University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 410-420

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/ebsx5618

Genetic Polymorphism of ASB15 gene and Its Relationship with Productive Performance, Growth Traits, and Skeletal Muscles in Rose 308 broiler chicken

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Abstract

The present study aimed to analyze the polymorphisms of the ASB15 gene in broiler chickens and to determine the effects of single nucleotide polymorphisms (SNPs) within the gene sequence, as well as their impact on the peptide chain of the ASB15 protein. This gene was selected due to its important role in regulating skeletal muscle growth and improving production traits in chickens. The study included 50 broiler chickens from a homogeneous commercial population of the Ross 308 strain. Genomic DNA was extracted from blood samples collected from the wing vein, and a specific fragment of the gene (exon 7, 740 bp) was amplified using Polymerase Chain Reaction (PCR) with the following primers: forward (GGTGCTTCTGTGTTAGGATTTT) and reverse (GGCTAACGGAAAGAAGAAAGTG). The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 10 minutes. Direct sequencing analysis was performed by Macrogen Company (Korea) to detect genetic variations.

The results revealed the presence of two point mutations, 287 A>G and 374 T>C, resulting in three genotypes at each locus. For the 287 A>G mutation, genotype frequencies were AA (0.66), AG (0.08), and GG (0.26), with allele frequencies of A (0.70) and G (0.30). For the 374 T>C mutation, genotype frequencies were TT (0.64), TC (0.10), and CC (0.26), with allele frequencies of T (0.69) and C (0.31). Allele frequency analysis indicated that alleles A and T were more frequent compared to the alternative alleles, suggesting their predominance in the studied population. The Chi-square test showed a significant deviation from Hardy–Weinberg equilibrium at both loci ($\chi^2 = 32.76$ and $\chi^2 = 29.35$, respectively; $P<0.05$), which may be attributed to artificial selection, environmental factors, or the relatively small sample size used in this study.

Furthermore, amino acid sequence analysis demonstrated that both detected mutations were synonymous substitutions at the codon level, classifying them as silent mutations, as they did not lead to any change in the amino acid sequence (proline and asparagine remained unchanged), indicating no direct effect on protein structure. However, it is hypothesized that these polymorphisms may potentially influence gene expression levels through possible effects on mRNA stability or splicing efficiency, which could be reflected in growth and production traits; this hypothesis requires further experimental validation through gene expression studies.

The study concludes that the ASB15 gene has significant potential as a molecular marker that can be utilized in genetic selection programs to improve growth traits and increase meat production in broiler chickens. However, several limitations should be acknowledged, including the relatively small sample size (n=50), the analysis of only one exon (exon 7) of the gene, and the absence of gene expression measurements, which restrict the generalizability of the findings. It is recommended that further studies be conducted with larger and more diverse populations to better understand the functional effects of these mutations and their impact on gene expression.

These findings also highlight the importance of integrating molecular analyses with production data in modern breeding programs. The use of molecular markers associated with growth-related genes, such as

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ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 410-420

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/ebsx5618

ASB15, may accelerate genetic improvement processes and enhance selection accuracy, thereby supporting sustainable and efficient poultry production.

***Keywords**: Allele Frequency, SNP : ASB15 gene ,Genetic polymorphism , Broiler chickens ,Growth traits ,Productive performance*

I. Introduction

Poultry production is a major animal industry worldwide due to its role in supplying high-quality animal protein and supporting food security. The short production cycle and rapid growth of broiler chickens contribute to strong economic returns and make poultry meat and eggs widely accessible sources of nutrition (Abdul Karim et al., 2016). Nutritionally, poultry products provide complete protein containing all essential amino acids, along with relatively low saturated fat and high levels of unsaturated fatty acids that benefit cardiovascular health. They are also rich in micronutrients such as iron, zinc, selenium, and vitamins B6 and B12 (Connolly & Campbell, 2023).Over recent decades, the sector has advanced considerably through improvements in breeding programs, genetic selection, and nutrition, leading to clear gains in growth performance and meat yield (Luo et al., 2017). With progress in molecular genetics and biotechnology, research has increasingly focused on using molecular tools and genetic markers to identify genes linked to productive and growth traits. The aim is to apply these markers in early selection schemes to develop strains with higher efficiency (Burt, 2002). Among molecular markers, single nucleotide polymorphisms (SNPs) are now the most widely used in poultry studies because they account for a large proportion of genetic variation in economically important traits such as body weight, growth rate, feed efficiency, and carcass characteristics (Wang et al., 2015).Skeletal muscle mass is a key determinant of meat yield, since final body weight in broilers depends largely on the development of breast and leg muscles. Consequently, many studies have targeted genes that regulate muscle growth and differentiation, given their roles in muscle cell proliferation, differentiation, and protein synthesis (Luo et al., 2017). Skeletal muscle mass also has direct economic value, and improvements in muscle growth enhance both meat quality and production efficiency (Muir et al., 2008; Dekkers, 2012).One candidate gene in this context is _ASB15_, which belongs to the ankyrin repeat and SOCS box protein family and is highly expressed in skeletal muscle (McDaneld & Spurlock, 2006). The gene encodes a protein with ankyrin repeat domains involved in intracellular signaling and a SOCS box that participates in protein degradation pathways linked to growth and hormonal regulation. Evidence indicates that _ASB15_ influences muscle growth by affecting protein synthesis and muscle cell differentiation, and it is associated with muscle hypertrophy and fiber development (McDaneld & Spurlock, 2008).Genetic polymorphisms in _ASB15_ have been reported in chickens, with significant associations found between variants such as rs315759231 and rs312619270 and traits including hatch weight, carcass weight, body length, organ weight, and leg muscle mass (Wang et al., 2015). More recent work shows that _ASB15_ is involved in post-hatch skeletal muscle growth in broilers and operates within a gene network alongside genes such as _MYOD_ and _RPL3L_ (Lin et al., 2022). These findings suggest that _ASB15_ can serve as a molecular marker in marker-assisted selection programs aimed at improving feed efficiency and meat production in commercial broiler lines (Mohammadabadi et al., 2021Therefore, this study aimed to sequence the _ASB15_ gene, identify polymorphisms, and evaluate the potential effects of mutations on the peptide structure of the ASB15 protein.

II. Materials and Methods

In this study, 50 broiler chickens (*Gallus gallus*) were used. A total of 1 mL of blood was collected from the wing vein of each bird for DNA extraction, which was considered sufficient to yield adequate DNA quantities for downstream applications. DNA extraction was performed using a commercial kit provided by Geneaid. The purity and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer, and DNA integrity was further verified by agarose gel electrophoresis. The average DNA concentration ranged between 35–123 ng/µL, while the average purity ratio (A260/A280) was recorded at 1.75. A specific fragment of the ASB15 gene (740 bp) was amplified using the Polymerase Chain Reaction (PCR). The PCR reaction mixture consisted of 13 µL of 2× Green Master Mix (Promega), 1 µL of forward primer, 1 µL of

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https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/ebsx5618

reverse primer, and 5 µL of genomic DNA. Nuclease-free water was added to complete the final volume to 25 µL. Thermal cycling was performed using a thermal cycler (Multigene, Germany).

The target fragment spanning exon 7 of the *ASB15* gene was amplified via Polymerase Chain Reaction (PCR) using specific primers: forward (5′-GGTGCTTCTGTGTTAGGATTTT-3′) and reverse (5′-GGCTAACGGAAAGAAGAAAGTG-3′), as previously described by Wang et al. (2015). The thermocycling profile initiated with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 45 s, concluding with a final extension step at 72 °C for 10 min. Subsequently, the PCR amplicons were resolved by electrophoresis on a 1.5% (w/v) agarose gel and visualized under ultraviolet (UV) light using a high-resolution gel documentation system. Successfully amplified products were dispatched to Macrogen Inc. (Seoul, South Korea) for bidirectional Sanger sequencing analysis."

Multiple sequence alignment of nucleotide and amino acid sequences was performed using Clustal Omega via the European Molecular Biology Laboratory (EMBL) online platform. Sequence quality was verified through chromatogram inspection prior to downstream analysis. A negative control (no-template control) was included in each PCR run to verify the absence of contamination. In addition, BioEdit software version 7.2.6 was used for sequence analysis (Hall, 1999).

III. Results & Discussion

Figure (1) shows agarose gel electrophoresis of genomic DNA extracted from broiler chicken blood using a DNA extraction kit produced by Geneaid (Taiwan). The DNA samples were run on a 1% agarose gel. The electrophoresis results confirm the successful DNA extraction process. The DNA bands appeared at approximately the same level, indicating the homogeneity of the extracted DNA samples. In addition, the similar intensity of band fluorescence reflects comparable DNA concentrations among the different samples. No smearing or fragmented bands were observed, which confirms the high quality and purity of the extracted genomic DNA and indicates that it is intact without degradation. The clear and well-defined bands observed in the lower region of the gel also support the efficiency of the electrophoresis conditions used in this study. Overall, these results indicate accurate DNA extraction and successful preparation of high-quality genomic DNA suitable for subsequent molecular analyses.

In this study, a total of 300 broiler chickens of the Ross 308 strain were raised under standard commercial conditions. At 35 days of age, 100 birds were randomly slaughtered, and 50 birds were subsequently randomly selected for genomic DNA extraction and molecular analysis. This multi-stage random selection process ensured an unbiased representation of the studied population. However, it should be acknowledged that the sample size of 50 birds may limit the generalizability of the findings to broader broiler populations, and future studies with larger sample sizes are recommended.

All statistical analyses were performed at a significance level of $P<0.05$. Genotype and allele frequencies were calculated, and the Chi-square test was applied to assess deviation from Hardy–Weinberg equilibrium at each locus independently.

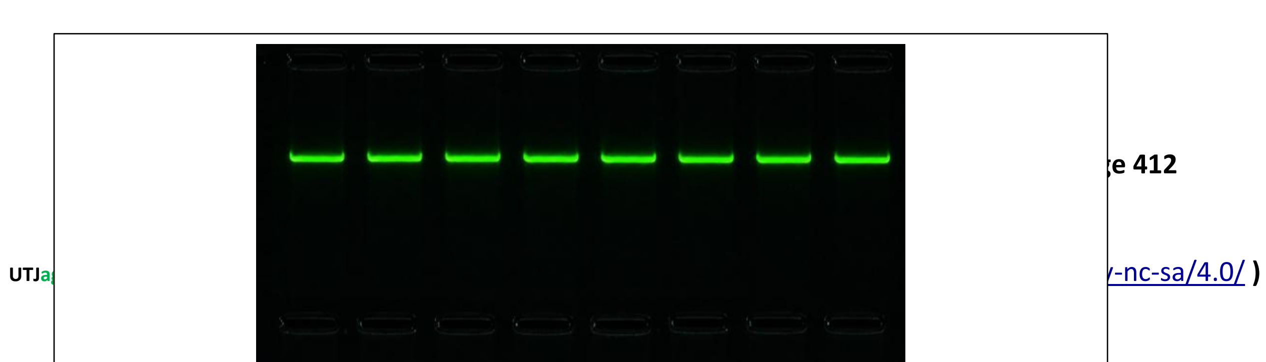

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Figure (2) shows the agarose gel electrophoresis of the PCR product of the ASB15 gene. The results indicate successful amplification of the target fragment, as evidenced by the presence of clear bands at approximately 740 base pairs when compared with a standard DNA ladder (100–3000 bp). As observed in the electrophoresis image (Figure 1), performed on a 1.5% agarose gel, the bands exhibit strong fluorescence intensity across all samples, suggesting good concentration and yield of the PCR product corresponding to exon 7 of the ASB15 gene.
Furthermore, no non-specific bands or smearing were detected, confirming the specificity and efficiency of the primers used to amplify the target fragment of the ASB15 gene, as reported by Wang et al. (2015). Based on these results, there is strong evidence supporting the success of the sequencing process for detecting nucleotide variations and identifying polymorphisms in the ASB15 gene.

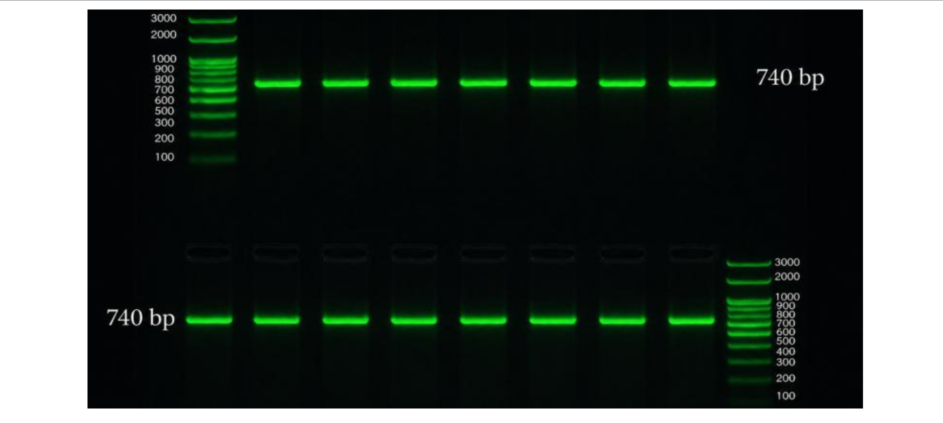

Figure (2): Agarose gel electrophoresis of the amplified fragment of the ASB15 gene (740 bp) on a 1.5% agarose gel (70 V / 85 mA for 45 minutes).

Figure (3) illustrates the position of the nucleotide substitution 287 A>G in the ASB15 gene sequence. This mutation resulted in three genotypes: AA, AG, and GG. A substitution of adenine (A) to guanine (G) is observed in sequences with the GG genotype compared to the AA genotype, while the heterozygous genotype (AG) contains both nucleotides A and G, respectively.

Upon examining the effect of this mutation on the peptide sequence of the ASB15 protein, it was found that the mutation occurs within the codon CCA, which is altered to CCG. Both codons encode the amino acid proline (P) at position 378 of the peptide chain, as shown in Figure (5). Therefore, the 287 A>G mutation is classified as a synonymous substitution at the codon level, representing a silent mutation with no direct effect on the amino acid sequence. However, it is important to note that silent mutations are not necessarily

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functionally neutral. Such synonymous substitutions may potentially influence gene expression through effects on mRNA stability, splicing efficiency, or translation efficiency, even in the absence of amino acid changes. These potential regulatory effects warrant further investigation through gene expression studies to fully elucidate the functional significance of this mutation in relation to growth and production traits in broiler chickens.

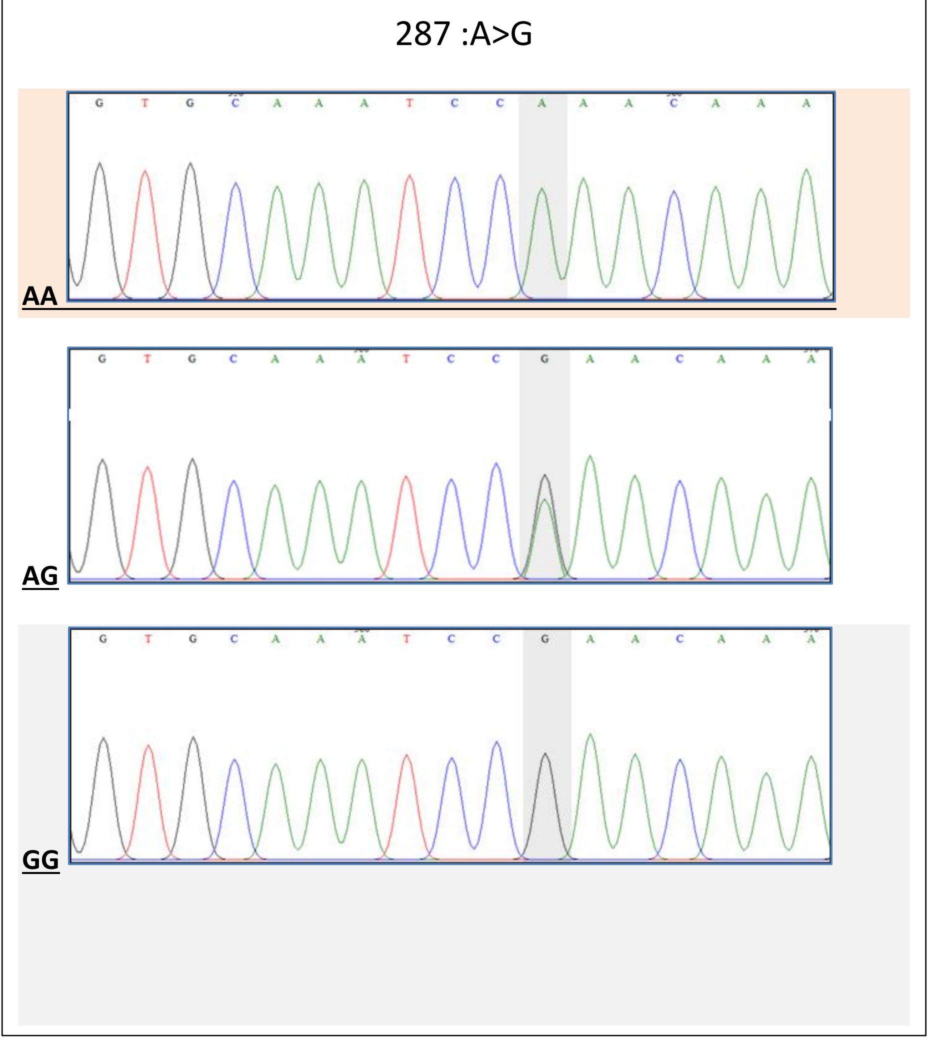

Figure (3): Position of the nucleotide substitution 287 A>G in the ASB15 gene sequence in broiler chickens.

Figure (4) illustrates the position of the second nucleotide substitution 374 T>C in the ASB15 gene sequence in broiler chickens. This mutation resulted in three genotypes: TT, CT, and CC. A substitution of thymine (T) with cytosine (C) is observed in sequences with the CC genotype compared to the TT genotype, while the heterozygous genotype (CT) contains both nucleotides T and C.

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https://jam.utq.edu.iq/index.php/main   https://doi.org/10.54174/ebsx5618

Analysis of this mutation in relation to the peptide sequence of the ASB15 protein showed that it occurs within the codon AAT, which is changed to AAC. Both codons encode the amino acid asparagine (N), as illustrated in Figure (5). This amino acid is located at position 407 of the ASB15 peptide chain. Therefore, the 374 T>C mutation is considered a silent mutation.

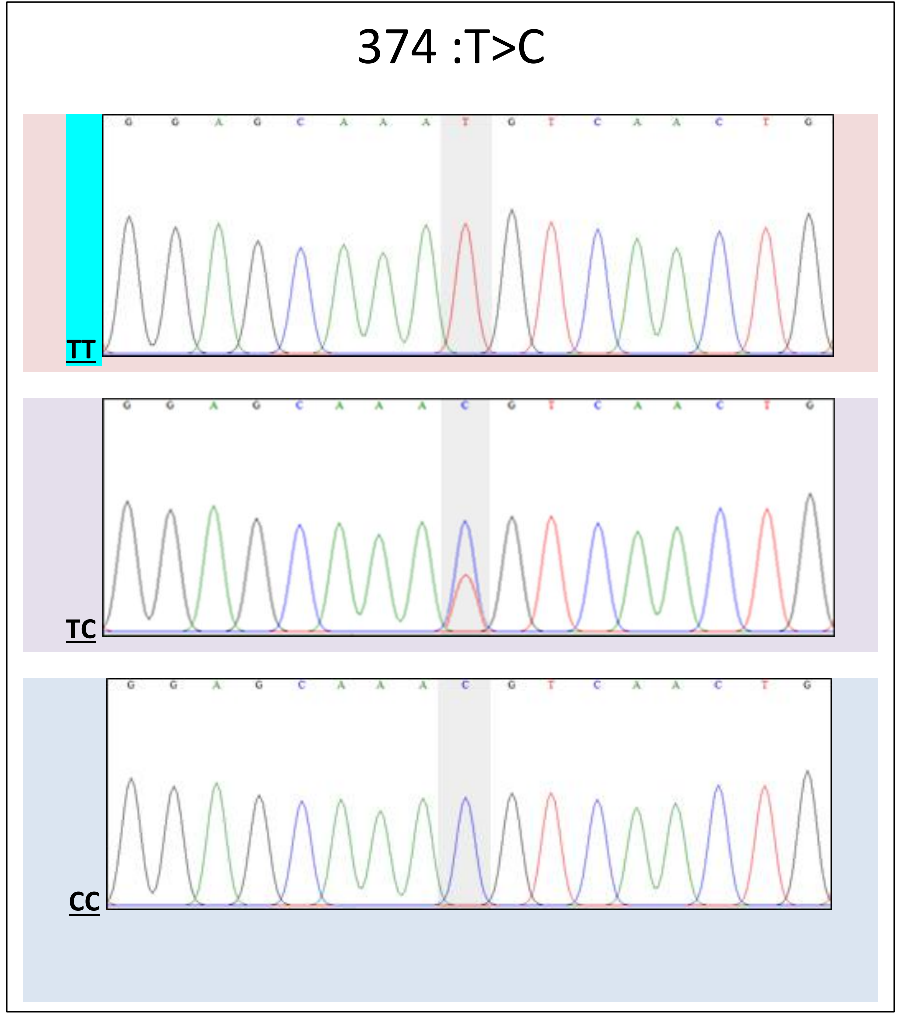

Figure (4): Position of the nucleotide substitution 374 T>C in the ASB15 gene sequence in broiler chickens.

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Amino_Acid1      TSQTAIQKSGISPVHSAADGQNSQCLELLIESDFDVNTVLDDHISNSYDDERKTALYFAV 360
Amino_Acid2      TSQTAIQKSGISPVHSAADGQNSQCLELLIESDFDVNTVLDDHISNSYDDERKTALYFAV 360
                 ************************************************************

Amino_Acid1      SNNDILCTEILLKAGANPNKDPLNCLLVAVRGGNHEIVRLLLSYGANVNCYFMMVNDTHF 420
Amino_Acid2      SNNDILCTEILLKAGANPNKDPLNCLLVAVRGGNHEIVRLLLSYGANVNCYFMMVNDTHF 420
                 ************************************************************

Amino_Acid1      PSAIQYALNDEVMLRLLLNHGYNVELCFDCMHQDVFGNSFVWSTPEEEILPGWTSSVIRD 480
Amino_Acid2      PSAIQYALNDEVMLRLLLNHGYNVELCFDCMHQDVFGNSFVWSTPEEEILPGWTSSVIRD 480
                 ************************************************************

Amino_Acid1      NPFCDFISVPWLKHLAGKVVRIFIDYMDYVPLCTKIKFVLETQKEWTEIRQILDNPRPLK 540
Amino_Acid2      NPFCDFISVPWLKHLAGKVVRIFIDYMDYVPLCTKIKFVLETQKEWTEIRQILDNPRPLK 540
                 ************************************************************

Amino_Acid1      HLCRLKIRKLLGLWRLQKLSSMKKFPLPPVLQNYILYKEYDLYGKEIHSE     590
Amino_Acid2      HLCRLKIRKLLGLWRLQKLSSMKKFPLPPVLQNYILYKEYDLYGKEIHSE     590
                 **************************************************
```

Figure (5): Alignment of the amino acid sequences of the ASB15 protein and the targeted region of this gene.

Table (1) presents the distribution of genotypes and allele frequencies of the ASB15 gene in broiler chickens. The results revealed clear genetic variation at the two mutation sites, 287 A>G and 374 T>C. At the first locus, the AA genotype was the most predominant, with a high frequency of the A allele, indicating its dominance in the studied samples, while the AG and GG genotypes were observed at lower frequencies, respectively.

At the second locus (374 T>C), the TT genotype was also the most frequent, with a high dominance of the T allele. The elevated Chi-square values indicate a deviation from Hardy–Weinberg equilibrium, which may be attributed to factors such as sample size, artificial selection, or environmental pressures. The ASB15 gene is considered one of the genes associated with the regulation of skeletal muscle growth, as it belongs to the ankyrin repeat and SOCS box protein family, which plays a significant role in muscle cell differentiation as well as protein degradation processes. Previous studies have shown that this gene is primarily expressed in muscle tissues and is strongly associated with muscle growth, increased muscle mass, and improved growth traits in poultry, making it an important candidate for genetic selection programs (McDaneld et al., 2006; Li et al., 2017).

Furthermore, genetic polymorphisms in this gene may influence gene expression levels, which can be reflected in productive performance traits such as growth rate and feed conversion efficiency (Zhou et al., 2019). Based on these findings, variations in allele and genotype frequencies of the ASB15 gene in broiler chickens may play an important role in explaining phenotypic variation in growth traits, highlighting its importance as a molecular marker for improving commercial poultry breeds.

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Table (1): Genotype and allele frequencies resulting from the mutations 287 A>G and 374 T>C in the ASB15 gene.

Chi Square	Allele frequency	Allele	Polymorphism frequency	Polymorphism		SNP	GENE
32.76 *	0.70	A	0.66	33	AA	**287A>G**	**ABS15 Gene**
	0.30	G	0.08	4	AG		
			0.26	13	GG		
29.35 *	0.69	T	0.64	32	TT	**374 T>C**	
	0.31	C	0.10	5	TC		
			0.26	13	CC		

Table (2). Association of ASB15 Gene Genotypes with Weekly Live Body Weights of Broiler Chickens (g ± Standard Error)

Overall mean	**374 T>C**				**287 A>G**				Weekly live body weight (g ± SE)
	p	**CC**	**TC**	**TT**	**P**	**GG**	**AG**	**AA**	
50		**13**	**5**	**32**		**13**	**4**	**33**	
168.54 ± 4.18	N.S	177.14 ± 8.75	185.36 ± 10.92	162.41 ± 5.018	*	183.44 ± 9.91 a	166.00 ± 15.46 b	162.97 ± 4.43 b	Week 1
435.63 ± 8.31	N.S	452.74 ± 19.05	472.16 ± 24.86	422.96 ± 9.31	*	468.32 ± 21.75 a	439.05 ± 33.69 ab	422.33 ± 7.73 b	Week 2
942.01 ± 14.68	N.S	972.63 ± 32.57	1020.44 ± 60.48	920.44 ± 15.73	*	999.06 ± 37.66 a	955.05 ± 69.36 ab	917.95 ± 13.31 b	Week 3
1607.67 ± 23.72	N.S	1662.22 ± 55.26	1702.26 ± 97.33	1570.73 ± 24.30	*	1700.34 ± 61.18 a	1648.42 ± 117.60 ab	1566.23 ± 20.73 b	Week 4
2408.58 ± 36.38	N.S	2458.05 ± 85.27	2562.78 ± 162.83	2364.39 ± 37.09	N.S	2533.60 ± 98.51	2475.17 ± 167.88	2351.26 ± 31.70	Week 5

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Table (3) Relationship between ASB15 Gene Genotypes and Weekly Feed Conversion Efficiency in Broiler Chickens ± Standard Error

المتوسط الكلي	374 T>C				287 A>G				Weekly Weight Gain (g) ± Standard Error
	p	CC	TC	TT	P	GG	AG	AA	
50		13	5	32		13	4	33	
1.35 ± .020	N.S	1.30 ± .0480	1.24 ± .030	1.39 ± .030	N.S	1.28 ± .050	1.42 ± .090	1.37 ± .040	First Week (Day 1–7)
1.71 ± .010	N.S	1.71 ± .010	1.67 ± .040	1.71 ± .010	N.S	1.72 ± .020	1.73 ± .010	1.72 ± .010	Second Week (Day 8–14)
1.70 ± 0.01	N.S	1.69 ± .010	1.72 ± .030	1.70 ± .010	N.S	1.70 ± .020	1.71 ± 0.02	1.72 ± .010	Third Week (Day 15–21)
1.71 ± .010	N.S	1.70 ± .01	1.70 ± .01	1.72 ± .01	N.S	1.71 ± .01	1.69 ± .02	1.72 ± .01	Fourth Week (Day 22–28)
1.62 ± .020	N.S	1.69 ± 0.03	1.58 ± .050	1.60 ± .020	N.S	1.65 ± .030	1.59 ± .040	1.61 ± .030	Fifth Week (Day 29–35)

- * Significant differences at $P \leq 0.05$
- N.S. indicates no significant differences.

It is important to acknowledge that growth traits in broiler chickens are influenced not only by genetic factors but also by environmental and managerial conditions. In this study, birds were raised under controlled temperature conditions that were gradually reduced from 35°C during the first week to 24°C by the fifth week, in accordance with standard broiler management practices. Birds were fed a standard three-phase diet consisting of a starter diet (0–14 days), a grower diet (15–28 days), and a finisher diet (29–42 days), with corn and soybean meal as the main ingredients and varying levels of protein and energy across phases. These controlled conditions were applied uniformly to all birds, which minimizes the confounding effects of diet and environment on genotype-phenotype associations.

IV. Conclusion

The ASB15 gene showed clear genetic polymorphism in broiler chickens, with two SNPs (287 A>G and 374 T>C) resulting in distinct genotypes, although both mutations were identified as silent at the protein level. The predominance of specific alleles and deviation from Hardy–Weinberg equilibrium suggest possible effects of selection pressure and environmental factors on genetic structure. Overall, ASB15 represents a promising molecular marker for improving growth and production traits, with further studies needed to clarify its role in gene expression and functional performance.

V. References

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ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 410-420

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