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The interaction between the milk production, milk components with a low frequency of analysis and factors affecting the milk composition in dual-purpose Simmental cows

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Abstract: The aim of this study was to improve the understanding of the contribution of components typically found in milk, but which are not often included in analyses, to the nutritional status of dairy cows. This was undertaken by analysing the amount and composition of milk produced by Simmental dairy cows, a dual-purpose breed farmed in the Czech Republic. Apart from the more frequently analysed group of components in milk, a less frequently analysed group of components were also investigated. This group, typically, consists of the following components: urea, casein, citric acid, beta hydroxy butyrate (BHB) ketones and free fatty acids. The average content of urea, casein and citric acid in milk is 25.75 mg/100 ml, 2.96%, 0.15%, respectively. The influence of environmental factors on these indicators was evaluated, as well as the degree of hereditary establishment. These less frequently analysed components (indicators), as well as the more frequently analysed components of milk, are subject to several external influences, especially the influence of the breeder, the year and the season of calving. The content of these components varied significantly statistically during the lactation period and also in the order of lactation. The influence of a cow's individuality and the degree of additive genetic background are evident, with estimated heritability coefficients ranging from 0.04 for the BHB ketone content to higher values, e.g., 0.28 for citric acid or 0.31 for the lactose content in milk. The relationships between the components of milk and the daily milk yield were also evaluated, and statistically significant negative correlations were found between the content of casein and the daily milk yield (–0.47) and between the lactose content and the number of somatic cells (–0.37).

Keywords: Czech Fleckvieh; dairy; heritability; parity; calving season

An analysis of the composite and individual components of milk provides an indication of the health status of dairy cows, and provides information on possible deficiencies in their nutrition (Zavadilova et al. 2021). Hanus et al. (2011) state that these are the so-called major or "more frequently analysed"

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milk components, but the components with a lower frequency of analysis are also very important. These more frequently analysed milk components mainly include the milk proteins, fat, and sugar, as well as the number of somatic cells (somatic cell count). Monitoring of the "less frequently analysed" milk components (urea, casein, ketones, citric acid, free fatty acids, and others) gives a strong indication of the nutritional status of dairy cows and can serve as a basis for the prevention of production disorders, and, thus, also for the promotion of animal health (Stadnik et al. 2022). Literature reviews suggest a very low frequency of analysis of these components (Hanus et al. 2011; Melfsen et al. 2012).

An analysis of the casein and urea content is used to assess the energy balance of dairy cows. If the casein content of the milk falls below the physiological range, it can be concluded that nutritional deficiencies can occur in the energy content of the rations of the dairy cow or herd. This may result in their malnutrition. It is, however, necessary to take the level of performance of the dairy cow into account. Amongst other things, the casein content is also analysed in the assessment of the cheese and protein yield capacity of milk (Hanus et al. 2011).

The citric acid (CIT) content affects the suitability of milk for cheese-making (Khaled et al. 1999). Knowledge of the CIT content can, therefore, be used to manage the nutritional level of dairy cows, and provide evidence of the processability of the milk. An optimal CIT content is between 8–10 mmol/l. Lower values (below 6 mmol/l) indicate the already mentioned energy deficit in the dairy metabolism. Conversely, higher values (above 12 mmol/l) induce excess energy.

In addition to CIT, the energy status of dairy cows is also indicated by the fatty acid and urea contents in the milk (Duchacek et al. 2010). Urea is the final product of protein metabolism, and the urea content can serve as an indicator for the suitability of the feed ration. Higher concentrations of urea in the milk are associated with excess protein in the diet (Hojman et al. 2004). The amount of urea contained in the milk is also an indication of the supply of nitrogenous substances (like protein concentrates) in the rations of cows (Horky 2014). The physiological value for the urea content is 20–30 mg/100 ml of milk (Kubesova et al. 2009). However, in dairy cows with higher performance, a value of up to 35 mg/100 ml is tolerated (Horky 2014). If there is an excessive amount of nitrogen in the feed ration, it will cause excessive ammonia formation in the rumen, which the rumen microflora cannot process. Excess ammonia then passes into the blood through the rumen wall and the liver detoxifies it to urea. Thus, the urea content indicates the nutritional and health status of dairy cows (Baker et al. 1995) and, according to its content, we can assess the balance of nutrition, i.e., nutrients of the feed ration. The urea content is also related to the longevity, reproduction, and last, but not least, to the technological and quality indicators of the milk, where its higher content weakens these indicators (Butler et al. 1996). As for free fatty acids (FFA), they are localised in the fat and slightly aqueous phase and can occur in small amounts that do not esterify into triglycerides and are freely dispersed. The FFA content found in milk fat is 0.5–1.2 mmol/g, but a maximum content of up to 1.3 mmol/100 g of fat is permitted (Bobbo et al. 2020). Higher levels may also induce health problems in dairy cows related to the increased somatic cell counts or to mammary gland disease. Amongst other things, these higher levels result in reduced technological properties (Vyletelova et al. 2000), including reduced sensory properties, i.e., smell and taste (= bitterness), of the milk. This aspect is also well discussed and explained in the article by Stolcova et al. (2021).

The calving season has an important effect on the variation and prominence of the less frequently analysed components as well as with the changes in the environmental characteristics of the seasonal variation, which directly impact the calving behaviour of cows, and, therefore, the changes in the levels of the less frequently analysed components. Specific examples, as indicated in the findings of this study, also suggest that changes in the seasonality of the calving behaviour and environmental conditions will impact the stability of the lactose content of the milk during the productive life of the cow, creating unstable somatic cell counts during lactation. Climate change could influence the availability and cost of the feed used for the rations that influence the rationing formulations and, eventually, the nutritional status and metabolic stress in the animal. This will directly influence the milk quality and health status of the lactating animal while putting a strain on the milk yield of the cow. Climate change, therefore, represents an important risk factor when it comes to the

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future sustainability of the milk production of the cow and the milk producing enterprise through increased costs of maintain the animal's welfare and feeding. The less frequently analysed milk components will provide important and vital information that can effectively be used in dairy management decision making to ensure the efficient production of good volumes and quality milk while ensuring high standards in the management of the dairy herd health and reproductive performance (Stadnik et al. 2022). During times of climatic change, the less frequently analysed components are very important indicators in tracking the successes and failures of dairy managers to support the health and nutritional status of lactating cows and provide indicators of the welfare status and resilience of such cows (Kasna et al. 2022).

In this study, it is therefore expected that the careful and accurate analyses of such less frequently analysed milk components will provide important and vital information that can effectively be used in dairy management decision making to ensure the efficient production of good volumes and quality milk while ensuring high standards in the management of the dairy herd's health and reproductive performance. An important objective of this study will, therefore, be to establish the quantum and significance of the relationships between the milk production and the presence of such less frequently analysed components with the intention to include them in dairy herd management decision making.

MATERIAL AND METHODS

Data

Milk samples within the dairy test-day recording scheme in Czech Republic were used for the analysis. In total, 21 962 milk samples from 2 569 purebred Czech Simmental cows were analysed. Cows from three high-production dairy farms (the mean value of the daily milk yield was 27.56 kg), located in the regions of the Czech-Moravian highlands and South Moravia, were included in the study. The average number of dairy cows for the included farms was about 650. These cows calved during 2019–2021 and the parity of these cows ranged from the 1st to 12th lactation. For the purpose of our analysis, the 4th to 12th parity were merged. The minimum days in milk (DIM) in the dataset was six days after calving and maximum DIM was 350 days.

The dependent variables, which were assessed in this study, were the daily milk yield (DMY; kg), fat percentage (FAT), protein percentage (PRT), lactose percentage (LAC), somatic cell count (1 000/ml; SCC), urea content in the milk (URE; mg/100 ml), casein percentage (CAS), citric acid percentage (CIT), ketone beta hydroxy butyrate (BHB) content (KET; mmol/l) and free fatty acid content (FFA; mmol/100 g of fat). All the variables, except for SCC, KET and FFA, showed normal frequency distributions. Therefore, $\log_{10}$ transformation was performed in the case of SCC, KET and FFA to reach an approximate normal frequency distribution. In the further statistical analyses, only the log-transformed values (logSCC, logKET and logFFA) were used.

Samples were analysed in a nationally accredited milk laboratory (CSN EN ISO/IEC 17025:2018) by the Czech-Moravian Breeders Association via MilkoScan^{TM} FT+ (FOSS A/S, Hillerød, Denmark). The samples were prepared in accordance with the ISO 707:2008 requirements (IDF 50: 2008). All the samples (21 962) were analysed for FAT, PRT, LAC, SCC and URE. The CAS was analysed in 14 218 samples, the CIT was analysed in 15 321 samples, the KET was analysed in 12 042 samples and the FFA was analysed in 16 975 samples.

Statistical analysis

Any missing data and erroneous observations were removed from the primary database containing all the data collected on the farms. All the analytical procedures were performed using SAS v9.1 statistical software (SAS Institute, Inc., Cary, NC, USA). The relationships between all the traits were expressed using Pearson's correlation coefficients using procedure PROC CORR. The influence of the effects on the milk components were assessed using a general linear model (PROC GLM) and analysis of variance (PROC ANOVA) procedures. The Tukey-Kramer method was used for the post hoc analyses to assess the differences between least squares (LS) means within each effect. Estimating the heritabilities was performed using PROC GLM, PROC ANOVA and matrix language PROC IML. All the traits were tested for normality and the data were adjusted for outliers, both using PROC UNIVARIATE.

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Influence of the selected factors on the milk components

The General Linear Model procedure was used to evaluate the effect of each factor entered into the model. The statistical significance of all the influences was tested via the TYPE III Sum of Squares at a level of significance of $P < 0.05$ (*) and $P < 0.01$ (**). The differences between the individual least square means (LSM) for the effect of the calving season and parity were assessed using the Tukey-Kramer method at a level of significance of $P < 0.05$ (Table 1). The following linear model was used to test the effects of the factors on the milk components:

$$y_{ijklm} = \mu + herd_i + cyear_j + seas_k + par_l + cow_m + b^*dim + e_{ijklm} \quad (1)$$

where:

y_{ijklm} – dependent variable;
μ – intercept;
$herd_i$ – i^{th} herd effect (i = 1–3);
$cyear_j$ – j^{th} effect of the calving year (j = 1–3);
$seas_k$ – k^{th} effect of the calving season (k = 1–4, 1: December to February, 2: March to May, 3: June to August, 4: September to November);
par_l – l^{th} effect of the parity (l = 1–4, 1: first lactation, 2: second lactation, 3: third lactation, 4: 4^{th} to 12^{th} lactation);
cow_m – m^{th} effect of the cow (m = 1–2 569);
b^*dim – (covariate), regression coefficient b expressing the relationship between y and the days in milk;
e_{ijklm} – random residual error.

The effect of the DIM was expressed as a covariate for reducing the Sum Square of the Error in the abovementioned model. This results in a regression relationship between the continuous explanatory variable (DIM) and the dependent variable (y). For a better illustration of the changes in the milk components during lactation, we made an additional analysis as follows: the categorical phase effect of the lactation was created according to the DIM and tested using a one-way ANOVA (Table 2):

Table 1. Least square (LS) means of the calving season and parity

	LS means	n	DMY	FAT	PRT	LAC	logSCC	Urea	Casein	CIT	logKET	logFFA
Calving season	Dec–Feb	5 593	27.57^{a}	4.09^{a}	3.69^{a}	4.89^{a}	5.02^{a}	28.18^{a}	3.13^{b}	0.15^{a}	3.66^{ac}	5.30^{a}
	Mar–May	5 463	28.45^{b}	4.07^{a}	3.61^{b}	4.93^{b}	5.01^{a}	25.00^{b}	3.16^{ab}	0.16^{b}	3.71^{bd}	5.02^{b}
	Jun–Aug	5 781	25.64^{c}	4.15^{b}	3.68^{a}	4.90^{a}	5.09^{b}	20.62^{c}	3.18^{a}	0.14^{c}	3.62^{a}	4.94^{c}
	Sep–Nov	5 125	24.92^{d}	4.30^{c}	3.75^{c}	4.84^{c}	5.07^{b}	23.82^{d}	3.12^{b}	0.13^{d}	3.69^{cd}	5.08^{d}
Parity	1	6 463	19.94^{a}	4.19^{a}	3.68^{b}	4.94^{a}	5.02^{b}	24.49^{a}	3.19^{a}	0.16^{a}	3.66^{a}	5.15^{a}
	2	5 881	26.41^{b}	4.13^{b}	3.71^{a}	4.87^{b}	5.03^{b}	24.56^{a}	3.15^{b}	0.14^{c}	3.67^{a}	5.08^{b}
	3	4 405	29.79^{c}	4.13^{b}	3.67^{b}	$4..88^{b}$	5.03^{b}	24.33^{a}	3.12^{c}	0.14^{c}	3.67^{a}	5.04^{c}
	4 to 12	5 213	30.45^{d}	4.15^{ab}	3.68^{b}	4.87^{b}	5.10^{a}	24.24^{a}	3.12^{c}	0.15^{b}	3.67^{a}	5.07^{bc}

CIT = citric acid; DMY = daily milk yield; FAT = fat content; LAC = lactose; logFFA = free fatty acids (logarithm); logKET = ketone beta hydroxy butyrate (logarithm); logSCC = somatic cell count (logarithm); n = number of observations; PRT = protein content

[a–d]Means with different superscript are significantly different at the $P < 0.05$ within each effect

Table 2. Means for the different phases of lactation

Lactation phase	n	DMY	FAT	PRT	LAC	logSCC	Urea	Casein	CIT	logKET	logFFA
DIM 1–100	7 282	31.92^{a}	3.95^{a}	3.36^{a}	5.00^{a}	4.98^{a}	24.06^{a}	2.69^{a}	0.15^{a}	3.66^{a}	4.99^{a}
DIM 101–200	7 575	28.08^{b}	3.90^{b}	3.62^{b}	4.94^{b}	5.02^{b}	26.07^{b}	2.99^{b}	0.14^{b}	3.62^{b}	5.03^{b}
DIM 201–350	7 105	22.53^{c}	4.12^{c}	3.83^{c}	4.88^{c}	5.12^{c}	27.13^{c}	3.23^{c}	0.14^{c}	3.64^{c}	5.05^{b}

CIT = citric acid; DIM = days in milk; DMY = daily milk yield; FAT = fat content; LAC = lactose; logFFA = free fatty acids (logarithm); logKET = ketone beta hydroxy butyrate (logarithm); logSCC = somatic cell count (logarithm); n = number of observations; PRT = protein content

[a–c]Means with different superscript are significantly different at the $P < 0.05$ within each effect

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$$y_i = \mu + \text{phase}_i + e_i \quad (2)$$

where:
y_i – dependent variable;
μ – intercept;
phase_i – i^{th} effect of the lactation phase (i = 1–3, 1: DIM 1–100, 2: DIM 101–200, 3: DIM 201–350);
e_i – random residual error.

Estimate of the heritability coefficients of the milk components

The primary dataset included information about the sires of the cows. There were 198 sires (at least three observations per sire). An analysis of variance (ANOVA) was used to estimate the genetic and environmental variance of the milk components and it is used to calculate the heritability coefficients (Meier et al. 2021). For this purpose, the mean square among the sire half-sib groups (MSB) and the mean square within the sire half-sib groups (MSW) were read from the ANOVA Table Type I.

The ANOVA analysis was preceded by the adjustment of the dependent variables for the fixed environmental factors via the GLM model:

$$y_{ijkl} = \mu + \text{herd}_i + \text{cyear}_j + \text{seas}_k + \text{par}_l + b^*\text{dim} + e_{ijkl} \quad (3)$$

where:
y_{ijkl} – dependent variable;
μ – intercept;
herd_i – i^{th} herd effect (i = 1–3);
cyear_j – j^{th} effect of the calving year (j = 1–3);
seas_k – k^{th} effect of the calving season (k = 1–4, 1: December to February, 2: March to May, 3: June to August, 4: September to November);
par_l – l^{th} effect of the parity (l = 1–4, 1: first lactation, 2: second lactation, 3: third lactation, 4: 4$^{\text{th}}$ to 12$^{\text{th}}$ lactation);
b^*dim – (covariate), regression coefficient b expressing the relationship between y and the days in milk (DIM);
e_{ijkl} – random residual error.

The corrected dependent variables (adjy) from the GLM model were used to estimate the genetic and environmental variance using an ANOVA:

$$\text{adjy}_i = \mu + \text{sire}_i + e_i \quad (4)$$

where:
adjy_i – adjusted dependent variable;
μ – intercept;
sire_i – i^{th} effect of the sire of the cows (i = 1–198);
e_i – random residual error.

From the ANOVA sum of squares table, the MSB and MSW values were taken and used to calculate the coefficient of heritability (h^2):

$$h^2 = \frac{4(\text{MSB} - \text{MSW})}{\text{MSB} + (R - 1)\,\text{MSW}} \quad (5)$$

MSW corresponds to environmental variance (σ_e^2) and (MSB – MSW) corresponds to the genetic variance (σ_g^2). The coefficient R was calculated using the degree of freedom within the sire half-sib groups (DFW) and the degree of freedom among the sire half-sib groups (DFB), also both taken from the ANOVA table sum of squares: R = DFW/(DFB + 1). The estimation of the heritability coefficients was provided for all the milk components (Table 3).

RESULTS

Table 4 indicates the basic components of the analysed milk. The average daily milk yield was 27.56 kg of milk with a fat content of 3.99% and a protein content of 3.60%. The lactose content averaged 4.94%. The proportion of casein in the milk was 2.96%, which thus makes up the majority of the total protein in milk (more than 80%). Citric acid was present in the evaluated milk with an average value of 0.15%. The average number of somatic cells in the milk was 232.19/ml (median of 91.00 and mode of 61.00).

The average urea content (Table 4) was 25.75 mg/100 ml and the average BHB ketone content was 0.06 mmol/l. The free fatty acid content averaged to 2.08 mmol/100 g of fat. The lowest coefficients of variability were achieved for the lactose (4.72%), protein content (9.74%) and the logarithmic values of the SCC, FFA and BHB ketones. On the contrary, the number of somatic cells and the content of free fatty acids showed the highest variability. Medium variability was achieved in the daily milk yield (26.12%), urea content (28.82%) and citric acid content (22.88%).

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Table 3. Significance of the selected factors on the milk components and heritability coefficients of the milk components

Effect	Levels	DMY	FAT	PRT	LAC	logSCC	Urea	Casein	CIT	logKET	logFFA
Herd	3	**	**	**	**	**	**	**	**	n.s.	**
Calving year	3	**	**	**	**	**	**	**	**	**	**
Calving season	4	**	**	**	**	**	**	**	**	**	**
Parity	4	**	**	**	**	**	n.s.	**	**	n.s.	**
DIM × cow	2 569	**	**	**	**	**	**	**	**	**	**
R-square	–	0.7	0.27	0.67	0.61	0.41	0.33	0.62	0.43	0.25	0.46
RMSE	–	4.15	0.54	0.22	0.16	0.35	0.47	0.28	0.03	0.32	0.38
h^2	–	0.22	0.10	0.32	0.31	0.19	0.10	0.23	0.28	0.04	0.07

CIT = citric acid; DIM = days in milk; DMY = daily milk yield; FAT = fat content; LAC = lactose; logFFA = free fatty acids (logarithm); logKET = ketone beta hydroxy butyrate (logarithm); logSCC = somatic cell count (logarithm); n.s. = not significant effect; PRT = protein content; RMSE = root mean square error; *R*-square = coefficient of determination of the model

$^{**}P < 0.01$

Table 4. Descriptive statistics of the analysed milk samples

Variable	*n*	Mean	Min.	Max.	SD	SE	CV
Daily milk yield (kg)	21 962	27.56	3.30	55.80	7.199 0	0.048 6	26.12
Fat (%)	21 962	3.99	1.14	9.93	0.590 1	0.004 0	14.80
Protein (%)	21 962	3.60	2.46	7.78	0.350 7	0.002 4	9.74
Lactose (%)	21 962	4.94	2.96	5.54	0.233 3	0.001 6	4.72
Somatic cell count (SCC, 1 000/ml)	21 962	232.19	2.00	9 792.00	601.518 0	4.058 9	259.06
$\log_{10}$(SCC)	21 962	5.04	3.30	6.99	0.430 6	0.002 9	8.54
Urea (mg/100ml)	21 962	25.75	5.10	49.90	7.419 4	0.050 1	28.82
Casein (%)	14 218	2.96	1.67	5.63	0.416 8	0.003 5	14.06
Citric acid (%)	15 321	0.15	0.06	0.33	0.033 3	0.000 3	22.88
Ketone beta hydroxy butyrate (BHB, mmol/l)	12 042	0.06	0.01	0.49	0.044 4	0.000 4	77.52
$\log_{10}$(ketone BHB)	12 042	3.64	2.78	4.69	0.332 6	0.003 0	9.14
Free fatty acids (mmol/100 g of fat)	16 975	2.08	0.00	27.42	3.577 5	0.027 5	172.24
$\log_{10}$(free fatty acids)	16 975	5.02	2.48	6.44	0.472 0	0.003 6	9.39

CV = coefficient of variability (%); *n* = number of observations; SD = standard deviation; SE = standard error of the mean

The relationship between the individual components with a low frequency of analysis in the milk samples, expressed by the correlation coefficient, is given in Table 5. The highest correlation coefficient value is, as expected, reflected between the content of the total protein and casein (0.85). The casein content also has a moderate correlation with the daily milk yield (–0.47). The number of somatic cells showed the strongest correlation with the daily milk yield (–0.22), and shows almost no correlation with other indicators. The urea, citric acid, BHB ketone and free fatty acid content show low correlation coefficient values to all the observed components, including the daily intake. In the lactose, the strongest correlation coefficient was observed in relation to the number of somatic cells (–0.37). An important observation is the negative correlation coefficient observed between the lactose and the fat (–0.14) and protein content in the milk (–0.28). The correlation between the daily intake and the lactose content is 0.27. The correlation between the fat and the protein content in the milk was 0.32. The daily milk showed a moderately negative correlation with the protein content (–0.55) and the casein content in the milk (–0.47). The correlation coefficient values reflect a complex picture

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Table 5. Pearson's correlation coefficients reflecting the relationships between the milk components with a low frequency of analysis in the study

Trait	FAT	PRT	LAC	logSCC	Urea	Casein	CIT	logKET	logFFA
DMY	–0.231 1	–0.549 5	0.270 5	–0.215 3	–0.135 5	–0.474 3	0.127 2	–0.008 4	–0.080 6
FAT		0.324 2	–0.142 6	0.128 2	0.191 4	0.309 2	0.078 8	0.014 6	0.135 1
PRT			–0.281 6	0.182 7	0.163 3	0.852 0	–0.175 8	–0.108 7	0.172 8
LAC				–0.368 8	–0.016 0	–0.113 9	0.161 6	–0.146 3	–0.062 6
logSCC					–0.017 4	0.138 0	–0.103 6	0.105 1	–0.052 6
Urea						0.194 7	–0.015 0	–0.096 5	0.167 5
Casein							–0.140 8	–0.021 3	0.213 8
CIT								0.107 6	0.184 8
logKET									0.079 4

CIT = citric acid; DMY = daily milk yield; FAT = fat content; LAC = lactose; logFFA = free fatty acids (logarithm); logKET = ketone beta hydroxy butyrate (logarithm); logSCC = somatic cell count (logarithm); PRT = protein content

of interrelationships between the different milk components, suggesting very sensitive interactions.

Table 3 indicates the influence of the individual environmental factors and the heritability coefficients for the different milk components with a generally low frequency of analysis. The influence of the herd, the calving season, the year of calving, the order of lactation, the day of lactation and the effect of the dairy cow were evaluated. All the effects had a highly statistically significant effect ($P < 0.01$) on the evaluated milk components and the milk yield. The exception was the non-significant effect of the order of lactation on the urea and BHB ketone content in the milk. A non-significant effect of the herd on the BHB ketone content in the milk was also observed.

The heritability coefficients of the respective properties are also calculated and presented in Table 3. The heritability coefficients ranged from 0.04 for the BHB ketone content and 0.07 for the free fatty acid content to 0.32 for the total protein content. A similarly high value was achieved for the lactose content in the milk (0.31). The casein content showed lower values of heredity compared to the total protein content (0.23).

A lower heritability coefficient (Table 3) value was also recorded for the fat and urea content of the milk (both 0.10). In the case of the daily yield, a heritability coefficient of 0.22 was calculated and a slightly lower value was estimated for the number of somatic cells in the milk (0.19).

Table 1 shows the LS means for each level of the calving season effect and lactation order. Consistent with the results in Table 3, statistically significant differences ($P < 0.05$) were recorded for all the observed properties, except for parity in the case of BHB and urea ketones, where no statistically significant difference between the lactations was observed. The daily milk yield reached the highest value during the calving season in the period of March–May (28.45 kg of milk) and, conversely, the lowest milking was achieved by the cows calved in the period of September–November (24.92 kg of milk). This corresponds to the lowest percentage of fat and protein in the period of March–May (4.07% and 3.61%) and the highest in the period of September–November (4.30% and 3.75%).

On the contrary (Table 1), in terms of the lactose content, which shows the same trend as the daily milk yield, i.e., the highest lactose content in the milk was recorded with the cows calved in the period of March–May (4.93%) and the lowest in the period of September–November (4.84%). The highest number of somatic cells was observed in the summer (5.09) and autumn (5.07) periods. These numbers were statistically significantly different from the winter (5.02) and spring (5.01) periods. The average urea content was statistically significantly different in all the calving periods, with the highest recorded in the winter (28.18 mg/100 ml) and the lowest in the summer (20.62 mg/100 ml).

In contrast to the total protein content (Table 1), the casein content in the milk was highest in the summer (3.18%) and lowest in the autumn (3.12%). The citric acid content was very balanced in all the periods of calving, the highest was in the spring (0.16%) and the lowest was in the autumn (0.13%).

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The BHB ketone content in the milk was highest in the cows that calved in the spring (3.71) and lowest in the cows that calved in the summer (3.62). The free fatty acid content reached the highest values in the dairy cows calved in the winter (5.30), and conversely, in the summer, it was the lowest (4.94).

Regarding the effect of the lactation order (Table 1), a non-significant effect of the lactation order was observed for the urea and BHB ketone content in the milk. The average daily yield was statistically significantly different in all the observed lactations, the lowest was reached in the first lactation (19.94 kg), then in the second, where an average 26.41 kg of milk was reached, in the third lactation, 29.79 kg of milk was reached and, in the 4th group (4th to 12th lactation), the average daily yield was 30.45 kg of milk. The fat content was highest in the first lactation (4.19%), the second highest content was observed in the 4th and subsequent lactations (4.15%) and the lowest levels were measured equally in the second and third lactations (4.13%). The highest protein content was recorded in the second lactation (3.71%), in the other lactations, the protein content in the milk was very similar (3.67% to 3.68%).

In contrast, the casein content showed the highest values in the first lactation (3.19%), it decreased in the second lactation to 3.15%, and, in the 3rd, 4th and subsequent lactations, it was at a value of 3.12% (Table 1). The lactose content was the highest in the cows after the first calving (4.94%) and, furthermore, the values were again very balanced and ranged from 4.87% to 4.88%. The somatic cell count (logSCC) was almost unchanged in the first three lactations (5.02 to 5.03), while the last group (4th to 12th lactations) showed a significantly higher average somatic cell count, specifically 5.10. The citric acid content in the milk changed only slightly depending on the order of lactation, the highest average content was found in the first lactation (0.16%), then in the 4th to 12th lactation (0.15%), and, in the second and third lactation, the citric acid content was identically equal to 0.14%. The free fatty acid content was again the highest in the milk of the cows after the first calving (5.15), then the value of 5.08 was measured at the second lactation, the lowest FFA content was 5.04 seen in the third lactation and, in the last group, the FFA content was again slightly higher at 5.07.

Table 2 shows the average values of the daily milk yield and milk content depending on the lactation phase. Lactation was divided into the first hundred days in milk, the second hundred days and a section of 210 to 350 days of lactation (maximal DIM of the basic set). With the exception of the free fatty acid content, the averages within all the observed phases of the lactation significantly differed statistically. The free fatty acid content is almost identical in the second and third stage of lactation, while only showing significantly lower values in the first 100 days of lactation, the FFA content was significantly lower. The daily milk yield was the highest in the first hundred days of lactation (31.92 kg), then dropped to 28.08 kg and was lowest in the last stage of lactation (22.53 kg).

In the case of the fat, there was an increase from 3.95% to 4.12% in the final stage of lactation (Table 2). The protein increased from 3.36% in the first stage of lactation to 3.83% in the last stage of lactation. The casein content in the milk also gradually increased from 2.69% at the beginning of lactation to 3.23% in the last phase of lactation. In contrast, with an increasing lactation phase, the lactose decreased slightly from 5.00% to 4.94% in the second stage of lactation, to 4.88% in the last stage of lactation. The somatic cell count was the lowest in the first hundred days of lactation (4.98) and gradually increased to 5.12 in the third phase of lactation. Similarly, the urea content in the milk increased with the advancing lactation phase, starting at 24.06 mg/100 ml in the first lactation phase and ending at 27.13 mg/100 ml in the final lactation phase. The citric acid content in the milk did not change significantly in the cows directly after calving or later in the other stages of lactation.

During the first hundred days of lactation, the citric acid content reached 0.15% and, in the following stages of lactation, it was the same at 0.14%. Moreover, the BHB ketone content did not show any significant fluctuations, the highest BHB ketone content occurred in the first phase of lactation (3.66), then decreased to 3.62 in the second phase of lactation and increased again to 3.64 (Table 2) in the last phase of lactation.

DISCUSSION

Daily milk yield and main components

The average recorded daily milk yield corresponds to the current average milk yield record-

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ed for the Simmental breed (Stadnik et al. 2017). In 1 189 observed dairy cows of the Simmental breed, the average daily milk yield was 27.56 kg, i.e., the same value as in our research. In the case of the daily yield, Stadnik et al. (2017) observed a different increase in production. Their study showed an average daily milk yield of dairy cows increases until the 3rd lactation (from 22.83 kg in the first lactation to 29.62 kg in the third lactation) and then a decrease in production occurs. This study recorded a continuous increase in the average daily milk yield from 19.94 kg for the first lactation up to 30.45 kg in the fourth category (lactation group) where the 4th to 12th lactation merged.

Tozshizi (2016) found no significant differences in the estimated values of the maximum daily milk yield in his study using Wood's model. The lowest milk yield was in agreement with our study and was measured in the cows calved in the summer. However, the highest milk yield was reported by Torshizi (2016) for cows calved in the fall, which contradicts our results, where the highest daily milk yield was achieved by the dairy cows calved in the spring months. In our research, all the differences in the daily milk yield between the individual calving seasons were statistically significant.

Costa et al. (2018) reported the heritability of the milk yield in Simmental cows of 0.29, which is similar value to the heritability of 0.22 calculated in this study. Bobbo et al. (2020) reported a heritability coefficient value for the daily milk yield of only 0.06 for Holstein cattle in Italy.

Yoon et al. (2004) evaluated the amount of milk components in Holstein cattle according to the calving season and lactation order. For the fat and protein content, they reported the highest values in the autumn (3.78% fat and 3.14% protein) and the lowest in the spring (3.68% fat and 3.06% protein). This is in agreement with our results, where the highest fat (4.30%) and protein (3.75%) content were reached in the autumn calving season. The lowest values in our case, were also reached in the spring calving season (4.07% fat and 3.61% protein). This approach to the analysis of the heritability of less frequently analysed milk components is supported in the study of Acosta-Balcazar et al. 2022. Regarding the lactation order effect, Yoon et al. (2004) stated for both the fat and protein content, a continuous decrease in the percentage of the fat and protein content with an increasing lactation order. This is contrary to our results, where, in the case of fat content, we found the highest values in the first lactation and then, in the second lactation, the fat content remained at the same value, and, in the 4th and higher lactations, the values were even slightly higher than in the second and third lactations. The protein content even increased in the second lactation compared to the first lactation and then decreased to the same level as the first lactation. The mentioned differences are undoubtedly conditioned by the different breed, while Simmental cows, as a dual purpose breed, have a lower level of milk production and this is reflected in the higher stability of the milk components during the whole life of these dairy cows.

In the case of heritability, Bobbo et al. (2020) reported a heritability for the milk fat content at the level of 0.17, 0.27 for the protein and 0.21 for the lactose content. The level of heritability for protein and lactose was higher in our study, 0.32 for the protein content and 0.31 for the lactose content in milk. In contrast, our estimate of the heritability of the fat percent content is substantially lower than that reported by Bobbo et al. (2020), where it was only 0.10. On the other hand, Costa et al. (2018) reports much higher heritability values for these main milk components in the Simmental breed in Austria, namely 0.58 for the fat, 0.57 for the protein and 0.69 for the lactose content.

Findings on the components not frequently analysed

Bobbo et al. (2020), in their research, achieved an average casein content of 2.68% with a protein content of 3.43%, which represents approximately 78% of the casein from the total protein.

In a similar study comparing different breeds, Manuelian et al. (2019), reported similar observations on the influence of the parity, stage of lactation and breed as important sources of variation for these milk components not frequently analysed, including the fatty acid composition, casein and range of fatty acids.

Bobbo et al. (2020) reported a casein heritability of 0.28, which is a value slightly higher than that found by us in the Simmental breed. Duchemin et al. (2020) estimated a heritability coefficient for the total casein of 0.29 in Swedish Red cattle.

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Other authors have reported different heritability coefficients for the SCC, e.g., Bobbo et al. (2020) at 0.05, Poulsen et al. (2015) at 0.18 and Duchemin et al. (2020) at 0.17. The heritability coefficient of 0.19 found by us corresponds most closely to the studies of Duchemin and Poulsen.

Kubesova et al. (2009) reported that the highest achieved concentration of citric acid was found in the Holstein breed in the first five weeks of lactation, while its lowest content was diagnosed in the 9th week of lactation when the achieved value was 6 mmol/l. Garnsworthy et al. (2006) confirms the mentioned results of the author Kubesova et al. (2009) and states that the significantly higher proportion of citric acid in dairy cows is at the beginning of lactation than in the middle of lactation. Its value was 11.3 mmol/l at the beginning of lactation, while, in the middle of lactation, the CIT value was only 9.7 mmol/l. Duchacek et al. (2010), reported a CIT content of 13.16 mmol/l in the first three weeks of lactation, while, in the 16th week, the value was only 6.95 mmol/l. Khaled et al. (1999) state that the correlation of citric acid concerning the plasma glucose contained in the blood is positive, while the correlation is negative with the total plasma protein.

Duchemin et al. (2020) estimated the heritability for the citric acid content in the milk to be 0.64, which significantly exceeds our estimate value of 0.28. Also, Poulsen et al. (2015), in their study, found a much higher heritability coefficient for citric acid, specifically 0.82 in Jersey cattle and 0.59 in Holstein cattle.

The physiological range of urea is usually 18–35 mg/100 ml. Its content in milk is affected by the energy and protein intake, where excess energy usually reduces the urea content in milk, while the excess crude protein in the feed results in its higher content in milk. Another factor is the intake of dry matter and water when the lack of water in the body increases the urea levels. We can also mention dairy cows animal health status (especially kidney and liver function), grazing, when using it we observe an increased urea content or even the time of sampling concerning the interval between sampling and feeding (Jilek et al. 2006). Its amount in the body, therefore, changes considerably during the day.

Pedraza et al. (2006) reported statistically significant differences in the urea content in dairy cows in different calving seasons, specifically 33.24 mg/100 ml in spring, 30.76 mg/100 ml in summer, 29.86 mg/100 ml in autumn and 30.97 mg/100 ml in the winter season. The achieved values are, in all cases, higher than proven in our research, but their study used a different breed and different climatic conditions.

Mitchell et al. (2005) reported a heritability coefficient for the urea content in milk of 0.22–0.23, i.e., a value higher than that found by us. Poulsen et al. (2015) reported a heritability of 0.80 for Danish Jersey cattle and 0.29 for Danish Holstein cattle. Bobbo et al. (2020) estimated a heritability coefficient value for urea of 0.14, which is very close to our results.

Among the causes of higher content of free fatty acids, multiple milking, shorter intervals between milkings or poor quality of bulk feeds, and poorer milking hygiene can be mentioned. However, we can also include mechanical stress during milking, including milk storage after milking. This is mainly the transport of milk through the milk pipeline, careless cooling or pumping of milk, which destroys the fat component in the milk. The amount of FFA, as a mixture of detached fatty acids from fat in the milk employing lipolysis, is also affected by the season, nutrition, and health status of the animals (Hanus et al. 2011).

Garnsworthy et al. (2010) reported a heritability of various free fatty acids from 0.05 to 0.27. Our total group of free fatty acids reached the lower borders of the heritability coefficient, specifically 0.07. Bobbo et al. (2020) also reported a large range for different groups of free fatty acids, from 0.02 to 0.23.

The ketone content is affected by a metabolic disease – ketosis, which occurs mainly in high-production dairy cows (Stolcova et al. 2021). As mentioned in the introduction, subclinical ketosis can be determined by the amount of acetone in the milk ranging from 4 to 40 mg/l (Gustafsson and Emanuelson 1996). However, values for ketones higher than 15 mg/l and acetone higher than 10 mg/l may be more accurate. The acetone content is also influenced by the season or by bulk canned feeds (Hanus et al. 2011).

Ranaraja et al. (2018) found a heritability coefficient for BHB ketones of 0.19, i.e., much higher values than our value 0.04. Lee et al. (2016) observed values similar to our results in Holstein cattle, where the heritability coefficient ranged from 0.08 to 0.11. The review submitted by Zavadilova et al.

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(2021) on the health traits in dairy cattle breeding, largely supports these findings.

CONCLUSION

In conclusion, it can be stated that environmental factors, such as the breeder and the calving period, but also the cow's own internal factors, have an influence (can be used as indicators of) on the presence, composition and quantities of milk components that are generally less frequently analysed. This is also confirmed by the estimated heritability coefficients. Furthermore, it can be concluded that the order of lactation, i.e., the age of the animals, has an effect on those milk components, it often corresponds to the amount of produced milk and the overall metabolic development of the individual animal during lactation and with the age of the individual. Also, the lactation phase, and days in milk, have a highly significant effect on the content of the individual milk components in this evaluated breed. All the observed indicators show characteristics similar to those found in other cattle populations by other researches.

At the same time, the analyses of the broader content of the main milk components shows a noticeable stability depending on the order of lactation, which underlines the production stability and longevity of the dairy cows of this breed, whose milk production is at a high level, on average only 2 000 kg lower than that of the top dairy breed – Holstein dairy cows. The Simmental breed, therefore, offers a very reliable alternative for the economic production of milk, especially with regard to current trends in animal production, where the emphasis is placed on ecological and sustainable production systems. It, therefore, stands to reason that regular assessments of these less frequently analysed milk components can productively and accurately be used to collect, test and even verify information on the efficiency of current dairy management practices and their impact on milk production. In some cases, information from these less frequently analysed components can be effectively applied as typical early warning mechanisms for the close evaluation and timely adjustment of milk production management practices to optimise the efficiency of dairy production systems.

Based on the findings regarding the generally less frequently analysed components of milk, these outputs can be recommended for official publication and further analyses within the control of the performance of the Simmental breed in the Czech Republic. The monitoring and analysis of these generally less frequently analysed components of milk in the Czech Simmental breed will help to improve the herd management and the body of knowledge in the field of dairy cattle breeding.

For future research on the same theme for Simmental breeds, for the purpose of establishing more reliable economic indicators, genetic evaluations should be closely and more purposefully conceptualised for the analysis, e.g., the heritability of casein is 0.23, the citric acid content reached a heritability of 0.28. Here, however, it will become necessary to perform analyses using the best linear unbiased prediction and restricted maximum likelihood methods and the SNP analysis.

Conflict of interest

The authors declare no conflict of interest.

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