Relationship of β-casein genotypes (A1A1, A1A2 and A2A2) to the physicochemical composition and sensory characteristics of cows’ milk

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ABSTRACT
This study determines the relationship between β-casein CSN2 genotypes (A1A1, A1A2, A2A2) and milk’s biochemical and sensory traits. Ninety-six cow’s milk from the same herd was investigated. Animals were grouped according to the β-casein genotype variants A1A1, A1A2 and A2A2, with thirty-two animals each. A1A1 milk had higher monounsaturated fatty acids MUFA (<0.001) and lower levels of saturated fatty acids SFA (<0.001) fatty acids content. A1A2 milk had higher amino acids and higher SFA (<0.001) fatty acid content in the milk. Furthermore, A1A2 milk had the highest lightness index (L*) (<0.001), which we found correlated to increased redness index (a*) (<0.001) and yellowness index (b*) (<0.01) values. A2A2 milk was higher in polyunsaturated fatty acids PUFA (<0.001), omega-3 (0.001) and omega-6 (<0.001) and lower saturated fatty acids SFA (<0.001) fatty acids content. We concluded that the amino acid content, fatty acid content and colour of the milk could be influenced by CSN2 genotypes A1A1, A1A2 and A2A2. As a result, the selective breeding of genotypes with preferred qualities may improve milk and dairy products.

Introduction
Milk from different mammal species has differences in composition, but its primary components are proteins, fats and carbohydrates. Milk proteins consist of 80% casein, which is divided into four fractions as1-, as2- β- and κ- caseins (Andič et al. 2021). β-casein contains 209 amino acids and has two variants A1 and A2, based on the presence of amino acid at 67th position. A1 type consists of histidine at the 67th position, whereas A2 consists of proline at the same position (Geeta Chauhan and Kumar 2021). This difference affects the digestion of the primary proteins, meaning A1 milk will produce the bioactive peptide β-casomorphin during digestion; β-casomorphin is linked to significant opioid activity (Massella et al. 2017; Sebastiani et al. 2020). β-casomorphin impairs the immune system, increases the risk of diabetes (Şahin et al. 2018), and influences the health of the cardiovascular system (Miluchová et al. 2016). Increased production of A2 milk could be beneficial for milk consumers to have access to healthier products. A better understanding of the benefits of A2 milk production and product manufacturing processes would allow the short-chain family farms to apply cattle husbandry plans that could create cattle capable of producing milk containing only the A2 genetic variant β-casein. Quantitative genetics have extensive applications in animal husbandry because the main goal in animal breeding is to select breeding stock that can produce offspring with desired phenotypes. For effective breeding programmes, for the production of A2 milk, it is necessary to identify the genetic inheritance of a specific specimen (Bugeac et al. 2013). Such strategies, paired with nongenetic factors, like housing conditions (Polsky and von Keyserlingk 2017) and nutrition (Kudlinskiene et al. 2020), could represent new methods for dairy processors to achieve the desired biochemical (Gustavsson et al. 2014; Perna et al. 2016; Albarella et al. 2020) and visual (colour) quality of milk products, such as cheese and butter (Comin et al. 2008; Scarsio et al. 2017), without relying on artificial additives. To our knowledge, the link between milk biochemical (DM, pH, protein, fat, lactose, amino and fatty acids) composition and sensory traits (appearance, taste, smell and colour of the milk) and the β-casein gene CSN2 has not previously been investigated. Therefore, this study aimed to determine the relationship between β-casein CSN2 genotypes (A1A1, A1A2 and A2A2) and milk’s biochemical and sensory traits.

Materials and methods
The study from the relationship between β-casein CSN2 genotypes (A1A1, A1A2, A2A2) and milk's biochemical and sensory traits was carried out following the methodology of the Law on the welfare of the farm Animals of the Republic of Lithuania...
and complied with the Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes. This research was conducted at the Lithuanian University of Health Sciences, Veterinary Academy Tiližas 18, Kaunas. Ninety-six crossbreed Simmental × Holstein dual-purpose cows from the same herd were grouped into three groups (n = 32 per group). Animals were grouped according to the β-casein genotype variants A1A1, A1A2 and A2A2, and were selected following milk sampling by the ISO guideline ISO 707/IDF 50C:2008 (2008). All cows were kept in the same housing conditions and fed according to their body weight. The Lithuanian national milk testing laboratory performed fat, protein and lactose analysis. Fatty acid content, DM, pH and colour of the milk were determined at the Lithuanian University of Health Sciences, Faculty of Animal Sciences, Institute of Animal Husbandry Technology, Tiližas 18, Kaunas. Milk sensory evaluation was performed at the Lithuanian University of Health Sciences, Faculty of Animal Sciences, Department of Animal Nutrition, Tiližas 18, Kaunas. Amino acid quantification of the milk was conducted at the Lithuanian University of Health Sciences, Institute of Animal Science R. Žebenkos 12, Baisiogala.

### Laboratory work

A summary of the sample replicates of the milk is given in Table 1. Two hundred and eighty-eight sample reps (200 mL) for determining the quantitative parameters (fat, protein, lactose, dry matter (DM), pH, colour, fatty acid composition and amino acid composition) of the milk. Additionally, nine (1000 mL) sample reps were collected for sensory evaluation of the milk; samples were refrigerated to 4°C and evaluated the next day.

DM, fat, protein and lactose were analyzed using the mid-infrared LactoScope FT infrared (FTIR) milk analyzer (model FT400, Delta Instruments, Drachten, the Netherlands) equipped with a Work-IR optical bench (ABB Bomem, Montreal, Canada), a standard CaF₂ cuvette (23 µm) using a fixed virtual filter calibration approach.

pH was measured with an INOLAB 3 (WTW GmbH, Weilheim in Oberbayern, Germany) pH metre, and a SenTix electrode. Milk sensory evaluation was undertaken to evaluate the following three characteristics of milk: appearance, taste and smell, and was performed as follows. The sensory panel comprised ten experienced assessors selected (five females and five males, aged 32–55 years), trained, and monitored per the ISO standard 8586:2012 (ISO 2012) guidelines. The analyses took place in a purpose-built sensory laboratory. The panel was trained in the definition and intensity of each attribute using milk with varying sensory properties (a sample stored in the dark and a sample exposed to light for 18 h). Each assessor was served with samples (50 mL each) in 200 mL plastic cups. The temperature of the samples was approximately 10°C. The samples were served twice, and the serving order was randomized. Water and crackers were served for cleansing the palate between samples. The intensity of the odors and flavours was evaluated. A 15-point hedonic scale was used to evaluate sensory attributes, ranging from very bad (value 1) to very good (value 15) for each attribute. Each assessor evaluated the samples at individual speed on the evaluation scale.

Colour evaluation of the milk was performed using a colorimeter. Colour coordinates (lightness (L*), red/green (a*) and blue/yellow (b*)) were detected using a Minolta Chroma Meter colorimeter (CR-200; Minolta Camera, Osaka, Japan). A 10 mL sub-sample of each milk sample was measured in a cuvette and expressed using the CIE-L*a*b* uniform colour space (CIELAB 1976). The CIE-L*a*b* plots the colour coordinates in a uniform colour space, which has an L*, a* and b* axis, with L* (lightness; on a scale from 0 to 100, where 0 = black and 100 = white), a* (where −a* has a green colour and + a* has a red colour) and b* (where −b* has a blue colour and +b* has a yellow colour) (Zhang et al. 2007). The further from zero or the more significant the absolute value is, the more intense the colour (i.e. a sample with a total value close to zero has a lighter colour than a sample with an absolute value close to 100). The colours of the milk of β-casein SN2 genotypes (A1A1, A1A2 and A2A2) were compared to each other and a milk gold standard (McDermott et al. 2016).

The essential and non-essential AA (g kg⁻¹) (in the study reported significant differences) profiles of three kinds of cow milk samples were determined. Milk samples were defatted by centrifuging at a rate of 4,000 r/min. Hydrolysis of the defatted samples was performed according to the Commission Regulation (EC) No 152/2009 of 27 January 2009. Amino acid analysis was performed using a Shimadzu high-performance liquid chromatography low-pressure gradient system (Shimadzu Corp., Kyoto, Japan), composed of an LC-10ATVP vacuum, RF-10AXL fluorescent detector, SIL-10ADVP automatic sample injec- tor, CTO-10ACVP automatic thermostat, SCL-10AVP systemic control module, and a DGU-14A degasser. Programming equipment was used to effectively manage the data acquisition and liquid chromatography system (Shimadzu Corp.). Amino acids were isolated for derivatization processes using a Nova-Pak C18 (4 µm, 150 × 3.9 mm × 150 mm; Waters Corp., Milford, MA, USA) chromatographic column at 37°C. A sample of 10 μL of amino acid extracted during the derivatization process was used for the analysis, whose signals were then measured at 250/395 nm ex/em wavelengths. To separate the amino acid extract, a three-component gradient was used: cut AccQ-Tag™ Eluent A Concentrate (A), acetonitrile (B) and water (C). A flow rate of 1.000 mL min⁻¹ was chosen for the process.

The quantitative and qualitative determination (in the study reported significant differences) of the unsaturated and saturated fatty acid (% composition of the milk were analyzed by Shimadzu single quadrupole GCMS-QP2010 SE gas chromatograph-mass spectrometer (Shimadzu Corporation Technolo- ogies, Inc., Tokyo, Japan) equipped with a split/splitless injector, according to the method described by Simionato et al. (2010) and ISO standards (ISO 12966-2:2011). Methyl esters of the fatty acids were injected into the gas chromatograph. The fatty acid esters were isolated using a Stabilwax®-MS column (30 m length, 0.25 mm internal diameter and a

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**Table 1.** Summary of sample replicates of the milk, n-numbers of animals.

| Sample reps | A1A1 Cows (n = 32) | A1A2 Cows (n = 32) | A2A2; Cows (n = 32) |
|-------------|--------------------|--------------------|--------------------|
| 96          | DM, %, fat, %, protein, %, lactose, % | pH, fatty acids, %, colour | amino acids, % |
| 96          | Taste              | Appearance         | Smell             |

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thickness of 0.25 μm). The carrier gas used for the chromatography was helium. The operating temperature ranged from 50 to 240°C. The temperature of the injector, ion source, and interface was set to 240°C, and the duration of analysis was 55 min. The detector used a specific analysis programme, and the scan parameters were 33–400 m/z. The standards used were Supelco 37 component FAME Mix and Linoleic acid conjugated methyl ester (Supelco Analytical, MilliporeSigma, St. Louis, MO, USA). The fatty acid content (percentage of total fatty acid) was calculated using the chromatograph GCMSsolution (Shimadzu Corp.).

Statistical analysis was performed with the IBM SPSS statistical package (version 27.0.1.0, IBM, Corp.). The data were analyzed using descriptive statistics (Explore) and analysis of variance (ANOVA) methods. For all statistical evaluations, the means and the mean squared errors (SEM) were used. In the case of a significant difference (P < 0.05), groups were compared by Fisher’s LSD criterion.

Results and discussion

Milk production traits and sensory evaluation

The composition in the milk of fat (%), protein (%), lactose (%), DM (%), pH, appearance (points 11–15), taste (points 11–15), smell (points 11–15), and calculated indexes evaluation according to genotype are given in Table 2. According to our findings, the various CSN2 genotypes did not affect the appearance, taste, or smell of the milk, supported by the lack of differences in the chemical composition (DM, fat, protein, lactose and pH) of the different CSN2 genotype milk.

Studies on the sensory properties of milk with genetics have been conducted on goat’s milk (Dagnachew et al. 2011; Björk 2018), but cow’s milk has not been extensively characterized in this manner by other researchers. Conversely, the presence of different β-casein polymorphisms has been discovered to influence the chemical composition of milk in various aspects. Nguyen et al. (2019) found no significant differences between the CSN2 genotypes (A1A1, A1A2, A2A2) and fat, protein, lactose, or total dry matter content; however, Albarella et al. (2020) found that A2A2 milk had higher protein content and total solids when compared to those of A1A1 milk.

The results of our study promote the breeding programmes of cows producing milk containing the β-casein variant A2 exclusively. A2A2 milk is a potentially healthier alternative to milk containing the A1 variant of beta-casein.

Colour of milk

Summary statistics of the colour of CSN2 genotype milk (L* = lightness; a* = greenness; b* = yellowness), as well as the gold standard colour (Mc Dermott et al. 2016), are provided in Table 3. In our research, phenotypic correlations between the milk colour parameters, found when all genotypes were compared to each other, A1A2 milk was statistically the lightest (L*) when compared to A1A1 (+2.55) and A2A2 (+2.95). Furthermore, A1A2 (−2.39) milk was also statistically redder (i.e. higher a*) than that of A1A1 (−3.36) and A2A2 (−3.66) milk. Additionally, A1A2 cows had statistically yellower milk than A1A1 milk, with a b* value of +2.84 and A2A2 milk, with a b* value of +3.31. This corroborates with our findings that a higher L* (lightness index) in milk correlated with an increase in a* (redness index) and b* (yellowness index) values. The lightness index (L*) of A1A1, A1A2 and A2A2 milk were statistically higher in all our milk samples when compared to the gold standard. Regarding the redness (a*) index, statistically significant differences were found between A1A1 and A1A2, which were both redder than the gold standard. Furthermore, the yellowness (b*) index varied between all milk genotypes, but they were all statistically more yellow than the gold standard. Overall, A2A2 milk was the closest to the gold standard. Our findings showed clear correlations between the CSN2 genotypes and the colour of the milk but found scarcely any literature with which to compare these results and suggest that A2A2 milk has more commercial potential than the A1A1 and A1A2 variants as it adheres closer to the Gold Standard for colour, making it more appealing to consumers without artificial food colouring.

Amino acids in milk

Our study found significant differences in the amino acid composition in milk protein (g kg⁻¹ of total proteins), calculated indexes evaluation according to genotype are reported in Table 4. According to the data we collected during our experiment, β-casein genotypes A1A1, A1A2 and A2A2 influenced

| Table 2. The composition in the milk of fat (%), protein (%), lactose (%), DM (%), pH, appearance (points 11–15), taste (points 11–15), smell (points 11–15), and calculated indexes evaluation according to genotype. |
|---------------|----------------|----------------|----------------|
|               | A1A1            | A1A2            | A2A2            |
| Fat (%)       | 5.53 ± 0.35     | 5.00 ± 0.97     | 5.72 ± 0.27     | 0.699 |
| Protein (%)   | 3.60 ± 0.06     | 3.80 ± 0.08     | 3.59 ± 0.13     | 0.220 |
| Lactose (%)   | 4.52 ± 0.07     | 4.43 ± 0.09     | 4.38 ± 0.05     | 0.380 |
| DM (%)        | 14.67 ± 0.65    | 14.23 ± 1.01    | 14.69 ± 1.28    | 0.591 |
| pH            | 6.49 ± 0.19     | 6.53 ± 0.26     | 6.49 ± 0.29     | 0.937 |
| Appearance    | 14.30 ± 0.31    | 14.50 ± 0.26    | 14.30 ± 0.26    | 0.459 |
| Taste         | 14.20 ± 0.33    | 14.15 ± 0.33    | 14.35 ± 0.28    | 0.773 |
| Smell         | 14.50 ± 0.24    | 14.25 ± 0.26    | 14.15 ± 0.28    | 0.494 |

Table 3. The colour milk and calculated indexes evaluation according to genotype.

| Phenotype | Mean     | Std. Dev. | Min   | Max   | P-value |
|-----------|----------|-----------|-------|-------|---------|
| Colour L* | A1A1     | 87.30 *** | 0.05  | 87.24 | a < 0.001 |
|           | A1A2     | 89.85 *** | 0.16  | 89.67 | b       |
|           | A2A2     | 86.90 **  | 0.83  | 85.94 | a       |
| #Gold     | 81.57    | 1.83      | 47.05 | 74.05 | 87.78   |
| Colour a* | A1A1     | −3.36 *   | 0.10  | −3.44 | −3.25 a < 0.001 |
|           | A1A2     | −2.39 *** | 0.03  | −2.42 | −2.37 b |
|           | A2A2     | −3.66 **  | 0.15  | −3.77 | −3.49 c |
| #Gold     | −3.88 a  | 0.53      | −5.78 | −2.10 |         |
| Colour b* | A1A1     | 14.38 **  | 0.87  | 13.37 | 14.94 a < 0.01 |
|           | A1A2     | 17.21 *** | 0.06  | 17.15 | 17.25 b |
|           | A2A2     | 13.90 *** | 1.19  | 12.58 | 14.90 a |
| #Gold     | 8.09     | 2.94      | −0.90 | 20.08 |         |

Values are expressed mean value ± standard deviation minimum (Min) and maximum (max); Means in the same row followed by different inline letters (a, b and c) are significantly different according to fishers LSD criterion test (P < 0.05).

* – P < 0.05; ** – P < 0.01; *** – P < 0.001 – the statistical significance of the difference from the mean value of the gold standard (Fisher’s LSD criterion).

#-Gold standard according to McDermott et al. (2016).
The heritability estimates of CSN2 genotypes (A1A1, A1A2 and A2A2) are not significantly different according to the fisher's LSD criterion test (P < 0.05).

Amino acid content in milk, significantly supporting our hypothesis that the genotypes influence the chemical composition of milk.

A1A2 milk had the highest content of amino acids, with Leucine being the only exception. A1A2 milk had significantly higher histidine (P < 0.001), lysine (P < 0.01), isoleucine (P < 0.05), methionine (P < 0.001) and valine (P < 0.01) essential amino acid content in comparison to the other researched genotypes. Additionally, A1A2 milk was the richest in conditionally non-essential proline (P < 0.001), serine (P < 0.05) and tyrosine (P < 0.01) amino acids, as well as non-essential aspartic acid (P < 0.001). Conversely, A2A2 milk had significantly higher Leucine (P < 0.001) content compared to that of both A1A1 and A1A2 milk, but overall had the lowest amino acid content of the three milk types. β-casein variants A1 and A2 have been shown to contribute to a difference in casein micelle assembly and molecular chaperone activity (Raynes et al. 2015). These results suggest that β-casein variants can influence milk composition, even from only a part of the protein fraction.

Furthermore, Nichols et al. (2019) found that the expression of CSN2, which encodes β-casein, did not have any direct ties to extra energy from either fat or protein; this supports the idea that β-casein genotypes influence amino acids in milk. While there is a lack of literature examining how β-casein haplotype CSN2 genotypes (A1A1, A1A2 and A2A2) affect the amino acid composition of milk proteins, some studies have, however, demonstrated that milk protein composition is related strongly to genetic factors. The heritability estimates for the six major milk protein groups, caseins (αs1-, αs2-, β- and κ-casein), and soluble proteins (α-lactalbumin and β-lactoglobulin), are in line with this hypothesis (Bonfatti et al. 2011). This would reaffirm the significance of the link between protein variants and the protein composition of milk (Visker et al. 2011).

**Fatty acids in milk**

Although research has been conducted on the heritability and genetic correlations between protein and fat content and milk yield (Samoré et al. 2012), studies attempt to accurately estimate the correlation between β-casein genotypes and fatty acid composition in milk are limited.

### Monounsaturated fatty acids (MUFA) in milk

The MUFA composition in milk fat (% of total fats) and calculated indexes evaluation according to genotype are reported in Table 5. The MUFA content of A1A1 milk was significantly higher (P < 0.001) than that of A2A2 and A1A2 by 0.52% and 1.21%, respectively. We cannot corroborate this with other studies; literature on this subject is limited. Perna et al. (2016) reported that among the different allelic combinations of loci αs1-, β- and κ-casein, BB-A2A2-AB was linked to an increase in MUFA fatty acid content, with a significantly higher oleic acid compared with the other genotypes. Islam et al. (2014) showed that palmitoleic acid content stayed constant in the milk of animals of the same genotype that were fed different diets; this corroborates with the findings of Devle et al. (2012), who have also reported that the fatty acid composition of milk might be affected by genotype.

### Polyunsaturated fatty acids (PUFA) in milk

The PUFA composition in milk fat (% of total fats) and calculated indexes evaluation according to genotype are reported in Table 6. The PUFA content of A2A2 milk was significantly higher (P < 0.001) than that of A1A1 and A1A2 milk by 0.52% and 1.21%, respectively. In our study, the omega-3 (P < 0.001), omega-6 (P < 0.001), linoleic acid (18:2-n6) (P < 0.001), α-linolenic acid (18:3-n3) (P < 0.001), Eicosatetraenoic acid (20:4-n6) (P < 0.01) and eicosapentaenoic acid (20:5-n3) (P < 0.001) content of A2A2 milk was significantly higher than those of A1A1 and A1A2 milk. The docosadienoic acid (22:2-n6) (P < 0.001) content of A1A1 milk was significantly higher than those of A1A2 and A2A2. The conjugated linoleic acid (18:2-9c) (P < 0.005) and docosahexaenoic acid (22:6-n3) (P < 0.01) content of A1A2 milk was significantly higher than that of A1A1 and A1A2 milk.

### Table 4. According to genotype, the amino acid composition in milk protein (g kg⁻¹ of total proteins) and calculated indexes evaluation.

| Amino acid | Genotype | SEM | P-value |
|------------|----------|-----|---------|
| Aspartic acid | A1A1 | 2.43a | 0.060 | <0.001 |
|             | A1A2 | 2.52b |       |         |
|             | A2A2 | 2.23b |       |         |
| Histidine   | A1A1 | 1.24a | 0.033 | <0.001 |
|             | A1A2 | 1.25b |       |         |
|             | A2A2 | 1.10b |       |         |
| Lysine      | A1A1 | 2.69a | 0.065 | <0.01  |
|             | A1A2 | 2.77b |       |         |
|             | A2A2 | 2.51b |       |         |
| Isoleucine  | A1A1 | 2.69a | 0.081 | <0.001 |
|             | A1A2 | 2.76b |       |         |
|             | A2A2 | 2.55b |       |         |
| Leucine     | A1A1 | 3.14a | 0.048 | <0.001 |
|             | A1A2 | 3.29b |       |         |
|             | A2A2 | 3.88c |       |         |
| Methionine  | A1A1 | 1.83a | 0.058 | <0.001 |
|             | A1A2 | 1.88b |       |         |
|             | A2A2 | 1.50b |       |         |
| Proline     | A1A1 | 3.15a | 0.053 | <0.001 |
|             | A1A2 | 3.36b |       |         |
|             | A2A2 | 3.11a |       |         |
| Serine      | A1A1 | 1.71a | 0.055 | <0.05  |
|             | A1A2 | 1.84b |       |         |
|             | A2A2 | 1.69a |       |         |
| Valine      | A1A1 | 2.11a | 0.069 | <0.01  |
|             | A1A2 | 2.21b |       |         |
|             | A2A2 | 1.96b |       |         |
| Tyrosine    | A1A1 | 1.62a | 0.045 | <0.01  |
|             | A1A2 | 1.70b |       |         |
|             | A2A2 | 1.54b |       |         |

Statistically significant differences are reported. Values are expressed mean value ± SEM; Means in the same row followed by different inline letters (a, b and c) are significantly different according to fisher’s LSD criterion test (P < 0.05).

### Table 5. The MUFA composition in milk fat (% of total fats) and calculated indexes evaluation according to genotype.

| Fatty acid | Genotype | SEM | P-value |
|------------|----------|-----|---------|
| Myristoleic 14:1n5 | A1A1 | 1.7a | 0.037 | <0.001 |
|             | A1A2 | 1.97b |       |         |
|             | A2A2 | 1.47c |       |         |
| Pentadecanoic 15:1c10 | A1A1 | 0.012a | 0.001 | <0.05 |
|             | A1A2 | 0.014b |       |         |
|             | A2A2 | 0.012a |       |         |
| Palmitoleic 16:1n7 | A1A1 | 0.170a | 0.016 | <0.001 |
|             | A1A2 | 0.161b |       |         |
|             | A2A2 | 0.286a |       |         |
| Heptadecanoic 17:1c10 | A1A1 | 0.256a | 0.025 | <0.01 |
|             | A1A2 | 0.304b |       |         |
|             | A2A2 | 0.360c |       |         |
| Oleic 18:1c9 | A1A1 | 18.06a | 0.073 | <0.001 |
|             | A1A2 | 16.13b |       |         |
|             | A2A2 | 17.19c |       |         |
| Elaidic 18:1t9 | A1A1 | 2.33a | 0.149 | <0.001 |
|             | A1A2 | 2.94b |       |         |
|             | A2A2 | 3.58c |       |         |
| MUFA* 23.62 | A1A1 | 21.68b | 0.050 | <0.001 |
|             | A1A2 | 23.04c |       |         |

Statistically significant differences are reported. Values are expressed mean value ± SEM; Means in the same row followed by different inline letters (a, b and c) are significantly different according to Fisher's LSD criterion test (P < 0.05). MUFA = monounsaturated fatty acids; 20:1n9 = eicosanoic acid; 22:1n9 = methyl erucate; 24:1n9 = nervonic acid.

*MUFA = C14:1c9 + C15:1c10 + C16:1c9 + C17:1c10 + C18:1c9 + C18:1t9 + C20:1n9 + C22:1n9 + C24:1n9.
Table 6. The PUFA composition in milk fat (% of total fats) and calculated indexes evaluation according to genotype.

| Fatty acid                  | Genotype     | A1A1 | A1A2 | A2A2 | SEM | P-value |
|-----------------------------|--------------|------|------|------|-----|---------|
| Conjugated linoleic 18:2-c9 |              | 0.23a| 0.23a| 0.27b| 0.005| <0.01  |
| Linoleic 18:2-n6            |              | 3.26a| 2.66b| 3.64a| 0.053| <0.001 |
| α-linolenic 18:3-n3         |              | 1.15a| 1.01b| 1.19c| 0.011| <0.001 |
| Eicosatetraenoic 20:4-n6    |              | 0.112| 0.139b| 0.174c| 0.014| <0.001 |
| Eicosapentaenoic 20:5-n3    |              | 0.052a| 0.061b| 0.092c| 0.002| <0.001 |
| Docosadienoic 22:2-n6       |              | 0.250a| 0.125b| 0.088c| 0.007| <0.001 |
| Docosahexaenoic 22:6-n3     |              | 0.014a| 0.023b| 0.011a| 0.003| <0.01  |
| Omega-3*                   |              | 1.22a| 1.10b| 1.29c| 0.012| <0.001 |
| Omega-6**                  |              | 3.52a| 2.95b| 3.98c| 0.060| <0.001 |
| PUFA***                    |              | 4.97a| 4.28b| 5.49c| 0.071| <0.001 |

Statistically significant differences are reported. Values are expressed mean value ± SEM; Means in the same row followed by different inline letters (a, b and c) are significantly different according to fishers LSD criterion test (P < 0.05). PUFA – polyunsaturated fatty acids; C18:3-n6 – gamma-linolenic acid; C20:2-n6 – eicosadienoic acid; C20:3-n6 – dihomogamma-linolenic acid; + C20:4-n6 – arachidonic acid.

Table 7. The SFA composition in milk fat (% of total fats) and calculated indexes evaluation according to genotype.

| Fatty acid | Genotype     | A1A1 | A1A2 | A2A2 | SEM | P-value |
|------------|--------------|------|------|------|-----|---------|
| Butyric 4:0|              | 3.71a| 3.23b| 2.99b| 0.104| <0.001 |
| Caproic 6:0|              | 3.23a| 2.95b| 2.90b| 0.102| <0.05  |
| Undecylic 11:0|            | 0.049a| 0.081b| 0.067b| 0.002| <0.001 |
| Lauric 12:0|              | 3.95a| 4.06b| 4.32b| 0.096| <0.01  |
| Tridecanoic 13:0|          | 0.120a| 0.156b| 0.117b| 0.009| <0.01  |
| Myristic 14:0|             | 11.93a| 12.83b| 12.04a| 0.092| <0.001 |
| Pentadecanoic 15:0|        | 1.47a| 1.80b| 1.41b| 0.030| <0.001 |
| Palmitic 16:0|            | 27.94a| 29.61b| 27.89a| 0.106| <0.001 |
| Margaric 17:0|            | 0.663a| 0.757b| 0.865b| 0.014| <0.001 |
| Stearic 18:0|             | 12.43a| 12.70a| 13.04b| 0.199| <0.05  |
| Behenic 22:0|             | 0.033a| 0.040b| 0.028b| 0.003| <0.01  |
| Linoleic acid 24:0|       | 0.061a| 0.058b| 0.047b| 0.005| <0.001 |
| SFA*        |              | 71.41a| 74.04b| 71.47a| 0.070| <0.001 |

Statistically significant differences are reported. Values are expressed mean value ± SEM; Means in the same row followed by different inline letters (a, b and c) are significantly different according to Fisher’s LSD criterion test (P < 0.05). SFA – saturated fatty acids; C8:0 – caprylic acid; C10:0 – capric acid.

higher than those of A1A1 and A2A2 milk. Additionally, the butyric acid (4:0) (P < 0.001), caproic acid (6:0) (P < 0.05) and lignoceric acid (24:0) (P < 0.05) content of A1A1 milk was significantly higher than those of A1A2 and A2A2 milk. The lauric acid (12:0) (P < 0.01), margaric acid (17:0) (P < 0.001) and stearic acid (18:0) (P < 0.05) content of A2A2 milk was significantly higher than those of A1A2 and A1A1 milk.

Milk and dairy products are known for their high nutritional value. However, milk is low in PUFA fatty acids and high (60%) in SFA fatty acids (George and Struthers 2009), such as lauric, myristic and palmitic acid, of which palmitic acid is the most abundant, constituting roughly 40% of the total SFA content (Islam et al. 2014; Junior et al. 2019).

Our study concluded that A2A2 milk had the statistically lowest SFA content compared to that of the other tested genotypes. On the contrary, Perna et al. (2016) reported that BB-A2A2-AB, BB-A2A2-BB and BB-A1A2-AA milk had the highest concentration of butyric caprylic and capric acids, as well as saturated short-chain fatty acids. BB-A2A2-AA milk had the highest palmitic acid content; lauric and myristic acid content was highest in BB-A1A2-AA milk, BB-A2A2-AA and BB-A1A2-AA milk had the lowest stearic acid content.

Our findings highlight the possibility of altering the concentration of fatty acids in milk and dairy products through breeding, which could be beneficial to the milk production chain.

Table 7. The SFA composition in milk fat (% of total fats) and calculated indexes evaluation according to genotype.

Conclusion

Our findings suggest an influence of β-casein variants (A1A1, A1A2 and A2A2) on amino-fatty acid composition and milk colour. Briefly, A1A1 milk had higher MUFA (<0.001) fatty acids content and lower levels of SFA (<0.001) fatty acids content in milk fats. A1A2 milk had higher levels of essential amino acids (histidine (<0.001), lysine (<0.01), isoleucine (<0.05), methionine (<0.001), valine (<0.01)) and non-essential amino acids (aspartic (0.001), proline (<0.001), serine (<0.05), tyrosine (<0.01)) in the milk proteins and highest SFA (<0.001) fatty acid content in the milk’s fats. A1A2 milk had the highest lightness index (L*) (<0.001), which we found correlated to increased redness index (a*) (<0.001) and yellowness index (b*) (<0.01) values. A2A2 milk was higher in PUFA (<0.001), omega-3 (0.001) and omega-6 (<0.001) and lower SFA (<0.001) fatty acids content in milk fats. However, owing to the limited literature available and the relatively small sample size of this study, which may affect the generalizability, further research with larger sample sizes is warranted.

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Data availability statement

Upon a reasonable request, the data supporting this study’s findings are available from the corresponding author, K. de Vitte.

Disclosure statement

No potential conflict of interest was reported by the authors.

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