Indian Journal of Poultry Science (2024) 59(1): 3–6; Research Article
https://doi.org/10.56093/IJPS.v59i1.154893

Association of chicken growth hormone (*CGH*) gene polymorphism with growth traits in RIR

RUHI MEENA*[1], SANJEEV KUMAR[2], S. K. BHANJA[3], MANISH MEHRA[1], CHIRAG CHOUDHARY[1] AND ARUNA T.S.[1]

[1]*ICAR-Central Avian Research Institute, Izatnagar-243122 (UP), India.*
[2]*Principal Scientist & Registrar (skgicar@gmail.com), Bihar Animal Sciences University, Patna, Bihar, India.*
[3]*Principal Scientist & Head Regional Station (skbhanja@radiffmail.com), ICAR-DPR, Bhubaneswar, India.*

(Received on January 15, 2024; accepted for publication on February 22, 2024)

ABSTRACT

Meena, R., Kumar, S., Bhanja, S.K., Mehra, M., Choudhary, C. and Aruna, T.S. 2024. Association of chicken growth hormone (CGH) gene polymorphism with growth traits in RIR. Indian Journal of Poultry Science 59(01): 3-6.

The aim of study was to assess allelic polymorphism of chicken growth hormone *(cGH)* gene and their association with growth traits in RIR chicken. Jugular vein was used to collect blood samples from 120 pullets belonging to two hatches. Data were recorded on body weight at 20 and 40 weeks of age (BW20 and BW40). Amplified product was digested with *SacI* restriction enzyme to reveal the polymorphic pattern. Allele frequencies were observed as found 0.17 (A), 0.76 (B) and 0.07 (C). Genotypic frequencies of AA, BC and BB genotypes were 0.17, 0.13 and 0.7, respectively. Significant association was found of *cGH -RFLP* genotype with BW20 and BW40 ($P<0.15$). The overall least squares means along with standard error of BW20, BW40 were observed as 1214.69±12.99 and 1567.63±16.66 g. The birds with BC genotype achieved higher BW20 and BW40 as 1431.84±70.04 g and 1750.64±76.31 g, respectively. The study suggested *cGH* locus BC RFLP genotype can be used as a potential marker to enhance growth of RIR birds.

Keyword: Chicken, Growth traits, *cGH gene*

INTRODUCTION

Poultry is the most expeditious growing segment of agricultural sector in India. Eggs and broilers have a growth rate of 8-10% per annum. According to the 20th livestock census (2020), the total poultry population of our country is 851.81 million, which has revealed an increase 16.81 million over the previous census. India has the 3rd rank for egg production (EP) and 6th in poultry meat production in the world. Annual egg and meat production is approximately 122.49 billion and 8.80 million tons, respectively which has an increase of 10.19% in EP in comparison to last year. The availability of meat is 6.52 Kg/annum per person, respectively against ICMR Recommendations of 11.5 Kg/annum (BAHS & FS, 2021-22). This huge gap may be filled by genetic improvement in the productivity of birds through selection. Genetic progress has slowed down over generations under a phenotype-based long-term breeding program and resulted in loss of genetic variation. This may be overcome by genomics using genetic markers or DNA markers. Molecular markers offer new possibilities to accelerate the selection of commonly measured traits or to select for new traits which are costly or hard to record. Various molecular markers are applied for DNA polymorphism evaluation such as AFLP (Amplified Fragment Length Polymorphism), RAPD (Randomly Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), Microsatellites and SNP (Single Nucleotide Polymorphism), etc. The PCR-RFLP is a very simple, powerful gel-based technique for recognition and differentiation of species, extensively employed to assess DNA polymorphism and used for genotyping by restriction enzyme treatment and electrophoretic separation (Saini *et al.*, 2007). RFLP markers are most widely used for genomic mapping, systematic evolution studies and markers-aided breeding. This study was conducted to assess the polymorphism in growth associated candidate gene as Chicken growth hormone (*cGH*) in selected strain of RIR chicken. *cGH* gene is most important candidate gene which has important impact on growth and metabolism (Vasilatos-Younken *et al.*, 2000). *cGH* gene is 4,101 long base pairs which contains 5 exons and 4 introns.. *SacI* polymorphic restriction sites have been detected inside *cGH* gene (Nie *et al.*, 2005). *Sac I* locus of *cGH* gene in intron 4 has an association with laying rate and egg number (Makhsous *et al.*, 2013). Allele PS1 (+), linked to egg production can be utilized as genetic marker for selection for egg production (Kansaku *et al.*, 2003). Regression analysis in White Leghorn strain found a significant association of hen day rate of lay (HDR) and AFE which is depend on growth hormone (GH) genotype (Feng *et al.*, 1997).

MATERIAL AND METHODS

One hundred twenty RIR birds (60 selected from high EP and 60 from low production group) maintained at experimental layer farm of CARI, Izatnagar were selected on basis of their 40-weeks egg production for

*Corresponding author Email: ruhimeena12@gmail.com

Table 1: Primer sequences, restriction enzymes, expected products of the amplicon of *cGH* candidate genes

Gene	Forward/ Reverse Sequence	Annealing temp (T_a) (°C)	Predicted Amplicon size (bp)	Predicted fragment size	RE	Reference
cGH	F:5'CTAAAGGACCTGGAAGAAGGG R:5'AACTTGTCGTAGGTGTGGGTCTG	62	1164	144/ 450/570/1020	Sac *I*	Makhsous *et.al.,* 2013

candidate gene polymorphism studies using PCR-RFLP. Blood collected with a 24 gauge needle in autoclaved heparinized (50 IU/ml) 1.5 ml microfuge tubes from each experimental bird. Genomic DNA was extracted through phenol:chloroform extraction method by using blood sample. Genomic DNA Purity and concentration was evaluated by using a nanodrop spectrophotometer and samples purity was assessed by absorbance ratio (260 nm / 280 nm). Extracted genomic DNA quality was evaluated on 0.7% horizontal submarine agarose gel electrophoresis (AGE) and sample showing intact band were considered as good for further analysis.

Primer sequence set for *cGH* candidate gene was reported by Makhsous *et al.,* 2013. Primer sequences, restriction enzymes and expected products of the amplicon of *cGH* candidate gene are depicted in table 1. PCR rea* were prepared in 0.2 ml clear, thin-walled sterile PCR tubes with clear flat caps and genomic DNA amplification was done in programmable thermal cycler after optimization.

PCR reaction was conducted in 0.2 ml nuclease-free PCR tubes with 25 µl volume by gentle mixing of 12.5 µl master mix, 2 µl of each primer and 1 ng template into NFW (nuclease free water) and all steps were done in ice box. To get reactant at bottom, mixing and spinning for 5-10 seconds at 3000 rpm was used. The PCR amplification programme was optimized for *cGH* candidate loci: initial denaturing at 95°C with 5min, followed by 30 cycles of (i) denaturation at 94°C for 1 minute, (ii) Annealing at T_a°C (T_a= optimized annealing temperature) with 1 minute (iii) Extension at 72°C for 1 minute followed by final extension at 72°C for 10 min. Genotypes of candidate gene were determined by PCR-RFLP technique after amplification of DNA. Product of PCR was digested with *SacI* restriction enzyme under water bath at 37°C for overnight and checked through 2% agarose gel under gel documentation with fast low range DNA ladder. Genotypes were assigned based on digested band patterns. Observed alleles and genotype in every sample for *cGH /SacI* loci were recorded and Locus specific alleles identified based on their molecular sizes. Allele and genotype frequencies were calculated via following this formula:

Genotype frequency= number of specific genotype/ total genotypes number in population

Gene frequency= 2D + H / 2N

Where, D= Frequency of homozygote, H= frequency of heterozygote, N= number of birds

Statistical analysis

Data recorded on growth traits were analyzed for association with genotypes of *cGH* via *SacI–RFLP.* Association between growth traits and genotypes at candidate gene loci were determined via least-square analysis (Harvey, 1990) using following model:

$$Y_{ijkl} = m + S_i + H_j + G_k + e_{ijk}$$

Where, Y_{ijkl}= value of traits measured on ijk^{th} individual, m= overall mean, S_i= random effect of i^{th} sire, H_j= fixed effect of j^{th} hatch, G_k= effect of k^{th} genotype of particular candidate gene associated candidate gene in layer of individual Y_{ijkl}, e_{ijk}= Random error associated with each observed Y_{ijkl} and assumed to be NID (0, s_2)

RESULTS AND DISCUSSION

Allelic profile of *cGH* with 1164 bp amplicon size was digested via restriction enzyme *SacI*. Three different alleles, C (570/450), B (1164/1020/144) and A (1020/ 144) have been resolved after digestion. The samples with only B allele or A were identified as BB and AA genotype. The heterozygotes that possessed both alleles, B and C and have been identified as BC genotypes. Allelic patterns observed in RIR breed was depicted in the Fig. 1. The samples with genotype AA reveled two fragments of 1024 bp and 144 bp sizes whereas samples with genotype BB had shown three fragments of 1164 bp, 1020 bp and 144 bp sizes. Genotype BC has resolved five fragments with 1164 bp, 1020 bp, 570 bp, 450 bp and 144 bp sizes. The restricted digested fragments showed that *cGH* was polymorphic with *SacI*. Allele and genotype frequencies for polymorphic region was depicted in Table 2. As seen in result of *cGH/SacI* RFLP study, gene frequencies of A, C and B alleles were estimated as 0.17, 0.07 and 0.76, respectively and genotype frequencies of AA, BC and BB were 0.17, 0.13 and 0.7, respectively. Chethan (2018) studied polymorphism in Wh ite Leghorn chicken and reported *SacI* polymorphism on *cGH*, individuals of four genotypes were found, i.e., AA, BB, CC and AB.

Table 2: Number of allele and genotypes and their frequencies at regions of *cGH* candidate gene in RIR chicken

Gene	Genotypes	Genotypes frequency	Alleles	Gene frequency
cGH	AA	0.17	A	0.17
	BB	0.7	B	0.76
	BC	0.13	C	0.07

According to current study of *cGH/SacI* PCR-RFLP analysis were found three alleles (A, C and B) along with three genotype (AA, BB and BC) in RIR birds which indicated existence of new restriction site in this tested population compared than the genotype reported earlier in this farm. It was observed in Chi square test that RIR population under selection were found not in H-W equilibrium with *cGH*.

Association of cGH/SacI genotype with growth traits

In RIR breed, least squares mean of growth traits for different *cGH/SacI* -RFLP genotypes was depicted in table 3 and 4. The effects of RFLP genotypes on growth traits were analyzed by using LS-ANOVA technique. RFLP genotype was considered to be independent fixed variable within model and LS-ANOVA and LS means estimates of traits were determined. Genotypes significantly affected BW20, BW40 at 15% significance level. In RIR birds with the BC genotype, it was observed that their BW was significantly higher at 20 weeks i.e. 1431.84±70.04 g as compared to birds with AA and BB genotype i.e. 1252.73±57.65 g and 1377.95±46.11 g. However, birds having BC genotypes were also related to significantly higher (P≤0.15) BW40 as 1750.64±76.31 g which was greater than other genotypes. Ghormade *et al.* (2012) reported similar results i.e. observed significant effect of *cGH* genotypes (Genotype AA, BB, AB) on BW20 and BW40 in

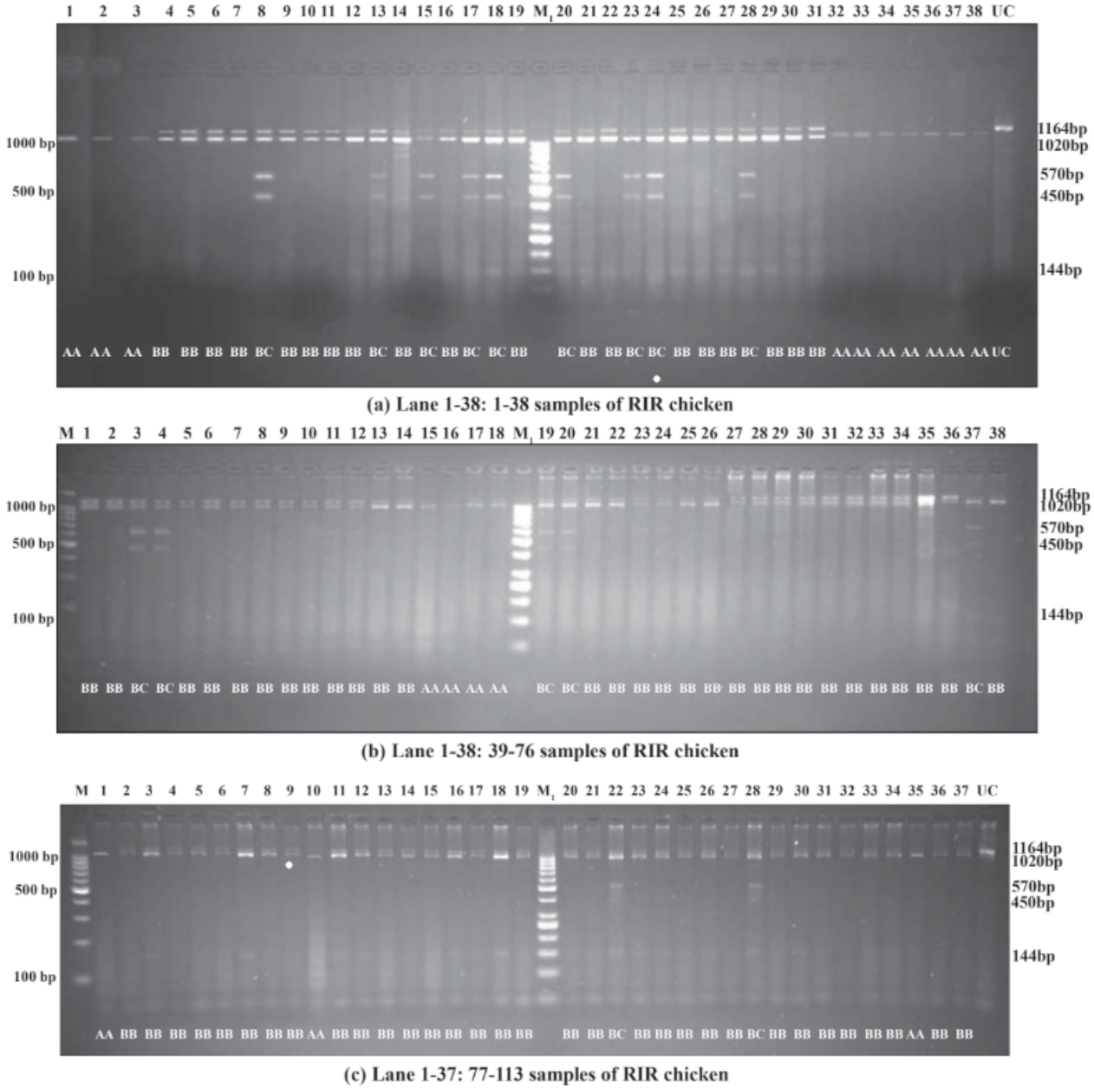

Fig. 1: *Sac I PCR-RFLP pattern of cGH gene in RIR chicken. (a –c) M: 100 bp DNA ladder, M1: 50bp DNA ladder, UC: uncut PCR product. Various genotypes have been indicated below the lanes. Genotypes with allele size have been shown on the right side; size of marker on the left side and the genotype below the amplicons in each lane.*

Kadaknath. Chethan *et al.* (2018) also reported *cGH SacI* were significantly associated with BW16 in RIR chickens. *cGH/SacI* polymorphism showed that pullet with CC´ had highest BW16 when compare to other genotypes (P≤0.15).

Comparing scientific studies for the association of *cGH/SacI* RFLP genotypes among RIR chickens was not available. Therefore, future research on other chicken breeds may be use the current study of *cGH/SacI* RFLP as baseline information.

Table 3: least squares ANOVA for *cGH (SacI*-RFLP*)* genotypes on various growth traits in Rhode Island Red (RIR)

Source	DF	P value	
		BW20	BW40
Sire	38	29342.56	63940.59
Hatch	1	10005.92	167640.93
cGH (SacI) Genotype	2	79672.88	88673.73

Figure in parenthesis is the degree of freedom

Table 4: Least squares means and standard errors of various growth traits for different genotypes at *cGH (SacI-RFLP)* in Rhode Island Red (RIR)

Genotypes	**N**	BW20 (g)	BW40 (g)
µ		1354.18±27.37	1630.16±39.97
AA	15	1252.73±57.65[a]	1537.19±64.93[a]
BB	44	1377.95±46.11[b]	1602.63±54.75[b]
BC	12	1431.84±70.04[c]	1750.64±76.31[c]

Figure in parenthesis is the number of observations; Means with different superscripts in a column differ significant

CONCLUSION

In conclusion, the association between growth traits and *SacI-RFLP* genotypes was determined. Present finding suggest that *cGH* locus BC RFLP genotype demonstrated a potential marker to enhance growth and this loci may be used as further studies on association of *SacI-RFLP* genotype with growth traits in RIR chicken. Furthermore, the association with this trait may be advantageous for elaboration of chicken breeding programmes in molecular marker-assisted selection (MAS). There were no scientific studies for comparing the association of *cGH/SacI* RFLP genotypes among RIR chickens with growth traits. So, this current study can used as baseline information for future research on other chicken breeds.

ACKNOWLEDGEMENT

The author thanks to incharge of Experimental Layer Farm of the Institute, CARI, Izatnagar for providing help during sample and data collection and for providing me all the necessary facilities to conduct the research and also thankful to the Director, ICAR-CARI, Izatnagar and Director, ICAR-IVRI for providing all the necessary facilities to carry out the experiment.

REFERENCES

20th Livestock Census, 2020. Department of Animal Husbandry, Dairying & Fisheries Annual Report, Ministry of Agriculture, Govt. of India, New Delhi.

BAHS, 2021-22. Basic Animal Husbandry and Fisheries Statistics. Ministry of Agriculture and farmers welfare, Government of India.

Chethan, R.R. 2018. Polymorphism in egg production linked genes and their association with economic traits of White Leghorn chicken. PhD Thesis, Deemed University, IVRI, Izatnagar.

Feng, X.P., Kuhnlein, U., Aggrey, S.E., Gavora, J.S. and Zadworny, D. 1997. Trait association of genetic markers in the growth hormone and the growth hormone receptor gene in a White Leghorn strain. *Poultry Science.*, **76**(12):1770-1775.

Ghormade, V., Parmar, S.N.S., Thakur M.S., Mahajan V., Sharma G. and Patel M. 2012 Chicken growth hormone gene polymorphism and its associations with growth traits. *Indian Journal of Animal Sciences*, **82** (1): 92-94

Harvey, W.R. 1990. User's guide for LSMLMW, mixed model least squares and maximum likelihood computer programme. Ohio State University (Monograph).

Kansaku, N., Nakada, A., Okabayashi, H., Guémené, D., Kuhnlein, U., Zadworny, D. and Shimada, K. 2003. DNA polymorphism in the chicken growth hormone gene: Association with egg production. *Animal Science Journal*, **74**(3): 243-244.

Makhsous, S.G., Mirhoseini, S.Z., Zamiri, M.J. and Niazi, A. 2013. Polymorphisms of growth hormone gene in a native chicken population: association with egg production. *Bulletin of the Veterinary Institute in Pulawy*., **57**(1):73-77.

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 Hereditory*, **96**(6): 698-703.

Saini, M., Das, D.K., Dhara, A., Swarup, D., Yadav, M.P. and Gupta, P.K. 2007. Characterisation of peacock *(Pavo cristatus*) mitochondrial 12S rRNA sequence and its use in differentiation from closely related poultry species. *British Poultry Science*, **48**(2):162-166.

Vasilatos-Younken, R., Zhou, Y., Wang, X., McMurtry, J.P., Rosebrough, W., Decuypere, E., Buys, N., Darras, M., Geyten, V., Tomas, F. 2000. Altered chicken thyroid hormone metabolism with chronic GH enhancement in vivo: consequences for skeletal muscle growth. *Journal of Endocrinology*. 166: 609-620.