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Effect of restricted grazing or feeding with total mix ration environments on the properties of milk quantity and quality from dairy cows of different genotypes

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Abstract

The effect of different feeding technologies, i.e., restricted grazing and non-grazing, on milk properties such as protein, fat, lactose, fatty acid content, somatic cell count, urea, and colour, in different β -casein *CSN2* genotypes (*A1A1*, *A1A2*, and *A2A2*) were analysed in this study. It was found that the physicochemical composition of cow milk was affected by both non-grazing and grazing environment and genotype. Compared with the other genotypes, the *A2A2* genotype cow milk showed a high polyunsaturated fatty acid and decreased the content of saturated fatty acid in the milk of restricted grazing and non-grazing groups and decreased the content of monounsaturated fatty acid in the restricted-grazing group. The *A1A2* genotype cow milk resulted in a higher content of monounsaturated fatty acid in the non-grazing group compared with the milk of cows in the restricted grazing group. The results of our experiment show that restricted grazing positively affects the properties of milk quantity and quality and that the cows with the *A2A2* genotype were well-suited to both restricted grazing and non-grazing systems.

Keywords: milk yield, milk colour, fatty acid, β -casein.

Introduction

Grass is the primary feed for cattle, both in its fresh and preserved form. However, with the numbers of cattle in farms gradually rising, grazing was slowly phased out by the ever-increasing production levels and advancement of technology. This trend is evident in many European countries (Hennessy et al., 2020).

One of the primary reasons for phasing out grazing was the increased nutritional demand for modern dairy production. Bruinenberg et al. (2002) concluded that the intake of dry matter, and, hence, the nutrient intake, can be expected to reach a maximum of 110 to 120 g (kg body weight)^{-0.75} in cows that were fed a grass-only diet and were sufficient to meet the requirements of maintenance and milk production of 22 to 28 kg. Hennessy et al. (2020) also reported similar results and concluded that increased milk production capacity of modern dairy cows necessitates supplementary feeding, without which it would be impossible to meet their high nutritional requirements. When offered supplementation, cows graze for shorter periods.

Grazing is becoming more difficult as the herd size and the grazing area grow proportionally. This increases the distance cows must walk from the grazing area to the milking parlour, a situation that is further exacerbated by the proliferation of robotic milking systems, as the cows must travel to and from the robotic milking system (Scott et al., 2014). On the other hand, grazing has also been linked to multiple health benefits in dairy cows (Di Grigoli et al., 2019) and reduced environmental impact (Box et al., 2017; Beukes et al., 2019; Molnar et al., 2020). Furthermore, consumers prefer dairy products from the grazed cattle (Wilkinson et al., 2020).

Milk proteins consist of up to 80% of casein, which is further divided into α s1-, α s2-, β -, and kappa-casein (Andic et al., 2021). β -casein consists of 209 amino acids and can be further subdivided into two variants, A1 and A2, determined by a variance in the amino acid at the 67th position. This variance is the presence of histidine in *A1* genotype and proline in *A2* genotype (Garg et al., 2021). This change is linked to health risks during digestion. The milk of *A1* genotype carriers breaks down into β -casomorphin, a bioactive peptide that has been linked to significant opioid activity (Massella et al., 2017; Sebastiani et al., 2020). β -casomorphin has been associated with vascular health (Fekete et al., 2013), heart health (Miluchova et al., 2016), inferior sleep quality (Brennan et al., 2013), and issues in the immune system (Konstantinou et al., 2014). During digestion, milk, and milk products of *A2* genotype carriers do not break down into β -casomorphins (Massella et al., 2017).

According to Morris et al. (2005), *A2A2* genotype carriers outperform *A1A1* and *A1A2* genotype carriers in a non-restricted-grazing system. It was hypothesised that dual-purpose *A2A2* genotype carrying cattle perform better in a restricted-grazing system than in a non-grazing one. Due to a lack of research into the effects of the chemical composition of feed on the lactation performance of different bovine *CSN2* genotypes, here the focus was on restricted-grazing and non-grazing systems, as there is.

This study was aimed at the evaluation of the effect of different feeding systems on the properties of milk quantity and quality: protein, fat, lactose, fatty acid content, somatic cell count, urea, and colour of milk, and the lactation performance of different bovine *CSN2* genotypes.

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Materials and methods

Research facilities. The research was conducted at the Veterinary Academy of the Lithuanian University of Health Sciences. The experiment was conducted between the 21 May and 21 July (three months) in 2021. The Animal Nutrition Department performed feed evaluation and prepared a ration structure. Fat, protein, and lactose analysis of milk was carried out at the Lithuanian National Milk Testing Laboratory. The content of fatty acid (FA) and the colour of the milk were determined at the Institute of Animal Rearing Technologies of the Faculty of Animal Science at the Lithuanian University of Health Sciences.

Experimental animals. Sixty cross-bred Simental × Holstein dual-purpose cows (average body weight 650 kg, aged 3–6 years) from the same herd were divided into two groups (n = 30 per group): 1) the restricted-grazing (experimental),

and 2) the non-grazing (control). Both groups were divided into subgroups, ten for each β-casein genotype: A1A1, A1A2, and A2A2. All cows were in the early lactation stage (up to three months after calving). Housing conditions were the same for both groups. The only exception was that the restricted-grazing group could enter the grazing pad, aside from the milking time. Cows were permanently housed in loose house barns with ad libitum water and roughages. The intakes were adjusted to have at least 5–10% of weigh-backs. Concentrates were offered twice daily in the milking parlour.

Chemical composition of the experimental diets and concentrate supplements is shown in Table 1. Non-grazing group was offered roughages containing the silage of young grass (50%) and whole crop maize (50%) at 14.16 kg day⁻¹ DM. The estimated DM intake for cattle grazed for 19 h day⁻¹ was 10 kg, implying that the remaining DM (±4 kg day⁻¹ DM) was obtained from the roughage mentioned above.

Table 1. Chemical composition of the experimental diet and concentrate supplement fed to restricted grazing and non-grazing lactating cows

Feed composition	Diet		
	roughages	grazed grass	concentrate supplement
Dry matter (DM) %	23	20	90
Metabolizable energy / megajoules (ME/MJ) DM kg	11.2	12.2	14.48
Crude protein %	14.8	15.6	21.7
Total fat %	5.4	5.5	3.7
Neutral detergent fibre (NDF) %	51.8	57.2	10.9
Acid detergent fibre (ADF) %	29.1	28.9	3.18
Ash %	5.45	10.05	2.81

All cows were offered a concentrated supplement containing flaked maize (65%) and soybean meal (35%) at 9.44 kg day⁻¹ DM. Additionally, they were given an adjusted mix of minerals and vitamins 200 g day⁻¹ per cow. The cows were offered hay *ad libitum*.

Grazing areas. For the experiment, 10 ha of a second-year pasture field was used. The field was divided into two parts, of which 6 ha were used to make haylage, while the remaining 4 ha were used for grazing. The grass seed mix consisted of various species: (1) white clover (*Trifolium repens* L.), 10%, red clover (*T. pratense* L.), 15%, timothy grass (*Phleum pratense* L.), 20%, perennial ryegrass (*Lolium perenne* L.), 35%, meadow fescue (*Festuca pratensis* Huds.), 10%, and Kentucky bluegrass (*Poa pratensis* L.), 10%, (2) Italian ryegrass (*Lolium multiflorum* Lam.), and (3) alfalfa (*Medicago sativa* L.), 14 kg of each, totalling 42 kg ha⁻¹. After testing the soil, the field was fertilised in spring using N₅₀P₄₀K₆₀ fertiliser per hectare.

Laboratory analyses. Ten samples of the total mix ratio feed were taken from the bunk at early, middle, and late feed-out. The total mix ratio feed was evaluated using an AgriNIR portable NIR analyser (Dinamica Generale, Italy). Nutritional values of the diets were calculated using the feeding software Hybrimin Futter 2008 (Hybrimin GmbH & Co., Germany). Milk samples from individual cows were collected once a month during the morning and afternoon milking, and the productivity of the animals was recorded according to standard ISO 707:2008 [IDF 50:2008].

In total, 192 milk samples (200 mL) from carriers of different (A1A1, A1A2, and A2A2) genotypes from restricted grazing and non-grazing groups were collected and analysed. The samples were kept at 4°C temperature and analysed the following day. The quantitative parameters of the milk: fat, protein, lactose, somatic cell count (SCC), urea, colour, and FA content, were evaluated. The fat, protein, lactose, SCC count, and urea content were determined by analysing the milk using a mid-infrared LactoScope Fourier-Transform Infrared Spectroscopy (FTIR) milk analyser FT 400 (Delta Instruments B.V., The Netherlands), which was equipped with a Work-IR optical bench (ABB Bomem, Canada) and a standard calcium fluoride cuvette (23 µm) utilising a fixed virtual filter calibration approach.

To detect the colour coordinates of the milk: lightness (L*), red/green (a*), and blue/yellow (b*), a Minolta Chroma Meter colourimeter CR-200 (Konica Minolta Inc., Japan) was used. Sub-samples (10 mL) of each milk sample were 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 the green colour and +a* has the red colour), and b* (where -b* has the blue colour and +b* has the yellow colour) (Zhang et al., 2007). The distance from 0 or the significance of the absolute values describes the intensity of the colour, i.e., a sample with a total value close to 0 has a lighter colour than a sample with an absolute value close to 100. The colours of the milk of bovine CSN2 genotype carriers (A1A1, A1A2, and A2A2) were compared.

Samples of FA were prepared following the standard ISO 12966-2:2011 and according to the procedure provided by Simonato et al. (2010). Milk lipids were extracted using chloroform, methanol, and water (2:1:1), and 150 mg of lipids were mixed with 5.0 mL of 0.25 mol L⁻¹ sodium methoxide in methanol-diethyl ether (1:1) and vigorously agitated for about 3 min. Next, 3.0 mL of isooctane and 15 mL of saturated sodium

chloride was added. The tube was vigorously agitated again and rested until phase separation. The supernatant was collected for chromatographic analysis. The FA content was evaluated using a gas chromatograph Shimadzu GC-2010 Plus with a mass spectrometer GCMS-QP2010 (Shimadzu Corp., Japan). The samples were separated using a mass spectrometer (MS) capillary column Restek Stabilwax, 30 m length, 0.25 mm I.D., and 0.25 µm d. (Bellefonte, USA). Full scan mode was selected on the spectrometer. The samples were injected in split mode with a split ratio of 1:60. The following parameters were used: 240°C, MS ion source 240°C, MS interface 240°C, helium (carrier gas) flow 0.90 ml min⁻¹, injector temperature 240°C, oven temperature 50°C (4 min), 10°C min⁻¹ to 110°C (1 min), 15°C min⁻¹ to 160°C (2 min), 2.5°C min⁻¹ to 195°C (1 min), 2°C min⁻¹ to 230°C (1 min), and 2°C min⁻¹ to 240°C (12 min). The concentration of fatty acid methyl esters (FAME) was calculated using a calibration curve and expressed as a percentage of the total FAME content in the sample. As a standard, Supelco 37 Component FAME Mix (MilliporeSigma, USA) was used.

Statistical analysis was performed using the software SPSS, version 28.0.10 (IBM Corp., USA). The Kolmogorov-Smirnov test confirmed the normality of the data distribution. The means and pooled standard error of means difference (PSEM) of gene effects and the effect of a restricted grazing/non-grazing environment on the milk protein, fat, lactose, FA content, SSC, urea, and the colour are given. In the case of a significant difference (P < 0.05), the Bonferroni post hoc criterion was used to assess the genotype influence.

Results

Properties of milk quantity and quality. Values expressed as the mean ± PSEM between the restricted grazing and non-grazing groups. The differences in the properties of milk quantity and quality between different genotypes are shown in Table 2. In the grazing group, significant differences (P < 0.05) in the properties of milk quantity and quality between A1A1, A1A2, and A2A2 genotype carriers (hereinafter, A1A1 cows, A1A2 cows, and A2A2 cows, respectively) were found; specifically, A2A2 cows produced 6.27 kg more milk per day than A1A1 cows.

Summary statistics on the milk colour of bovine CSN2 genotypes (L* – lightness; a* – greenness; b* – yellowness) in the two groups are provided in Table 3. Significant differences in milk colour parameters between the CSN2 genotypes were determined. The parameters also differed between the restricted- or non-grazing groups. The A1A1 cow milk was significantly the lightest (L*) (P < 0.001) between the CSN2 genotypes and the restricted grazing and non-grazing groups. Furthermore, A1A1 (-2.93) cow milk was also significantly redder (i.e., higher a*) than A1A2 (-5.40) and A2A2 (-3.89) cow milk of restricted-grazing group, whereas A2A2 (-3.86) cow milk was redder than the A1A1 (-4.61) and A1A2 (-4.50) cow milk in the non-grazing group. Additionally, A1A1 cows (P < 0.001) had significantly yellower milk among all CSN2 genotypes (P < 0.001) and between restricted- (P < 0.001) and non-grazing (P < 0.001) groups.

Monounsaturated fatty acid (MUFA) content in milk fat (% of total fat) and calculated index evaluation by genotype in restricted grazing and non-grazing groups are shown in Table 4. It was found that the MUFA content in cow milk differed significantly between the restricted grazing and non-grazing groups. The MUFA content in the restricted-grazing A1A1 cow milk was higher by 1.52% (P < 0.001), in A1A2

Table 2. Properties of milk quantity and quality and calculated indexes according to the genotype of restricted grazing and non-grazing groups of lactating cows

Property	Feeding system	Mean			PSEM	P-value** (gene effect)
		<i>A1A1</i>	<i>A1A2</i>	<i>A2A2</i>		
Milk kg	grazing	37.75 a	40.25	44.02 b	2.700	< 0.05
	non-grazing	41.79	45.36	42.94	2.732	0.203
	P-value*	0.225	0.052	0.659		
Fat	restricted grazing	5.20	5.54	5.46	0.378	0.369
	non-grazing	5.19	5.36	5.51	0.412	0.444
	P-value*	0.987	0.589	0.900		
Protein	restricted grazing	3.81	3.51	3.59	0.183	0.115
	non-grazing	3.54	3.30	3.49	0.148	0.104
	P-value*	0.189	0.228	0.438		
Lactose	restricted grazing	4.41	4.41	4.52	0.099	0.267
	non-grazing	4.39	4.52	4.56	0.109	0.148
	P-value*	0.902	0.180	0.770		
SSC	restricted grazing	162.6	149.8	113.7	39.322	0.225
	non-grazing	131.8	120.4	115.4	47.110	0.730
	P-value*	0.541	0.539	0.958		
Urea	restricted grazing	18.3	17.9	17.8	1.318	0.707
	non-grazing	20.2	19.0	19.6	1.606	0.461
	P-value	0.176	0.435	0.293		

Note. Values are expressed as the mean \pm PSEM; means in the same row followed by different inline letters (a, b, and c) are significantly different according to the Bonferroni criterion ($P < 0.05$); * – between restricted grazing and non-grazing groups, ** – gene effects on the properties of milk quantity and quality.

Table 3. Summary statistics on the milk colour of different bovine *CSN2* genotypes in restricted grazing and non-grazing groups of lactating cows

Colour	Feeding system	Mean			PSEM	P-value** (gene effect)
		<i>A1A1</i>	<i>A1A2</i>	<i>A2A2</i>		
L* – lightness	restricted grazing	102.4 a	102.2 a	100.6 b	0.243	<0.001
	non-grazing	104.8 a	103.2 b	103.3 c	0.013	<0.001
	P-value*	<0.001	<0.05	<0.001		
a* – greenness	restricted grazing	-2.93 a	-5.40 b	-3.89 c	0.025	<0.001
	non-grazing	-4.61 a	-4.50 b	-3.86 c	0.015	<0.001
	P-value*	<0.001	<0.001	<0.001		
b* – yellowness	restricted grazing	27.3 a	20.1 b	19.7 c	0.041	<0.001
	non-grazing	23.7 a	20.8 b	21.0 c	0.049	<0.001
	P-value*	<0.001	<0.001	<0.001		

Explanation under Table 2

Table 4. Monounsaturated fatty acid (MUFA) content in milk fat (% of total fats) and index evaluation in the genotype in grazing-restricted and non-grazing groups of lactating cows

Fatty acid	Feeding system	Mean			PSEM	P-value** (gene effect)
		<i>A1A1</i>	<i>A1A2</i>	<i>A2A2</i>		
Myristoleic C14:1 n-5	restricted grazing	0.82 a	1.62 b	1.36 c	0.03	<0.001
	non-grazing	2.22 a	2.23 a	0.95 b	0.029	<0.001
	P-value*	<0.001	<0.001	<0.001		
Pentadecenoic C15:1 n-5	restricted grazing	0.00	0.00	0.00	0.00	<0.05
	non-grazing	0.012 ab	0.019 a	0.000 b	0.005	<0.05
	P-value*	0.188	<0.001	<0.001		
Palmitoleic C16:1 n-7	restricted grazing	1.89 a	2.74 b	2.72 b	0.037	<0.001
	non-grazing	3.77 a	3.34 b	2.06 b	0.029	<0.001
	P-value*	<0.001	<0.001	<0.001		
Cis-10-heptadecanoic C17:1 n-7	restricted grazing	0.289	0.438	0.532	0.118	0.086
	non-grazing	0.288	0.405	0.35	0.117	0.358
	P-value*	0.998	0.147	<0.001		
Oleic C18:1 n-9	restricted grazing	19.18 a	17.07 b	24.24 c	0.385	<0.001
	non-grazing	14.43 a	15.18 b	15.51 c	0.110	<0.001
	P-value*	<0.01	<0.001	<0.001		
Eicosanoic C20:1 n-9	restricted grazing	0.069	0.091	0.061	0.014	0.183
	non-grazing	0.029 a	0.037 ab	0.055 b	0.008	<0.05
	P-value*	<0.05	<0.01	0.67		
MUFA***	restricted grazing	22.27 a	21.95 a	28.90 b	0.405	<0.001
	non-grazing	20.75 a	21.21 b	18.93 c	0.155	<0.001
	P-value*	<0.05	<0.01	<0.001		

Note. Explanation under Table 2; significant differences are reported; *** – content of FA included in MUFA = C14:1 n-5 + C15:1 n-5 + C16:1 n-7 + C17:1 n-7 + C18:1 n-9 + C18:1 trans n-9 (elaidic) + C20:1 n-9 + C22:1 n-9 (methyl erucate) + C24:1 n-9 (nervonic).

cow milk it was higher by 0.74%, and in *A2A2* cow milk it was higher by 9.97% compared with that in the non-grazing *A1A1*, *A1A2*, and *A2A2* cow milk, respectively. The restricted-grazing *A1A1* cow milk had a significantly higher content of oleic (C18:1 n-9) ($P < 0.01$) and eicosanoic (C20:1 n-9) ($P < 0.05$) FA when compared with non-grazing *A1A1* cow milk. The non-grazing *A1A1* cow milk had a higher content of myristoleic (C14:1 n-5) ($P < 0.001$) and palmitoleic (C16:1 n-7) ($P < 0.001$) FA than that in restricted-grazing *A1A1* cow milk. Restricted-grazing *A1A2* cow milk was significantly higher in oleic (C18:1 n-9) ($P < 0.001$) and eicosanoic (C20:1 n-9) ($P < 0.01$) FA content compared with non-grazing *A1A2* cow milk. Meanwhile, non-grazing *A1A2* cow milk had a higher content of myristoleic (C14:1 n-5) ($P < 0.001$) and palmitoleic (C16:1 n-7) ($P < 0.001$) FA when compared to the restricted-grazing *A1A2* cow milk. Restricted-grazing *A2A2* cow milk had a significantly higher content of myristoleic (C14:1 n-5) ($P < 0.001$), palmitoleic (C16:1 n-7) ($P < 0.001$), heptadecanoic (C17:1 n-7) ($P < 0.001$), and oleic (C18:1 n-9) ($P < 0.001$) FA compared with that in the non-grazing *A2A2* cow milk.

The gene effect on MUFA content in restricted-grazing *A2A2* cow milk was significantly higher ($P < 0.001$) than in *A1A1* and *A1A2* cow milk, by 8.15% and 6.95%, respectively. The MUFA content in non-grazing group cow milk was the highest in *A1A2* cow milk compared with *A1A1* and *A2A2* cow milk by 0.46% and 2.28%, respectively. The highest content of palmitoleic (C16:1

n-7) FA within the groups was reported in the non-grazing *A1A1* ($P < 0.001$) and restricted-grazing *A1A2* ($P < 0.001$) cow milk. Furthermore, in the group of non-grazing cows, compared with that in the other genotypes, the *A1A2* cow milk had the highest content of myristoleic (C14:1 n-5) ($P < 0.001$) FA, whereas the *A2A2* one had the highest content of oleic (C18:1 n-9) ($P < 0.001$) and eicosanoic (C20:1 n-9) ($P < 0.05$) FA. In the restricted-grazing cow's milk, compared with that from the other genotypes, the *A2A2* cow milk had the highest content of myristoleic (C14:1 n-5) and oleic (C18:1 n-9) FA.

Polyunsaturated fatty acid (PUFA) content in milk fat (% of total fat) and calculated index evaluation by genotype in restricted grazing and non-grazing groups are shown in Table 5. Significant differences were observed between the PUFA content in the milk of restricted grazing and non-grazing groups. The PUFA content in restricted-grazing *A2A2* cow milk was by 0.99% ($P < 0.001$), in *A1A1* cow milk – by 0.38% ($P < 0.01$), and in *A1A2* cow milk – by 0.55% ($P < 0.01$) higher than that in the non-grazing *A2A2*, *A1A1*, and *A1A2* cow milk, respectively. The restricted-grazing *A1A1* cow milk was significantly higher in α -linolenic (C18:3 n-3) ($P < 0.001$) and eicosapentaenoic (C20:5 n-3) ($P < 0.001$) FA content. At the same time, the non-grazing cow's milk had the highest content of dihomo-gamma-linolenic (C20:3 n-6) ($P < 0.05$) FA. The restricted-grazing *A1A2* cow milk was richest in linoleic (C18:2 n-6) ($P < 0.05$), α -linolenic (C18:3 n-3) ($P < 0.001$), and dihomo-gamma-linolenic

Table 5. Polyunsaturated fatty acid (PUFA) milk fat (% of total fats) content and calculated index evaluation by genotype of restricted-grazing and non-grazing groups of lactating cows

Fatty acid	Feeding system	Mean			PSEM	P-value** (gene effect)
		A1A1	A1A2	A2A2		
Linoleic C18:2 n-6	restricted grazing	2.78 a	3.18 b	3.72 c	0.083	<0.001
	non-grazing	2.74 a	2.92 b	2.94 b	0.049	
	P-value*	0.599	<0.05	<0.001		
α -linolenic C18:3 n-3	restricted grazing	0.869 a	0.719 b	0.753 c	0.011	<0.001
	non-grazing	0.584	0.588	0.598	0.016	
	P-value*	<0.001	<0.001	<0.001		
Eicosadienoic C20:2 n-6	restricted grazing	0.011 a	0.054 b	0.079 b	0.016	<0.001
	non-grazing	0.01	0.019	0.019	0.005	
	P-value*	0.873	0.103	<0.01		
γ -linoleic C20:3 n-6	restricted grazing	0.072 a	0.121 b	0.097 ab	0.011	<0.01
	non-grazing	0.092	0.097	0.092	0.003	
	P-value*	<0.05	<0.05	0.682		
Eicosatetraenoic C20:4 n-6	restricted grazing	0.232 a	0.184 b	0.200 ab	0.013	<0.01
	non-grazing	0.215	0.209	0.207	0.011	
	P-value*	0.382	<0.05	0.465		
Eicosapentaenoic C20:5 n-3	restricted grazing	0.153 a	0.015 b	0.050 c	0.009	<0.001
	non-grazing	0.035	0.035	0.037	0.01	
	P-value*	<0.001	<0.05	0.182		
Docosadienoic C22:2 n-6	restricted grazing	0.026 a	0.233 b	0.096 a	0.038	<0.01
	non-grazing	0.102	0.07	0.099	0.027	
	P-value*	0.142	0.071	0.722		
PUFA***	restricted grazing	4.18 a	4.52 b	5.01 c	0.073	<0.001
	non-grazing	3.80 a	3.96 b	4.02 b	0.055	
	P-value*	<0.01	<0.01	<0.001		

Note. Explanation under Table 2; significant differences are reported; *** – content of FA included in PUFA = C18:2 n-6 + C18:2 c-9 (conjugated linoleic) + C18:3 n-3 + C18:3 n-6 (gamma-linolenic) + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C20:5 n-3 + C22:2 n-6 + C22:6 n-3 (docosahexaenoic).

(C20:3n-6) ($P < 0.05$) FA. The non-grazing A1A2 cow milk had a significantly higher content of eicosatetraenoic (C20:4 n-6) ($P < 0.05$) and eicosapentaenoic (C20:5 n-3) ($P < 0.05$) FA when compared with that in the milk of restricted-grazing cows. Statistically, the restricted-grazing A2A2 cow milk was the richest in linoleic (C18:2 n-6) ($P < 0.001$), α -linolenic (C18:3 n-3) ($P < 0.001$), and eicosadienoic (C20:2 n-6) ($P < 0.01$) FA when compared with that from non-grazing group A2A2 cows.

PUFA was significantly affected by genotype; the A2A2 cow milk had the highest content in restricted-grazing and non-grazing groups. In the restricted-grazing group, the A2A2 cow milk was higher in PUFA by 0.83% in comparison with A1A1 cow milk and by 0.49% compared with A1A2 cow milk. The non-grazing A2A2 cow milk was 0.22% and 0.06% higher than A1A1 and A1A2 one, respectively. In the restricted-grazing

group, the A1A1 cow milk showed significant differences compared to other genotypes, which had the highest content of α -linolenic (C18:3 n-3) ($P < 0.001$) and eicosatetraenoic (C20:4 n-6) ($P < 0.01$) FA. Significant differences were found in restricted-grazing A1A2 cow milk when compared to other genotypes in dihomogamma-linoleic (C20:3 n-6) ($P < 0.01$) and docosadienoic (C22:2 n-6) ($P < 0.01$) FA content. The restricted-grazing A2A2 cow milk had significantly higher content of linoleic (C18:2 n-6) ($P < 0.001$) and eicosadienoic (C20:2 n-6) ($P < 0.01$) FA.

Saturated fatty acid (SFA) content in milk fat (% of total fat) and calculated index evaluation by genotype of restricted grazing and non-grazing groups are shown in Table 6. Significant differences were observed in the SFA content.

Table 6. Saturated fatty acid (SFA) content in milk fat (% of total fats) and index evaluation by genotype in restricted grazing and non-grazing groups of lactating cows

Fatty acid	Feeding system	Mean			PSEM	P-value** (gene effect)
		A1A1	A1A2	A2A2		
Butyric C4:0	restricted grazing	4.30 a	2.43 b	2.98 b	0.266	<0.001
	non-grazing	4.12 a	3.19 b	4.28 a	0.206	
	P-value*	0.666	<0.05	<0.001		
Caproic C6:0	restricted grazing	2.69 a	1.76 b	1.93 b	0.149	<0.001
	non-grazing	2.82 a	2.33 b	2.84 a	0.11	
	P-value*	0.59	<0.01	<0.001		
Lauric C12:0	restricted grazing	4.41 a	6.13 b	4.10 c	0.089	<0.001
	non-grazing	5.38 a	6.16 b	5.50 c	0.04	
	P-value*	<0.001	0.239	<0.001		
Caproic C6:0	restricted grazing	0.095 a	0.315 b	0.203 c	0.009	<0.001
	non-grazing	0.256 a	0.285 a	0.146 b	0.014	
	P-value*	<0.01	<0.05	<0.001		
Myristic C14:0	restricted grazing	12.98 a	14.30 b	11.58 c	0.127	<0.001
	non-grazing	13.44 a	14.30 b	13.41 a	0.074	
	P-value*	<0.05	0.935	<0.001		
Pentadecanoic C15:0	restricted grazing	1.22 a	2.67 b	1.97 c	0.033	<0.001
	non-grazing	2.26 a	2.28 a	1.49 b	0.033	
	P-value*	<0.001	<0.001	<0.001		
Palmitic C16:0	restricted grazing	28.58 a	33.25 b	29.37 a	0.51	<0.001
	non-grazing	33.85 a	31.82 b	32.33 b	0.339	
	P-value*	<0.01	<0.001	<0.001		
Margaric C17:0	restricted grazing	1.05 a	0.92 b	1.07 a	0.018	<0.001
	non-grazing	0.806 ab	0.765 a	0.871 b	0.027	
	P-value*	<0.01	<0.001	<0.001		
Stearic C18:0	restricted grazing	12.46 a	5.74 b	7.96 c	0.102	<0.001
	non-grazing	6.04 a	7.19 b	9.45 c	0.156	
	P-value*	<0.001	<0.001	<0.001		
Behenic C22:0	restricted grazing	0.039 a	0.055 b	0.057 b	0.003	<0.01
	non-grazing	0.029 a	0.041 b	0.036 ab	0.003	
	P-value*	<0.05	<0.05	<0.01		
SFA***	restricted grazing	73.56 a	73.53 a	66.09 b	0.427	<0.001
	non-grazing	75.44 a	74.83 b	77.05 c	0.199	
	P-value*	<0.05	<0.001	<0.001		

Note. Explanation under Table 2; significant differences are reported; *** – content of FA included in SFA = C4:0 + C6:0 + C8:0 (caprylic) + C10:0 (capric) + C11:0 (undecylic) + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 (eicosanoate) + C21:0 (heneicosanoic) + C22:0 + C24:0 (lignoceric).

The SFA content in restricted-grazing A2A2 cow milk was lower by 10.96% ($P < 0.001$) compared with that in the non-grazing A2A2 cow milk. In the A1A1 cow milk, the SFA content was lower by 1.88% ($P < 0.05$) in the restricted-grazing group than in the non-grazing one. The SFA content in A1A2 cow milk was by 1.3% ($P < 0.001$) lower in the restricted-grazing group than that in the non-grazing one. The restricted-grazing A1A1 cow milk had the lowest content of lauric C12:0 ($P < 0.001$), tridecanoic (C13:0) ($P < 0.001$), myristic (C14:0) ($P < 0.05$), pentadecanoic (C15:0) ($P < 0.001$), and palmitic (C16:0) ($P < 0.01$) FA. Non-grazing A1A1 cow milk had the lowest content of margaric (C17:0) ($P < 0.01$), stearic (C18:0) ($P < 0.001$), and behenic (C22:0) ($P < 0.05$) FA. The restricted-

grazing A1A2 cow milk had the lowest content of butyric (C4:0) ($P < 0.05$), caproic (C6:0) ($P < 0.01$), and stearic (C18:0) ($P < 0.001$) FA. The non-grazing A1A2 cow milk had the lowest content of tridecanoic (C13:0) ($P < 0.05$), pentadecanoic (C15:0) ($P < 0.001$), palmitic (C16:0) ($P < 0.001$), margaric (C17:0) ($P < 0.001$), and behenic (C22:0) ($P < 0.05$) FA. In the restricted-grazing group, the A2A2 cow milk had the lowest content of butyric (C4:0) ($P < 0.001$), caproic (C6:0) ($P < 0.001$), lauric (C12:0) ($P < 0.001$), myristic (C14:0) ($P < 0.001$), palmitic (C16:0) ($P < 0.001$), and stearic (C18:0) ($P < 0.001$) FA among other genotypes. The non-grazing A2A2 cow milk had the lowest content of tridecanoic (C13:0) ($P < 0.001$), pentadecanoic (C15:0) ($P < 0.001$), margaric (C17:0) ($P < 0.001$), and behenic (C22:0) ($P < 0.01$) FA.

SFA was significantly affected by genes. The restricted-grazing *A2A2* cow milk had the lowest SFA content compared with the *A1A1* and *A1A2* cow milk by 7.47% and 7.44%, respectively. The SFA content in the non-grazing *A1A2* cow milk was lower than in *A1A1* and *A2A2* cow milk by 0.61% and 2.22%, respectively. The gene effect on the restricted grazing and non-grazing *A1A1* cow milk was significant: restricted-grazing *A1A1* cow milk had the lowest content of tridecanoic (C13:0) ($P < 0.001$), pentadecanoic (C15:0) ($P < 0.001$), palmitic (C16:0) ($P < 0.001$), and behenic (C22:0) ($P < 0.01$) FA. The non-grazing *A1A1* cow milk had the lowest content of lauric (C12:0) ($P < 0.001$), stearic (C18:0) ($P < 0.001$), and behenic (C22:0) ($P < 0.05$) FA. The gene effect on the restricted-grazing and non-grazing *A1A2* cow milk was significant. The restricted-grazing *A1A2* cow milk had the lowest content of butyric (C4:0) ($P < 0.001$), caproic (C6:0) ($P < 0.001$), margaric (C17:0) ($P < 0.001$), and stearic (C18:0) ($P < 0.001$) FA among other genotypes. The non-grazing *A1A2* cow milk had the lowest content of butyric (C4:0) ($P < 0.01$), caproic (C6:0) ($P < 0.01$), and margaric (C17:0) ($P < 0.01$) FA among other genotypes. The gene effect on the restricted grazing and non-grazing *A2A2* cow milk was significant: the restricted-grazing *A2A2* cow milk had the lowest content of lauric (C12:0) ($P < 0.001$) and myristic (C14:0) ($P < 0.001$) FA among all genotypes in both groups. The non-grazing *A2A2* cow milk had the lowest content of tridecanoic (C13:0) ($P < 0.001$), myristic (C14:0) ($P < 0.001$), and pentadecanoic (C15:0) ($P < 0.001$) FA among all other genotypes in both groups.

Discussion

The daily milk yield from the restricted grazing group cows was significantly affected by genes when comparing different bovine *CSN2* genotypes (*A1A1*, *A1A2*, and *A2A2*) with one another. In the restricted-grazing group, *A2A2* cows ($P < 0.05$) produced more milk than the *A1A1* cows. Similarly, Ikonen et al. (2001) reported that the casein A2 allele was associated with higher milk and protein yield and lower fat percentage. This correlates with the findings of Morris et al. (2005), who found that the grazed *A2A2* cow milk had higher content of milk protein than the *A1A1* cows. However, they also found that the *A2A2* cow milk was significantly higher ($P < 0.05$) in milk fat than that of *A1A2* cows. Furthermore, Morris et al. (2005) reported that SCC was higher ($P < 0.05$) in *A1A2* cow milk than in *A1A1* one.

No significant differences in the properties of milk quantity and quality were found between the A1 and A2 alleles. Nguyen et al. (2018) and Citek et al. (2019) found no differences between the fat, protein, lactose, and total DM content of the two genotypes. However, Nguyen et al. (2018) found slight differences in the physical properties of *A1A1* and *A2A2* cow milk. Their notion of the topic requires further study.

The colour of milk was significantly affected between the restricted grazing and non-grazing groups. Unfortunately, our findings are not mirrored in the literature, as, to our knowledge, no studies have addressed the influence of grazing on milk colour. This is important, because the colour of milk and milk products affects consumer reception (Chudy et al., 2020). Finding other methods of manipulating the colour of milk without relying on artificial colouring would be highly beneficial for the dairy industry (Scarso et al., 2017).

The milk from the restricted-grazing cows displayed an increased MUFA content compared with the non-grazing cows. Similar findings were reported by Benbrook et al. (2018), who found that pasture feeding systems or including fresh pasture in a cows' diet were linked to an increase in an array of beneficial MUFAs. Morris et al. (2005) reported similar results on grazing cows with different genotypes of milk; in the *A2A2* cow milk, MUFA was higher ($P < 0.05$) than that in *A1A1* cow milk. Perna et al. (2016) reported that *BB-A2A2-AB* cow milk showed the highest content ($P < 0.05$) of oleic acid (C18:1 n-9) (18.40%), whereas *BB-A2A2-BB* cow milk showed the lowest content of oleic acid (C18:1 n-9) (16.19%) compared to other haplotypes. In the current experiment, the content of oleic acid (C18:1 n-9) was the highest ($P < 0.001$) in *A2A2* cow milk in both restricted- (24%) and non-grazing (15.51%) groups. The content of oleic acid (C18:1 n-9) was higher in the milk of restricted-grazing cows than in the non-grazing group.

PUFA content in milk fat of restricted-grazing cows was higher than that in the non-grazing cow's milk, similar to the results reported by Kučević et al. (2016) and Benbrook et al. (2018). The grazing cow's milk had a higher unsaturated FA content with a higher content of PUFA. Bonanno et al. (2013) concluded that grazing affects the quality of dairy products resulting in more unsaturated FA, including conjugated linoleic acid (C18:2 n-9). Although there is a lack of literature on the effect of the *A1A2*, *A1A2*, and *A2A2* cows on PUFA content in milk, a study by Perna et al. (2016) offered some insight: it was found that *BB-A2A2-AA* cow milk had the lowest content (3.13%; $P < 0.05$) of linoleic acid (C18:2 n-6) compared with all other genotypes. Between all groups of cows, linoleic acid

(C18:2 n-6) showed no significant differences. Perna et al. (2016) found that content of the α -linolenic acid (C18:3 n-3) was significantly lower (0.66%, $P < 0.05$) in *BB-A2A2-AB* cow milk than in other haplotypes.

In our experiment, in contrast, *A1A2* cow milk showed the lowest content of α -linolenic acid compared with that in other genotypes of the restricted-grazing group. Furthermore, it was found that restricted-grazing group milk had a higher overall content of the α -linolenic acid (C18:3 n-3) when compared with that in the non-grazing cow milk. Perna et al. (2016) found that *BB-A2A2-AA* cow milk had the lowest content ($P < 0.05$) of eicosapentaenoic (C20:5 n-3) and docosahexaenoic (C22:6 n-3) FA. The content of eicosapentaenoic acid (C20:5 n-3) was the lowest in the restricted-grazing *A1A2* cow milk compared with that in the cow milk of other (*A1A1* and *A2A2*) genotypes ($P < 0.001$). In comparison, the content of docosahexaenoic acid (C22:6 n-3) did not differ significantly between milk of any group or genotype.

Bovine *CSN2* genotypes significantly affected the SFA content of milk. This result contrasts with that reported by Perna et al. (2016), who found significant differences in individual SFA content, which did not affect total SFA content. Non-grazing cows had an elevated SFA in comparison to restricted-grazing cow milk, and similar results were reported by Kučević et al. (2016), in which non-grazing feeding resulted in higher SFA content than grazing cow's milk. Perna et al. (2016) found that the content of butyric acid (C4:0) was lower in the *BB-A2A2-BB* and *BB-A1A1-AA* cow milk than in the milk of other haplotypes ($P < 0.05$). The content of butyric acid (C4:0) was the highest in *A1A1* cow milk and the lowest in *A1A2* cow milk in restricted grazing. Furthermore, in the non-grazing cow milk, the content of butyric acid (C4:0) was the lowest in *A1A2* cow milk and the highest in the *A2A2* cow milk. Between the groups, the content of butyric acid (C4:0) was higher in the non-grazing cow milk when compared with the *A1A2* and *A2A2* cow milk. Perna et al. (2016) reported that the *BB-A2A2-AB* and *BB-A2A2-BB* cow milk had the highest content of caproic (C6:0), caprylic (C8:0), and capric (C10:0) FA. Only the content of caproic acid (C6:0) was significantly higher in *A1A1* cow milk than in the other genotype cow's milk in restricted-grazing. Regarding non-grazing group, content of caproic acid (C6:0) was higher in *A2A2* cow milk than in the *A1A2* cow milk. Between the groups, the content of caproic acid (C6:0) was higher in the non-grazing group milk than in *A1A2* and *A2A2* cow milk. Perna et al. (2016) reported that the *BB-A2A2-AA* haplotype showed the highest content (22.43%; $P < 0.05$) of palmitic acid (C16:0), which is in contrast with the results of our experiment, wherein the *A1A2* cow milk had the highest content (33.25%; $P < 0.001$) of palmitic acid (C16:0) in restricted-grazing group and in the non-grazing *A1A1* cow milk (33.85% $P < 0.001$). Between the groups, non-grazing cows' milk had a higher content of palmitic acid (C16:0) than the milk of restricted-grazing cows. Perna et al. (2016) reported that the content of stearic acid (C18:0) was the lowest in the *BB-A2A2-AA* cow milk (9.41%; $P < 0.05$). The content of stearic acid (C18:0) was the highest in the restricted-grazing *A1A1* cow milk (12.46%; $P < 0.001$) and in the non-grazing *A2A2* cow milk (9.45%; $P < 0.001$).

Our findings are important due to the lack of research on the role the genes play when it comes to the properties of milk quantity and quality in grazing cows. These properties include milk yield, colour, and FA content (% of total milk fat). All the properties mentioned above are critical to the profitability of milk industry; however, not all farms can provide a permanent grazing environment to their cattle. Our research has merit specifically for those farms that cannot ensure permanent grazing. These results will be important in decision-making regarding grazing possibilities in farms that are incapable of using permanent grazing. There is a lack of concrete research into the role the genes play in how grazing affects the properties of milk quantity and quality, and it would be pertinent to investigate this link further.

Conclusion

The physicochemical composition of restricted grazing and non-grazing cow milk was affected by both group and genotype (*A1A1*, *A1A2*, and *A2A2*). The *A2A2* genotype cow milk had elevated polyunsaturated fatty acid (PUFA) content and lowered saturated fatty acid (SFA) content in both groups and decreased monounsaturated fatty acid (MUFA) content in restricted-grazing cow milk. The *A1A2* genotype cow milk exhibited a higher MUFA content in the non-grazing group when compared to the milk of the restricted grazing group.

Results of our experiment lead us to the conclusion that restricted grazing positively affects the properties of milk quantity and quality and that *A2A2* genotype cows are well-suited to both grazing and non-grazing systems.

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Riboto ganymo arba šerimo viso raciono pašarų mišiniu poveikis skirtingų genotipų karvių pieno savybėms

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Santrauka

Tyrimo metu įvertinta galvijų pašarų energinė ir mitybinė vertė. Nustatyta skirtingo šerimo įtaka įvairių CSN-2 genotipų (A1A1, A1A2 ir A2A2) karvių pieno ir pieno riebalų kiekybiniais rodikliais: baltymų, riebalų, laktozės kiekiui, riebalų rūgščių sudėčiai, somatinių ląstelių skaičiui, urėjai ir spalvai. Ribotos ganiavos šerimo sistema A2A2 genotipo karvių piene padidino mononesočiųjų ($P < 0,001$) ir polinesočiųjų ($P < 0,001$) riebalų rūgščių kiekį, reikšmingai padidino pieno kiekį ($P < 0,05$) ir sumažino sočiųjų riebalų rūgščių kiekį. A1A2 genotipo karvių, šertų tik viso raciono pašarų mišiniu, piene reikšmingai padidėjo mononesočiųjų ($P < 0,001$) ir sumažėjo sočiųjų ($P < 0,01$) riebalų rūgščių kiekis.

Reikšminiai žodžiai: pieno kiekis, pieno spalva, riebalų rūgštys, β-kazeinas.