

## Evaluation of changes in milk composition in winter and summer period and its influence on rennet coagulation time of milk

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*Abstract:* Objective of this research was evaluation of changes in milk composition in winter and summer period and its influence on rennet coagulation time of milk. During the period of 24<sup>th</sup> January 2013 to 11<sup>th</sup> March 2013 (winter period - WP; n=47) and 30<sup>th</sup> June 2013 to 30<sup>th</sup> September 2013 (summer period - SP; n=47) were analysed bulk milk samples obtained in herd of Czech Fleckvieh cows from farm GenAGRO Říčany, a.s. (GPS 49°12'32.319"N, 16°23'42.666"E). Monitored parameters were: average diurnal temperature (ADT), content of fat, protein, lactose, Ca, Mg, Cl, somatic cell count, chloride-lactose ratio, titratable acidity, rennet coagulation time (RCT). Between WP and SP were found in ADT, RCT and content of Ca statistically very highly significant differences (P<0.001) and in content of Cl statistically significant difference (P<0.05). In the evaluation of effect of ADT and milk composition on RCT was in WP found statistically highly significant (P<0.01; r = -0.38) correlation relationship between RCT and titratable acidity. In SP was found statistically highly significant (P<0.01; r = 0.43) correlation relationship between RCT and ADT.

*Key-Words:* Milk composition, rennet coagulation time, season, average diurnal temperature

### Introduction

There is a great interest in changes in milk composition. The composition of milk varies with stage of lactation, feeding, health status or genetic factors [1]. No less important factors are climatic conditions [2]. Cows' thermoneutral zone is defined as air temperature range from 3 to 12°C. Heat stress starts already from temperature above 25°C [3]. Chládek [4] considers thermoneutral zone from 10 to 16°C. Heat stress has a negative effect on feed intake, leading to a reduction in milk yield [5]. On the contrary longer day in the summer feed intake increases [6]. Low temperatures in winter can also cause problems. Dairy cows tolerate low temperatures better than high, but extreme cold also affects them negatively [7]. Heck et al. [8] reported that the percentage of fat or protein was influenced by the seasonal variations. In winter is usually achieved the highest percentages of fat and protein, in the summer, these values are lower. Changes in lactose content during the year are usually small [9]. In the case of normally fed and reared cows salt content has certain regularity. When diseases of the mammary gland are detected content of calcium, potassium, magnesium, phosphorus and lactose decrease and content of sodium, chloride and

somatic cells count increase [10]. To compensate osmotic pressure in the mammary gland, because of low lactose content, there is an increased transfer of sodium chloride from blood to milk [11]. Koestler (1920) used the ratio of chloride ions and lactose to indicate normal or mastitis milk [12].

Due to changes in milk composition are affected its technological properties [13]. Milk coagulation properties are an important aspect in assessing cheese-making ability and are influenced by many factors. The most important factors are the content of casein, calcium and phosphorus, acidity of milk or health. An important factor is also temperature [14, 15].

The aim of this study was evaluation of changes in milk composition in winter and summer period and its influence on rennet coagulation time of milk. A part of the research was also evaluation of changes in titratable acidity and its effect on rennet coagulation time.

### Material and Methods

#### Characterization of the farm where the research was carried out and experimental design

During the period of 24<sup>th</sup> January 2013 to 11<sup>th</sup> March 2013 (winter period - WP; n=47) and 30<sup>th</sup> June 2013 to 30<sup>th</sup> September 2013 (summer period - SP; n=47) were analysed bulk milk samples obtained in herd of Czech Fleckvieh cows. Animals were kept on farm GenAGRO Říčany, a.s. with free stall barn (GPS 49°12'32.319"N, 16°23'42.666"E). Samples from WP were sampled daily, from SP were sampled every other day. Cows were fed total mixed ration *ad libitum* and were in various stage of lactation.

### Description of the method of measurement and laboratory methods

Analysis of milk samples were performed in the laboratory of Department of Animal Breeding at Mendel University in Brno. **Lactose (L)**, **fat (F)**, **protein (P)** content were measured on instrument Julie C5 Automatic (Scope Electric) working on the principle of thermo analysis. **Chloride (Cl)** content in milk was determined after the addition of nitric acid by titration argentometric. Chlorides were precipitated by excess silver nitrate solution and for reverse titration was used a solution of ammonium thiocyanate. For the determination of **calcium (Ca)** and **magnesium (Mg)** content was used complexometric titration with EDTA, 2Na. **Titrateable acidity (TA)** was detected by the methodology Soxhlet-Henkel titration of the sample with sodium hydroxide. **Rennet coagulation time (RCT)** was measured using nefelo-turbidic sensor of milk coagulation according to the methodology [16]. **Average diurnal temperature (ADT)** represents the arithmetic mean of the temperatures (detected all day before the date of sampling), measured every 15 minutes using 3 sensors (located in stable) with HOBO data logger (Onset Computer). **Somatic cell count (SCC)** was determined by apparatus NucleoCounter SCC-100 (Chemometec), which allows objective determination of somatic cells based on their automatic counting using a fluorescence microscope. **Chloride-lactose ratio (Cl/L)** number (x) was determined according to the formula:

a...chloride ions content (g.l<sup>-1</sup>)

b...lactose content in milk (g.100g<sup>-1</sup>)

$$x = \frac{a.100}{b.10}$$

For statistical analysis were used programs MS Office Excel 2010 and STATISTICA 10.

## Results and Discussion

### Evaluation of milk composition in winter and in summer period

Table 1 provides relationship between both monitored periods within measured properties of milk. The average diurnal temperature (ADT) in winter period (WP) was 0.58 °C in summer period (SP) was measured (ADT) 18.61 °C. As expected, between ADT in the monitored periods was found statistically very highly significant difference (P<0.001). Average calcium (Ca) content in WP was 1.04 g.l<sup>-1</sup> and in SP this value was 1.14 g.l<sup>-1</sup>. There was found statistically highly significant difference (P<0.001) between observed periods. Ozrenk and Inci [9] found higher content of salts in summer period too. On the contrary Lukášová and Smrčková [17] found higher content of Ca in WP than in SP, Moreno-Rojas et al. [18] found in their research highest contain of Ca in November and lowest in May. Gaucheron [19] states that Ca content stay during the season almost unchanged, great impact on its content have phase of lactation, illness or diet. Average rennet coagulation time (RCT) in WP was 207 sec. and in SP was 190 sec. Between the monitored periods was found statistically highly significant difference (P<0.001). This result corresponds with data published by Chládek et al. [20]. On the other hand Polák et al. [14] in their study published opposite results that lower value of RCT were found in the SP. Regarding the chloride content (Cl) in WP was detected 0.88 g.l<sup>-1</sup> and 0.91 g.l<sup>-1</sup> in SP. With regard to these parameters was detected statistically significant difference (P<0.05). Castillo et al. [21] observed lowest Cl content in January and February. Gaucheron [19] states that Cl content is during season constant.

Between WP and SP in content of fat (F), protein (P), lactose (L), magnesium (Mg), somatic cell count (SCC), chloride-lactose ratio (Cl/L) and titrateable acidity (TA) was not found statistically significant differences (P>0.05). Many authors observed statistically significant differences between content of F or P and season due to differences between temperature [20, 14, 22, 23, 24]. With statistically not significant differences between both monitored periods in TA agree Chládek et al. [20]. In case of SCC Rajčević et al. [25] found higher SCC in winter, on the contrary Heck et al. [8] came to the opposite result. To L content same authors state that during year is its content relatively constant. In normal bulk milk samples is Cl/L 1.7–2.2. Values greater than upper limit points to secretory disorders in the mammary gland [26]. McSweeney and Fox [12] present upper limit to 3.

Table 1 Average values of the measured parameters within the monitored periods

Parameter	Unit	Average values			Significancy
		Total	WP	SP	
n	-	94	47	47	-
ADT	°C	9.60	0.58	18.61	***
TA	°SH	6.50	6.52	6.47	N.S.
F	g.100g <sup>-1</sup>	3.84	3.87	3.81	N.S.
P	g.100g <sup>-1</sup>	3.20	3.18	3.21	N.S.
L	g.100g <sup>-1</sup>	4.78	4.75	4.8	N.S.
SCC	ths.ml <sup>-1</sup>	147	156	137	N.S.
Ca	g.l <sup>-1</sup>	1.09	1.04	1.14	***
Mg	g.l <sup>-1</sup>	0.27	0.16	0.37	N.S.
Cl	g.l <sup>-1</sup>	0.90	0.88	0.91	*
Cl/L	-	1.88	1.86	1.9	N.S.
RCT	sec.	199	207	190	***

N.S. = non-significant ( $P > 0.05$ ), \* =  $P < 0.05$ , \*\*\* =  $P < 0.001$

**Effect of ADT and milk composition on RCT**

Table 2 provides correlation relationships of measured parameters and RCT in the monitored

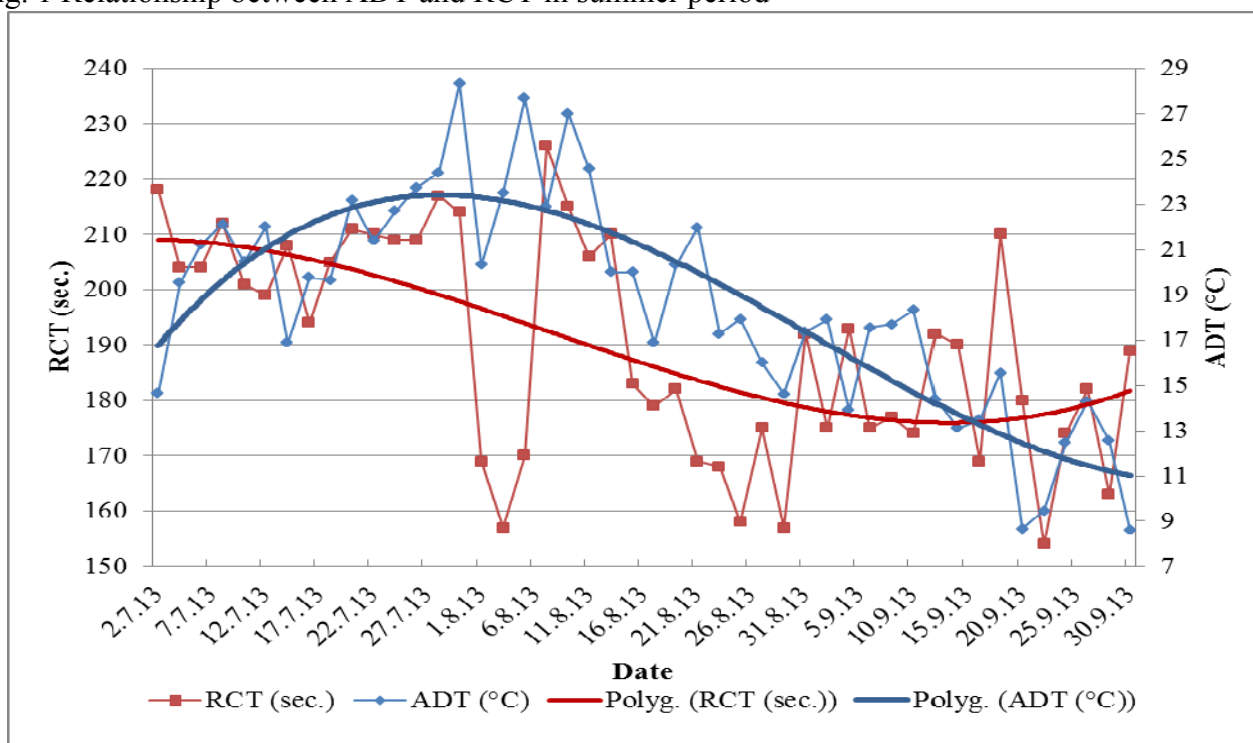
periods. In WP was not found statistically significant relationship between ADT and RCT ( $P > 0.05$ ;  $r = -0.15$ ).

Table 2 Correlation relationship of RCT and measured parameters within the monitored periods

period/parameter	ADT	TA	T	P	L	SCC	Ca	Mg	Cl	Cl/L
WP	-0.15	-0.38**	0.04	-0.21	-0.23	-0.30	-0.09	0.08	-0.05	0.10
SP	0.43**	-0.21	0.03	0.14	0.14	-0.06	-0.08	0.10	0.27	0.24

N.S. = non-significant ( $P > 0.05$ ), \*\* =  $P < 0.01$

Fig. 1 Relationship between ADT and RCT in summer period



In WP was not found statistically significant relationship between ADT and RCT ( $P > 0.05$ ;  $r = -0.15$ ). On the other hand, in SP (see Fig. 1) was found statistically highly significant relationship between this two measured parameters ( $P < 0.01$ ;  $r = 0.43$ ). Polák et al. [14] as well as Daviau et al. [27] found that the higher value of ADT means the shorter RCT. Our results in SP correspond with data published by Velecká et al. [28] who found similar relationship between ADT and RCT ( $P < 0.01$ ;  $r = 0.73$ ). In WP was found statistically significant relationship between TA and RCT ( $P < 0.01$ ;  $r = -0.38$ ) In SP was found similar trend but not statistically significant ( $P > 0.05$ ;  $r = -0.21$ ). Chládek and Čejna [29] found very similar correlation relationship to ours findings ( $r = -0.50$ ). Between RCT in both periods (WP and SP) within other measured parameters (F, P, L, SCC, Ca, Mg, Cl and Cl/L) was not found statistical significant relationship. In case of P ( $P > 0.05$ ;  $r = -0.21$ ) and L ( $P > 0.05$ ;  $r = -0.23$ ) in WP was found not statistically significant trend, that with increasing P and L in this period RCT decreased. A similar result reached Jůdu et al. [30]. On the other hand Daviau et al. [27] argue that the shorter RCT was in their research associated with a decrease in P content. Statistical not significant trend in WP was also found between SCC ( $P > 0.05$ ;  $r = -0.30$ ) and RCT which means that with increasing SCC RCT decreased. But Chládek and Čejna [29] found opposite result ( $r = 0.36$ ). In case of research carried out by Jůdu et al. [30]. was correlation relationship very weak ( $r = 0.02$ ). In SP was in relationship of RCT and Cl content also found statistically not significant trend ( $P > 0.05$ ;  $r = 0.27$ ).

## Conclusion

Aim of this paper was evaluation of changes in milk composition in winter and summer period and its influence on rennet coagulation time of milk. Regarding the comparison of the composition of milk in the monitored periods were determined statistically very highly significant differences ( $P < 0.001$ ) in calcium content and rennet coagulation time of milk. Logically, was also found statistically very highly significant difference ( $P < 0.001$ ) between temperature in winter and summer period. Between summer and winter period was also found statistically significant difference ( $P < 0.05$ ) in chloride content. In the case of determining the effect of the observed period (represented by average diurnal temperature in stable) and composition of milk on its rennet coagulation time was in summer period

found statistically significant effect ( $P < 0.01$ ) in case of temperature. In winter period was found statistically significant ( $P < 0.01$ ) relationship between rennet coagulation time and titratable acidity. We can assume about statistically not significant ( $P > 0.05$ ) trend that in the winter period there is a relationship between rennet coagulation time and milk protein, lactose, somatic cell count.

Our research proved fact that during the year there are significant and not significant changes in milk composition. These changes affect the technological properties of milk. An awareness of these changes is important for primary producers as well as processors.

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## References:

- [1] Fox PF, McSweeney PLH, *Dairy Chemistry and Biochemistry*, Blackie Academic and Professional, London, 1998.
- [2] Kubeková K, Obsah mléčných složek jako kritérium výživy a zdraví, (dle přednášky dr. Richardta), *Náš chov*, Profi Press s. r. o., Praha No.11, 2004, P26–P28.
- [3] Hanuš O, Vyletělová M, Genčurová, V, Jedelská R, Kopecký J, Nezval O, Hot stress of Holstein dairy cows as substantiv factor of milk composition, *Scientia Agriculturae Bohemica*, Vol.39, No.4, 2008, pp. 310–317.
- [4] Chládek G, Vliv chovatelského prostředí na kvalitu mléka, In KUCHTÍK, J. *Farmářská výroba sýrů a kysaných mléčných výrobků* (1<sup>st</sup> edition), Brno: MZLU v Brně, 2004, pp. 11–13.
- [5] Kudrna V et al., Tepelný stres a výživa, *Farmář*, Profi Press s.r.o., Praha, Vol.8, 2004, pp. 44–46.
- [6] Mudřík Z, Doležal P, Koukal P, *Základy moderní výživy skotu*, vědecká monografie zpracovaná v rámci řešení VZ MSM 6046030901 (1<sup>st</sup> edition), Česká zemědělská univerzita v Praze, 2006.
- [7] Hömberg D, Dojení v zimě: Jak lze zabránit poškozením z chladu?, *Náš chov*, Profi Press s. r. o., Praha, Vol.2, 2011, pp. 53–55.
- [8] Heck JML et al., Seasonal variation in the Dutch bovine raw milk composition, *Journal of Dairy science*, Vol.92, No.10, 2009, pp. 4745–4755.
- [9] Ozrenk E, Inci SS, The effect of seasonal variation on the composition of cow milk in



- Van Province. *Pakistan Journal of Nutrition*, Vol.7, No.1, 2008, pp. 161–164.
- [10] Gajdušek S, *Laktologie* (1<sup>st</sup> edition), MZLU Brno, 2003.
- [11] Harding F, *Milk quality* (1<sup>st</sup> edition), Wolters Kluwer Law & Business, 1996.
- [12] McSweeney PLH, Fox PF, *Advanced Dairy Chemistry. Volume 3: Lactose, Water, Salts and Minor Constituents* (3<sup>rd</sup> edition), Springer Science+Business Media XXIV, 2009.
- [13] Walstra P, Wouters, JTM, Geurts, T J), *Dairy science and technology* (2<sup>nd</sup> edition), Boca Raton: CRC/Taylor & Francis, New York, 2006.
- [14] Polák, O, Falta D, Hanuš O, Chládek G, Effect of barn airspace temperature on composition and technological parameters of bulk milk produced by dairy cows of Czech Fleckvieh and Holstein breeds. *Acta univ. agric. et silvic. Mendel. Brun.*, LIX, No.6, 2011, pp. 271–280.
- [15] Ikonen T, Morri S, Tyrisevä AM, Ruottinen O, Ojala M, Genetic and Phenotypic Correlations Between Milk Coagulation Properties, Milk Production Traits, Somatic Cell Count, Casein Content, and pH of Milk. *Journal of Dairy Science*, Vol.87, No.2, 2004, pp. 371–376.
- [16] Příbyla L, Čejna V, Porovnání vizuální a nefelo-turbidimetrické metody pro měření syřitelnosti mléka, In *Den mléka*, ČZU Praha, 2006, pp. 110–111.
- [17] Lukášová J, Smrčková A, Obsah vápníku v mléce a jeho význam, *Veterinářství*, Vol. 53, 2003, pp. 192–193. Available at: [http://www.vetweb.cz/informace-z-oboru/hygiena-technologie/Obsah-vapniku-v-mlece-a-jeho-vyznam\\_\\_s1496x50823.html](http://www.vetweb.cz/informace-z-oboru/hygiena-technologie/Obsah-vapniku-v-mlece-a-jeho-vyznam__s1496x50823.html)
- [18] Moreno-Rojas R, Zurera-Cosano G, Amaro-Lopez MA, Concentration and seasonal variation of calcium, magnesium, sodium and potassium in raw cow, ewe and goat milk. *International Journal of Food Sciences and Nutrition*, Vol.45, 1993, pp. 99-105.
- [19] Gaucheron F, The minerals of milk. *Reprod. Nutr. Dev.*, Vol.45, 2005, pp. 473–483.
- [20] Chládek G, Čejna V, Falta D, Máchal L, Effect of season and herd on rennet coagulation time and other parameters of milk technological quality in Holstein dairy cows, *Acta universitatis agriculturae et silviculturae Mendeliana Brunensis*, Vol.59, No.5, 2011, pp. 113–118.
- [21] Castillo LS, Trimberger GW, Herrington BL, Henderson CR, Turk KL, Factors Affecting Chloride Content and Relationship of Chloride, Protein, and Solids-Not-Fat in Guernsey Milk, *Journal of Dairy Science*, Vol.44, No.10, 1961, pp. 1961–1965.
- [22] Drevjany, Kozel, Padrůněk, *Holštýnský svět* (1<sup>st</sup> edition), Zea Sedmihorky, s. r. o., Zemědělským týdeník, 2004.
- [23] Doležal O, Abramson S, Výživa a krmení při eliminaci tepelného stresu (1.), *Náš chov*, Profi Press s. r. o., Praha, Vol.8, 2009, pp. 26–28.
- [24] Rusek A, Problémy s mléčnými složkami u dojnic – obsah tuku, *Náš chov*, Profi Press s. r. o., Praha, Vol.5, 2006, pp. 40–41.
- [25] Rajčević M, Potočník K, Levstek J, Correlations Between Somatic Cells Count and Milk Composition with Regard to the Season, *Agriculturae Conspectus Scientificus*, Vol.68, No.3, 2003, pp. 221–226.
- [26] Šustová K, *Laktologie (návody do cvičení)*, 2005, In press
- [27] Daviau C, Famelart, MH, Pierre A, Gouedranche H, Maubois JL, Rennet coagulation of skim milk and curd drainage: Effect of pH, casein concentration, ionic strength and heat treatment. *Lait*, Vol.80, No.4, 2000, pp. 397–415
- [28] Velecká, M, Javorová J, Falta D, Večeřa M, Andrýsek J, Chládek G, Change of stable microclimate during the spring and summer as a criterion of welfare and its effect on milk yield, composition and qualitative indicators as holstein dairy cows, *Animal Welfare, Ethology and Housing Systems*, Vol.9, No.3, 2013, pp. 47–52.
- [29] Chládek G, Čejna V, The effect of lactation phase on coagulation time and titratable acidity in milk of Czech Fleckvieh cows, In *III. mezinárodní seminář-aktuální problémy ve šlechtění kombinovaného skotu*, Svaz chovatelů českého strakatého skotu, 2004, pp. 23–25.
- [30] Jõudu I, Henno M, Kaart T, Pussa T, Kara O, The effect of milk protein on the rennet coagulation properties of milk from individual dairy cows. *International Dairy Journal*, Vol.18, No.9, 2008, pp. 964–967.