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ANALYSE OF RELATIONSHIPS BETWEEN FREEZING POINT AND SELECTED INDICATORS OF UDDER HEALTH STATE AMONG COW, GOAT AND SHEEP MILK

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Abstract

HANUŠ, O., GENČUROVÁ, V., KUČERA, J., VYLETĚLOVÁ, M., TŘINÁCTÝ, J.: *Analyse of relationships between freezing point and selected indicators of udder health state among cow, goat and sheep milk*. Acta univ. agric. et silvic. Mendel. Brun., 2009, LVII, No. 5, pp. 103–110

Milk freezing point (MFP) is important quality indicator. Aim was to analyse the relationships of MFP to selected udder health milk indicators (MIs) by comparison between cows (reference), goats and sheep. Bulk milk samples came from 3 herds of Czech Fleckvieh (B, n 93) and 1 goat herd and sheep flock (White short-haired, W, n 60; Tsigai, C, n 60). Animal nutrition was performed under the typical country conditions. MIs which were investigated: DM, dry matter; SNF, solid non fat; L, lactose (all in %); SCC, somatic cell count (10^3 ml^{-1}); EC, electrical conductivity (mS cm^{-1}); MFP (°C); Na and K (in mg kg^{-1}). W MFP was –0.5544 ± 0.0293, B –0.5221 ± 0.0043 and C –0.6048 ± 0.0691 °C. The B MFP was related to L (–0.36; $P < 0.01$), W was not related to L (–0.07; $P > 0.05$) and C was related to L (0.40; $P < 0.01$). These facts could be explainable by worse SCC geometric averages for used W (3,646 10^3 ml^{-1}) and C (560 10^3 ml^{-1}) milk as compared to B (159 10^3 ml^{-1}). Only 0.5 and 10.5% of variations in MFP were explainable by variations in DM and SNF in B, 32.7 and 12.8% in W but already 49.4 and 45.0% in C. Higher C values were caused by high MFP variability, 11.8% (C) versus 0.8% (B). There is possible to derive the more reliable MFP qualitative limits for more efficient monitoring rules of milk quality problems in B, W and C.

ruminant, raw milk, milk freezing point, udder health state, lactose, somatic cell count, electrical conductivity, natrium

Raw milk quality is very important for food chain safety. That is reason why mentioned problem was studied in terms of different point of views by row of papers during last time (Vyletělová et al., 1999, 2000, 2001; Cempírková and Thér, 2000; Hanuš et al., 2004 a; Cempírková, 2001, 2002, 2007). Milk freezing point (MFP) is an important polyfactorial physical and technological indicator. This is used for control of milk food chain quality (Buchberger, 1994; Kološta, 2003). More papers were carried out about MFP measurement principles (Koops et al., 1989; Bauch et al., 1993; Buchberger and Klostermeyer, 1995). More authors (Demott 1969; Brouwer, 1981; Walstra and Jenness, 1984; Hanuš et al., 2003 b) reported that lactose content causes 53.8% of the MFP depression in cows. Further the MFP is determined in declining order by K^+ 12.7%, Cl^- 10.5%, Na^+ 7.2%, citrates 4.3%, urea 1.9% and other components such as fat and protein 6.9%. The main practical effect on cow MFP deterioration could be a foreign water addition. Possible influence of the first automatic milking system (AMS) on MFP deterioration was also recently published (Rasmussen and Bjerring, 2005). The MFPs were stabilized after technical improvement of the AMS. The frequency of MFPs above –0.516 °C was 23% in the first year with use of AMS and declined to 2.2% in the last year. Nevertheless, besides foreign drinking watter addition more factors exist, which can influence the cow MFP (Freeman and Bucy 1967; Eisses and Zee, 1980; Buchberger, 1990 a, b, 1991, 1994, 1997; Wiedemann et al., 1993). In general there can be farm impacts such as dairy cow herd, breed, herd milk yield, year season, pasture, nutrition and feeding of dairy cows, their lactation stage and their state of health in terms of production disorder occurrence (mastitis, ketosis, acidosis, alkalosis), too (Hanuš et al., 2003 b). Also animal species is an essential factor. Therefore, it is very important to differ in the right way between the mentioned impacts and real foreign water addition in terms of objective milk quality determination for purposes of milk payment and milk foodstuff chain quality control. However, it is not always clear under the practical conditions. There are other technological negative impacts on the freezing point of pasteurized milk during its processing like drinking water addition and protein heat stress (Rohm et al., 1991; Roubal et al., 2004; Janštová et al., 2007). All cow milk deliveries for processing into dairy plants contain foreign water to a certain degree in dairy developed countries in terms of machine milking existence.

Sheep and goat farming is returning back into the Czech Republic (CR) because of confirmed positive effects of alternative milk consumption on human health. The importance of raw milk quality control is still growing not only in cows but also in goats and sheep (Antunac et al., 2001; Hejtmánková et al., 2002; Kuchtík and Sedláčková, 2003; Paape et al., 2007; Raynal-Ljutovac et al., 2007). Therefore the aim of this paper was to analyse the relationships of freezing point to milk components or properties which are closely linked with health state of mammary gland by interspecific comparison between cow, goat and sheep bulk milk.

MATERIAL AND METHODS

Breed, herd, animals and milk samples

The details of experimental ruminant keeping were described also in our corresponding works (Hanuš et al., 2005, 2008 a, b; Genčurová et al., 2008 a, b; Macek et al., 2008) including climate conditions. Bulk milk samples (BMSs, small groups of animals, from 4 to 6 animals in one sample, which is valid for all species) were collected in three dairy cow herds with Czech Fleckvieh cattle (B; *Bos primigenius* f. *taurus*, L, 1758; n = 93 BMSs), one goat herd (W; *Capra aegagrus* f. *hircus*, L, 1758; White short-haired breed; n = 60 BMSs) and one sheep flock (C; *Ovis aries*, L, 1758; Tsigai; n = 60 BMSs). The animals in herds and flock were sampled in spring and summer seasons repeatedly for 3 years (2005–2007). The herds were kept in altitudes from 360 to 475 (B) and 572 m (W and C) over the sea level. The goat herd and sheep flock were kept in one stable in less favourable area. The nutrition of animals and species was carried out in typical ways under the the CR conditions. The dairy cow herds were fed by total mixed ration (TMR), which consist of: maize silage 13 kg; trifolium silage 9 kg; whole spindle maize silage (LKS) 5 kg; brewery draff 3 kg; concentrates 6 kg per cow and day. Feeding was performed in agreement with standard demands and according to current daily milk yield. Goat herd and sheep flock were fed by the natural grass and herb pasture (composition with the dominance: *Poa pratensis, Dactylis glomerata, Elytrygia repens, Trifolium repens* and *Taraxacum officinale*) and by the grain supplement with daily ration 0.6 kg for goat and 0.3 kg for sheep (mixture of grains and mineral components). All sampled animals were in first two third of their lactation and had typical milk yield for relevant breed in the country (B 20.04; W 1.75; C 0.36 kg per day). All animals were milked twice a day by machine milking. The cows were kept in tie stable (pipeline milking equipment), the goats and sheep in free stables (milking parlours). Similar file but with lower number of BMSs was obtained also from Holstein cows (H) and this file was partly simultaneously evaluated (Hanuš et al., 2008 a, b) as well.

Investigation of chemical-compositional, physical, health and technological milk indicators

Milk analyses were performed regularly in accredited testing laboratory in Rapotin (n. 1340, EN ISO 17025, accreditation certificate No. 124/2004) according to valid standard operation manuals. The following abbreviations were used for investigated MIs: DM = dry matter (total solids, %); SNF = solid non fat (%); L = lactose (monohydrate %); SCC = somatic cell count ($10^3 ml^{-1}$); EC = electrical conductivity (mS cm^{-1}); MFP = milk freezing point (°C); macroelements such as Na, natrium and K, kalium (in mg kg^{-1}); RIS = residues of inhibitory substances in milk = antibiotica drugs (positive, negative). DM, SNF and L contents were determined by apparatus Milko-Scan 133 B with relevant calibration (Foss Electric). SCC was investigated by instrument Fossomatic 90 (Foss Electric). Both previously mentioned instruments took part in relevant proficiency testing regularly. EC was measured by conductometer Radelkis OK 102/1 with using of glass bell electrode and standard NaCl solution for calibration. MFP was determined by cryoscope CryoStar automatic (Funke-Gerber; Brouwer, 1981; Koops et al., 1989; Bauch et al., 1993; Buchberger and Klostermeyer, 1995). For Na and K (Hejtmánková et al., 2002) the used analytical instrument was atom absorption spectrophotometer SOLAAR S4 plus GFS97 (Graphite Furnace).

Statistical data processing

The processing of the results included calculation of basic statistical parameters, regression analyse and correlation coefficients (Programme Excel). Cow results were used as reference for comparison to small ruminant milk results. SCC values were logarithmically transformed because of no normal frequency distribution in most of cases (Ali and Shook, 1980; Raubertas and Shook, 1982; Shook, 1982; Reneau, 1986; Reneau et al., 1988; Meloun and Militký, 1994; Hanuš et al., 2001;) and after that also geometrical averages were used in evaluation of results.

RESULTS AND DISCUSSION

Evaluation of main statistical results in ruminant milk

The average goat MFP (Tab. I) was –0.5544 ± 0.0293 °C and differed significantly ($P \leq 0.001$) from B cow MFP –0.5221 ± 0.0043 (also from –0.532 ± 0.005 Holstein cows; Hanuš et al., 2008 a, b) and from C sheep MFP –0.6048 ± 0.0691 °C (Macek et al., 2008). There were result similarities to other published values (Rohm et al., 1991; Roubal et al., 2004; Janštová et al., 2007). The other average values of investigated MIs in various ruminant species moved in normal ranges (Hanuš and Foltys, 1991; Antunac et al., 2001; Hejtmánková et al., 2002; Kuchtík and Sedláčková, 2003; Hanuš et al., 2003 a, 2004 b) with exception of SCC in goats and sheep (Kuchtík and Sedláčková, 2003; Paape et al., 2007; Raynal-Ljutovac et al., 2007). Our values were higher, especially goat results, in comparison to some reported results. However, it is known fact that SCC results are commonly higher in small ruminants in comparison to cows. RIS results were negative in all data sets for all three ruminant species. It means that values of other MIs were not interferentially influenced by animal treatment with antibiotica drugs. Only with exception of lactose difference between goats and sheep all shown interspecific differences within MIs were significant (Tab. I; $P < 0.01$ and $P < 0.001$). Right this L accordance between goats and sheep and by contraries their significant differences as compared to cow milk could be also caused due to lower cow SCCs and higher goat and sheep SCCs in used data sets.

As it is shown in Table II, with regard to the relationship between cow MFP and L (Fig. 1; –0.36; $P < 0.01$) the goat MFP was not correlated to lactose content (–0.07; $P > 0.05$) and sheep MFP was actually related positively to L (0.40; $P < 0.01$). As in cow milk this fact is in accordance with declared participation of lactose on freezing point depression effect (54%; Demott, 1969; Brouwer, 1981; Walstra and Jenness, 1984; Koops et al., 1989; Wiedemann et al., 1993; Buchberger, 1990, 1991, 1994; Hanuš et al., 2003 b) these results were quite another and surprising in goat and sheep milk. There were 12.6% variations in cow MFP explainable by L variations. Especially this relationship is opposite in sheep. It could be explainable by worse geometric averages of SCC (poorer udder health state) for used goat (3,646 $10^3\,ml^{-1}$) and sheep (560 $10^3\,ml^{-1}$) data set in comparison to cows (159 $10^3\,ml^{-1}$). A little higher SCC at relatively good udder health state did not reduce L (cows). It had not to be compensated by Na ion secretion into milk or only restrictedly in terms of preservation of osmotic pressure balance and L could participate on freezing point depression without a disturbance. After that the MFP could be a little better along thin SCC decrease (Tab. II; Fig. 1; $P > 0.05$). Also Na concentration had not to show a negative effect on MFP (Tab. II; Fig. 1; 0.23; $P < 0.05$). In the case of higher or high SCC and worse udder health state (sheep and especially goats in this case) the lactose was probably a little reduced and osmotic pressure was balanced by higher Na ion secretion simultaneously with improvement of MFP (especially in sheep; Tab. II; Fig. 1). The participation of L content on freezing point depression was limited under such circumstances. Nevertheless, the MFP was more tightly connected by negative relationships with main milk components such as DM and SNF in small ruminants than in cows (–0.07 and –0.32; $P > 0.05$ and $P < 0.05$; Tab. II). It is clear especially in sheep (–0.70 and –0.67; $P < 0.001$; Tab. II) with typically higher DM and SNF average

I: *Averages and differences in MFP and other selected MIs according to ruminant species, goat (W), cow (B) and sheep (C)*

MI	Unit	B x ± sd	W x ± sd	C x ± sd	B – W	B – C	W – C
MFP	°C	–0.5221 ± 0.0043	–0.5544 ± 0.0293	–0.6048 ± 0.0691	***	***	***
SNF	%	8.95 ± 0.208	8.24 ± 0.437	11.4 ± 0.547	***	***	***
DM	%	12.35 ± 0.546	12.82 ± 1.091	18.98 ± 1.930	***	***	***
L	%	5.06 ± 0.117	4.43 ± 0.287	4.44 ± 0.380	***	***	ns
SCC	$10^3\,ml^{-1}$	230.1 ± 222.7	4,267.4 ± 2,297.9	948.5 ± 1,404.7	***	***	***
log SCC		2.2012 ± 0.3694	3.5618 ± 0.2589	2.7479 ± 0.4012	***	***	***
EC	$mS\,cm^{-1}$	3.57 ± 0.320	5.67 ± 0.398	4.36 ± 0.348	***	***	***
Na	$mg\,kg^{-1}$	395.6 ± 80.0	438.6 ± 98.1	740.1 ± 157.8	**	***	***
K	$mg\,kg^{-1}$	1,629.5 ± 71.8	2,013.8 ± 202.7	1,296.8 ± 123.8	***	***	***

(MFP – milk freezing point; SNF – solid non fat; DM – dry matter; L – lactose; SCC – somatic cell count; EC – electrical conductivity; macroelements Na – natrium and K– kalium; x – arithmetical mean; sd – standard deviation; *, ** and *** – statistical significance $P \leq 0.05$, ≤ 0.01 and ≤ 0.001; ns $P > 0.05$.)

Evaluation of relationships of MFP to other milk indicators among ruminants

Logically DM and SNF showed (Tab. II) the mostly significant negative relationships to MFP in all ruminant species. There were investigated less close relationships in cow milk and it was quite lucid especially in sheep milk (–0.70 and –0.67; $P < 0.001$).

II: *Comparison of relationships of MFP to selected MIs (with linke to udder health state) among goats (W), cows (B) and sheep (C)*

Species	MI	Regression equation	R^2	r	Significance
B	L	y = –9.64x + 0.0239	0.1262	–0.36	**
	SNF	y = –15.671x + 0.7728	0.1050	–0.32	**
	DM	y = –9.0088x + 7.6486	0.0051	–0.07	ns
	log SCC	y = 6.6604x + 5.6784	0.0060	0.08	ns
	EC	y = 0.7854x + 3.9836	0.0001	0.01	ns
	Na	y = 4,287.8x + 2,634.1	0.0534	0.23	*
	K	y = –523.07x + 1,356.5	0.0010	–0.03	ns
W	L	y = –0.6457x + 4.0745	0.0043	–0.07	ns
	SNF	y = –5.3269x + 5.2875	0.1282	–0.36	**
	DM	y = –21.273x + 1.0218	0.3273	–0.57	***
	log SCC	y = –1.6751x + 2.633	0.0360	–0.19	ns
	EC	y = –1.5857x + 4.7882	0.0137	–0.12	ns
	Na	y = 322.76x + 617,5	0.0093	0.10	ns
	K	y = –1,128.4x + 1,388.1	0.0267	–0.16	ns
C	L	y = 2.1723x + 5.7579	0.1561	0.40	**
	SNF	y = –5,3086x + 8,1902	0.4495	–0.67	***
	DM	y = –19,631x + 7,1098	0.4944	–0.70	***
	log SCC	y = –3.5673x + 0.5904	0.3778	–0.61	***
	EC	y = –2.2523x + 2.9941	0.2006	–0.45	**
	Na	y = –368.87x + 517.04	0.0261	–0.16	ns
	K	y = 914.82x + 1,850	0.2607	0.51	***

(R^2 – determination coefficient; r correlation coefficient.)

values (Tab. II). Mentioned relationships could explain the obtained apparently antagonistic results.

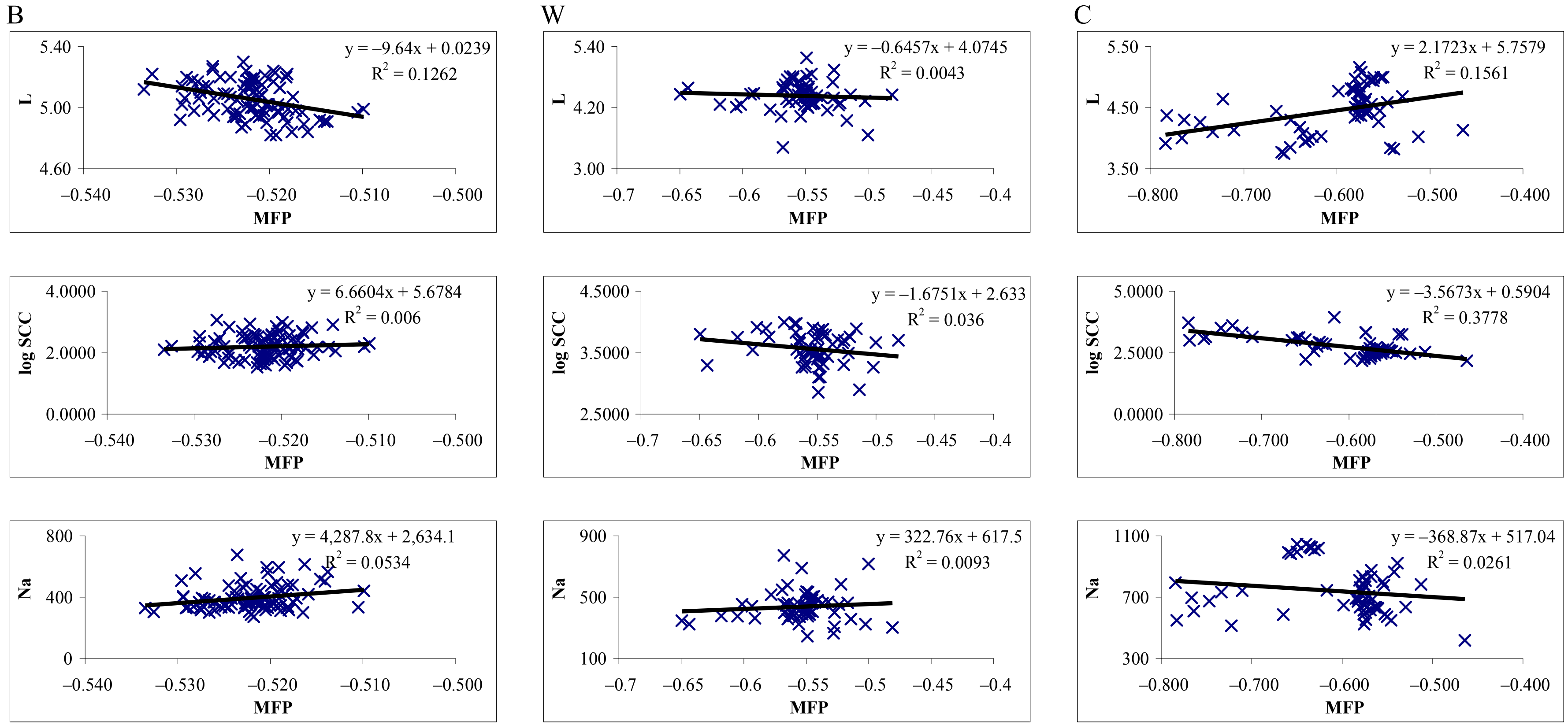
However, as the evaluated results showed, the principles of creation of milk freezing point depression by effects of other milk commponents are quite various among different ruminant species. Only 0.5 and 10.5% of MFP variations were explainable by variations in main milk components DM and SNF in cows but already 32.7 and 12.8% in goats and even 49.4 and 45.0% in sheep. The mostly significant relationships of MFP to the other MIs in sheep were confirmed probably especially because of proven high variability of MFP as compared to the other ruminants 11.8% (C) versus 5.3 (W), 0.8 (B) and 0.9% (H; Macek et al., 2008; Genčurová et al., 2008 a).

CONCLUSION

More relationships between MFP and other udder health milk indicators in goats, cows and sheep were investigated, compared and interpreted in this paper. The results showed that milk freezing point depression was created by various factors and rules as compared among more ruminant species. On the basis of obtained results it could be possible to derive the more reliable MFP qualitative discrimination limits in various ruminant species for standardization purposes and also for more efficient rules in monitoring and prevention of possible milk quality problems in cows and small ruminants.

SUMMARY

Sheep and goat farming is returning back into the Czech Republic (CR) because of possible positive effects of alternative milk consumption on human health. That is reason why this milk quality control is important. Milk freezing point (MFP) is important physical indicator. MFP is used for control of milk food chain quality. Aim of this paper was to analyse the relationships of MFP to selected milk indicators (MIs; with link to udder health) by comparison between cows (reference), goats and sheep. Bulk milk samples came from 3 dairy herds of Czech Fleckvieh cattle (B, n = 93) and 1 goat herd and sheep flock (White short-haired, W, n = 60; Tsigai, C, n = 60). The goat herd and sheep flock were situated in less favourable area. Animal nutrition of all species was performed under the typical CR country conditions. Following MIs were investigated: DM, dry matter (total solids); SNF, solid non fat; L, lactose (all in %); SCC, somatic cell count (10^3 ml^{-1}); EC, electrical conductivity (mS cm^{-1}); MFP, milk freezing point (°C); Na, natrium and K, kalium as macroelements (in mg kg^{-1}). Goat MFP was –0.5544 ± 0.0293 °C and differed ($P \leq 0.001$) from B MFP –0.5221 ± 0.0043 and C MFP –0.6048 ± 0.0691 °C. The MIs in rumi-



1: *Selected linear regression relationships between milk freezing point (MFP in °C) and lactose (L in %), somatic cell count (log SCC) and natrium concentration (Na in mg kg⁻¹) as compared among more ruminant species, cow (B), goat (W) and sheep (C)*

nant species were relatively normal with exception of higher SCCs in goats and sheep. The cow MFP was related to L (–0.36; P < 0.01). Goat MFP was not correlated to lactose content (–0.07; P > 0.05) and was related to L (0.40; P < 0.01) in sheep. As cow milk was in accordance with declared participation of L on MFP depression (54%) the unlike results were surprisingly observed in goats and sheep. This fact could be explainable by worse SCC geometric averages (poorer udder health) for used goat (3,646 10^3 ml^{-1}) and sheep (560 10^3 ml^{-1}) set in comparison to cow (159 10^3 ml^{-1}). MFP was more tightly connected with DM and SNF in small ruminants (C –0.70 and –0.67; P < 0.001) than in B (–0.07 and –0.32; P > 0.05 and P < 0.05) due to higher DM and SNF. Only 0.5 and 10.5% of variations in MFP were explainable by variations in DM and SNF in cow but already 32.7 and 12.8% in goat and even 49.4 and 45.0% in sheep. Higher sheep values were caused by high MFP variability, 11.8% (C) versus 5.3% (W) and 0.8% (B). According to obtained results, there could be possible to derive the more reliable MFP qualitative discrimination limits for standards and more efficient rules for monitoring and prevention of milk quality problems in cow, goat and sheep.

SOUHRN

Analýza vztahů mezi bodem mrznutí a vybranými ukazateli zdravotního stavu vemene mezi kravským, kozím a ovčím mlékem

Chov ovcí a koz se vrací do České republiky (ČR) kvůli možným pozitivním vlivům spotřeby alternativních mlék na lidské zdraví. To je důvod, proč je důležitá kontrola kvality tohoto mléka. Bod mrznutí mléka (MFP) je významný fyzikální ukazatel a je používán pro konrolu kvality mléčného potravinového řetězce. Cílem této práce bylo analyzovat vztahy MFP k vybraným ukazatelům kvality mléka (MIs; s vazbou na zdraví vemene) prostřednictvím srovnání mezi kravami (reference), kozami a ovcemi. Bazénové vzorky mléka pocházely od tří mléčných stád krav plemene České strakaté (B, n = 93) a jednoho kozího a ovčího stáda (Bílá krátkosrstá, W, n = 60; Cigája, C, n = 60). Kozí a ovčí stádo bylo chováno v méně využitelné oblasti (LFA). Výživa zvířat všech druhů byla provedena za podmínek typických v ČR. Byly vyšetřeny následující MIs: DM, celková sušina; SNF, sušina tukuprostá; L, laktóza (vše v %); SCC, počet somatických buněk (10^3 ml^{-1}); EC, elektrická vodivost (mS cm^{-1}); MFP, bod mrznutí mléka (°C); Na, sodík a K, draslík jako makroprvky (v mg kg^{-1}). Kozí MFP byl –0,5544 ± 0,0293 °C a lišil se (P $\leq$ 0,001) od B MFP –0,5221 ± 0,0043 a C MFP –0,6048 ± 0,0691 °C. MIs u druhů přežvýkavců byly relativně normální s výjimkou vyšších SCC u koz a ovcí. Kravský MFP byl vztažen k L (–0,36; P < 0,01). Kozí MFP nebyl korelován k obsahu L (–0,07; P > 0,05) a ovčí MFP byl vztažen k L (0,40; P < 0,01). Zatímco kravské mléko bylo v souladu s deklarovanou účastí L na depresi MFP (54 %), překvapivě nestejné výsledky byly pozorovány u koz a ovcí. Tato skutečnost by mohla být vysvětlitelná zhoršenými geometrickými průměry SCC (horší zdravotní stav vemene) pro použitý kozí (3 646 10^3 ml^{-1}) a ovčí (560 10^3 ml^{-1}) soubor ve srovnání ke kravskému (159 10^3 ml^{-1}). MFP byl u malých přežvýkavců těsněji spojen s DM a SNF (C –0,70 a –0,67; P < 0,001) než u krav (–0,07 a –0,32; P > 0,05 a P < 0,05) v důsledku vyšší DM a SNF. Pouze 0,5 a 10,5 % variací v MFP u krav bylo vysvětlitelných prostřednictvím variací v DM a SNF, ale již 32,7 a 12,8 % u koz a dokonce 49,4 a 45,0 % u ovcí. Vyšší hodnoty u ovcí byly zapříčiněny prostřednictvím vysoké variability MFP, 11,8 % (C) oproti 5,3 % (W) a 0,8 % (B). Podle získaných výsledků by bylo možné odvodit věrohodnější kvalitativní diskriminační limity MFP pro normy a účinnější pravidla pro monitoring a prevenci problémů kvality mléka u krav, koz a ovcí.

přežvýkavec, syrové mléko, bod mrznutí mléka, zdravotní stav vemene, laktóza, počet somatických buněk, elektrická vodivost, sodík

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