Contamination of cow's raw milk by psychrotrophic and mesophilic microflora in relation to selected factors

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ABSTRACT: The objective of the paper was to analyse the influence of dairy cow management technology, milking method, predipping and summer grazing on the contamination of cow's raw milk by mesophilic (TBC), psychrotrophic (PBC), lipolytic (PLiBC) and proteolytic (PPrBC) bacteria. The values of TBC, PBC, PLiBC and PPrBC in bulk milk samples were determined by the culture method according to IDF standards. Investigations were carried out in nine stables of seven dairy farms from January 2005 to June 2006. Summer grazing has the most marked influence on the values of studied parameters. Farms with summer grazing had a lower microbial contamination of milk compared to farms without grazing and the difference was statistically highly significant in all studied parameters ($P < 0.001$). A positive effect of predipping on a reduction in the values of milk microbial contamination was proved while the difference between farms with predipping and those without it was on a significance level $P < 0.05$ to 0.001 except PLiBC. A comparison of the influence of dairy cow management technology indicated the lowest values of all microbiological indicators in loose cubicle littered housing, higher values were determined in stanchion littered housing and the highest in loose slatted-floor housing. A statistical difference between the technologies was proved mainly in TBC ($P < 0.001$). Farms with milking in milking parlours had a lower microbial contamination of milk compared to farms that used the in-stall milking pipeline system but the difference was statistically significant only in TBC ($P < 0.05$).

Keywords: milk; total bacterial count; psychrotrophic bacteria count; lipolytic bacteria count; proteolytic bacteria count; management technology; predipping; grazing

Cold storage of milk on farms minimises the growth of mesophilic microflora but it has brought about a new problem because low temperatures allow the growth of psychrotrophic microflora in milk (Burdová et al., 2002). An increase in the counts of psychrotrophic bacteria in cow's raw milk is problematic because they produce thermoresistant extracellular proteolytic and lipolytic enzymes that pose a qualitative risk during milk processing and cause the spoilage of final products during storage (Choi and Jeon, 1993; Matta and Punj, 1999; Vyletělová et al., 1999; Chen et al., 2003). Vyletělová et al. (2000a) documented the effects of thermostable lipolytic enzymes *Pseudomonas fluorescens* 66 ZB in pasteurized milk on the concentration of free fatty acids in milk. It is important to carry out prevention already during raw milk production by farm hygienic measures (Hanuš et al., 2004).

The rate of microbial contamination of cow's raw milk is influenced by the health status and hygiene of dairy cows, hygiene of the environment in which dairy cows are housed and milked, methods of udder preparation and milking techniques, methods used for the cleaning and disinfection of milking machines and milk tanks, hygiene of the attendant staff. Other important factors are the rate of milk chilling to the required temperature and the length of milk storage (Wiking et al., 2002). Regulation

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6007665806).

No. 853/2004 of the European Parliament and of the Council (EC) sets down the hygienic limit ≤ 100 000 CFU/ml milk for the total bacteria count (TBC) in cow's raw milk. TBC is one of the main indicators of hygienic quality of cow's raw milk that is also used to set the purchase price of milk.

Among the additional traits of microbial milk quality the hygienic limit ≤ 50 000 CFU/ml milk was determined for psychrotrophic bacteria count (PBC). The determination of PBC is required by some dairies because of their technology (Vyletělová et al., 1999). Vyletělová et al. (2000b) reported the limit 45×10^3 CFU/ml for psychrotrophic proteolytic and/or psychrotrophic lipolytic bacteria count (PPrBC and/or PLiBC) as a risky limit for milk processing into high-processed dairy products. The presence of psychrotrophic bacteria in milk significantly correlates with the occurrence of mesophilic bacteria (Vyletělová et al., 1999, 2000b; Cempírková, 2002).

In operational conditions mainly a failure to observe the hygienic rules of milking process contributes to the impairment of microbial quality of bulk samples of cow's raw milk (Jayarao et al., 2004). It was also confirmed by the identification of microflora in bulk milk samples with higher bacterial counts than 3.0×10^4 CFU/ml in Denmark. Microorganisms primarily associated with poor hygiene dominated in 64% of samples; bacteria also associated with poor hygiene and growth at low temperatures (psychrotrophic bacteria) were dominant microflora in 28% of samples and bacteria connected mainly with mastitis were dominant microflora in 8% of samples (Holm et al., 2004).

The system of dairy cow housing and milking technology create different conditions for the hygienic acquirement of milk. Regula et al. (2002) stated that the bacterial count in milk was lower in loose housing compared to the system of stanchion housing. Gonzalo et al. (2006) also reported lower microbial contamination of milk in loose cubicle littered housing with milking in a milking parlour in comparison with stanchion littered housing and an in-stall milking pipeline system. In addition, it is known that loose housing provides cows with more comfort and welfare (Brouček et al., 2006).

Teat disinfection before milking – predipping is an important factor that reduces TBC or somatic cell count (SCC) in bulk milk samples (Ingawa et al., 1992). Blowey and Collis (1992), who tested the effect of predipping by using an iodophore disinfectant, concluded that the occurrence of clinical mastitis was reduced by 57% while TBC was reduced by 70%.

The rate of microbial contamination of teats and milk can be significantly influenced by dairy cow grazing. McKinnon et al. (1990) reported that housed dairy cows with obviously clean udders might contribute to contamination by more than 10 000 CFU/ml of milk while grazing dairy cows with clean teats might contribute less than 100 CFU/ml of milk. Lower values of TBC in grazing management systems in dairy cows were also reported by Goldberg et al. (1992) and Regula et al. (2002).

The objectives of the present paper were to analyse the relationships between individual groups of microorganisms and to analyse the factors influ-

Table 1. Characteristics of the studied farms

Milking	Milking parlour					In-stall milking pipeline system			
Housing	loose cubicle littered				lsl*	stanchion littered			
Farm	Vj	Cho	Hd	Zu1	Cd1	Zu2	Cd2	Te	Ry
Altitude above sea level	800	520	420	600	410	600	410	700	650
Number of dairy cows	120	290	120	315	320	50	74	146	123
Breed (%)	C 92, H 8	H 70, H × C 30	H 90, L 10	H 70, C 30	H 100	H 70, C 30	H 100	C 100	C 60, H 40
Average daily milk yield (l)	19	16	17.8	19.8	12.5	14.8	12	13.5	18
Predipping	no	yes	no	yes	no	no	no	no	no
Summer grazing	yes	no	no	no	no	no	no	yes	yes

*lsl = loose slatted-floor litterless
C = Czech Pied cattle; H = Holstein cattle; L = Czech Red cattle

encing the values of TBC, PBC, PLiBC and PPrBC in bulk samples of cow's raw milk in nine stables of seven selected dairy farms. It is the influence of management technology, milking method, predipping and summer grazing.

MATERIAL AND METHOD

From January 2005 to June 2006 we monitored TBC, PBC, PLiBC and PPrBC in bulk samples of cow's raw milk in nine stables (places of collection) of seven farms situated in mountain and foothills areas of Southern and Western Bohemia. A total of 345 milk samples were examined. Loose cubicle littered housing with milking in a milking parlour was used on four farms (Vj; Cho; Hd; Zu1; Table 1), and loose litterless slatted-floor housing with milking in a milking parlour was used on one farm (Cd1; Table 1). On another four farms (Zu2; Cd2; Te; Ry; Table 1) stanchion littered housing with an in-stall milking piping system was used. Three farms practised summer grazing (Vj; Te; Ry; Table 1). Predipping was applied on two farms (Cho; Zu1; Table 1). These disinfectants were used for the sanitation of milk machines: Demyro A, K (Vj farm); Despon A, K (Cho farm); Mikal 94D and Mikasan D (Hd and Ry farms); Dosyl A, K (Zu1, Zu2, Te farms); Bilo sp and Bilo rd-p (Cd1, Cd2 farms). The toilet of the mammary gland on farms with milking in milking parlours consisted in shower with subsequent wiping of the udder with a cloth. Only on the farms using predipping (i.e. Cho and Zu1) dry toilet was done in clean udders (wet toilet in dirty udders) that were wiped with a disposable tissue cloth, and Dermaline product (Cho) or Triolet disinfection cloths (Zu1) were used for predipping. On farms with an in-stall milking pipeline system the toilet of the udder consisted in washing with subsequent wiping of the udder with a cloth. All farms used these products for postdipping: Lactobarier (Vj); Filmadine (Cho, Ry, and in the summer macroclimatic season Zu1, Zu2); Diemacid Direct (Hd); Mikasan JD (winter macroclimatic season Zu1, Zu2); Iodonal (Cd1, Cd2); Deosan Teat Care Plus (Te). Milk marketability was 95% on the majority of the farms, only farm Vj had 98% milk marketability and farm Zu2 80%.

Bulk samples of cow's raw milk were collected into sterile sample flasks with Heeschen's preservative (Heeschen et al., 1969) at a 10:1 ratio (30 ml milk, 3 ml Heeschen's agent) and they were transported in thermos cool boxes. They were processed as soon as they were delivered to a laboratory. Sterile physiological saline with peptone was used for sample dilution. A medium tempered to 45°C was added to 1 ml of the inoculum of the respective dilution. Samples were inoculated always after three consecutive dilutions, in two replications. The plate count skim milk agar (MERCK) was used to determine total counts of mesophilic (TBC) and psychrotrophic (PBC) bacteria. Incubation was done at 30°C for 72 hours in TBC and at 6.5°C for 10 days in PBC. Plates with the number of colonies 10 to 300 were read off. Milk agar (OXOID) was used for the culture of psychrotrophic proteolytic bacteria (PPrBC) while the culture of psychrotrophic lipolytic bacteria (PLiBC) was done on Tributyrin agar (Merck). Incubation was carried out at 6.5°C for 10 days. Colonies with the clear lytic zone were read off.

From the actual values of TBC, PBC, PLiBC and PPrBC arithmetical means and standard deviations were calculated and the variance range was determined by the software Microsoft Excel 97. For the groups of microorganisms proportional indexes (p_i) were determined that were calculated as the ratio of real values (PBC/TBC; PLiBC/PBC; PPrBC/PBC; PLiBC/TBC; PPrBC/TBC) and correlation coefficients (r) were calculated from logarithmically transformed data. Statistical evaluation of data was done by the software Statistica ver. 6. Before the statistical analysis the values of TBC, PBC, PLiBC and PPrBC were logarithmically transformed in order to approach normal distribution. Tukey's test was used for a comparison of the housing technologies whereas t-test was applied to compare two groups, i.e. milking in a milking parlour and in-stall milking pipeline system, use of predipping and without predipping, grazing and no grazing.

RESULTS AND DISCUSSION

Total count of mesophilic bacteria (TBC) in the whole set of bulk milk samples ranged from 3.5×10^3 to 1.9×10^5 CFU/ml with the arithmetical average of 2.4×10^4 CFU/ml and the count of psychrotrophic bacteria (PBC) was in the range of 3.0×10^2 CFU/ml to 6.0×10^4 CFU/ml with the average of 4.2×10^3 CFU/ml (Table 2). The proportional index PBC/TBC was 0.18 in both studied groups (Table 3). A statistically highly significant coefficient of correlation $r = 0.75$ ($P < 0.001$) (Ta-

Table 2. Values of TBC, PBC, PLiBC, PPrBC (CFU/ml) of the whole set (n = 365)

Parameter	TBC	PBC	PLiBC	PPrBC
Mean	23 592	4 187	881	1 038
S.D.	24 728	6 580	1 588	1 890
Max	188 636	60 000	13 500	17 500
Min	3 500	300	50	50

ble 3) between the logarithmically transformed values of TBC and PBC was calculated, confirming the previous finding (Vyletělová et al., 1999, 2000; Cempírková, 2002) that the occurrence of psychrotrophic bacteria is in significant correlation with the occurrence of mesophilic bacteria, and so it is possible to assume the identical source of contamination.

The variation range of psychrotrophic lipolytic bacteria (PLiBC) was from 5.0×10^1 CFU/ml to 1.4×10^4 CFU/ml with the average of 8.8×10^2 CFU/ml, and in psychrotrophic proteolytic bacteria (PPrBC) it was from 5.0×10^1 CFU/ml to $1.8\ 10^4$ CFU/ml with the average of 1.0×10^3 CFU/ml (Table 2). The values of proportional indexes (p_i) PLiBC/PBC p_i = 0.21 and PPrBC/PBC p_i = 0.25 are different from the values of proportional indexes for these groups of bacteria reported by Vyletělová et al. (2000b), but the values of proportional indexes PLiBC/TBC p_i = 0.04 and PPrBC/TBC p_i = 0.04 (Table 3) are nearly identical (0.03 and 0.05; Vyletělová et al., 2000b). From the values of proportional indexes (0.04) it is possible to calculate that the hygienic limit for TBC ≤ 100 000 CFU/ml corresponds with the values 4 000 CFU/ml for PLiBC and/or PPrBC. By statistical evaluation of the relation between log PBC and log PLiBC the correlation coefficient r = 0.89 in log PBC and r = 0.88 in log PPrBC was calculated while the values of correlation coefficients for mesophilic and psychrotrophic lytic bacteria were lower (log TBC and log PLiBC r = 0.69; log TBC and log PPrBC r = 0.68; Table 3), and all values of correlation coefficients were on a statistically highly significant level ($P < 0.001$).

The comparison of the microbial contamination of milk in relation to the housing technology (Table 4) indicated the lowest values of all groups of investigated microorganisms in the technology of loose cubicle littered housing (lcl) compared to the stanchion littered technology (sl) and loose slatted-floor litterless housing (lsl). A marked difference was observed mainly in TBC, where the average value of TBC in lcl housing was $1.6\ 10^4$ CFU/ml, in sl housing it was 2.8×10^4 CFU/ml and in lsl housing 4.1×10^4 CFU/ml (Table 4). A difference in ln TBC between the technologies was statistically highly significant ($P < 0.001$; Table 5). We also proved a statistically significant difference ($P < 0.01$; Table 5) in the contamination by psychrotrophic microorganisms (PBC) between the technology lcl (2.9×10^3 CFU/ml) and lsl (6.2×10^3 CFU/ml; Table 4). The average values of lipolytic (PLiBC) and proteolytic (PPrBC) bacteria were the lowest in technology lcl (Table 4) but this difference was significant only in the parameter PLiBC for technologies lcl and lsl (Table 5). Our results confirm the previous findings (Regula et al., 2002; Gonzalo et al., 2006) that loose cubicle littered housing is more favourable for the acquirement of milk of microbially higher quality compared to stanchion littered housing. Loose slatted-floor litterless housing

Table 3. Relations between the technological and physiological groups of bacteria of the whole set (n = 365)

Correlation coefficient r (ln TBC × ln PBC)	0.75***
Correlation coefficient r (ln PBC × ln PLiBC)	0.89***
Correlation coefficient r (ln PBC × ln PPrBC)	0.88***
Correlation coefficient r (ln TBC × ln PLiBC)	0.69***
Correlation coefficient r (ln TBC × ln PPrBC)	0.68***
Proportional index p_i (PBC/TBC)	0.18
Proportional index p_i (PLiBC/PBC)	0.21
Proportional index p_i (PPrBC/PBC)	0.25
Proportional index p_i (PLiBC/TBC)	0.04
Proportional index p_i (PPrBC/TBC)	0.04

***Significance level $P < 0.001$

Table 4. The values of total bacterial count of TBC, PBC, PLiBC, PPrBC (CFU/ml) in relation to selected factors

Variable		TBC		PBC		PLiBC		PPrBC		*n*
		mean	SD	mean	SD	mean	SD	mean	SD	
Technology of housing	loose cubicle littered (lcl)	15 718	10 753	2 906	3 659	654	1 263	849	1 820	165
	loose slatted-floor litterless (lsl)	40 692	31 449	6 203	9 519	1 087	1 230	1 179	1 662	39
	stanchion littered (sl)	27 519	29 638	5 226	7 691	1 063	1 903	1 197	1 991	161
Milking method	milking parlour	20 493	19 469	3 536	5 462	737	1 269	912	1 796	204
	in-stall milking pipeline system	27 519	29 638	5 011	7 691	1 063	1 903	1 197	1 991	161
Predipping	yes	14 250	8 637	2 853	3 585	654	1 041	821	1 601	92
	no	26 740	27 442	4 636	7 264	975	1 728	1 111	1 972	273
Grazing	yes	14 649	10 664	2 560	10 664	500	858	609	843	107
	no	27 301	27 766	4 862	7 279	1 039	1 783	1 215	2 156	258

was connected with the insufficient environmental hygiene and with the subsequent higher fouling of dairy cows, which was reflected, together with a failure to observe the hygienic principles of milking process, in the highest milk contamination by all groups of the investigated microorganisms. But the results cannot be generalised because this technology was used on one farm only in our investigation (Cd1). Nevertheless, the influence of deficiencies in herd management and milking on the microbial quality of milk was confirmed (Holm et al., 2004; Jayarao et al., 2004).

The influence of milking technology on the rate of microbial contamination of milk (Table 4) confirmed the finding (Gonzalo et al., 2006) that milking in a milking parlour is done under more hygienic conditions of milk acquirement compared to the in-stall milking pipeline system. Although the most problematic farm Cd1 belonged to the group of farms with milking in milking parlours, the farms with milking in milking parlours had lower average values of all studied parameters (TBC 2.0×10^4 CFU/ml; PBC 3.5×10^3 CFU/ml; PLiBC 7.3×10^2 CFU/ml; PPrBC 9.1×10^2 CFU/ml; Table 4) compared to farms with in-stall milking pipeline systems (TBC 2.8×10^4 CFU/ml; PBC 5.0×10^3 CFU/ml; PLiBC 1.1×10^3 CFU/ml; PPrBC 1.2×10^3 CFU/ml; Table 4). Statistical evaluation detected a significant difference only in TBC ($P < 0.05$; Table 6) while in PBC, PLiBC and PPrBC the difference was not significant. Contrary to generally used postdipping, predipping is carried out only in some dairy herds in Czech conditions. We compared the values of microbial contamination of milk on farms using predipping with those not applying this practice. Farms with predipping in the udder preparation had markedly lower average values of all groups of microorganisms (TBC 1.4×10^4 CFU/ml; PBC 2.9×10^3 CFU/ml; PLiBC 6.5×10^2 CFU/ml; PPrBC 8.2×10^2 CFU/ml; Table 4) compared to farms without predipping (TBC 2.7×10^4 CFU/ml; PBC 4.6×10^3 CFU/ml; PLiBC 9.8×10^2 CFU/ml; PPrBC 1.1×10^3 CFU/ml; Table 4). Statistical differences on a significance level $P < 0.001$ were proved for TBC; on a significance level $P < 0.05$ for PBC and PPrBC, and only for PLiBC the difference between predipping and no predipping was not significant (Table 6). Our results confirm data on the positive role of predipping in a reduction in TBC (Blowey and Collis 1992; Ingawa et al., 1992) and contamination of milk by psychrotrophic bacteria.

Table 5. Statistical differences in ln TBC, ln PBC, ln PLiBC, ln PPrBC in relation to the technology of housing

Parameter	ln TBC			ln PBC			ln PLiBC			ln PPrBC		
Housing	lcl	lsl	sl	lcl	lsl	sl	lcl	lsl	sl	lcl	lsl	sl
lcl		0.001	0.001		0.01	NS		0.05	NS		NS	NS
lsl	0.001		0.001	0.01		NS	0.05		NS	NS		NS
sl	0.001	0.001		NS	NS		NS	NS		NS	NS	

lcl = loose cubicle littered
lsl = loose slatted-floor litterless
sl = stanchion littered

Table 6. Statistical differences in ln TBC, ln PBC, ln PLiBC, ln PPrBC in relation to other factors

Parameter	ln TBC	ln PBC	ln PLiBC	ln PPrBC
Milking parlour × in-stall milking pipeline system	0.050	NS	NS	NS
Predipping × without predipping	0.001	0.050	NS	0.050
Summer grazing × without grazing	0.001	0.001	0.001	0.001

The last factor studied in relation to the microbial contamination of milk was the effect of summer grazing. Farms practising summer grazing proved a statistically highly significant difference ($P < 0.001$; Table 6) in all tested groups of microorganisms (average values: TBC 1.5×10^4 CFU/ml; PBC 2.6×10^3 CFU/ml; PLiBC 5.0×10^2 CFU/ml; PPrBC 6.1×10^2 CFU/ml; Table 4) from farms without summer grazing (average values: TBC 2.7×10^4 CFU/ml; PBC 4.9×10^3 CFU/ml; PLiBC 1.0×10^3 CFU/ml; PPrBC 1.2×10^3 CFU/ml; Table 4). The stay of dairy cows in pasture markedly contributes to the higher cleanness of dairy cows and their udders, and naturally to the lower microbial contamination of milk. Lower values of TBC in pasture management of dairy cows were also reported by McKinnon et al. (1990), Goldberg et al. (1992) and Regula et al. (2002). Our results document that besides the reduction in TBC in pasture management the contamination of milk by psychrotrophic bacteria and technologically risky proteolytic and lipolytic bacteria also decreases.

The rate of microbial contamination of milk by mesophilic and psychrotrophic bacteria is mainly influenced by the level of herd hygiene and by the observation of hygienic principles of milk acquirement and storage. The technology of loose cubicle littered housing with milking in a milking parlour, use of predipping in the udder preparation and especially summer grazing of dairy cows are considered as factors positively influencing the values of mesophilic, lipolytic and proteolytic bacteria in cow's raw milk.

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Received: 2007–06–11
Accepted after corrections: 2007–09–07

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