

*Original Research***Polymorphisms in Exon-1 and Partial Intron-1 of Growth Hormone Gene and its Association with Body Weight Traits in Broiler****Sunita Meena, G. C. Gahlot, U. Pannu, Durga Devi, Hemlata Chouhan, M. Ashraf and Hiteshwar Singh Yadav**

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Abstract

In this study chicken growth hormone (*cGH*) gene polymorphism has been investigated in broiler by PCR-RFLP. The genomic DNA was extracted from 150 samples by using modified spin column technique. The DNA fragment of the growth hormone gene with 776 bp was amplified by PCR using specific primers. Then the PCR products were digested with *MspI* restriction enzyme and analyzed on 8% polyacrylamide gel. The allelic frequency of exon-1 and partial intron-1 locus for A and B allele were 0.39 and 0.61, respectively. The genotypic frequency for AA, AB and BB in Broilers were 0.22, 0.28 and 0.50, respectively. The result showed that the exon-1 and partial intron-1 of *cGH* is polymorphic.

Key words: Chicken Growth Hormone, Polymorphism, PCR-RFLP

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Introduction

Poultry production is an important and diverse component of agriculture all over the world. Today, more attention has been given to poultry due to their quality of meat and sustainable production (Kaya and Yıldız, 2008). Growth hormone (GH) is secreted from the anterior pituitary gland and affects a wide variety of physiological functions in birds (Kansaku *et al.*, 2008). The chicken growth hormone (*cGH*) gene is one of the effective gene that influenced the chicken performance traits because it plays a crucial role in growth and metabolism (Vasilatos-Younken *et al.*, 2000; Nie *et al.*, 2005). Polymorphisms in the *cGH* gene were widely studied by restriction fragment length polymorphisms (RFLPs) or sequencing (Mou *et al.*, 1995; Tanaka *et al.*, 1992). Molecular technology has provided new opportunities to evaluate genetic variability at the DNA level (Kaya and Yıldız, 2008). Many candidate genes associated with poultry productivity have

been identified (Abdi *et al.*, 2014; Fatemi *et al.*, 2012; Xu *et al.*, 2011a; Xu *et al.*, 2011b). A significant correlation was reported between GH gene and meat quality in Anka and Rugao hens (Sheng *et al.*, 2008). Polymorphism in the chicken growth hormone gene has positive correlation with resistance and egg production (Kuhnlein *et al.*, 1997). The purpose of the present research was to estimate genetic variability of *cGH* gene and to investigate their associations with the growth traits of the broiler in India.

Material and Methods

Ethical Approval

This experiment was conducted by Animal Genetics and Breeding department, College of Veterinary and Animal Science, Bikaner with all essential procedures of sample collection were performed strictly as specified by Institutional Ethical Committee with minimal stress to Birds.

Sample Material

For present investigation blood samples were collected from the 150 unrelated broilers were selected from poultry farm situated in the campus of College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Science, Bikaner and approximately 0.5-1.0 ml of venous blood samples were collected in sterile vacutainer tubes containing 0.5 M EDTA as anticoagulant. The blood samples were gently mixed with EDTA and kept in ice pack to prevent cell lysis and stored at 4°C until the isolation of genomic DNA.

DNA Extraction and PCR

Genomic DNA was extracted using the spin column method (Sambrook and Russell, 2001) under manufacture protocols with slight modification through genomic DNA Isolation kit (Himedia Pvt. Ltd). The quality of the extracted DNA was checked by Agarose gel electrophoreses. Based on *cGH* gene sequence (GenBank accession number: EF452679) primers were designed through online primer-3 software. The specificity of primer pairs was confirmed on the basis of available sequence of *cGH* gene at the National Center of Biotechnology Information (NCBI). The forward and reverse primers were 5'ATCCCCAGGCAACATCCTC3' and 5'CCTCGAC ATCCAGGTCACAT3', respectively. The PCR reactions were performed with a total volume of 25 ml. The cycling conditions were as follow: 5 min at 95°C for initiation of denaturation, then 45 s at 94°C (denaturation), 1 min at 55°C (annealing), 60 s at 72°C for extension (35 cycles), followed by 10 min at 72°C for final extension.

Polymorphism Detection

The polymorphism of *cGH* was detected with PCR-RFLP. The 30 µl reaction mixture was prepared to adding 17 µl nuclease free water, 2 µl 10X Buffer and 10 µl PCR product were digested with 1 µl *MspI*

restriction enzyme. This enzyme acts on 5'...C↓CGG...3' and 3'...GGC↑C...5'. Then it kept for digestion in incubator at 37°C overnight. After the digestion, heat inactivation of the enzyme was done at 80°C for 20 min. The restriction fragments were resolved by polyacrylamide gel electrophoresis on 8% gel in 1X TBE buffer at 120V for 1.5 hrs.

Traits and Statistical Analysis

The genotypes were detected by seeing the RFLP patterns of each sample in the RFLP gels. The frequency of exon-1 and partial intron-1 of growth hormone genotypic patterns were estimated by standard procedure (Falconer and Mackey, 1996).

$$\text{Genotyping patterns frequencies} = \frac{\text{Total no.of individuals of a particular genotype}}{\text{Total no.of individuals of all genotype}}$$

$$\text{Gene frequencies} = \frac{2D+H}{2N}$$

Where, D = number of birds homozygous for a particular allele, H = number of heterozygote birds and N = total number of birds

Analysis of Association between PCR-RFLP Genotypes Variants and Body Weight Traits

The association between genotypic patterns of chicken growth hormone gene (*cGH*) with body weight traits in broiler was carried out through General Linear Model (GLM) procedure of SPSS by following statistical mode -

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

Where, Y_{ij} = body weight of the i^{th} Genotype, μ = Overall mean, G_i = Fixed effect of j^{th} genotype, e_{ij} = Random error NID (0, σ^2).

Result and Discussion

PCR Amplification of *cGH* gene

The PCR products with 776 bp length which run on 1.5% agarose gel were presented in Fig. 1.

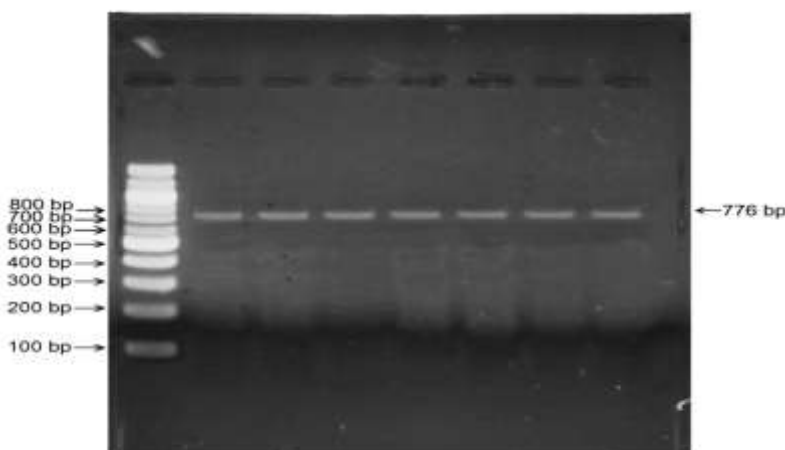


Fig. 1: Amplification of *cGH* (exon-1 and partial intron-1) gene using the specific primer of polymerase chain reaction in broiler visualize under UV Illuminator stained with *EtBr*. A commercial DNA marker was used for size analysis.

RFLP Analysis of *cGH* Gene

Three genotypic patterns *viz.* AA (235 and 541 bp fragments), AB (126, 148, 235, 267 bp fragments) and BB (148, 235, and 393 bp fragments) were detected by digesting the 776 bp chicken growth hormone gene region by *MspI* restriction endonuclease which has C[^]CGG as its restriction site. These genotypes were found in all the strains. These restriction patterns were in concurrence with the observations made earlier by Kaur *et al.* (2008) in Panjabi broiler and Singh *et al.* (2008) in Rhode Island red and White Leghorn layer (Fig. 2).

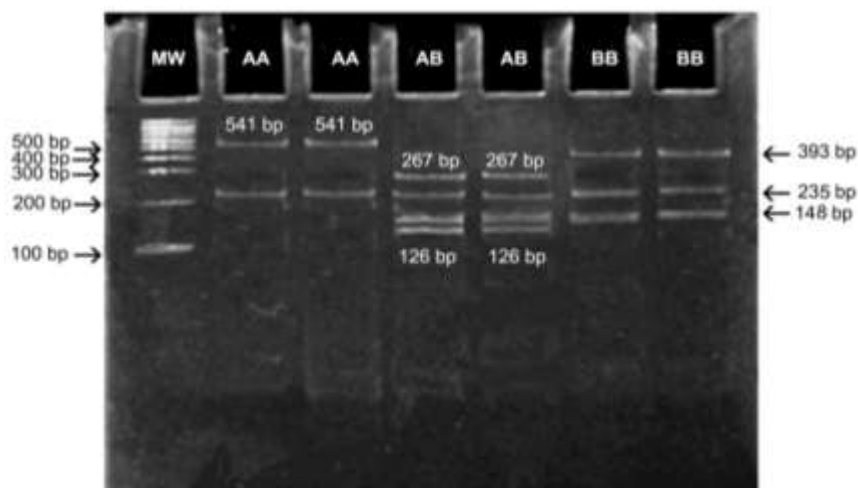


Fig. 2: 8% polyacrylamide gel electrophoresis and restriction fragment length polymorphism (RFLP) analysis of polymerase chain reaction (PCR) amplified product of exon-1 and partial intron-1 in Chicken Growth Hormone (*cGH*) gene digested with *MspI* in broiler. Lane 1: DNA marker, Lane 1-2: genotype AA, Lane 3-4: genotype AB, Lane 5-6: genotype BB.

Gene and Genotypic Frequency of Chicken Growth Hormone (*cGH*) Gene

The gene and genotypic frequency were observed for RFLP restricted genotypic patterns. The three genotypes AA, AB and BB were distributed in the frequency of 0.22, 0.28 and 0.5, respectively. The genotypic pattern 'BB' was observed in most (n=75) of the birds. The results indicated that genotypic patterns BB were dominant in the chicken population. The genotypic patterns 'AA' and 'AB' were present in low number. The estimated allele frequency of A and B allele as observed is 0.39 and 0.61, respectively. The allele A is found to be less frequent in comparison to allele B.

Table 1: Association study of genotypic patterns of *cGH* gene with body weights in broiler

Genotypic Patterns	Body Weight (gms)			
	Mean \pm SE			
	Day-14	Day-21	Day-28	Day-35
	NS	NS	NS	NS
AA	225.47 \pm 5.39	331.72 \pm 8.34	561.35 \pm 13.00	929.08 \pm 17.84
AB	226.20 \pm 6.25	333.88 \pm 9.67	578.63 \pm 15.07	950.57 \pm 20.68
BB	232.04 \pm 4.08	343.29 \pm 6.31	591.845 \pm 9.86	964.02 \pm 13.51

NS: Non significant

The statistical analysis involving association of genotypic patterns of chicken growth hormone gene with body weight parameters in broiler was carried out using General Linear Model (GLM) procedure of SPSS. The three genotypic patterns 'AA', 'AB' and 'BB' of *cGH* exon-1 and partial intron-1 gene in broiler did not reveal any significant association with body weight at any stage of life. However, non-significantly higher body weight was observed for 'BB' genotypic pattern at day-14, day-21, day-28 and day-35 of age.

Conclusion

The results of the current study indicated that exon-1 and partial intron-1 of *cGH* is polymorphic in broiler. Therefore, the *cGH* gene could be exploited as a candidate gene for marker-assisted selection (MAS) in broiler. Further studies are required to evaluate the association of production traits with *cGH* gene polymorphism broiler.

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References

1. Abdi, M., Seyedabadi, H. and Gorbani, A. (2014). Prolactin and NPY Gene Polymorphism and its Associations with Production and Reproductive traits in West-Azarbaijan Native chicken. *Bulletin, Enviromental Pharmacology Life Science*, 3, 39-45.

2. Falconer, D. S. and Mackay, F.C. (1996). *Introduction to quantitative genetics*. Longman group Ltd. ISBN 0-321: 04347-2.
3. Fatemi, S. A., Mehrabani-Yeganeh, H., Nejati-Javaremi, A. and Niknafs, S. (2012). Association of neuropeptide Y and gonadotrophin-releasing hormone receptor gene SNPs with breeding value for growth and egg production traits in Mazandaran native chickens. *Genetic Molecular Research 11*(3), 2539-2547.
4. Kansaku, N., Hiyama, G., Sasanami, T. and Zadworny, D. (2008). Prolactin and growth hormone in birds: Protein structure, gene structure and genetic variation. *The journal of poultry science*, 45(1), 1-6.
5. Kaur, T., Kumar, G. R., Bajwa, I. S. and Trehan, P. K. (2008). PCR-RFLP of growth hormone gene in meat type chicken. *Indian Journal of Poultry Science*, 43(2), 129-133.
6. Kaya, M. and Yıldız, M. A. (2008). Genetic diversity among Turkish native chickens, Denizli and Gerze, estimated by microsatellite markers. *Biochemical genetics*, 46(7-8), 480-491.
7. Kuhnlein, U., Ni, L., Zadworny, D. and Fairfull, W. (1997). DNA polymorphisms in the chicken growth hormone gene response to selection for disease resistance and association with egg production. *Animal genetics*, 28(2), 116-123.
8. Mou, L., Liu, N., Zadworny, D., Chalifour, L. and Kuhnlein, U. (1995). Presence of an additional PstI fragment in intron 1 of the chicken growth hormone-encoding gene. *Gene*, 160(2), 313-314.
9. Nie, Q., Sun, B., Zhang, D., Luo, C., Ishag, N. A., Lei, M. 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.
10. Sambrook, J. and Russell, D. W. (2001). *Molecular Cloning: A Laboratory Manual*. 3rd Edn. Cold spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.
11. Sheng-Long, W., Musa, H. H., Wen-Bin, B., Ke-Hua, W., Guo-Qian, Z. and Guo-Hong, C. (2008). Mutation in exon 4 apo VLDL-II gene is a candidate for meat tenderness in chickens. *Journal of Animal and Veterinary Advances*, 7(12), 1624-1627.
12. Singh, S. P., Brah, G. S., Amrita, C. and Kumar, G. R. (2008). PCR-RFLP of chicken growth hormone gene in egg-type chickens. *Indian Journal of Poultry Science*, 43(1), 1-4.
13. 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), 235-239.
14. Vasilatos-Younken, R., Zhou, Y., Wang, X., McMurtry, J. P., Rosebrough, R. W., Decuypere, E. and Tomas, F. (2000). Altered chicken thyroid hormone metabolism with chronic GH enhancement in vivo consequences for skeletal muscle growth. *Journal of Endocrinology*, 166(3), 609-620.
15. Xu, H., Zeng, H., Luo, C., Zhang, D., Wang, Q., Sun, L., Yang, L., Zhou, M., Nie, Q. and Zhang, X. (2011a) Genetic effects of polymorphisms in candidate gens and the QTL region on chicken age at first egg. *BMC Genetics 12*:33. DOI: 10.1186/1471-2156-12-33.
16. Xu, HP., Zeng, H., Zhang, DX, Jia, XL, Luo, CL, Fang, MX, Nie, QH, Zhang and XQ (2011b) Polymorphisms associated with egg number at 300 days of age in chickens. *Genetics and Molecular Research*, 10: 2279-2289. DOI: 10.4238/2011.October.3.5