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Chicken growth hormone gene polymorphism and its associations with growth traits

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The chicken growth hormone (cGH) gene is considered one of the most candidate genes that can influence chicken performance traits because of its crucial function in growth, egg production, body composition, appetite control, ageing, reproduction and metabolism (Vasilatos-Younken *et al.* 1997). Polymorphism in the cGH gene was widely studied by restriction fragment length polymorphism (RFLP) or sequencing. RFLP was characterized in the introns of cGH gene of White Leghorn and it has been suggested that the alleles identified were linked to egg production traits, resistance to Marek's disease and avian leukosis (Yan *et al.* 2003). PCR-RFLP was also studied in various populations of Chinese native chickens and it was suggested that an allele present in intron1 might be linked to laying performance (Stephen *et al.* 2001). PCR-RFLP analysis of cGH in Kadaknath chicken suggested the role of A allele at cGH1 locus for higher egg production (Thakur *et al.* 2009).

Kadaknath, a native breed of poultry inhabiting Jhabua and Dhar districts in western parts of Madhya Pradesh, lays around 80–90 eggs annually and is not a good brooder. This is the only poultry breed having black flesh which is considered not only a delicacy of distinctive taste, but also of aphrodisiac value. The meat and eggs are reckoned to be a rich source of protein (25.47% in flesh) and iron (Thakur *et al.* 2006). Synthetic colored dual type birds are developed from randomly bred multicolored population as dual purpose birds. It has the ability to grow faster and the bird produces good number of eggs with heavier weight. Kadaknath cross is developed by crossing and backcrossing of pure bred Kadaknath male with black plumage dual type colour birds. The bird is bigger in size and body weight, earlier in sexual maturity and superior in egg production than pure bred Kadaknath. So, the present study was undertaken to detect polymorphisms in the intron I of the c*GH* gene using the RFLP method and to analyze the associations of these polymorphisms with growth traits in Kadaknath, Kadaknath cross and Synthetic colored dual type birds.

Blood samples of 50 unrelated female birds each of Kadaknath, Kadaknath crosses and synthetic colored dual type birds selected randomly were collected from All India Coordinated Research Project (AICRP) on Poultry, College of Veterinary Science and Animal Husbandry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur along with their growth records. DNA was isolated as per John *et al.* (1991) with slight modifications. The purity and quantity of the extracted DNA was checked by Nanodrop spectrophotometer and quality of DNA was assessed on 0.8% horizontal submarine agarose gel electrophoresis. Intron 1 of cGH gene was amplified using a set of primers as described by Kuhnlein *et al.* (1997). The PCR conditions used comprised initial denaturation at 94°C for 10 min followed by 31 cycles of denaturation at 94°C for 1 min then annealing at 55°C for 45 sec and extension at 72°C for 1 min and final extension at 72°C for 10 min. The 770 bp amplified PCR product digested with *MspI* restriction endonuclease (RE). 10 µl PCR product of each sample was digested with 1 µl of *MspI* in a final reaction volume of 30 µl. The reaction mixture was incubated at 37°C for 15 min in water bath. Restriction digests were electrophoresed on 2% of agarose gel along with 100 bp DNA ladder as a molecular size marker. Genotype frequencies, gene frequencies and genetic equilibrium at different loci were estimated using software POPGENE 32 version 1.32, the user-friendly software for population genetic analysis. (Yeh *et al.* 1999) and the association of various polymorphic variants of cGH gene with economic traits were analyzed by mixed model least squares and maximum likelihood computer programme PC-2 (Harvey 1990).

The 770 bp amplicon of cGH gene on digestion with *Msp*I revealed 3 different patterns (AA, AB and BB). The 3 different RFLP patterns revealed restriction fragments of 529, 373, 241 and 156 bp sizes with 2 restriction site at 373 bp

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Table 1. Least squares means and standard errors of genotypes for adult body weight at 20 and 40 weeks (g)

Genotype	BW (20 week)			BWC (40 week)		
	Breed			Breed		
	Kadaknath	Kadaknath crosses	Synthetic colored dual type birds	Kadaknath	Kadaknath crosses	Synthetic colored dual type birds
AA	954.00±15.43[b] (15)	1112.40±11.95[b] (25)	1772.14±15.97[b] (14)	1452.67±18.92[b] (15)	1699.20±14.66[b] (25)	2065.71±19.59[b] (14)
AB	966.84±13.71[a] (19)	1153.57±15.97[a] (14)	1824.44±14.09[a] (18)	1471.05±16.81[a] (19)	1754.29±19.59[a] (14)	2128.33±17.28[a] (18)
BB	967.19±14.94[a] (16)	1119.09±18.02[b] (11)	1755.56±14.09[b] (18)	1458.75±18.32[b] (16)	1705.46±22.09 [b] (11)	2051.11±17.28[b] (18)
Overall	962.68±8.49 (50)	1128.35±8.96 (50)	1784.05±8.51 (50)	1460.82±10.42 (50)	1719.65±10.99 (50)	2081.72±10.44 (50)

Figures in parenthesis shows number of birds in each genotype, values with different superscript in columns differ significantly (P<0.05).

(site A) and 529 bp (site B) position in 770 bp amplicon. An allele with presence of only 1 site at 529 bp was considered as 'A' allele and presence of 2 restriction sites at 373 and 241bp was considered as 'B' allele. Genotypic frequencies in indigenous Kadaknath, Kadaknath crosses and synthetic colored dual type birds for AA genotype were 0.30, 0.50 and 0.28; for AB genotype 0.38, 0.28 and 0.36 and for BB genotype 0.32, 0.22 and 0.36 respectively. The allelic frequencies in Kadaknath, Kadaknath crosses and synthetic colored dual type birds for A allele were 0.49, 0.64 and 0.46 and for B allele 0.51, 0.36 and 0.54 respectively. The highly significant Chi-square value (7.69, P<0.05) in Kadaknath crosses showed that the population was not in Hardy Weinberg equilibrium, however nonsignificant Chi-square value in other 2 breeds showed that the population was in equilibrium.

Association of growth hormone-gene polymorphic variants with growth traits: To find out the association between various polymorphic variants/genotypes with adult body weight at 20 and 40 weeks, least squares analysis of variance was conducted. The result of least squares analysis of variance showed highly significant differences among breeds and genotypes, where as the effect of breed × genotype interaction was nonsignificant. The least squares means of genotype for adult body weight at 20 weeks (g) in Kadaknath, Kadaknath crosses and synthetic colored dual type birds were 962.68±8.49, 1128.35±8.96 and 1784.05±8.51 respectively. The least squares means for adult body weight at 40 weeks (g) in Kadaknath, Kadaknath crosses and synthetic colored dual type birds were 1460.82±10.42, 1719.65±10.99 and 2081.72±10.44 respectively. The breed-wise genotypic least squares means for adult body weight at 20 and 40 weeks (g) for each genotype among all the 3 breeds are presented in Table 1.

The overall genotypic least squares mean for adult body weight at 20 and 40 weeks was highest for synthetic colored dual type birds followed by Kadaknath crosses and Kadaknath birds showing lowest adult body weight at 20 and 40 weeks. The genotypic least squares means among all the 3 breeds and within breed genotypic least squares means for AA, AB and BB genotypes among all the 3 breeds were highly significant (P<0.01). Significant mean differences in body weight were also reported by Kaur *et al.* (2008) in meat type chicken strain PB-2. Significant differences were also observed in least squares means among various genotypes among all the breeds. In all breed, AB genotype was found to be superior to both AA and BB genotypes where as no significant difference is observed in AA and BB genotypes.

SUMMARY

Present study was conducted on chicken growth hormone (cGH) gene polymorphism in Kadaknath, Kadaknath crosses and synthetic colored dual type birds using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. The 770 bp PCR product was obtained and digested with *MspI* restriction enzyme. Three different RFLP patterns were obtained at 2 restriction sites corresponding to AA, AB and BB genotypes. The allelic frequencies in indigenous Kadaknath, Kadaknath crosses and synthetic colored dual type birds for A allele were found to be 0.49, 0.64 and 0.46 and for B allele 0.51, 0.36 and 0.54 respectively. The highly significant Chi-square value in Kadaknath crosses showed that the population is not in Hardy Weinberg equilibrium, however non significant. Chi-square value in other two breeds showed that the population is in equilibrium. Among the traits considered the least squares analysis of variance for different genotypes in all the 3 breeds revealed significant differences for adult body weight at 20 weeks and 40 weeks. Genotype AB was superior to AA and BB for adult body weight at 20 weeks and 40 weeks. The significant differences between genotypic least squares means at 20 and 40 weeks of age among all the 3 breeds indicate the effect of

growth hormone gene locus on growth traits. Higher body weight was observed for AB genotype among all the 3 breeds clearly indicating the superiority of AB genotypes than AA and BB genotypes. Hence it is recommended that birds having AB genotype may be selected as parents for future breeding.

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