International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 272-274

Received: 15-05-2018
Accepted: 16-06-2018

Sunita Meena
Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

GC Gahlot
Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

M Ashraf
Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

U Pannu
Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

H Chouhan
Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Garima Choudhary
Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Correspondence
Sunita Meena
Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Study of genetic variability of chicken growth hormone gene in broiler strain (vencobb-400) by PCR-RFLP

Sunita Meena, GC Gahlot, M Ashraf, U Pannu, H Chouhan and Garima Choudhary

Abstract
Present study to estimate the genetic variability of Chicken growth hormone gene in broiler strain (Vencobb-400). In order to study and investigate chicken growth hormone gene polymorphism, blood samples were collected from 150 individuals of broiler and Genomic DNA was extracted from whole blood using spin column method as per manufacture protocol with slight modification. The purity of extracted genomic DNA was determined by 0.8% agarose gel electrophoresis. DNA fragment with 776 bp at Chicken growth hormone gene was amplified using Polymerase Chain Reaction (PCR). The PCR product was digested with restriction enzyme *Msp*I and subsequently electroforesed on 8% polyacrylamide gel with ethidium bromide. In this population, heterozygosis was estimated 0.28 and obtained genotypes frequency of *Msp*I enzyme on 776 bp fragment for AA, AB and BB genotype were .22, .28, and .50, respectively. The gene frequency of A and B alleles were .39 and .61, respectively.

Keywords: Chicken growth hormone, heterozygosity, polymorphism, gene and genotype

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) [4]. The chicken growth hormone *(cGH)* gene is one of the effective genes that influenced the chicken performance traits because it plays a crucial role in growth and metabolism (Vasilatos-Younken *et al*., 2000; Que *et al*., 2005) [1]. The *cGH* is a 22-kDa protein, containing 191 amino acid residues (Hrabia *et al.,* 2008) [5]. In poultry, *cGH* consists of 4,101 base pairs, having five exons and four introns (Kansaku *et al.,* 2008) [3], known as a polypeptide, hormone produced, and secreted by pituitary gland; *cGH* affects a variety of physiological functions in growth performance (Byatt *et al*., 1993; Apa *et al*., 1994) [6, 7]. Molecular technology has provided new opportunities to evaluate genetic variability at the DNA level (Kaya and Yildiz, 2008) [4]. The purpose of the present research was to estimate genetic variability of Chicken growth hormone gene of the broiler strain (Vencobb-400).

Material and Methods
150 unrelated birds of broiler strain (vencobb-400) were selected from poultry farm situated in the campus of Rajasthan University of Veterinary and Animal Sciences, Bikaner. Blood samples (0.25-0.5 mL) were collected from the wing vein using EDTA as an anti-coagulating agent. Blood samples were stored at −20 ◦C. 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. Genomic DNA (2.5 µl) was amplified with 8.9 µl Nuclease Free water (NFW), 13 µl PCR master mixture and 0.3 µl of each primer in total volume of 25 µl. The PCR reaction mixture was subjected to the an initial 5 min denaturation at 95 °C, followed by 35 cycles of denaturation at 94 °C for 45 sec, annealing at 55 °C for 1 min, extension at 72 °C for 60 sec. and final extension at 72 °C for 10 min. After the addition of 5 µl of amplification product were loaded on 1.5% denaturing agarose gels. To visualize the PCR product, gels were stained with ethidium bromide. The stained gels were scanned and genotypes were scored. Then, for digestion by enzyme and determination of RFLP, 10 µl of PCR product (776 bp) was added to 1 µl of *Msp*I enzyme and

2 µl 10X Buffer and final volume was 30 µl with dilution 17 µl Nuclease Free Water and digested in incubater at 37 °C overnight. Digested samples were loaded by 8% polyacrylamide gel electrophoresis at 120V for 1.5 hours. Then, stained with ethidium bromide the fragments were visualized by UV Transilluminator. Then, genotypes were scored.

Results and discussion

DNA extraction and quality determination with electrophoresis and UV Transilluminator was done obtained result was acceptable (fig.1). The PCR amplification carried out using the forward and reverse primers were 5'ATCCCCAGGCAACATCCTC3' and 5'CCTCGAC ATCCAGGTCACAT3' respectively. The PCR products with 776 bp length which run on 1.5% agarose gel were presented in Figure 2. With electroforesing of digested samples, 3 types of genotype were diagnosed. In AA genotype 235 and 541 bp band were registered. In AB genotype 126, 148, 235 and 267 bp bands were registered and BB genotype 148, 235, and 393 bp bands were determined. In total, 43 samples have AA genotype, 32 samples have AB genotype and 75 samples shown BB genotype (fig.3). These restriction patterns were in concurrence with the observations made earlier by Kaur *et al*. (2008) [8] in Panjabi broiler and Singh *et al*. (2008) [9] in Rhode Island red and White Leghorn layer (Figure 2). The genotype and gene frequency in population is shown in Table 1. Calculations were done with standard formula (Falconer and Mackay, 1996) [10]. The observed heterozigosity was 0.28 in this study.

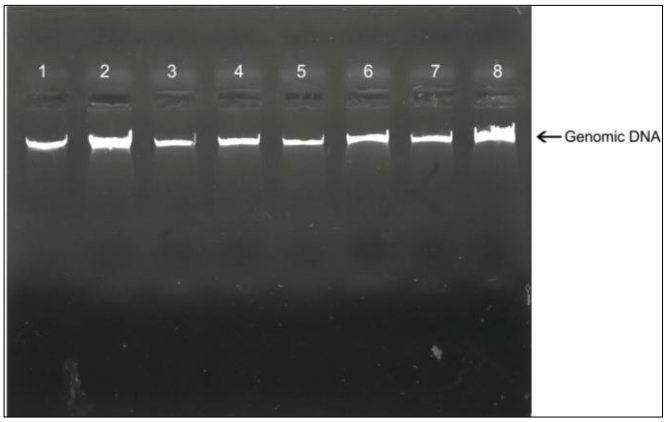

Fig 1: DNA extracted samples

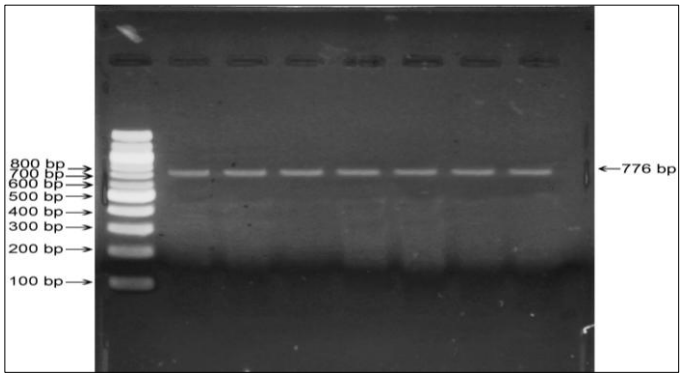

Fig 2: Electrophoresed PCR product on agarose gel; Marker 100 bp

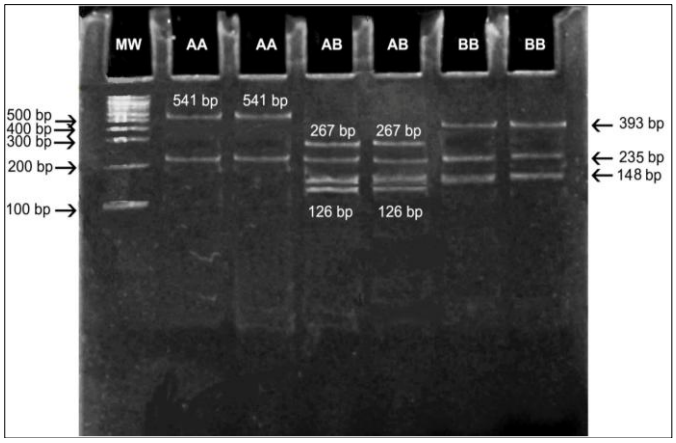

Fig 3: Eletroforesing digested product on polyacrylamide gel: Marker 100 bp

Table 1: Obtained genotype and gene frequency of MspI enzyme on 776 bp fragment.

Genotype	Number	Frequency
AA	43	0.22
AB	32	0.28
BB	75	0.50
Allele		
A	118	.39
B	182	.61

Acknowledgments

The authors are thankful to dean and Faculty Chairman of the Institute, for providing all facilities and financial support to carry out the work. The helps rendered by the staffs of Poultry Farm are also duly acknowledged.

References

1. Vasilatos-Younken, Zhou Y, Wang X, Mc Murtry JP, Rosebrough W, Decuypere E *et al*. Journal Endocrinol. 2000; 166:609-620.
2. Q Nie, B Sun, D Zhang, C Luo, NA Ishag, M Lei, G Yang, Zhang X. Journal of Heredity. 2005; 96:698-703.
3. Kansaku N, Hiyama G, Sasanami T, Zadwomy D. Prolactin and growth hormone in birds: Protein structure, gene structure and genetic variation. J Poultry Sci. 2008; 45:1-6.
4. Kaya M, Yıldız MA. Genetic diversity among Turkish native chickens, Denizli and Gerze, estimated by microsatellite markers. Biochemical genetics. 2008; 46(7-8):480-491.
5. Hrabia A, Paczoska-Eliasiewicz HE, Berghman LR, Harvey S, Rząsa J. Expression and localization of growth hormone and its receptors in the chicken ovary during sexual maturation. Cell and tissue research. 2008; 332(2):317-328.
6. Byatt JC, Staten NR, Salsgiver WJ, Kostelc JG, Collier RJ. Stimulation of food intake and weight gain in mature female rats by bovine prolactin and bovine growth hormone. American Journal of Physiology-Endocrinology and Metabolism. 1993; 264(6):E986-E992.
7. Apa R, Lanzone A, Miceli F, Mastrandrea M, Caruso A, Mancuso S *et al*. Growth hormone induces *in vitro* maturation of follicle-and cumulus-enclosed rat oocytes. Molecular and cellular endocrinology. 1994; 106(1):207-212.

8. Kaur T, Kumar GR, Bajwa IS, Trehan PK. PCR-RFLP of growth hormone gene in meat type chicken. Indian Journal of Poultry Science. 2008; 43(2):129-133.
9. Singh SP, Brah GS, Amrita C, Kumar GR. PCR-RFLP of chicken growth hormone gene in egg-type chickens. Indian Journal of Poultry Science. 2008; 43(1):1-4.
10. Falconer DS, Mackay FC. Introduction to quantitative genetics. Longman group Ltd. ISBN. 1996; 321:04347-2.
11. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. 3rd Edn. Cold spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press, 2001.