Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1297156

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 6 - Issue 6: 649-654 / November 2023

GENOTYPIC EFFECTS OF B-CASEIN IN MILK COMPOSITION IN JERSEY COWS

Leila BEN FARHAT[1,2], Amanda HOARAU[3], Violetta TÓTH[2]*, Agnes SULI[2], Kata Sara LABAS[4], Ferid ABIDI[1], Edit MIKÓ[2]

[1]*University of Carthage, INSAT (Institut National des Sciences Appliquées et de Technologie, Laboratory of Protein Engineering and Bioactive Molecules (LR11ES24), BP 676-1080, Tunis, Tunisia*
[2]*University of Szeged, Faculty of Agriculture, Institute of Animal Sciences and Wildlife Management, H-6800 Hódmezovásárhely, Hungary*
[3]*University of Reunion Islands, Faculty of Sciences and Technology, Engineering School (Esiroi), Agri-Food, St Pierre, Reunion Island*
[4]*University of Szeged, Faculty of Agriculture, Institute of Economics and Rural Development Management, H-6800 Hódmezovásárhely, Hungary*

Abstract: The aim of this study was to investigate the relationship between the β-casein CSN2 genotypes (A1A1, A1A2, A2A2) and the biochemical characters and fatty acid composition of milk. Twenty-three milk samples from Jersey cows from the same herd from a farm in Hungary were studied. Animals were grouped according to β-casein genotype variants A1A1, A1A2 and A2A2. A1A1 milk had a significantly higher content of monounsaturated fatty acids (P<0.001) and a lower content of saturated fatty acids (<0.001). A2A2 milk had a higher content of polyunsaturated fatty acids (P<0.001) in milk. Moreover, the three varieties of milk show no significant difference for the composition of the polyunsaturated between CSN2 genotypes A1A1, A1A2 and A2A2. Also, no significant differences were observed in physicochemical composition of the milk. Accordingly, selective selection of genotypes with preferred qualities can improve milk and dairy products. In conclusion the fatty acid content the milk could be influenced by CSN2 genotypes A1A1, A1A2 and A2A2.

Keywords: A1 milk, A2 milk, Beta-casein, Fatty acid

***Corresponding author:** University of Szeged, Faculty of Agriculture, Institute of Animal Sciences and Wildlife Management H-6800 Hódmezovásárhely, Hungary
E mail: toth.violetta@szte.hu (V. TÓTH)

Leila BEN FARHAT https://orcid.org/0000-0002-6912-6088
Amanda HOARAU https://orcid.org/0000-0001-9665-4110
Violetta TÓTH https://orcid.org/0000-0002-1394-716X
Agnes SULI https://orcid.org/0000-0003-4939-2423
Kata Sara LABAS https://orcid.org/0009-0007-7439-7994
Ferid ABIDI https://orcid.org/0000-0002-7896-1624
Edit MIKÓ https://orcid.org/0000-0001-7584-445X

Received: May 29, 2023
Accepted: October 18, 2023
Published: November 01, 2023

Cite as: Ben Ferhat L, Hoarau A, Tóth V, Suli A, Labas KS, Abidi F, Mikó E. 2023. Genotypic effects of b-casein in milk composition in Jersey cows. BSJ Agri, 6(6): 649-654.

1. Introduction

The composition of milk from different mammal species and in particular bovine such as lipid protein, lactose, fatty acids, and nitrogen fractions, were influenced by several factors, including nutrition, age, stage of lactation, breed and genetic variation (Baer, 1991; Carroll et al., 2006). Several research confirms that bovine milk is a nutritionally valuable food. Indeed, caseins and whey milk proteins constitute more than 95% of all proteins (Ivanković et al., 2021). Caseins are one of the major proteins in cow's milk, which includes four forms: alpha s1 casein (39-46% of total caseins), alpha s2 casein (8-11%), beta-casein (25-35%) and kappa casein (8- 15%) (Roginski et al., 2003; Ivanković et al., 2021). Research was focused on the genetic polymorphism of proteins and their great interest, because of its relations with the, the physicochemical composition, the quality and other important traits. Milk casein is encoded by genes CSN1S1, CSN2, CSN1S2 and CSN3 located on chromosome 6 with 250 Kb of length (Ferretti et al., 1990; Ahmed et al., 2017). The Beta-casein protein is encoded by the CSN2 gene which is 8.5 kb in length, and contains five exons and eight introns (Bonsing et al., 1988). Beta-casein includes 12 known genetic variants (A1, A2, A3, B, C, D, E, F, G, H1, H2, I; (Farrel et al., 2004; Cui et al., 2012). The A1 and A2 variants represent the most common protein types of beta-casein which differ in the presence of the amino acid histidine (CAT) at position 67 in milk A1 and proline (CCT) in milk A2, due to a single nucleotide difference in the sequence of the exon VII bovine CSN2 gene at position 8101 (Bonsing et al., 1988). This genetic modification has a direct effect on the proteolytic digestion of the primary protein structure of the caseins, which allows the production of different peptides. Indeed, the enzymatic digestion of variants of β-casein A1 lead to the formation of the peptide B-casomorphin

which is characterized by significant opioid activity. This peptide can seep into the bloodstream more easily and cause various health problems, such as gastrointestinal disorders, insulin-dependent diabetes, atherosclerosis, ischemic heart disease and sudden infant death syndrome (Vougiouklaki et al., 2020).

Nowadays, the choices of the consumers have changed, they have become based not only on the nutritional aspects and organoleptic qualities of food, but also on products known to promote for a good health and prevent disease. In this regard, although milk represents an important element in the human diet all over the world, a new debate on the type of milk is followed by the heterozygous A1A2 genotype and the homozygous A2A2 and A1A1. Studies have shown that A2 milk has a beneficial effect on health than A1 milk, which can cause several diseases. Therefore, this milk is marketed as a healthier choice than A1 milk (Kumar et al., 2017)

Although the evaluation of the genetic variation of the genes encoding for the β-casein protein has been widely tackled by researchers. But, the study of the correlation between the biochemical composition of milk (DM, pH, proteins, fat, lactose, acidity) and the sensory traits (appearance, taste, odor and color of milk) and the CSN2 gene of β-casein has been rarely studied (Samoré et al., 2012). Also, studies that accurately correlate genetic polymorphism with milk fatty acid composition are limited (De Vitte et al., 2022).

This study aims to determine the link between milk protein genotypes (A1A1, A1A2 and A2A2) and biochemical traits of milk.

2. Materials and Methods

2.1. Sampling

The raw milk samples from Jersey cows collected from a local single farm in Hódmezővásárhely, Hungary, in order to see its physico-chemical characteristics for further investigation. Jersey dairy cow is selected as the study subject because they had the highest conception rates (59.6%) and higher percentages of cows pregnant in 75 d (78.1%) in Hungary (Washburn et al., 2002). All the cows at lactation times used in this study are from the same herd and were kept under the same housing and feeding conditions. The animals were classified according to the β-casein genotype variants A1A1, A1A2 and A2A2. 23 samples of 120 ml of milk from the previously chosen cows were collected in sterile polypropylene plastic bottle, then transported to the laboratory to be analyzes (Xiao et al., 2022). It is important to emphasize that the collection of samples from the Sampling stations fully comply with the recommended hygiene and asepsis rules in microbiology. Indeed, during the collection of cow's milk by breeders, washing and rinsing the teats of the udders of the cows with water mixed with the chlorine, followed by the elimination of the first milk jets, are carried out before each milking, therefore before the recovery of the sample (Mayer et al., 2021). For fatty acids analysis, the samples were frozen until use.

2.2. Physico-Chemical Properties

The physico-chemical properties of the milk were determined using the LactoScope FT-A infrared (FTIR) mid-infrared milk analyzer (model FT 400, Delta Dairy Analyzer, Budapest, Hungary). The concentrations of fat, protein, dry substance and lactose and also the density, in g/ml, were determined. The samples were stored at − 20 °C for further analysis (DiGiacomo et al., 2022).

2.3. Titrable Acidity

To measure Dornic acidity of milk we append a drop of alcoholic solution of 1% phenolphthalein as an indicator of the color change point in 1 mL of milk. 0.01 mL of sodium hydroxide (NaOH) were added, until the sample changed color from white to light pink and the color change was maintained. Titratable acidity was expressed in Dornic acidity (°D) as described by (Vázquez-Román et al., 2013).

2.4. Quantitative Analysis of Fatty Acids, Fatty Acids Extraction

The extraction of fatty acid was carried out following the conventional method of (De Jong and Badings, 1990) with a slight modification. Milk samples were homogenized by shaking with 10.75 mL of sulfuric acid (18%) and 1 mL of amyl alcohol. After centrifugation at 5000 rpm for 10 min, a volume of 50 µl of the supernatant was returned to a round bottom flask then mixed with 2 mL sodium methoxide at a concentration of 25%. The mixture was incubated for 40 min at 95°C in a water bath. Afterwards, 200 µl of methanolic sulfuric acid at a concentration of 3% was added until the coloring of the solution was observed. A second incubation of 5 min at 95 °C in water bath was performed. After liquid cooling, 4 mL of saturated NaCl and 1 mL of concentrated hexane was added. Samples were stored at −20 °C until analysis.

2.5. Capillary Gas Chromatography with Flame Ionization Detector: GC-FID

Before analysis, Samples were thawed at in room temperature, then filtered using 0.2 µm pore diameter (MiniSart Syringe Filter, Satorius, Goettingen, Germany), and a 1 µL filtered sample was subsequently kept in 2 mL GC vials for analysis step. Fatty acid content and profile were determined by gas chromatography (Nexis GC-2030, Shimadzu Scientific Instruments Inc., Kyoto, Japan) equipped with a polyethylene glycol column (ZB-WAX; 30 m × 0.25 mm inner diameter, 0.25 µm film thickness; Zebron, Phenomenex, CA, USA), and flame ionization detector. Helium was used as carrier and make-up gas. The run time per sample was 8.71 min. The oven temperature was programmed at 145 °C for 3 min and then increased from 145 °C to 245 °C at 16.6 °C/min. The injector and the flame ionization detector were maintained at 220 and 250 °C. The gas flows were 24, 32 and 200 ml/min for Helium. A standard curve was made using a mixture of volatile fatty acids from Sigma Aldrich (St. Louis, MO, USA) (Barnsteiner et al., 2011; Eisenstecken et al., 2021).

2.6. Statistical Analysis

All data were expressed as mean ± standard Error (SE). Variance analysis (one-way ANOVA) of the experimental data was done using Origin Pro 8.0 software (OriginLab Corporation, MA, USA), using Tukey's test, at a significance level of 95% (P<0.05). In addition, correlation analysis was used to investigate the relationship between the different genotypic b-casein and the biological activities using SPSS software (SPSS, version 23.0, USA).

3. Results and Discussion

The physicochemical composition in the milk of Partial Least Squares (PLS, %), protein (%), lactose (%), pH (%), Solids (%), SNF (Solids Not Fat, %), Conductivity (%) and FFD (Freezing Point Depression, %) was investigated using LactoScope FT-A and the result was given in Table1. The data shows No significant difference between all samples (P<0.05) (Table 1 and Supplementary Table 2). According to our results, the different cow's genotypes affect the appearance, taste or smell of milk, supported by the presence of significant differences (P<0.05) in chemical composition of lactose, and pH.

These results are consistent with the work of Nguyen et al. (2019) and De Vitte et al. (2022) which showed no significant (P<0.05) difference between the CSN2 genotypes (A1A1, A1A2, A2A2) and the composition of the milk. While the data of Albarella et al. (2020) finding that A2A2 milk had a higher protein and higher total solids than A1A1 milk. While for the percentage of lactose no significant difference between the different genotypes was observed (Samoré et al., 2012). Regarding A1A2 samples showed a slightly higher percentage of lactose, solids, FFD and fat content than the samples of the other two genotypes. For the A2A2 genotype milk samples show significantly (P<0.05) higher values in total protein

3.1. Titrable Acidity

In our study, titrable acidity results reported in Table 1, were not significantly correlated with β-casein genotypes (A1A1, A1A2, A2A2).

3.2. Quantitative Analysis of Fatty Acids

Several studies grant a correlation between genetic variation and protein and fat content and milk production (Samoré et al., 2012). However, studies that accurately correlate genetic polymorphism with milk fatty acid composition are limited (De Vitte et al., 2022).

3.3. Saturated Fatty Acids in Milk

The results of the monounsaturated fatty acid composition in milk fat (% of total fat) are presented in Table 3 and Supplementary Table 4. The saturated fatty acid content of the majority of genotype samples A1A1 milk was significantly higher (P<0.05) than that of A2A2 and A1A2. Except for saturated fatty acids Undecylic acid, Tridecylic acid, Margaric acid, and Behenic acid which are significantly higher in A2A2 gene type milk. In present research, saturated fatty acid was significantly correlated with β-casein genotypes (A1A1, A1A2, A2A2) expected the data of Undecylic acid. These results are similar to the study performed by De Vitte et al. (2022) which found that genetic polymophism plays an important role in fatty acid composition in milk. Indeed, following their studies on the comparison between saturated fatty acids, they concluded that undecyl acid (11:0) (P<0.001), tridecanoic acid (13:0) (P<0.01), Myristic acid (14:0) (P<0.001), pentadecanoic acid (15:0) (P<0.001), palmitic acid (16:0) (P<0.001) and behenic acid (22:0)) (P<0.01) of A1A2 milk was significantly higher than those of A1A1 and A2A2 milks.

3.4. Monounsaturated Fatty Acids in Milk

The composition of monounsaturated fatty acids in milk fat (% of total fat) according to genotype are reported in Table 5 and Supplementary Table 6. The monounsaturated fatty acid content of milk A2A2 was significantly higher (P<0.05) than of A1A1 and A1A2, with a content of Tetradecenoic acid, Palmitoleic acid and cis-Vaccenic acid of A1A1 milk significantly higher (P<0.05) than that of A1A2 and A1A1 milk. But, the Oleic acid content (P<0.05) of A1A2 milk was significantly higher than that of A1A1 and A2A2 milks. De Vitte et al. (2022), correlates the monounsaturated fatty acid content significantly with the different genotypic combination of milk β-casein genotypes (A1A1, A1A2, A2A2). On the other hand, Perna et al. (2016) reported in their study that the significant difference between the contents of monosaturated fatty acids are linked to the different allelic combinations of αS1-, β- and κ-casein loci, BB-A2A2-AB.

Table 1. Characterization of raw milk

	A2	A1A2	A1	Sig
Fat Partial Least Squares (PLS)	5.32±0.92	5.04±0.96	4.92±1.63	0.791
Protein	3.71±0.35	3.52±0.47	3.38±0.15	0.13
Lactose	4.70±0.23	4.69±0.25	5.02±0.23	0.047
Solids	14.47±1.08	13.93±1.18	13.98±1.59	1.03
Solids Not Fat (SNF)	9.12±0.39	8.93±0.39	9.11±0.09	0.135
Conductivity	6.02±0.45	6.45±0.58	5.7±0.11	0.179
Freezing Point Depression (FPD)	506.462±6.53	514.546±7.47	510.15±0.92	37.319
pH	6.485±0.02	6.486±0.05	6.45±0.09	0.003
Fat	5.363±0.96	5.056±0.98	4.945±1.61	0.809
Titrable acidity TA (°D)	37.77±4.37	36.933±4.50	39±10.60	16.73

Note: Data are expressed as mean ± standard Error of three replicates.

Table 3. Saturated fatty acid composition

		A2	A1A2	A1	Sig.
Butyric acid	C:4	1.451±0.169	1.342±0.258	1.381±0.007	0.025
Caproic acid	C:6	1.692±0.186	1.524±0.262	1.672±0.118	0.031
Caprylic acid	C:8	1.039±0.147	0.948±0.176	1.106±0.060	0.019
Capric acid	C:10	2.463±0.393	2.253±0.529	2.709±0.021	0.137
Undecylic acid	C:11	0.053±0.016	0.059±0.036	0.065±0.014	0.001
Lauric acid	C:12	3.268±0.449	3.015±0.822	3.566±0.143	0.179
Tridecylic acid	C:13	0.113±0.017	0.105±0.038	0.124±0.005	0.001
Myristic acid	C:14	11.521±0.742	10.822±1.481	11.978±0.540	0.489
Pentadecylic acid	C:15	1.228±0.127	1.112±0.359	1.238±0.125	0.014
Palmitic acid	C:16	33.983±0.349	32.043±4.091	32.228±1.416	0.943
Margaric acid	C:17	0.628±0.095	0.656±3.485	0.574±0.017	0.008
Stearic acid	C:18	14.840±1.678	13.950±0.090	15.027±1.152	2.501
Arachidic acid	C:20	0.217±0.021	0.191±0.047	0.191±0.014	0.002
Behenic acid	C:22	0.076±0.014	0.065±0.020	0.054±0.017	0.001

Note: Data are expressed as mean ± standard Error of three replicates.

Table 5. Fatty acid composition

Samples			A1	A1A2	A1	Sig.
Monoun saturated fatty acid	Tetradecenoicacid	C14:1	0.688±0.105	0.685±0.259	0.745±0.000	0.010
	Palmitoleicacid	C16:1	1.148±0.186	1.308±0.287	1.123±0.000	0.031
	Oleicacid	C18:1n9c	19.992±2.287	23.595±3.298	20.785±1.126	4.651
	cis-Vaccenicacid	C18:1n7	2.950±0.934	3.205±0.804	3.453±0.012	0.775
Polyun saturated fatty acid	Linoleicacid	C18:2n6c	1.591±0.305	1.811±0.307	1.311±0.025	0.083
	γ-Linolenic	C18:3n6	0.028±0.012	0.027±0.010	0.016±0.000	0.227
	α-Linolenic	C18:3n3	0.288±0.076	0.313±0.061	0.262±0.000	0.005
	CLA	CLA	0.605±0.160	0.789±0.315	0.832±0.000	0.023
	Homo-γ-Linolenic	(C20:3n6)	0.044±0.011	0.040±0.018	0.038±0.000	0.021
	Arachidonic	(C20:4n6)	0.027±0.033	0.041±0.044	0.086±0.000	0.001
	EPA	(C20:5n3)	0.032±0.008	0.044±0.021	0.025±0.000	0.139
	Dpan-6	(C22:5n3)	0.064±0.014	0.065±0.025	0.080±0.000	0.020

Table 7. Matrix of correlation between samples and milk composition

		Fat (PLS)	Protein	Lactose	Solids	SNF	Conductivity	(FPD)	pH	Fat(0101)
r	FatPLS	1.000	0.352	-0.144	0.944	0.287	-0.420	0.207	-0.524	0.999
	Protein	0.352	1.000	-0.447	0.585	0.799*	-0.085	-0.118	-0.150	0.393
	Lactose	-0.144	-0.447	1.000	-0.086	0.180	-0.697	0.137	-0.223	-0.168
	Solids	0.944	0.585	-0.086	1.000	0.583*	-0.532	0.153	-0.548	0.953
	SNF	0.287	0.799	0.180	0.583	1.000	-0.560	-0.036	-0.312	0.315
	Conductivity	-0.420	-0.085	-0.697	-0.532	-0.560	1.000	0.197	0.477	-0.410
	FPD	0.207	-0.118	0.137	0.153	-0.036	0.197	1.000	-0.155	0.201
	pH	-0.524	-0.150	-0.223	-0.548	-0.312	0.477	-0.155	1.000	-0.510
	Fat(0101)	0.999	0.393	-0.168	0.953	0.315	-0.410	0.201	-0.510	1.000
Sig.	FatPLS		0.050	0.256	0.000	0.092	0.023	0.171	0.005	0.000
	Protein	0.050		0.016	0.002	0.000	0.349	0.296	0.247	0.032
	Lactose	0.256	0.016		0.349	0.206	0.000	0.266	0.153	0.222
	Solids	0.000	0.002	0.349		0.002	0.004	0.243	0.003	0.000
	SNF	0.092	0.000	0.206	0.002		0.003	0.436	0.074	0.071
	Conductivity	0.023	0.349	0.000	0.004	0.003		0.184	0.011	0.026
	FPD	0.171	0.296	0.266	0.243	0.436	0.184		0.241	0.179
	pH	0.005	0.247	0.153	0.003	0.074	0.011	0.241		0.006
	Fat(0101)	0.000	0.032	0.222	0.000	0.071	0.026	0.179	0.006	

PLS= partial least squares, SNF= solids no tfat, FPD= freezing point depression, r=correlation coefficient.

3.5. Polyunsaturated Fatty Acids in Milk

The composition of polyunsaturated fatty acids in milk fat (% of total fat) is reported in Table 5. The results of the polyunsaturated fatty acid content showed that there is no significant difference between the three varieties. Perna et al. (2016) and De Vitte et al. (2022) found that the A1A1, A1A2 and A2A2 genotypes showed a significant difference in polyunsaturated fatty acids in

the milk samples tested. But in their studies, they reported that A2A2 and A1A2 milk had the lowest percentage of polyunsaturated fatty acid and essentially α-linolenic acid, linoleic acid, eicosapentaenoic acid, docosahexaenoic acid and therefore n-6 PUFA in milk.

4. Conclusion

Our results suggest that there is no influence of β-casein variants (A1A1, A1A2 and A2A2) on the physicochemical composition of milk in fat (%), protein (%), lactose (%), pH (%), Solids (%), SNF (%), Conductivity (%) and FFD (%). On the other hand, this genetic variation influences the fatty acid composition. Briefly, A1A1 milk had a higher content of saturated fatty acids and a lower content of monounsaturated fatty acids in milk fat. Whereas, A2A2 milk was higher in polyunsaturated fatty acid in milk fat. However, due to the limited literature available and the relatively small sample size of this study, which is limited to a single farm, which may affect generalizability, further research with larger sample sizes is recommended.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	L.B.F	A.H.	V.T.	A.S.	K.S.L	F.A.	E.M.
C	20	10		50		20	
D	30	30	30		10		
S				30		30	40
DCP	40	40	10		10		
DAI	20	10		30		20	20
L	40	40	10		10		
W	40	30	10	20			
CR				30		40	30
SR	50		50				
PM	10			20		40	30
FA			10	40	10		40

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans (the data was taken from the farm, there was no experimental application on the animals).

Acknowledgments

We warmly thank the entire team of the Faculty of Agriculture, H-6800 Hódmezovásárhely for their contribution to this work. We are also aware that without their contributions this special issue would not be possible.

References

Ahmed AS, Rahmatalla S, Bortfeldt R, Arends D, Reissmann M, Brockmann GA. 2017. Milk protein polymorphisms and casein haplotypes in Butana cattle. J Appl Genet, 58: 261-271.

Albarella S, Selvaggi M, D'Anza E, Cosenza G, Caira S, Scaloni A, Fontana A, Peretti V, Ciotola F. 2020. Influence of the casein composite genotype on milk quality and coagulation properties in the endangered Agerolese cattle breed. Anim, 10: 892.

Baer RJ. 1991. Alteration of the fatty acid content of milk fat. J Food Prot, 54: 383-386.

Barnsteiner A, Lubinus T, di Gianvito A, Schmid W, Engel KH. 2011. GC-based analysis of plant stanyl fatty acid esters in enriched foods. J Agric Food Chem, 59: 5204-5214.

Bonsing J, Ring JM, Stewart AF, Mackinlay AG. 1988. Complete nucleotide sequence of the bovine beta-casein gene. Aust J Biol Sci, 41: 527-537.

Carroll SM, DePeters EJ, Taylor SJ, Rosenberg M, Perez-Monti H, Capps VA. 2006. Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. Anim Feed Sci Tech, 131: 451-473.

Cui Y, Liu B, Qu X, Dong A, Cao Y, Ma Y. 2012. Detection of β-casein variants in yak (Bos grunniens) by PCR-SSCP. Eur Food Res Technol, 234: 551-556.

De Jong C, Badings HT. 1990. Determination of free fatty acids in milk and cheese procedures for extraction, clean up, and capillary gas chromatographic analysis. J High Resol Chromatogr, 13: 94-98.

De Vitte K, Kerziene S, Klementavičiūtė J, De Vitte M, Mišeikienė R, Kudlinskienė I, Čepaitė J, Dilbiene V, Stankevičius R. 2022. Relationship of β-casein genotypes (A1A1, A1A2 and A2A2) to the physicochemical composition and sensory characteristics of cows' milk. J App Anim Res, 50: 161-166.

DiGiacomo K, Zamuner F, Sun Y, Dunshea FR, Raynes JK, Leury BJ. 2022. Effects of raw and pasteurized camel milk on metabolic responses in pigs fed a high-fat diet. Anim, 12: 1701.

Eisenstecken D, Stanstrup J, Robatscher P, Huck CW, Oberhuber M. 2021. Fatty acid profiling of bovine milk and cheese from six European areas by GC-FID and GC-MS. Int J Dairy Technol, 74: 215-224.

Ferretti L, Leone P, Sgaramella V. 1990. Long range restriction analysis of the bovine casein genes. Nucleic Acids Res, 18: 6829-6833.

Ivanković A, Pećina M, Ramljak J, Pašić V. 2021. Genetic polymorphism and effect on milk production of CSN2 gene in conventional and local cattle breeds in Croatia. Mljekarstvo, 71: 3-12.

Kumar S, Dahiya SP, Kumar S, Magotra A. 2017. Type of milk (A1/A2) and human health attributes: A review. Indian J Health Wellbeing, 8: 1268-1270.

Mayer HK, Lenz K, Halbauer EM. 2021. "A2 milk" authentication using isoelectric focusing and different PCR techniques. Food Res Int, 147: 110523.

Nguyen QV, Malau-Aduli BS, Cavalieri J, Nichols PD, Malau-Aduli AE. 2019. Enhancing omega-3 long-chain polyunsaturated fatty acid content of dairy-derived foods for human consumption. Nutrients, 11: 743.

Perna A, Intaglietta I, Simonetti A, Gambacorta E. 2016. The influence of casein haplotype on morphometric characteristics of fat globules and fatty acid composition of milk in Italian Holstein cows. J Dairy Sci, 99: 2512-2519.

Roginski H, Fuquay JW, Fox PF. 2003. Encyclopedia of dairy sciences. Volumes 1-4. Academic press, London, UK, pp: 122.

Samoré A, Canavesi F, Rossoni A, Bagnato A. 2012. Genetics of

casein content in Brown Swiss and Italian Holstein dairy cattle breeds. Ital J Anim Sci, 36: 196-202.

Vázquez-Román S, Garcia-Lara NR, Escuder-Vieco D, Chaves-Sánchez F, De la Cruz-Bertolo J, Pallas-Alonso CR. 2013. Determination of dornic acidity as a method to select donor milk in a milk bank. Breastfeeding Medic, 8: 99-104.

Vougiouklaki D, Antonopoulos D, Allexeli S, Houhoula D. 2020. Identification of polymorphisms of gene CSN2 of B casein in Greek cow breeds (Holstein) by restriction fragment length polymorphism. J Agri Sci, 12(11): 32.

Washburn SP, White SL, Green Jr JT, Benson GA. 2002. Reproduction, mastitis, and body condition of seasonally calved Holstein and Jersey cows in confinement or pasture systems. J Dairy Sci, 85(1): 105-111.

Xiao S, Wang Q, Li C, Liu W, Zhang J, Fan Y, Zhang S. 2022. Rapid identification of A1 and A2 milk based on the combination of mid-infrared spectroscopy and chemometrics. Food Control, 134: 108659.