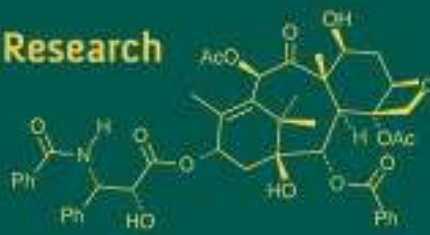
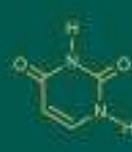
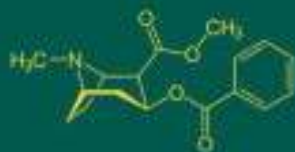


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Polymorphism in the promoter region of *ACACB* gene and its association with egg fat content in white leghorn layer chicken

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Abstract

The present study aimed to investigate polymorphisms in the promoter region of the *ACACB* gene and their association with egg fat content in IWI and IWK lines of White Leghorn layer chickens. The results revealed that the promoter region was highly polymorphic, exhibiting 12 haplotypes and 18 haplogroups across two fragments identified through SSCP analysis. Egg fat percentage was significantly higher in the IWI line. In IWI the least square means for total egg fat in h10h10 haplogroup birds was the highest (25.94 ± 0.95) and in the h11h11 haplogroup it was found to be the lowest (20.35 ± 1.12) total egg fat. In case of IWK, the h6h6 haplogroup birds were the highest (24.24 ± 1.01) and in the h2h2 haplogroup it was found to be the lowest (22.06 ± 0.70) total egg fat. Overall, the findings indicate that the promoter region of the *ACACB* gene is highly polymorphic and significantly influences egg fat content in White Leghorn layer chickens.

Keywords: *ACACB* gene, promoter, SSCP, polymorphism, egg fat percentage

Introduction

Chicken eggs and meat are relatively inexpensive and accessible sources of protein for human consumption. However, traditional within-population selection has been less effective, particularly when multiple traits with unfavorable genetic correlations are involved. The use of genetic markers has been shown to enhance selection efficiency, potentially increasing the genetic gain for growth rate in chicken populations by up to 15% compared to conventional family-based selection (Pongpisantham, 1994) [14]. Molecular markers exhibit distinct genetic characteristics and methodological advantages, making them more effective and suitable for genetic analysis than traditional markers. Various types of molecular markers, including RFLP, microsatellites, and single-strand conformation polymorphism (SSCP), have been widely used in the past to study complex genetic traits (Badola *et al.*, 2004; Bhattacharya *et al.*, 2011; Bhattacharya *et al.*, 2015b; Bhattacharya *et al.*, 2016) [2, 6, 5, 7]. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), developed by Orita *et al.* (1989) [13], is a rapid, simple, and reproducible technique widely used for detecting DNA mutations, including deletions, insertions, and substitutions (Kakavas *et al.*, 2008) [10]. SSCP identifies single-base sequence variations by detecting differences in the electrophoretic mobility of one or both single-stranded DNA fragments in a non-denaturing polyacrylamide gel (Gruszczynska *et al.*, 2005) [9]. Early applications of the technique relied on radioactive labeling, which restricted its broader use. To improve simplicity and efficiency, alternative staining methods such as ethidium bromide (Yap and McGee, 1992) [22] and silver staining (Ainsworth *et al.*, 1991) [1] were later introduced. High fat content in chicken meat poses potential health risks to humans (Milićević *et al.*, 2014) [12]. In laying hens, excessive fat deposition can adversely affect reproductive performance (Xing *et al.*, 2009) [21]. The genetic regulation of egg production and quality, particularly in relation to fat metabolism and fat composition, has been elucidated through genome-wide association studies in chickens. Fatty acid degradation primarily occurs through the mitochondrial β -oxidation pathway. The Acetyl-CoA Carboxylase Beta (*ACACB*, also known as *ACC- β* or *ACC2*) gene plays a crucial role in

regulating this pathway. As the *ACACB* gene is involved in fatty acid metabolism in chickens, it may influence egg quality. The enzyme encoded by *ACACB* produces malonyl-CoA, which inhibits carnitine/palmitoyl-transferase 1 (CPT1) and restricts the transport of fatty acyl groups into the mitochondria via the carnitine shuttle system for β -oxidation (McGarry *et al.*, 1977; Ruderman *et al.*, 1999; Wakil and Abu-Elheiga, 2009) [11, 16, 20]. Consequently, this results in increased glucose utilization, decreased fatty acid oxidation, and enhanced synthesis of fatty acids and triglycerides (TG). Understanding genetic variation in the chicken *ACACB* gene may aid in identifying variants associated with egg fat content. In particular, characterization of variation in the regulatory region of this gene could support future breeding strategies aimed at improving egg quality in chickens. Therefore, the present study was undertaken to detect polymorphisms in the promoter region of the *ACACB* gene and to evaluate their association with egg fat content in IWI and IWK lines of White Leghorn layer chickens.

Materials and Methods

Experimental birds

The present study was conducted on 500 birds, belonging to IWI (250 birds) and IWK (250 birds) lines of the White Leghorn breed. The IWI and IWK lines are improved egg producing lines laying 298 and 276 eggs annually (DPR Annual Report 2018-19). Both the lines are maintained at the ICAR-Directorate of Poultry Research (ICAR-DPR), Hyderabad, India.

Management

Day-old chicks from both lines were vent-sexed, wing-banded, and reared in an open-sided house on deep litter until 16 weeks of age. They were fed a layer chick starter diet containing 2,600 kcal/kg metabolizable energy (ME) and 18% crude protein (CP) up to 8 weeks, followed by a grower diet with 2,500 kcal/kg ME and 16% CP from 9 to 16 weeks. After 16 weeks, the birds were transferred to individual cages to enable recording of individual production. From the onset of egg production until the end of the experiment, they were provided a layer diet containing 2,600 kcal/kg ME and 16% CP. A photoperiod of 16 hours, including natural daylight, was maintained during the laying period. All birds were maintained under uniform management conditions with *ad libitum* access to water. On the day of hatch, chicks were vaccinated against Marek's disease, and subsequently immunized against major diseases such as Ranikhet disease, Infectious Bursal Disease, and fowl pox following a standard vaccination schedule. During the summer season, cooling was provided by sprinkling water on the roof, and adequate lighting arrangements were ensured in the shed. Additionally, all routine management practices, including strict bio-security measures, were implemented in and around the sheds to provide a conducive environment for optimal performance of the birds.

Sample collection

Blood samples were collected from the wing vein, and genomic DNA was extracted following the standard protocol described by Sambrook and Russell (2001) [17]. Eggs were collected daily to record egg fat content.

Estimation

Whole eggs were dried at 60°C temperature at the BOD incubator. The total fat (%) of an egg was estimated using Soxhlet extraction method following standard protocol.

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 were designed based on the chicken *ACACB* gene sequence (Accession No. NC_006102) to amplify these fragments (Table 1). Polymerase chain reaction (PCR) amplification was performed in 0.2 ml PCR tubes with 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 template, and nuclease-free water to make up a final volume of 10 μ l. The PCR 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, with a final extension at 72 °C for 10 minutes.

Single strand conformation polymorphism (SSCP)

The amplicons of both fragments were denatured at 95 °C for 5 minutes, followed by rapid cooling on ice for 15 minutes. The samples were then separated on a 12% polyacrylamide gel prepared using 9.6 ml Acrylamide: Bisacrylamide (49:1), 28.3 ml 1 $\times$ Tris-borate-EDTA (TBE) buffer, 1.5 ml glycerol, 100 μ l of 10% ammonium persulfate (APS), and 75 μ l tetramethylethylenediamine (TEMED) to detect genetic variants. The PCR products were loaded onto the gel, and electrophoresis was carried out at 200 V for 12 hours. Following electrophoresis, the gel was stained with silver nitrate, as described by Vohra *et al.* (2006) [19], to visualize the banding patterns and determine genotypes. Different fragment size combinations were scored as distinct genotypes.

Haplotypes

The SSCP variants observed in the ACACB-S1 and ACACB-S2 fragments were recorded for each individual. The ACACB-S1 fragment exhibited three distinct patterns, while the ACACB-S2 fragment showed six different patterns. The combination of patterns from both fragments in each bird was used to infer its haplotype. In total, 12 haplotypes (designated h1 to h12) were identified based on these combinations. The frequencies of haplotypes and haplogroups were estimated using the direct counting method (Bhattacharya *et al.*, 2013). In diploid individuals, the pair of haplotypes constituted the haplogroup.

Statistical analysis

Allelic and genotypic frequencies for each fragment were estimated using the POPGENE software (Yeh *et al.*, 1997) [23]. A Univariate General Linear Model was applied to assess the association between traits across the two lines using the Statistical Package for Social Sciences (SPSS) version 17.0. Haplogroups with fewer than three observations were excluded from the analysis.

Results

Polymorphism in ACACB gene promoter

The ACACB-S1 (301 bp) fragment exhibited six distinct banding patterns in both IWI and IWK lines, while the ACACB-S2 (233 bp) fragment showed three different patterns in both populations. The combination of patterns from these two fragments resulted in the identification of twelve haplotypes (h1 to h12) and eighteen haplogroups, namely h1h1, h1h2, h2h2, h3h3, h3h4, h4h4, h5h5, h5h6, h6h6, h7h7, h7h8, h8h8, h9h9, h9h10, h10h10, h11h11, h11h12, and h12h12.

Association of haplogroups with total egg fat content

In IWI lines within group there is a significant ($p \leq 0.05$) effect on total egg fat. Whereas In IWK lines within group there is a non-significant ($p \leq 0.05$) effect on total egg fat was observed. The haplogroups of ACACB gene promoter had non-significant ($p \leq 0.05$) effect on total egg fat between the group of IWI and IWK lines with the exception of h10h10 and h11h11. In general h11h11 haplogroup birds was showed lowest total egg fat and h6h6 haplogroup birds was showed highest total egg fat. In IWI and IWK lines the haplogroups were associated with the total egg fat (%), which was the most important indicators depicting the egg quality. While performing the association, the haplogroups which had the less number were excluded. In IWI the least square means for total egg fat in h10h10 haplogroup birds was the highest (25.94 ± 0.95) and in the h11h11 haplogroup it was found to be the lowest (20.35 ± 1.12) total egg fat. In case of IWK, the h6h6 haplogroup birds were the highest (24.24 ± 1.01) and in the h2h2 haplogroup it was found to be the lowest (22.06 ± 0.70) total egg fat (Table 2)

Discussion

The ACACB gene has been reported to be associated with conditions such as nephropathy, obesity, diabetes, and end-stage renal disease (Tang *et al.*, 2010; Riancho *et al.*, 2011; Zain *et al.*, 2017) [18, 15, 24]. Polymorphisms in the promoter region may influence gene function, thereby affecting its association with various traits in chickens (Cui *et al.*, 2006; Zhu and Jiang, 2014) [8, 25]. For the present polymorphism analysis, two genetic lines of White Leghorn layer chickens, IWI and IWK, were selected. The IWI line has been developed for egg production, whereas the IWK line has been selected for both egg production and egg weight. Both the fragments in the present study revealed polymorphism based on SSCP analysis. The 301 bp fragment revealed higher polymorphism compared to 233 bp fragment. Based on analysis of both the fragments, 12 haplotypes were identified. These 12 haplotypes formed 18 haplogroups in both the populations studied. Polymorphism in the promoter region of genes in chicken is common phenomenon for several genes. Promoter polymorphism is also observed in matrix metalloproteinase 9 gene (Zhu and Jiang 2014) [25], prolactin gene (Cui *et al.*, 2006; Bhattacharya *et al.*, 2015a) [8, 4] etc. in chicken. Generally the variation is associated with difference in function. The total egg fat% is significantly higher within IWI line, when they have h10h10 haplogroup. The same haplogroup also showed higher total fat in IWI line compared to IWK line. But the h11h11 haplogroup is associated with higher total fat% in IWK line. Since ACACB gene plays a key role in fatty acid synthesis and fatty acid oxidation, it would be interesting how this

particular haplogroup is influencing the total fat% in IWI line.

Table 1: Details of primers used for amplification of ACACB gene promoter.

Primer name	Primer Sequences (5'-3')	Amplicon Size (bp)
ACACB-S1	F: CAGTCCTGCTTTGCTTCCGTG R: TTGTGGGACAAAGGTGCCATG	301
ACACB-S2	F: AGGTGAGCTCCATGGCACTG R: GACAGCCAGACCTCTCATAT	233

Table 2: Association of haplogroups with egg fat content in IWI and IWK lines.

Haplogroups	Total egg fat content	
	IWI	IWK
h2h2	22.00 ± 1.32^{ab}	22.06 ± 0.70
h5h6	-	22.82 ± 0.89
h6h6	24.09 ± 0.96^{ab}	24.24 ± 1.01
h7h7	21.20 ± 0.54	-
h9h9	22.79 ± 2.38^{ab}	24.20 ± 0.69
h9h10	21.92 ± 1.12^{ab}	22.07 ± 1.04
h10h10	25.94 ± 0.95^{bx}	22.67 ± 0.53^y
h11h11	20.35 ± 1.12^{ax}	23.87 ± 0.64^y
h11h12	-	22.37 ± 0.78
h12h12	-	23.70 ± 1.48

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)

Conclusion

From the results obtained in this study, it can be concluded that the promoter region of ACACB gene is polymorphic and certain haplogroups are associated with egg fat percentage. It would be interesting to further validate the specific mutations to understand their precise role in the egg fat percentage in the two lines, which would help in marker assisted selection.

Declaration of interests

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

References

- Ainsworth P, Surh L, Coulter-Mackie M. Diagnostic single strand conformation polymorphism (SSCP): a simplified non-radioisotopic method as applied to Tay-Sachs B1 variant. *Nucleic Acids Res.* 1991;19(2):405-406. doi:10.1093/nar/19.2.405.
- Badola S, Bhattacharya TK, Biswas TK, Shiva Kumar, Kumar P, Sharma A. A comparison on polymorphism of beta-lactoglobulin gene in *Bos indicus*, *Bos taurus* and indicine $\times$ taurine crossbred cattle. *Asian-Australas J Anim Sci.* 2004;17(5):733-736.
- Bhattacharya TK, Chatterjee RN. Polymorphism of the myostatin gene and its association with growth traits in chicken. *Poult Sci.* 2013;92(4):910-915. doi:10.3382/ps.2012-02736.
- Bhattacharya TK, Chatterjee RN, Raj Kumar U, Rajaravindra KS, Niranjana M. Genetic polymorphism in the promoter of prolactin gene in layer chicken. *Indian J Anim Res.* 2015a;49(6):875-877. doi:10.18805/ijar.7056.

5. Bhattacharya TK, Chatterjee RN, Dushyanth K, Paswan C, Shukla R, Shanmugam M. Polymorphism and expression of insulin-like growth factor 1 (IGF1) gene and its association with growth traits in chicken. *Br Poult Sci.* 2015b;56(4):398-407.
6. Bhattacharya TK, Chatterjee RN, Sharma R, Niranjana M, Rajkumar U, Reddy BLN. Polymorphism in prolactin promoter and their association with growth traits in chicken. *Biochem Genet.* 2011;49(5-6):385-394.
7. Bhattacharya TK, Shukla R, Chatterjee RN, Dushyanth K. Knockdown of the myostatin gene by RNA interference increased body weight in chicken. *J Biotechnol.* 2016;241:61-68.
8. Cui JX, Du HL, Liang Y, Deng XM, Li N, Zhang XQ. Association of polymorphisms in the promoter region of chicken prolactin with egg production. *Poult Sci.* 2006;85(1):26-31. doi:10.1093/ps/85.1.26.
9. Gruszczynska J, Brokowska K, Charon KM, Swiderek WP. Restriction fragment length polymorphism of exon 2 Ovar-DRB1 gene in Polish Heath sheep and Polish Lowland sheep. *J Appl Genet.* 2005;46(3):311-314.
10. Kakavas VK, Plageras P, Vlachos T, Papaioannou A, Noulas AV. PCR-SSCP: a method for the molecular analysis of genetic diseases. *Mol Biotechnol.* 2008;38(2):155-163. doi:10.1007/s12033-007-9006-7.
11. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system: from concept to molecular analysis. *Eur J Biochem.* 1997;244(1):1-14. doi:10.1111/j.1432-1033.1997.00001.x.
12. Miličević D, Vranic D, Masic Z, Parunovic N, Trbovic D, Nedeljkovic-Trailovic J, *et al.* The role of total fats, saturated/unsaturated fatty acids and cholesterol content in chicken meat as cardiovascular risk factors. *Lipids Health Dis.* 2014;13:42-42. doi:10.1186/1476-511X-13-42.
13. Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics.* 1989;5(4):874-879.
14. Pongpisantham B. Applying genotype and marker-assisted selection for the improvement of quantitative traits in poultry [MSc thesis]. University of New England; 1994. p. 1-121.
15. Riancho JA, Vázquez L, García-Pérez MA, Sainz J, Olmos JM, Hernández JL, *et al.* Association of ACACB polymorphisms with obesity and diabetes. *Mol Genet Metab.* 2011;104(4):670-676.
16. Ruderman NB, Saha AK, Vavvas D, Witters LA. Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol Endocrinol Metab.* 1999;276(1):E1-E18.
17. Sambrook J, Russell DW. Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2001.
18. Tang VTM, Leung LY, Chan SSH, Wong DWS, Chu JCK, Leung YWH, *et al.* The acetyl-coenzyme A carboxylase beta (ACACB) gene is associated with nephropathy in Chinese patients with type 2 diabetes. *Nephrol Dial Transplant.* 2010;25(12):3931-3934.
19. Vohra V, Bhattacharya TK, Dayal S, Kumar P, Sharma A. Genetic variants of beta-lactoglobulin gene and its association with milk composition traits in riverine buffalo. *J Dairy Res.* 2006;73(4):499-503. doi:10.1017/S0022029906002044.
20. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. *J Lipid Res.* 2009;50(Suppl):S138-S143.
21. Xing J, Kang L, Hu Y, Xu Q, Zhang N, Jiang Y. Effect of dietary betaine supplementation on mRNA expression and promoter CpG methylation of lipoprotein lipase gene in laying hens. *J Poult Sci.* 2009;46(3):224-228.
22. Yap E, McGee J. Nonisotopic SSCP detection in PCR products by ethidium bromide staining. *Trends Genet.* 1992;8(2):49-49.
23. Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. POPGENE: the user-friendly shareware for population genetic analysis. Edmonton: University of Alberta; 1997.
24. Zain M, Awan FR, Najam SS, Islam M, Khan AR, Ahmed B, *et al.* Association of ACACB gene polymorphism (rs2268388, G>A) with type 2 diabetes and end-stage renal disease in Pakistani Punjabi population. *Meta Gene.* 2017;12:109-112.
25. Zhu G, Jiang Y. Polymorphism, genetic effect and association with egg production traits of chicken matrix metalloproteinases 9 promoter. *Asian-Australas J Anim Sci.* 2014;27(11):1526-1531. doi:10.5713/ajas.2014.14209.