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Research Paper Veterinary

Dna Polymorphism Studies On Ghrelin Gene In Chicken

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ABSTRACT

Ghrelin is a key actor in the hypothalamic melanocortin system. The present investigation was carried out to study nucleotide sequencing and DNA polymorphism by PCR-RFLP of ghrelin gene in four strains of chicken. Genomic DNA was isolated from a total of 200 birds belonging to four Indian strains of chicken namely new Genotype, Punjab Broiler, Indian Cornish and University Male Line. A fragment of ghrelin gene, comprising of a partial of intron 3, complete exon 4 and partial intron 4, was amplified. The products from each variety were digested with Hinf I, which recognizes the 5'- G^ANTC-3' sequence. The RFLP pattern revealed three genotypes (LL, VV and GG) and three different alleles (L, V and G).

Keywords : GHRL gene, Polymorphism, Hinf I, PCR-RFLP

Ghrelin is a novel endogenous ligand for the growth hormone receptor (GHS-R) and is expressed primarily in the stomach and hypothalamus with the probable function of stimulating GH secretion and food intake both in mammals and poultry. Ghrelin was first detected in stomach and was identified as a peripheral metabolic signal informing the brain about nutrient load in the stomach. Ghrelin secreted by the stomach has paracrine or endocrine effects on feeding behavior and GI motility or it circulates in the blood and acts on other target tissues. When ghrelin levels are high, the animal will also increase its feed intake (Barsh et al., 2002).

Ghrelin stimulates feeding in rats, however, intracerebroventricular injection of ghrelin strongly

suppressed feeding in neonatal chicks (Furuse et al., 2001). This anorexic effect was almost identical when chicken or rat ghrelin was administered. Administration of chicken ghrelin increased plasma GH levels in both rats and chicks, with a potency similar to that of rat or human ghrelin. In addition, chicken ghrelin also increased plasma corticosterone levels in growing chicks at a lower dose than in mammals (Kaiya et al., 2002). Analysis of the chicken genome indicates that the ghrelin gene is located on chromosome 12 and spans 5 kb (Richards et al., 2006). The avian ghrelin gene consists of five exons and four introns. This structure is similar to the human gene with the exception of the first exon which is larger in birds. The nucleotide sequence of the ghrelin gene and intron: exon organization has been reported from chicken (Richards et al., 2006) and turkey (Richards et al., 2006). . The complete sequences of ghrelin gene have been reported in humans and mice (Lall et al., 2001). Chicken ghrelin is 26 amino acids long and possesses 54% sequence identity with human ghrelin (Kaiya et al., 2002). The serine residue at position 3 (Ser3) is conserved between the chicken and mammalian species. Chicken ghrelin mRNA is predominantly expressed in the stomach, where it is present in the proventriculus but absent in the gizzard. Meixi et al. (2006) investigated an 8 bp polymorphism in exon 1 of the chicken Ghrelin (cGHRL) gene and was genotyped in a F2 designed full-sib population to analyze its associations with chicken growth rate and body composition traits. Result showed that this 8 bp indel was significantly associated with body weight at the age of 28 days and 56 days. The AA genotype was superior for all the traits. He et al. (2005) used PCR-RFLP and sequencing techniques for studying ghrelin gene in chicken. In addition, the mRNA data tested earlier were also used in this study to evaluate the effect of ghrelin gene expression. Three genotypes were identified and C2100T was confirmed to be the key reason for inducing this polymorphism with evidence from sequencing profiles. Linkage analyses showed that C2100T was significantly associated with body weight. The present study was undertaken with the objective to find out DNA polymorphism, sequence, characterize and investigate GHRL gene polymorphism in meat type chickens.

Materials and methods

Experimental birds

A total of 200 chickens from four strains (50 from each strain) were used. Samples collected randomly from the four chicken genetic groups (New Genotype, Punjabi Broiler-1, Indian Cornish-3 and University Male Line) produced and maintained at poultry farm, veterinary college Bangalore.

DNA extraction

About 1 ml of venous blood was collected from vein of the wing of every bird and mixed with 37µl anticoagulant and 50 µl of whole blood was transferred into 1.5 ml microcentrifuge tubes and basal tip of the calamus from the superior umbilicus were separated from each feather and processed for DNA isolation by phenol/chloroform method as described by sambrook et al. (2001) and Horvath et al. (2005) from blood and feather samples respectively. DNA samples were checked for its quality and concentration.

PCR-RFLP and Sequencing

An 878 bp fragment of cGH gene consisting of the intron-3 (341 bp), exon-4 (109 bp) and intron-4 (428 bp) was amplified by using a set of forward (5' AAGGACACGTGGAAACTGCCAGC3`) and reverse (5' AAGCAGCCTGAGGTGACTGCAA 3') primers were designed using the Primer 3 program. For amplification of all the fragments PCR reactions of 25 ml were prepared separately as follows: 2.5 ml PCR buffer, 10 mM of each primer, 1.5 mM MgCl2, 200 mM of each dNTPs, 3.0 U Taq DNA polymerase and 100 ng of genomic DNA. The amplification was carried out using a MJ Mini Gradiant Thermalcycler, Hot Master Mix with the following conditions: 34 amplification cycles were carried out using a pre-programmed thermal cycler. The initial denaturation was done at 94°C for 5 min, annealing at 62°C for 50 sec, extension at 72°C for 1

min and final extension at 72°C for 10 min. The PCR products of 10ml from each tube were digested with Hinf I restriction enzymes along with the appropriate buffer supplied with the enzyme. The restriction enzyme digested PCR products were electrophoresed in 2.2% agarose gel containing ethidium bromide as staining agent in 1 TBE buffer for 60-75 minute at 100V. The digested products were visualized and documented under gel documentation system.

RESULTS AND DISCUSSION

The fragment of cGH gene (878 bp) has been characterized and successfully amplified from the DNA of each sample (200 samples) used in the present study (Fig. 1). Digestion of 878 bp target region of GHRL gene exon- 4 was carried out with Hinf I endonuclease enzyme (Fig. 2) . Digestion of the PCR product with Hinf I restriction endonuclease which has G^ANTC as its restriction site revealed three different patterns (LL, VV and GG) created by a combination of polymorphic Hinf I cut sits present in intron-3 (one site) and in intron-4 (two sites) (Fig. 3). The LL genotype had 89, 196 and 593 bp fragments and was observed in all the four strains. VV genotype resolved 34, 89 and 755 bp fragments and was found in NG and PB1 strains. GG genotype was a homozygous uncut PCR product and found in IC3 and UM1 strains. There were no heterozygous patterns in these strains. Polymorphism of different regions of ghrelin gene of

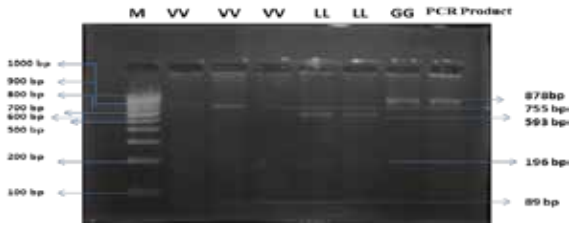

Fig 1: Hinf1 -RFLP pattern of 878 bp fragment of GHRL gene in chicken

M- Molecular marker (100 bp), GG - IC3, UM1, LL - NG, PB1, VV- NG, PB1

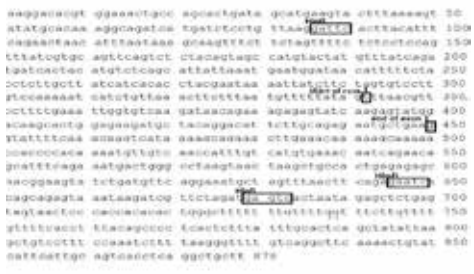

Fig 2. A 878 bp amplicon of GHRL gene and Hinf I cut sites

different product sizes have been reported in 12 Chinese indigenous chickens (Li and Li. 2006) and four other chicken strains (Nie et al., 2009). The overall gene frequencies for L, V and G alleles in four strains of chicken were 0.845, 0.100 and 0.055 respectively. The highest genotype frequency was observed for LL in IC3 (0.96). The LL genotype seems to be favorable one in all the strains studied. Nie et al. (2005) reported, for a different region of ghrelin gene, allelic frequencies of C and T alleles as 0.6552 and 0.3448 in Leghorn strain.

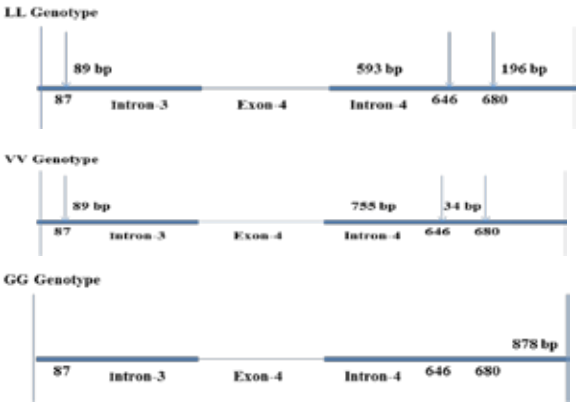

Fig 3. Hinf I Restriction map of LL, VV and GG genotypes of GHRL gene in chicken

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