# THE RELATIONS OF SHEEP'S AND COW'S FREEZING POINT OF MILK TO ITS COMPOSITION AND PROPERTIES*

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Keeping of small ruminants increases in many countries. More information about small ruminant milk properties are needed. Aim of this work was to investigate the sheep and cow milk composition, properties and milk freezing point (MFP) as an important physical and technological milk indicator (MI) and to evaluate their mutual relationships. Data sets of sheep (Tsigai /C/, $n = 60$) and cows (Czech Fleckvieh cattle /B/, $n = 93$) bulk milk samples (BMSs) were investigated. BMSs (4 – 8 animals in sample) originated from the first two thirds of the lactation and the winter and summer season. Sheep milk showed the lower MFP value (–0.6048 °C; C) than cow milk (–0.5221 °C, B). It is caused by specific physiological differences between species. Mentioned differences influenced also the contents of some components: fat (7.58% C vs. 3.40% B, $P < 0.001$); urea (63.6 mg.100 mL$^{-1}$ C vs. 40.4 mg.100 mL$^{-1}$ B, $P < 0.001$); calcium (1915 mg.L$^{-1}$ C vs. 1172 mg.L$^{-1}$ B, $P < 0.001$) etc. Lactose content as a main source of MFP depression in cow milk is in opposite constellation 4.44% C vs. 5.06% of monohydrate B ($P < 0.001$). Some significant relations (correlation coefficients or indexes from linear or non linear regressions) were observed between sheep MFP and: lactose (0.395, $P < 0.01$, C vs. –0.355, $P < 0.01$, B, which is quite unusual); solids non fat (–0.670, $P < 0.001$, C vs. –0.324, $P < 0.01$, B); titration acidity (–0.491, $P < 0.001$, C vs. –0.329, $P < 0.01$, B); pH (0.926, $P < 0.001$, C vs. –0.455, $P < 0.001$, B) etc. More significant correlations about freezing point were found in sheep than in cow milk.

ruminant; sheep; cow; milk composition; milk freezing point; milk properties

## INTRODUCTION

Freezing point is an important polyfactorial physical and technological milk indicator. It is used for control of milk foodstuff chain quality (Buchberger, 1994; Košta, 2003). More papers were carried out about measurement principles of MFP (Koops et al., 1989; Bauch et al., 1993; Buchberger, Klostermeyer, 1995). The main effect on cow MFP could be a foreign water addition.

Possible influence of the automatic milking system (AMS) on MFP deterioration has been published recently (Rasmussen, Bjerring, 2005). The MFPs were stabilized after improvement of the AMS. The frequency of MFPs above –0.516 °C was 23% in the first year with AMS and declined to 2.2% in the last year. Nevertheless, more factors exist besides foreign drinking watter addition, which can influence the cow MFP (Freeman, Bucy, 1967; Eisses, Zee, 1980; Buchberger, 1990a, b, 1991, 1994, 1997; Wiedemann et al., 1993). In general it can be farm impacts such as cow herd, breed of dairy cows, herd milk yield, year season, pasture, nutrition and feeding of dairy cows and their health state in terms of production disorder occurrence, too.

It is very important to differ between the mentioned impacts and real foreign water addition in terms of objective milk quality determination for milk payment purposes and milk foodstuff chain quality control in the right way. However, it is not always clear under the practical conditions. There are other technological negative impacts on freezing point of pasteurized milk during its processing like drinking water addition and protein heat stress (Rohm et al., 1991; Roubal et al., 2004; Hanuš et al., 2006; Janštová et al., 2007). In fact all cow milk deliveries into dairy plants for processing contain certain degree of foreign water in dairy developed countries in terms of machine milking existence and its impacts. Beside incidental addition of foreign water or some compounds with effects on coligative properties into milk the MFP is influenced by milk yield, nutrition, season, lactation stage and animal health state, of course (Hanuš et al., 2003).

As a result of numerous political and economical changes the sheep livestock population was expressively reduced in the CR during last 20 years (429 714 head in 1990 vs. 84 108 head in 2000). Not until last period (2006) the certain increase of livestock population to 148 412 head was noticed (Bucek et al., 2004; Holá, 2006).

There are not so much information about milk freezing point and its relationships to milk composition and properties in the small ruminants in the Czech Republic. That is probably a general state. Some facts about small ruminant milk (in sheep) studied Hanuš et al. (2005a, b) including MFP. Sheep MFP (breed Tsigai) was lower (better) by 13.4%, 24.1% and 21.3% as compared to goat (breed White short-haired) and cow (Czech Fleckvieh cattle and Holstein) MFPs.

Consequently, because of reason of the another features in keeping of all ruminants, such as changeovers in

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reproduction indicators, longevity (length of production life), technological equipment of dairy cow keeping, frequency of free stable and milking parlour technology, the aim of this paper was to analyse and compare the relationships of freezing point to milk components and properties in cows and sheep.

## MATERIAL AND METHODS

The Czech Republic inheres in temperate earth-zone in middle Europe. Climate conditions are various and they are influenced by the elevation above sea-level (mean is 450 m) and for instance grasslands are situated in the Jeseníky region. One of the main agriculture investigation activities in the Research Institute for Cattle Breeding (RICB) is cattle keeping in the less favourable areas (LFAs).

Bulk milk samples (/BMSs/ one sample from 4 to 8 animals) were collected in three dairy cow herds with Czech Fleckvieh cattle (B; *n* = 93 BMSs) and one sheep herd (C; Tsigai breed; *n* = 60 BMSs). The animals were sampled during spring and summer seasons for three years (2005, 2006 and 2007). The studied ruminant herds were kept in altitudes from 360 to 475 (B) and 572 (C) m above see level with total precipitation 700 (B) and 1 200 (C) mm and mean air temperature 7.0 and 3.7 °C, it means under typical climate conditions in the CR. There were observed three cow herds (Bohemian Spotted cattle; B) and one sheep herd (Tsigai; C). BMSs were analyzed in the RICB in the accredited testing laboratory according to relevant operation manuals. Cow results were used as reference to milk results of small ruminants, namely sheep, under the identical environmental and technological conditions.

The nutritions of both animal species were carried out in typical ways for the CR conditions. The cow herds were fed by the total mixed ration (TMR), it is maize silage and red clover and alfalfa silage with mineral and concentrate supplements. The sheep herd was fed by the natural grass and herb pasture and by the grain supplement with daily ration 0.3 kg for individuum (that was mixture of wheat,

Table 1. Survey of some important MIs and basic statistic results for cow (B) and sheep (C), textual discussed

| Milk indicator | Unit | Breed | Statistical characteristics | | | | |
|---|---|---|---|---|---|---|---|
| | | | $x$ | $s_d$ | $v_x$ | minimum | maximum |
| MFP | °C | B | –0.5221 | 0.0043 | 0.8 | –0.5335 | –0.5099 |
| | | C | –0.6048 | 0.0685 | 11.3 | –0.7843 | –0.4645 |
| F | % | B | 3.40 | 0.471 | 13.9 | 2.42 | 4.70 |
| | | C | 7.58 | 1.876 | 24.7 | 1.28 | 11.44 |
| L | % | B | 5.06 | 0.116 | 2.3 | 4.82 | 5.30 |
| | | C | 4.44 | 0.377 | 8.5 | 3.75 | 5.16 |
| SNF | % | B | 8.95 | 0.207 | 2.3 | 8.55 | 9.52 |
| | | C | 11.40 | 0.543 | 4.8 | 9.33 | 12.73 |
| U | mg.100 $mL^{-1}$ | B | 26.73 | 5.618 | 21.0 | 17.85 | 44.76 |
| | | C | 63.55 | 10.085 | 15.9 | 43.67 | 83.78 |
| A | $mg.L^{-1}$ | B | 6.10 | 3.981 | 65.3 | 0.19 | 26.49 |
| | | C | 11.10 | 7.574 | 68.2 | 0.82 | 34.41 |
| CA | $mmol.L^{-1}$ | B | 8.35 | 1.330 | 15.9 | 3.96 | 10.63 |
| | | C | 6.77 | 1.463 | 21.6 | 2.49 | 10.22 |
| TA | ml 0.25 $mol.L^{-1}$ NaOH x 100 $mL^{-1}$ | B | 8.19 | 0.587 | 7.2 | 6.79 | 9.54 |
| | | C | 12.26 | 3.300 | 26.9 | 6.91 | 20.51 |
| pH | a.u. | B | 6.68 | 0.070 | 1.0 | 6.48 | 6.82 |
| | | C | 6.50 | 0.402 | 6.2 | 5.31 | 7.02 |
| CP | % | B | 3.33 | 0.214 | 6.4 | 2.99 | 3.89 |
| | | C | 6.32 | 0.542 | 8.6 | 4.67 | 7.47 |
| CAS | % | B | 2.66 | 0.087 | 3.3 | 2.47 | 2.85 |
| | | C | 4.96 | 0.370 | 7.5 | 3.93 | 5.81 |
| TP | % | B | 3.17 | 0.118 | 3.7 | 2.91 | 3.41 |
| | | C | 5.91 | 0.555 | 9.4 | 4.44 | 7.08 |
| Ca | $mg.kg^{-1}$ | B | 1300 | 190.2 | 14.6 | 906 | 2107 |
| | | C | 1915 | 294.5 | 15.4 | 980 | 2287 |
| P | $mg.kg^{-1}$ | B | 1017 | 89.0 | 8.8 | 757 | 1232 |
| | | C | 1597 | 127.2 | 8.0 | 938 | 1800 |

$x$ = arithmetical mean, $s_d$ = standard deviation, $v_x$ = coefficient of variation

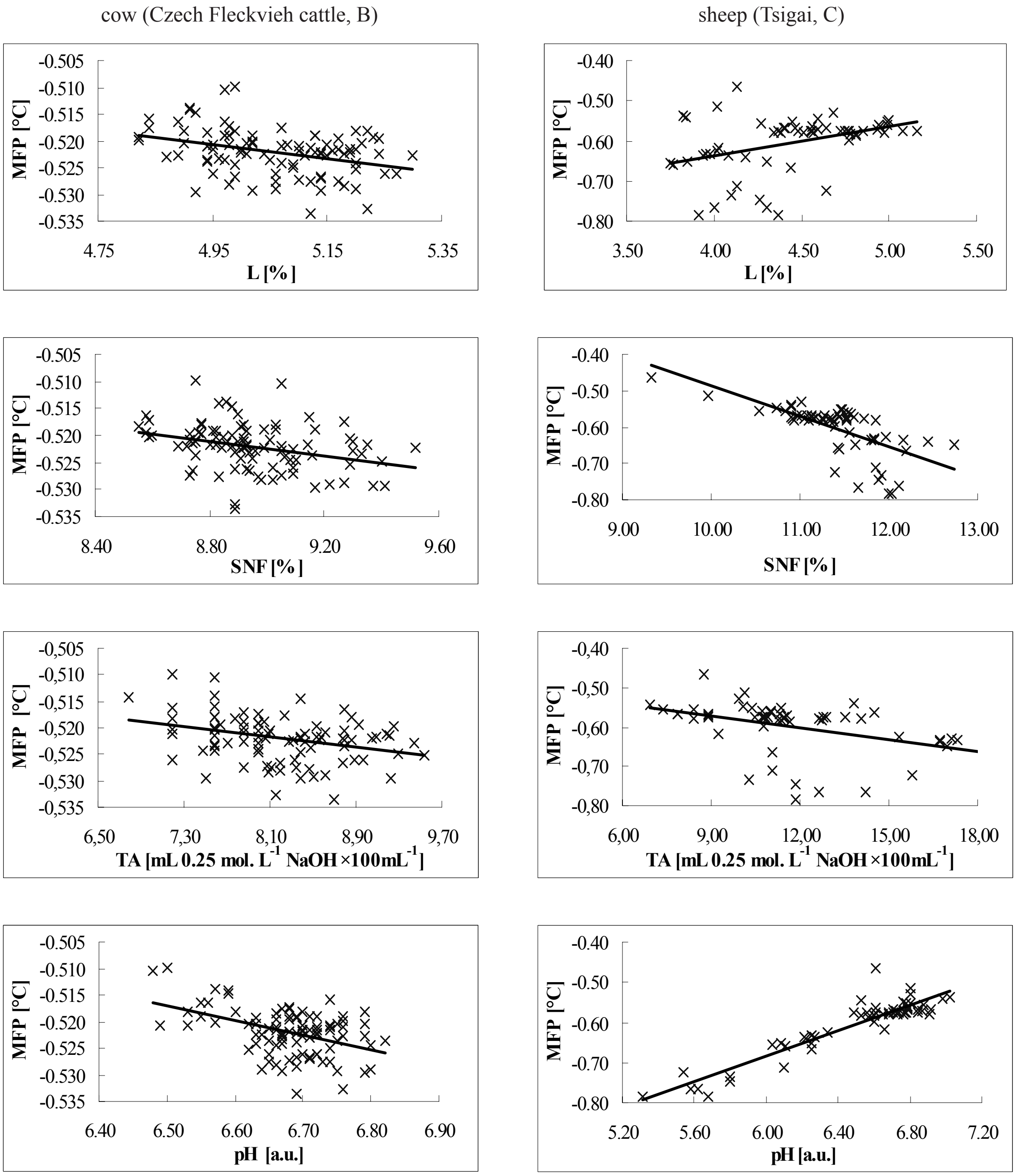


Fig. 1. The graphic chart of the relations between MFP and the other important quality MIs in Czech Fleckvieh cattle (B) as compared to Tsigai (C) sheep

maize, barley and rape seed-oil and mineral components). All animals included into sampling were in their first two thirds of the lactation and they were milked twice a day by machine equipment in milking parlour. The daily milk yields were typical for studied and compared species and breeds of animals, 20.04 kg for B and 0.36 kg for C under mentioned climatic and technological conditions. 37 milk quality indicators (MIs) were measured and calculated for each of BMSs.

The following listed abbreviations of MIs were used and thereinafter commentated: F = fat (g.100g$^{-1}$ ≈ %); L = lactose (monohydrate, g.100g$^{-1}$ ≈ %); SNF = solids non fat (g.100g$^{-1}$ ≈ %); U = urea concentration (mg.100 mL$^{-1}$); A = acetone concentration (mg.L$^{-1}$); CA = citric acid concentration (mmol.L$^{-1}$); MFP = milk freezing point (°C); TA = titration acidity (mL 0.25mol.L$^{-1}$ NaOH x 100 mL$^{-1}$); pH = active acidity (a.u.); CP = crude protein content (Kjeldahl, total N×6.38; g.100 g$^{-1}$ ≈ %); CAS = casein content (Kjeldahl, casein N×6.38, g.100 g$^{-1}$ ≈ %); TP = true protein content (Kjeldahl, protein N×6.38, g.100 g$^{-1}$ ≈ %); macroelements = Ca and P (mg.kg$^{-1}$).

The milk samples were analysed on the MFP values by the top cryoscopic instrument Cry-Star automatic Funke-Gerber (Germany). The selected measurement mood was Plateau Search (with parameters: interval = 23 sec. and Δt = 0.4m °C). The instrument was under regular calibration by the standard NaCl solutions (Funke-Gerber) and took part in the national proficiency testing with successful results regularly. The other investigated milk indicators such as the L and SNF were measured by the instrument MilkoScan 133B (Foss Electric, Denmark), which was regularly calibrated according to the reference method results. F was measured by Gerber's method. The nitrogen protein fractions such as CP, TP and CAS were determined by the reference Kjeldahl's method via the instrument line Tecator with Kjeltec Auto Destillation unit 2200 (Foss-Tecator AB, Sweden). The milk U concentration was determined by the spectrophotometric methode at the 420 nm of the wavelength. The specific reaction solution was prepared as sour mixture with the p-dimethylaminobenzaldehyde. The Specol 11 instrument (Carl Zeiss Jena, Germany) was calibrated by the six samples in the scale with the increased U concentrations from 1 to 10 mmol.L$^{-1}$. The milk A content was investigated by the spectrophotometric measurement at 485 nm of the wavelength. The A was adsorbed into alcali solution of KCl with the salicylaldehyde due to 24 hours' microdiffusion in the special vessels (at 20 °C in the darkness). The Specol 11 was calibrated by the five points on the scale with the increased A concentrations from 1 to 20 mg.L$^{-1}$. The milk CA concentration was determined by the spectrophotometric measurement at 428 nm of the wavelength. Milk was coagulated by the trichloracetic acid and after it the adventitious filtrate reacted with the pyridin and acetanhydride (30 min at 32 °C). The CA generates with the pyridin a yellow-coloured complex in acetanhydride medium. The Specol 11 was calibrated by seven points of the concentrations from 1.5 to 20.0 mmol.L$^{-1}$, it means from 0.03 up to 0.36%. The pH was measured by the pH-meter CyberScan 510 (Eutech Instrumets, the Netherlands) at 20 °C. The mentioned instrument was regularly calibrated by the standard buffer solutions (pH 4.0 and 7.0 Hamilton Duracal Buffer, Switzerland) at the each milk sample set measurement. The TA was measured with the milk titration (100 mL) by alcaline solution up to the light pink colour of the mixture. The macro- and microelement milk contents (except P) were investigated (after mineralization) by the atom absorption spectroscopy via the equipment Spectrometer Solaar S4 (Thermoelemental, England). The P content was determined as a molybdenum-blue (with ammonium, ascorbic and sulfuric acid). The Specol 11 was calibrated by the five points on the scale with increased P concentration from 2 to 20 mg.L$^{-1}$ (at 750 nm).

The processing of the results included the calculation of basic statistical parameters, regression analysis and correlation coefficients by Excel programme.

## RESULTS AND DISCUSSION

The main statistical characteristics of investigated milk components and properties for introduced data sets are shown in Table 1. The table offers the comparison of both breeds of species (B vs. C). Practically, we take notice of substantial differences of all listed values for individual MIs, namely MFP (–0.5221 °C vs. –0.6048 °C), F (3.40% vs. 7.58%), SNF (8.95% vs. 11.40%), U (26.73 mg.100 mL$^{-1}$ vs. 63.55 mg.100 mL$^{-1}$), CP (3.33% vs. 6.32%), CAS (2.66% vs. 4.96%), Ca (1172 mg.kg$^{-1}$ vs. 1915 mg.kg$^{-1}$), P (1017 mg.kg$^{-1}$ vs. 1597 mg.kg$^{-1}$) and so on. It is evident that all of selected characteristic differences are statistically significant. The principal reason is the interspecific distinctness, so the physiological and morphological singularity. Regression equation, the coefficient of determi-

Table 2. Summary of the interesting relations between MFP and the other MIs for cow (B) and sheep (C), regression characteristics included

| Relationship between MFP and | Breed | Regression analysis | | | |
|---|---|---|---|---|---|
| | | equation | coefficient of determination | coefficient of correlation | significance |
| F | C | $y = -14.323x - 1.0804$ | 0.2740 | –0.5235 | *** |
| L | B | $y = -9.64x + 0.0239$ | 0.1262 | –0.3552 | ** |
| | C | $y = 2.1723x + 5.7579$ | 0.1561 | 0.3951 | ** |
| SNF | B | $y = -15.671x + 0.7728$ | 0.1050 | –0.3240 | ** |
| | C | $y = -5.3086x + 8.1902$ | 0.4495 | –0.6704 | *** |
| U | C | $y = -75.148x + 18.103$ | 0.2609 | –0.5108 | *** |
| A | C | $y = -30.967x - 7.6307$ | 0.0785 | –0.2802 | * |
| TA | B | $y = -44.973x - 15.285$ | 0.1081 | –0.3288 | ** |
| | C | $y = -23.652x - 2.0456$ | 0.2414 | –0.4913 | *** |
| PH | B | $y = -7.4725x + 2.7791$ | 0.2066 | –0.4545 | *** |
| | C | $y = 5.4252x + 9.778$ | 0.8579 | 0.9262 | *** |
| CP | C | $y = -6.4548x + 2.4186$ | 0.6667 | –0.8165 | *** |
| CAS | C | $y = -4.4642x + 2.2582$ | 0.6842 | –0.8272 | *** |
| TP | C | $y = -6.3699x + 2.0557$ | 0.6184 | –0.7864 | *** |
| Ca | C | $y = 1901.5x + 3064.5$ | 0.1959 | 0.4426 | ** |
| P | B | $y = -4943.4x - 1563.8$ | 0.0567 | –0.2381 | * |
| | C | $y = -579{,}22x + 1246{,}4$ | 0.0975 | –0.3122 | * |

*, ** and *** = statistical significance $P < 0.05$, $< 0.01$ and $< 0.001$, ns = $P > 0.05$

nation, the coefficient of correlation and its significance are included. It is possible to observe the statistical significant relations between MFP and: L ($r$ = 0.395, $P$ < 0.01, C vs. $r$ = –0.355, $P$ < 0.01, B); SNF ($r$ = –0.670, $P$ < 0.001, C vs. $r$ = –0.324, $P$ < 0.01, B); TA ($r$ = –0.491, $P$ < 0.001, C vs. $r$ = –0.329, $P$ < 0.01, B); pH ($r$ = 0.926, $P$ < 0.001, C vs. $r$ = –0.455, $P$ < 0.001, B); P ($r$ = –0.312, $P$ < 0.05, C vs. $r$ = –0.238, $P$ < 0.05, B) for sheep and cows, simultaneously. It quite unusual that the MFP x L and MFP x pH relations for C have the opposite trend (the MFP increases with L or pH values) than for B. The mentioned relations are demonstrated in Fig. 1. The other relations (MFP x F, MFP x U, MFP x A, MFP x CP, MFP x CAS, MFP x TP and MFP x Ca) are significant for C (Table 2), but non-significant for B. Perhaps, these differences in relationships between MFP and last mentioned MIs could explain the opposite trend about relationship MFP x L in sheep and cows. MFP was better with increase of L in cows and worse in sheep (Table 2, Fig. 1). More authors (Demott, 1969; Brouwer, 1981; Walstra, Jenness, 1984; Hanuš et al., 2003) reported that lactose content causes 53.8% of the MFP depression in cows. Further, in declining approx. order, $K^+$ 12.7%, $Cl^-$ 10.5%, $Na^+$ 7.2%, citrates 4.3%, urea 1.9% and other components 6.9%. Then lower (better) average MFP in sheep (Table 1) should be caused by other negative relationships between MFP and other sheep milk components such as P, but especially protein fractions, CP or TP and CAS. 68.4% and 66.7% of variations in MFP could be caused by the variations in casein and crude protein contents. It is evident that MFP depression in sheep milk is created due to principles and relationships of MFP to other milk components, which are somewhat different as compared to these in cow milk (Freeman, Bucy, 1967; Demott, 1969; Eisses, Zee, 1980; Brouwer, 1981; Walstra, Jenness, 1984; Buchberger, 1990a, b, 1991, 1994, 1997; Wiedemann et al., 1993; Hanuš et al., 2003). This phenomenon that sheep MFP worsened (elevated) with increasing lactose content in opposite trend to cow milk (Table 2; Fig. 1) could be explainable by this fact that there was higher geometric mean of milk somatic cell count (SCC) in sheep as compared to cows (560 > 159 $10^3$ $ml^{-1}$). Higher SCC as indicator of secretion disorders could reduce lactose content and consequently elevate $Na^+$ and $Cl^-$ ion concentrations, which could improve MFP. The correlation coefficient between sheep log SCC and MFP also was –0.61 ($P$ < 0.001) and between $Na^+$ concentration and MFP – 0.16 ($P$ > 0.05).

## CONCLUSIONS

There were found some relevant relations between milk freezing point and the other investigated parameters, such as fat, lactose, solid non fat, pH etc. in ruminant milk samples. In general, the sheep data set features better significance of relations than the cow one. Simultaneously were observed the expressive interspecific differences in mean values of MIs and in their relationships between MFP and other MIs as well (cows vs. sheep). Probably it could be possible to derive the more effective rules for better monitoring and prevention against milk quality problems in small ruminants by obtained results.

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**Vztahy bodu mrznutí ovčího a kravského mléka k jeho složení a vlastnostem.**

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Vzhledem k tomu, že chov malých přežvýkavců vzrůstá v mnoha zemích, je potřeba více informací o vlastnostech mléka malých přežvýkavců. Cílem této práce bylo vyšetřit složení a vlastnosti ovčího a kravského mléka a bod mrznutí mléka (MFP) jako významný fyzikální a technologický ukazatel mléka (MI) a vyhodnotit jejich vzájemné vztahy. Byly vyšetřeny datové soubory bazénových vzorků mléka (BMSs) ovcí (cigája /C/, $n = 60$) a krav (český strakatý skot /B/, $n = 93$). BMSs (4–8 zvířat ve vzorku) pocházely z prvních dvou třetin laktace a zimní a letní sezony. Ovčí mléko vykázalo nižší hodnotu MFP (–0,6048 °C; C) než mléko kravské (–0,5221 °C, B). To je zapříčiněné specifickými mezidruhovými fyziologickými rozdíly. Tyto rozdíly ovlivnily také obsahy některých složek: tuk (7,58 % C vs. 3,40 % B, $P < 0{,}001$); močovina (63,6 mg.100 mL$^{-1}$ C vs. 40,4 mg.100 mL$^{-1}$ B, $P < 0{,}001$); vápník (1915 mg.L$^{-1}$ C vs. 1172 mg.L$^{-1}$ B, $P < 0{,}001$) atd. Obsah laktózy jako hlavní zdroj deprese MFP v kravském mléce je v opačné konstelaci – 4,44 % C vs. 5,06 % monohydrátu B ($P < 0{,}001$). Byly pozorovány některé významné vztahy (korelační koeficienty nebo indexy z lineárních nebo nelineárních regresí) mezi ovčím MFP a: laktózou (0,395, $P < 0{,}01$, C vs. –0,355, $P < 0{,}01$, B, což je poněkud neobvyklé); sušinou tukuprostou (–0,670, $P < 0{,}001$, C vs. –0,324, $P < 0{,}01$, B); titrační kyselostí (–0,491, $P < 0{,}001$, C vs. –0,329, $P < 0{,}01$, B); pH (0,926, $P < 0{,}001$, C vs. –0,455, $P < 0{,}001$, B) atd. U bodu mrznutí ovčího mléka bylo nalezeno více významných korelací než u kravského mléka.

přežvýkavec; ovce; kráva; složení mléka; bod mrznutí mléka; vlastnosti mléka

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