR product were used to conduct sequence analysis of the FGFBP2 gene. The nitrogenous base sequences were compared against those at the National Center for Biotechnology Information (NCBI) to detect sequence variations in the FGFBP2 gene. The variation obtained for the FGFBP2 gene for broiler chicken samples was located in exon two by comparing it with the NCBI data. The occurrence of substitution mutations is noticed by detecting the variants in the FGFBP2 gene (Figure 4a, b, c).

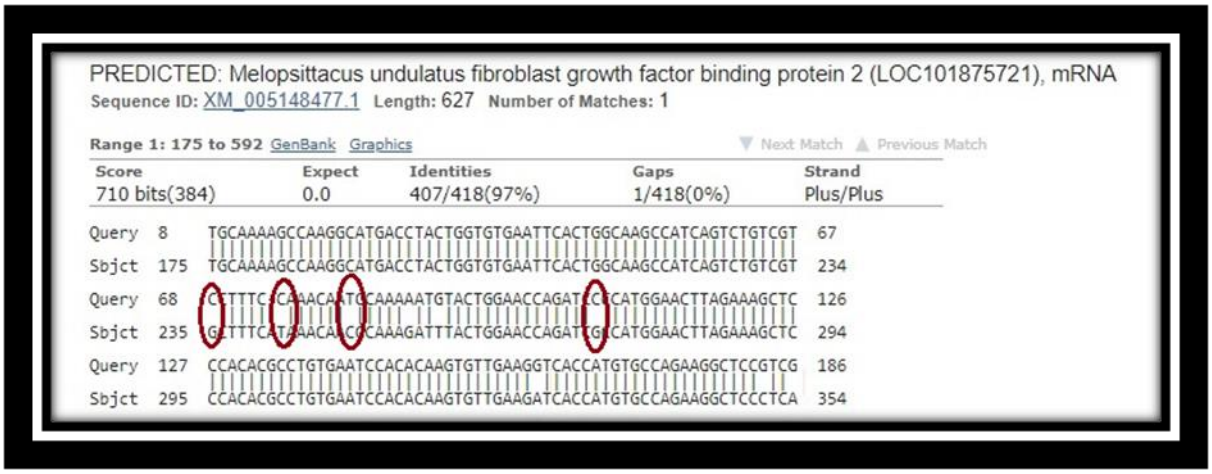

Figure 4a: Difference in the FGFBP2 gene sequence where the C/G 235, C/T 242, T/C 250, and C/G 275 nitrogenous base substitutions were found after comparing with NCBI.

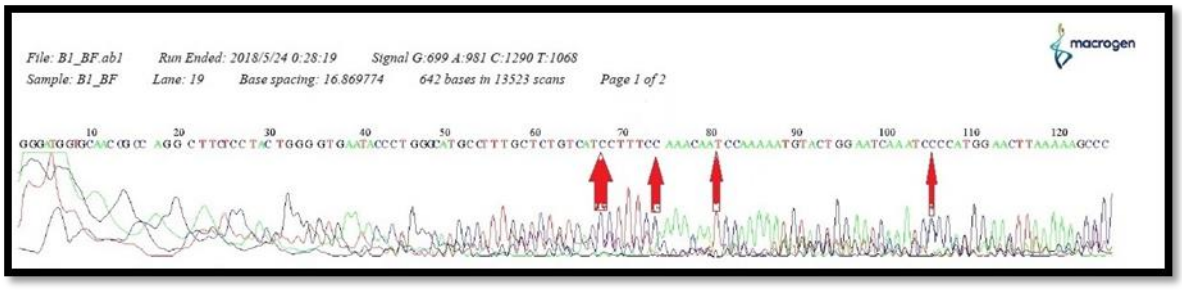

Figure 4b: Comparison of the FGFBP2 gene with the control from the NCBI.

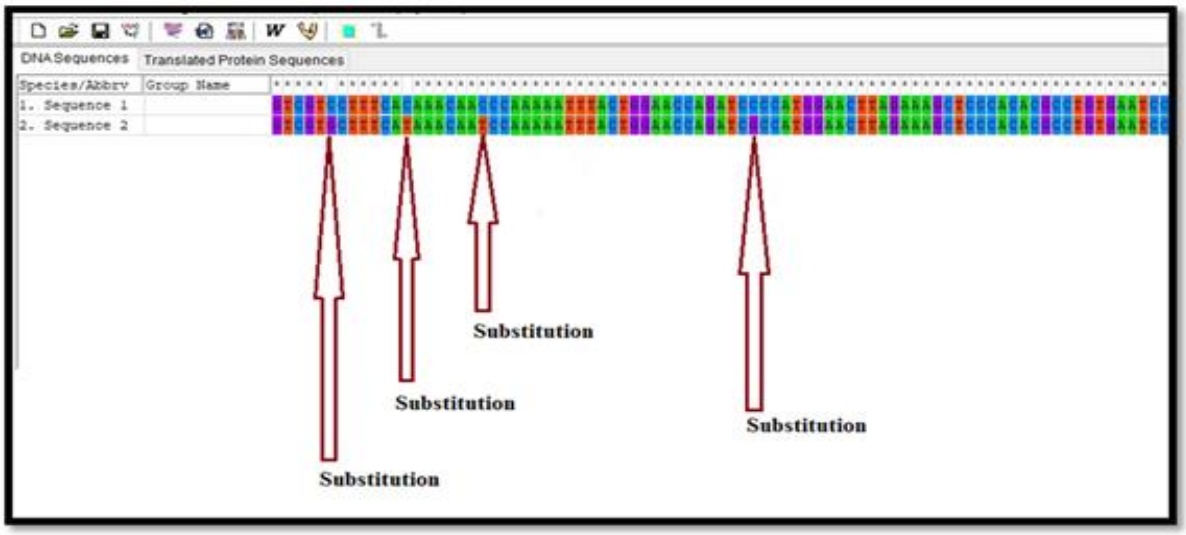

Figure 4c: Sequences of the nitrogenous bases with the locations of the mutations (substitution).

Sequencing analysis of the FGFBP2 gene revealed four single nucleotide polymorphisms (SNPs) within exon 2, each potentially influencing the structure and function of the resulting protein. The first SNP involved a substitution from cytosine (C) to guanine (G) at nucleotide positions 234–236, altering the codon TGC and leading to an amino acid change from serine (Ser) to cysteine (Cys). This modification is functionally relevant, as serine contains a hydroxyl group involved in phosphorylation, while cysteine has a thiol group capable of forming disulfide bonds, which may impact protein folding and stability. The second SNP involved a substitution from thymine (T) to cytosine (C) at positions 241–243, ATA = Ile •

ACT / ACC / ACA / ACG = Thr, a non-polar, hydrophobic amino acid. Such a change may affect the protein's local environment, especially in binding or functional domains.

The third SNP, also a T to C substitution at positions 248–251, altered the codon ACC, reversing the previous change and resulting in a shift from isoleucine (Ile) back to threonine (Thr), suggesting possible selective pressure at this site. The fourth SNP was identified as a substitution from C to G at positions 274–276, modifying the codon CGC and changing the encoded amino acid from proline (Pro) to arginine (Arg). Since proline is structurally rigid and often induces kinks in polypeptide chains, while arginine carries a positively charged side chain that participates in hydrogen bonding and electrostatic interactions, this substitution may significantly alter protein structure or interactions.

Collectively, these SNPs are classified as missense mutations, as they result in changes to the amino acid sequence of the FGFBP2 protein. These findings differ from (4), which reported only silent mutations that did not affect the amino acid sequence. Such discrepancies may stem from variations in sample populations, genetic background, or environmental factors. The observed mutations underscore the potential biological significance of FGFBP2 variation and suggest a need for further investigation using molecular modeling and in silico analysis to better understand their structural and functional implications.

Conclusions

SNP1, SNP2, SNP3, and SNP4 sites in the FGFBP2 gene were associated with carcass traits of broiler chickens, as the C/G, T/C, T/C, and C/G point mutations were observed at sites 235, 242, 249, and 274, respectively. These sites showed amino acid changes that improved carcass quality, especially breast cut. Therefore, single nucleotide polymorphisms can be studied to select genotypes that affect genetic improvement in broiler chickens and rely on artificial selection for these genetic phenotypes to obtain good carcass traits in broiler chicken strains.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

A. H. Essa: methodology, writing—original draft preparation; Safaa S. Hussein writing A. Q. Aswad review and editing M. A. Shareef. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement:

Not applicable.

Data Availability Statement:

Data available upon request.

Conflicts of Interest:

The authors declare no conflict of interest.

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