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ALLELIC AND GENOTYPIC FREQUENCIES OF THE CSN-2 GENE IN A POPULATION OF DAIRY CATTLE IN THE NORTHERN REGION OF THE STATE OF TOCANTINS

FREQUÊNCIAS ALÉLICAS E GENOTÍPICAS DO GENE CSN-2 EM UMA POPULAÇÃO DE BOVINOS LEITEIROS NA REGIÃO NORTE DO ESTADO DO TOCANTINS

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ABSTRACT

The present study aimed to verify allelic and genotypic frequencies of the β-casein genes in a population of dairy cattle in the north of the state of Tocantins in order to identify animals capable of producing β-casein A2 in preference to its counterpart. To this end, five herds belonging to the cities of Araguaína, Arapoema, Araguatins, Colinas do Tocantins, and Palmas were selected, totaling 1,109 dairy cattle of mixed and/or Gir dairy breed. Samples for two markers of polymorphic regions were characterized and confirmed by real-time PCR, using an ABI Prism® 7500 sequence detection system (Applied Biosystems). Allelic and genotypic frequencies were determined using the TaqMan ™ detection system, in which the primer and probe emit different fluorescence signals for each allele of the polymorphism. The observed frequencies indicated a prevalence of the A2 allele (72.59%) compared to the A1 allele (27.41%). Considering the genotypic constitution of the animals, there was a greater correspondence for A2A2 (54.55%) compared to A1A1 (9.38%) and A1A2 (36.06%). This denotes a promising future for the development of economic activities focused on the production of type-A2 milk in the northern region of the state of Tocantins.

Keywords: Beta casein A1. Beta casein A2. Bovine milk. Hypersensitivity.

RESUMO

O presente estudo teve como objetivo verificar as frequências alélicas e genotípicas dos genes da β-caseína em uma população de bovinos leiteiros no norte do estado do Tocantins, a fim de identificar animais capazes de produzir β-caseína A2 preferencialmente à sua contraparte. Para tanto, foram selecionados cinco rebanhos pertencentes aos municípios de Araguaína, Arapoema, Araguatins, Colinas do Tocantins e Palmas, totalizando 1.109 bovinos leiteiros da raça leiteira mestiça e/ou Gir. Amostras para dois marcadores de regiões polimórficas foram caracterizadas e

confirmadas por PCR em tempo real, utilizando um sistema de detecção de sequência ABI Prism® 7500 (Applied Biosystems). As frequências alélicas e genotípicas foram determinadas usando o sistema de detecção TaqMan ™, no qual o primer e a sonda emitem diferentes sinais de fluorescência para cada alelo do polimorfismo. As frequências observadas indicaram prevalência do alelo A2 (72,59%) em relação ao alelo A1 (27,41%). Considerando a constituição genotípica dos animais, houve maior correspondência para A2A2 (54,55%) em relação a A1A1 (9,38%) e A1A2 (36,06%). Isso indica um futuro promissor para o desenvolvimento de atividades econômicas voltadas para a produção de leite tipo A2 na região norte do estado do Tocantins.

Palavras-chave: Beta caseína A1. Beta caseína A2. Leite bovino. Hipersensibilidade.

INTRODUCTION

In its composition, bovine milk has lipids, minerals, and proteins of high biological value, being considered an important source of nutrients for all stages of human life and therefore forming the basis of the diet of Brazilian consumers. In this sense, dairy production transcends market values, placing itself as an important social agent responsible for various spillover effects along the production chain, fostering employment links, and attracting sectoral investments (Polastrini 2020).

Bovine milk has several protein groups, which can be subdivided into Alpha S1 and S2, Kappa, and Beta casein (Hanusová et al. 2010). The beta fraction has two manifestations: A1 β-casein, and A2 β-casein. What differentiates these two genetic variants is the substitution of just one amino acid at position 67 of the 209 amino acids that make up this protein. A1 β-casein has a histidine residue (His67), while A2 β-casein has a proline residue (Pro67) (Nguyen et al. 2015).

A1 β-casein causes cleavage and breakage of the peptide bond as a result of the digestion process, releasing bioactive β-casomorphin-7 (BCM-7) peptides responsible for causing allergic reactions and gastrointestinal discomfort. The presence of the A2 allele, on the other hand, prevents the hydrolysis of the peptide bond and inhibits the release of BCM-7 (Kamiński et al. 2007; Sharma et al. 2013).

The expression of different types of β-casein in milk, however, derives from the genotypic composition of the animal. Cows of the A1A1 and A1A2 genotypes translate A1 β-casein and A2 β-casein as proteins, while cows of the A2A2 genotype express only A2 β-casein. The latter, therefore, are neglected to produce more digestible bovine milk, the A2-type milk. It is an alternative that counteracts the hypersensitivity effects caused by BCM-7 and, therefore, different types of allergies, including cow's milk protein allergy (CMPA) (Sousa et al. 2019).

Additionally, studies suggest that cows genotyped as A2A2 produce higher protein content, but, on the other hand, the results regarding fat content are controversial (Olensko 2010; Nilsen 2009). Such characteristics result in a product with higher added value when compared to conventional milk, making it an option to increase income with dairy production.

In this way, type-A2 milk acts as a product that is not only more digestible than its counterpart, type-A1 milk, but which, as an innovative technology, converses with proposals for food inclusion and economic attractiveness. In short, an extremely promising combination of factors has given rise to an increasingly informed and critical consumer market, especially attentive to health and well-being issues (Souza et al. 2022).

Thus, the present study aimed to evaluate the genotypic profile and allele frequencies for the expression of β-casein, A1, and A2 in dairy herds in the north of the state of Tocantins.

MATERIALS AND METHODS

This experiment was approved by the Animal Experimentation Ethics Committee (CEUA) of the Federal University of Tocantins under protocol number 23.101.002.456/2020-23. The sample population consisted of 1,109 animals characterized as dairy types from five rural properties located in the municipalities of Araguaína, Arapoema, Araguatins, Colinas do Tocantins, and Palmas. The productive profile of the properties corresponds to medium-sized dairy farms with an average daily production of 700 liters. The animals, for the most part, came from crossbred

animals and/or from the dairy Gir breed. The sample collection period for genotyping was from August 2020 to September 2022.

A sample of approximately 80 hairs from the tail broom containing a hair follicle was collected for the extraction of genetic material. The samples were placed in a properly identified sealed envelope, and the DNA extraction from the hair follicle was performed at the Animal Improvement Laboratory (LMA) of the Veterinary Medicine course at the Federal University of Northern Tocantins (UFNT), using the kit EXTRACTS 96 from hair DNA (Loccus, Cotia, São Paulo, Brazil) according to the manufacturer's instructions. DNA concentration and purity were analyzed by a One/OneC nanodrop spectrophotometer (Thermo Scientific). Samples with an OD ratio (A260/A280 nm) between 1.7 and 2.0 were considered suitable and used for real-time polymerase chain reaction (PCR).

Primers and probes were designed based on the bovine beta-casein gene sequence (CSN2 gene) ID 281099 (GenBank, NCBI), as described by Giglioti et al. (2020). The sequences of the primers used were:

Forward - 5’ CCCAGACACAGTCTCTAGTCTATCC 3’
Reverse - 5’ GGTTTGAGTAAGAGGAGGGATGTTT3’
And the fluorescence probe by the sequences:
Forward - 5’ CCCATCCCTACAGCCT 3’
Reverse - 5’ CCCATCCATACAGCCT 3’

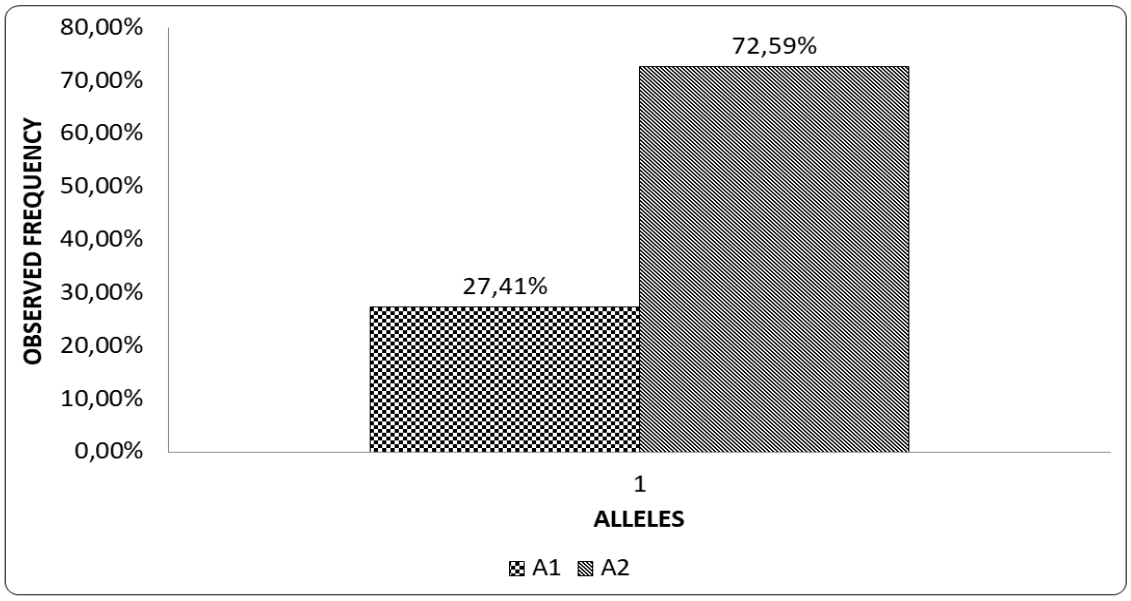
The allele and genotype frequencies were determined using the TaqManTM detection system. The real-time polymerase chain reaction (PCR) was set up for a volume of 10 µL consisting of 20 ng of genomic DNA, 0.25 µL Assay Mix® (Applied Biosystems), and 5.0 µL Taqman® Master Mix Universal PCR (Applied Biosystems). The reaction was carried out in a QuantStudio 12K thermocycler (Applied Biosystems) under the following conditions: 95 ºC for 10 min, followed by 45 cycles of denaturation at 92 ºC for 15 seconds and annealing at 60 ºC for 1 minute, and finally 60 ºC for 30 seconds.

The Chi-square test to determine whether the distribution of the genotype frequencies was in the Hardy–Weinberg equilibrium was carried out using the PopGene32 (ver.1.32) statistical program (Yeh et al., 1997). Polymorphism information content (PIC) was calculated for CSN2 according to Botstein et al. (1980) and Anderson et al. (1993).

RESULTS

When analyzing the genetic composition of the 1109 animals studied (Graph 1), we observed a predominance of the A2 allele to the detriment of the A1 allele (72.59% and 27.41%, respectively). Furthermore, it denotes favorable selection pressure for the trait analyzed given the high frequency observed for the desired gene.

Graph 1. Distribution of observed frequencies for the A1 and A2 alleles in dairy herds in the northern region of Tocantins, 2022.

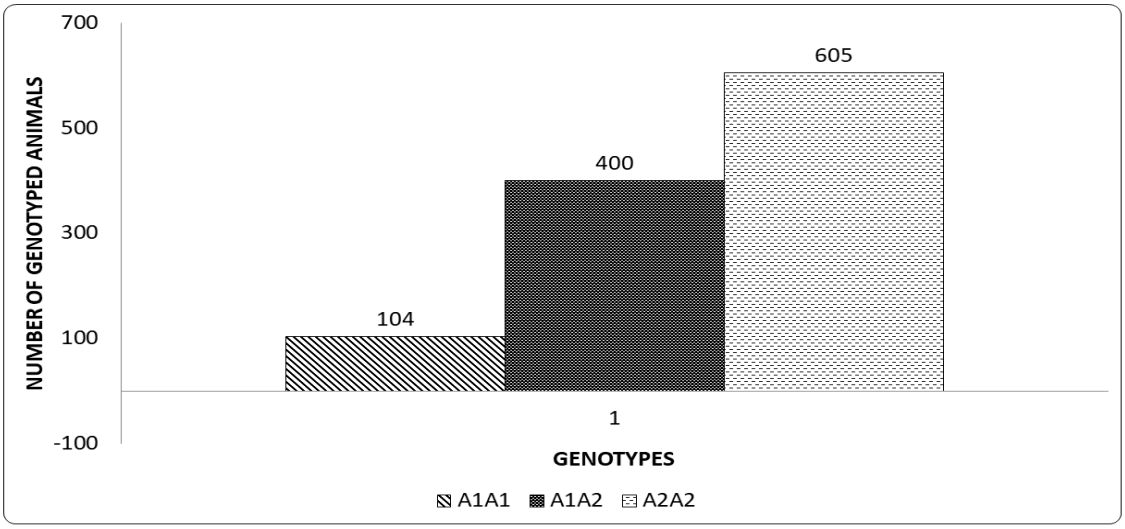


Fonte: Os autores.

It was also observed that 104 animals (9.38%) had the A1A1 genotype, 400 (36.06%) corresponded to A1A2, and 605 (54.55%) were genotyped as A2A2 (Graph 2). In this sense, it is understood that most of the analyzed population is efficient in

producing milk free of A1 β-casein and, therefore, protein generators are unable to degrade into compounds potentially allergenic to the human body.

Graph 2. Demonstration of the number of animals genotyped as A1A1, A1A2 and A2A2 in dairy herds in the northern region of the State of Tocantins, 2022.



Based on the chi-square test ($\chi 2$), considering the observed and expected genotypic frequencies for the β-casein polymorphism, a significant difference was found ($P < 0.05$). Thus, it is concluded by the Hardy-Weinberg Theorem that the population is not in genetic equilibrium.

DISCUSSION

There is a significant number of animals that, due to the A1A2 mixed genotype (36.06%), are capable of translating A1 β-casein and A2 β-casein. In this sense, the proteins present in the milk produced by these animals can be converted into opioid compounds during digestion, such as BCM-7, triggering hypersensitivity reactions and gastrointestinal discomfort since this process is associated with the presence of the A1 allele, but absent when related to the homozygosity of the A2 allele.

Such results and observations come from the high degree of zebu blood present in Brazilian herds since there is a greater natural expression of the A2 allele in animals belonging to the tested group. Thus, the successive crossings between zebu and taurine

animals to obtain animals more adapted to the tropical climate act as a determining factor in explaining the frequencies observed in the studied population.

In this sense, it is possible to infer that the panorama for the production of type-A2 milk in the northern region of Tocantins is favorable. However, it is necessary to evaluate a larger number of animals to prove this potential, also addressing the economic viability of the product. Thus, relating the expression of β-casein A2 in milk to an attractive commercial return due to the offer of a product with greater added value.

According to Souza et al. (2022), in a study on the behavior of milk consumers and the level of knowledge about A2 milk in the population of Tocantins, found that the majority of the Tocantins population is still unaware of the benefits of type-A2 milk. However, among individuals with prior knowledge about the product, there was a predisposition to pay more for it. In addition, the authors observed that brand, price, and packaging standards correspond to the main criteria used by the consumer when making a purchase, denoting the importance of bringing the consumer closer to the product.

Therefore, it is necessary to understand the consumer as a complex element (Schiffman & Kanuk, 2000) and weave strategic marketing actions with assertive marketing, generating expectations regarding the benefits of the product and its added values (Engel et al. 2000). In such a way, bringing knowledge about the physiological properties of type-A2 milk and its potential to contribute to a healthier life.

The prevalence of the A2A2 genotype and A2 allele observed in the studied population sample prevails over the A1 genotypes and alleles in the population. It denotes a promising future for the development of economic activities aimed at the production of type-A2 milk in the northern region of the state of Tocantins. However, there is still a need for further studies, especially in the areas of marketing, public policy aimed at the development of the sector, and animal improvement.

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