Genetic Polymorphism of Beta-Lactoglobulin in Holstein-Gir Crosses in the State of Pernambuco, Brazil

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Introduction

The future of the national livestock industry and its progress is related to the genetic potential and genetic variability found among the animals (Egito et al., 2004). Genetic variability is found in different species, breeds and individuals within breeds. Brazilian milk production is mostly from crossbred animals, 80% represented by different crosses of the Holstein and Gir breeds (*Bos taurus taurus* × *Bos taurus indicus*) (Machado, 2001; McManus et al., 2008).

Advances in molecular biology have made possible the mapping and identification of genes responsible for variations in milk yield and composition, facilitating the process of genetic selection for productivity of dairy animals (Regitano & Coutinho, 2001). Genetic polymorphism of main milk proteins, such as caseins, β-Lactoglobulin and α-lactoalbumin, are interesting markers for milk production, milk composition and yield of dairy products. Some of these markers are already available or close to have a commercial application in dairying and dairy processing, as it is the case of β-Lactoglobulin (Regitano & Coutinho, 2001).

As known, milk is composed by two groups of proteins: caseins (αs1, αs2, β and k-casein) and whey proteins (alfa-lactoalbumin and beta-lactoglobulin). β-Lactoglobulin (β-Lg) represents almost 50% of the whey proteins and 12% of the total proteins in bovine milk (Fox e McSweenwey, 1998). β-Lg is formed by a sequence of 162 amino acids (Hambling et al., 1992), and according to Hill et al. (1996) main genotypes of β-LG (AA, AB, e BB) result in different protein codifications, provoking variations in the physicochemical properties and composition of milk.

The objective of this preliminary study was to verify the existence of polymorphism at the locus of the β-Lg gene in different genetic groups of the Holstein-Gir crosses, to be used as genetic markers in genetic improvement programs. This is one of a series of studies aiming the identification of molecular markers for traits of economic importance in one of the most important breed crosses in Brazil.

Material and Methods

The genetic polymorphism of the β-Lactoglobulin (β-Lg) gene was determined from blood samples in 165 animals belonging to various genetic groups of Holstein-Gir crosses reared in three farms, two experimental and one commercial, in the state of Pernambuco, Brazil. The observed genetic groups were 1/2, 3/4, 5/8 Holstein and 5/8 Holstein with I, II, III and IV

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inter-se generations. A located fragment was amplified in the areas of exons II and III of β-Lg gene using the PCR technique and RFLP analysis.

Descriptive and dispersion of allele frequencies were performed by direct counting from the genotype identified in the photo documentation of electrophoresis of PCR-RFLP.

Results and discussion

Distribution of the different genetic groups in the population studied is shown in table 1.

Table 1. Distribution of the different genetic groups in the population studied.

Genetic group	Percentage (%)
½ Holstein	6
¾ Holstein	12
5/8 Holstein	7
5/8 Holstein, I *inter-se* Generation	12
5/8 Holstein, II *inter-se* Generation	31
5/8 Holstein, III *inter-se* Generation	17
5/8 Holstein, IV *inter-se* Generation	1
Unidentified	14

As shown in Table 1, 61% of the animals studied belong to the synthetic breed Girolando (5/8 *inter se* I, II, III and IV generations).

Digestion of β-Lg fragments by *Hae*III endonuclease generated the same electrophoretic pattern for all individuals, producing fragments of 400, 220, 190, and 151pb molecular weight for all animals, revealing just the presence of the heterozygous genotype (100% AB) of β-Lg in all genetic groups (Table 2). A and B allelic frequencies were 0.5 for each allele. The frequencies observed clearly show (supported by a chi-square test) that this sample was not on Hardy-Weinberg equilibrium. This fact could be justified by the existence of various evolutionary factors affecting this population (Beiguelman, 2008).

Most studies on the literature report the presence of three genotypes for this gene (AA, AB e BB), with higher frequency of genotype AB (Ng-Kwai-Hang et al., 1990; Aleandri et al., 1990; Sabour et al., 1993; Hill et al., 1996; Paterson et al., 1999; Celik et al., 2003; Tsiaras et al., 2005; Oner & Elmaci, 2006; Neves et al., 1998). This study is in disagreement with those reports, because only heterozygous individuals (AB) were found in the sample studied. Some selection strategies applied to this population may be responsible for this situation (Giannoni & Giannoni, 1983).

In all electrophoretic patterns a constant fragment of 400pb was found, confirming the efficiency of the digestion using *Hae*III endonuclease. Additionally, a non digested sample was added as a way of controlling the enzymatic digestion.

Table 2. Distribution of gene and genotypic frequencies, observed and expected, for the β-Lactoglobulin gene studied

Genotypes	Genotypic frequencies	N observed	N expected	Allele frequency
AA	---	--	41.25	A (0.5)
AB	100	165	82.50	
BB	---	--	41.25	B (0.5)
Total		165	165	

Conclusion

The sample studied showed a frequency of 100% for the heterozygous genotype (AB) of the beta-lactoglobulin gene in all genetic groups of the Holstein-Gir crosses. Further research is needed to elucidate the polymorphism of the β-Lg *locus* in the different Holstein-Gir genetic groups in a larger population sample, together with development of specific primers for Holstein-Gir crosses. Following research will study the relationship of the β-Lg polymorphism with several traits of economic importance, with the purpose of evaluating the efficiency of those polymorphisms as markers for genetic improvement programs of the Holstein-Gir crosses in Brazil.

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