

Growth Hormone Gene Polymorphism and Its Effect on Birth Weight in Cattle and Buffalo

T. K. Biswas, T. K. Bhattacharya*, A. D. Narayan, S. Badola, Pushendra Kumar and Arjava Sharma
Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P.-243 122, India

ABSTRACT : The study was carried out in Sahiwal, Holstein Friesian, Jersey and crossbred cattle and Murrah, Bhadwari, Jaffarabadi, Nagpuri and Surti buffaloes maintained at different organized herds to work out the polymorphism at growth hormone locus and study its effect on birth weight. A 223 bp fragment of the gene was amplified and digested with *Afu* I restriction enzyme. Two alleles, L and V with three genotypes LL, LV and VV were observed in Jersey, Holstein and cross bred cattle. Sahiwal cattle and buffalo were monomorphic for this locus producing only one genotype LL and one allele L. The frequency of L allele was comparatively higher in Holstein and crossbred cattle while in Jersey breed, the frequency of this allele was intermediate. The effect of genotype on birth weight was significant and LV genotype had higher birth weight than other genotypes. Hence, LV genotype in Holstein Friesian favored higher birth weight. (*Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 4 : 494-497*)

Key Words : Association, Birth Weight, Buffalo, Cattle, Growth Hormone Gene, Polymorphism

INTRODUCTION

Significant association of polymorphic candidate gene with economic traits will help the breeders to search out some genetic marker for economic traits. To identify genetic marker for birth weight, studies on polymorphism of growth hormone gene have gained much importance. Allelic variations of bovine growth hormone gene have been reported to be associated with the variation in milk yield (Sabour et al., 1997); carcass traits like carcass gain, meat value, live weight etc. (Grochowska et al., 2001) and reproductive traits (Lechniak et al., 1999). The study of birth weight is gaining more importance, as birth weight is directly related to growth rate and mature live weight of animals. Biologically, growth hormone is directly or indirectly involved in regulating various physiological processes, thereby, influences certain traits of economic importance. It helps in body growth through rapid cell division and skeletal growth and metabolism (Neathery et al., 1991), in mammogenesis, galactopoiesis, lipolysis etc. (Bauman and McCutcheon, 1986). Growth hormone influences reproductive functions (Spiteri and Neischlag, 1993) and aids in body's immune response, wound healing and haematopoiesis (Golde et al., 1977; Gelato, 1993). Hence, the present investigation was undertaken to find out polymorphism at growth hormone locus and its association with birth weight in cattle and buffalo.

MATERIALS AND METHODS

Animals

The study was carried out on 372 individuals of four

* Corresponding Author: T. K. Bhattacharya, Tel: +95-581-447382, Fax: +95-581-457284, E-mail: tarun@ivri.up.nic.in

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breeds of cattle and five breeds of buffaloes. Cattle breeds included 34 Jersey (Bull Mother farm, Lucknow), 33 Holstein Friesian (Cattle and Buffalo farm, Indian Veterinary Research Institute, Izatnagar), 31 Sahiwal (Livestock Research Centre, Pantnagar, Uttaranchal) and 82 crossbred animals maintained at Cattle and Buffalo farm, IVRI, Izatnagar. Crossbred animals consisted of three distinct lines each with a particular level of inheritance viz. (1/2 Haryana×1/2 Holstein Friesian), (1/2 Holstein Friesian×1/4 Brown Swiss×1/4 Haryana) and (1/2 Holstein Friesian×1/4 Jersey×1/4 Haryana). The buffalo breeds under the study were 70 Murrah (Cattle and Buffalo Farm, IVRI, Izatnagar and Buffalo Farm, Lakhimpur-Kheri, U.P.), 32 Bhadwari (Bhadwari Farm, Etawah, U.P.), 30 Jaffarabadi (Cattle Breeding Farm, Gujrat Agricultural University, Junagarh, Gujrat), 30 Surti (GAU, Anand, Gujrat) and 30 Nagpuri (Farmers' Herds, Maharashtra).

Sample and data

About 10 ml venous blood was collected, under sterile conditions, from the jugular vein of the animals into a sterile 50 ml polypropylene vial containing 0.5 ml of 0.5 M EDTA as anticoagulant. After collection of blood, tubes were capped tightly and shaken gently to facilitate thorough mixing of blood with anticoagulant. About 5 straws of frozen semen per A.I. bull were also collected and stored at -20°C.

Data on birth weight and date of birth of animals were noted from daily farm register maintained at the farm/herd.

Genomic DNA

Genomic DNA was isolated from blood samples following phenol-chloroform extraction method described by Sambrook et al. (1989) and from frozen semen samples

following the protocols of Lien et al. (1990). After isolation, DNA pellet present in eppendorf tube was dissolved in TE buffer and was kept in water bath at 60°C for 2 h to dissolve pellet properly in buffer. Quality of DNA was checked under spectrophotometry by taking O.D. ratio at 260 and 280 nm. The samples lying in the range of O.D. ratio between 1.7 and 1.9 were considered as good and used for further study. The samples beyond this range were re-extracted with phenol-chloroform extraction method.

DNA amplification

A 223 bp fragment of growth hormone gene spanning over fourth quarter of 4th intron and almost whole of the fifth exon except last triplet codon was amplified with forward (5'-GCTGCTCCTGAGGGCCCTTCG-3') and reverse (5'-GCGGCGGCACTTCATGACCCT-3') primers. A total volume of 25 µl of PCR reaction mixture containing 80-100 ng DNA, 2.5 µl 10X PCR assay buffer, 100 µM of each dNTP, *Taq* DNA polymerase and 20 pM of each primer was set up for amplifying individual DNA sample. A negative control, containing all reaction components except DNA was also made to check any contamination of foreign DNA. PCR reaction was carried out in PTC-200 programmable thermal cycler (MJ Research Inc., USA). The cycling conditions were hot start PCR at 94°C for 5 min with 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min followed by final extension at 72°C for 10 min.

Endonuclease digestion

The 223 bp amplicon was treated with *Alu*I enzyme to identify polymorphism at growth hormone gene. A volume of 5 µl PCR product was digested with 6 U *Alu* I enzyme and 10×digestion buffer at 37°C for overnight. The reaction was stopped by adding 0.5 M EDTA.

Electrophoresis

The digested product was electrophoresed in 3% w/v agarose gel at 50 V for 2 h at 4°C temperature. The gel was stained with ethidium bromide (0.5 µg/ml), visualized under UV-transilluminator and finally documented in the gel doc system.

Statistical analysis

Gene and genotype frequency was estimated as per the method described by Falconer (1998). The effect of genotype on birth weight was tested by analysis of variance using linear models (Snedecor and Cochran, 1967). The model used for the study was : $Y_{ijk} = \mu + G_i + S_j + E_{ijk}$ where, Y_{ijk} = kth observation of birth weight

μ = Overall mean

G_i = Fixed effect of ith genotype

S_j = Fixed effect of jth season of calving

E_{ijk} = Random error with NID (0, σ^2_e)

The season was grouped into three categories i.e. summer (March to July), rainy (August to September) and winter (October to February). In case of crossbred animals, genetic group was included in the model. Besides, chi-square test was also performed and all the animals were classified into two groups i.e. high and low birth weight.

RESULTS

Genotyping

In cattle the restriction digestion analysis of 223 bp fragment of growth hormone gene indicated the presence of three types of restriction patterns. The first pattern produced two fragments 171 and 52 bp whereas second pattern showed only one band of 223 bp in the gel. The third pattern was in between the first and second pattern (223, 171 and 52 bp). Hence, the first pattern was assigned as genotype LL, second pattern was genotype VV and the third pattern as genotype LV (Figure 1). In buffalo only one pattern was found, which corresponds to the first pattern of cattle. However, all buffalo breeds were monomorphic. Besides, we have amplified the same gene fragment in sheep and goat and restriction digestion indicated the presence of monomer (LL genotype).

Gene and genotype frequency

Three types of genotypes LL, LV and VV with two alleles L and V were observed in cattle while only LL genotype was found in buffalo. Perusal of Table 1 shows the



Figure 1. Growth hormone genotypes of Jersey cattle.

Lane M: 100 bp DNA ladder

Lane 1 and 2: LL genotype

Lane 3 and 4: LV genotype

Lane 5 and 6: VV genotype

Table 1. Distribution of gene and genotype frequency of growth hormone gene and genotype-wise birth weight in different cattle and buffalo breeds

Species	Breeds	Gene frequency		Genotype frequency			Birth weight (kg)		
		L	V	LL	LV	VV	LL	LV	VV
Cattle	Jersey	0.50	0.50	0.18	0.64	0.18	20.22	17.00	14.67
	Holstein	0.85	0.15	0.73	0.24	0.03	22.00 ^a	26.75 ^b	26.10 ^b
	Friesian								
	Crossbred	0.91	0.09	0.83	0.17	0.00	22.98	21.14	-
	Sahiwal	1.00	0.00	1.00	0.00	0.00	21.00	-	-
Buffalo	Murrah	1.00	0.00	1.00	0.00	0.00	25.08	-	-
	Bhadwari	1.00	0.00	1.00	0.00	0.00	21.35	-	-
	Jaffarabadi	1.00	0.00	1.00	0.00	0.00	22.41	-	-
	Surti	1.00	0.00	1.00	0.00	0.00	23.65	-	-
	Nagpuri	1.00	0.00	1.00	0.00	0.00	22.89	-	-

Different superscript indicates significance at $p \leq 0.01$.

distribution of LL, LV and VV genotypes and L and V alleles in different cattle and buffalo breeds. Frequency of VV genotype was high in all breeds of cattle except Jersey where frequency of both LL and VV were similar. Consequently, the frequency of L allele was highest in all the genetic groups of cattle except Jersey where intermediate frequency of both the alleles were found. In all the buffalo breeds the frequency of LL genotype was 1.00 with frequency of L allele as 1.00.

Effect of genotype

In Holstein Friesian cattle, the LV heterozygote had significantly higher birth weight ($p \leq 0.01$) than LL genotype (26.75 vs 22.00 kg). In higher birth weight group (27.5 kg) of Holstein Friesian cattle, the frequency of V allele was found to be significantly ($p \leq 0.01$) higher than lower birth weight group (18.75 kg). But, the effect of L/V genotype on birth weight in Jersey and crossbred cattle was observed as non-significant (Table 1). The average birth weight of Sahiwal cattle was 21.00 ± 0.53 kg while value of birth weight in different buffalo breeds were estimated to be 25.08 kg (Murrah), 21.35 kg (Bhadwari), 22.41 kg (Jaffarabadi), 23.65 kg (Surti) and 22.89 kg (Nagpuri). As the gene was not polymorphic in Sahiwal cattle and all the buffalo breeds, the effect of genotype on birth weight did not have a scientific meaning.

DISCUSSION

In both cattle and buffalo, the size of amplified product was 223 bp, which is the indicative of strong conservation of DNA in the structural gene. Such a nature of conservation is not only restricted in bovine and bubaline species but is also found in ovine and caprine species (Schlee et al., 1994; Chickumi et al., 1991; Zwierzchowski et al., 2001).

The two types of alleles differ only in terms of restriction site of *Alu* I endonuclease enzyme. The L allele indicated the presence of restriction site while its absence

was assigned as allele V. In L allele the restriction site contained the nucleotide C while a transition with G at the same site indicated the absence of *Alu* I restriction site. The total length of amino acid in growth hormone is 191. The presence of nucleotide C at triplet codon encodes the amino acid leucine while the nucleotide, G encodes the amino acid valine. This leucine/valine substitution was found in 127th position of the polypeptide. However, in Sahiwal cattle and all buffalo breeds, the growth hormone gene had amino acid, leucine at 127th position of the polypeptide.

Polymorphism at L/V locus is a common phenomenon for different breeds of cattle like Holstein Friesian, Hereford, Ayrshire, Korean cattle as reported by several workers (Chikumi et al., 1991; Sabour et al., 1997; Citek et al., 2000). Since the crossbred animals were the crosses of indicine and taurine cattle, polymorphism at this locus was expected. But, the reports of growth hormone gene polymorphism in Indian cattle and riverine buffalo are very scanty.

It is a fact that birth weight is the true indicator of future body weight of mature animals. Several workers have predicted the mature body weight of animals on the basis of birth weight as the correlation of birth weight and mature body weight is significantly high. Significant association of birth weight and growth hormone genotype indicates the highest birth weight for LV genotype. There was no report on the association of growth hormone gene polymorphism and birth weight. Several investigators have however, reported significant association between the genotypes and meat traits, carcass gain (Schlee et al., 1994; Grochowska et al., 2001). If birth weight is considered as live weight at 1st day of age, the present study corresponds well with the findings of Zwierzchowski et al. (2001).

In conclusion, it may be stated that growth hormone gene is polymorphic in exotic and crossbred cattle whereas it is monomorphic in indicine cattle and riverine buffalo. The genotype had significant effect on birth weight where LV genotype had higher birth weight in Holstein Friesian. Hence, this genotype may be favored in the farm to get

calves of very high birth weight as the calf is the future of the herd.

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