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# SEASONAL CORRELATIONS BETWEEN HEAT STABILITY AND OTHER RAW BULK COW MILK QUALITY INDICATORS

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## Abstract

Heat milk stability (thermostability, TES) is important technological feature which can contribute to create higher added value in the dairy industry. The aim of this paper was to evaluate the seasonal dynamics and relationships of TES to other milk qality indicators in the results of an exceptionally large data set of bulk samples without technological compositional modification and acidity adjustment, just with native raw cow milk. There were carried out 2,634 of TES measurements including other milk indicators during 3 years under controlled farm conditions. Results were processed by polyfactorial linear model of variance analysis and linear and nonlinear regression method. Correlation indexes of seasonal dependence of milk indicators such as fat content (F), crude protein content (CP), lactose monohydrate concentration (L), solids non-fat content (SNF), total solids (TS), urea concentration (U), F/CP ratio, F/L ratio, milk freezing point (MFP), somatic cell count (SCC), total count of mesophilic microorganisms (TCM) and coli-form bacteria count (COLI) were significant ($P < 0.05$ and $< 0.01$) including TES ($r = 0.869$; $P < 0.01$), with the exception of F/CP ($P > 0.05$). The seasonal dynamics of milk TES values corresponds positively with the trends of U and L. The negative seasonal trends are between milk TES and F, CP, SNF, TS, F/CP, F/L and MFP. Significant ($P \le 0.1$) negative seasonal correlations were between TES and F, CP, TS and F/L (–0.56, –0.55, –0.54 and –0.57). Significant ($P \le 0.05$) positive seasonal correlations were between TES and U and TCM (0.62 and 0.58). Insignificant ($P > 0.1$) negative seasonal correlations were found between TES and SNF, F/CP and MFP (–0.49, –0.29 and –0.16). Insignificant (P > 0.1) positive seasonal correlations were between TES and L, SCC and COLI (0.44, 0.42 and 0.43). Performed explanation of possible effects of chosen factors on raw cow milk TES can allow efficient selection of raw material for its processing by technological treatment under high temperature into relevant dairy products.

Keywords: dairy cow, Czech Fleckvieh, Holstein, environmental and technological factors, protein, urea, somatic cell count

## INTRODUCTION

The good heat milk stability (thermostability, TES) of the raw material is important for high quality of the durability of dairy products. TES determines the changes of the colloidal milk system during the heat treatment and thus also during the subsequent storage of the product (Štětina *et al.*, 2016). The TES may be impaired by a decrease in milk quality, for instance due to occurrence of production disorders such as mastitis (Feagan *et al.*, 1966). Most of the papers have determined the active acidity of milk pH (Miller and Sommer, 1940; Kailasapathy, 2008; Singh, 2004) as the main factor of its heat stability. However, the pH variability of native milk is multiply lower than the TES variability due to the natural buffering capacity of the milk (Hanuš *et al.*, 2019). Therefore, it is important to analyze the practical environmental and technological factors of TES variability of untreated original milk for the possibility of technological selection of raw material for specific dairy products. An important factor in raw milk quality indicators, including technological indicators such as TES, is the season (Godič-Torkar and Golc-Teger, 2008; Barłowska *et al.*, 2014; Chen *et al.*, 2015; Hanuš *et al.*, 2018, 2019). However, there are a number of other farm factors influencing milk quality, such as the conventional and organic farming system (Sharifi *et al.*, 2017; Chung *et al.*, 2018). TES can be influenced also by biological species differences for instance among cow, sheep, goat, camel or yak milk (Raynal-Ljutovac *et al.*, 2007; Metwalli *et al.*, 2013; Li *et al.*, 2014; Chramostová *et al.*, 2016). Another important factor of milk TES can be dairy breed (Barłowska *et al.*, 2014; Litwińczuk *et al.*, 2016).

In terms of previous text the milk TES, which is important technological feature (Huppertz, 2016), can contribute to create higher added value in the dairy industry. That is reason why it is important to know the sources of TES variability under practice conditions. Good raw milk TES is required in the production of durable (with long shelf live) products (condensed and sterilized UHT milk; Singh, 2004; Huppertz, 2016). In a wider interpretation, a number of technological indicators such as titration acidity, cheeseability or fermentationability are also part of the quality (Gajdůšek *et al.*, 1989). Also, these technological indicators are important for milk processing.

Probably for labor and time demanding of TES test the data sets for TES study are of lesser scale in tens of samples as maximum (Chramostová *et al.*, 2014, 2016; Peroutková *et al.*, 2016; Štětina *et al.*, 2016). Therefore the determination of raw milk TES is often replaced by less work demanding measurement and this is milk alcohol stability test which results can be positively correlated with TES. Sources of milk alcohol stability were analysed as well (Horne and Parker, 1980, 1981, 1982; Gajdůšek, 1989; Horne and Muir, 1990; Genčurová *et al.*, 1993; Horne, 2016). Therefore the database used here is exceptional for its number of measurements and its length of experimental period.

The aim of this work was to evaluate seasonal relationships of TES to other milk quality indicators and assess the practice (environmental and technological) sources of variability for milk indicators which can be immediately linked with TES on the results of an exceptionally wide data set of bulk milk samples without technological compositional modification and acidity adjustment, just on original milk with natural composition in East Bohemia.

## MATERIALS AND METHODS

### Cow herd conditions and bulk milk samples

Forty eight dairy cow herds (Czech Fleckvieh and Holstein breed, 35 and 10 and 3 (mixed) herds of both breeds) were included in this evaluation. These were milked twice a day and housed in free stables (35) and in binding cowsheds (13) under East Bohemia regional conditions. Dairy cow milking was performed by machine in the milking parlor (35) in free stables and into pipeline in binding cowsheads (13) including automatic milking system. The covered total number of animals was 8,928 (heads). 3,310 (n, all samples analysed including TES measurement absence) bulk milk samples (2,829 (n) for TES determination) were collected in two-weekly or monthly intervals during the 3-year experimental period. The grazing was applied in the summer feed season in some herds. Feeding of animals was mostly carried out in the form of total mixed ration (TMR) using a mobile feeding mixer. The frame, total, average composition of the feeding rations (these cows were fed with volume modifications according to lactation phase and milk yield) was supplemented with the consumption of forage cereal concentrates according to the feed tables for the current milk yield. The quality of the applied TMRs in the observation can be considered as moderate overall and especially with regard to the roughage feeding portion. Other environmental and technological figures about dairy cow herds were: - average number of animals in the herd, 186 ± 164 heads; - altitude of herd, 347.7 ± 68.8 m; - average of annual sum of rainfall, 554 ± 143 mm; - average raw milk delivery into dairy plant, 4,454 ± 4,095 kg; - mean milk yield per dairy cow and standard lactation, 6,728 ± 2,488 kg.

## Bulk milk sample analysis

The milk samples were preserved with bronopol (0.03%), transported under cold conditions (< 8 °C) to an accredited dairy laboratory (LRM Buštěhrad, ČMSCH a.s.) and then analyzed. Values for milk quality indicators were determined as follows: fat (F); crude protein (CP, total N × 6.38); lactose monohydrate (L); solids non-fat (SNF); urea (U); milk freezing point (MFP); somatic cell count (SCC); total count of mesophilic microorganisms (TCM); count of coli-form bacteria (COLI); milk thermostability (TES). In addition, energy (ketose) milk (cow) coefficients F/CP and F/L (Steen *et al.*, 1996; Siebert and Pallauf, 2010; van Knegsel *et al.*, 2010; Hanuš *et al.*, 2013; Manzenreiter *et al.*, 2013) were calculated. Milk analyzes were performed according to relevant methods with calibrated and controlled analytical techniques according to standard operating procedures. The milk components (F, CP, L, SNF, U) and properties (MFP) were determined by the indirect method of MIR-FT infrared spectroscopy (in mid range with interferometer and Fourier's transformation, in case of MFP with electrical conductivity measurement) using CombiFoss FT+ (Foss Electric, Hilleröd, Denmark). The SCC was determined by flow cytometry using the same device. TCM was also determined by flow cytometry using IBC FC (Bentley Instruments, Chaska, Minnesota, USA). The COLI count was determined by plate cultivation method (VRBL agar, 37 ± 1 °C, abbreviated cultivation period 24–48 hours). The TES was determined in minutes in non-preserved milk (Janštová and Navrátilová, 2014). The time was determined up to visual denaturation (flocculation) of milk proteins when heated in an oil bath at 135 °C. The procedure was carried out with 2.5 ml of milk in a relevant thick-walled glass tube in the Bohemilk Opočno laboratory. There are existing more technological analytical methods for milk TES measurement. Also shortened test method with smaller milk volume consumption was developed (Kasinos *et al.*, 2015) for milk heat stability determination, nevertheless, in this paper the classical mentioned procedure was used because of its better distinguishing ability towards heat coagulation of milk proteins (towards their denaturation).

## Data treatment and statistic evaluation of results

Data file was completed by records about dairy cow herds and their environmental and technological conditions. Because of some record absence the data file was reduced to the record complete form. The milk indicators (such as SCC, TCM, COLI, TES) with usual or confirmed absence of normal data frequency distribution (Ali and Shook, 1980; Reneau, 1986; Hanuš *et al.*, 2010) were logarithmized on a decimal basis ($\log_{10}$). After that also geometric means (xg) can be used in results evaluation in addition to arithmetic (x). The multifactorial analysis of variance was performed with data file in number reduced range and in complete form of matrices from mathematical point of wiev (n = 2,634 for TES). The SAS v. 9 programme package was used for the calculation. Means and GLM procedures were performed. This was carried out by linear model with fixed effects and with random effect in formula as follows:

$$Y_{ijklmnopqrst} = \mu + YE_i + S_j + M_k + A_l + W_m + NH_n + MY_o + B_p + TM_q + LS_r + SP_s + FA_t + e_{ijklmnopqrst},$$

where:

Y = investigated milk indicator; μ = general average; $YE_i$ = year effect for i from 1 to 3;

$S_j$ = season effect for j from 1 to 2 (1 = summer (from May to October); 2 = winter (from November to April)); $M_k$ = calendar month effect for k from 1 to 12; $A_l$ = altitude effect for l from 1 to 3 (1 = < 300; 2 = 300–450; 3 = > 450 m); $W_m$ = effect of total annual sum of rainfall for m from 1 to 3 (1 = < 450; 2 = 450–650; 3 = > 650 mm); $NH_n$ = the effect of number of dairy cows in the herd for n from 1 to 3 (1 = < 100; 2 = 100–400; 3 = > 400 of heads); $MY_o$ = the effect of milk yield level in milk recording for o from 1 to 3 (1 = < 6,000; 2 = 6,000–9,000; 3 = > 9,000 kg); $B_p$ = breed effect for p from 1 to 3 (1 = Czech Fleckvieh (CF); 2 = Holstein (H); various crossbreeds between CF and H); $TM_q$ = the effect of type of milking for q from 1 to 3 (1 = machine milking into can and pipeline; 2 = milking parlor; 3 = automatic milking system); $LS_r$ = the effect of litter type in the stable for r from 1 to 3 (1 = straw; 2 = rubber mattress; 3 = manure separation (liquid excrements)); $SP_s$ = the effect of summer pasture application, sometimes with green forage feeding next to silages in the mixture for s from 1 to 2 (with and without only by preserved roughage feeding rations (silages)); $FA_t$ = farm effect for t from 1 to 29; $e_{ijklmnopqrst}$ = random effect.

Various additional statistical calculations were performed with results of analysis of variance by method of linear (correlation coefficients and their significance for seasonal relations between TES and other milk indicators) and nonlinear (correlation indexes and their significance for relations of TES seasonal trends to other milk indicators) regression (using MS Excel, Microsoft, Redmond, Washington, USA).

## RESULTS AND DISCUSSION

### Seasonal relationships between TES and other milk indicators

General statistic results are shown in Tab. I. In general, there is showen good quality of raw cow milk in experimental data file. Variability explanation of included milk indicators through the linear model is shown also in Tab. I. The relevant value for the log TES was 41.1%. For all milk indicators the effectiveness of variability explanation was significant (P < 0.0001). The majority of the fixed effects had a significant effect on most of selected milk indicators (Tab. II). The geometric mean (xg) of TES was 18.8 minutes (Tab. I).

I: *Main statistic characteristics of raw cow milk indicators and explanation of linear model of variance analysis*

| IND | unit | n | x | xg | $s_d$ | vx (%) | $R^2$ (%) |
|---|---|---|---|---|---|---|---|
| **F** | % | 2,993 | 3.89 | - | 0.282 | 7.2 | 46.3 |
| **CP** | % | 3,015 | 3.4 | - | 0.128 | 3.8 | 56.0 |
| **L** | % | 3,015 | 4.89 | - | 0.08 | 1.6 | 54.8 |
| **SNF** | % | 3,015 | 8.89 | - | 0.144 | 1.6 | 59.5 |
| **TS** | % | 2,993 | 12.79 | - | 0.332 | 2.6 | 49.4 |
| **U** | $mg.100ml^{-1}$ | 1,804 | 24.5 | - | 5.0 | 20.4 | 43.5 |
| **F/CP** | - | 2,993 | 1.15 | - | 0.083 | 7.2 | 45.8 |
| **F/L** | - | 2,993 | 0.8 | - | 0.063 | 7.9 | 47.7 |
| **MFP** | °C | 3,015 | –0.526048 | - | 0.005521 | 1.0 | 23.7 |
| **log SCC** | - | 3,013 | 2.329102 | 213[a] | 0.188909 | - | 34.4 |
| **log TCM** | - | 3,069 | 1.486766 | 30.6[b] | 0.355881 | - | 30.2 |
| **log COLI** | - | 3,069 | 0.666409 | 4.6[c] | 0.749125 | - | 24.4 |
| **log TES** | - | 2,634 | 1.273654 | 18.8[d] | 0.144189 | - | 41.1 |

IND indicator; n sample number; x arithmetic mean; xg geometric mean; $s_d$ standard deviation; vx variation coefficient (%); $R^2$ determination coefficient of linear model of variance analysis (%); F fat content; CP crude protein content; L lactose monohydrate concentration; SNF solids non-fat content; TS total solids; U urea concentration; F/CP ketosis and energy balance milk coefficient fat/crude protein; F/L ketosis and energy balance milk coefficient fat/lactose; MFP milk freezing point; SCC somatic cell count, SCC $log_{10}$; TCM total count of mesophilic microorganisms, TCM $log_{10}$; COLI coli-form bacteria count, COLI $log_{10}$; TES raw cow bulk milk thermostability, TES $log_{10}$; [a] in $10^3.ml^{-1}$; [b] in $10^3CFU.ml^{-1}$ (CFU colony forming unit); [c] in $CFU.ml^{-1}$; [d] in minutes.

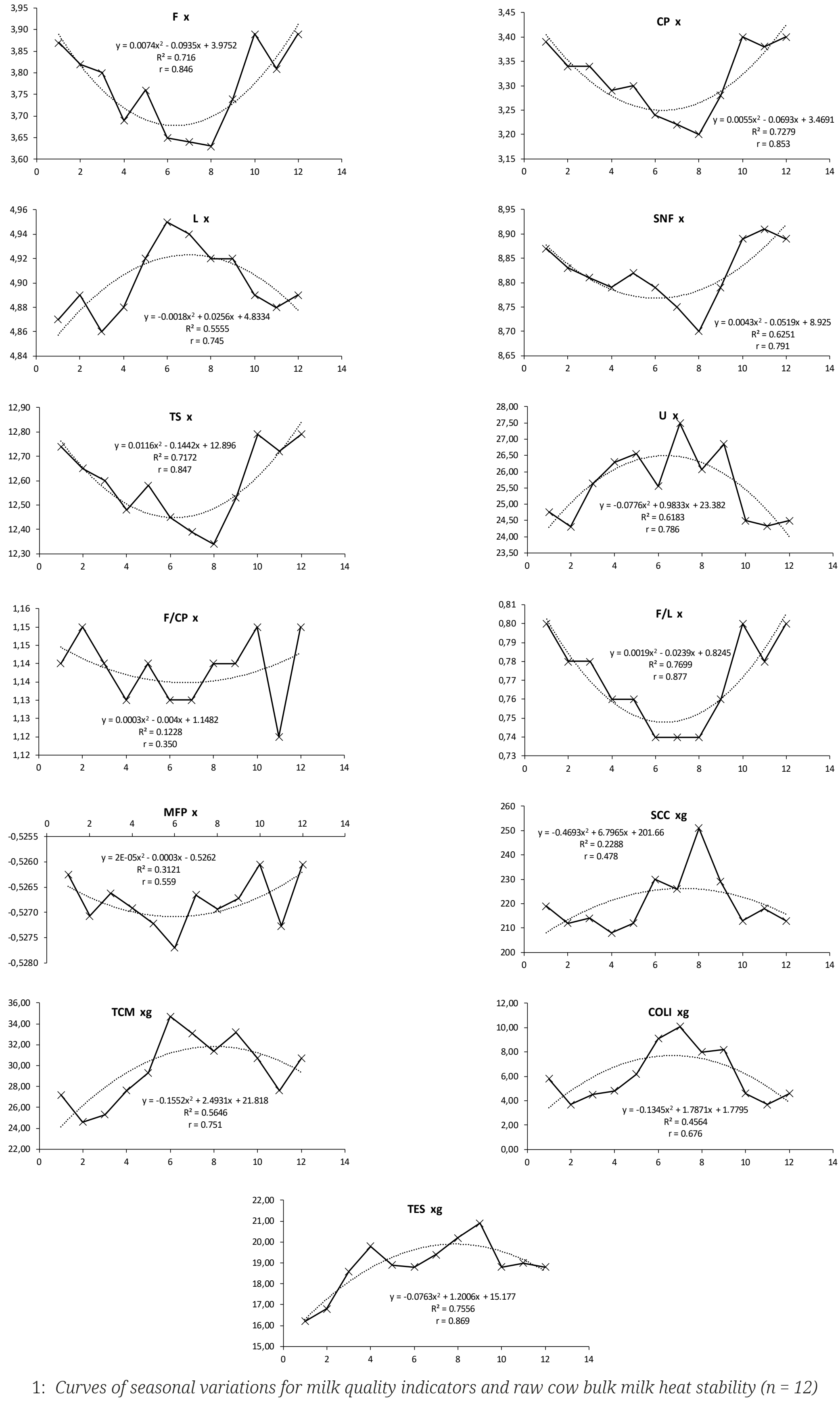
The average monthly values of all milk indicators show the characteristic seasonal dynamics expressed as a line of monthly means (x and xg) and the relevant nonlinear function (Fig. 1). Correlation indexes of functions expressing the seasonal dependence (x or xg) of milk indicators were significant ($P < 0.05$ and $< 0.01$) including TES ($r = 0.869$; $P < 0.01$), with the exception of F/CP ($P > 0.05$). The effect of the calendar month (M) on the primary measured values was also significant for all selected milk indicators (Tab. II) and especially for milk TES ($P < 0.0001$). Seasonal trends in microbiological raw milk quality indicators are largely in accordance with the findings by Godič-Torkar and Golc-Teger (2008). It is evident that the seasonal dynamics of milk TES values (xg; Fig. 1) corresponds positively with the trends of SCC, TCM, COLI (Fig. 1). However, this link is slightly paradoxical with regard to the general interpretation of milk quality. This phenomenon can be explained primarily by a known, parallel correspondence of seasonal temperatures with the hygienic milk values (SCC, TCM, COLI) in terms of fact that higher environmental temperatures are corresponding with higher (worse) hygienic values, as Godič-Torkar and Golc-Teger (2008) mentioned as well. A similar positive correspondence exists between TES trends and urea and lactose concentrations (Fig. 1) where a more realistic basis for this relationship can be expected. Chládek and Čejna (2005) did not capture the effect of urea on TES ($P > 0.05$) in individual milk samples similarly as Chen *et al.* (2015) did not find correlation between urea and TES in bulk milk samples. Nevertheless, van Boekel *et al.* (1989, cit. Čejna, 2006) noted such a pattern and it was explained by higher protein dissociation for higher urea contents, where casein molecules are more susceptible to flocculation. Chramostová *et al.* (2014) did not find a significant impact of basic milk indicators including urea on TES. The opposite seasonal trends, negative TES correspondence to other milk indicators, such as F, CP, SNF, TS, F/CP, F/L and MFP are shown in Fig. 1. These seasonal relationships of milk components to TES can logically be considered to be more substantial compared to the mentioned TES reciprocal correspondence to hygienic indicators (Fig. 1). Peroutková *et al.* (2016) recorded a worsening of milk TES when supplemented with certain types of milk proteins as a retentate, whey protein concentrate and milk protein concentrate.

The mentioned significant, positive and negative correspondence of seasonal dynamics between TES and other monitored milk indicators can also be documented by additional calculations of seasonal correlation (Tab. III and Fig. 2 and 3). In this mentioned sense it can be stated: - significant ($P \le 0.1$) negative seasonal correlations were found between TES and F, CP, TS and F/L (–0.56, –0.55 (Fig. 2), –0.54 and –0.57); - significant ($P \le 0.05$) positive seasonal correlations were found between TES and U and TCM (0.62 (Fig. 3) and 0.58); - insignificant ($P > 0.1$) negative seasonal correlations were found between TES and SNF, F/CP and MFP (–0.49, –0.29 and –0.16); - insignificant ($P > 0.1$) positive seasonal correlations were found between TES and L, SCC and COLI (0.44, 0.42 and 0.43). This can be interpreted in such a way that it is practically unlikely the deeper connection of improved technological milk quality (TES) with deteriorated hygienic values (SCC, TCM and COLI) in the summer months. Here a systematic overlapping of the period of higher summer environmental temperatures is likely playing the role with a slight deterioration in these indicators at a time when TES is better, as it has already been mentioned. On the other hand, there is more likely the deeper causal link between seasonal changes in the milk

II: *Significance of fixed effects regarding selected milk indicators according to analysis of variance*

| IND | YE | S | M | A | W | NH | MY | B | TM | LS | SP | FA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CP** | <.0001 | <.0001 | <.0001 | <.0001 | 0.0001 | <.0001 | <.0001 | <.0001 | 0.0023 | 0.8489 | 0.8016 | <.0001 |
| **SNF** | <.0001 | <.0001 | <.0001 | <.0001 | 0.2389 | <.0001 | <.0001 | <.0001 | 0.3563 | 0.8043 | 0.2625 | <.0001 |
| **U** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0003 | 0.0948 | <.0001 | <.0001 |
| **F/CP** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0004 | <.0001 | 0.2148 | <.0001 | <.0001 |
| **log TES** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0001 | 0.0033 | <.0001 |

The fffect of: YE year; S season; M calendar month; A altitude; W total annual sum of rainfall; NH number of dairy cows in the herd; MY milk yield level in milk recording; B breed; TM type of milking; LS litter type in the stable; SP summer pasture application, sometimes with green forage feeding next to silages in the mixture; FA farm; figure means probability of zero hypothesis according to F value; normal letters, statistic significant; italics letters, insignificant.




1: *Curves of seasonal variations for milk quality indicators and raw cow bulk milk heat stability (n = 12)*
x = arithmetic mean; xg = geometric mean; $R^2$ = determination coefficient of function;
r = correlation index.

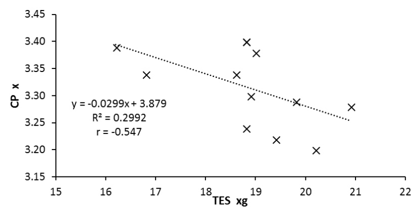
composition indicators, which means their decline in the summer period, and TES improvement.

Thus, although between TES and other milk quality indicators, especially component ones, have not usually been documented the significant relations in the assessment of individual measurements (primary data, Chramostová *et al.*, 2014; Hanuš *et al.*, 2018), in evaluating seasonal correlations between monthly mean values (derived characteristics) such relationships are already significantly manifested (Tab. III and Fig. 2 and 3). Their consideration may be relevant in methods of prediction of suitable locations (dairy herds) and selective collection of specific quality of raw material for processing into heat stressed dairy products with advantage in the form of better heat stability. In general, a higher cow milk yield is known in the summer period when better milk TES was also recorded. Fig. 1 (TES)

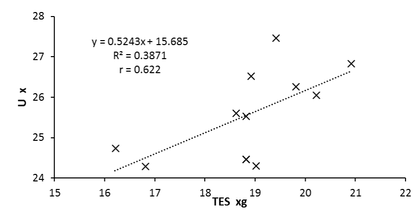
III: *Results of linear regressions with seasonal (for means of 12 calendar months) correlation coefficients between milk heat stability (TES; y axis) and other bulk raw cow milk indicators (x axis)*

| IND | linear equation | $R^2$ (%) | r | P | opposite linear equation (TES; x axis) |
|---|---|---|---|---|---|
| **F** | y = –7.5914x + 47.4379 | 31.65 | –0.5626 | * | y = –0.0417x + 4.5518 |
| **CP** | y = –10x + 52 | 29.92 | –0.547 | * | y = –0.0299x + 3.879 |
| **L** | y = 19.9625x–78.9829 | 19.10 | 0.437 | ns | y = 0.0096x + 4.7205 |
| **SNF** | y = –10.1643x + 108.4993 | 23.73 | –0.4871 | ns | y = –0.0233x + 9.26 |
| **TS** | y = –4.5676x + 76.3489 | 29.35 | –0.5418 | * | y = –0.0643x + 13.7996 |
| **U** | y = 0.7384x–0.0282 | 38.71 | 0.6222 | ** | y = 0.5243x + 15.6847 |
| **F/CP** | y = -40.3448x + 64.7759 | 8.48 | –0.2912 | ns | y = –0.0021x + 1.178 |
| **F/L** | y = -31.6667x + 43.2333 | 32.43 | –0.5695 | * | y = –0.0102x + 0.9631 |
| **MFP** | y = –399.2747x–191.4829 | 2.41 | –0.1552 | ns | y = –0.0001x–0.5257 |
| **log SCC** | y = 0.0455x + 8.8148 | 17.73 | 0.4211 | ns | y = 3.8949x + 146.9982 |
| **log TCM** | y = 0.235x + 11.8903 | 34.0 | 0.5831 | ** | y = 1.4469x + 2.3426 |
| **log COLI** | y = 0.2546x + 17.2946 | 18.66 | 0.432 | ns | y = 0.7329x–7.7065 |

$R^2$ determination coefficient; r = correlation coefficient; P probability of zero hypothesis: ns (no significant) P > 0.1; * (significant) P ≤ 0.1; ** P ≤ 0.05.




2: *Seasonal correlation as linear relationship of month means between crude protein content (CP; %) and milk heat stability (TES; minute; n = 12)*
r = correlation coefficient.




3: *Seasonal correlation as linear relationship of month means between urea concentration (U; mg.100ml⁻¹) and milk heat stability (TES; minute; n = 12)*

IV: *Raw cow milk indicators as CP, SNF, U, F/CP under influences of various environmental and technology farm factors according to variance analysis results*

| MI | | | CP | | SNF | | U | | F/CP | |
|---|---|---|---|---|---|---|---|---|---|---|
| FAFA | FAFAT | IFA | F criterion | t value/probability | F criterion | t value/probability | F criterion | t value/probability | F criterion | t value/probability |
| YE | i | 1 | 14.3 | 1.41/0.1593 | 223.9 | -9.19/< .0001 | 26.6 | -3.71/0.0002 | 33.0 | 1.68/0.0926 |
| | | 2–3 | | 3.64/0.0003 | | -3.71/0.0002 | | - | | –2.13/0.0334 |
| S | j | 1–2 | 558.8 | –1.37/0.1716 | 161.4 | -0.63/0.5279 | 43.8 | 0.52/06007 | 85.5 | –3.95/< .0001 |
| M | k | 1–12 | 60.1 | - | 41.2 | - | 4.7 | - | 3.97 | - |
| A | l | 1 | 47.7 | –8.32/< .0001 | 57.5 | –9.57/< .0001 | 35.3 | 4.83/< .0001 | 38.7 | –7.04/< .0001 |
| | | 2–3 | | –5.61/< .0001 | | –3.99/< .0001 | | 7.92/< .0001 | | –12.25/< .0001 |
| W | m | 1 | 9.21 | –3.17/0.0016 | 1.4 | –3.16/0.0016 | 55.7 | 6.57/< .0001 | 44.7 | 6.06/< .0001 |
| | | 2–3 | | –3.34/0.0008 | | –4.25/< .0001 | | 4.69/< .0001 | | 2.76/< .0001 |
| NH | n | 1 | 228.7 | 1.45/0.1479 | 314.2 | 2.12/0.0344 | 49.0 | –8.74/< .0001 | 418.1 | 3.1/0.0019 |
| | | 2–3 | | 3.65/0.0003 | | 2.46/0.014 | | –4.58/< .0001 | | 6.27/< .0001 |
| MY | o | 1 | 262.0 | –9.34/< .0001 | 350.9 | –11.79/< .0001 | 19.0 | 0.81/0.4162 | 40.0 | –4.32/< .0001 |
| | | 2–3 | | –3.56/0.0004 | | –5.48/< .0001 | | –3.27/0.0011 | | –5.73/< .0001 |
| B | p | 1 | 115.4 | 5.6/< .0001 | 115.4 | 10.15/< .0001 | 8.1 | –0.73/0.463 | 7.9 | –2.81/0.0049 |
| | | 2–3 | | –3.64/0.0003 | | –2.72/0.0066 | | –4.97//< .0001 | | –2.02/0.0439 |
| TM | q | 1 | 6.11 | 0.71/0.4792 | 1.0 | –0.49/0.6223 | 2.36 | 0.29/0.7685 | 9.9 | 9.88/< .0001 |
| | | 2–3 | | 2.96/0.0031 | | 5.16/< .0001 | | –0.64/0.5209 | | 5.86/< .0001 |
| LS | r | 1 | 0.2 | 0.67/0.527 | 0.2 | 0.15/0.8805 | - | - | 1.5 | –0.11/0.9089 |
| | | 2–3 | | 0.44/0.6592 | | 0.55/0.5792 | | - | | 0.13/0.8997 |
| SP | s | 1–2 | 0.1 | 7.81/< .0001 | 1.3 | 11.71/< .0001 | 45.0 | 1.51/0.1302 | 38.3 | –4.46/< .0001 |
| FA | t | 1–29 | 44.3 | - | 58.6 | - | 30.3 | - | 40.4 | - |

MI milk indicator; F criterion value as an influence power; FAFA = farm factor; FAFAT = farm factor type; IFA = identification of farm factor type; t = t criterion value; $YE_i$ = year effect for i from 1 to 3; $S_j$ = season effect for j from 1 to 2 (1 = summer (from May to October); 2 = winter (from November to April)); $M_k$ = calendar month effect for k from 1 to 12; $A_l$ = altitude effect for l from 1 to 3 (1 = < 300; 2 = 300–450; 3 = > 450 m); $W_m$ = effect of total annual sum of rainfall for m from 1 to 3 (1 = < 450; 2 = 450–650; 3 = > 650 mm); $NH_n$ = the effect of number of dairy cows in the herd for n from 1 to 3 (1 = < 100; 2 = 100–400; 3 = > 400 of heads); $MY_o$ = the effect of milk yield level in milk recording for o from 1 to 3 (1 = < 6,000; 2 = 6,000–9,000; 3 = > 9,000 kg); $B_p$ = breed effect for p from 1 to 3 (1 = Czech Fleckvieh (CF); 2 = Holstein (H); various hybrids between CF and H); $TM_q$ = the effect of type of milking for q from 1 to 3 (1 = machine milking into can and pipeline; 2 = milking parlor; 3 = automatic milking system); $LS_r$ = the effect of litter type in the stable for r from 1 to 3 (1 = straw; 2 = rubber mattress; 3 = manure separation (liquid excrements)); $SP_s$ = the effect of summer pasture application, sometimes with green forage feeding next to silages in the mixture for s from 1 to 2 (with and without only by preserved roughage feeding rations (silages)); $FA_t$ = farm effect for t from 1 to 29.

hypothetically suggests a slightly positive seasonal trend of milk TES to milk yield dynamics. Here, the monthly milk yield was not been recorded, however, this hypothetical consideration can be based on the demonstrable fact of the existence of a regular positive correlation between the monthly lactose content in milk (Fig. 1 (L)) and milk yield due to mastitis and lactation-osmotic reasons (Renner, 1972; Körner *et al.*, 1977; Bergmann, 1978, 1979; Schneeberger a Leuenberger, 1981; Famigli-Bergamini, 1987; Danuser, 1991; Hanuš *et al.*, 1992, 1993, 1994).

## Influence of farm factors on choosen milk indicators which are in relationship to milk TES

The effects of the farm factors on TES according to variance analysis evaluation have been already described in the previous papers (Hanuš *et al.*, 2018 and 2019). This work builds on the newly established relationships between the monthly means of bulk cow milk TES and some other milk indicators. This can refine the methods of TES estimation and prediction for bulk milk. Therefore, also the farm effects on the chosen milk indicators are shown as follows: CP; SNF; U; F/CP. The interesting results are then commented in more detail.

The year of the experimental period had a significant impact on all selected indicators (Tab. II (P < 0.0001) and IV; CP, SNF, U and F/CP). The values of the test criterion F of the variance analysis were 14.3, 223.9, 26.6 and 33.0. The SNF values were most strongly affected. The season impact was also significant for all indicators (Tab. II (P < 0.0001) and IV; CP, SNF, U and F/CP) at F criterion values 558.8, 161.4, 43.8 and 85,5 and the strongest impact on CP values. This was lower in summer (3.38%) as compared to winter (3.4%). Logically, the calendar month had a significant effect on all indicators (Tab. II (P < 0.0001) and IV; CP, SNF, U and F/CP) at F criterion values 60.1, 41.2, 4.7 and 4.0 and the strongest impact on CP values. The monthly trends of these chosen indicators can be deduced in Fig. 1, including a significant seasonal dependence of milk TES (this is in trend line with the results by Barłowska *et al.* (2014) in Polish Black and White Holstein Friesian breed). A significant influence of the altitude of dairy cow herd was also observed on all monitored milk indicators (Tab. II (P < 0.0001) and IV; CP, SNF, U and F/CP). The relevant F criterion values were 47.7, 57.5, 35.3 and 38.7. The power of influence was fairly balanced on all indicators. At the lowest altitude < 300 m the lowest CP values were found by 0.25%, as well as SNF by 0.32%, a higher U value by 7.23 mg.100ml$^{-1}$, and a lower F/CP value by 0.13. The sum of the annual water precipitation (W) for the monitored milk indicators was significant for CP, U and F/CP (Tab. II (P = 0.0001 and P < 0.0001) and IV). At the lowest W values < 450 mm the lowest CP values by 0.07% the highest U values by 7.1 mg.100ml$^{-1}$ and the highest F/CP values by 0.09 were recorded.

The herd size had a significant effect on all indicators (Tab. II (P < 0.0001) and IV; CP, SNF, U and F/CP). The F criterion values were 228.7, 314.2, 49.0 and 418.1 with the strongest impact on the F/CP values. The largest herds (> 400 of the heads) had the lowest CP values by 0.06 and 0.11% and the SNF by 0.1 and 0.08%, the highest U values by 18.8 and 6.9 mg.100ml$^{-1}$ and the lowest F/CP values by 0.09 and 0.12. Milk yield had a significant effect on all indicators (Tab. II (P < 0.0001) and IV; CP, SNF, U and F/CP). The F criterion values were 262.0, 350.9, 19.0 and 40.0 with the strongest effect on SNF values. The breed of dairy cows had a significant effect on all indicators (Tab. II (P < 0.0001 and = 0.0004) and IV; CP, SNF, U and F/CP). The F criterion values were 115.4, 115.4, 8.1 and 7.9 with the strongest effect on CP and SNF values. CF breed showed higher CP by 0.25%, SNF by 0.35%, U by 8.45 mg.100 ml$^{-1}$ (versus H breed). Results for CP, SNF and U are consistent with previous evaluations (Sojková *et al.*, 2010 a, b; Bendelja *et al.*, 2011).

A significant effect of the milking type was recorded in CP, U and F/CP (Tab. II (P = 0.0023, 0.0003 and < 0.0001) and IV) at F values of 6.1, 2.4 and 9.9. There was no significant impact of the litter type in the stable on the monitored indicators (Tab. II (P > 0.05) and IV). The significant effect of summer grazing and summer fresh green forage addition was recorded in U and F/CP (Tab. II (P < 0.0001) and IV). The F criterion values were 45.0 and 38.3 with a stronger influence on milk U. The grazing application showed higher U values by 2.3 mg.100ml$^{-1}$ and lower F/CP by 0.09. The finding for U is in line with the opinion by Trevaskis and Fulkerson (1999), Rajala-Schultz and Saville (2003) and Rzewuska and Strabel (2013). The significant farm impact (a combination of environmental conditions and technology) was recorded for all milk indicators (Tab. II (P < 0.0001) and IV; CP, SNF, U and F/CP) according to expectation. The values of the F test criterion of the variance analysis were fairly balanced 44.3, 58.6, 30.3 and 40.4 with the strongest effect on SNF values.

## CONCLUSION

Due to the marked stability (relatively low variability) of milk thermostability results on farms (Hanuš *et al.*, 2018) and their wide variability among farms there is possible to predict the raw material selection with advantageous thermostability value at milk collection for dairy plant according to historical farm values in collection area. Regarding results of this study there is necessary to derive these selection limits for thermostability mean value and its variability according to predictable seasonal changes. These factors are explained by this paper. At the same time, it has been shown that, unlike the original individual values of analytical measurements, at monthly averages there are significant relationships between the thermostability of raw bulk cow milk and a number of milk indicators such as fat content, crude protein content, content of total solids, urea concentration and fat/lactose ratio. This could help to improve the reliability of thermostability prediction and the estimation of raw material selection limits.

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