Proceedings, 10th World Congress of Genetics Applied to Livestock Production

SNP Analysis of the Growth Hormone Gene in Indigenous Philippine Cattle, Ilocos Genetic Group by PCR-RFLP

P.J.C. Icalia[12], A. J. Salces[1], M.S. Mendioro[1], C.C. Sevilla[1], K.S. Seo[3] and T.S. Kim[3].
[1]University of the Philippines Los Baños, Laguna, Philippines, [2]Mariano Marcos State University, City of Batac, Philippines [3]Sunchon National University, Suncheon City, South Korea

ABSTRACT: Polymorphism in the bovine growth hormone (GH) gene was detected in indigenous Philippine cattle,Ilocos genetic group using PCR-RFLP. There has been an association made between the GH gene and growth. A SNP site at the position 127 of the amino acid chain has been associated to variability in economically important traits. Digestion of the PCR products of the GH gene with the restriction enzyme AluI showed alleles L and V. The most frequent genotype was LV. The frequency of the L allele was 0.43 while V allele was 0.57. The number of genotypes and allele frequencies for this polymorphism of GH gene so that three patterns were observed and frequencies were 0.20 (n=11), 0.45 (n=25), and 0.35 (n=19) for LL, LV, and VV genotypes, repectively. Based on the observed and expected genotype frequencies the population was in Hardy-Weinberg equilibrium.
Keywords: Cattle; Growth Hormone Gene; Polymorphism; PCR-RFLP

Introduction

Bovine growth hormone (GH) is a single chain polypeptide with 190 or 191 amino acids and molecular weight 22 kDa. This hormone is produced in the anterior pituitary gland under the hypothalamic controls of two hormones: growth hormone releasing factor, which increases the secretion of GH, and somatotropin release-inhibiting factor which inhibits its secretion (Nicoll et al., 1986). Growth hormone exerts its effects on growth and metabolism by interacting with specific receptor on the surface of target cells. GH plays a vital role in regulating body weight by decreasing the synthesis of lipids and, therefore, decreased concentrations of GH would increase synthesis of lipids (McMahon et al., 2001). Therefore it has this important relationship; GH can be used as a candidate gene marker for improving growth, meat or milk production and for marked-assisted selection programs in cattle either. The GH gene with approximately 1800 bp length, five exons and four introns is a part of multiple gene family that contains prolactin and placental lactogenes and assigned with chromosome region 19q26 in bovine genome. Flanking repeat sequences of GH gene regulate the expression of a gene (Hediger et al., 1990). The aim of this study was to identify the polymorphisms of growth hormone gene (*AluI* loci) in Indigenous Philippine Cattle, Ilocos Genetic Group.

Materials and Methods

Animals and Blood Collection. The 55 blood samples used in this study were collected from Northern Luzon specifically obtained fromIlocos Norte and Ilocos Sur. The animals were randomly chosen from the different municipalities of the three provinces. Blood (1cc) was collected from the jugular vein of the animals using a syringe and dropped in a concentric circular motion to the labeled FTA(Flinders Technology Associates) cards.

DNA Isolation and Purification. A total of 40 FTA sample discs for each sample were placed in a PCR amplification tube and 200µl of lysis solution and 10µl of proteinase were added. Samples were vortexed for 10 seconds be incubated for 20 minutes at room temperature. The reagent was discarded using a pipette. Same step was repeated once without the adding the proteinase. Washing solution with a volume of 200µl was added to each PCR tube and was incubated for 20 minutes at room temperature. All spent washing solution was removed and discarded using a pipette. The same step was repeated twice and the discs were heat assisted at 56^{o}C for 10 minutesfor drying.

DNA Quantification and Quality Check. DNA was eluted from the dried discs by adding 50µl of sterile nanopure water and water bathed in 60°C for 30 minutes. The eluted DNA concentration and quality was determined using Biotek Epoch™ UV-VIS microplate spectrophotometer.

PCR Amplification. PCR primers designed and used to amplify *GH* and *PRKAG3* gene fragments are presented in Table 1. PCR was performed using a programmable BIOTEK Thermal Cycler (Applied Biosystems) in a volume of 15 µl containing the DNA template (about 10-100 ng), 1 U Taq DNA polymerase (Vivantis), 1X PCR Buffer, 2.5 mMdNTPs, 10 pmol of each primer and 1.0-2.0 mM of MgCl2. PCR profile for the analysis of individual samples were as follows: 5 min at 95°C; 35 amplification cycles of 30 sec at 95°C, 30 sec at the specific annealing temperature for each primer pair (Table 1), 30 sec at 72°C; 10 min at 72°C. PCR of the template DNA extracted from Ilocos and Batanes cattle were carried out with 40 amplification cycles.

Table 1.Primer sequence for GH gene for PCR-RFLP analysis.

Gene	Sequence (5' → 3')	Enzymes
GH for	CGGACCGTGTCTATGA-GAAGCTGAAG	ALU1
rev	GTTCTTGAG-CAGCGCGTCGTCA	

These PCR fragments obtained with the primers were analyzed by means of restricted fragment length polymorphism (RFLP). Results from the PCR amplification were scored according to the bands present basing from a 100bp ladder. Samples with same band sizes indicate similarity, otherwise, dissimilarity. RFLP was performed using a volume of 20 µl for *GH* containing 2 µl PCR product, 1 µl restriction enzyme, 2 µl 10x buffer and 16 µl distilled water. A total volume of 10 µl was used for *PRKAG3* containing 2 µl PCR product, 0.5 µl restriction enzyme, 1 µl BSA, 1 µl 10x buffer and 6.5 µl distilled water. All enzymes were allowed to react in a water bath with a temperature of 37°C for six hours. Enzymes used for the PCR-RFLP are indicated in table 1. The digestion products were separated by horizontal electrophoresis in 3% agarose gel in 0.5 x TBE (135 V for 20 min) stained with RedSafe prior to visualization under UV light.

Results and Discussion

SNP Analysis of Growth Hormone Gene in Ilocos Cattle by PCR-RFLP Method. 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). 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). 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). 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). 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.

Table 2. Observed gene and genotypic frequencies of GH *AluI* loci in Ilocos cattle.

Cattle (n=55)	Genotype			Gene	
	LL	LV	VV	L	V
Number	11	25	19		
Frequency	0.20	0.45	0.35	0.43	0.57

Tables 2 presents the observed gene and genotype frequencies of Ilocos cattle for the GH gene. The most prevalent genotype is the heterozygous condition of LV with a gene frequency of 0.45 (n=25). It is followed by the VV genotype with gene frequency of 0.35 (n=19) then the homozygous dominant LL recording a gene frequency of 0.20 (n=11). The recorded allele frequencies are 0.57 for valine and 0.43 for leucine. Ilocos cattle is known to have originated from cattle of *B. indicus*. Contrary to the report of Jakaria et al. (2009) that cattle of *B. taurus* origin has a higher gene frequency for leucine but for this study allele V showed higher gene frequency.

Table 3. Expected gene and genotypic frequencies of GH *AluI* loci in Ilocos cattle.

Cattle (n=55)	Genotype			Gene	
	LL	LV	VV	L	V
Number	10	27	18		
Frequency	0.19	0.49	0.32	0.44	0.56

The most dominant in the population was the heterozygous genotype LV wherein 45% of the randomly selected population (n=55) of Ilocos cattle carry this genotype. Moreover, 35% are homozygous recessive for this gene while the remaining 20% are homozygous dominant. Comparing the observed (Table 2) and expected (LL=0.19, LV=0.49, VV=0.32) genotypic frequencies (Table 3), it is

inferred that the population is under the Hardy-Weinberg genetic equilibrium. Results of this study is relatively different from the frequencies observed in the reports of Jakaria et al. (2009) that cattle of *B. indicus* origin such as the Ilocos cattle has a higher frequency for L allele.

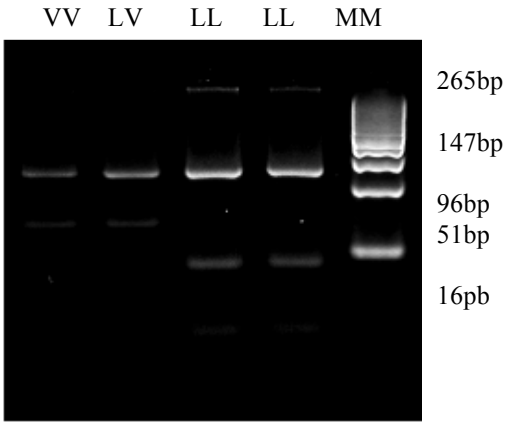

Figure 1. Representative results PCR-RFLP analysis *GH AluI loci* on 3% agarose gel Line 1 is VV genotype (265, 147 and 16 bp), line 2 is LV genotype (265, 147, 96, 51 and 16 bp), line 3 and 4 are LL genotype (265, 96, 51 and 16 bp), line 5 is a marker of molecular weight (Fermentas, 100 bp).

Conclusion

Genotyping using PCR-RFLP method revealed three genotypes and two alleles in the polymorphic sites of growth hormone gene (*Alu*I loci). In the studied population of 55 Ilocos cattle, the frequency of the L allele was 0.43 while V allele was 0.57. The number of genotypes and allele frequencies for this polymorphism of GH gene so that three patterns were observed and frequencies were 0.24 (n=11), 0.45 (n=25), and 0.35 (n=19) for LL, LV, and VV genotypes, respectively. The population was under the Hardy-Weinberg genetic equilibrium. These results can be used for correlation analysis of the observed alleles with economically important traits in cattle.

Literature Cited

Hediger, R. V., Johnson, S. E., Brendse, W. et al. (1990). Genomics, 8, 171-174

Jakaria, R.R., Noor, H. et al. (2009).1st International Seminar in Animal Industry and Food Safety, Bogor- Indonesia. November 22-24, 2009. P 42-47

McMahon, C. D., Lookingland, R. P., Tucker, H. A. . (2001). Dom. Anim. Endocrinol. 20: 65-87

Saitou, N., Nei, M. (1987). MolBiolEvol 4(4): 406-25.

Schlee, P., Gramil, R. et al. (1994). , M., Madsen, P., Jensen, J., et al. (2003). J. Anim. Breed. Genet. 111:253-256

Svennersten-Sjauna, K. and Olsson, K. (2005).Endocrinol., 29, 241-258