FACTORS NEGATIVELY INFLUENCING MICROBIAL CONTAMINATION OF MILK

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Abstract

The objective of this study was to analyse the influence of selected factors in relation to total bacterial counts (TBC) in bulk milk samples. Milk samples were tested in nine cowsheds of seven farms for a period of one year. The TBC values were determined by the automated estimation of bacterial counts in cow's raw milk by directly counting the bacterial cells on a BactoScan 8000 apparatus. The highest average values of TBC were determined in loose bedding-free slatted-floor housing ($42.122 \cdot 10^3 \cdot ml^{-1}$) while the difference in lnTBC compared to loose bedded cubicle housing (TBC average $7.354 \cdot 10^3 \cdot ml^{-1}$) and stanchion bedded housing (TBC average $10.735 \cdot 10^3 \cdot ml^{-1}$) was statistically highly significant ($p < 0.001$). We also proved a statistically highly significant difference in lnTBC ($p < 0.001$) between farms using predipping (TBC average $8.443 \cdot 10^3 \cdot ml^{-1}$) and farms without predipping (TBC average $14.518 \cdot 10^3 \cdot ml^{-1}$); the difference in lnTBC between farms with summer grazing (TBC average $10.04 \cdot 10^3 \cdot ml^{-1}$) and farms without grazing (TBC average $13.959 \cdot 10^3 \cdot ml^{-1}$) was statistically significant ($p < 0.05$).

Key words: cow's raw milk; total bacterial counts (TBC); factors influencing TBC.

INTRODUCTION

The values of microbial contamination of cow's raw milk are influenced by dairy cow's health and hygiene, by the hygiene of the environment where dairy cows are housed and milked, by methods of udder preparation and milking technique, methods of cleaning and sanitation of milking machines and milk cisterns, tenders' hygiene, speed of milk cooling to a required temperature and milk storage time (BRAMLEY and McKINNON, 1990).

By the analysis of dominant microflora in downgraded bulk milk (TBC > $3.0 \cdot 10^4 \cdot ml^{-1}$) HOLM et al. (2004) found out that 64% of samples contained microorganisms connected with poor hygiene; 28% of samples was contaminated by the microflora also connected with poor hygiene, and in addition, growing at low temperatures (psychrotrophic bacteria) and in 8% of samples bacteria connected with mastitis were predominant.

Based on TBC determination in milk, time consumption and costs RYŠÁNEK et al. (1993) considered the use of a disinfected terry cloth, dry terry cloth and individual terry cloth as the most effective method of udder preparation. To reduce the mastitis spread KRUZE (1998) recommended to use individual disposable tissue cloths for the udder wiping. In agreement with previous findings (SKRZYPEK et al., 2003) KAMIENIECKI et al. (2004) reported that the udder and teat cleaning with a dry cloth (contrary to their washing with water) was one of the factors leading to a decrease in TBC.

Teat disinfection before milking by predipping seems to be an important factor reducing TBC or somatic cell counts (SCC) in bulk milk samples (INGAWA et al., 1992). BLOWEY and COLLIS (1992), who tested the effect of predipping in a iodophore disinfectant, found that the occurrence of clinical mastitis was reduced by 57% and TBC were reduced by 70%. SIUGZDAITE et al. (2005) reported that the use of Dermisan and Profilaclopre solutions before milking resulted in a lower bacterial contamination of teats while the antiseptics did not influence the total bacterial contamination of milk and no inhibitory substances were found in milk. Predipping is significantly efficient in the prevention of new intramammary infections (RUEGG and DOHOO, 1997; OLIVER et al., 2001). Products for predipping contain a small amount of pliable-making additives that may reduce erosions on the teat (HEMLING 2002).

While predipping is done only on some farms in this country, teat disinfection after milking – postdipping is a normally used method of mastitis prevention and its positive effects on a SCC reduction in bulk milk samples were proved (JORDAN and FOURRAINE, 1993; JAYARO et al., 2004).

The environment of dairy cows significantly influences microorganism counts on teats and in milk. McKINNON et al. (1990) reported that dairy cows with obviously clean udders kept in a cowshed might contribute to contamination more than 10 000 CFU/ml milk whereas grazing dairy cows with clean teats might contribute less than 100 CFU/ml milk. A similar conclusion was drawn by COOK (2002), who explained this fact by highly contaminated bedding even though it might look relatively clean and dry. The stay of dairy cows in the pasture is also good from the aspect of a decrease in SCC values and a reduction in the risk of clinical mastitis (GOLDBERG et al., 1992; WAAGE et al., 1998). Lower values of TBC in grazing systems were also reported by GOLDBERG et al. (1992), REGULA et al. (2002) and KAMIENIECKI et al. (2004).

The evaluation of microbiological quality of milk in relation to the output yield of milk showed that farms producing more than 60 000 litres of milk per year, equipped with high-tech milking machines and cooling facilities, produce milk of the highest microbial quality (DANKOW et al., 2004). JAYRAO et al. (2004) also stated that herd size and management practices had a great influence on TBC and SCC in bulk milk samples.

Based on the study of the influence of dairy cow management technology and milking method on the level of microbial contamination of milk a system of loose bedded cubicle housing with milking in a milking parlour is preferred that is more suitable for the production higher-quality milk in microbial terms compared to stanchion housing and an in-stall milking pipeline system (REGULA et al., 2002; CEMPÍRKOVÁ, 2004; GONZALO et al., 2006).

The objective of this study was to analyse factors influencing TBC in bulk milk samples of cow's raw milk in nine cowsheds of seven farms in the course of a calendar year. We investigated mainly the influence of management technology, milking method, udder preparation method, and the influence of summer grazing and dairy cow herd size.

MATERIAL AND METHOD

In 2005 we examined total bacterial counts in bulk milk samples of cow's raw milk in nine cowsheds (sampling places) of seven farms situated in mountain and sub-mountain areas of Southern and Western Bohemia. In total 346 samples was examined for TBC. In four cowsheds (Vj; Cho; Hd; Zu1) loose bedded cubicle housing with milking in a milking parlour was used, and one cowshed (Cd1) had loose bedding-free slatted-floor housing with milking in a milking parlour. In another four cowsheds (Zu2; Cd2; Te; Ry) stanchion bedded housing with an in-stall milking pipeline system was used. On three farms (Vj; Te; Ry) summer grazing was practiced.

Farm Vj – South Bohemian Region, altitude 800 m; loose bedded cubicle housing (straw bedding); 120 dairy cows; dominant breed C (110 cows) and remaining cows of H breed (10 cows); average daily milk yield 19 l; tandem milking parlour (2 x 4), disinfection of milking machines – Demyro A, K; udder toilet – teat shower, wiping with a cloth (one cloth per 10 dairy cows), no predipping; postdipping with a barrier disinfectant Lactobarier; very good zoohygienic conditions; milk marketability 98%; individual somatic cell count (ISCC) determined 1x a month; mastitis prevention – Orbenin Dry Cow applied in the period of drying off; in the case of mastitis occurrence laboratory diagnostics of the originator including an antibiotic sensitivity test was done; grazing in 2005 from 4.5. to 27. 9.

Farma Cho – South Bohemian Region, altitude 520 m; loose bedded cubicle housing; 290 dairy cows; H breed is dominant (70%), the remaining cows are crossbreds H x C; average daily milk yield 16.1 l; milking parlour 2 x 10 De Laval; disinfection of milking machines with Despon A, K; udder toilet – warm shower followed by the wiping with a disposable tissue cloth of only dirty teats and by predipping, clean teats – predipping with the foam disinfectant Dermaline and subsequent wiping with a disposable tissue cloth; after milking – barrier disinfectant Filmadine; very good zoohygienic conditions; NK-tests performed 1x a month; ISCC is determined 1x a month as a part of performance testing; Orbenin Dry Cow is applied in the dry period; three weeks before parturition the vitamin preparation Duphafral is applied; 1x a week homoeopathics for mastitis prevention are added to feeding water; milk marketability 95%.

Farm Hd – South Bohemian Region, altitude 420 m; loose bedded cubicle housing in five sections; 120 dairy cows; dominant breed H (90%), C (10%); average daily milk yield 17.8 l; herringbone milking parlour (2 x 6); disinfection of milking machines with Mikal 94 D and Mikasan D; udder toilet – warm shower, wiping with a cloth (one cloth per about 20 dairy cows); no predipping; postdipping – barrier disinfectant Diemacid Direct; less good zoohygienic conditions during littering – high dust nuisance because a Jentil straw separator is used for littering, therefore it was imposed to litter when dairy cows are not in the cowshed); milk marketability 95%; NK-tests performed only in the case of suspicion of mastitis; ISCC determined 1x a month as a part of performance testing; Orbenin Dry Cow applied in the dry period; Axetocal applied to all cows 1x to 2x a year;

Farm Zu1 – South Bohemian Region, altitude 600 m; loose bedded cubicle housing (straw bedding); 315 dairy cows; dominant breed H (70 %), and C (30 %); average daily milk yield 19.80 l; herringbone milking parlour (2 x 10), disinfection of milking machines with Dosyl A, K in summer, Mikal 94 D and Mikasan D in winter; udder toilet – teat shower of dirty udders only, wiping with a disposable tissue cloth, teats of clean udders are wiped with a disposable tissue cloth Drycel soaked in a Triolet disinfection solution; postdipping – Filmadine in summer and Mikasan JD in winter; good zoohygienic conditions; milk marketability 95%; NK-tests performed irregularly; ISCC determined 1x a month; during drying off Orbenin Dry Cow applied to all cows, alternated with Mamin after six months; application of organic Se, Zn and vitamin E in DOVP mixture 1x a year.

Farm Cd1 – South Bohemian Region, altitude 410 m; loose bedding-free slatted-floor housing; 320 dairy cows; H breed; average daily milk yield 12.5 l; herringbone milking parlour 2 x 10; disinfection of milking machines – Bilo sp and Bilo rd-p; udder toilet – warm shower and wiping with a felt cloth (used for several dairy cows); no predipping; postdipping – Jodonal; zoohygienic conditions – less good; milk marketability 94%; NK-tests performed 2x a month; determination of individual somatic cell count (ISCC) 1x a month; Axetocal application to all cows 1x a year;

Farm Zu2 – South Bohemian Region, altitude 600 m; stanchion bedded housing; 50 dairy cows; C + H breed; average daily milk yield 14.74 l; in-stall milking pipeline system, disinfection of milking machines with Dosyl A, K in summer, Mikal 94 D and Mikasan D in winter; udder toilet – teat washing with warm water, wiping with a cloth (one cloth per 15 dairy cows); no predipping; postdipping – Filmadine in summer, Mikasan JD in winter; average zoohygienic conditions; milk marketability 80%; monthly incidence of clinical forms of mastitis in 7 to 15% of dairy cows; NK-tests and ISCC determination performed 1x a month; during drying off Orbenin Dry Cow applied to all cows, alternated with Mamin after six months; Zn, Se and vitamin E in DOVP mixture applied 1x a year.

Farm Cd2 – South Bohemian Region, altitude 410 m; stanchion bedded housing; 74 dairy cows; H breed; average daily milk yield 12 l; in-stall milking pipeline system; disinfection of milking machines – Bilo sp and Bilo rd-p; udder toilet – washing with warm water, wiping with a felt cloth (used for several dairy cows); no predipping; postdipping – Jodonal; less good zoohygienic conditions; milk marketability 94%; NK-tests performed 2x a month; ISCC determined 1x a month; Axetocal applied to all cows 1x a year;

Farm Te – West Bohemian Region, altitude 700 m; stanchion bedded housing; 146 dairy cows; C breed; average daily milk yield 12 to 15 l; in-stall milking pipeline system; disinfection of milking machines – Dosyl A, K; udder toilet – washing with warm water and wiping with a special cloth; no predipping; postdipping – Deosan Teat Care Plus; milk marketability – 95%; very good zoohygienic conditions; NK-tests performed in the case of suspicion of mastitis; ISCC determined 1x a month; during drying off Orbenin Dry Cow applied to all cows; grazing from 16. 5. to 14. 10. 2005.

Farm Ry – South Bohemian Region, altitude 650 m; stanchion bedded housing; 123 dairy cows; breed C (60%) and H (40%); average daily milk yield 18 l; in-stall milking pipeline system; disinfection of milking machines – Mikal A, K; udder toilet – in the non-grazing period teat washing with a 0.5 to 1% solution of Dermisan in warm water using a synthetic cloth, and wiping with an individual cloth; in the grazing season no predipping (Dermisan) is used, only washing with warm water and wiping with an individual cloth; postdipping – barrier disinfectant Filmadine; milk marketability – 95%; very good zoohygienic conditions; NK-tests only in dairy cows with high values of ISCC 1x a fortnight, after calving and at the change of milk; ISCC determined 1x a month; Naftpensal applied during drying off to cows with chronic mastitis; vitamin D2 applied at parturition; grazing from 27. 4. to 20. 11. 2005.

Sample collection and determination of TBC values were done by the company MADETA Agro a.s., accredited Central Laboratory in České Budějovice, and for one farm (Te) by MLEKOLAB s.r.o., accredited Central Laboratory in Pardubice. TBC were determined in accordance with the standard CSN 57 0539 (Automated Estimation of the Bacterial Count in Cow's Raw Milk by Direct Counting of Bacterial Cells) on a BactoScan 8000 apparatus.

The resultant TBC values were categorised as the percentage representation of results in intervals. Arithmetic means and standard deviation were calculated from the actual values and the range of variation was determined by Microsoft Excel 97. Statistical evaluation of data was done in Statistica ver. 6 programme. Before the statistical analysis the TBC values were logarithmically transformed (lnTBC) to approach normal distribution. As heteroscedasticity in the particular groups (comparison of farms, housing technologies, comparison according to the herd size) was significant, Kruskal-Wallis test was used. The traditional t-test was used for a comparison of two groups (milking parlour and in-stall milking pipeline system, use of predipping and without predipping, summer grazing and without grazing).

RESULTS AND DISCUSSION

The microbial contamination of milk on the particular farms expressed by the annual arithmetic mean (Tab. 1) shows that farm Cd1 had the highest average of TBC values ($42.122 \cdot 10^3 \cdot ml^{-1}$) in 2005; it also had the highest fluctuation of TBC actual values, and as the only farm it exceeded the permissible hygienic limit for TBC, i.e. $100 \cdot 10^3 \cdot ml^{-1}$, in 2.44% of samples (Tab. 3). On the contrary, farm Zu1 produced milk with the lowest microbial contamination with average TBC $5.447 \cdot 10^3 \cdot ml^{-1}$ (Tab. 1) while the particular actual values of TBC were steadily low (97.37% of the values < $10 \cdot 10^3 \cdot ml^{-1}$; Tab. 3). Kruskal-Wallis test proved a statistically highly significant difference in lnTBC in relation to the farm $H = 121.922$; $p < 0.001$. The multiple comparison of farms shows a statistically highly significant difference in lnTBC between farm Cd1 and all the other farms, between farm Zu1 and Cd2 ($p < 0.001$ to 0.005; Tab. 4), and a statistically significant difference ($p < 0.05$) between farm Cd2 and Te, and Cd2 and Ry (Tab. 4). The higher values of microbial contamination of milk on farm Cd1 were connected with the enormously soiled environment of the cowshed and dairy cow udders with faeces and with the insufficient drying of udders with a synthetic cloth after their premilking washing, i.e. with deficiencies in the hygiene of the cowshed and milking that were consequently reflected in TBC values (McKINNON et al., 1990; HOLM et al., 2004).

In line with previous findings (REGULA et al., 2002; CEMPÍRKOVÁ, 2004; GONZALO et al., 2006) lower values of the microbial contamination of milk were determined for farms with loose bedded cubicle housing compared to stanchion bedded housing. The farms with the technology of loose bedded cubicle housing had the annual average of TBC values $7.252 \cdot 10^3 \cdot ml^{-1}$ while this average on farms with stanchion housing was 10.739·10^3·ml^{-1}. The highest average annual value of TBC was found out on the farm with loose bedding-free slatted-floor housing: 42.122·10^3·ml^{-1} (Tab. 2). According to Kruskal-Wallis test the influence of the factor of housing on lnTBC was statistically highly significant H = 94.60284; $p < 0.001$. The multiple comparison of differences between the types of housing proved differences between loose bedded cubicle housing and loose bedding-free slatted-floor housing ($p < 0.001$; Tab. 5), and between stanchion bedded housing and loose bedding-free slatted-floor housing ($p < 0.001$; Tab. 5).

Farms with milking parlours, compared to farms with in-stall milking pipeline systems, paradoxically had the higher average value of TBC (Tab. 2) as a consequence of higher TBC values on farm Cd1, where the cows were also milked in a milking parlour. As for the influence of milking method on lnTBC, we could not prove a statistically significant difference at a 95% reliability level between groups with milking parlours and those with the in-stall milking pipeline system (Tab. 7). The positive influence of milking parlour on TBC values, proved in previous studies (REGULA et al., 2002; DANKOW et al., 2004), was negated in our observations by the farm with loose bedding-free slatted-floor housing (Cd1) where serious deficiencies in housing and milking hygiene were observed.

For the categorisation of farms according to the number of dairy cows we compared large farms with the number of cows > 150 head (Cho; Zu1; Cd1), medium-sized farms with 120 – 150 dairy cows (Vj; Hd; Te; Ry) and small farms with the number of cows < 120 head (Zu2; Cd2) in relation to the lnTBC values. Kruskal-Wallis test H = 8.632696; p = 0.0133 proved a statistically significant difference in lnTBC values in relation to the herd size. The multiple comparison of differences in lnTBC in relation to the herd size proved a statistically significant difference between small and large farms ($p < 0.001$; Tab. 6) and between small and medium-sized farms ($p < 0.001$; Tab. 6). The influence of the factor of herd size on TBC values should be evaluated carefully, which is documented by the average values of TBC in the group > 150 cows if the technology of housing were also considered (Tab. 2). In this case in the category of herds > 150 cows the lowest values of TBC would be measured in herds with loose bedded cubicle housing (7.354·10^3·ml^{-1}) while the highest average value would be recorded in the herd with loose bedding-free slatted-floor housing (42.122·10^3·ml^{-1}). The influence of herd size on the microbiological quality of milk should always be evaluated along with management practices (DANKOW et al., 2004; JAYRAO et al., 2004).

The farms using predipping and at the same time disposable cloths for udder toilet (Cho; Zu1; Ry) had the lower average value of TBC (8.443·10^3·ml^{-1}; Tab. 2) compared to the other farms (14.518·10^3·ml^{-1}; Tab. 2) that did not apply predipping and used one cloth for several dairy cows. The test for the presence of inhibitory substances in milk was negative on all studied farms. The effect of predipping is instantaneous, reflected in a decrease in the microbial contamination of milk (BLOWEY and COLLIS, 1992) and on the teat skin (SIUGZDAITE et al., 2005). The analysis of lnTBC of both groups (farms using predipping and farms without predipping) proved a statistically significant difference between the groups ($p < 0.001$; Tab. 7). Our finding confirmed a previous report (BLOWEY and COLLIS, 1992; INGAVA et al., 1992; HEMLING, 2002) on the positive influence of predipping on a decrease in the microbial contamination of milk. The application of predipping was connected with the use of individual cloths for udder toilet, and their use also leads to a decrease in the microbial contamination of milk and reduces especially the risk of mastitis spread (KRUZE, 1998). The use of one cloth for several dairy cows is risky from the aspect of mastitis prevention and spread.

The influence of summer grazing on TBC values was the last of the evaluated factors. Grazing was used on three farms (Vj; Te; Ry). The average value of TBC on these farms was 10.04·10^3·ml^{-1} while the average values of TBC determined on farms without grazing amounted to 13.959·10^3·ml^{-1} (Tab. 2). Lower values of TBC measured in grazing systems of dairy cow management were also reported by McKINNON et al. (1990), GOLDBERG et al. (1992), COOK (2002), and REGULA et al. (2002). The evaluation of significance of the difference in lnTBC between the group of farms with grazing and the group of farms without summer grazing showed a statistically significant difference between both groups ($p < 0.05$; Tab. 7). Grazing contributes to better cleanness of teats compared to cows kept in cowsheds (McKINNON et al., 1990) because bedding is always highly contaminated even though it may look clean and dry (COOK, 2002).

Based on our survey the factors negatively influencing the resultant microbial contamination of cow's raw milk are the system of loose bedding-free slatted-floor housing that was connected with deficiencies in herd and milking hygiene, the system where neither predipping nor individual cloths are used for udder toilet, and the system of dairy cow management without grazing.

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Tab. 1. : Total bacterial counts (TBC$\cdot 10^3 \cdot ml^{-1}$) in bulk milk samples on the examined farms in 2005

milking	milking parlour					in-stall milking pipeline system			
housing	loose bedded cubicles				*lbfsf	stanchions			
farm	Vj	Cho	Hd	Zu1	Cd1	Zu2	Cd2	Te	Ry
N	41	43	41	38	41	39	43	23	37
mean	6.61	8.558	8.195	5.447	42.122	8.872	10.14	16.304	9.946
S.D.	5.678	7.124	8.699	1.831	75.402	6.607	7.684	20.025	18.324
maximum	34	35	54	16	485	29	30	82	116
minimum	5	5	5	5	5	5	5	6	5

*lbfsf - loose bedding-free slatted floor

Tab. 2. : The values of total bacterial counts (TBC$\cdot 10^3 \cdot ml^{-1}$) in relation to selected factors

variable			mean	S.D.	*N*
technology of housing	loose bedded cubicles (lbc)		7.252	6.546	163
	loose bedding-free slatted floor (lbfsf)		42.122	75.402	41
	stanchions (s)		10.739	13.732	142
milking method	milking parlour		14.26	37.043	204
	in-stall milking pipeline system		10.739	13.731	142
herd size *(number of dairy cows)*	> 150		18.869	46.956	122
		> 150 (lbc)	7.354	5.984	81
		> 150 (lbfsf)	42.122	75.402	41
	120 - 150		9.507	13.93	142
	< 120		9.537	7.22	82
predipping	yes		8.443	12.317	97
	no		14.518	34.153	249
grazing	yes		10.04	15.528	101
	no		13.959	33.944	245

Tab. 3. : Interval distribution of the values of total bacterial counts (TBC$\cdot 10^3 \cdot ml^{-1}$) in bulk milk samples, 2005

farm	TBC < 10	11 - 30	31 - 50	51 - 70	71 - 100	101 - 200	201 - 300	301 - 400	> 400	*N*
Vj	92.68	4.88	2.44							41
Cho	79.07	18.60	2.33							43
Hd	82.93	14.63		2.44						41
Zu1	97.37	2.63								38
Cd1	24.39	43.90	12.19	4.88	9.76	2.44			**2.44**	41
Zu2	74.36	25.64								39
Cd2	67.77	30.23								43
Te	69.56	17.39		8.70	4.35					23
Ry	83.78	13.52				2.70				37

Tab. 4. : Statistical differences in lnTBC in relation to the farm

farm	Vj	Cho	Hd	Zu1	Cd1	Zu2	Cd2	Te	Ry
Vj		ns	ns	ns	< 0.001	ns	ns	ns	ns
Cho	ns		ns	ns	< 0.001	ns	ns	ns	ns
Hd	ns	ns		ns	< 0.001	ns	ns	ns	ns
Zu1	ns	ns	ns		< 0.001	ns	< 0.005	ns	ns
Cd1	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	< 0.005	< 0.001	< 0.001
Zu2	ns	ns	ns	ns	< 0.001		ns	ns	ns
Cd2	ns	ns	ns	< 0.005	< 0.005	ns		< 0.05	< 0.05
Te	ns	ns	ns	ns	< 0.001	ns	< 0.05		ns
Ry	ns	ns	ns	ns	< 0.001	ns	< 0.05	ns	

Tab. 5. : Statistical differences in lnTBC in relation to the technology of housing

housing	lbc	lbfsf	s
lbc		< 0.001	ns
lbfsf	< 0.001		< 0.001
s	ns	< 0.001	

lbc - loose bedded cubicles
lbfsf - loose bedding-free slatted floor
s - stanchions

Tab. 6. : Statistical differences in lnTBC in relation to the herd size

N	> 150	120-150	< 120
> 150		ns	< 0.001
120-150	ns		< 0.001
< 120	< 0.001	< 0.001	

N - number of dairy cows

Tab. 7. : Statistical differences in lnTBC in relation to other factors

milking parlour x in stall milking pipeline system			ns
predipping x without predipping			< 0.001
summer grazing x without grazing			< 0.05