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Genetic polymorphism within *Chicken growth hormone* and significant association of *Perilipin* gene with growth traits in Kadaknath chicken

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ABSTRACT

The research work was planned to identify polymorphisms within *Chicken growth hormone (cGH)* and *Perilipin (PLIN1)* gene in Kadaknath chicken and test association of polymorphism with growth and carcass traits. Genomic DNA from 118 Kadaknath birds was estimated using DNA extraction kit. Restriction digestion of intron-3 region of *cGH* gene fragment was polymorphic with 'G' allele frequency as 0.85 and 'A' allele frequency as 0.15. Restriction digestion of 545 bp gene fragment of exon-6 region of *PLIN1* gene using Tsp4CI enzyme, revealed polymorphism at *PLIN1*KC1-Tsp4CI locus with frequency of C1 allele as 0.65 and C2 allele as 0.35. The *PLIN1* 'C2C2' genotype was associated with body weight at BW8, BW10 and BW12 weeks and ADG at 6-8 weeks and 10-12 weeks of age at *PLIN1*KC1- Tsp4CI PCR-RFLP locus in Kadaknath population. The 5′flanking region of *PLIN1* using direct sequencing of birds revealed four SNPs. The study revealed polymorphisms in *cGH* and *PLIN1* genes loci in experimental Kadaknath population. The identified association of *PLIN1*KC1-Tsp4CI with body weight and average daily gain may be used for selection of chicks for growth traits and for genetic improvement after validation in larger dataset.

Keywords: *cGH*, Kadaknath chicken, *PLIN1*, Polymorphism

India possesses around 851.81 million poultry birds consisting 317.07 million backyard chickens and 534.74 million commercial chickens nationwide, respectively (20th Livestock Census 2019). The poultry meat production was 9.77 million tons in 2022-23, which contributed about 51.14% of total meat production (BAHS 2023). Indian poultry sector has exhibited increase in popularity at 45% of all meat consumption (Chatterjee and Rajkumar 2015). Meat of indigenous breeds of poultry is costlier and in high consumer demand. However, genetically these breeds show slow growth and low bodyweight as compared to the commercial chicken breeds. In view of this, it is essential to exploit the existing variability in bodyweight and growth rate to improve productivity potential (Singh *et al.* 2023). Indigenous poultry breeds with their unique characteristics and universal adaptivity have proven to be useful for Indian poultry landscape, creating a huge scope for improvement using selective breeding for its sustainable development and conservation (Goli *et al.* 2024).

In high-quality chicken breeding programmes, improved meat quality and increased muscle yield are particularly desired. The body weight and weight gain are straightforward and useful metrics used to assess the growth of chickens. The body weight gain is important quantitative characteristics that greatly affects the economic value of the poultry (Sartika *et al.* 2008). Kadaknath is most appreciated indigenous poultry breed with black pigmentation due to excessive buildup of eumelanin, a form of melanin pigment. The single autosomal fibromelanosis (*Fm*) gene, which is incompletely dominant, is responsible for this pigmentation condition (Mishra *et al.* 2008, Haunshi and Prince 2021). The breeding tract of Kadaknath breed is from Chhattisgarh and Dhar and Jhabua districts of Madhya Pradesh with an additional prevalence in few districts of Rajasthan and Gujarat. The Kadaknath meat quality, texture, and flavour are renowned with a geographical indication tag with remarkable therapeutic properties. Kadaknath can thrive in any setting with incredible level of disease resistance and is comparatively higher in protein, lower in fat, and lower cholesterol. The Kadaknath breed exhibits greater heritability estimates for juvenile growth parameters like body weight at 4 and 6 weeks of age (Haunshi *et al.* 2012). These results revealed that enough additive genetic variation for growth traits exist, which opens the door for improving native chickens for body weight.

The candidate gene approach using single nucleotide polymorphisms (SNPs) analysis has been successfully employed to identify some DNA markers. Exploring a candidate gene approach may yield higher efficiency in

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detecting the desired traits essential to improve production performance. Recent advances in DNA technology have opened new opportunities to analyze genetic variability at the DNA level (Mutum and Wangkheimayum 2023). The results of gene polymorphism within candidate genes like chicken growth hormone (*cGH*) gene and perilipin (*PLIN1*) gene studies could be linked to a gene significantly implicated for growth and carcass traits in chicken, which will be helpful to establish marker-assisted selection in chickens' selection and breeding programs.

The anterior pituitary gland secretes chicken growth hormone, a significant hormone that is vital to the development and growth of chickens (Yan *et al.* 2003). The length of the *cGH* is approximately 4.1 Kb comprising 4 introns and 5 exons (Tanaka *et al.*1992). *cGH* gene affects a wide range of physiological processes, including development, body composition, egg production, ageing, reproduction, and immunological responsiveness as it is extremely polymorphic. For marker-assisted selection to enhance performance, the *cGH* gene has been employed as a candidate gene (Meena *et al.* 2019). *PLIN1* is a candidate gene for fat and carcass traits in ducks (Zhang *et al.* 2013) and plays an essential role in lipid metabolism and fat deposition (Li *et al.* 2017). Polymorphisms of the *PLIN1* gene are highly associated with adiposity in mammals and are potential molecular markers for improving meat quality and carcass traits in chickens. *PLIN1* gene polymorphisms are likely genetic indicators for enhancing meat quality and carcass features in chickens since they are strongly associated with adiposity in mammals (Zhang *et al.* 2015).

Identified DNA markers with traditional selection methods, needs to be used to enhance production potential while preserving its unique traits. Systematic and precise body weight data record generation by expert as phenomic data base for genomic studies is essential for DNA marker development. In view of this, it is necessary to analyze candidate genes like *cGH* and *PLIN1* genes for relating polymorphism with economic traits for future selection and breeding decisions.

MATERIALS AND METHODS

Compliance with ethical standards and experimental birds: Sampling from Kadaknath chicken was performed as per ethical standards approved by institute animal ethical committee (NVC/IAEC/01/2023). Blood samples were collected from 118 unrelated birds of Kadaknath breed, reared at Nagpur Veterinary College, Nagpur from day old age up to a period of 15 weeks from the wing vein under aseptic conditions. Blood samples were transported to the laboratory in an icebox and stored at -20°C till the isolation of genomic DNA. All the birds were wing tagged and were subjected for individual recording of body weight and carcass traits.

Measurement of variables: Under growth traits, body weight (BW) and average daily gain (ADG) were measured. Body weight of each individual bird was measured on a weekly basis for the first month, and then fortnightly for the next 2 months. The ADG was calculated fortnightly at 2 to 4, 4 to 6, 6 to 8, 8 to 10 & 10 to 12 weeks of age. The growth traits were recorded as BW at 1, 2, 3, 4, 6, 8, 10 and 12 weeks of age. Under carcass traits, the observations were recorded as live weight (g), dressed weight (g), eviscerated weight (g), cut up parts -breast, thigh, drumstick, back, neck, wing weight (g) and giblet: liver, heart, gizzard weight (g).

DNA isolation: The genomic DNA was isolated from blood samples using HiPurA SPP Blood DNA isolation kit (Himedia) with certain modifications. The DNA samples were processed for quantity and quality check using Biospetrometer (Eppendorf) and were also evaluated on 0.5% agarose gel electrophoresis. The compact and intact consistency without smearing were regarded satisfactory. The samples exhibiting absorbance ratio between 1.7 and 1.9 were considered of good quality and were used in further downstream applications.

PCR amplification: For the current study, four primers were selected (Table 1) for study of 2 regions *cGH* and 2 regions of *PLIN1* gene were amplified using specific primers reported in research literature.

The PCR reaction with total volume of 25 µL was performed for the four primers representing *cGH* and *PLIN1* genes using 12.5 µL 2X DreamTaq Green PCR Mastermix, 1 µL volume of each forward and reverse primer with 12.5 pM concentration for *cGH*KC1 amplified region, whereas 10.0 pM primer concentration was optimized for the remaining primers. Template DNA with 50 ng/ µL concentration and 8.0 µL molecular biology grade water was utilised for all the primers. The annealing

Table 1. Name of the primers, region, sequence and product size of amplicons used in *cGH* and *PLIN1* gene polymorphism study in Kadaknath population

Primer name/ Region	Primer sequence	Product size (bp)	Temperature
cGHKC1/ intron 3	F-5'TCCCAGGCTGCGTTTTGTTACTC3' R-5'ACGGGGGTGAGCCAGGACTG3'	429	64°C
cGHKC2/ intron1	F-5'AAGCAACACCTGAGCAAC3' R-5'CTCTCTGGGACACACCTG 3'	621	59°C
PLIN1KC1/ Exon-6	F-5'AGCCAAAGGGCAGGAA3' R-5'AAACCCACAAACCAACAACT3'	545	54.1°C
PLIN1KC2/ 5'Flanking region	F-5'TCCAGCAGAGGAGGAGTGTGT3' R-5'TTCTGCAAAGGCTGATTCTT3'	239	56.9°C

temperatures for four primers representing *cGH* and *PLIN1* genes were optimized in the laboratory as cited in Table 1. The amplified PCR products were resolved in 1.5% agarose gel electrophoresis which were later documented for optimizing quality amplification using the Gel Doc system (Bio-era).

PCR-RFLP analysis: For *cGHKC1*, the PCR products of 8 µL from each tube were digested with 0.3 µL (3 units) of *EcoRV* restriction enzymes along with the appropriate buffer supplied with the enzyme. For *PLIN1KC1*, the 8 µL of PCR products were digested with 0.5 µL (5 units) of *Tsp4CI* restriction enzymes along with the appropriate buffer supplied with the enzyme. The reaction mixture was kept for digestion in incubator at 37°C for 16 h. After restriction enzyme digestion, digested PCR products of *cGHKC1* and *PLIN1*KC1 were electrophoresed on 2% and 2.5% agarose gel respectively. The bands were visualized under UV light and documented by gel documentation system.

Direct sequencing, bioinformatics analysis and statistical analysis: The PCR products representing to the birds exhibiting relatively better phenotypic records were sequenced (Eurofins Genomics India Pvt. Ltd., Bangalore). The obtained sequence was analysed using BLAST, CLUSTAL-W etc. in view of identification of nucleotide-based substitutions within the region of studied genes. Genotype and allele frequencies were estimated using POPGENE Version 1.31 (Yeh *et al.* 1997). The obtained gene & genotype frequencies were tested for deviation from Hardy-Weinberg equilibrium (HWE) by using a Chi-square test (Devlin and Risch 1995, Nielsen *et al.* 1998). The effect of *cGH* and *PLIN1* genotypes on growth traits (Body weight, Average daily gain) and Carcass traits was tested by General linear model (GLM) using software SPSS (Version 20 IBM, USA). The model is as follows,

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

Where: Y_{ij} is the observed trait value for the j^{th} animal;
μ= population mean value
$G_{i\,=}$ Fixed effect of i^{th} Genotype of either gene
e_{ij}= random error.

RESULTS AND DISCUSSION

cGH- EcoRV PCR-RFLP analysis: For 429 bp intron-3 region of *cGH* gene, two alleles specific patterns were obtained after EcoRV digestion, allele G including 429 bp DNA fragments and two DNA fragments of 295 bp and 134 bp for allele A. The PCR-RFLP was found to be polymorphic (Fig. 1) in the current Kadaknath chicken population with 03 genotypes which were represented as GG (429 bp), GA (429, 295 and 134 bp) and AA (295 and 134 bp). The PCR-RFLP analysis of Kadaknath chicken population in the intron-3 region of the *cGH* gene segregated at a frequency of 0.85 for 'G' allele and 0.15 for 'A' allele.

The genotype frequency for the genotype GG, GA and AA was found as 0.71,0.27 and 0.02 respectively (Table 2). The chicken growth hormone genotype frequency distributions

Table 2. Genetic diversity measures at *cGH*- EcoRV locus in intron-3 region of *Chicken Growth Hormone* gene in Kadaknath chicken population

Genotype	n	Frequency		χ2	*p* value	n_e	H_o	H_e	*I
		Genotypic	Allelic						
GG (84)	118	0.71	0.85 (G)	0.24	0.62	1.35	0.27	0.26	0.43
GA (32)		0.27	0.15 (A)						
AA (02)		0.02	-						

* Indicates significant at 0.05 (p<0.05) level, Where, H_o : Observed heterozygosity, H_e : Expected heterozygosity, ne: Effective number of alleles, *I: Shannon's information index.

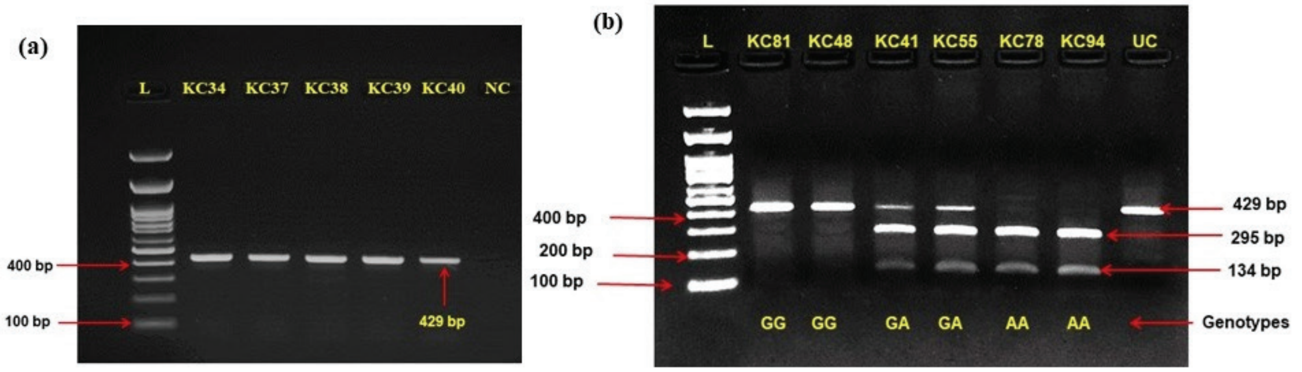

Fig.1 Electrophoresis profiles (a) *cGH*KC1 PCR amplification of 429 bp intron-3 region of *cGH* gene fragment (b) Polymorphic *cGH*KC1-EcoRV PCR-RFLP in intron-3 region of *cGH* resolved in 2.5% agarose gel electrophoresis in Kadaknath chicken population. Where, L: 100 bp DNA Ladder (Himedia), KC81-94: Kadaknath Chicken Numbers, U: Uncut, Genotype GG: 429 bp. GA: 429 bp, 295 bp and134 bp. AA: 295 and134 bp

Table 3. Genetic diversity measures at *PLIN1*KC1- Tsp4CI locus in exon-6 region of Perilipin gene in Kadaknath chicken population

Genotype	Frequency			$\chi2$	*p* value	n_e*	H_o	H_e	*I
	n	Genotypic	Allelic						
C1C1 (34)		0.40	0.65 (C1)						
C1C2 (42)	85	0.49	0.35 (C2)	0.49	0.45	1.84	0.49	0.46	0.65
C2C2 (09)		0.11	-						

* Indicates statistically significant at 0.05 ($p<0.05$) level, where, H_o: Observed heterozygosity, H_e: Expected heterozygosity, ne*: Effective number of alleles, *I: Shannon's information index.

were all in Hardy-Weinberg equilibrium expectations ($\chi2$ =0.24, $p>0.05$) in studied Kadaknath population.

Various researchers had also earlier reported the frequency of the G and A allele in accordance with the results obtained in the present study. Three genotypes i.e. GG, GA, and AA with frequencies of 0.77, 0.19, and 0.04, respectively were reported by Nie *et al.* (2005) in an F_2 resource population produced by crossing a slow-growing line called Xinghua (X) with a fast-growing line called White Recessive Rock (WRR). Anh *et al.* (2015) genotyped four Thai broiler lines i.e., PS×KM, PS×KT, PS×SN, and PS×SP, consisting around 408 samples at the same locus and observed three genotypes and reported the frequency of G allele as 0.80, 0.61, 0.71, and 0.73, correspondingly, and the frequency of A allele as 0.20, 0.30, 0.29, and 0.27 correspondingly with the four lines. Some researchers have also reported high frequency of GG genotypes as compared to the present findings. Utama *et al*. (2018) studied chicken growth hormone gene polymorphism at intron-3 region in relatively small population of 28 samples of back-crossed hybrid chicken, found two genotypes i.e. GG with frequency of 0.89 and GA with frequency of 0.11.

In contrast with results of the current study, some researchers have reported less frequency of the GG genotype. Al-khatib and Al-Hassani (2016) studied the G1705A SNP in the third intron of the *cGH* gene in two commercial broiler hybrids and reported frequency of genotype GG, GA, and AA as 0.47, 0.46, and 0.07 in Cobb500 and 0.61, 0.37, and 0.02 in Hubbard, respectively. They also found that the G allele was superior to A allele with frequencies as 0.70 and 0.30 for Cobb500 and 0.79 and 0.21 for Hubbard, respectively. Lie *et al*. (2007) reported frequency of genotype GG, GA, and AA as 0.45, 0.44, and 0.11 in Xinghua chickens. In conclusion, it can be observed that most of the researchers have reported dominance of GG genotype and G allele in the studied populations.

*cGH*kc1-ecorv *polymorphism and association with bodyweight, average daily gain and carcass traits*: The results of the analysis of variance, revealed no significant differences ($p>0.05$) between identified *cGH* genotypes at *cGH*KC1-EcoRV locus and growth traits (bodyweight, Average daily gain) and carcass traits. It can be concluded that dominance of G allele was observed; however, it has been observed that nearly every growth attribute exhibited strong association with G+1705A, and the A allele having beneficial influence on chicken growth.

SNP detection in intron-1 region of cGH gene: In present study, *cGH*KC2 amplified products (Fig. 2) representing the bird exhibiting relatively superior and relatively inferior bodyweight trait means were selected for sequencing which revealed SNP C>T/C at 278th position in the query sequence (Intron-1 region of *cGH* gene in Kadaknath**)**. The nucleotide BLAST results of the *cGH*KC2 amplicon query sequence revealed 99.82% percent identity with GenBank id: CP100581.1 representing Huxu breed of poultry. The query sequence revealed 99% percent identity with GenBank id: MW654249.1 representing growth hormone (GH) gene, partial cds of Ross breed.

*PLIN1*KC1-*Tsp4CI* *PCR-RFLP* *analysis*: The

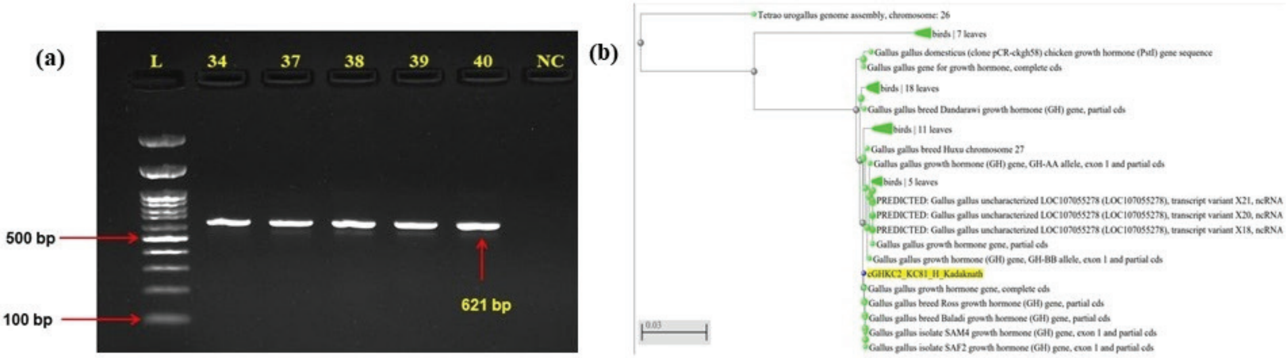

Fig. 2 Electrophoresis profiles (a) cGHKC2 PCR amplification of 621 bp intron-1 region of *cGH*) gene fragment resolved in 1.5% agarose gel electrophoresis (b) Phylogenetic tree based on 574 bp sequence of cGH gene depicting closer relation of Kadaknath chicken with *Gallus gallus* growth hormone gene in other poultry germplasm, where, 34-40: Kadaknath chicken numbers, NC: Negative Control, L: 100 bp DNA Ladder (Himedia)

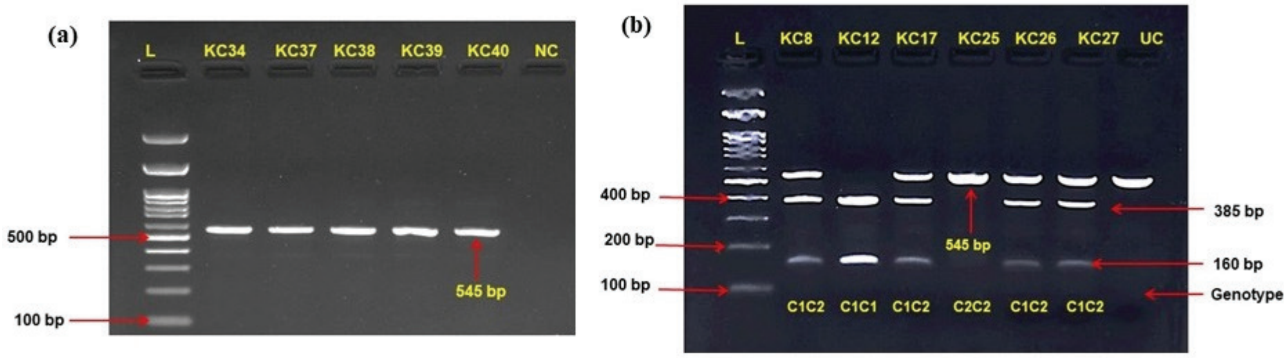

Fig. 3. Electrophoresis profiles (a) 4.8 PLINIKCI PCR amplification Tsp4CI PCR-RFLP in exon-6 region of Perilipin gene (*PLIN1*) population, where, L: 100bp DNA Ladder (Himedia), KC8-27 bp, 160 bp, C * C(1,2): 545 bp, 385 bp and 160 bp, C * C(2,2): 545bp of 545 bp exon6 region of PLINI gene (b) Polymorphic PLINIKCI-resolved in 2.5% agarose gel electrophoresis in Kadaknath chicken Kadaknath Chicken Numbers, U: Uncut, Genotype C * C(1,1): 385

*PLIN1*KC1 analysis of 545 bp PCR product representing exon 6 of *PLIN1* revealed a polymorphic pattern with 3 genotypes. i.e. C1C1 with 385 and 160 bp band size, C1C2 with 545, 385 and 160 bp band sizes and C2C2 with 545 bp band sizes studied in Kadaknath chicken population (Fig. 3).

The *PLIN1*KC1-Tsp4CI PCR-RFLP analysis of Kadaknath chicken population in the exon-6 region of the *PLIN1* gene segregated at a frequency of 0.65 for 'C1' allele and 0.35 for 'C2' allele. The genotype frequency for the genotype C1C1, C1C2 & C2C2 was estimated as 0.40,0.49 and 0.11, respectively (Table 3). The *Perilipin* genotype frequency distributions were all in Hardy-Weinberg equilibrium expectations (χ2 =0.49, p>0.05) at *PLIN1*KC1-Tsp4CI locus in studied Kadaknath chicken population.

The results of the study at the same locus by other researchers were in accordance with the current study for the gene frequency but were not in accordance for the genotype frequency. Yan *et al*. (2014) used the Tsp4CI enzyme for PCR-RFLP analysis in a population of 384 Luqin chickens. They identified three genotypes: C1C1, C1C2, and C2C2, with frequencies of 0.58, 0.35, and 0.07, respectively. The allele frequency of 2 alleles was reported as C1=0.75 and C2= 0.25. At the polymorphic loci, where C1 is the dominant allele, the C1C1 genotype exhibited the highest frequency at G.2272 C>T, followed by the heterozygous genotype C1C2, and the C2C2 genotype exhibited the lowest frequency. However, in present study the C1C2 heterozygous genotype was predominant followed by C1C1 and C2C2.

*PLIN1*KC1-Tsp4CI *polymorphism and association with bodyweight, average daily gain and carcass traits*: The results of ANOVA revealed significant differences (p<0.05) between *PLIN1* gene C1C1, C1C2 and C2C2 genotypes with bodyweight (BW) at eight-week, tenth week and twelfth week of age. The multiple traits mean comparison revealed that *PLIN1* genotype C2C2 (399.11±31.23) exhibited relatively more body weight at 8th week (Table 4) as compared to the genotype C1C2 (318.57±14.97) and C1C1 (310.29± 10.75). The multiple traits mean comparison revealed that *PLIN1* genotype C2C2 (535.56±39.33) exhibited relatively more body weight at 10th week as compared to the genotype C1C2 (437.86±19.69) and C1C1 (417.94±15.01). The multiple traits mean comparison revealed that *PLIN1* genotype

Table 4. Least square means along with standard errors for body weight trait for *Perilipin* gene (*PLIN1*KC1- Tsp4CI) in Kadaknath chicken

Geno-type	Body weight (gm) at different weeks of age							
	First Week^{NS}	Second Week^{NS}	Third Week^{NS}	Fourth Week^{NS}	Sixth Week^{NS}	Eight Week*	Tenth Week*	Twelth Week*
C1C1 (34)	42.12±1.07	71.13±2.03	89.95±2.66	123.62±4.05	219.04±8.22	310.29± 0.75	417.94±15.01	539.97±17.63
C1C2 (42)	41.76±0.97	71.71±1.61	90.81±2.65	124.69±4.17	221.95±9.90	318.57±14.97	437.86±19.69	585.50±26.17
C2C2 (09)	44.33±2.65	79.00±5.72	101.63±7.95	145.76±11.28	265.50±21.28	399.11±31.23	535.56±39.33	696.22±51.91
Total (85)	42.18±0.70	72.25±1.29	91.61±1.89	126.49±2.93	225.40 ±6.41	323.79±9.50	440.24±12.59	579.01±16.31
p value	0.56	0.20	0.19	0.08	0.1	0.02	0.02	0.02

*p<0.05, NS: Non significant, values bearing different superscript in column differ significantly and figures in parentheses are number of observations.

Table 5. Least square means along with standard errors for average daily gain (ADG) for *Perilipin* gene (*PLIN1*KC1- Tsp4CI) in Kadaknath chicken

Genotype	Average daily gain (gm) at different weeks of age				
	02-04 Weeks^{NS}	04-06 Weeks^{NS}	06-08 Weeks*	08-10 Weeks^{NS}	10-12 Weeks*
C1C1 (34)	3.75±0.19	6.82±0.33	6.52±0.42	7.69±0.60	8.72±0.62
C1C2 (42)	3.78±0.21	6.95±0.48	6.90±0.54	8.52±0.49	10.55±0.62
C2C2(09)	4.77±0.45	8.55±0.81	9.54±0.93	9.75±0.79	11.48±1.12
Total (85)	3.80±0.14	7.07±0.29	7.03±0.34	8.32±0.35	9.91 ±0.42
p value	0.09	0.21	0.03	0.21	0.049

* $p<0.05$, NS: Non-significant, values bearing different superscript in column differ significantly and figures in parentheses are number of observations.

C2C2 (696.22±51.91) exhibited relatively more body weight at 12th week as compared to the genotype C1C2 (585.50±26.17) and C1C1 (539.97± 17.63).

The results of ANOVA revealed significant differences ($p<0.05$) between *PLIN1* gene C1C1, C1C2 and C2C2 genotypes with average daily gain (ADG) during 06-08 weeks and 10-12 Weeks of age. The multiple traits mean comparison revealed that *PLIN1* genotype C2C2 (9.54±0.93) exhibited relatively more average daily gain during 06-08 weeks (Table 5) as compared to the genotype C1C2 (6.90±0.54) and C1C1 (6.52± 0.42). The multiple traits mean comparison revealed that *PLIN1* genotype C2C2 (11.48±1.12) exhibited relatively more average daily gain during 10-12 weeks of age as compared to the genotype C1C2 (10.55±0.62) and C1C1 (8.72± 0.62). The results of the analysis of variance, revealed no significant differences ($p>0.05$) between *PLIN1* gene C1C1, C1C2 and C2C2 genotypes with carcass traits. The multiple traits mean comparison test revealed that *PLIN1* genotype C2C2 (944.00±77.65) exhibited relatively more live weight at slaughter as compared to the genotype C1C2 (830.29±53.12) and C1C1 (684.30± 50.69). In current study at *PLIN1*KC1- Tsp4CI PCR-RFLP locus, the *PLIN1* 'C2C2' genotype was found to be associated ($p<0.05$) with body weight at BW8, BW10 and BW12 weeks and ADG at 6-8 weeks and 10-12 weeks of age in Kadaknath population. However; no association was found between the *PLIN1* genotypes and carcass traits. In other studies, at the same locus, reported association of the genotypes with carcass traits. The results of the association analysis

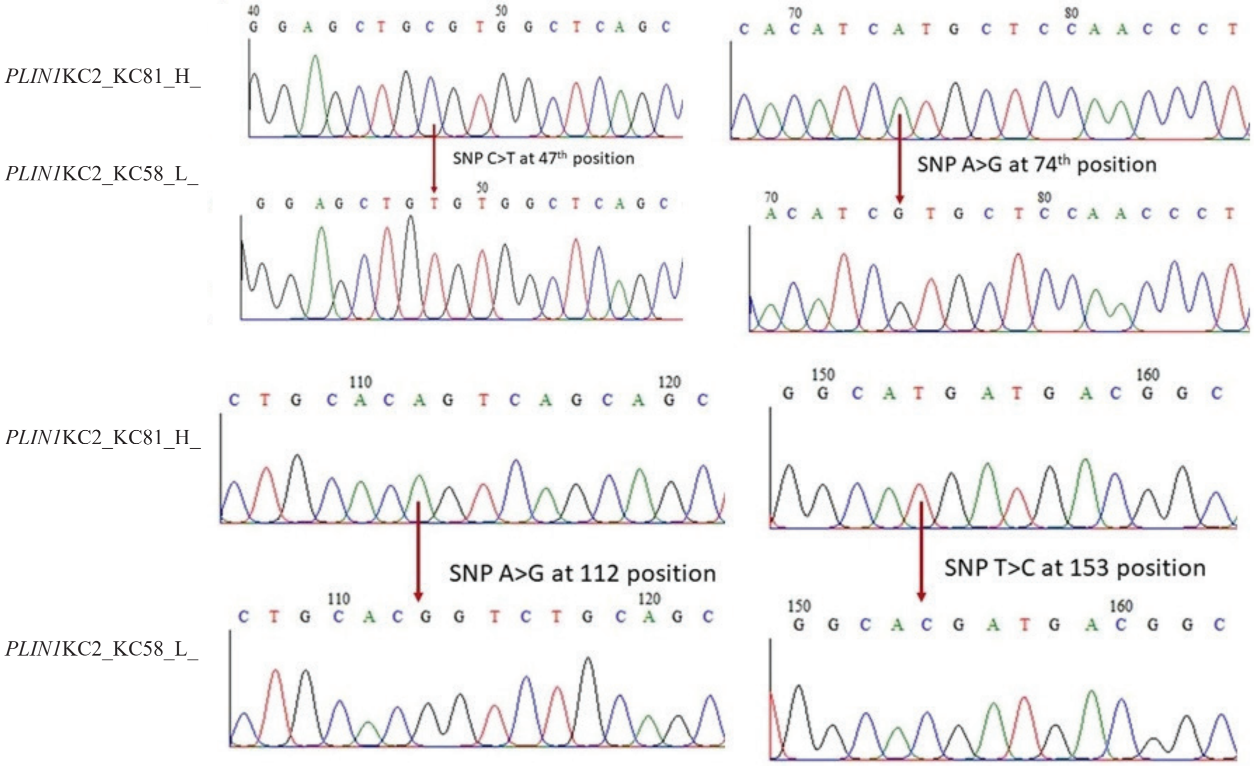

Fig.4 Identified SNP C>T at 47th, SNP A>G at 74th. A>G at 112th and SNP T>C at 153rd position in 188 bp sequence of *PLIN1*KC2 amplified 5'flanking region of *Perilipin* Gene in Kadaknath chicken population

conducted by Yan *et al*. (2014) at g.2272C>T locus of the PLIN gene showed a near-significant effect on living body weight (*p*=0.05), carcass weight (P=0.05), breast intramuscular fat content (*p*=0.06) and a non-significant effect on other carcass traits.

SNP detection in 5'flanking region of PLIN1 gene: In present study, *PLIN1*KC2 amplicon representing the bird exhibiting relatively superior and relatively inferior carcass trait means were selected for sequencing which detected four SNPs i.e. SNP C>T at 47th , SNP A>G at 74th, A>G at 112th and SNP T>C at 153rd position (Fig. 4). The nucleotide BLAST results of the *PLIN1*KC2 amplicon query sequence (5'flanking region of *PLIN1* gene in Kadaknath**)** revealed 98.38%, 96.76% and 97.87% percent identity with GenBank ids: XM_046899060.1, CP100564.1 and XM_015292004.4 representing *Gallus gallus* sequences in the database.

In conclusion, it is observed that, the current study will surely fill the information gap with respect to identified polymorphisms in *cGH* and *PLIN1* genes in the Kadaknath chicken. The *cGH*KC1-EcoRV locus was polymorphic, however it was not related with growth traits. The current study identified polymorphism at *PLIN1*KC1- Tsp4CI locus and found association of *PLIN1* 'C2C2' genotype with body weight at 8th, 10th and 12th week and average daily gain at 6-8 and 10-12 weeks of age. The results after validation in larger data set; can be recommended for marker assisted selection of Kadaknath chicks for genetic improvement for growth traits along with traditional selection methods.

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REFERENCES

20th Livestock Census. 2019. Department of Animal Husbandry, Dairying & Fisheries Annual Report, Ministry of Agriculture, Govt. of India, New Delhi.

Al-Khatib B G M and Al-Hassani D H H. 2016. Effect of G1705A SNP in growth hormone gene on the productive and physiological performance in broiler chicken. *Iraqi Journal of Biotechnology* **15**(1): 33-45.

Anh N T L, Kunhareang S and Duangjinda M. 2015. Association of chicken growth hormones and insulin-like growth factor gene polymorphisms with growth performance and carcass traits in Thai broilers. *Asian-Australasian Journal of Animal Sciences* **28**(12): 1686–95.

Basic Animal Husbandry Statistics, 2023. Department of Animal Husbandry & Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, Krishi Bhawan, New Delhi.

Chatterjee R N and Rajkumar U. 2015. An overview of poultry production in India. *Indian Journal of Animal Health* **54**(2): 89–108.

Devlin B and Risch N. 1995. A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* **29**(2): 311-22.

Goli R C, Sukhija N, Rathi P, Chishi K G, Koloi S, Malik A A, Sree C C, Purohit P B, Shetkar M and Kanaka K K. 2024. Unraveling the genetic tapestry of Indian chicken: A comprehensive study of molecular variations and diversity. *Ecological Genetics and Genomics* **30** (5):100220.

Haunshi S, Murugesan S, Padhi M K, Niranjan M, Ullengala R, Reddy M R and Panda A K. 2012. Evaluation of two indian native chicken breeds for reproduction traits and heritability of juvenile growth traits. *Tropical Animal Health and Production* **44**(5): 969–73.

Haunshi S and Prince L L L. 2021. Kadaknath: a popular native chicken breed of India with unique black colour characteristics. *World's Poultry Science Journal* **77**(2): 427–40.

Lei M, Luo C, Peng X, Fang M, Nie Q, Zhang D, Yang G and Zhang X. 2007. Polymorphism of growth-correlated genes associated with fatness and muscle fiber traits in chickens. *Poultry Science* **86**(5): 835–42.

Li T, Ling J, Duan L, Xue Q and Wang J. 2017. Association between perilipin gene polymorphisms and body weight traits in Jinmao Hua chickens. *Archives Animal Breeding* **60**(3): 327-33.

Meena S, Gahlot G C, Pannu U, Devi D, Chouhan H, Ashraf M and Yadav H S. 2019. Polymorphisms in exon-1 and partial intron-1 of growth hormone gene and its association with body weight traits in broiler. *International Journal of Livestock Research* **9** (4): 114-19.

Mishra S K, Arora G, Pratap S O, Singh D P, Narayan R and Beura C K. 2008. Interaction of fibromelanosis gene with various genetic backgrounds affecting carcass pigmentation in crossbred Kadaknath chicken. *Indian Journal of Poultry Science* **43** (3): 267–71.

Mutum R S and Wangkheimayum V D. 2023. DNA barcoding of indigenous fowl of Manipur, Kaunayen (*Gallus gallus domesticus*). *Animal Biotechnology* **34**(9): 4430–34.

Nie Q, Sun B, Zhang D, Luo C, Ishag N A, Lei M, Yang G and Zhang X. 2005. High diversity of the chicken growth hormone gene and effects on growth and carcass traits. *Journal of Heredity* **96**(6): 698-703.

Nielsen D M, Ehm M G and Weir B S. 1998. Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. *The American Journal of Human Genetics* **63**(5): 1531-40.

Sartika, T, Wati D K, Rahayu H S I and Iskandar S. 2008. Comparison of external genetic of Wareng and Kampung chicken, observed from introgression rate and genetic variability. *Jurnal Ilmu Ternak dan Veteriner* **13**(4): 279-87.

Singh M K, Kumar S, Singh S K, Sharma R K, Prakash A, Prasad S, Singh Y and Singh D N. 2023. Body weights and growth rates in indigenous chicken breeds of India. *World's Poultry Science Journal* **79**(4): 867-78.

Tanaka M, Hosokawa Y, Watahiki M and Nakashima K. 1992. Structure of the chicken growth hormone-encoding gene and its promoter region. *Gene* **112**(2): 235–39.

Utama I V, Perdamaian A B I and Daryono B S. 2018. Plumage uniformity, growth rate and growth hormone polymorphism in indonesian hybrid chickens. *International Journal of Poultry Science* **17**(10): 486-92.

Yan B, Deng X, Fei J, Hu X, Wu C and Li N. 2003. Single nucleotide polymorphism analysis in chicken growth hormone gene and its associations with growth and carcass traits. *Chinese Science Bulletin* **48**(15): 1561–64.

Yan Z, QiuXia L, HaiXia H, FuWei L, Jin-Bo G, Wei L, Yan L and DingGuo C. 2014. Association on single nucleotide polymorphism of perilipin gene (*PLIN*) with carcass and fatness traits in Luqin chicken (*Gallus gallus*). *Journal of Agricultural Biotechnology* **22**(8): 1001–08.

Yeh F C, Yang R C, Boyle T B, Ye Z H and Mao J X. 1997. POPGENE, the user-friendly shareware for population genetic analysis. *Molecular Biology and Biotechnology Centre, University of Alberta, Canada* **10**: 295- 301.

Zhang H L, Fan H J, Liu X L, Wu Y and Hou S S. 2013. Molecular cloning of the *perilipin* gene and its association with carcass and fat traits in Chinese ducks. *Genetics and Molecular Research* **12**(2): 1582-92.

Zhang L, Zhu Q, Liu Y, Gilbert E R, Li D, Yin H, Wang Y, Yang Z, Wang Z, Yuan Y and Zhao X. 2015. Polymorphisms in the *perilipin* Gene May affect carcass traits of Chinese meat-type chickens. *Asian-Australasian Journal of Animal Sciences* **28**(6): 763–70.