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Genetic polymorphisms in the promoter region of the ACACB gene and their effects on egg quality traits in White Leghorn layer chickens

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Abstract

The present study investigated genetic polymorphisms in the promoter region of the *ACACB* gene and their effects on egg quality traits in IWI and IWK lines of White Leghorn layer chickens. The promoter region exhibited a high degree of polymorphism. Egg weight did not vary significantly within lines across most ages, except for the h1h2 haplogroup in the IWI line at 72 weeks of age, which was associated with increased egg weight. Analysis of haplogroup associations with shell weight revealed no significant effects in the IWI line; however, in the IWK line, haplogroups h6h6 and h9h9 were associated with higher shell weight. Additionally, haplogroups h9h9 and h9h10 showed positive associations with increased shell weight in the IWK line. Overall, the results indicate that polymorphisms in the promoter region of the *ACACB* gene are highly prevalent and exert significant effects on egg quality traits in White Leghorn layer chickens.

Keywords: C *ACACB* gene, promoter, SSCP, polymorphism, egg quality

1. Introduction

The poultry sector is one of the fastest-growing livestock industries worldwide. Chicken eggs and meat are affordable sources of protein for humans. Traditional selection methods have been less effective, especially when improving multiple traits with negative genetic relationships. Using genetic markers can improve selection efficiency, increasing growth rate by up to 15% compared to family-based selection^[1]. Molecular markers exhibit distinct genetic characteristics and methodological advantages, making them highly suitable for genetic analysis compared to conventional markers. Various molecular marker systems, including restriction fragment length polymorphism (RFLP), microsatellites, and single-strand conformation polymorphism (SSCP), have been extensively employed for the analysis of complex traits^[2, 3, 4, 5].

Polymerase chain reaction–single strand conformation polymorphism (PCR–SSCP) is a rapid, sensitive, and reproducible technique for detecting genetic variations, including insertions, deletions, and single nucleotide substitutions^[6]. Originally developed by^[7], this method has been widely utilized for mutation screening and DNA polymorphism analysis. SSCP enables the detection of single-base sequence variations based on altered electrophoretic mobility of single-stranded DNA fragments under non-denaturing polyacrylamide gel conditions, resulting from conformational changes in the DNA strands^[8]. High lipid content in poultry meat has been identified as a potential risk factor for human health^[9]. Moreover, excessive adiposity in laying hens adversely impacts reproductive efficiency and overall production performance^[10]. The genetic regulation of egg production and quality particularly traits associated with lipid metabolism and fatty acid composition has been increasingly elucidated through genome-wide association studies (GWAS) in chickens. Fatty acid catabolism is primarily mediated through the mitochondrial β -oxidation pathway, a central metabolic process governing energy homeostasis. The *Acetyl-CoA Carboxylase Beta* (*ACACB*; also known as *ACC- β* or *ACC2*)

gene encodes a critical regulatory enzyme in this pathway, modulating the balance between fatty acid synthesis and oxidation. Given its pivotal role in lipid metabolism, the chicken *ACACB* gene represents a strong candidate for influencing egg quality traits, particularly those related to lipid composition and nutritional value. *ACACB* catalyzes the formation of malonyl-CoA, a key metabolic intermediate that acts as a potent inhibitor of carnitine palmitoyltransferase 1 (CPT1), thereby regulating the transport of long-chain fatty acyl groups into the mitochondrial matrix via the carnitine shuttle system for β -oxidation^[11, 12, 13]. Elevated malonyl-CoA levels consequently suppress mitochondrial fatty acid oxidation, promote a metabolic shift toward increased glucose utilization, and enhance de novo lipogenesis, resulting in augmented fatty acid and triglyceride (TG) synthesis. Characterization of genetic variation within the chicken *ACACB* gene may facilitate the identification of functionally relevant variants associated with egg quality traits. In particular, polymorphisms within regulatory regions, such as the promoter, are likely to influence gene expression and downstream metabolic phenotypes. Therefore, elucidating such variations could provide valuable insights for marker-assisted selection and the genetic improvement of egg quality. In this context, the present study was undertaken to identify polymorphisms in the promoter region of the *ACACB* gene and to evaluate their association with egg quality traits in IWI and IWK lines of White Leghorn laying chickens.

2. Materials and Methods

2.1 Experimental birds

The present study was conducted on a total of 500 birds comprising 250 individuals each from the IWI and IWK lines of the White Leghorn breed. Both lines are selected and improved egg-type populations, with average annual egg production records of 298 and 276 eggs, respectively (DPR Annual Report 2018–19). The IWI and IWK lines are maintained under controlled breeding and management conditions at the ICAR–Directorate of Poultry Research (ICAR–DPR), Hyderabad, India.

2.2 Management

At 16 weeks of age, birds were transferred to individual laying cages to facilitate precise recording of individual performance, and were subsequently fed a layer ration containing 2,600 kcal/kg ME and 16% CP from the onset of egg production until completion of the experiment. A 16-hour lighting regimen (including natural daylight) was maintained throughout the laying period. All birds were managed under uniform husbandry conditions with ad libitum access to clean drinking water. On the day of hatch, chicks were vaccinated against Marek's disease and were subsequently immunized as per standard vaccination schedule against major poultry diseases, including Newcastle disease (Ranikhet disease), infectious bursal disease, and fowl pox. During summer months, thermal comfort was maintained through roof water sprinkling-based cooling systems, and appropriate artificial lighting was provided within the sheds. Standard biosecurity and management protocols were strictly followed to ensure optimal health status and to support expression of the birds' genetic production potential.

2.3 Sample collection

Blood samples were collected from the brachial (wing) vein, and genomic DNA was extracted from whole blood using a standard phenol–chloroform-based protocol¹⁴. Eggs were

collected on a daily basis throughout the experimental period for subsequent assessment of egg quality traits.

2.4 Estimation

Egg weight at 28th week, 40th week, 52nd week, 64th week and 72nd week, were measured in the electronic weighing balance on three consecutive days for each bird. The egg shell weights of all eggs were also measured.

2.5 PCR

The 5' upstream region of the transcription start site of the *ACACB* gene, spanning 534 bp, was divided into two non-overlapping fragments: *ACACB*-S1 (301 bp) and *ACACB*-S2 (233 bp). Primers for amplifying these fragments were designed based on the chicken *ACACB* gene sequence (Accession No. NC_006102). PCR amplification was performed in 0.2 ml tubes using a reaction mixture containing 1 μ l of 10 $\times$ DreamTaq buffer, 0.5 μ l of 2.5 mM dNTP mix, 1 μ l each of forward and reverse primers (20 ng/ μ l), 0.2 μ l of Taq DNA polymerase (5 U/ μ l), approximately 100 ng of genomic DNA as the template, and nuclease-free water to a final volume of 10 μ l. The thermal cycling conditions included an initial denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 64°C for the S1 fragment and 66°C for the S2 fragment for 30 seconds, and extension at 72°C for 30 seconds. A final extension step was carried out at 72°C for 10 minutes.

2.5.1 Single strand conformation polymorphism (SSCP)

The PCR amplicons of both fragments were first denatured at 95°C for 5 minutes and then immediately snap-cooled on ice for 15 minutes. The samples were subsequently resolved on a 12% polyacrylamide gel prepared using 9.6 ml acrylamide: bisacrylamide (49:1), 28.3 ml of 1 $\times$ Tris-borate-EDTA (TBE) buffer, 1.5 ml glycerol, 100 μ l of 10% ammonium persulfate (APS), and 75 μ l tetramethyl ethylenediamine (TEMED) for variant detection. The denatured products were loaded onto the gel and electrophoresis was carried out at 4°C for 12 hours at a constant voltage of 200 V. Following electrophoresis, the gel was stained using silver nitrate to visualize the banding patterns, as described by^[15], for genotype determination. Different banding patterns corresponding to varying fragment sizes were scored as distinct genotypes.

2.5.2 Haplotypes

Each SSCP variant observed in the *ACACB*-S1 and *ACACB*-S2 fragments was recorded. The *ACACB*-S1 fragment exhibited three distinct banding patterns, while the *ACACB*-S2 fragment displayed six different patterns. The combination of patterns from both fragments in each individual bird was used to infer its haplotype. A total of 12 haplotypes, designated h1 to h12, were identified based on these pattern combinations. The frequencies of haplotypes and haplogroups were estimated using the direct counting method^[16]. In diploid individuals, the pair of haplotypes together constitutes the haplogroup.

2.6 Statistical analysis

Allelic and genotypic frequencies for each fragment were estimated using POPGENE software^[17]. The association between traits across the two lines was analyzed using a univariate General Linear Model mkk implemented in the Statistical Package for the Social Sciences (SPSS) version 17.0. Haplogroups represented by fewer than three individuals

were excluded from the association analysis.

3. Results

3.1 Polymorphism in ACACB gene promoter

The ACACB-S1 fragment (301 bp) exhibited six distinct banding patterns in both the IWI and IWK lines, while the ACACB-S2 fragment (233 bp) showed three banding patterns in each population. By combining the patterns from these two fragments, a total of twelve haplotypes (h1–h12) and eighteen haplogroups (h1h1, h1h2, h2h2, h3h3, h3h4, h4h4, h5h5, h5h6, h6h6, h7h7, h7h8, h8h8, h9h9, h9h10, h10h10, h11h11, h11h12, and h12h12) were identified.

3.2 Association of haplogroups with egg weight

Both IWI and IWK lines didn't show significant association with any of the haplogroups observed except for IWI line at 72nd week. At 72nd week, the haplogroup h1h2 showed significant association ($P < 0.05$) with egg weight in IWI line (Table 6). Certain haplogroups revealed significant association with egg weight between IWI and IWK lines at different age groups (Table 6). The h1h1 haplogroups showed higher egg weight at 28th, 40th and 72nd weeks. The haplogroups, h2h2 and h10h10 showed highest egg weight at 52nd week. The haplogroup, h9h9 showed highest egg weights at 64th and 72nd weeks (Table 1).

3.3 Association of haplogroups with shell weight

In general, the IWK line showed higher shell weight

compared to IWI line. This difference is significant ($P < 0.05$) for the haplogroup h9h9 and h9h10. Within IWI line, there was non-significant association for shell weight. But, haplogroup, h6h6 showed higher shell weight in IWK line (Table 2).

4. Discussion

The ACACB gene has been reported to be associated with conditions such as nephropathy, obesity, diabetes, and end-stage renal disease [18, 19, 20]. Polymorphisms in the promoter region can influence gene function, thereby contributing to variations in its association with economically important traits in chickens [21, 22]. For the present polymorphism study, two genetic lines of White Leghorn layer chickens, IWI and IWK, were selected. The IWI line has been developed primarily for egg production, whereas the IWK line has been selected for both egg production and egg weight. Both fragments examined in the present study exhibited polymorphism as detected by SSCP analysis. The 301 bp fragment showed greater polymorphic variation compared to the 233 bp fragment. Combined analysis of the two fragments led to the identification of 12 haplotypes, which further grouped into 18 haplogroups across the populations studied. Association analysis the egg weight in general is higher in IWK line, when compared to IWI line which would be due to the fact that the line is selected for higher egg weight in addition to production. The haplogroups h1h1, h2h2, h6h6, h9h9, h10h10 and h11h11

Table 1: Association of Haplogroups with egg weight at different age in IWI and IWK lines

Haplo Haplo group	Egg weight (g)									
	28 th week		40 th week		52 nd week		64 th week		72 nd week	
	IWI	IWK	IWI	IWK	IWI	IWK	IWI	IWK	IWI	IWK
h1h1	47.60±2.11 ^x	53.37±0.81 ^y	48.93±1.11 ^x	53.57±1.31 ^y	55.35±1.72	55.33±1.29	55.85±0.80	58.35±1.61	54.45±0.85 ^{ax}	58.68±0.55 ^y
h1h2	50.53±1.60	47.87±1.35	47.80±0.53	50.20±1.02	52.13±2.08	54.96±3.09	55.72±1.25	56.37±1.34	61.20±2.98 ^c	58.68±0.59
h2h2	47.04±0.82	49.87±0.54	50.01±0.61	51.74±1.74	53.65±0.73 ^x	59.53±0.93 ^y	54.91±0.92	54.21±1.95	57.58±0.88 ^{abc}	59.36±0.89
h3h3	48.26±2.94	48.15±1.15	49.15±1.32	51.37±0.78	54.53±1.35	55.30±0.60	55.83±1.82	53.67±0.91	58.02±1.48 ^{abc}	57.60±1.60
h3h4	52.50±0.50	49.72±0.99	44.00±2.40	49.01±1.11	55.00±0.90	54.76±1.45	52.35±0.65	55.88±2.00	60.40±2.80 ^{bc}	59.16±1.26
h5h6	-	49.58±1.94	-	52.54±0.88	-	57.44±1.44	-	55.70±2.73	-	57.72±1.05
h6h6	47.25±0.96	49.80±1.64	50.70±0.94	54.08±2.43	55.87±0.88	58.17±0.40	52.41±1.66	55.98±1.58	55.50±1.46 ^{abx}	59.94±0.63 ^y
h7h7	49.04±0.89	49.00±1.20	47.95±1.45	49.00±0.70	53.50±1.24	55.63±0.75	54.58±0.89	57.13±1.25	55.61±1.48 ^{ab}	59.20±1.80
h8h8	47.80±1.90	52.70±1.77	51.10±1.40	53.50±0.50	57.50±2.70	58.03±1.13	54.85±0.15	60.20±3.29	55.10±1.10 ^a	58.50±0.60
h9h9	50.66±0.78	50.20±0.49	49.64±0.99 ^x	52.97±1.00 ^y	55.08±1.33	58.36±1.03	54.90±1.04 ^x	59.62±0.79 ^y	55.97±0.60 ^{abx}	58.99±0.75 ^y
h9h10	49.08±1.47	50.48±0.87	49.14±1.67	52.27±1.01	52.47±0.83	55.09±1.63	55.38±1.54	55.54±1.07	58.53±0.91 ^{abc}	58.46±0.75
h10h10	45.87±3.20	49.72±0.77	50.09±0.84	52.28±1.65	52.66±1.13 ^x	58.14±0.77 ^y	54.70±1.33	57.05±1.35	57.12±0.71 ^{abc}	58.67±0.63
h11h11	47.35±0.78 ^x	51.37±1.34 ^y	50.17±0.89 ^x	53.81±0.84 ^y	54.29±0.76 ^x	57.92±0.73 ^y	53.33±1.01 ^x	59.06±0.94 ^y	56.64±0.67 ^{abc}	58.72±1.27
h11h12	46.15±3.35	49.65±0.84	50.88±1.95	54.22±2.15	56.28±1.84	55.43±1.56	58.70±2.62	59.06±1.68	57.05±1.42 ^{abc}	57.91±0.80
h12h12	46.13±1.60	48.86±1.43	48.38±1.95	53.85±2.19	51.72±1.41	55.78±1.76	53.21±1.71	55.23±2.05	55.60±1.45 ^{ab}	57.03±1.88

Means with different superscripts (a-c) within each column differ significantly ($P \leq 0.05$) (Duncan's MRT)

Means with different superscripts (x, y) in each age group differ significantly ($P \leq 0.05$) (Independent sample t-test)

Table 2: Association of haplogroups with egg weight traits in IWI and IWK lines.

Haplo- group	Shell weight (g)	
	IWI	IWK
h2h2	4.33±0.71	4.94±0.27 ^{ab}
h5h6	-	4.94±0.23 ^{ab}
h6h6	5.04±1.27	5.73±0.22 ^c
h7h7	5.07±0.63	-
h9h9	4.59±0.27 ^x	5.61±0.14 ^{cy}
h9h10	4.64±0.32 ^x	5.37±0.09 ^{bcy}
h10h10	5.00±0.85	5.22±0.16 ^{abc}
h11h11	5.05±0.53	4.91±0.12 ^{ab}
h11h12	-	4.78±0.14 ^a
h12h12	-	5.36±0.11 ^{bc}

Means with different superscripts (a-c) within each column differ significantly ($P \leq 0.05$) (Duncan's MRT);

Means with different superscripts (x, y) in each row for each trait differ significantly ($P \leq 0.05$) (Independent sample t-test) are likely to be the haplogroups associated with egg weight at different ages. Within IWI line, the haplogroup h1h2 is a promising haplotype associated with higher egg weight. The egg quality is measured in terms of shell weight (g). The shell weight is not particularly associated with any of the haplotypes in IWI line, but within IWK line, the h6h6 and h9h9 haplogroups showed higher shell weight. Between the lines, the h9h9 and h9h10 haplogroups are associated with higher shell weight in IWK line compared to IWI line.

5. Conclusion

The findings of this study indicate that the promoter region of the *ACACB* gene exhibits polymorphism, with certain haplogroups showing associations with egg weight and shell weight. Further validation of the specific mutations would be valuable to clarify their exact role in egg quality traits across the two lines, which could ultimately support their application in marker-assisted selection.

6. Declaration of interests

All the authors declare that they have no competing interests. All authors read and approved the final manuscript.

Conflict of Interest

Not available

Financial Support

Not available

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