Original Research

Growth Hormone Gene Polymorphism in Indigenous Cattle of North East India *vis-a-vis* Crossbred Cattle

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Abstract

The study was conducted to explore polymorphism in bovine growth hormone gene in 210 indigenous cattle of North East India (viz. Manipur, Meghalaya, Mizoram, Nagaland, Assam and Tripura) and crossbred cattle. Two different variants of bGH gene viz. A and T were detected from DNA using PCR-RFLP. The frequency of A allele was predominant (0.667) among indigenous cattle. On the contrary, the frequency of T allele was predominant (0.867) in crossbred cattle. Among the genotypes, AA genotype was found in intermediate to high frequency among the local cattle of North East India. While the genotype AA was completely absent in the crossbred population. The population conforming to equilibrium indicated lack of selection pressure in these cattle population.

Key words: Crossbred Cattle, Growth Hormone Gene, Gene Frequency, Heterozygosity**,** Indigenous Cattle, North East India

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Introduction

The eight north east (NE) states of India contribute around 6.9% of total cattle population of the country. The proportion of indigenous and exotic cattle in these states as compared to the whole country is 8.19% and 2.28%, respectively (Livestock Census, 2012). The local cattle of North East India are mostly of non-descript type except Tho-Tho, Siri and Lakhimi cattle of Nagaland, Sikkim and Assam respectively. The reproductive efficiency has a direct influence on productivity. Cow fertility is affected by the age at puberty as later is the age at puberty later is the first calving reflecting low reproductive efficiency (Faure and Morales, 2003). Starting in the 1970's, the advent of the era of molecular genetics provided new

opportunities to improve breeding programs in livestock by allowing the use of DNA markers to identify genes or genomic regions that control traits of interest (Khare and Khare, 2017).

The bovine growth hormone gene play a key role in the regulation of growth and development; indirectly influencing the reproductive efficiency of cattle (Abolfazl *et al*., 2009; Arango *et al*., 2014). Growth hormone (GH) gene is a member of multigene family 47 approximately 1800 bp in length (Gordon *et al*., 1983) and assigned with chromosome region 19q26 in bovine genome (Hediger *et al.*, 1990). GH is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior pituitary in a circadian and pulsate manner, the pattern of which plays an important role in postnatal longitudinal growth and development, tissue growth, lactation, reproduction, as well as protein, lipid and carbohydrate metabolism (Ayuk and Sheppard, 2006).

Material and Methods

Experimental Animal and Blood Sampling

The study was conducted on a total of 210 unrelated indigenous cattle (*Bos indicus*) of North East India *viz*. Lakshmi (Assam), Meitei san (Manipur), Meghalaya, Zobawng (Mizoram), Tho tho (Nagaland) and Tripura and crossbred cattle. A total of 30 animals each were selected from the indigenous animals of each state and crossbred cattle of Mizoram. These animals were randomly selected from field(s), private farm(s), institute(s) and organized herd(s) maintained in these states of North East India. Blood samples were collected aseptically from the jugular vein of the selected animals in vacutainer tubes containing EDTA. Cold chain was maintained during the transit of the sample from farm to laboratory and stored in deep freezer at –20˚C till further use.

Genomic DNA Isolation

Genomic DNA was extracted using GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) according to the instruction manual. The quantity and quality of DNA were checked with a NanoDrop MultiscanGo Spectrophotometer (Thermo Scientific, USA). The primers and restriction enzyme used for PCR-RFLP analysis are given in Table 1.

Table 1: Gene location of locus, size of PCR product, primer sets, annealing temperature and restriction enzyme used for RFLF analysis

Name of Primers		Primer sequence (5´ - 3´)	RE	Location within gene	Product size (bp)	T^{A} (°C)	Reference
GH	F	CCCACGGGCAAGAATGAGGC	*Msp*I	Intron 3	329	56	Dybus *et al.*, 2003
	R	TGAGGAACTGCAGGGGCCCA					

PCR and RFLP

The PCR amplification was carried in a 25 µl of 10X PCR buffer, 2mM of $Mgcl_2$, 200 µM of each dNTPs, 5 pM each of primers, 2 U Taq DNA polymerase and 60 ng genomic DNA. The following cycles were

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applied: at 95ºC for 5 min, followed by 35 cycles of 95ºC for 30 sec, 56ºC for 45 sec, 72ºC for 30 sec and final synthesis at 72ºC for 10 min. The amplified DNA was digested with *Msp*I enzyme by incubating at 37ºC for 3 hours. The digested products were separated in 3% agarose gel in 0.5 X TAE containing 1.0 µM ethidium bromide and visualized under UV trans-illuminator and photograph were taken using Gel Doc system.

Statistical Analysis

The allele and genotype frequency calculation as well as the chi-square test were carried out by using the Popgene32 software (Yeh *et al.*, 1997).

Results and Discussion

GH/*Msp*I analysis of 329 bp amplicon revealed three different genotypic patterns (Fig. 1). The A allele showed 329 bp fragment due to the absence of restriction site for *Msp*I. The digested T allele produced 224 bp and 105 bp fragments. The heterozygote AB genotype yielded a restriction pattern of three (329 bp, 224 bp and 105 bp) fragments.

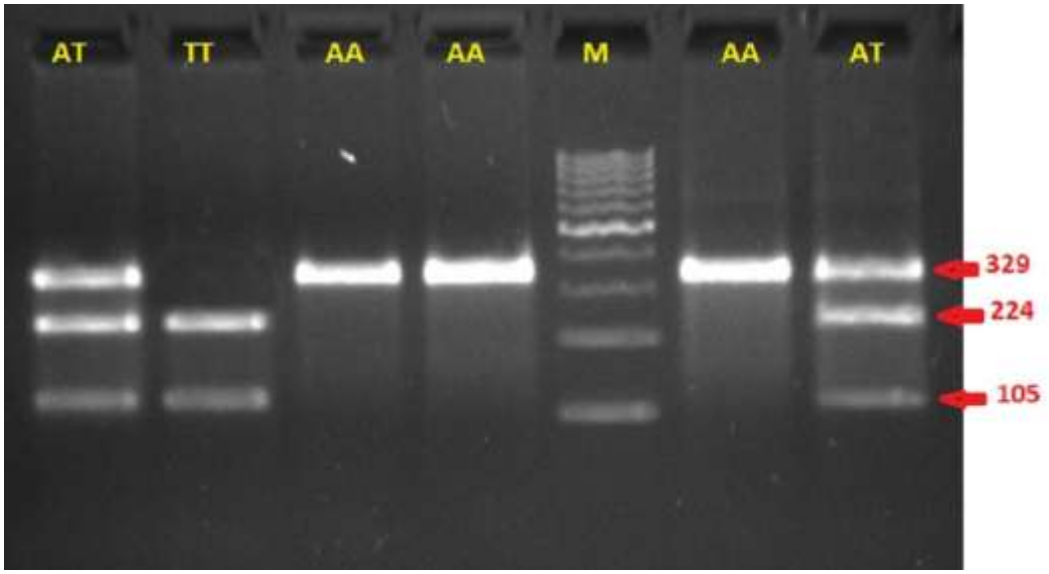

Fig.1: Genotype of GH gene digested with *Msp*I in 2.5% agarose gel

The genotypic frequency distributions of GH in Meitei san (Manipur), Zobawng (Mizoram), Tho tho (Nagaland), Lakshmi (Assam), Tripura and Meghalaya local cattle of North East India and crossbred cattle are presented in Table 2. Among the genotypes, AA genotype was found in intermediate to high frequency among the local cattle of North East India. While the genotype AA was completely absent in the crossbred population. Highest (0.63) and lowest (0.40) frequency of AA genotype was prevalent in Meitei san and Tho tho, and Lakshmi, respectively.

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Table 2: Genotype frequencies of GH gene in Meitei san (MS), Zobawng (Zo), Tho tho (TT), Lakshmi (LM), Tripura (TR) and Meghalaya (ML) local cattle of North East India and crossbred (CB) cattle

Genotype	Types of Cattle						
	MS (n=30)	Zo (n=30)	TT (n=30)	LM (n=30)	TR (n=30)	ML (n=30)	CB (n=30)
AA	0.63 (19)	0.6 (18)	0.63 (19)	0.4 (12)	0.6 (18)	0.47 (14)	0 (0)
AT	0.37 (11)	0.4 (12)	0.37 (11)	0.5 (15)	0.4 (12)	0.53 (16)	0.27 (8)
TT	0 (0)	0 (0)	0 (0)	0.1 (3)	0 (0)	0 (0)	0.73 (22)
Observed heterozygosity	0.367	0.4	0.367	0.5	0.4	0.533	0.267
Expected heterozygosity	0.305	0.325	0.305	0.463	0.325	0.398	0.235
χ^2 value	1.512 ^{NS}	1.875 ^{NS}	1.512 ^{NS}	0.293 ^{NS}	1.875 ^{NS}	3.967*	0.710 ^{NS}

*n = Number of animals; NS = Not significant, * Significant at 5% level (P<0.05); the values within the parenthesis are the number of animals*

The TT genotype was almost absent in the indigenous cattle population except Lakshmi where it was observed in 3 animals. However, the TT genotype was a predominant genotype in crossbred population with a frequency of 0.73. The heterozygote, AT genotype was found in low to intermediate frequency across the population under study. The highest (0.50) and lowest (0.27) frequency of AT genotype was observed in Lakshmi and crossbred cattle, respectively.

Table 3: Allele frequency of GH in Meitei san, Zobawng, Tho tho, Lakshmi, Tripura and Meghalaya local cattle of North East India and crossbred cattle

Allele	Cattle	Number	Frequency	Overall
A	Meitei san	50	0.817	0.667(288)
	Zobawng	48	0.8	
	Tho tho	50	0.817	
	Lakshmi	40	0.65	
	Tripura	48	0.8	
	Meghalaya	44	0.733	
	Crossbred	8	0.133	0.019 (8)
T	Meitei san	10	0.183	0.190(80)
	Zobawng	12	0.2	
	Tho tho	10	0.183	
	Lakshmi	20	0.35	
	Tripura	12	0.2	
	Meghalaya	16	0.267	
	Crossbred	52	0.867	0.124 (52)
Total		420		1

The values within the parenthesis are the number of alleles

In the present study, the A allele was predominantly (0.667) prevalent among local cattle of North East India (Table 3) whereas the T allele was predominant (0.867) in crossbred cattle. The highest (0.817) and lowest (0.650) frequency of A allele in local cattle was observed in Meitei san and Tho tho, and Lakshmi cattle, respectively. In crossbred cattle, the frequency of A allele was 0.133. Frequency pattern of bGH/ *MspI* alleles obtained in this study was similar to that reported in earlier studies. Similar A allele frequency of 0.86, 0.82 and 1 were reported in Sahiwal (Mitra *et al.*, 1995), Brazilian Nellore and Ongole (Lagziel *et*

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al., 2000) cattle respectively. On the other hand low A allele frequencies were reported in Taurine breeds *i.e.*, Holstein, 0.26 (Zhang *et al*., 1993); Angus, 0.14; Brown Carpathian of Ukraine, 0.26; Hereford, 0.00; Jersey, 0.15; Limousin, 0.39 (Lagziel *et al.*, 2000), and Red Danish, 0.05 (Høj *et al.*, 1993) which is in agreement to our finding in crossbred cattle.

Similarly, in Holstein Friesian heifers high (83.75%) A and low (16.25 %) B allele frequencies and AA (70.00%), AB (27.50%) and BB (2.50%) genotype frequencies were reported by Oner *et al.* (2017). Intermediate findings of 53.3% and 46.7% for A and T alleles, respectively in Pesisir cattle of Indonesia were reported by Hartatik *et al.* (2018). It has been proposed that the A allele of bGH originated in the Indian subcontinent because frequencies of this allele in relation to geographic origin of breeds showed a cline pattern, decreasing with distance from the Indian subcontinent (Lagziel and Soller, 1999; Lagziel *et al*., 2000).

In the present study observed and expected heterozygosity of bGH locus were calculated from allele frequencies, considering the population in Hardy-Weinberg equilibrium. Its unbiased estimate was calculated by taking the number of allele into account. The populations showed intermediate observed heterozygosity and less difference between observed and expected heterozygosity (Table 2). Highest (0.500) and lowest (0.267) observed heterozygosity were found in Lakshmi and crossbred cattle, respectively. The observed and expected heterozygosity of GH gene locus were within Hardy-Weinberg expectation in all the populations except Meghalaya cattle as revealed by the chi-square values. Close to present finding, Oner *et al.* (2017) reported moderate observed and expected heterozygosity as 0.275 and 0.273, respectively in Holstein cattle.

Conclusion

It can be concluded from above findings that PCR-RFLP can be used successfully for identification of the variants in bGH locus. The finding reveals the probability of origin of A allele in India as its frequency is predominant in indigenous cattle (Zebu), however needs further studies for its confirmation. The population conforming to equilibrium indicates lack of selection pressure in these cattle population as most of the animals were let loose in the field for grazing. And it was expected that random mating took place in the population.

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References

1. Abolfazl G, Rasoul T, Mortaza B and Cyrus A. 2009. Restriction fragment length polymorphism of bovine growth hormone gene intron 3 and its association with testis biometry traits in Iranian Holstein bull. *Afr. J. Microbiol. Res*. 3(11): 809-814.
2. Arango GJ, Echeverri ZJ and Lopez HA. 2014. Association of the bovine growth hormone gene with Holstein cattle reproductive parameters. *Revista MVZ Cordoba*. 19(3): 4249-4258.
3. Ayuk J and Sheppard MC. 2006. Growth hormone and its disorder. *Postgrad. Med. J*. 82(63): 24-30.
4. Dybus A, Kmieć M, Sobek Z, Pietrzyk W and Wiśniewski B. 2003. Associations between polymorphism of growth hormone releasing hormone (GHRH) and pituitary transcription factor 1 (PIT1) genes and production traits of Limousine cattle. *Arch. Tierz., Dummerstorf*. 46: 527-534.
5. Faure R and Morales C. 2003 La pubertad de la hembra bovina: I. aspectos fisiologicos. *Rev Salud Anim*. 25(1): 13-19.
6. Gordon DG, Quick DP, Erwin CR, Donelson JE and Maurer RA. 1983. Nucleotide sequence of the bovine growth hormone chromosomal 134 gene. *Mol. Cell. Endocrinol*. 33(1): 81-95.
7. Hartatik T, Putra DE, Volkandari SD, Kanazawa T and Sumadi, 2018. Genotype analysis of partial growth hormone gene (GH891⏐MspI) in Pesisir cattle and Simmental-Pesisir crossbred cattle. *J. Indonesian Trop. Anim. Agric.* 43 (1): 1-8.
8. Hediger R, Johnson SE, Barendse W, Drinkwater RD, Moore SS and Hetzel J. 1990. Assignment of the growth hormone gene locus to 19 q26-136 qter in cattle and to 11 q25-qter in sheep by in situ hybridization. *Genom.* 8: 171-174.
9. Høj S, Fredholm M, Larsen NJ and Nielsen VH. 1993. Growth hormone gene polymorphism associated with selection for milk fat production in lines of cattle. *Anim. Genet*. 24: 91-96.
10. Khare V and Khare A. 2017. Modern Approach in Animal Breeding by Use of Advanced Molecular Genetic Techniques. *Intl J Livestock Res*. 7(5): 1-22
11. Lagziel A and Soller M. 1999. DNA sequence of SSCP haplotypes at the bovine growth hormone (bGH) gene. *Anim. Genet*. 30: 362-365.
12. Lagziel A, Denise S, Hanotte O, Dhara S, Glazko V, Broadhead A, Davoli R, Russo V and Soller M. 2000. Geographic and breed distribution of an *Msp*I PCR-RFLP in the bovine growth hormone (bGH) gene. *Anim. Genet*. 31: 210-213.
13. Livestock Census (2012). The 19th Livestock Census- 2012 All India Report. http://dahd.nic.in/sites/default/files/Livestock%20%205.pdf.
14. Mitra A, Schlee P, Balakrishnan CR and Pirchner F. 1995. Polymorphisms at growth hormone and prolactin loci in Indian cattle and buffalo. *J. Anim. Breeding Genet.* 112: 71-74.
15. Öner Y, Yılmaz O, Okut H, Ata N, Yılmazbaş-Mecitoğlu G and Keskin A. 2017. Associations between GH, PRL, STAT5A, OPN, PIT-1, LEP and FGF2 polymorphisms and fertility in Holstein-Friesian heifers. *Kafkas Univ. Vet. Fak Derg.* 23(4): 527-534.
16. Yeh FC, Yang RC, Boyle TBJ, Ye ZH and Mao JX. 1997. POPGENE the user friendly shareware for population genetic analysis. University of Alberta, Canada.
17. Zhang HM, Brown DR, Denise SK and Ax RL. 1993. Polymerase chain reaction restriction fragment length polymorphism analysis of the bovine somatotropin gene. *J. Anim. Sci*. 71:2276.

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