

Effect of A1 and A2 alleles of CSN2 gene on yield, composition traits, and somatic cell count of milk from cows of contrasting genotypes in Brazil

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ABSTRACT. This study was developed to identify possible effects of the A1 and A2 alleles of β -casein (β -CN) on the yield, composition traits, and somatic cell count (SCC) of milk from cows with the A1A1, A1A2, and A2A2 genotypes from a high-yielding Holstein herd in Brazil. Genotypic and phenotypic analyses were carried out. Genotypic analysis involved information from 1,816 Holstein cows between the years 2015 and 2019 and the characterization of allele (A1 and A2) and genotype (A1A1, A1A2, and A2A2) frequencies associated with 1,816 lactations. The different genotypes were also related to the following phenotypic traits: total milk yield; milk yield adjusted to 305 days; protein, fat, lactose, total solids, and solids-not-fat contents; and SCC. There was no difference between the genotypes for milk composition or SCC, and all genotypes showed values within the range established by Brazilian law. However, animals with the A1A1 genotype exhibited a higher 305-day lactation yield. In addition, the

herd is in Hardy-Weinberg Equilibrium, indicating that these animals are responsive to genetic selection for the A2 allele.

Key words: A2 milk; β -casein; Genotype; Phenotype.

INTRODUCTION

Caseins account for approximately 78% of the total protein in bovine milk and, within them, β -casein represents 30% (BROOKE-TAYLOR et al., 2017; KASKOUS, 2020). Dairy cattle have 13 β -casein variants (A1, A2, A3, A4, B, C, D, E, F, H1, H2, I, and G) (KAMIŃSKI et al., 2007) however, only seven of these (A1, A2, A3, B, C, I, and E) have been detected in European cattle breeds (Jiménez-Montenegro et al., 2022). It can occur in different genetic variants, although the A1 and A2 variants are the most common (Guantario et al., 2020). The A2 variant is considered the oldest variant, from which the others originated via mutation. The most common variants are A1 and A2; the B variant is less common in most breeds (Kamiński et al., 2007; Sebastiani et al., 2020). β -casein has 209 amino acid residues in its protein chain, and is controlled by a single gene, located on chromosome 6, called CSN2 (Caroli et al., 2009). The mutation that causes differences in the β -casein protein is a result of a single nucleotide polymorphism at codon 67 in exon 7 of the gene CCT (A2, proline) which results in CAT (A1, histidine) (Farrell et al., 2004).

The A1 β -casein differs from the A2 β -casein by a different amino acid at position 67 of the peptide chain; this is histidine (His67) in the A1 variant and proline (Pro67) in the A2 variant. His67 within A1 has been associated with an increased susceptibility of A1 β -casein to enzymatic hydrolysis by gastric enzymes such as pancreatic elastase, pepsin, and leucine aminopeptidase (Thiruvengadam et al., 2021; Li et al., 2022). These changes in digestion patterns associated with A1 β -casein lead to the release of β -casomorphin 7 (during gastrointestinal digestion (Jiménez-Montenegro et al., 2022)).

BCM-7 is Bioactive peptide (Kamiński et al., 2007; Jarmołowska et al., 1999), which originates mostly from β -casein variants A1 (Kostyra et al., 2004; Jinsmaa et al., 1999), may be a significant risk factor in human ischemic heart disease, arteriosclerosis, type I diabetes, sudden infant death syndrome, and autism (Kamiński et al., 2007; Cieślińska et al., 2015; Reichelt et al., 2012).

Many reports have presented research results on β -casein gene polymorphisms, given the potential importance of A2 milk for public health and its apparent commercial potential. However, literature on the relationship between β -casein variants, productivity and milk composition of cows with β -casein genotype A2 in tropical conditions is scarce. The objective of the current study was to identify possible effects of the A1 and A2 alleles of β -casein (β -CN) on the yield, composition traits, and SCC of milk from cows with the A1A1, A1A2, and A2A2 genotypes in a high-yielding Holstein herd in Brazil.

MATERIAL AND METHODS

The data used in this study were obtained from a dairy farm located in São Paulo State, Brazil. Daily milking records were available for 1,718 cows.

Genotypic analysis of the cows

For the genotypic analysis, genotypic information of 1,816 cows was used, during the years 2015 to 2019, extracted from the Clarifide Zoetis© program and provided by the dairy farm. The allele (A1 and A2) and genotypic (A1A1, A1A2 and A2A2) frequencies of β -casein in the herd cows were characterized according to Falconer (1987).

Genotype effects relative to the evaluated phenotypic effects

To observe the relationship between the different genotypes and phenotypes of the cows, information associated with the 1,816 lactations was used, with 391 information from cows carrying the A1A1 genotype for the β -casein gene, 863 information from cows carrying the A1A2 genotype for the β -casein gene and 562 information on cows carrying the A2A2 genotype for the β -casein gene.

The phenotypic traits used in this study were total milk yield (1,816 data), milk yield adjusted to 305 days (1,816 data), and protein, fat, lactose, total solids (TS), solids-not-fat (SNF), and SCC (1,556 data) in the milk of these cows.

Analysis of milk yield, composition, and somatic cell count (SCC)

Milk samples for composition analysis (protein content, fat content, lactose content, TS, SNF, and SCC) were sent monthly to “Clínica do Leite” (ESALQ/USP) and analyzed with infrared equipment for the determination of the protein, fat, lactose, TS, and SNF contents (ISO 9622/IDF 141 method) and flow cytometry for the quantification of SCC (flow cytometry protocol, according to ISO 13366-2/IDF 148-2 anchored by calibration on method ISO 13366-1/IDF 148-1) (Brasil, 2018).

To determine the total yield and 305-day milk yield, the milk of all lactating cows was weighed monthly and results were tabulated into performance-control spreadsheets.

STATISTICAL ANALYSIS

Data were submitted to descriptive statistical analysis to verify the influence of the three different genotypes for the β -casein gene on production, composition and somatic cell count of milk from Holstein cows. Total milk production (TMY), adjusted milk production at 305 days of lactation (MY305), fat (%F), protein (%P), lactose (%L), total solids (% TS), non-fat solids (%SNF) and somatic cell count (SCC, in log cells/mL⁻¹) were analyzed as dependent variables and the A1A1, A1A2 and A2A2 genotypes were analyzed as independent variables. In the mixed linear model, year-calving season, lactation number, genetic composition and genotypes were the fixed effects, and sire was the random effect. In which, for the calving-season year, five year classes were used (2015, 2016, 2017, 2018 and 2019); for the season class, four season classes were used (autumn, winter, spring and summer), with 20 experimental units; for the lactation number, six classes were used (1, 2, 3, 4, 5 and 6); for genetic composition, two classes were used (crossbred and purebred cattle); for the genotype, three classes were used (A1A1, A1A2 and A2A2), with 11 experimental units. Considering the four different variables (year-season, lactation number, genetic composition and genotype) for the genotypic and phenotypic characterization of the herd, it totaled

31 experimental units, with 1,816 numbers of observations for the TMY and MY305 variables and with 1,556 numbers of observations for the %F, %P, %L, %TS, %SNF, and SCC (log cells/mL⁻¹) variables.

For the genotypic and phenotypic characterization of the herd, the normality of the residues was verified by the Shapiro-Wilk and Kolmogorov-Smirnov tests and homogeneity between the variances was verified by the Levene and Bartlett test, adopting a significance level (α) of 5% ($P < 0.05$). For the mean test, Tukey's test was used, adopting a significance level (α) of 5% ($P < 0.05$). Data were analyzed by ANOVA with the aid of the MIXED procedure of the Statistical Analysis System® version 9.4 computer program (SAS, 2008), adopting significance levels (α) of 5% ($p \leq 0.05$) and 1% ($p \leq 0.01$). The statistical model used was:

$$Y_{ijklmn} = \mu + AE_i + N_j + C_k + G_l + R_m + e_{ijklmn}$$

wherein,

Y_{ijklmn} = is the observed value for the phenotypic trait in question;

μ = overall average;

AE_i = year-calving season fixed effect i , where $i = 1$ (2015-summer), 2 (2015-autumn), ..., 20 (2019-spring);

N_j = fixed effect of lactation number k , where $k = 1, 2, 3, 4, 5, 6$;

C_k = fixed effect of genetic makeup k , where $j = 1$ (GC) and 2 (PO);

G_l = fixed effect of genotype i , where $i = 1$ (A1A1), 2 (A1A2) and 3 (A2A2);

R_m = random effect of Player m , with mean 0 and s^2R ;

e_{ijklmn} = random effect associated with each observation, with mean 0 and s^2e .

RESULTS

In the studied herd, the population frequencies were 21.53% (391 cows) for the A1A1 genotype; 47.52% (863 cows) for the A1A2 genotype; and 30.95% (562 cows) for the A2A2 genotype, indicating that the majority of cows in the herd have the A1A2 genotype (Table 1).

In terms of allele frequency, results show a situation in which the A1 allele had a frequency of 45.29%, whereas the frequency of A2 allele was 54.71%, totaling 100% of the herd. Therefore, the A2 allele occurs at a higher frequency in the evaluated herd (Table 2).

The cows in the studied herd have a total production volume and a 305-day lactation yield characteristic of high-yielding Brazilian commercial herds. The levels of fat, protein, lactose, TS, SNF, and SCC (Table 3) were within what is considered normal for the composition of milk as established by NI76 (Brasil, 2018).

Based on the test of means, there was no statistically significant difference ($P < 0.05$) for total milk yield; fat, protein, lactose, TS, or SNF contents; or SCC between the three different genotypes (Table 4).

In the same row, different letters (^a and ^b) differ statistically from each other and common letters (^a and ^a) do not differ statistically from each other. “^a” represents the highest means at a significance level lower than or equal to 5% ($P < 0.05$).

Effect of A1 and A2 alleles of CSN2 gene on yield, composition traits, and somatic cell count of milk from cows 5 of contrasting genotypes in Brazil

Table 1. Genotype frequency in the population and total number of Holstein cows, observed between the years 2015-2019.

| Frequency | Genotype | | | Number of cows |
|-----------------------|----------|-------|-------|----------------|
| | A1A1 | A1A2 | A2A2 | |
| Absolute ¹ | 391 | 863 | 562 | 1,816 |
| Relative (%) | 21,53 | 47,52 | 30,95 | 100 |

Table 2. Allele frequency of the A1 and A2 alleles in Holstein cows, observed between the years 2015-2019.

| Frequency | Allele | | Total |
|--------------|--------|--------|-------|
| | A1 | A2 | |
| Relative (%) | 0,4529 | 0,5471 | 1 |

Table 3. Estimates of the mean, minimum (MIN), and maximum (MAX) values of yield, composition traits, and SCC of milk from Holstein cows, observed between the years 2015-2019.

| Trait | N of cows | Mean | Min | Max |
|----------------------------------|-----------|-----------|----------|-----------|
| Total milk yield (kg) | 1,816 | 13,660.11 | 5,520.27 | 37,638.30 |
| MY305 (kg) ¹ | 1,816 | 11,021.49 | 6,092.00 | 17,380.00 |
| Fat (%) | 1,556 | 3.64 | 1.95 | 6.51 |
| Protein (%) | 1,556 | 3.06 | 2.33 | 3.94 |
| Lactose (%) | 1,556 | 4.66 | 2.81 | 5.37 |
| Total solids (%) | 1,556 | 12.35 | 9.97 | 15.42 |
| Solids-not-fat (%) | 1,556 | 8.70 | 7.41 | 9.71 |
| SCC (log cells /mL) ² | 1,556 | 1.91 | 0.92 | 3.69 |

Source: authors' creation.

¹Milk yield adjusted to 305 days of lactation; ²Logarithm of somatic cell count/mL of milk.

The number of cows refers to the number of observations used on a clear basis.

Table 4. Mean estimates and standard error of the mean of the three different genotypes (A1A1, A1A2, and A2A2) for the CSN-2 gene compared with the phenotypic traits of a commercial dairy herd, between the years 2015-2019.

| Trait | A1A1 | | A1A2 | | A2A2 | | p-value |
|---------------------------------|---------------------|--------|---------------------|--------|----------------------|--------|---------|
| | Mean | SEM | Mean | SEM | Mean | SEM | |
| Total milk yield (kg) | 13,805 | 372.72 | 14,269 | 347.91 | 14,435 | 367.71 | 0.0639 |
| MY305 (kg) ¹ | 11,260 ^a | 160.55 | 10,966 ^b | 149.10 | 10,995 ^{ab} | 158.15 | 0.0183 |
| Fat (%) | 3.55 | 0.061 | 3.47 | 0.05 | 3.50 | 0.05 | 0.1522 |
| Protein (%) | 3.06 | 0.024 | 3.07 | 0.02 | 3.08 | 0.02 | 0.2909 |
| Lactose (%) | 4.57 | 0.020 | 4.55 | 0.01 | 4.56 | 0.01 | 0.1396 |
| Total solids (%) | 12.17 | 0.077 | 12.09 | 0.07 | 12.13 | 0.07 | 0.1539 |
| Solids not fat (%) | 8.61 | 0.033 | 8.61 | 0.03 | 8.63 | 0.03 | 0.4804 |
| SCC (log cells/mL) ² | 2.26 | 0.05 | 2.19 | 0.05 | 2.23 | 0.05 | 0.1449 |

Source: authors' creation.

SEM: standard error of the mean.

¹Milk yield adjusted to 305 days of lactation; ²Logarithm of somatic cell count/mL of milk.

Table 5. Estimate of the additive effect of the A1 allele on the A2 allele for CSN-2, relative to yield and composition traits and SCC of milk from cows, between the years 2015-2019.

| Trait | Mean | SD | p-value |
|---------------------------------|---------|--------|---------|
| Total milk yield (kg) | 629.90 | 274.51 | 0.0219 |
| MY305 (kg) ¹ | -265.06 | 118.98 | 0.0260 |
| Fat (%) | -0.048 | 0.045 | 0.2821 |
| Protein (%) | 0.026 | 0.017 | 0.1163 |
| Lactose (%) | -0.014 | 0.014 | 0.3183 |
| Total solids (%) | -0.037 | 0.054 | 0.4967 |
| Solids not fat (%) | 0.015 | 0.023 | 0.5118 |
| SCC (log cells/mL) ² | -0.030 | 0.041 | 0.4646 |

Source: authors' creation.

SD: standard deviation.

¹Milk yield adjusted to 305 days of lactation; ²Logarithm of somatic cell count/mL of milk.

Significance level less than or equal to 5% ($P < 0.05$).

Table 6. Estimate of the effect associated with dominance deviation for the CSN-2 gene, relative to yield, composition traits and SCC of milk from cows, between the years 2015-2019.

| Trait | Mean | SEM | p-value |
|---------------------------------|----------|---------|---------|
| Total milk yield (kg) | 149.420 | 194.720 | 0.4430 |
| MY305 (kg) ¹ | -162.290 | 81.617 | 0.0469 |
| Fat (%) | -0.050 | 0.029 | 0.0821 |
| Protein (%) | 0.002 | 0.010 | 0.8145 |
| Lactose (%) | -0.016 | 0.009 | 0.0680 |
| Total solids (%) | -0.065 | 0.034 | 0.0599 |
| Solids not fat (%) | -0.014 | 0.015 | 0.3522 |
| SCC (log cells/mL) ² | -0.050 | 0.026 | 0.0573 |

Source: authors' creation.

SEM: standard error of the mean.

¹Milk yield adjusted to 305 days of lactation; ²Logarithm of somatic cell count/mL of milk.

Significance level less than or equal to 5% ($P < 0.05$).

Table 7. Estimates of the gene substitution effects of the A1 allele on the A2 allele for the CSN-2 gene, relative to yield, composition traits, and SCC of milk from cows, between the years 2015-2019.

| Trait | Mean | SD | p-value |
|---------------------------------|----------|---------|---------|
| Total milk yield (kg) | 302.260 | 136.240 | 0.0266 |
| MY305 (kg) ¹ | -119.360 | 59.203 | 0.0439 |
| Fat (%) | -0.019 | 0.022 | 0.3888 |
| Protein (%) | 0.013 | 0.008 | 0.1202 |
| Lactose (%) | -0.005 | 0.007 | 0.4402 |
| Total solids (%) | -0.012 | 0.027 | 0.6529 |
| Solids not fat (%) | 0.009 | 0.012 | 0.4370 |
| SCC (log cells/mL) ² | -0.010 | 0.020 | 0.6168 |

Source: authors' creation.

SEM: standard error of the mean.

¹Milk yield adjusted to 305 days of lactation; ²Logarithm of somatic cell count/mL of milk.

Significance level less than or equal to 5% ($P < 0.05$).

As for 305-day lactation yield, according to the results of the test of means, there was a statistically significant difference ($P < 0.05$) between genotypes A1A1 (11,260 kg), A1A2 (10,966 kg), and A2A2 (10,995 kg), with A1A2 exhibiting the lowest values. The A2A2 genotype did not show a statistically significant difference in relation to the other two genotypes.

Fat, protein, lactose, TS, and SNF contents and SCC showed no statistically significant difference ($P < 0.05$) between the A1 and A2 alleles (Table 5).

Total milk yield and 305-day lactation yield, however, differed significantly ($P < 0.05$) between the alleles, with better results seen for both production traits when the A1 allele was introduced into the herd (Table 5).

Total milk yield; fat, protein, lactose, TS, and SNF contents; and SCC showed no statistically significant difference ($P < 0.05$) between homozygous and heterozygous cows (Table 6).

For total yield and 305-day lactation yield, there was a statistically significant difference ($P < 0.05$) between homozygotes and heterozygotes, with the heterozygous cows exhibiting higher values.

Fat, protein, lactose, TS, and SNF contents and SCC did not differ significantly ($P < 0.05$) with the gene substitution of effects of the A1 allele on the A2 allele (Table 7).

Total milk yield and 305-day lactation yield showed a statistically significant difference ($P < 0.05$) between alleles A1 and A2, with both variables increasing when the A1 allele was introduced into the herd (Table 7).

DISCUSSION

Rahimi et al., (2015) studied the allele and genotype frequencies of the CSN-2 gene in 131 native cattle in Iran and observed that the allele frequency of A1 and A2 was 38.2% and 61.8%, respectively, and that genotype frequency was 16.8% for A1A1, 42.7% for A1A2, and 40.5% for A2A2, which our results corroborate.

In a herd of Holstein cows, in 1989, Heck et al., (2009) found that the A1 allele represented 0.462%, whereas the A2 allele represented 0.498% of the herd. In 2005, when the authors evaluated 1,912 cows from 398 different herds, the frequency of the A2 allele was 0.692%, whereas the A1 allele represented 0.285% of the population. The authors emphasize that Holland had no selective breeding program that selected protein variants, and that these changes in the percentage of allele frequencies could have been due to random deviation.

Although the present study was carried out in a herd on which selective breeding is already practiced and the A2 allele is selected in the population with the objective of producing milk from cows with the A2A2 genotype, our results corroborate those found by the aforementioned authors.

Random selection of animals in a herd causes the genes to be distributed randomly within a population. This evolution happens over time and cannot be controlled; everything will depend on which animals are being used to reproduce within the herd and their genes. This will result in changes in allele and genotype frequencies. In this case, the gene distribution was favorable for the A2 allele, which may explain the similar results.

Chessa et al., (2013) analyzed the allele frequency of Holstein herds in two different years (2003 and 2012), in a total of 100 cows. Among the milk protein genes evaluated, CSN-2 showed

0.392% A1, 0.533% A2, 0.005% A3, 0% I, and 0.070% B in the year 2003; and 0.395% A1, 0.530% A2, 0% A3, 0.035% B, and 0.040% I in 2012. In the discussion, the authors emphasized that gene selection can strongly affect the polymorphism of milk protein genes and that changes in the frequency of variants can also change the composition of milk, interfering with its properties. According to the consulted literature, it is believed that genetic selection, the evolution of species, and random mating are responsible for the polymorphism of the CSN-2 gene.

Daí et al., (2016), Massella et al. 2017, and Kumar et al. (2019) found allele frequencies of 0.432% for A1 and 0.453% for A2; 0.371% for A1 and 0.546% for A2; and 0.432% for A1 and 0.563% for A2, respectively. As for genotype frequency, the authors found 0.203% for A1A1, 0.353% for A1A2, and 0.226% for A2A2; 0.139% for A1A1, 0.400% for A1A2, and 0.301% for A2A2; and 0.175% for A1A1, 0.515% for A1A2, and 0.310% for A2A2, respectively, in Holstein herds. Daí et al., 2016 also examined the frequency of other allelic variants and found 0.060%, 0.045%, and 0.004% for alleles B, I, and A3, respectively, and genotype frequencies of 0.068% for A1B, 0.060% for A2I, 0.052% for A2B, 0.030% for A1I, and 0.007% A1A3, demonstrating that the A1 and A2 variants continue to be the most common and that the A1A2 genotype occurs more frequently in the population.

It is known that an important factor for the conduct of studies such as the present is the number of animals and that a greater number of animals can better represent reality. Nonetheless, although the number of animals evaluated by Dai et al., (2016) and Kumar et al., (2019) was lower than the 1,816 cows evaluated in the present study and also than the population studied by Massella et al., (2017), all results are in line with our findings.

The frequency of alleles and genotypes in small or large herds can be similar, since what is taken into account in these situations is the number of genes present in the population. Selective breeding is a tool that can be used in both herd sizes and contributes to increasing the frequency of the chosen allele in the population more quickly and efficiently.

Another factor to be considered is the animal breed, as some authors believe that around ten thousand years ago, the total cattle herd had only the A2 allele for the production of β -CN and that factors such as the evolution of the species and selective breeding forced polymorphism and the formation of the A1 allele. After that, animals of the taurine breed have had a higher frequency of the A1 allele; however, according to the consulted literature, Holstein herds have a higher frequency of the A2 allele even when they are not genetically selected and selection happens randomly.

Hanusová et al., (2010) described results that disagree with the current findings, reporting allele frequencies for the CSN-2 gene of 0.54% A1 and 0.46% A2 in cows and 0.60% A1 and 0.40% A2 in bulls. Genotype frequencies in these animals were 0.13% A1A1, 0.83% A1A2, and 0.04% A2A2 in cows and 0.20% A1A1 and 0.80% A1A2 in bulls. Cieślińska et al., (2019) also reported discordant results, with allele frequencies of 0.63% A1 and 0.37% A2 in cows and 0.42% A1 and 0.58% A2 in bulls and genotype frequencies of 36.7% A1A1, 52.5% A1A2, and 10.7% A2A2 in cows and 12.5% A1A1, 58.3% A1A2, and 29.2% A2A2 in bulls.

The high frequency of the A1 allele in cows and bulls can generate a higher frequency of homozygous (A1A1) and even heterozygous (A1A2) genotypes, which would explain the absence of the A2A2 genotype in the bulls and the low frequency of the A2A2 genotype in the cows. Furthermore, the balance of allele frequency between cows and bulls can also increase the frequency of heterozygotes in the population, which suggests that in a herd where animals are mated at random,

the increase in alleles and genotypes also occurs randomly, but it is not possible to predict how this interferes with the genetic evolution of the herd. In a study with 32 cows (16 Holstein and 16 Brown Swiss), Deitos et al. (2011) observed a milk yield of 30.02 kg/cow/day, 2.85% fat, 3.15% protein, 4.65% lactose, 11.72% TS, 8.77% SNF, and a SCC of 297.83 log cells/mL in the Holstein animals. In the Brown-Swiss cows, milk yield was 29.17 kg/cow/day, the fat content was 3.43%, the protein content was 3.17%, the lactose content was 4.58%, TS was 12.17%, SNF was 8.73%, and SCC was 142.12 log cells/mL.

Ludovico et al., (2019) examined the effects of different genetic groups related to heat stress and production traits in Holstein animals and found 3.14% protein, 4.56% lactose, 12% TS, and 8.61% SNF in their milk.

The milk composition results found in the present study (3.64% fat, 3.06% protein, 4.66% lactose, 12.35% TS, 8.70% SNF, and SCC of 1.91 log cells/mL) corroborate those reported by Ludovico et al., (2019) and comply with NI76 (Brasil, 2018).

Environmental, nutritional, and genetic factors present in animal production can cause these indices to vary upwards or downwards, and the synchronization of all these indices, along with the use of animals of good genetics and aptitude for milk production and other components, can favor reproduction, generating higher-yielding animals and bringing positive results to the farm's economy.

By estimating the means of the three different genotypes (A1A1, A1A2, and A2A2), we observed that only milk yield adjusted to 305 days of lactation differed between the genotypes and that animals with the A1A1 genotype were higher-yielding. These results disagree with those found by Hanusová et al., (2010), who examined the production profile of 92 primiparous Holstein cows and observed that, for total milk yield, the A1A1, A1A2, and A2A2 genotypes did not exhibit a statistically significant difference. As for fat, genotypes A1A2 (3.90%) and A2A2 (3.90) showed a higher percentage than genotype A1A1 (3.77%). Regarding the protein content, genotypes A1A1 (3.17%) and A1A2 (3.20%) produced less when compared with genotype A2A2 (3.28%). The authors emphasized the importance of studies with a greater number of animals to determine whether these results follow the same pattern.

Molee et al., (2011) analyzed the effects of the β -CN and k-casein gene on the yield and composition of milk from Holstein cows and also found discordant results. According to the authors, the only statistically significant difference was found in A1A2 heterozygous animals, which showed a significantly higher fat content when compared with homozygous A1A1 and A2A2 animals.

This information may be of interest to the industry, whose main focus is the amount of milk purchased. The introduction of animals with the A1A1 genotype into the herd can increase the amount of milk produced at 305 days, thereby increasing the amount of milk supplied to the dairy industry and, consequently, the revenues of the producing farm.

Results regarding the additive effect, dominance deviation, and gene substitution effect related to the CSN-2 gene were not discussed, as no studies with this specific gene and its alleles of interest (A1 and A2) were found in the consulted literature. However, similar articles using other genes of interest for milk composition will be discussed.

Oleński et al., (2012) analyzed the additive effect of the A2 allele of the CSN-2 gene and the C allele of the BCE129 gene and found that when animals with the A2 allele were introduced into the herd, there was an increase in total milk yield and protein content and that the C allele was related to higher milk fat contents. The authors concluded that the A2 allele can be used as a

molecular marker for milk production traits and protein content and that the two alleles (A1 and A2) are useful for assisted selection on farms that intend to work with selective breeding for this gene.

These results differ from the current findings regarding the additive genetic effect of the A1 allele on the A2 allele, which revealed that when the A1 allele was added to the herd, there was an improvement in total yield and 305-day lactation yield.

Considering the 305-day lactation yield of the analyzed herd, it is observed that A1A1 cows have an average production of 11,260 kg and that A2A2 cows produce, on average 10,995 kg, i.e., the introduction of the A1 allele into the herd increases 305-day lactation yield by 206.06 kg.

According to the results, from an economic point of view, considering only the price of a liter of conventional milk of BRL 3.75 for the consumer (market quotation of March 10, 2022), this herd could be failing to earn about BRL 772.50 per animal with the A2 allele, and A1A1 animals could generate about BRL 42,225.00 per animal in one lactation according to the average. However, it is worth mentioning that milk from cows with the A2A2 genotype is a niche market and that the liter of this milk can reach BRL 7.20 (market quotation of March 10, 2022), Additionally if multiplied by the average production of A2A2 animals, this value would generate an amount of BRL 79,164.00 per animal in one lactation. Therefore, even if A1A1 cows produce more milk at 305 days, in economic terms, if we consider only the value of a liter of milk, the production of A2 milk is apparently more profitable.

Tabaran et al., (2015) worked with Holstein animals and found that the K allele is related to higher concentrations of milk fat, compared with the A allele.

Like Tabaran et al., (2015), we also investigated the additive genetic effect of the A1 allele on the A2 allele and observed that when the A1 allele was introduced into the herd, better results for traits such as total milk yield and 305-day lactation yield could be attained in comparison with the A2 allele.

Heck et al., (2009) examined the estimated effects of CSN-2 gene variants in 1,912 Holstein cows and observed that the A1 allele is related to lower protein production and higher concentrations of α_{s1} when compared with the A2 allele. Variant B was related to a higher concentration of κ -casein when compared with alleles A1 and A2.

These results demonstrate the importance of the interaction between genes and how the addition or subtraction of a gene can influence milk production, since it is known that genes that have an inheritance of economic interest for these traits, both in terms of production and composition, are polygenic.

CONCLUSION

Animals with the A1A1 genotype showed higher lactation performance at 305 days, while for total production, milk composition (fat, protein, lactose, total solids or non-fat solids) and SCC there was no difference between the genotypes, in addition, the Herd is in Hardy-Weinberg Equilibrium, indicating that these animals are responsible for genetic selection for the A2 allele.

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Effect of A1 and A2 alleles of CSN2 gene on yield, composition traits, and somatic cell count of milk from cows¹² of contrasting genotypes in Brazil

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