Research Article



Association Analysis of Polymorphisms in *PLIN1* Gene with the Body Weight of Ross-308 Broiler Chickens

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Abstract | *Perilipin 1* (*PLIN1*) gene is one of the candidate gene to improve the body weight of chicken. This study aimed to determine the association between chicken *PLIN1* gene polymorphisms with body weight of Ross-308 Broiler chicken. The blood samples from one-hundred unsexed birds were used in the present study for the DNA sequencing analysis. The sequencing analysis revealed two transition mutation sites of g.1912C>T and g.2308C>T in the *PLIN1* gene of birds under study. The polymorphic informative content (PIC) in both mutation sites were classified in low category (PIC<0.30). The C allele was the common allele (±80%) in both mutation sites. The polymorphisms of *PLIN1* gene were not significantly associated with body weight. In conclusion, the polymorphisms of *Perilipin 1* gene cannot be used as a genetic indicator for body weight of chickens.

Keywords | Body weight, Genetic polymorphisms, PLIN1 gene, Chicken

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INTRODUCTION

One significant and varied aspect of agriculture worldwide is the production of poultry (Prince *et al.*, 2020; Al-Saeedi *et al.*, 2024; Salman *et al.*, 2024a,b). The growing global demand for broiler meat high in animal protein has led to the expansion of genetic improvement programs at the production level of broiler chicken flocks in order to meet human demands for high-quality meat and bridge the gap in food shortages, resulting in competition among global

production companies (AL-Jaryan et al., 2023; Ajafar et al., 2024a,b). As a result, essential breeders' major objective was to choose breeds that provide the best commercial performance (meat yield, feed conversion, and growth rate) with a tall financial return to their clients (Seyedabadi et al., 2010; Al-Jebory et al., 2024). In broiler progression, advancement viability is a fundamental budgetary quality that is regulated by intricate attributes (Dadousis et al., 2021). According to Zhang et al. (2015), growth is regulated by a complex process by multiple neuroendocrine mechanisms. Apply-

ing candidate gene approaches increases the likelihood of identifying traits needed for breeding progress (Chethan and Kanaka, 2018; Al-Behadili et al., 2024). Molecular markers can be used to improve productive and reproductive qualities in field animals (Faraj et al., 2023; Jaffar and Abdulkareem, 2022), as well as molecular characterization, because they disclose genetic variations at the DNA level (Dekkers, 2004). The impacts of environmental and genetic determine animal production (Tavaniello et al., 2014) to enhance the surrender level, it is essential to maximize natural conditions and improve the genetic structure of the organism through selection and crossbreeding (Cilek and Tekin, 2005). The hereditary structure of animals can be depicted employing an assortment of intermediary atomic markers agreeing to the point of examination (Buzala et al., 2015). One of these has become widely used in this field: the analysis of single nucleotide polymorphisms (SNP) as an intrinsic marker. In light of this, the application of SNPs or whole genome sifting may alter the conclusions of earlier studies concerning the evaluation of innate assortments and genetic determinants of animal breeds, thus providing a more insightful understanding of the nuclear precursor of genetic disparities (Faraj et al., 2021; Gao et al., 2023). According to Dou et al. (2019) and Ateya et al. (2023), SNP markers can also be employed to improve the accuracy of determination and, consequently, the hereditary advancement of significant financial traits.

The previously mentioned studies already demonstrated the Perilipin 1 (PLIN1) gene's influence on B.W and fat expression in livestock (Shijun et al., 2020; Kang et al., 2022; Liu et al., 2023). A diverse spectrum of eukaryotic species, including vertebrates and amebae, contain the lipid droplet coat protein perilipin (Miura et al., 2002). According to Kimmel et al. (2010), perilipins have a highly organized domain and a strong affinity for the membranes of lipid cytoplasmic droplets, which are intracellular lipid storage organelles. The chicken (Gallus gallus) chromosome 10 contains the PLIN1 gene, which is made up of seven exons totaling 4,555 bp (GenBank: NC_052541.1). Adipogenesis in chickens depends on the PLIN1 gene (Sun et al., 2019; Sun et al., 2020; Zhai et al., 2022). Therefore, the current study aims to investigate the PLIN1 gene in Ross-308 Broiler chicken and its relationship with their body weight.

MATERIALS AND METHODS

ETHICAL APPROVAL

This research was approved by The Office of Science, College of Science, College of Misan, Maysan, Iraq at 16/07/2023 (Certificate No: 373).

BIRD'S MANAGEMENT AND DATA COLLECTION A total of 100 one-day-old Ross-308 broiler chickens (unsexed) were used in the present study. The experimental birds were brooded together in the brooding unit (deep litter system) under the same environmental conditions as one group for 6 days for adaptation using 100 W electric bulb, 30 °C brooding temperature and 14 h of light per day and the feeding trial lasted for 42 days. Good ventilation and fresh air were provided to reduce ammonia concentration in the house. Chickens were fed a concentrate diet formulated according to NRC (1994) and clean fresh water was available all times. Vitamin E and selenium, AD3E, prophylactic antibiotics and anti-coccidian drugs were also provided. Body weight for each chicken was recorded at the end of the experiment using digital balance (Sartorius).

DNA EXTRACTION

Blood samples were collected from wing vein of each chicken into tubes containing an anticoagulant disodium EDTA for DNA extraction. DNA was isolated from whole blood using a genomic DNA extraction kit (Thermo Scientific, Lithuania) following the manufacturer's instructions.

PCR OF PLIN1 GENE

Polymerase chain reaction (PCR) was used to amplify a fragment of the chicken PLIN1 gene (GenBank: NM_001127439) with an expected amplicon size of 806 bp belonging to exon 4 to exon 6 (Figure 1). The primer pairs in this study were designed by Primer3plus program (https://www.primer3plus.com) as follows: forward: 5'- GAC CAC AGC AAG GTA CAC GA -3'; reverse: 5'- TCA CCA CTA TCT CCA TTT TTC CCT-3'. The 50-μL PCR amplification volume contained: 3 μl genomic DNA as a template, 0.5 µl each of forward and reverse primer, 25 µl PCR master mix, and 21 µl d. d. water. The cycling technique includes an initial denaturation at 95°C for 3 minutes, 30 cycles of 95°C for 1 minute, annealing at 58°C for 1 minute, and an initial denaturation at 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes (Al-Saad and Al-Zaalan, 2019).



Figure 1: The primer position (underline) and target sequence of chicken *PLIN1* gene (GenBank: NC_052541.1) A fragment 806 bp length with the coding region (grey shading) of exon 5 (1628th - 1749th nucleotide), exon 6 (1961th - 2190th nucleotide) and exon 7 (2264th - 2307th nucleotide).

DETECTION OF POLYMORPHISMS

For DNA sequencing, PCR products containing target regions were processed using the chain-termination method (Sanger *et al.*, 1977). These items were sequenced in directions and switch directions using an ABI 3730XL DNA (Connected Biosystems, USA). Using Chromas 1.45 and Impact 2.0, DNA sequencing data were examined (Altschul *et al.*, 1990). On the basis of a comparison between the PCR items of the *PLIN1* gene and the reference grouping available in GenBank, contrasts were categorized as SNPs, as shown in Table 1.

DATA ANALYSIS

Genotype frequency, allele frequency, observed heterozygosity ($H_{\rm o}$), expected heterozygosity ($H_{\rm e}$), polymorphic informative content (PIC), number of effective allele ($n_{\rm e}$) and Chi-square (χ^2) values were estimated following to Falconer and Mackay (1996) and Nei and Kumar (2000). Therefore, the association analysis was performed with general linear model (GLM) method using SAS 9.2 (SAS Founded, Inc.). The mathematical model for association analysis as follows:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where;

 Y_{ij} is the observed trait; μ is the common mean; G_i is the effect of i^{th} genotype; e_{ij} is the experimental error.

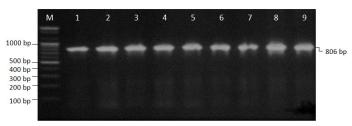


Figure 2: The amplification of *PLIN1* gene (806 bp) in Ross-308 Broiler chicken on 1% agarose gel. **M:** DNA ladder 100 bp; **Line 1-9:** Amplified PCR samples.

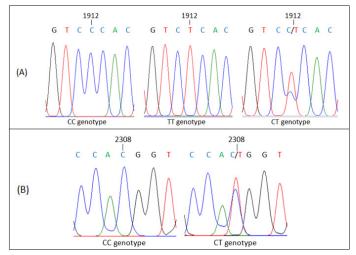


Figure 3: Two novel SNPs in the *PLIN1* gene of Ross-308 Broiler chicken i.e. g.1912C>T (A) and g.2308C>T (B).

Table 1: Genetic diversity in the two SNPs in the *PLIN1* gene of Ross-308 Broiler chicken.

	SNP	Genotype frequency (N)				н, н	H _e	I _e PIC	n _e	χ^2	
		CC	CT	TT	C	T					
	g.1912C >T	0.73 (73)			0.85	0.15	0.23	0.26	0.23	1.35	0.34*
,		0.77 (77)	(23)	(0)		0.12	0.23	0.20	0.18	1.26	0.44*

N: number of birds; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic informative content; n_e : number of effective allele; χ^2 : Chi-square. *under in genetic equilibrium.

RESULTS AND DISCUSSION

A fragment of 806 bp for chicken PLIN1 gene was successfully amplified as shown in Figure 2. The sequencing analysis revealed that two transition mutation of transition mutation sites (C>T) were detected in the 1912th and 2308th nucleotide in PLIN1 gene of birds under study (Figure 3). The mutation of g.1912C>T revealed three genotypes of CC (0.73), CT (0.23) and TT (0.04). While, a mutation site of g.2308C>T revealed two genotype of CC (0.77) and CT (0.23). The CC genotype (±70%) and C allele (±80%) were detected in the most birds under investigation. The polymorphic informative content (PIC) value in both mutation sites were in low category (PIC<0.30). According to the number of effective allele (n) value, both mutation sites have a common allele of C (n < 1.50). However, the evidence of mutation sites of PLIN1 gene was in genetic equilibrium (χ^2 <5.99). The polymorphisms of *PLIN1* gene were not significantly associated with body weight (Table 2). Nonetheless, the body weight in heterozygous birds was higher than homozygous ones. Furthermore, the body weight of birds with CT/CT haplotype was higher than other haplotype as shown in Table 3.

Table 2: Association of SNPs in the *PLIN1* gene with body weight of Ross-308 Broiler chicken.

SNP	Genotype	N	Body weight (g)
	CC	73	1951.10±149.09
g.1912C>T	TT	4	1770.00±137.42
	CT	23	1962.20±140.80
g.2308C>T	CC	77	1942.00±150.66
	CT	23	1962.20±140.80

N: number of birds.

Improved body weight is a common goal in the chicken breeding programs (Tarsan *et al.*, 2019). It takes a lot of work to produce chickens with a lower slaughter age, high meat production and growth, and superior feed efficiency. Intense selection on the growth speed issue will lead to an increase in growth and body fat deposition features (Pe-

tracci and Cavani, 2012). Excessive body fat formation can affect the carcass's performance as well as the meat's quality (Zhao et al., 2020). Numerous quantitative trait loci (QTL) studies have shown that different animals growth and carcass traits can be influenced (Clark, 2004; Zhang et al., 2015; Seger et al., 2023). Genetic selection of individuals is more precise and has a long-lasting effect on the progeny (Lande and Thompson, 1990), and breeding programs can be run on a molecular level (Sun and Wang, 2006).

Table 3: Effect of haplotype in the two mutation sites in the *PLIN1* gene with body weight of Ross-308 Broiler chicken.

Haplotype*	N	Body weight (kg)
CC/CC	73	1951.05±149.09
CT/CT	23	1962.17±140.80
TT/CC	4	1770.00±137.42

N: number of birds; *g.1912C>T / g.2308C>T.

Two mutation sites in the *PLIN1* gene of the Ross-308 Broiler chicken (g.1912C>T and g.2308C>T) were polymorphic with the moderate PIC value (0.10 – 0.30). However, the PIC value in the g.1912C>T was higher than g.2308C>T (0.23 vs 0.18). According to Nei and Kumar (2000), the PIC value of less than 0.10 and more than 0.30 were classifies as low or high, respectively. In addition, two mutation sites under study were under genetic equilibrium and indicated that no previous selection affecting the genetic diversity in both mutation sites. Genetic equilibrium can be disrupted by migration, crossbreeding, inbreeding, and selection (Falconer and Mackay, 1996).

Interestingly, the g.2308C>T in the *PLIN1* gene of chicken under study was located at exon 7 region but this mutation is detected as the nonsense or synonymous mutation (H284H). It's critical to stress that, when compared to the relevant datasets obtained from GenBank, the polymorphisms discovered and made available in this context offer new information for the examined *PLIN1* indicator. The PLIN1 gene polymorphisms and body weight were not substantially correlated. No significance association between PLIN1 gene polymorphisms with body weight in birds under study can be caused by genetical factor and less number of population size for investigation. Ross-308 Broiler chicken is a commercial strain that produced from line-breeding. Hence, the Ross-308 Broiler chickens had the standard performance with less variation. In this study, the genetic diversity in the PLIN1 gene was moderate and it's not significantly associated with body weight of birds under study. It can be concluded that several candidate genes may be caused the growth of chicken.

To the best of our knowledge, little is known about the relationship between the *PLIN1* gene and chicken growth features. Zhang et al. (2015) obtained three mutation sites

in the *PLIN1* gene (GenBank: NC_052541.1) of Chinese meat-type chickens *i.e.* g.243T>C (5'UTR); c.253A>G (exon 1) and c.268C>T (exon 1). Chinese meat-type chicken carcass features were highly correlated with the g.243T>C mutation. The entire set of genes in the genome that are consistently and effectively expressed under various physiological situations is referred to as the "transcriptome" (Kukurba and Montgomery, 2015). Kang *et al.* (2022) discovered the *PLIN1* gene expression abundances in the fat of abdominal and liver cells of various chicken breeds. Age, breed, and tissue all had an impact on how the *PLIN1* gene was expressed. The results showed that breed-specific variations in adipocyte formation and adipose deposition are caused by genetic variables.

Previous studies on *PLIN1* gene polymorphisms in chickens used PCR-RFLP, which has lower resolution than the direct sequencing method employed here (Zhang et al., 2015; Zhou et al., 2020). Uncovered DNA grouping SNP hereditary markers revolutionize past accomplishments in preservation choices, biodiversity evaluation, and hereditary characterization of breeds, driving to a more profound information of the genomic premise of utilitarian differences (Dhorne-Pollet et al., 2020). Also, compared to other markers, DNA grouping SNP ponder may be able to clarify the history of European cattle with more noteworthy precision (Socol et al., 2015). SNPs are too well-known to be of much use when searching for connections between a marker at a known genomic region and an unknown gene locus. It is important to look for these linkages because a phenotypic impact can be evaluated by determining its hereditary establishment (Svensson et al., 2007). Additionally, we looked for genetic variants in PLIN1 exons. Exons of studied genes, which are a conserved portion of the genes, can be sequenced using PCR-DNA technology to accurately characterize the genes and determine the physiological variations in breed-specific components (Marwal and Gaur, 2020).

It is vital to notice that, *PLIN1* gene polymorphisms in ducks were identified using PCR-RFLP analysis (Susanti, 2021). In this study, PCR-RFLP investigation of local ducks explain a polymorphism in exon 5 of the *LPL* gene and a monomorphism in exon 2 of the *PLIN* gene. *PLIN1* was also shown by Li *et al.* (2018) to be a crucial gene that might influence the amount of intramuscular fat in pigs based on transcriptome analysis.

CONCLUSIONS AND RECOMMENDATIONS

Two mutation sites in the chicken *PLIN1* gene (g.1912C>T and g.2308C>T) were found in the studied Ross-308 broiler chicken by PCR-DNA sequencing analysis. How-



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ever, both mutation sites had the moderate genetic diversity. The polymorphisms of the PLIN1 gene in Ross-308 Broiler chickens were not significantly to body weight, indicating that they cannot be used as a genetic marker for chicken body weight. In-depth study to determine the genetic marker from each gene region of PLIN1 gene is important for developing molecular selection in Ross-308 Broiler chicken. Furthermore, a genome-wide association study (GWAS) can be performed to detect the potential candidate genes for the growth traits of Ross-308 Broiler chickens accurately.

Detecting the mutation sites in each region of chicken PLIN1 gene is important to manage in the future. Despite this, using large number of birds for investigation is important to confirm the genetic marker with significance statistical value.

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NOVELTY STATEMENT

The novelty in this study is the analysis of Polymorphisms in PLIN1 gene with the body weight of Ross-308 broiler chickens.

AUTHORS'S CONTRIBUTIONS

Ali A. Abdulkareem: Contributed to the data collection, data analysis and manuscript preparation.

Salah H. Faraj and Mohammed Sabeeh Majeed: Had the role of designing and generating the concept, supervising, monitoring and controlling the research, data analysis and interpretation, and manuscript preparation.

Widya P. B. Putra and Ratna S. Harahap: Contributed to supervising the research.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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