The analysis of relationships between chemical composition, physical, technological and health indicators and freezing point in raw cow milk

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ABSTRACT: The milk freezing point (MFP) is used for the control of milk food chain quality especially for possible adulteration with water. A crucial issue is the acceptance of the legislative discrimination limit (RLDL) of MFP for standard quality. The aim was to explain the relations between MFP and spectrum of milk indicators (MI) and possible impacts of MFP on technological milk properties. 76 bulk milk samples (BMS) from Holstein (1, n = 36) and Czech Fleckvieh (2, n = 40) cattle were analyzed for 48 MIs. The dairy cows were relatively healthy as for the occurrence of production disorders. BMSs were taken from February to June. Extraneous water was excluded. 44 MIs were correlated with the MFP. The relations were not regularly consistent between breeds. Milk yield was connected with MFP (r = 0.40; $P < 0.05$). It shows the necessity of modification of RLDL of MFP in dependence on dairy cow breeding. Further relations ($P \leq 0.05$) were among MFP and: total milk solids (r = –0.50); solids-non-fat (–0.33); crude protein (–0.32); true protein (–0.43); whey protein (–0.47); milk fat (–0.46); electrical conductivity (–0.35); lactose (–0.35); somatic cell count (–0.36); fat/protein ratio (–0.36); milk citric acid (0.47); Na (–0.34). The poor relations ($P > 0.05$) were among MFP and casein, milk urea and acetone. The cheese-making indicators were not affected by MFP. The MFP was related to milk fermentation indicators (r = from –0.34 to –0.39, $P < 0.05$). It is important for the control of milk food chain quality by MFP and for the estimation of its RLDL.

Keywords: dairy cow; health state; secretion disorder; milk components; physical indicator; technological property

Milk freezing point legislative standard limits

The milk freezing point (MFP) is an important physical qualitative indicator of milk. It is mostly used for the control of raw or pasteurized milk quality with regard to incidental milk adulteration with water (Figure 1). It means for the control of the technological discipline of milk producers or processors. Water can penetrate into milk from milking machines as a necessary addition or due to bad milking practice. Currently, some European Union

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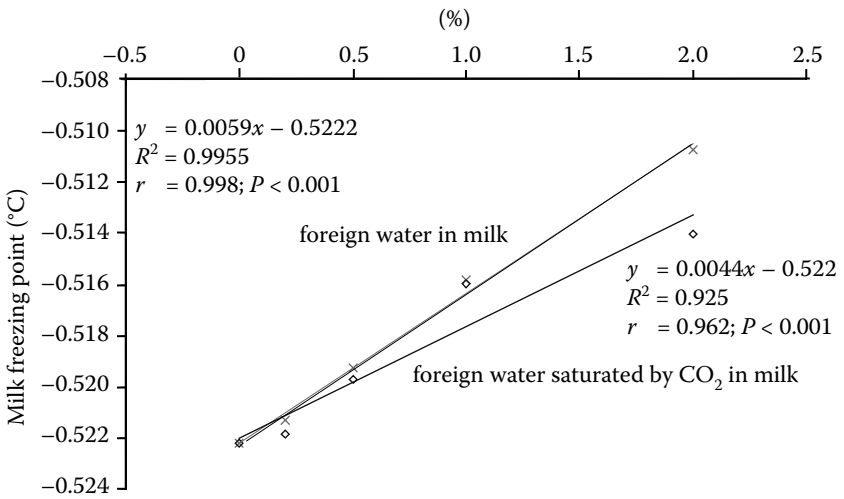

Figure 1. The impact of additions of water and water saturated by carbon dioxide into raw cow milk on its freezing point depression (according to Hanuš et al., 2006)

countries use the legislative discrimination limits of MFP for standard milk quality from ≤ –0.520°C to ≤ –0.505°C (Rohm et al., 1991; Buchberger, 1994). In the Czech Republic (CR) a discrimination limit ≤ –0.515°C was valid previously, now it is ≤ –0.520°C for raw and pasteurised cow milk in accordance with EEC 92/46 and Regulation (EC) No. 853/2004 (No. 638/2004 Sb.). However, 20.1% of deliveries into dairy plants do not meet this limit in the CR. Such an amount cannot be caused by the poor technological discipline. It is evident that a discrepancy exists in the limit determination.

Effects on milk freezing point

The main effect on MFP could be an extraneous water addition. A possible influence of the first automatic milking system (AMS) on MFP deterioration was published recently (Rasmussen and Bjerring, 2005). MFPs were stabilized after an improvement of the AMS. The frequency of MFPs above –0.516°C was 23% and declined to 2.2%. Nevertheless, there exist more factors besides the addition of extraneous drinking water which can influence the MFP (Freeman and Bucy, 1967; Eisses and Zee, 1980; Wiedemann et al., 1993; Buchberger, 1994; Kološta, 2003). In general, it can be farm impacts such as animal species (Janštová et al., 2007; Genčurová et al., 2008; Hanuš et al., 2008a,b; Macek et al., 2008), cow herd, breed, milk yield, year season and pasture, animal nutrition, feeding and health state related with the occurrence of production disorders. It is important to distinguish between the above-mentioned impacts and the real addition of extraneous water when determining the objective milk quality for milk payment and for the control of milk food chain quality. There are other technological negative impacts on pasteurised MFP during its processing like drinking water addition and protein heat stress (Rohm et al., 1991). De facto all milk deliveries for processing contain a certain amount of extraneous water if machine milking is used.

Milk composition and properties and milk freezing point

There are more components which influence the MFP value, mainly due to their osmotic pressure. Many authors (Demott, 1969; Brouwer, 1981; Walstra and Jenness, 1984) reported that the lactose content caused 53.8% of the MFP depression. Further, in declining approximate order, K^+ 12.7%, Cl^- 10.5%, Na^+ 7.2%, citrates 4.3%, urea 1.9% and other components 6.9%. The effect of the milk content of carbonic acid gas and its evaporation were studied not only by the carbon dioxide exhausting but also by the milk oversaturation (Figure 2). Carbon dioxide affects the freezing point of raw and pasteurised milk in the processing chain because of its current decrease due to mixing, shaking and heating. Its volumetric content decreases approx. from 7% down to 2%.

Aim of the paper in terms of MFP interpretation

There has been a discussion about the discrimination value of MFP for standard milk quality un-

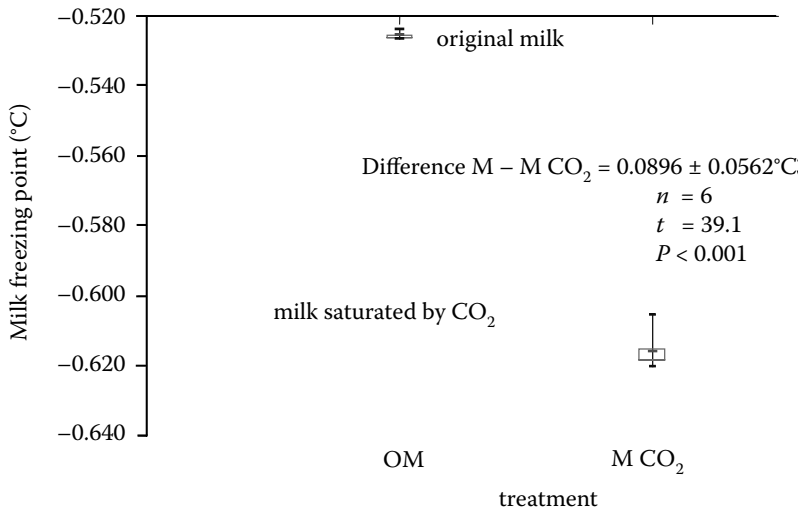

Figure 2. The impact of the saturation of milk by to carbon dioxide on its freezing point (according to Hanuš et al., 2006)

der Czech legislative conditions. In case that MFP could be influenced by various factors, the quantification of such impacts could contribute to the more precise estimation of MFP legislative limit and also support the milk quality control. The goal of this study was to find significant relations between MFP values and a large spectrum of other indicators in raw cow milk without addition of extraneous water. It is important for the control of the dairy food chain quality.

MATERIAL AND METHODS

Animals and milk samples

All sampled dairy cows were in the first half of lactation. Milk samples were collected regularly from February to June (1st set in winter and spring and 2nd set in spring and summer). There were two milked breeds, Holstein (1st, H) and Czech Fleckvieh (2nd, B). Daily milk yield (DMY) was measured with electronic flow milk meters (Fullwood, tandem milking parlour 2 × 5) in the first herd, which was kept in a free stable, and with the classic flow milk meters (Tru-Test; New Zealand) in the second herd, which was kept in a tie stable. The sampled dairy cows were in the relatively good health state from the aspect of mastitis of their mammary glands. All animals received the total mixed ration which consisted of: (1st) maize silage 15, lucerne silage 10, whole cob maize silage (LKS) 5, brewery draff 3, lucerne hay 1, dried whey 0.3 and concentrates with yeasts 5 kg; (2nd) maize silage 13, clover silage 9, whole cob maize silage (LKS) 5, brewery draff 3, concentrates 6 kg per cow and day. Concentrates, mineral and vitamin supplements were fed in the accordance with cow DMY. Both herds were milked twice a day.

Milk samples (1st set = 36 and 2nd = 40) were obtained at regular milking. Each was prepared as a bulk milk sample by mixing from four or eight animals. Milk was taken as an original liquid without any extraneous water addition. Therefore the results are valid for native milk free from technological impacts and suitable for the real quality control. The samples were completed in accordance with DMY of cows and transported immediately for analysis to an accredited testing laboratory under cooling conditions (< 10°C), without any preservation substance. Only bacteriological samples were preserved by Heeschen's agent.

Investigated milk indicators with relevant units

Milk indicators (MI) were as follows: MFP milk freezing point (°C); DMY daily milk yield (kg of milk per day); F milk fat content (g/100 g; %); L lactose content (monohydrate; g/100 g; %); SNF solids non-fat content (g/100 g; %); DM dry matter (total solids; g/100 g; %); SCC somatic cell count (ths/ml); F/CP ratio between fat and crude protein; U urea (mmol/l); A acetone (mg/l); CA citric acid (mmol per l); AS alcohol stability (ml, consumption of 96% ethanol to protein coagulation in 5 ml of milk); TA titratable acidity according to Soxhlet-Henkel (ml of 0.25 mol/l NaOH solution, which was used for the titration of 100 ml of milk); pH actual milk acidity

(H ion concentration); EC electrical conductivity (mS/cm); RCT rennet coagulation time (seconds); CQ subjective estimation of curds cake quality determined by the aspection and touch from 1st (excellent) to 4th (poor) class; CF cheese curds firmness, depth of the penetration of a corpuscle after fall into curds cake under standard conditions, the measured value shows the opposite relationship to firmness (cm); WV whey volume, obtained during the process of enzymatic cheese-making from curds cake (ml); SW specific weight (g/cm^3); CP crude protein (total N × 6.38; g/100 g; %); TP true protein (protein N × 6.38; g/100 g; %); CAS casein (casein N × 6.38; g/100 g; %); WP whey protein (difference TP-CAS; g/100 g; %); NNM non-protein nitrogen matters (nitrogen CP-TP × 6.38; g/100 g; %); UNPN ratio of urea nitrogen in non-protein nitrogen (%); CN-CP and CN-TP casein numbers for CP and TP as ratios of casein in protein fractions (%); FAM-T fermentation ability of milk, it means a yoghourt test with microbial culture (by the titratable acidity of yoghourt in ml of 0.25 mol/l NaOH/100 ml); FAM-pH (by the actual acidity of yoghourt pH); FAM-TCM (by the total count of the fermenting noble microorganisms in CFU/ml); FAM-CL (by the count of lactobacilli in CFU/ml); FAM-CS (by the count of streptococci in CFU/ml); FAM-RSL (by the ratio between streptococci and lactobacilli), all the previous parameters at FAM were measured after the yoghourt test fermentation; RIS residues of inhibitory substances (mostly antibiotic drugs, microbiological Delvo-test, +/–); Ca, P, Na, Mg, K and I, Mn, Fe, Cu, Zn, Ni as milk macro- and microelements were expressed in mg/kg (with the exception of I in μg/l); SMME sum of the measured macro- and microelements expressed in mg/kg. The following microbiological species were investigated: *Streptococcus uberis*, *Streptococcus parauberis*, *Staphylococcus aureus* and *Staphylococcus haemolyticus*. The results are expressed in CFU/ml. The microbiological cultivation methods with likelihood identification were used.

Used milk analytical methods

The samples were analysed for MFP values with the top cryoscopic instrument Cryo-Star automatic (Funke-Gerber, Germany). The instrument was under regular calibration. The incidental interference effects were controlled (in accordance with Bauch et al., 1993; Koops et al., 1989; Buchberger and Klostermeyer, 1995). The other investigated indicators such as F, L and SNF were measured with a MilkoScan 133B instrument (Foss Electric, Denmark), which was regularly calibrated (standard ČSN 57 0536 and 57 0530). The SCC was determined with a Fossomatic 90 instrument (Foss Electric, Denmark) according to ČSN EN ISO 13366-3 (1998). The protein fractions such as CP, TP and CAS were determined by reference Kjeldahl's method on the instrument line Tecator with Kjeltec Auto Distillation unit 2 200 (Foss-Tecator AB, Sweden) according to ČSN 57 0530 (1973). The instrument was included in proficiency testing (APLAC and ICAR-CECALAIT). Milk nitrogen fractions were analysed using the previous experience (Hanuš et al., 1995). U, A and CA were determined spectrophotometrically: U at 420 nm of the wavelength (with *p*-dimethyl-aminobenzaldehyde); A at 485 nm (with salicylaldehyde); CA at 428 nm (with pyridin and acetanhydride). The Spekol 11 instrument (Carl Zeiss Jena, Germany) was calibrated by six (U), five (A) and seven (CA) concentration scale points (samples). The EC was measured with an OK 102/1(Radelkis, Hungary) conductometer, which was calibrated at each milk sample set. The pH was determined with a CyberScan 510 pH-meter (Eutech Instruments), which was regularly calibrated by the standard buffer solutions (pH 4.0 and 7.0 Hamilton Duracal Buffer, Switzerland) at each sample set. The TA was measured by milk titration (100 ml) with 0.25 mol/l NaOH/100 ml solution according to ČSN 57 0530 (1973). The AS was determined with the help of milk titration (5 ml) by 96% ethanol up to the formation of the first visible milk protein precipitated flakes (in alcohol ml). The macro- and microelements were investigated by atom absorption spectrophotometry on the equipment Spectrometer SOLAAR S4 and 6F S97 Thermo Elemental (England) according to standard operation procedures and literature sources (Hejtmánková et al., 2002). The FAM-TCMs (carried out according to ON 57 0534 with thermophilic yoghourt culture YC – 180 – 40 – FLEX = *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *L. d.* subsp. *bulgaricus*) were investigated by the calculation of the colony forming units (CFU) at the plate cultivation (at 30°C for 72 hours) with GTK M (Milcom Tabor) agar with glucose monohydrate, triptone-peptone, dehydrated yeast extract and skim milk powder (according to ČSN ISO 6610 (1996).

Table 1. Main statistical characteristics of indicators in small group bulk milk samples in Holstein cattle

Milk indicator	Statistical characteristic, n = 36					
	x	xg	SD	CV	minimum	maximum
MFP (°C)	–0.5320		0.0050	0.9	–0.5401	–0.5210
DMY (kg/day)	28.11		2.45	8.7	21.60	32.74
F (%)	4.06		0.42	10.3	3.22	4.93
L (%)	4.82		0.07	1.5	4.64	4.95
SNF (%)	8.77		0.11	1.3	8.51	9.00
DM (%)	12.83		0.47	3.7	11.91	13.76
SCC (ths/ml)	141		58	41.1	71	316
log SCC (–)	2.1186	131	0.1613	7.6	1.8513	2.4997
F/CP (–)	1.21		0.11	8.9	0.97	1.39
U (mmol/l)	6.73		0.90	13.4	4.76	8.11
A (mg/l)	2.03		1.11	54.5	0.08	4.61
log A (–)	0.2042	1.60	0.3720	182.2	–1.0969	0.6637
CA (mmol/l)	8.76		1.23	14.0	6.24	10.70
AS (ml/5 ml)	0.46		0.17	36.8	0.20	0.90
TA (ml 0.25M NaOH/100 ml)	7.17		0.52	7.3	5.93	8.00
pH (–)	6.70		0.05	0.8	6.62	6.80
EC (mS/cm)	4.31		0.34	7.8	3.56	4.92
RCT (s)	177		34	19.1	120	238
CQ (class)	2.81		0.79	28.0	1	4
CF (cm)	1.88		0.04	2.0	1.80	1.90
WV (ml)	32.78		1.76	5.4	29.0	36.0
SW (g/cm^3)	1.0324		0.0013	0.1	1.0296	1.0351
CP (%)	3.36		0.11	3.4	3.10	3.55
TP (%)	3.17		0.12	3.8	2.91	3.41
CAS (%)	2.66		0.09	3.3	2.47	2.85
WP (%)	0.51		0.05	10.2	0.43	0.64
NNM (%)	0.19		0.04	20.5	0.10	0.27

Table 1 to be continued

Milk indicator	Statistical characteristic, n = 36					
	x	xg	SD	CV	minimum	maximum
CN-CP (%)	79.01		1.01	1.3	77.10	81.20
CN-TP (%)	83.83		1.26	1.5	80.06	85.95
FAM-T (ml 0.25M NaOH/100 ml)	27.27		4.00	14.7	18.20	31.97
FAM-pH (–)	5.08		0.33	6.5	4.67	5.90
FAM-TCM (CFU/ml)	1 388 980 556		979 634 387	70.5	288 400 000	5 110 000 000
log FAM-TCM (–)	9.0460	1 111 731 727	0.3032	3.4	8.4600	9.7084
FAM-CL (CFU/ml)	43 702 778		24 120 785	55.2	7 600 000	110 000 000
log FAM-CL (–)	7.5524	35 677 959	0.3139	4.2	6.8808	8.0414
FAM-CS (CFU/ml)	1 345 277 778		961 417 068	71.5	280 000 000	5 000 000 000
log FAM-CS (–)	9.0303	1 072 259 740	0.3052	3.4	8.4472	9.6990
FAM-RSL (–)	33.08		13.83	41.8	11.41	60.00
Ca (mg/kg)	1 172.01		81.81	7.0	1 048.38	1 367.57
P (mg/kg)	950.06		55.14	5.8	823.24	1031.10
Na (mg/kg)	452.97		30.01	6.6	398.55	509.32
Mg (mg/kg)	107.41		3.47	3.2	99.54	113.82
K (mg/kg)	1 563.45		52.67	3.4	1 445.01	1 663.88
I (µg/l)	462.84		103.92	22.5	261.60	614.88
Mn (mg/kg)	0.02		0.01	25.0	0.02	0.03
Fe (mg/kg)	0.15		0.07	46.7	0.05	0.37
Cu (mg/kg)	0.08		0.02	28.8	0.05	0.15
Zn (mg/kg)	4.20		0.62	14.7	3.07	5.66
Ni (mg/kg)	0.04		0.02	42.5	0.01	0.08
SMME (mg/kg)	4 250.39		154.80	3.64	3 940.95	4 586.84

x = arithmetical mean; xg = geometrical mean; SD = standard deviation; CV = variation coefficient; n = number of cases

Table 2. Main statistical characteristics of indicators in small group bulk milk samples in Czech Fleckvieh

Milk indicator	Statistical characteristic, n = 40					
	x	xg	SD	CV	minimum	maximum
MFP (°C)	–0.5202		0.0043	0.8	–0.5294	–0.5099
DMY (kg/day)	26.17		4.79	18.3	17.73	35.20
F (%)	3.71		0.46	12.3	3.01	4.70
L (%)	4.98		0.07	1.3	4.84	5.20
SNF (%)	8.97		0.23	2.5	8.55	9.52
DM (%)	12.69		0.57	4.5	11.56	13.71
SCC (ths/ml)	281		212	75.4	39	864
log SCC (–)	2.3351	216	0.3220	13.8	1.5911	2.9365
F/CP (–)	1.08		0.13	11.8	0.90	1.38
U (mmol/l)	5.13		0.90	17.4	3.42	7.46
A (mg/l)	7.77		4.94	63.6	1.11	26.49
log A (–)	0.8018	6.34	0.2960	36.9	0.0453	1.4231
CA (mmol/l)	7.63		1.44	18.9	3.96	10.63
AS (ml/5 ml)	0.57		0.21	36.7	0.12	1.10
TA (ml 0.25M NaOH/100 ml)	7.88		0.54	6.9	6.79	9.19
pH (–)	6.66		0.09	1.3	6.48	6.82
RCT (s)	116		19	16.6	85	167
CQ (class)	2.00		0.45	22.7	1	3
CF (cm)	1.78		0.09	5.2	1.60	1.90
WV (ml)	35.65		1.27	3.5	34.0	39.0
SW (g/cm^3)	1.0315		0.0010	0.1	1.0297	1.0350
CP (%)	3.45		0.17	5.0	3.13	3.85
TP (%)	3.25		0.17	5.2	2.96	3.66
CAS (%)	2.79		0.15	5.4	2.52	3.14
WP (%)	0.47		0.07	15.3	0.32	0.66

Table 2 to be continued

Milk indicator	Statistical characteristic, n = 40					
	x	xg	SD	CV	minimum	maximum
UNPN (%)	46.98		11.67	24.8	29.81	86.50
CN-CP (%)	80.78		2.00	2.5	76.75	84.24
CN-TP (%)	85.61		2.01	2.3	80.59	89.68
FAM-T (ml 0.25M NaOH/100 ml)	28.79		3.33	11.6	19.77	33.75
FAM-pH (–)	5.01		0.19	3.8	4.76	5.66
FAM-TCM (CFU/ml)	1 132 800 000		768 902 254	67.9	280 000 000	3 147 000 000
log FAM-TCM (–)	8.9552	901 986 422	0.3003	3.4	8.4472	9.4979
FAM-CL (CFU/ml)	47 800 000		18 042 969	37.7	20 000 000	94 000 000
log FAM-CL (–)	7.6483	44 493 851	0.1697	2.2	7.3010	7.9731
FAM-CS (CFU/ml)	1 085 000 000		762 065 345	70.2	260 000 000	3 100 000 000
log FAM-CS (–)	8.9292	849 571 627	0.3113	3.5	8.4150	9.4914
FAM-RSL (–)	23.35		15.90	68.1	7.50	65.96
Ca (mg/kg)	1 417.89		208.10	14.7	1 099.20	2 107.30
P (mg/kg)	954.09		82.64	8.7	756.90	1 157.20
Na (mg/kg)	431.90		98.04	22.7	272.00	676.10
Mg (mg/kg)	136.91		9.69	7.1	119.40	154.80
K (mg/kg)	1 627.53		69.45	4.3	1 453.30	1 772.90
I (µg/l)	377.47		113.45	30.1	189.25	574.46
Mn (mg/kg)	0.07		0.01	20.0	0.05	0.12
Fe (mg/kg)	1.05		1.06	100.6	0.16	4.50
Cu (mg/kg)	0.12		0.03	25.8	0.06	0.19
Zn (mg/kg)	4.86		0.51	10.6	3.40	5.95
Ni (mg/kg)	0.04		0.02	55.0	0.02	0.13
SMME (mg/kg)	4 574.46		236.06	5.16	4 221.39	5 311.58

Table 3. The linear and incidentally nonlinear regression equations, determination coefficients and correlation coefficients or indexes of relations between MFPs of bulk milk samples and their other milk indicators in Holstein (1) and Czech Fleckvieh (2)

	Relationship between MFP and	Regression analyse			
		equation	coefficient of determination	coefficient or index of correlation	significance
1	DMY	y = 8.2E-4 x –0.55515	0.16239	0.40298	*
		y = –4.0E-5 x 2-1.18E-3 x–0.52802	0.16543	0.40731	*
2	DMY	y = 1.6E-4 x –0.52444	0.03254	0.18039	NS
		y = –4.0E-5 x 2 + 2.02E-3 x –0.54824	0.06497	0.25489	NS
1	F	y = –1.96E-3 x –0.52406	0.02686	–0.16389	NS
2	F	y = –4.26E-3 x –0.50445	0.20970	–0.45793	**
1	L	y = 1.063E-2 x –0.5833	0.02425	0.15572	NS
2	L	y = –2.233E 2 x –0.4091	0.12043	–0.34703	*
1	SNF	y = –1.445E-2 x –0.4054	0.10890	–0.33000	NS
		y = –2.146E-2 x^2+ 0.3613 x –2.04998	0.11454	0.33844	*
2	SNF	y = –6.28E-3 x –0.46388	0.10998	–0.33163	*
		y = –1.06E-3 x^2 + 1.281E-2 x –0.54996	0.11024	0.33202	*
1	DM	y = –2.38E-3 x –0.50151	0.05035	–0.22439	NS
		y = –3.83E-3 x^2 + 9.584E-2 x –1.13058	0.09191	0.30317	NS
2	DM	y = –3.72E-3 x –0.47308	0.24830	–0.49823	**
		y = –3.2E-4 x^2 +4.52E-3 x –0.52526	0.24896	0.49896	**
1	SCC	y = –3E-5 x –0.52765	0.12938	–0.35969	*
2	SCC	y = –1.4235E-6 x –0.51984	0.00502	–0.07085	NS
1	log SCC	y = –1.123E-2 x –0.50823	0.13090	–0.36180	*
2	log SCC	y = –1.19E-3 x –0.51747	0.00806	–0.08978	NS
1	F/CP	y = –2.96E-3 x –0.52845	0.00407	–0.06380	NS
2	F/CP	y = –1.188E-2 x –0.50744	0.12611	–0.35512	*
1	U	y = –4.3E-4 x –0.52916	0.00587	–0.07662	NS
2	U	y = –8.9E-4 x –0.5157	0.03465	–0.18615	NS
1	A	y = –5.711E-2 x –0.53003	0.04726	–0.21739	NS
2	A	y = –1.84E-3 x –0.52	0.00135	–0.03674	NS
1	log A	y = –1.89E-3 x –0.53498	0.01969	–0.14032	NS
2	log A	y = 3.8E-4 x –0.51988	0.00068	0.02608	NS
1	CA	y = –9.7E-4 x –0.52351	0.05683	–0.23839	NS
		y = 7.5E-4 x^2 –1.367E-2 x –0.47092	0.09936	0.31521	NS
2	CA	y = 1.39E-3 x –0.53084	0.22055	0.46963	**
		y = 1.4E-4 x^2 – 5.7E-4 x –0.52414	0.22987	0.47945	**
1	AS	y = 3.04E-3 x –0.53343	0.01054	0.10266	NS

Table 3 to be continued

	Relationship between MFP and	Regression analyse			
		equation	coefficient of determination	coefficient or index of correlation	significance
2	AS	y = 3.57E-3 x –0.5227	0.03074	0.17533	NS
1	TA	y = –1.4E – 0.53106	0.00020	–0.01414	NS
2	TA	y = –2.48E-3 x –0.5007	0.09950	–0.31544	*
1	pH	y = 5.095E-2 x –0.87332	0.27492	0.52433	**
2	pH	y = –2.471E-2 x –0.35573	0.24783	–0.49783	**
1	EC	y = –5.23E-3 x –0.5095	0.12458	–0.35296	*
	EC	y = –4.19E-3 x^2 + 3.085E-2 x –0.58677	0.13474	0.36707	*
1	RCT	y = –8.2267E-6 x –0.53057	0.00307	–0.05541	NS
2	RCT	y = 8.2197E-6 x –0.5212	0.00139	0.03728	NS
1	CQ	y = 1.54E-3 x –0.53636	0.05869	0.24226	NS
2	CQ	y = 2.08E-2 x –0.52439	0.04871	0.22070	NS
1	CF	y = 1.603E-2 x –0.56223	0.01465	0.12103	NS
2	CF	y = 1.047E-2 x –0.53885	0.05109	0.22603	NS
1	WV	y = 7.7E-4 x –0.55726	0.07490	0.27368	NS
2	WV	y = 5.4E-4 x –0.53951	0.02607	0.16146	NS
1	SW	y = –0.11337 x –0.415	0.00087	–0.02950	NS
2	SW	y = –0.9768 x +0.48736	0.07009	–0.26475	NS
1	CP	y = –2.23E-3 x –0.52456	0.00265	–0.05148	NS
		y = 4.226E-2 x^2 –0.2852 x –0.05138	0.01591	0.12614	NS
2	CP	y = –7.84E-3 x –0.4932	0.10082	–0.31752	*
		y = –6.98E-3 x^2 +4.071E-2 x –0.57741	0.10459	0.32340	*
1	TP	y = –1.802E-2 x –0.47493	0.18545	–0.43064	**
		y = –0.53561 + 52 741.5 exp(–x/0.18971)	0.22572	0.47510	**
2	TP	y = –8.39E-3 x –0.49295	0.11071	–0.33273	*
		y = –0.52358 + 12.06 exp(–x/0.39299)	0.09864	0.31407	*
1	CAS	y = –1.77E-3 x –0.52733	0.00101	–0.03178	NS
2	CAS	y = –5.81E-3 x –0.50406	0.04280	–0.20688	NS
1	WP	y = –4.524E-2 x –0.50882	0.22197	–0.47114	**
		y = 0.31096x^2 –0.36862x –0.42558	0.25262	0.50261	**
2	WP	y = –2.06E-2 x –0.51059	0.12012	–0.34658	*
		y = –0.14045x^2 +0.11294x –0.54147	0.18382	0.42874	**
1	NNM	y = 4.894E-2 x –0.54146	0.14387	0.37930	*
2	NNM	y = 8.98E-3 x –0.52199	0.00308	0.05550	NS
1	UNPN	y = –1.6E-4 x –0.52191	0.18451	–0.42955	**
		y = 3.4635E-6 x^2 –6.2E-4 x –0.50722	0.20163	0.44903	**
2	UNPN	y = –7E-5 x –0.51682	0.03977	–0.19942	NS
		y = –3.9827E-6 x^2 + 3.6E-4 x – 0.52767	0.07811	0.27948	NS

Table 3 to be continued

	Relationship between MFP and	Regression analyse			
		equation	coefficient of determination	coefficient or index of correlation	significance
1	CN–CP	y = 3.2E-4 x –0.55709	0.00407	0.06380	NS
2	CN–CP	y = 4E-4 x –0.55265	0.03540	0.18815	NS
1	CN–TP	y = 1.65E-3 x –0.66995	0.17184	0.41454	*
		y = 1.9E-4 x^2 –2.942E-2 x +0.62628	0.17919	0.42331	*
2	CN–TP	y = 5.4E-4 x –0.56668	0.06535	0.25564	NS
		y = –2.0E-4 x^2 + 3.471E-2 x–2.0249	0.13339	0.36523	*
1	FAM–T	y = –4.6E-4 x –0.51939	0.13695	–0.37007	*
2	FAM–T	y = –2.3E-4 x –0.51368	0.03171	–0.17807	NS
1	FAM–pH	y = 5.44E-3 x –0.55968	0.12959	0.35999	*
2	FAM–pH	y = 3.47E-3 x –0.53765	0.02437	0.15611	NS
1	FAM–TCM	y = –1.5573E-12 x –0.52987	0.09282	–0.30466	NS
2	FAM–TCM	y = –6.8332E-13 x –0.51947	0.01522	–0.12337	NS
1	log FAM–TCM	y = –6.48E-3 x –0.47344	0.15375	–0.39211	*
2	log FAM–TCM	y = –5.4E-4 x –0.51543	0.00144	–0.03795	NS
1	FAM–CL	y = –6.6851E-11 x –0.52911	0.10369	–0.32201	NS
2	FAM–CL	y = –2.8307E-12 x –0.52011	0.00014	–0.01183	NS
1	log FAM–CL	y = –5.37E-3 x –0.49149	0.11323	–0.33650	*
2	log FAM–CL	y = –3E-5 x –0.51998	1.8237E-6	–0.00135	NS
1	FAM–CS	y = –1.5748E-12 x –0.52991	0.09142	–0.30236	NS
2	FAM–CS	y = –6.9405E-13 x –0.51949	0.01543	–0.12422	NS
1	log FAM–CS	y = –6.43E-3 x –0.474	0.15335	–0.39160	*
2	log FAM–CS	y = –5E-4 x –0.51576	0.00134	–0.03661	NS
1	FAM–RSL	y = –2E-5 x –0.53131	0.00367	–0.06058	NS
2	FAM–RSL	y = –3E-5 x –0.51966	0.00875	–0.09354	NS
1	Ca	y = –6.7596E-6 x –0.52411	0.01220	–0.11045	NS
2	Ca	y = 1.5902E-6 x –0.5225	0.00604	0.07772	NS
1	P	y = –6.0872E-6 x –0.52625	0.00449	–0.06701	NS
2	P	y = 8.9489E-6 x –0.52878	0.03016	0.17367	NS
1	Na	y = –6E-5 x –0.50633	0.11562	–0.34003	*
2	Na	y = 3.1191E-6 x –0.52159	0.00516	0.07183	NS
1	Mg	y = –3.4E-4 x –0.49546	0.05570	–0.23601	NS
2	Mg	y = –8E-5 x –0.5092	0.03369	–0.18355	NS
1	K	y = 1E-5 x –0.55044	0.01534	0.12385	NS
2	K	y = 4.3591E-6 x –0.52734	0.00505	0.07106	NS
1	I	y = –6.4812E-6 x –0.52903	0.01809	–0.13450	NS
2	I	y = 1.5957E-6 x – 0.52084	0.00181	0.04254	NS

Table 3 to be continued

	Relationship between MFP and	Regression analyse			
		equation	coefficient of determination	coefficient or index of correlation	significance
1	Mn	y = –0.197 x –0.52722	0.03931	–0.19827	NS
2	Mn	y = 0.10402 x –0.52724	0.11626	0.34097	*
1	Fe	y = 1.811E-2 x –0.5348	0.06423	0.25344	NS
2	Fe	y = –6.9E-4 x –0.51951	0.02938	–0.17141	NS
1	Cu	y = 5.854E-2 x –0.53642	0.07381	0.27168	NS
2	Cu	y = –4.122E-2 x –0.51536	0.09062	–0.30103	NS
1	Zn	y = –2.37E-3 x –0.5221	0.08470	–0.29103	NS
2	Zn	y = –7.7E-4 x –0.51647	0.00873	–0.09343	NS
1	Ni	y = 2.256E-2 x –0.53294	0.00620	0.07874	NS
2	Ni	y = –3.12E-3 x –0.52011	0.00025	–0.01581	NS
1	SMME	y = –3.5324E-6 x –0.51702	0.01227	–0.11077	NS
2	SMME	y = 3.0167E-6 x –0.53404	0.02868	0.16935	NS

NS = insignificant; $P > 0.05$; *,**,*** = significant at probability levels $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$

Used statistical procedures

The statistical evaluations were carried out separately for each breed, because the data distribution (breed and nutrition effects) does not facilitate a common evaluation (Tables 1 and 2). Selected MIs were logarithmically transformed because of no normal data frequency distribution (FD; Meloun and Militký, 1992). The xg was used there. The FD of the whole MFP data set (1 and 2) was tested in terms of its normality by the Q and Q-Q graphs (Meloun and Militký, 1992, 1994). The 3rd and 4th (tercial and quarter) central statistical moments [the skewness and acuteness (excess)] were tested as well. The linear (the first record in a row, Table 3) and nonlinear (logarithmic, exponential, quadratic and polynomial in terms of the second or third degree of polynomial incidentally as the second record in a row, Table 3) regressions were used at the testing of the relationships between MFP and other MIs (Excel Programme). The MFP data were represented by box graphs showing milk urea and protein combination classes as the diagnostic indicator of cow nutrition in terms of their nitrogen matter and energy maintenance (overloading or malnutrition, Table 4). It means N/E nutrition balance (Kirchgessner et al., 1985, 1986; Bíro et al., 1992; Hanuš et al., 1993; Homola and Vencl, 1993; Jílek et al., 2006; Řehák et al., 2009). The nutrition class impact on the MFP was tested by Student's test.

RESULTS AND DISCUSSION

General description of important milk indicators in the data file

The statistical characteristics are shown in Tables 1 and 2. The DMY averages were 28.11 ± 2.45 kg (in H, 1) and 26.17 ± 4.79 kg (in B, 2) with *CV* 8.7 and 18.3%. The difference was insignificant ($P < 0.05$). Other MIs are mostly in accordance with the characteristics as known under Czech conditions in terms of mean values and their variability. According to the milk mean values (Tables 1 and 2) of F (4.06 ± 0.42% and 3.71 ± 0.46% with *CV* 10.3 and 12.3%), CP (3.36 ± 0.11% and 3.45 ± 0.17% with *CV* 3.4 and 5.0%), U (6.73 ± 0.90 mmol/l and 5.13 ± 0.90 mmol/l with *CV* 13.4 and 17.4%), A (2.03 ± 1.11 mg/l and 7.77 ± 4.94 mg/l with *CV* 54.5 and 63.6% and xg 1.60 mg/l and 6.34 mg/l)

Table 4. The grouping of milk samples in accordance with presupposed dairy cow nutrition balance (N/E) according to CP and U (the system was modified according to Kirchgessner et al. (1985, 1986) and adjusted towards the real data file distribution)

Milk		Protein (%)		
		< 3.10	3.10–3.45	> 3.45
Urea (mmol/l)	< 3.33	1 N – E – *n* = 0	2 N – E 0 *n* = 0	3 N – E + *n* = 0
	3.33–5.50	4 N 0 E – *n* = 0	5 N 0 E 0 *n* = 18	6 N 0 E + *n* = 16
	> 5.50	7 N + E – *n* = 0	8 N + E 0 *n* = 29	9 N + E + *n* = 13

n = number of observations; E = energy maintenance; N = nitrogen matter maintenance; + = surplus; 0 = balanced; – = insufficiency

and F/CP (1.21 ± 0.11 and 1.08 ± 0.13 with *CV* 8.9 and 11.8%) the cows were in good nutrition state considering the fact that they were mostly in the first half of lactation. However, the tendency to cover their slightly higher nitrogen loading of their metabolism by a slight body energy mobilisation was visible. The sampled dairy cows were in the relatively good state of health considering secretion disorders (Tables 1, 2): SCC means were 141 ± 58 and 281 ± 212 ths/ml (*CV* 41.1 and 75.4%); SCC xg means were 131 and 216 ths/ml; the EC x (Table 1) was 4.31 ± 0.34 mS/cm (*CV* 7.8%); the *x* and xg of the frequency of occurrence of *Streptococcus uberis*, *Streptococcus parauberis*, *Staphylococcus aureus* (*Sa*) and *Staphylococcus haemolyticus* as important mastitis pathogens were 78 ± 293 and 0.25 CFU per ml, 1 042 ± 1 847 and 1.84 CFU/ml, 56 ± 138 and 0.74 and 38 ± 115 and 0.46 CFU/ml in the whole file. In individual cases the *Sa* values were mostly (72%) below the critical limit for a herd suspected of mastitis of relevant aetiology (which is 200 CFU/ml in bulk milk samples), as compared to results by Benda et al. (1997).

Milk freezing point data file investigation

The MFP one-dimensional data file was investigated by the exploratory analysis method in terms of its FD normality testing. The real MFP data file was characterized by normal FD. After the skewness and acuteness calculation it was also confirmed graphically. The likelihood of the zero hypothesis considering the normality of the FD was $P > 0.05$ for both. The real skewness was –0.039 as compared to the normal value 0 and the real acuteness was 2.093 versus 3. Using classical statistical methods and *t*-testing is right in the case of MFP. Arithmetical mean and sd, which are –0.5320 ± 0.0050 and –0.5202 ± 0.0043°C with very low vx 0.9 and 0.8% (Tables 1, 2), are reliable representatives of the real MFP data files. It was surprising that there was a difference (0.0118°C; $P < 0.001$; *t* 10.9) between breeds. The MFP of H cows with a little higher DMY was better (lower) as compared to B cows. However, this investigation was not the aim of the study as the data file was not suitable for such a purpose. It could be caused by the interference effect of different nutrition and feeding conditions.

Relations between MFP and MIs in dependence on cow nutrition indicators

Deterioration of MFP with an increase in DMY under identical environmental conditions within both herds was confirmed (Table 3, also Hanuš et

al., 2003). The effect was significant in H breed (Table 3, $r = 0.40$; $P < 0.05$). The dependence of MFP on DMY is probably valid within both herds and breeds and among breeds or regions as well. The explanation of this fact can be that while the breed effect could be expressed by the closeness (correlation coefficient value) and character (steepness) of relationships (under identical conditions, only with different DMY, it means with changed genetic bases for DMY within breed and herd, respectively), the absolute shift (drift, bias) in MFP values (H and B data files) could be caused by nutrition differences between herds. In general it is possible to state that the legislative discrimination limit of freezing point for the raw milk quality control should be up-dated in dependence on the genetic improvement of cow populations. The intervals should depend on the milk yield increase. There are consistent relation tendencies between MFP and the other compositional MIs (which depend on the cow nutrition) such as all forms of milk proteins, fat, urea etc. both within and between the breeds. Components are essential in terms of MFP depression creation (Demott, 1969; Brouwer, 1981; Walstra and Jenness, 1984; Buchberger, 1994; Buchberger and Klostermeyer, 1995; Buchberger, 1997; Hanuš et al., 2003, 2006). Dependence of MFP on CP was determined first of all in B ($r = –0.32$; $P < 0.05$). Chládek and Čejna (2005) found a closer relation also in B breed ($r = –0.57$) in comparison with H ($r = –0.18$). The dependences of MFPs on TP ($P < 0.05$ for B and $P < 0.01$ for H) were observed in both breeds ($r = –0.33$ and $–0.43$) on quite a high closeness level (Table 3). However, the dependence of MFP on CAS was observed surprisingly only on an insignificant level ($P > 0.05$). The dependence of MFP on WP was observe also in both breeds ($P < 0.05$ for B and $P < 0.01$ for H, where $r = –0.35$ and $–0.47$).

The dependence of MFP on U was identically negative, but insignificant ($P > 0.05$; $r = –0.08$ for H and $–0.19$ for B; Table 3). Chládek and Čejna (2005) recorded two significant correlations ($r = –0.34$ for H and $–0.39$ for B), similarly like Kirchnerová and Foltys (2005) ($r = –0.45$). This is comprehensible in consideration of the fact that U is responsible for approx. 1.9% of MFP depression (Walstra and Jenness, 1984). However, it was noted that MFP had depended on UNPN (Table 3), first of all in H ($r = –0.43$; $P < 0.01$), where the U level was higher as compared to B (6.73 > 5.13 mmol/l). The difference depends rather on the feeding of herds than on breeds, of course. The same tendency was observed in both breeds. Therefore, high U could decrease (improve) MFP a little. Nevertheless, on the other hand it could be an indication of poor dairy cow nutrition (Kirchgessner et al., 1985, 1986; Hanuš et al., 2004a) in terms of the balance of protein/energy ratio to DMY. This fact should not lead to speculations how to ensure the improvement of MFP by feeding dairy cows upon higher U under practical conditions.

In B milk (Table 3) a closer relation was determined between MFP and F with the identical tendency in both breeds ($r = –0.46$; $P < 0.01$). This result is not in good accordance with the fact that F addition into and (F removing from) identical milk did not change the MFP significantly (Hanuš et al., 2003). Changes of F were performed by artificial manipulations. The above-mentioned relation could depend more on numerous physiological consequences in the whole milk composition besides F level, which simultaneously go along with F variations, and not so much on the content of its own components.

Relations between MFP and physical milk indicators

Contrary to the expectation, the MFP was not affected by the SW ($P > 0.05$) although the MFP was slightly improved due to SW increase in both breeds, first of all in B. However, the MFP was related to the EC (Table 3; $r = –0.35$; $P < 0.05$) in H. It is in accordance with the results of the authors (Koops et al., 1989; Buchberger and Klostermeyer, 1994) who reported the regression equations for recalculations of EC, L and the other main milk components onto the MFP value. The relationship between MFP and EC is in close correlation with the health state of the dairy cow mammary gland. It will be mentioned once more later. Considering the pH value the MFP was affected (Table 3; $r = 0.52$ for H and $–0.50$ for B; $P < 0.01$), but not in accordance with the breed tendencies. This finding was unexpected, inconsistent and not easy to explain.

Relations between MFP and technological MIs

The MFP was slightly related to the milk technological properties only sometimes. The MFP was affected due to TA fluctuations in B (Table 3; $r = –0.32$; $P < 0.05$). This finding could be affected in accordance with the above-mentioned TP and WP findings in terms of logical internal methodical

links. The AS as milk protein thermostability was not influenced due to MFP variations ($P > 0.05$). The milk rennet capacity (RCT, CQ, CF and WV; Table 3) was not affected by the MFP variations ($P > 0.05$). However, a tendency of higher WV and lower CF was observed in cheese-making from milk with a worse MFP in both breeds.

Significant relations were observed in H between MFP and yoghurt test indicators such as FAM-T, FAM-pH, log FAM-TCM, log FAM-CL and log FAM-CS (Table 3; $r = -0.37$, 0.36, −0.39, −0.34 and −0.39; $P < 0.05$). These relations are consistent and show the improvement of yoghurt processing with the MFP improving value in general. Similar tendencies were observed in B, but they were not significant. The better (lower) MFPs are connected with better raw cow milk quality for the production of higher-processed dairy products.

The casein numbers (on the basis of CP and TP) were in the identical relation to MFP value, but the significant dependences were observed only for CN-TP (Table 3; $r = 0.36$, and 0.42; $P < 0.05$ and 0.01). This fact is connected with previous findings about relations of MFP to various milk nitrogen fractions. The worse MFP values were connected with higher values of CN-TP. Of course, this fact does not prefer MFP as an indicator with advantageous relation to milk cheese-making yield in terms of milk transport efficiency to dairy plants.

Relations between MFP and udder health state milk indicators of dairy cows

Surprisingly antagonistic tendencies were observed between breeds for the relation between MFP and L. Logically (Walstra and Jenness, 1984; Buchberger, 1994), the relationship is significant for B only (Table 3; $r = -0.35$; $P < 0.05$). Chládek and Čejna (2005) reported the identical correlations ($r = -0.76$ and −0.11 for B and H). The decreased L causes the deterioration of MFP. This confirms that L could account for up to 53.8% of MFP depression (Walstra and Jenness, 1984). This correlation of L with MFP could also copy a decrease in L during lactation with a decrease in DMY and/or along with the increasing lactation number from the standpoint of lactation physiology (or pathology if mastitis occurs). The improved MFPs with EC and WP increase have already been mentioned. That is why the MFPs are generally improved due to higher SCCs in both breeds. Nevertheless, this dependence was found significant especially in H (Table 3; $r = -0.36$; $P < 0.05$). Chládek and Čejna (2005) also observed a slightly better MFP value with higher SCC ($r = -0.15$ in B) in the mentioned continuity, while Kovářová et al. (2005) described a closer relation. EC grows with the deteriorating state of the mammary gland health, where SCC also grows as a rule. Under such circumstances L usually decreases (Hanuš et al., 1992). This L decrease is replaced by salt ions (especially Na^+ and Cl^-) due to the decreased secretion epithelium function because of a possibility to compensate osmotic pressure and maintain milk production. This increases EC simultaneously with SCC growth due to an inflammatory process. Under the mentioned circumstances and as a paradox of the milk quality investigation, improved MFPs are observed with increased SCCs. Obviously it is not possible to speculate practically about possibilities how to improve the MFP by a benevolence to the higher frequency of milk secretion disorders. It is excluded not only because of deterioration of the other quality indicators but also in particular because of economic losses due to related losses in DMY.

Relations between MFP and energy metabolism MIs of cows

The A is a parameter of dairy cow energy metabolism during early lactation. The high values point to a risk of ketosis occurrence (Unglaub, 1983; Andersson, 1984, 1988; Andersson and Lundström, 1984ab; Gravert et al., 1986; Diekmann, 1987; Gustafsson and Emanuelson, 1993; Hanuš et al., 2004a,b). The MFP was related insignificantly to A (Table 3; $P > 0.05$). Nevertheless, there was a slight tendency for better MFPs concurrently with higher A ($r = -0.22$) in H. However, all values were in the physiological range in H (no energy deficiency). F and F/CP ratios are good indicators of dairy cow energy balance during the first third of lactation (Agabriel et al., 1990, 1991; Bíro et al., 1992; Schulz, 1997; Pechová et al., 2000). Higher values show energy deficiency (ketosis risk) and lower values indicate the deficiency of structural fibre in dairy cow nutrition. Other good indicators are U in relation to CP, further CA and A (Diekmann, 1987). There exists a good correlation between A (log A) and F/CP ratio in the first third of lactation (Hanuš et al., 2004a,b). The values were mostly significant and ranged between 0.18 and 0.48 ($P < 0.05$

or < 0.001) according to the effects such as breed, season, lactation number and stage. The overall correlation had the value 0.23 ($P < 0.001$). The F/CP ratio was negatively related to the MFP, especially in B (Table 3; $r = -0.36$; $P < 0.05$). The MFP was surprisingly improved in connection with the higher F/CP ratio, which means a presumption of certain energy deficiency of cows. However, all F/CP values were in the physiological range for the above-mentioned breeds (Hanuš et al., 2004a,b). Therefore, the presented results could be caused by the first half of cow lactation and due to this fact by the higher L as well. The CA is also one of the useable energy metabolism MIs in dairy cows. The MFP values were positively correlated with CA (Table 3; $r = 0.47$; $P < 0.01$) in B. In H an opposite, but insignificant tendency was observed. In B it means that MFPs were improved at lower CA. However, most CA values are in the physiological range (8–10 mmol/l) in both breeds. Nevertheless, in both breeds it is possible to see similar interesting tendencies in nonlinear regressions (Table 3). This suggests a deterioration of MFPs on both sides outside the CA physiological range.

The predicted mean nutrition state of cow groups in terms of protein/energy balance in relation to DMY was estimated according to combinations of U and CP in milk (Table 4). The U and CP physiological ranges were adapted to a better distribution of MFP values in Table 4. Four milk sample groups were obtained. The average MFPs of these groups were tested for their mutual differences over the whole data file. The results are shown in Figure 3. The MFPs were lower (better) with higher likelihood for the higher nitrogen matter loading of cow nutrition (–0.5216 > –0.5291 and > –0.5291°C; $P < 0.01$; –0.5221 > –0.5291 and –0.5291°C; $P < 0.01$). It is in connection with higher U, as mentioned above. Contrary to this fact, the MFPs were not different ($P > 0.05$) under the presupposed overloading of cows due to energy consumption in the framework of identical groups in terms of cow nutrition by nitrogen matters.

Relations between MFP and other chemical MIs

The NNM were related to MFP in a positive way (Table 3; $r = 0.38$; $P < 0.05$) in H. The MFPs were improved together with lower NNM values. In harmony with a logical expectation the MFPs were mostly deteriorated by lower values of DM and SNF in both breeds (Table 3; $r = -0.22$ and –0.50 for DM in H and B ($P > 0.05$ and $P < 0.01$) and –0.33 for SNF and both breeds ($P > 0.05$ and $P < 0.05$)). In some cases the MFPs were also related to macro- and microelements, but often inconsistently (Table 3) in terms of the breed. The MFPs were significantly related to Na, Mn and Cu (Table 3) from among eleven macro- and microelements ($P < 0.05$). The majority of relations were insignificant ($P > 0.05$). The most interesting relation was observed between MFP and Na concentration in H (Table 3; $r = -0.34$; $P < 0.05$). MFPs improved with higher Na. It could be caused by the osmotic pressure balancing due to the physiological function of mammary gland because of its slightly pathological state.

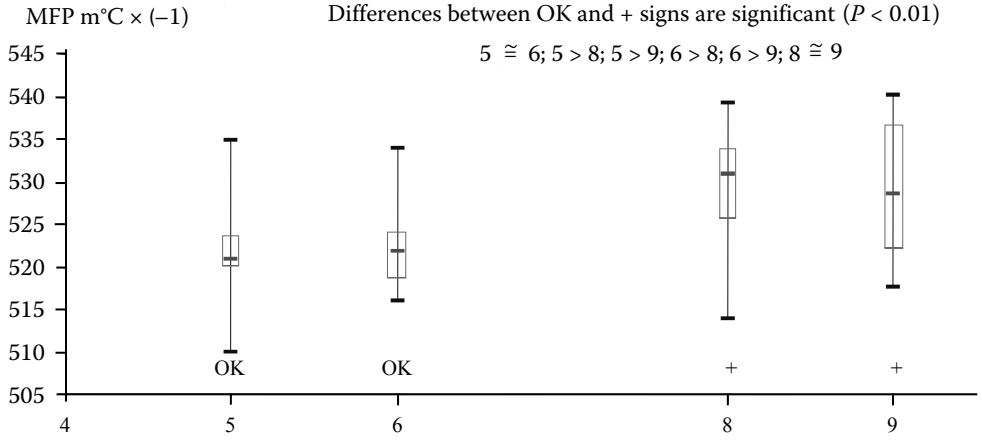

Figure 3. The box graphs showing the influence of combinations of milk urea and protein contents as indicators of the nitrogen and energy nutrition balance of dairy cows (according to Table 4) on MFPs in the bulk milk samples
(The boxes are ranked by the presupposed nitrogen matter loading of dairy cows; OK = in order; + = overloading)

Inconsistent relations between MFP and the other milk properties were found by comparison of the two breeds in indicators such as pH, L, CA, P, Ca, Na, Fe, Cu, Mn, and SMME, which was complicated for interpretation, in particular when the relations between both breeds were significant. Especially the findings in microelements, where there are very low values, could have been influenced by random effects.

CONCLUSION

There are some problems with ensuring the sufficient energetic nutritional sources for dairy cows in the framework of the natura basis of unpreserved or preserved forage, especially in the less favoured areas. There could be more difficult for a part of dairy herds to satisfy the legislative discrimination limit value of MFP of raw milk. It is therefore important to define this MFP limit in a right way for the specific conditions of the country. The above-mentioned results are useful for an improvement of such estimations. They are also important for the specification of relations of MFP without water addition to technological properties like fermentation ability of milk. Therefore the MFP monitoring is a competent method for controlling the dairy food chain quality. The confirmed relations of MFP to cow DMY and to some milk health indicators are also important for the support of other interpretations towards the right legislative discrimination limits in the country.

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