Variability of microbial teat skin flora in relation to farming practices and individual dairy cow characteristics

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ORIGINAL PAPER

Variability of microbial teat skin flora in relation to farming practices and individual dairy cow characteristics

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Abstract This study is the first that assessed the influence of farming practices and individual cow characteristics on a large number of microbial groups ($n=10$) and cow samples ($n=192$). Its aim was to establish how farming practices and intrinsic characteristics of dairy cows can influence the microbiota on teat skin. Microbial flora of 96 cow teat skin from 16 farms, sampled during milking and before washing, was counted on ten dairy-specific media. Gram-positive catalase-positive bacteria including coagulase-negative staphylococci, were at high level on teat skin (4.7 ± 1.5 log cfu.mL^{-1}) whereas lactobacilli, enterococci, Gram-negative bacteria, moulds and yeasts were at a level below 3 log cfu.mL^{-1}. Gram-positive catalase-positive bacteria and yeasts were lower in heifers and when milking hygiene practices were intensive. Higher *Lactobacillus* and *Enterococcus* counts were linked to a silage-based diet, free stalls with straw bedding and moderate milking hygiene but also to multiparous cows. This study showed that dairy cow characteristics could interact with farming practices to affect the counts of microbial flora on teat skin. It offered prospects to better control teat microbial balance taking into account the milking hygiene practices, the parturition and the type of animal housing.

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养殖方式和奶牛个体特征对牛乳头皮肤上微生物菌群的影响

摘要 ：本文首次评价了养殖方式和奶牛的个体特征对微生物的菌群(10)和奶牛(192)的影响。该研究的目的是确立养殖方式和奶牛固有的特征是如何影响奶牛乳头皮肤上微生物菌群的。来自16个牧场的96头奶牛的乳头皮肤上微生物在10种不同的微生物培养基上进行培养并计数，分别在乳头洗涤之前和挤奶过程中取奶样。实验结果表明：奶牛乳头皮肤上有大量的革兰氏阳性过氧化氢阳性菌，如凝固酶阴性葡萄球菌(4.7±1.5 log cfu.mL^{-1})，然而，乳酸杆菌，肠球菌，革兰氏阴性菌，霉菌和酵母的数量低于3 log cfu.mL^{-1}。对于小母牛来说，当挤奶卫生条件较好时，革兰氏阳性过氧化氢阳性菌和酵母菌的数量较低。高数量的乳酸菌(*Lactobacillus*)和肠球菌(*Enterococcus*)与饲喂奶牛的青贮饲料、散草堆底层草、挤奶卫生条件以及多胎次奶牛有关。这些研究表明奶牛的特征和养殖方式相互一起影响了奶牛乳头皮肤上微生物菌群的数量。本研究在考虑到奶牛挤奶的卫生条件、奶牛的分娩以及奶牛的圈养方式的情况下，对更好的控制奶牛乳头皮肤上微生物平衡提供了较好的发展前景

Keywords Teat skin · Microbial count · Farming practices · Cow characteristics

关键词 乳头皮肤· 微生物数量· 养殖方式· 奶牛特征

1 Introduction

In spite of some bans on raw milk cheese, these cheeses constitute an important economic niche, especially in Europe due to consumer interest for their distinctive flavour, to their role in rural development and land use and human values that they drive (Licitra 2010). The sensory qualities of ripened raw milk cheeses are determined by the initial microbial and biochemical characteristics of the milk and their changes during manufacturing and ripening. Milk microbial diversity contributes to the diversity of sensory properties in raw milk cheeses (Beuvier and Buchin 2004; Callon et al. 2005) and it can also inhibit the growth of pathogens in cheese (Millet et al. 2006). In France, the milk price paid to farmers is calculated according to total flora count, which must be as low as possible (70,000 cfu.mL^{-1}), with specific criteria for pathogenic bacteria (EC regulation no. 852-853/2004).

Therefore farmers tend to produce milk with low microbial flora, which leads to a decrease in levels of pathogenic bacteria but also in those of the microbial flora of interest in cheesemaking.

Milk in udder cells is sterile (Tolle 1980) but it can be inoculated with microorganisms during its passage through the teat canal (Gill et al. 2006). Biofilms on the milking machine (Laithier et al. 2005), water, air and animal environment, teat skin (Vacheyrou et al. 2011) and udder skin (Brisabois et al. 1997) can also be sources of milk inoculation.

Raw milk cheese producers have become aware that a change of strategy is needed to preserve the diversity of raw milk cheeses. Indeed, raw milk cheese is characterised by high species diversity belonging to lactic acid bacteria, ripening bacteria (*Corynebacteriaceae*, non-pathogenic *Staphylococcaceae* and *Micrococcaceae*), yeasts and moulds (Quigley et al. 2011) having an interest for cheesemaking.

A better understanding of the sources of microbial diversity in milk in relation to milk production practices on dairy farms may help to achieve this goal. Teat skin has already been described as a potential reservoir of microbial diversity for milk (Michel

et al. 2006). But only the study of Vacheyrou et al. (2011) described the microbial community naturally present on dairy cow teat skin in link with milk and other microbial farm communities. Other studies have focused mainly on detecting and finding ways to eliminate pathogenic bacteria responsible for human disease (Marino et al. 2000) or mastitis (Gibson et al. 2008; Supré et al. 2011), or undesirable bacteria involved in sensory defects in cheeses (Sorhaug and Stepaniak 1997). Few studies have suggested the importance of individual cow characteristics in the variability of bacterial counts on teat skin (Rendos et al. 1975). To take advantage of the natural microbial flora on teats, i.e. to maintain the flora of interest for cheesemaking while eliminating pathogenic bacteria, it is necessary to improve knowledge of this flora and its variation factors. The aim of this study was to establish how farming practices and dairy cows' individual characteristics can influence the composition of the microbial community on teat skin.

2 Materials and methods

2.1 Data collection of farm and animal characteristics

The study was conducted in 16 dairy farms (herd size ranging from 25 to 50 cows) selected in the Cantal department, Massif Central, France, by the departmental dairy performance monitoring service. These farms were representative of Cantal dairy herds with regard to cow numbers and cattle housing, feeding and milking practices. They were characterised by the housing type (free stalls or tie) and the type of bedding (straw or mats). The Holstein or Montbeliarde cows were fed on hay and silage or wrapping. Milking equipment were mostly parlour and pipelines.

Each farm was visited twice, at an interval of 1 week, between February and April 2009, when the cows were housed full-time. A survey was carried out on each farm at each visit, during milking time. Questions were asked and observations were made about (1) herd characteristics (milk quota, dairy cow numbers and breed), (2) the type of feed used in winter, (3) housing system (type of barn, number of stalls and bedding material) and (4) milking system (type of milking parlour and number of clusters). Special attention was paid to milking hygiene practices. According to the methodology detailed by Michel et al. (2006), six criteria describing the teat cleaning method carried out by the farmers were recorded from 0 to 2 as explained in Table 1. The quality of the milking hygiene practices was defined by the sum of the scores (between 0 and 12) for these six criteria. Using this score (Table 1), the farms were classified for milking hygiene practice as either “intensive” (score>8), “moderate” (score>4 and ≤8), or “non-intensive” (score≤4).

To study the dairy cows' characteristics, a visual assessment made by the scientist visiting the farms gave a score for udder and teat shape by a description of individual cows' udder and teat morphologies adapted from the method used by the Montbéliarde breed organisation (Organisme de Sélection de la Race Montbéliarde, in Roulans, France; http://www.montbeliarde.org/pdf/POINTAGE.pdf). Prim Holstein and Montbéliarde cows were tested. The individual cow's data were supplied by the departmental dairy performance monitoring service (parity, monthly cow SCCs and occurrence of mastitis were recorded). Teat skin health was assessed by the scientist

Table 1 Criteria for notation of teat cleaning practices

Criteria	1 Teat washing	2 Pre-dipping with disinfectant solution	3 Type of towel	4 Solution for towel	5 Teat drying	6 Post-milking disinfection
	Always=2	Yes=2	Paper=2	Water+soap=2	Paper=2	Always=2
	Sometimes=1	No=0	Individual towel cloth=1.5	Only water=1	Towel=1	Sometimes=1
	Never=0		Collective towel cloth=1	No use=0	No use=0	Never=0
			Dry towel=0.5			
			No use=0			

The sum of score for these six criteria (Σ) was calculated and teat hygiene practices were classified in three groups: $\Sigma \leq 4$=non-intensive, $4 < \Sigma \leq 8$=moderate, $\Sigma > 8$=intensive

by observations adapted from the guidelines for evaluating teat skin condition (http://nmconline.org/docs/teatskincondguide.pdf). A teat skin was considered as healthy when there were no cutaneous damage (abrasions, cuts and frostbite) and chaps.

2.2 Sample collection

At each visit, teat samples were taken from six randomly chosen cows per farm during the milking. The same cows were sampled at both visits. The anterior right and posterior left teats were sampled before pre-milking udder preparation by the farmer using one sterile swab per cow (ECOLAB Dermasoft) moistened with 5 mL of sterile NaCl (9 $g.L^{-1}$)–Tween 80 (1 $g.L^{-1}$) solution. After sampling, each teat swab was placed in an individual stomacher bag (BagFilter, Interscience, St. Nom la Bretèche, France) with 10 mL of NaCl–Tween 80 solution with 0.5% sterile milk added (Lait G, Standa industrie, Caen, France). Sterile gloves were used throughout the sampling procedures. In all, the 96 cows sampled provided 192 individual teat samples (16 farms×6 cows×2 visits). Each teat swab was stored at 4 °C during 12 h maximum until blending for 4 min with a stomacher (Bag System Interscience St. Nom la Bretèche, France). Individual teat swab suspensions were extracted, frozen with 10% glycerol added and kept at −20 °C until analysis.

2.3 Microbial analyses

After thawing at 25 °C, the teat suspensions were used for all microbial analyses. Appropriate dilutions of the teat suspensions were plated on different selective culture media (Millet et al. 2006). Since the media used were more or less specific for milk and cheese, the microbial flora enumerated were presumed to be as follows: total flora count on Plate Count Agar (PCA); Gram-negative bacteria on PCA with Gram-positive inhibitor (0.1% cristal violet, 0.05% vancomycin) added; coliforms on Violet Red Bile Lactose agar (VRBL); yeasts and moulds on Oxytetracyclin Glucose Agar medium; facultative heterofermentative lactobacilli on FH agar

medium; dextran-producing leuconostocs on Mayeux-Sandine-Elliker agar; enterococci on Slanetz and Bartley agar; ripening bacteria (Gram-positive and catalase-positive bacteria, G+C+bacteria) on Cheese-Ripening Bacterial Medium (CRBM); coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS) on Rabbit Plasma Fibrinogen agar and *Pseudomonas* on Cetrimin–Fucidin–Cephalosporin media plates. Ready-to-use media were purchased from Biokar Diagnostics (Biokar Diagnostics, Beauvais, France).

All microbial analyses were performed in duplicate using a Spiral Plater (Interscience, St. Nom la Bretèche, France), except the VRBL counts which were inoculated in the mass of the medium.

The Gram KOH technique according to Powers (1995) was performed on the CRBM colonies to differentiate Gram-positive bacteria from Gram-negative. On the same colonies, ability of bacteria to produce catalase was also assessed by using H_2O_2 30% (*w*/*w*). Then the Gram-positive catalase-positive bacteria (G+C+bacteria) on CRBM medium was counted and integrated in the statistical analysis (detection limit between 30 and 300 cfu.mL^{-1}).

2.4 Statistical analyses

Microbial data were converted to log10 and the two samplings on the same cows during the two visits were considered separately.

In total, we analysed twice the teat skin microflora of 96 cows reared in 16 farms. We classified the farms according to their practices and we classified the animals according to their characteristics.

To classify the farms, forage and barn type, bedding material and quality of milking hygiene practice were used as variables in a Multiple Correspondence Analysis (MCA) made with Statistica 8.1 software (StatSoft, 2000, Tusa, USA). An Ascending Hierarchical Classification (AHC) using Euclidean distances and Ward's method was then performed on the position of each farm on the first four axes of the MCA which represented 91% of variance. The dendrogram obtained made it possible to significantly display three groups at a threshold aggregation distance of less than 40. This analysis was followed by K-means clustering to establish the farm characteristics of the three groups. The Chi-square test was performed to assess their differences.

To classify the 96 cows, the parity, teat/hock position, teat length and teat shape were used as variables in a MCA. An AHC was then performed on the position of each cow on the first four axes of the MCA (87% of variance).

Then the statistical analysis of the data set for teat skin microbial composition was performed using the Mixed procedure of SAS version 8.6 (SAS Institute Inc., Cary, NC, 2003). Dextran-producing leuconostocs and CPS were not included in the statistical treatments because their counts were below the detection limit (<10 cfu.mL^{-1}). We used the groups of farms and animals as factors in an ANOVA in order to test both the effects of farming practices (groups of farms) and cows characteristics (groups of cows) and their interactions on teat skin microflora composition. The model included farm group, cow group, interaction between cow group and farm group as fixed effects, the individual cow within farm as random effect and the visit as repeated measure. Significance was declared at $P \leq 0.05$ and trends were considered at $0.05 < P \leq 0.10$. The estimation method was “REML” and

the covariance structure was "Compound Symetry". Values reported in the results (Table 4) are the estimates and standard error of the mean (marked SEM).

3 Results

3.1 Discrimination of farms by farming practices

The three groups of farms were distinguished as indicated in Table 2. The groups FA (six farms) and FC (five farms) were mainly composed of Montbéliarde cows fed with hay and tied in stalls whereas the group FB (five farms) was composed entirely of Holstein cows fed with silage (corn and grass) and housed in free stall with straw bedding. All FA farms applied "intensive" milking hygiene practices according to the criteria defined in Table 1 and checked for mastitis by eliminating first milk ejection. Farms of group FB used "moderate" milking hygiene practices: all always cleaned teats before milking and performed post-milking disinfection but they did not systematically wipe teats. The milking hygiene practices of group FC were "non-intensive" and the mastitis were never checked.

3.2 Classification of dairy cow

The 96 dairy cows were classified according to their characteristics to assess the effect of the cow's characteristics on teat skin microbial composition. Three groups of dairy cows were obtained (Table 3). All selected dairy cows were healthy as 90% had healthy teat skin (data not shown). The individual cow's somatic cell counts were under 200,000 cells.mL^{-1} for 77% of the cows commonly accepted as threshold for intramammary infection (De Vliegher et al. 2003). Moreover, the departmental dairy performance monitoring service did not indicate clinical mastitis in cow studied and the scientist did not suspect mastitis during the period of the study. The cows of group 1 (C1=27 cows) were mainly primiparous (93%), with udders above hock level, short (41%) or normal (52%) teat length and normal teat shape. The cows of group 2 (C2= 34 cows) were multiparous (3/4 of cows were≤4 lactations). Their udders were above hock level (59%) or hock-high (41%), with normal teat length and shape. The characteristics of the group 3 cows (C3=35 cows) were close to those of group 2, with multiparous dairy cows (46%>4 lactations), hock-high udders but with teats that were long (80%) and wide (77%).

3.3 Teat skin microbial counts

The levels of all microbial groups on teat skin were very variable (Fig. 1). On average, the total flora count of the 192 samples was 5.4±0.8 log cfu.mL^{-1}. Ripening bacteria on CRBM (4.7±1.5 log cfu.mL^{-1}) and CNS (4.6±1.5 log cfu.mL^{-1}) were dominant on teat skin; their counts were very similar and correlated (R^2=0.66; P<0.001) with each other and the count of G+C+bacteria was correlated with the total flora count (R^2=0.75 P<0.001). The levels of other populations were all under 3 log cfu.mL^{-1}, moulds (2.4±0.9 log cfu.mL^{-1}), yeasts (1.5±0.9 log cfu.mL^{-1}), enterococci (2.6±1.6 log cfu.mL^{-1}), *Lactobacillus* (2.0±1.1 log cfu.mL^{-1}), *Pseudomonas* (2.0±1.1

Table 2 Farm characteristics and practices

	Group of farming practices			
	FA	FB	FC	Total
Number of farms	6	5	5	16
Mean number of dairy cows (*n*)	43	47	41	
Mean dairy quota (10^3 L/farm)	245	297	225	
Dairy breed (% of farms)[a]				
Holstein	19	100	37	50
Montbéliarde	81	0	63	50
Type of forage (% of farms)[a, b]				
Silage (corn and grass)	0	100	0	31
Hay and wrapped forage	33	0	20	19
Hay	67	0	80	50
Type of barn (% of farms)[a, b]				
Free stalls	33	100	20	50
Tie stalls	67	0	80	50
Type of bedding (% of farms)[a, b]				
Straw	33	80	60	56
Mats without straw	67	20	40	44
Milking system (% of farms)[a]				
Milking parlour	17	100	0	38
Pipeline	67	0	100	56
Bucket	16	0	0	6
Quality of milking hygiene practice (% of farms)[a, b]				
Intensive (Σ 6 criteria>8)[c]	100	40	0	50
Moderate ($4<\Sigma$ 6 criteria ≤ 8)[c]	0	60	40	31
Non-intensive (Σ 6 criteria≤ 4)[c]	0	0	60	19
Check for mastitis (% of farms)[a]				
Never	0	20	100	38
Sometimes	33	40	0	25
Always	67	40	0	37

[a] The difference of distribution of farms between groups was significant at the level $P<0.001$

[b] Farming practices: active variables used in the MCA

[c] The six criteria used to calculate the level of teat hygiene are explained in Table 1

log cfu.mL^{-1}) and coliforms (1.0±0.6 log cfu.mL^{-1}). In 83% of teat samples, dextran-producing leuconostocs were below the detection limit (10 cfu.mL^{-1}) and in 98% coagulase-positive staphylococci were below that limit.

3.4 Teat skin microbial counts and farming practices

On average, as shown in Table 4, the counts of total bacteria, Gram-negative bacteria (including *Pseudomonas* and coliforms), Gram-positive catalase-positive bacteria,

Table 3 Dairy cow characteristics

	Group of cows			
	C1	C2	C3	Significance
Number of cows	27	34	35	
Groups of farming practices				**
A	30	32	49	
B	37	44	14	
C	33	24	37	
Breed (% of cows)				*
Holstein	56	59	37	
Montbéliarde	44	41	63	
Parity (% of cows)[a]				***
Primiparous	93	0	0	
Multiparous≤4 lactations	4	76	54	
Multiparous >4 lactations	4	24	46	
Udder symmetry (% of cows)				ns
Horizontal udder floor	56	50	49	
Front/hind asymmetry	44	50	51	
Teat/hock position (% of cows)[a]				***
Above hock level	81	59	9	
Hock-high	19	41	74	
Below hock level	0	0	17	
Teat Length (% of cows)[a]				***
Short (<5 cm)	41	21	0	
Normal (=5 cm)	52	74	20	
Long (>5 cm)	7	6	80	
Teat Shape (% of cows)[a]				***
Slim (<2.5 cm)	22	12	0	
Normal (=2.5 cm)	56	76	23	
Wide (>2.5 cm)	22	12	77	

ns not significant

*$P<0.05$; **$P<0.01$; ***$P<0.001$

[a] Cow characteristics: active variables used in the MCA

enterococci, lactobacilli, coagulase-negative staphylococci and yeasts, except moulds were the lowest in the group FA whereas all these microbial groups (except yeasts and lactobacilli) were the highest in the group FC. The count of moulds was the highest in groups FC and FA whereas the highest counts of yeasts and lactobacilli characterised the group FB. The coliform counts were under the detection limit (<10 cfu.mL^{-1}) for 88% of group FA, 72% of group FB samples and 53% of group FC samples.

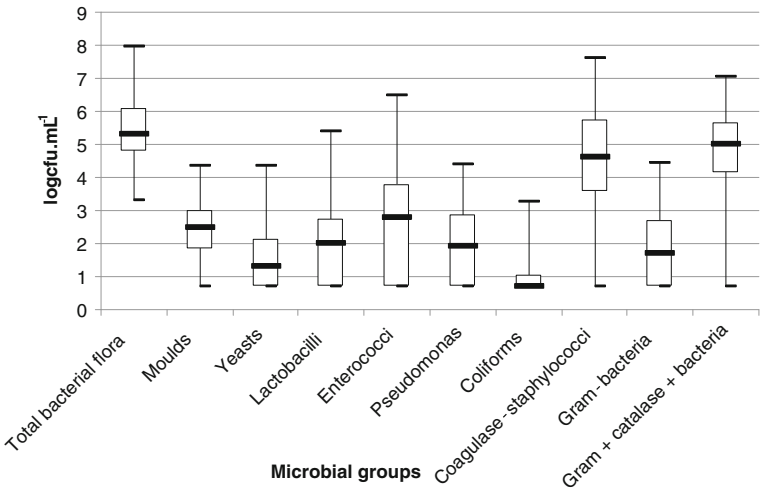

Fig. 1 Box plot of teat skin microbial levels (log colony-forming units per milliliter) using 192 values (6 cows per farm×16 farms×2 visits) *Boxes* denote the interquartile range between the first and the third quartile. *Whiskers* denote the lowest and highest values and the line between the whiskers denotes the median

Table 4 Teat skin microbial levels (log colony-forming units per milliliter) in relation to farming practices (FP), dairy cow characteristics (C) and interactions (C×FP): results of SAS Mix procedure

	Group of farming practices (FP)			Group of cows (C)				Significance		
	FA	FB	FC	C1	C2	C3	SEM	FP	C	C×FP
Number of dairy cows	36	30	30	27	34	35				
Total flora count	4.85^{c}	5.42^{b}	6.01^{a}	5.17^{b}	5.47^{a}	5.64^{a}	0.22	***	**	*
Moulds	2.47^{a}	2.17^{b}	2.59^{a}	2.31	2.30	2.62	0.31	****	ns	ns
Yeasts	1.13^{b}	1.85^{a}	1.58^{a}	1.26^{b}	1.36^{b}	1.93^{a}	0.30	***	***	***
Lactobacilli	1.61^{b}	2.41^{a}	1.86^{b}	1.50^{b}	2.04^{a}	2.34^{a}	0.36	**	***	*
Enterococci	1.99^{b}	2.11^{b}	3.82^{a}	2.29^{b}	2.47^{b}	3.16^{a}	0.50	***	*	*
Pseudomonas	1.55^{c}	1.99^{b}	2.53^{a}	1.82^{b}	1.98^{a}	2.27^{a}	0.33	***	****	ns
Coliforms	0.78^{b}	1.25^{a}	1.18^{a}	0.99	1.02	1.21	0.23	**	ns	ns
Coagulase-negative staphylococci	3.60c	4.37^{b}	5.98^{a}	4.23^{b}	4.73^{a}	4.99^{a}	0.37	***	**	****
Gram-negative bacteria	1.38^{b}	2.16^{a}	2.10^{a}	1.55^{b}	1.95^{ab}	2.13^{a}	0.35	***	*	ns
Gram-positive catalase-positive bacteria	3.89^{b}	4.86^{ab}	5.46^{a}	4.38	4.91	4.92	0.50	***	ns	*

The values are the means of 192 samples corresponding to 96 cows×2 visits

The farming practices (FP) are presented in Table 2 and the dairy cow characteristics (C) in Table 3.

Means within a row with different letters (a–c) differ

ns not significant

***$P<0.001$; **$P<0.01$; *$P<0.05$; ****$P<0.1$

3.5 Teat skin microbial counts and cow characteristics

The teat skin microbial counts of the three dairy cow groups (C1, C2 and C3) are described in Table 4. On average, there was no significant difference between the three groups of dairy cows for mould and coliform counts. However, the level of Gram-negative bacteria of group C1 cows was significantly lower than group C2 and C3 cows ($P<0.05$).

Overall, the levels of four other microbial groups (lactobacilli, enterococci, yeasts and G+C+bacteria) increased from cow group C1 to C3 but a significant interaction between farming practice groups and dairy cow groups was observed, as illustrated in Fig. 2. In farm group FC, the *Lactobacillus* count was highest in the C3 cows (2.7±0.4 log cfu.mL^{-1}). In group FB, the level was very similar for the three cow groups (C1, C2, C3) whereas in group FA, group C1 was significantly ($P<0.05$) distinguished by a lower *Lactobacillus* count (1.0±0.3 log cfu.mL^{-1}) than groups C2 and C3 (2.1±0.2 and 1.7±0.2 log cfu.mL^{-1}). As with lactobacilli, in group FC and to a lesser extent in FB, the enterococci count was highest for group C3 whereas in group FA, cow group C1 was distinguished from C2 and C3 by a lower enterococci count ($P<0.05$). The counts of G+C+bacteria were similar for groups C1, C2 and C3 in groups FB (4.80±0.5 to 5.23±0.5 log cfu.mL^{-1}) and FC (5.30±0.3 to 5.64±0.3 log cfu.mL^{-1}). In group FA, group C1 had a significantly ($P<0.05$) lower G+C+bacteria count (2.9±0.4 log cfu.mL^{-1}) than C2 and C3 (4.5±0.3 and 4.2±0.3 log cfu.mL^{-1} respectively). Yeast levels were similar for the three groups of cows (C1, C2 and C3) in group FA. But in groups FB and FC, group C3 had a significantly ($P<0.001$) higher count (2.4±0.3 log cfu.mL^{-1}) than C1 and C2 (1±0.2 to 1.3±0.2 log cfu.mL^{-1}).

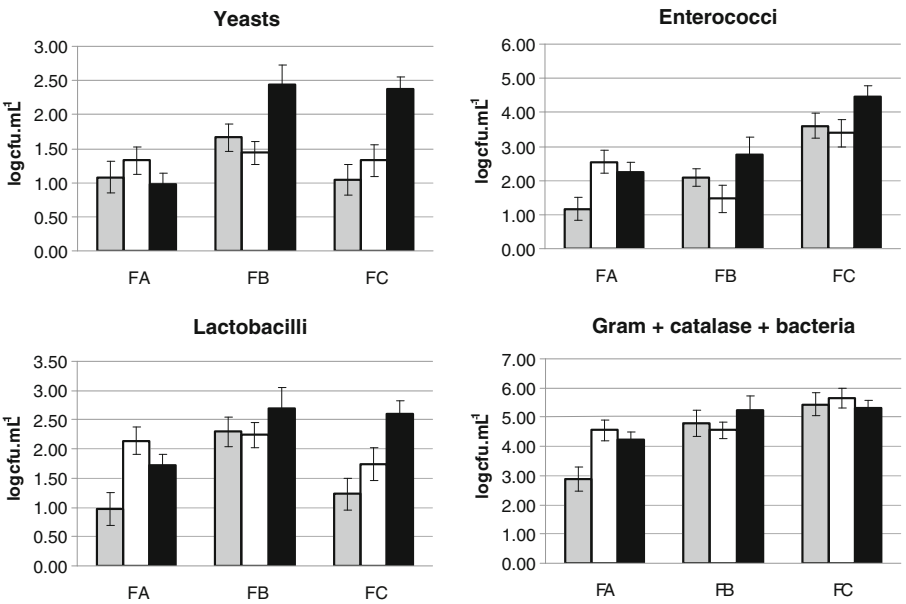

Fig. 2 Interaction between farming practices group (FA, FB and FC, see Table 2) and cows'group (C1, C2 and C3, see Table 3) on lactobacilli, enterococci, yeasts and G+C+bacteria levels (log colony-forming units per milliliter) on teat skin *grey box* C1; *white box* C2; *black box* C3

4 Discussion

To our knowledge, this is the first study that assessed the influence of farming practices and individual cow characteristics on teat skin. Whatever the farming practices and cow characteristics, healthy teats were reservoirs of microbial flora of interest for cheesemaking, especially Gram-positive catalase-positive bacteria including coagulase-negative staphylococci (CNS). It is well-known that these bacteria contribute to the sensory properties of raw milk cheeses (Irlinger et al. 1997). CNS are common bacteria on floor, in air and in bedding in farms (Piessens et al. 2011) and on animal skin, particularly in udder and a large number of species have been identified on teat skin (Rendos et al. 1975; Vacheyrou et al. 2011; Verdier-Metz et al. 2012). Nevertheless their clinical/pathogenic relevance for animals is still discussed. Attention must be paid to identification to CNS as some species such as *Staphylococcus chromogenes*, *Staphylococcus simulans* and *Staphylococcus xylosus* can be involved in bovine intramammary infection without causing clinical mastitis (Supré et al. 2011). *Pseudomonas* and coliforms and at less extent lactic acid bacteria including lactobacilli and enterococci were subdominant populations. *Lactobacillus* species and Gram-negative species were encountered on teat surface (Vacheyrou et al. 2011). Coagulase-positive staphylococci were not detected on teat skin in our study but were detected in that of Vacheyrou et al. (2011). This may be due to the fact that over 90% of the teats showed no cutaneous damage and that CNS can exert strong competition against *Staphylococcus aureus* on teats (Woodward et al. 1988; De Vliegher et al. 2003). Coagulase-negative *Staphylococcus* can also impede the counting of coagulase-positive staphylococci due to growth competition on the medium.

The results of this study were consistent with those of Michel et al. (2006) who showed that the flora of interest for cheesemaking (acidifying mesophilic bacteria and halophilic bacteria) gave counts 100 times greater than coliforms and *Pseudomonas* on healthy teat skin before teat washing by the farmer. However, our results cannot be compared to those from the literature because the sampling methods and the culture media used for microbial analyses were different. Furthermore results are not always expressed in the same units. Some authors have expressed the microbial flora count by total area of teat (Michel et al. 2006) or by swab (Rendos et al. 1975). In most studies, only the teat end was sampled (Woodward et al. 1988; De Vliegher et al. 2003) or one side of a teat per cow (White et al. 1989). In our study, the teat surface in contact with the teat-cup liner and the teat end were sampled to find out the potential of inoculation of milk by teats during milking. Sampling the anterior right and the posterior left teats had to take into account the heterogeneity of dirt on the four teats and to have a better view of the teat skin microbiota.

In the present study, Gram-negative bacteria, including coliforms and *Pseudomonas* did not represent a risk factor owing to their low counts, irrespective of cow characteristics and farming practices. For coliforms, only 47% of the samples presented counts above the detection limit (10 cfu.mL^{-1}) when straw bedding and less intensive milking hygiene practices were used (groups FB and FC).

Overall, teats with high levels of microbial flora, especially lactobacilli, enterococci, ripening bacteria and yeasts, were associated with farming practices FB and FC, based on the use of straw bedding, rather low-intensity milking hygiene practices

and multiparous cows with long, wide teats. Significantly higher levels of mesophilic bacteria were associated with straw bedding (Michel et al. 2006). The occurence of lactobacilli (Kagkli et al. 2007) and yeasts (Reboux et al. 2006) in silage may suggest that silage was a likely source of these microorganisms on FB farms, where the cow diet was silage. Enterococci, important in flavour development (Giraffa 2002), are common in the environment due to contamination by animal faeces, so teat contamination could occur when cows are lying down. Bedding material can be an important source of bacterial exposure for teats. Rendos et al. (1975) have reported a positive correlation between *Streptococcus* and *Staphylococcus* counts in bedding materials and those present on teat ends. It has been shown that CNS species composition on teat skins of heifers changes with the age from 1 day to 2 years (White et al. 1989) and may induce a protective effect against intramammary infection with *S. aureus* (De Vliegher et al. 2003). Straw bedding has often been described as an organic material which, unlike inorganic materials such as rubber mats, promotes the growth of microorganisms, particularly *Klebsiella pneumoniae* (Godden et al. 2007).

Low microbial levels on teat skin were mainly associated with primiparous cows and teats above hock level, particularly when milking hygiene practices were intensive. This suggests that milking practices could be adapted according to primiparous or multiparous animals having always in mind to preserve useful cheese-making bacteria on teats while eliminating pathogenic bacteria. Mastitis and bulk-milk somatic cell count must be controlled after this change. Although the sampling was done before washing the teats by the farmer, the hygiene practices of the previous milking had a residual effect, especially when they were drastic. It seems that with intensive milking hygiene practices, microorganisms do not have time to recolonize the teat skin between milkings. Gibson et al. (2008) showed that a pre-milking teat cleaning regime involving the washing of teats with an effective disinfectant and then drying was the most effective for removing bacteria and minimizing bacterial growth. Increasing levels of microbial flora with age could be due to a gradual establishment of microorganisms over time (Woodward et al. 1988) or progressive damage to the teat skin. Teat skin is very sensitive to weather changes (temperature, humidity and sunburn) and successive milkings could damage the hydrolipidic film covering the skin, preventing microbial colonization of the epidermal surface. Bacic et al. (1968) have reported significantly highest bacterial counts in both foremilk and total milk for cows in the third and subsequent lactations; this could be due to a gradual loss of teat integrity with increasing age.

5 Conclusion

In conclusion, the combinations of the practices (housing and milking) and the cow characteristics may influence the balance between the microbial groups at the surface of teat skin. Further studies should be conducted to better understand these interactions. Verdier-Metz et al. (2012) highlighted the large diversity of the bacterial community that may be found on teat skin and could be an interesting vector of biodiversity for milk. So it is important to manage it. Consequently, to advise the dairy farmers, we also need to learn about the flow of microorganisms from teat to milk by better measuring the effect of milking practices.

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