Allele and genotype frequency for milk beta-casein in dairy cattle in the northern region of Tocantins State, Brazil

Frequências alélicas e genotípicas para beta-caseína do leite em bovinos leiteiros da microrregião de Araguaína, Tocantins

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ABSTRACT
At present, there is a concern about the quality of milk and diseases related to its consumption, as it can generate discomfort and allergic reactions in some individuals due to its protein components. Thus, the present study was developed to identify the allele and genotype frequencies of genes for β-casein, A1 and A2, in dairy herds in the region of Araguaína-TO, Brazil. Genetic material from 421 animals (crossbred dairy cattle in lactation) was used. All animals were numbered for identification, and DNA samples were extracted from hair bulbs. Samples for two markers from the polymorphic regions were characterized and confirmed by real-time PCR using the ABI Prism™ 7500 Sequence Detection System (Applied Biosystems). Allele and genotype frequencies were determined using the TaqMan™ detection system, where the primer and probe release different fluorescence signals for each allele of the polymorphism. The sampled herd showed frequencies of 28.27% for the A1 allele and 71.73% for the A2 allele. Genotype frequencies were 52.96% (223/421) for A2A2; 37.53% (158/421) for the A1A2 genotype; and 9.50% (40/421) for the A1A1 genotype. The frequency of the A1 allele for β-casein in dairy herds from the northern region of Tocantins was low and is per the results of previous studies. Although the A2A2 genotype of β-casein had a high relative frequency, the A1A2 genotype is still rather frequent, warranting greater selection pressure.

Keywords: Beta-casein. Dairy cattle. Genotyping. Tocantins.

RESUMO
Atualmente existe uma preocupação em relação à qualidade de leite e doenças relacionadas ao consumo de leite, pois o mesmo pode gerar desconforto e reações alérgicas em alguns indivíduos devido ao efeito de seus constituintes proteicos. Assim, o presente estudo teve como objetivo identificar a frequência alélica e genotípica de genes para beta caseína, A1 e A2, em rebanhos leiteiros da região de Araguaína-TO. Foram utilizados material genético de 421 animais (bovinos leiteiros mestiços em lactação), e todos os animais foram numerados para identificação e amostras de DNA foram extraídas de bulbo de folículos pilosos. As amostras para dois marcadores das regiões polimórficas foram caracterizadas e confirmadas por PCR em tempo real, usando um sistema de detecção de sequências ABI Prism™ 7500 (Applied Biosystems). As frequências alélicas e genotípicas foram determinadas utilizando o sistema de detecção TaqMan™, no qual o primer e a sonda emitem diferentes sinais de fluorescência para cada alelo do polimorfismo. Observou-se frequência do alelo A1 de 28,27%, e do alelo A2 de 71,73% no rebanho amostral. A frequência genotípica de A2A2 foi de 52,96% (223/421), com genótipo A1A2 de 37,53% (158/421), e de 9,50% (40/421) animais com genótipo A1A1. A frequência do alelo A1 para beta-caseína em rebanhos leiteiros da região norte do Tocantins foi baixa e seguiu a mesma tendência já observada em estudos anteriores. Os genótipos A2A2 da beta-caseína apresentaram frequência relativa alta, entretanto o genótipo A1A2 ainda é bastante frequente, necessitando de maior pressão de seleção.

Palavras-chave: Beta-caseína. Bovino de leite. Genotipagem. Tocantins.

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Introduction

Tocantins State is the third-largest milk-producing state in the northern region of Brazil, with 405 million liters of milk produced per year, i.e., 1.20% of the national bovine milk production (Zoccal, 2020). Although this index is still low, milk holds significant importance for the regional economy, with employment and income-generating potential, especially in family farming.

In milk, β-casein represents 25 to 35% of total proteins, bearing two alleles: A1 and A2 (Barbosa et al., 2019). What differentiates these β-casein variants is the substitution of an amino acid at position 67 of the protein. A1 β-casein has a histidine residue (His67), whereas A2 β-casein has a proline (Pro67) (Ramakrishnan et al., 2020).

At present, there is a concern among the population regarding diseases related to the consumption of milk and dairy products (Siqueira, 2019), as it can cause discomfort for some people. According to Ingram et al. (2009), about 65% of the world population has some degree of allergy or intolerance to milk components. Data referring to food allergy in Brazil are scarce, but it is estimated that 2.2% of the world population has cow's milk protein allergy (CMPA), which affects 5.4% of children (Solé et al., 2018; Vieira et al., 2010).

This allergy can be due to any component of milk, the most common being the metabolite produced by the A1A1 genotype of β-casein. However, this can be corrected through the selection of A2A2 animals and, therefore, genotyping for that genotype may contribute to the non-production of this metabolite (Kay et al., 2021).

In some people, to digest milk with A1 β-casein, the organism breaks peptide bonds and releases the bioactive peptide β-casomorphin-7 (BCM-7), which causes allergic reactions. Nonetheless, the presence of the A2 allele prevents peptide bond hydrolysis and inhibits the release of BCM-7 (Kamiński et al., 2007; Sharma et al., 2013).

Compared with conventional milk, A2A2 milk is a product of greater added value that constitutes an option to increase income from milk production. In Brazil, studies related to the frequency of the presence of β-casein alleles are still scarce, especially in the northern region of the country. Thus, this study was undertaken to examine the allele and genotype frequencies for β casein, A1 and A2, in dairy herds in the microregion of Araguaína - TO, Brazil.

Material and Methods

The present experiment was carried out from August 2020 to February 2021. Three (03) herds were selected from the dairy production chain of the microregion of Araguaína - TO, Brazil, located in the municipalities of Arapoema (Farm 1), Colinas do Tocantins (Farm 2), and Araguaína (Farm 3).

A total of 421 hair bulb samples distributed across three herds, characterized as dairy crossbreeds in production, were collected. The sample number was calculated considering 500,000 lactating cows, a 95% confidence interval, and a 5% experimental error, using an electronic calculator (“Calculator.net”) with 95% confidence level (Z= 1.96 standard deviation) and 5% margin of error. This experiment was approved by the Ethics Committee on Animal Experimentation (CEUA) at the Federal University of Tocantins (approval n. 23.101.002.456/2020-23).

The selection of animals for sampling was based on the following criteria: 60% of the cows in the first, second, or third lactation, between 30 and 250 days in milk. Breed and age were not considered, due to the composition and genetic diversity of the herds. All properties were characterized as dairy farms. Sires and young service bulls were also sampled.

DNA extraction from the hair follicle was performed at the Animal Breeding Laboratory (LMA) of the Veterinary Medicine Program at the Federal University of Tocantins (UFT), following the protocol described by Olerup & Zetterquist (1992).

After the extractions of genetic material, the quantity and purity of DNA were determined using a NanoDrop 1000 spectrophotometer (ThermoScientific, Waltham, MA, USA). The genotype samples for two markers from the polymorphic regions were characterized and confirmed by real-time PCR using the ABI Prism® 7500 instrument (Applied Biosystems) (Carvalho et al., 2017).
The allele and genotype frequencies were determined using the TaqMan™ detection system, whereby the primer and probe release different fluorescence signals for each allele of the polymorphism and are paired in the target DNA region, allowing the identification of different alleles (A1 and A2) by reading the fluorescence of each sample. When only one fluorescent signal is detected, the sample is homozygous for one allele, and when two different fluorescent signals are detected, the sample is heterozygous, considering both possible alleles (Carvalho et al., 2017).

The probes were synthesized to selectively pair on the DNA template where the polymorphism of interest is located. The rate of heterozygous and homozygous individuals to one of their genotypes was estimated by the fluorescent signals of the probes (Carvalho et al., 2017).

To amplify the genotypes corresponding to the A1 and A2 alleles for β-casein, the area of the gene responsible for encoding the protein, a pair of primers previously designed with the regions of interest, containing the respective nucleotide sequences, was used:

**Forward -** 5’ CCCAGACACAGTCTCTTAGTCTATCC 3’
**Reverse -** 5’ GGTTTGAGTAAGAGGAGGGATGTTT 3’

And the fluorescence probe, by the following sequences:

**Forward -** 5’ CCCATCC[C]TACAGCCT 3’
**Reverse -** 5’ CCCATCC[A]TACAGCCT 3’

For the real-time polymerase chain reaction (PCR), approximately 15 ng of DNA were used for a reaction volume of 10 µL, containing 0.25 µL Assay Mix® (Applied Biosystems), and 5.0 µL of Taqman® Master Mix Universal PCR (Applied Biosystems), under reaction conditions of 10 min at 95 °C and 45 cycles of 15 s at 92 °C and 1 min at 60 °C (Carvalho et al., 2017).

By visualizing the genotypes’ curve pattern, it was possible to calculate the gene (xᵢ and xⱼ) and genotype (xᵢᵢ, xᵢⱼ and xⱼⱼ) frequencies, which were determined by directly counting the observed genotypes. To test the observed frequencies, Hardy-Weinberg equilibrium testing was performed (Falconer & Mackay, 1996).

**Results**

The frequencies of the A1 and A2 alleles in the sampled animals were 28.27% and 71.73%, respectively. Of the total 421 samples, 2.13% (09/421) corresponded to males, sires, of which 0.47% (02/421) showed the A1A1 genotype; 0.47% (02/421) A1A2; and 1.19% (05/421) the A2A2 genotype. Females represented 97.86% (412/421) of the analyzed herd, with 9.02% (38/421) having the A1A1 genotype; 37.05% (156/421) A1A2; and 51.79% (218/421) the A2A2 genotype.

At farm I, 67 animals were genotyped. Of these, 17.91% (12/67) showed the A1A1 genotype; 29.85% (20/67); A1A2 and 52.24% (35/67) the A2A2 genotype. At farm II, 149 animals were genotyped, consisting of 6.04% (9/149) with the A1A1 genotype; 40.94% (61/149) with A1A2; and 53.02% (79/149) with A2A2. Finally, oat farm III, 205 animals were genotyped, with 9.27% (19/205) showing the A1A1 genotype; 37.56% (77/205) A1A2; and 53.17% (109/205) A2A2 (Figure 1).

All properties were characterized as dairy farms, with average milk production of 15.8 L/animal and an average fat content of 4.11%. The herds exhibited a great variation in their breed composition, with 41.33% of the animals characterized as crossbred (Bos taurus × Bos indicus), 49.88% as Girolando breed, 7.60% as Gir breed, and the remaining 1.19% as other breeds (Jersey and Sindhi). A noteworthy trait of the crossbred animals is that, in phenotypic terms, the zebu genetic composition prevailed over other European phenotypes, although the contribution of each breed could not be defined.

This phenotypic characterization possibly explains the higher frequency of the A2A2 genotype, which represented 20.66% (87/421) of the total herd and 50% (87/174) of the group of animals from crossbreeding. Among the other crossbred animals, 9.77% (17/174) showed the A1A1 genotype and 40.23% (70/174) A1A2.

In all herds analyzed, the frequency of A2 alleles was higher than that of A1, with A2A2 genotype seen in 52.97% of the evaluated animals, A1A2 in 37.53%, and A1A1 in 9.50%. Figure 1 represents the absolute and relative frequencies of the genotypes for milk β-casein in each of the analyzed herds.
**Discussion**

This herd characterization somehow explains the non-observance of Wardy-Weinberg equilibrium in the studied population, since the variability is large and selection is present in the herds. Another characteristic that corroborates this statement is the high frequency of the A2A2 genotype (55.55%) in the service bulls of the farm (05/09). Yet another aspect that may have contributed is the use of 100% semen from bulls already genotyped for A2A2 on the females.

The presented results do not differ from those reported in the previous studies in Brazil (Lima et al., 2014; Paschoal et al., 2017; Vercesi, 2011), which overall indicate a higher frequency of the A2 allele and its A2A2 genotype over the A1 allele and the A1A1 genotype. Thus, it suggests that many breeders have already been directing mating to increase the frequency of A2A2 genotypes.

Lima et al. (2014) and Vercesi (2011) evaluated Gir cows and observed a higher frequency of the A2A2 genotype, which represented 0.85 for the first author and 0.96 for the second. The frequency of the A1 allele can vary across breeds, with some of them having a lesser predisposition for this allele and thus the A2 allele predominating. In this way, the high zebu composition of the herd may have contributed to this frequency, since zebu breeds have a higher frequency of the A2 allele (Paschoal et al., 2017).

In Brazil, studies investigating the frequency of the presence of alleles for β-casein are still rare, especially in Zebu herds. In a study with dairy Gir cows, Paschoal et al. (2017) found that 41% (7/17) had the A1A2 allele and 59% (10/17) the A2A2 allele.

It is known that all mammalian species once produced A2 β-casein only, but because of a genetic mutation that occurred approximately 10,000 years ago, some cows started to produce A1 β-casein. And, due to a selection process, this gene (A1) is found more frequently in European breeds. Kamiński et al. (2007) found a higher frequency of the A2 allele compared with the low frequency of the A1 β-casein allele in the northern region of Tocantins, which overall indicate that these herds have low production of BCM-7. Thus, the selection of animals for the A2A2 genotype is less likely to cause the same health problems as milk with a high amount of A1 β-casein (Pereira, 2018). Studies led by Jianqin et al. (2016) showed that individuals who consumed A1 milk tend to experience significantly greater symptoms of digestive discomfort, delayed gastrointestinal transit, and gastroenteritis and that it may trigger lactose intolerance when compared with A2 milk.

Beta-casomorphin-7, a metabolite of A1 β-casein, also increases predisposition to other diseases such as human ischemic heart disease (McLachlan, 2001), type-1 diabetes mellitus (Elliott et al., 1999), arteriosclerosis (Tailford et al., 2003), and autism (Sokolov et al., 2014).

Therefore, the genotyping of animals for this gene can contribute to reducing the incidence of these diseases as a result of the ingestion of milk and its derivatives. Additionally, studies suggest that cows genotyped as A2A2 produce milk with higher protein content. In contrast, results regarding fat content are controversial (Nilsen et al., 2009; Olenski et al., 2010; Paschoal et al., 2017). Such characteristics of A2A2 milk culminate in a product with greater added value compared with conventional milk, constituting an option to increase income from milk production.

Milk holds great economic importance to the State of Tocantins, with employment and income-generating potential. Currently, some farms have significantly invested in improving their herds and sought to invest in the sale of differentiated products. However, there is still a need for technical monitoring, with guidelines to promote an efficient selection and formation of a differentiated market.

**Conclusion**

The frequency of the A1 allele for β-casein in dairy herds in the northern region of Tocantins was low and is per the results of previous studies. The A2A2 genotypes of β-casein had a high relative frequency; however, the A1A2 genotype is still rather frequent, warranting greater selection pressure, since it is recommended to cull animals with this genotype.

**Conflict of interests**

All authors declare that there are no conflicts of interest.
**Ethics Statement**

The project entitled 'Frequencies of A1 and A2 beta-casein alleles in dairy cattle herds from the mesoregion of Araguaína – Tocantins', case n. 23101.002456/2020-23, under the responsibility of Jorge Luís Ferreira, complies with the ethical standards established by the procedural law for the scientific use of animals, of October 8th, 2008, and its execution was approved by the Ethics Committee on Animal Use at the Federal University of Tocantins. Araguaina, 06/10/2020.

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