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Are bedding materials a source of useful microorganisms for dairy cow and ewe milk?

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Abstract. Milk microbial diversity contributes to the sensorial diversity of raw milk cheeses. The milking is an important step for milk inoculation through teat skin, biofilm on the milking machine, water, air and animal environment. This study aims at evaluating microbial count of bedding materials usual in dairy cow and ewe farms. Four bedding materials (straw, mat, grass, slatted floor) from 48 farms in four French mountain areas of PDO cheese production were studied. Bedding surfaces, teats and bulk milk were sampled in winter (housed full-time) and summer (pasture) for cows, and during the grazing season for ewes. Microbial groups were counted on five culture media. For housed animals the microbial count (95 to 99% of ripening bacteria) of bedding material for cow and ewe was similar. In ewe farms, all microbial levels were higher on straw bedding than on slatted floor, and ripening bacteria levels on teat surface and in milk were significantly the highest with straw. On cow pasture surface, the ripening bacteria were less dominant (62%) than in winter and Gram negative bacteria (22%), yeasts (9%) and moulds (7%) proportions were higher. The season and the type of bedding material had similar effect on the teat skin but not in the milk microbial counts. The microbial flows from bedding to teat and teat to milk must be better understood to advise dairy farmers suggesting microbial transfer between bedding and teats.

Keywords. Bedding material – Teat skin – Microbial community – Raw milk.

Les litières sont-elles une source de micro-organismes utiles pour le lait de vache et de brebis ?

Résumé. *La diversité microbienne du lait contribue à la diversité sensorielle des fromages au lait cru. La traite est une étape importante pour l'inoculation du lait par la peau du trayon, le biofilm de la machine à traire, l'eau, l'air et l'environnement. Cette étude vise à évaluer les niveaux microbiens des litières de fermes laitières bovines et ovines. Quatre supports de couchage (paille, tapis, herbe, caillebotis) issus de 48 fermes de quatre zones fromagères françaises de montagne en AOP ont été étudiées. Les litières, les trayons et le lait ont été échantillonnés en hiver (logement en bâtiments) et en été (pâturage) pour les bovins, sur une saison à l'herbe pour les brebis. Les groupes microbiens ont été dénombrés sur cinq milieux de culture. Les niveaux microbiens (bactéries d'affinage>95%) des litières des animaux logés en bâtiment, dans les fermes bovines et ovines étaient semblables. En exploitation ovine, tous les niveaux microbiens étaient plus élevés sur les litières paille que sur les caillebotis et les niveaux de bactéries d'affinage sur la peau des trayons et dans le lait étaient significativement plus élevés avec la paille. Sur la surface de pâturage, ces bactéries étaient moins dominantes (62%) qu'en hiver au profit des bactéries à Gram négatif (22%), des levures (9%) et des moisissures (7%). Les effets de la saison (en vache) et litières sont aussi significatifs sur les niveaux microbiens des trayons, mais pas sur ceux du lait, suggérant des transferts microbiens entre litière et trayons.*

Mots-clés. *Litière – Peau des trayons – Communauté microbienne – Lait cru.*

I – Introduction

Milk in udder cells of a healthy lactating female is sterile. The composition of the milk microbiota depends on microbial sources directly in contact with the milk: the animal's teat (Vacheyrou *et al*., 2011; Verdier-Metz *et al*., 2012) and dairy equipment such as milking machine (Laithier *et al*., 2004) and tank. It also depends on the composition of the microbial indirect sources (feed, litter, drinking and washing water, stable and milking parlour air, milker (Montel *et al*., 2014). As there is little literature about the role of bedding material as sources of milk inoculation, this study aims at evaluating microbial composition of bedding materials usual in dairy cow and dairy ewe farms as reservoir of micro-organisms for milk. Preserving microbial diversity in milk at sufficient level is an important goal for traditional cheese production, since it plays a major role in the making of taste and typicality of raw milk cheeses.

II – Materials and methods

The study was carried out from January 2011 to July 2012 in 36 cow and 11 ewe farms in four French mountain areas producing PDO cheese (Pyrénées for ewe; Auvergne, Franche-Comté and Alpes, for cows). The cow farms' were selected according to the housing type: tie (18 farms) or free stalls (18 farms) and the bedding material: with straw (24 farms) or without straw (12 farms) and they were sampled at two periods: in winter, when animals housed full-time and in summer, at the pasture. Ewe farms' were chosen only according to bedding material: with straw (7 farms) and stalled floor (4 farms). Ewes pastured all the year but housed in the sheepfold (open housing) during the night. Bedding materials and teat surfaces were taken before morning milking and bulk tank milk at the end of milking. Cow teat surfaces were sampled before cleaning with a sterile swab as described by Monsallier *et al*. (2012) and ewe teat surfaces with sterile tips. Sampling was repeated 4 to 6 times in each farm.

Samples were plated on Plate Count Agar (PCA) medium with milk for total bacterial count; on PCA with Gram-positive inhibitor (0.1% cristal violet, 0.05% vancomycin) added for presumed Gram negative bacteria; on Cheese Ripening Bacterial Medium (CRBM) for presumed ripening bacteria (Gram positive and catalase positive bacteria, G+C+ bacteria), on Man Rogosa Sharp medium (MRS) for lactic acid bacteria, on Oxytetracyclin Glucose Agar medium (OGA) for yeasts and moulds as previously described (Verdier-Metz *et al*., 2012). The results are expressed in $\log_{10}$ cfu/cm^2 for bedding, /2 teats for teat and /mL for milk.

The effects of season, the types of housing and bedding material on the microbial levels and their interactions were evaluated by analysis of variance using XLStat© and Statistica Softwares. The Fisher test was used to compare difference in the means. Relationships between microbial counts were studied using the Pearson's correlation coefficient.

III – Results and discussion

1. Microbial characteristics of bedding materials

For cow and ewe (Tables 1 and 2), microbial count of bedding material was dominated by ripening bacteria (95 to 99% of total population). Lactic acid bacteria (1 to 3%) and the Gramnegative bacteria (0.1 to 2%) were at lower level. The levels of yeasts and moulds were the lowest. For all microbial groups, except for lactic acid bacteria, in both cow and ewe, and for ripening bacteria in cow, counts were significantly higher on straw bedding than on mat or slatted floor. Straw bedding material presented significantly more total population (+0.6 $\log_{10}$ cfu/cm^2 and +0.9 $\log_{10}$ cfu/cm^2 respectively for cow and ewe), Gram negative bacteria (+0.6 $\log_{10}$ cfu/cm^2 and +0.7 $\log_{10}$ cfu/cm^2), yeasts (+1.1 $\log_{10}$ cfu/cm^2 for cow) and moulds (+0.8 $\log_{10}$ cfu/cm^2 for cow) than bedding material without straw.

Ripening bacteria were dominant on cows' pasture, but at a lower level than in winter (62% total population), whereas lactic acid bacteria were less than 1% of total bacteria and yeasts and moulds raised up to 9% of population (Table 1).

Table 1. Mean value (± standard deviation) of microbial count (log_{10} cfu/cm^2) of: (i) straw and mat bedding in winter; and (ii) winter bedding and pasture in summer for cow

Seasons	Winter				Summer	
Bedding materials	**Straw (n = 30)**	**Mat (n = 30)**	**Straw *vs* without straw**	**Winter bedding (n = 60)**	**Grass (n = 52)**	**Winter bedding *vs* pasture**
Total count	6.5 ± 0.8	5.9 ± 0.9	**	6.2 ± 0.9	5.1 ± 0.8	***
Lactic acid bacteria	4.3 ± 1.6	3.8 ± 1.2	NS	4.0 ± 1.4	2.3 ± 0.9	***
Ripening bacteria	6.3 ± 1.1	5.9 ± 1.0	NS	6.1 ± 1.1	4.1 ± 1.1	***
Gram negative bacteria	3.6 ± 1.0	3.0 ± 0.9	**	3.3 ± 1.0	3.6 ± 0.9	*
Yeasts	3.4 ± 1.3	2.3 ± 1.2	***	2.9 ± 1.3	3.2 ± 1.6	**
Moulds	2.6 ± 0.9	1.8 ± 0.9	***	2.2 ± 1.0	3.1 ± 1.0	***

*P<0.05, **P<0.01, ***P<0.001, NS: non significant.

Table 2. Mean value ± standard deviation of microbial count (log_{10} cfu/cm^2) straw and slatted floor ewes' bedding. Comparison between bedding materials counts was made

Bedding materials	Straw (n = 42)	Slatted floor (n = 24)	Straw *vs* stalled floor
Total count	4.8 ± 1.0	3.9 ± 0.9	**
Lactic acid bacteria	1.4 ± 0.6	1.2 ± 0.6	NS
Ripening bacteria	5.4 ± 0.6	4.8 ± 0.9	**
Gram negative bacteria	2.6 ± 0.6	1.9 ± 0.6	***
Yeasts and Moulds	1.9 ± 0.6	1.4 ± 0.6	**

*P<0.05, **P<0.01, ***P<0.001, NS: non significant.

2. Relationships between microbial characteristics of bedding, teat and milk

A. From bedding to teat

As for bedding material, teat surface was dominated by ripening bacteria; 95% of total bacterial count (6.58 log_{10} cfu/2 teats). Lactic acid bacteria (4.42 log_{10} cfu/2 teats) and the Gramnegative bacteria (3.82 log_{10} cfu/2 teats) were at lower level. Yeasts and moulds have the lowest level (3.00 log_{10} cfu/2 teats). As already observed on the bedding material, the levels of microbial counts on the teat surface were higher in winter (cows housed full-time) than in summer (grazing cows). The levels of total population, ripening bacteria and lactic acid bacteria on the teat skin were 0.7 to 1 log_{10} cfu/2 teats higher in winter than in summer. This result can be explained by the cow claustration in stall during winter.

In winter the level of ripening bacteria in bedding material specially in tie stall with straw was well correlated with those on teat surfaces (r = 0.80).The level of lactic acid bacteria on teat was correlated with that in tie stall (r = 0.77) and yeast level on teats and in free stall, especially without straw, was also correlated (r = 0.74). The microbial count on pasture and teat surfaces were weakly correlated (r<0.57) except that of yeasts (r = 0.66).

All the teat surface counts of ewes in farms with slatted floor were lower than those with straw, but only the difference in ripening bacteria level was significant ($p<0.05$). The ripening bacteria count on teat were correlated with that on straw ($r = 0.5$) and lactic acid bacteria level was correlated with that on slatted floors ($r = 0.5$).

B. From bedding to milk

On cow farms, there was no significant difference (<0.5 cfu/ml) between milk microbial counts in summer *versus* winter, in free stall *versus* tie stall and on bedding with straw *versus* without straw. The level of ripening bacteria in milk and teat surface was correlated in farms using straw ($r = 0.44$).

On ewe farms, milk from farms using straw as bedding had significantly higher ripening acid bacteria level ($p<0,001$) than that of farms using slatted floors. There was no significant correlation between the microbial levels of milk and of bedding surfaces. However, ripening bacteria levels for both material beddings were correlated with those of milk ($0.5<r<0.6$). The highest correlation was observed with the level of ripening bacteria on farms using straw ($r = 0.44$).

IV – Conclusions

Bedding materials are a source of ripening bacteria potentially useful in cheesemaking. Straw in cow farms, as well as in ewe farms, can be interesting since its microbial level was higher than mat or stalled floors. Levels of few microbial groups from bedding and teat surfaces were correlated, but no corelation between bedding surfaces and milk count was found. It remains to understand why microbial balance in bedding surface, source of ripening bacteria and milk differ and to determine if some strains from bedding transfer to milk. The role of microbial biofilm of milking for the enrichment of the milking should be deeper studied.

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