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The association between bedding material and the bacterial counts of *Staphylococcus aureus, Streptococcus uberis* and coliform bacteria on teat skin and in teat canals in lactating dairy cattle

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Several mastitis-causing pathogens are able to colonize the bovine teat canal. The objective of this study was to investigate the association between the treatment of sawdust bedding with a commercial alkaline conditioner and the bacterial counts on teat skin and in the teat canal. The study used a crossover design. Ten lactating Holstein cows that were free of udder infections and mastitis were included in the study. The animals were bedded on either untreated sawdust or sawdust that had been treated with a hydrated lime-based conditioner. Once a day, fresh bedding material was added. After 3 weeks, the bedding material was removed from the cubicles, fresh bedding material was provided, and the cows were rotated between the two bedding material groups. Teat skin and teat canals were sampled using the wet and dry swab technique after weeks 1, 2, 3, 4, 5 and 6. *Staphylococcus aureus, Streptococcus uberis, Escherichia coli* and other coliform bacteria were detected in the resulting agar plate cultures. The treatment of the bedding material was associated with the teat skin bacterial counts of *Str. uberis, Esch. coli* and other coliform bacteria. An association was also found between the bedding material and the teat canal bacterial counts of coliform bacteria other than *Esch. coli*. For *Staph. aureus*, no associations with the bedding material were found. In general, the addition of a hydrated lime-based conditioner to sawdust reduces the population sizes of environmental pathogens on teat skin and in teat canals.

Keywords: Mastitis, bedding material, mastitis causing pathogens, teat flora.

The teat canal is the first barrier that microorganisms face when invading the bovine udder (Jain, 1979; Paulrud, 2005). Despite several non-specific defence mechanisms of the bovine teat canal in lactating cattle (Williams & Mein, 1985; Paulrud, 2005) the presence of free amino acids and intercellular lipids can support the microbial colonization of the teat canal's keratin layer (Nickerson, 1987; Paulrud, 2005).

The important mastitis-causing pathogens that have been isolated from the teat canals of lactating cattle include coagulase-negative staphylococci, *Staphylococcus aureus, Streptococcus uberis* and coliform bacteria (Du Preez, 1985; Zecconi et al. 1992; Paduch et al. 2012; Quirk et al. 2012). Du Preez (1985), Zecconi et al. (1992) and Haveri et al. (2008) have speculated that the microbial colonization of the teat canal may be associated with the development of intramammary infections.

New infection rates and mastitis rates may relate to the environmental bacteria counts in bedding materials (Bramley & Neave, 1975; Smith et al. 1985; Hogan et al. 1989). As postulated by Zadoks et al. (2001) and Munoz et al. (2007), bedding material and bedding management may play roles in the outbreaks of *Str. uberis* mastitis and *Klebsiella pneumoniae* mastitis. Rendos et al. (1975) noted that the bacterial populations in bedding may affect the teat skin populations in lactating cattle. In several studies, positive correlations between the bacterial counts in bedding materials and bacterial counts on teat skin have been found for Gram-negative bacteria, coliforms, *Klebsiella* spp., and streptococci (Hogan & Smith, 1997; Hogan et al. 1999; Zdanowicz et al. 2004). Currently, there is a lack of knowledge about associations between bedding material

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and bacterial populations on bovine teat skin and in bovine teat canals. The objective of the present study was to investigate whether the treatment of sawdust bedding with an alkaline conditioner is associated with the teat skin and teat canal bacterial counts of mastitis-causing pathogens in lactating dairy cattle.

Materials and Methods

Herd and animals

This field study was conducted on one commercial dairy farm in northern Germany with 145 German Holstein black pied cows. The average milk yield was 9620 kg (3·7% fat-corrected milk), and the mean bulk milk somatic cell count was 220 000 cells/ml. The cattle were housed in free-stall barns with sawdust-bedded cubicles and were milked twice a day. The farm was equipped with a rotary parlour.

Cows in the middle (100–200 days in milk) of their second lactation with normal udders, normal teats and round teat ends (Grunert, 1990) were included in this study. Further criteria included: four functional quarters without udder infections or signs of clinical mastitis (i.e. no detection of microorganisms in 0·01 ml milk, a somatic cell count < 100 000 cells/ml per quarter, no clotting or discolouration of milk, no swelling or udder redness and no heat upon udder palpation), clean udders (teat skin without splashing or plaques of manure), no visible udder lesions or trauma, teat tissue and skin that appeared normal, no excessively rough callous rings around the teat orifices of all four teats (Mein et al. 2001), and similar sizes of the four teats. These criteria were evaluated by one trained researcher immediately before the trial period. During the trial period, clinical signs of mastitis in animals that were included in the study were recorded by the farmer, who was trained by the researcher.

Experimental design

The present study used a crossover design with two groups of lactating dairy cattle (each consisting of 5 cows) to reduce confounding (Wellek & Blettner, 2012). The total duration of the trial period was 6 weeks. After 3 weeks, the animals rotated between the two bedding material groups [sawdust + alkaline conditioner (pH 9·8) *vs*. untreated sawdust (pH 6·6)]. The same diet was fed to all cows included in the study for the entire experimental period.

In both bedding material groups, the animals were kept in identical stalls with deep-bedded cubicles. The animal : cubicle ratio was 1 : 1. The height of the bedding was approximately 10 cm. At the beginning of the study, the stalls were filled with fresh bedding material. In one bedding material group, a commercial hydrated lime-based alkaline conditioner (pH 12) (Desical®, Hufgard GmbH, Rottenberg, Germany) was mixed with industrial sawdust from conifers (weight ratio 1 : 1). In the second group, the bedding material consisted of pure sawdust. The total mesophilic aerobic bacterial count of the fresh sawdust was 2800 cfu/g; the counts of coliform bacteria and aesculin-positive streptococci were below 10 cfu/g. Before filling the stalls, 800 l water was added to the bedding material of the 150 cubicles. Once a day during morning milking, the stalls were cleaned manually and 300 g of fresh bedding material [sawdust + alkaline conditioner (weight ratio 1 : 1) or untreated sawdust] was added per m^2. This bedding system is common in many European countries with deep bedded cubicles. After 3 weeks, the bedding material was removed from all stalls and the stalls were filled with fresh bedding material.

Sample collection

Immediately before the trial period, the udder health status of 20 animals complying with the inclusion criteria were evaluated on the basis of quarter foremilk samples. From the animals that were free of udder infections, 10 cows were randomly selected for the study. To evaluate the udder health status during the trial period, quarter foremilk samples were taken from all quarters of the animals included in the study at the end of weeks 3 and 6. After cleaning the teat ends with paper towels and disinfecting them with ethanol (70%), the first three streams of milk were discarded. From each quarter, approximately 10 ml of milk were collected aseptically into a sterile tube.

Teat skin and teat canal swab samples were collected and analysed microbiologically, as described by Paduch & Krömker (2011) and Paduch et al. (2012). The teat skin and teat canals of the right front teat and the left rear teat were sampled using the modified wet and dry swab technique at the end of weeks 1, 2, 3, 4, 5 and 6. The teat skin was sampled after pre-milking and pre-cleaning with dry paper towels, which was conducted by the dairy farmer. The first swab (ultrafine, Dry Swab, Check Diagnostics, Westerau, Germany) was moistened with ¼ Ringer's solution (Merck, Darmstadt, Germany) and rotated 360° around the teat canal orifice at a distance of 1 cm. The same procedure was carried out with the dry swab. Immediately after sampling, the tips of both swabs were transferred into one tube with 2 ml of sterile Ringer's solution.

The teat canals were sampled immediately after cluster detachment and before post-milking teat disinfection. Both swabs were inserted 5 mm into the teat canal and rotated 360 °C. The tips were transferred into one tube, as described for the teat skin swabs.

The quarter foremilk and swab samples were transported at 5 °C to the microbiology laboratory of the University of Applied Sciences and Arts Hannover (Germany) within 8 h. One trained researcher conducted all of the sampling.

Laboratory analysis

A subsample of 0·01 ml of each quarter foremilk sample was streaked onto a quadrant of an aesculin blood agar plate (Oxoid, Wesel, Germany). The plates were incubated

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aerobically at 37 °C and examined after 24 and 48 h. Colonies were identified by Gram staining, cell morphology, haemolysis patterns, and aesculin hydrolysis. Gram-positive cocci were differentiated by a catalase test. Presumptive *Staph. aureus* was identified with a tube test using Bactident® Coagulase EDTA rabbit plasma (Merck, Darmstadt, Germany) after subcultivation in a brain heart infusion broth (Merck, Darmstadt, Germany) at 37 °C for 24 h. Aesculin-positive streptococci were subcultivated on modified Rambach agar (Watts et al. 1993) at 37 °C for 24 h. Gram-positive, aesculin-positive, catalase-negative and β-D-galactosidase-positive cocci were identified as presumptive *Str. uberis*. Gram-negative and oxidase-negative (Bactident® Oxidase; Merck, Darmstadt, Germany) isolates were subcultivated on ChromoCult® coliform agar plates (Merck, Darmstadt, Germany). *Escherichia coli* produces blue to violet-coloured colonies, whereas other coliform bacteria produce salmon-coloured colonies. A sample was defined as contaminated if more than two different colony types of environmental mastitis-causing pathogens were observed. The somatic cell count was determined using a SomaScope Smart flow cytometer (Delta Instruments B.V., Drachten, The Netherlands). The udder health status was evaluated according to the recommendations of the German Veterinary Medical Society (2002) based on the results of the cyto-bacteriological investigation of the foremilk samples. Mastitis was diagnosed if the somatic cell count exceeded 100 000 cells/ml. If mastitis-causing micro-organisms were isolated from a quarter foremilk sample and the somatic cell count was below 100 000 cells/ml, a latent infection was inferred.

The swab sample material was vortexed with a mixer (type REAX 1 R, Heidolph, Schwabach, Germany) for 20 s before removing the swab tips from the tubes. The agar plates were inoculated in duplicate with either 0·1 ml of a swab solution or a dilution (−2, −3, −4 or −5) prepared with ¼ Ringer's solution. The inoculum was spread with a sterile Drigalski spatula onto the agar surface. *Staph. aureus* counts in the swab samples were determined with Baird-Parker agar plates (Merck, Darmstadt, Germany) with an egg yolk-tellurite emulsion (Oxoid, Wesel, Germany), and *Str. uberis* counts were evaluated with modified Rambach agar plates. ChromoCult® coliform agar plates (Merck, Darmstadt, Germany) were used for the detection of *Esch. coli* and other coliform bacteria. Baird-Parker agar plates were incubated aerobically at 37 °C for 48 h. The coagulase activity of isolates producing black colonies with clearing and precipitate zones on the Baird-Parker agar was determined by a coagulase tube test. The coagulase-positive isolates were defined as presumptive *Staph. aureus*. The inoculated modified Rambach agar plates and ChromoCult® coliform agar plates were incubated aerobically at 37 °C for 24 h. From the modified Rambach agar, one colony of each colony type of β-D-galactosidase-positive cocci was isolated and subcultivated on aesculin blood agar (Oxoid, Wesel, Germany) at 37 °C for 24 h to identify presumptive *Str. uberis*. The results from plates with 1–300 colonies were used to calculate bacterial counts in teat skin and teat canal swab solutions. The weighted arithmetic means were calculated for each of the pathogen groups included in the investigation (*Staph. aureus, Str. uberis, Esch. coli*, and other coliforms). Results were reported as cfu/ml of swab solution.

Statistical analysis

The data were recorded with Microsoft Excel 2003 software (Microsoft, USA). Bacterial counts were normalized by adding 1, followed by the $\log_{10}$-transformation ($\log_{10}$ cfu/ml). SPSS 19.0 software (IBM, USA) was applied for data analysis. Descriptive statistics were calculated, and linear mixed regression models for repeated measurements were used to determine associations between the bedding material and the $\log_{10}$-transformed bacterial counts (including *Staph. aureus, Str. uberis, Esch. coli*, and other coliforms) in the teat skin and teat canal swab samples. The subject was the teat. The bedding material (sawdust + alkaline conditioner *vs*. untreated sawdust), sampling week (1, 2, 3, 4, 5, or 6) and the interaction between these variables were treated as fixed factors, and the animal was considered a random factor. Pearson correlation coefficients between $\log_{10}$-transformed bacterial counts in teat skin and teat canal samples were calculated. Statistical significance was defined at $P<0{\cdot}05$.

Results

In total, 120 teat skin swab samples and 120 teat canal swab samples from 20 quarters of 10 cows were taken.

During the trial period, all animals included in the study were free of clinical mastitis; no changes in teat skin condition (e.g. irritation, dryness, chapping) were observed. Furthermore, all animals were free of intramammary infections and mastitis at the end of weeks 3 and 6.

Staphylococcus aureus

Overall, for the teat skin swab samples from the animals that were bedded on sawdust treated with the alkaline conditioner, the mean *Staph. aureus* count was 0·1 log units higher than in samples from animals bedded on untreated sawdust (Table 1). For the teat canal swab samples from the cows housed on sawdust treated with the conditioner, the mean *Staph. aureus* count was $2{\cdot}1 \pm 0{\cdot}2$ $\log_{10}$ cfu/ml. Similarly, in the untreated sawdust group, the mean bacterial count was $2{\cdot}0 \pm 0{\cdot}2$ $\log_{10}$ cfu/ml.

Streptococcus uberis

In the teat skin swab samples the mean *Str. uberis* counts were $0{\cdot}9 \pm 0{\cdot}1$ $\log_{10}$ cfu/ml (sawdust + alkaline conditioner) and $1{\cdot}4 \pm 0{\cdot}2$ $\log_{10}$ cfu/ml (untreated sawdust) (Table 1). For the teat canal swab samples from the cows housed on sawdust treated with the conditioner, the mean *Str. uberis*

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Table 1. Means (± SEM) (log_{10} cfu/ml) of bacterial counts (*Staphylococcus aureus, Streptococcus uberis, Escherichia coli*, coliforms other than *Esch. coli*) in teat skin swab samples ($n = 60$ per bedding material group) and teat canal swab samples ($n = 60$ per bedding material group). After week 3, the animals ($n = 5$ per bedding material group) rotated between the bedding material groups [sawdust + alkaline conditioner (alk), untreated sawdust (unt)]

Pathogen		Sampling week						Overall
Teat skin	Bedding	1	2	3	4	5	6	mean
Staph. aureus	alk	1·8 ± 0·4	1·9 ± 0·5	3·1 ± 0·3	3·0 ± 0·1	2·7 ± 0·3	1·5 ± 0·3	2·3 ± 0·2
	unt	2·0 ± 0·4	0·9 ± 0·3	2·2 ± 0·2	3·3 ± 0·1	2·7 ± 0·2	1·9 ± 0·5	2·2 ± 0·2
Str. uberis	alk	0·9 ± 0·5	1·6 ± 0·5	1·4 ± 0·3	0·4 ± 0·2	0·6 ± 0·2	0·4 ± 0·2	0·9 ± 0·1
	unt	1·9 ± 0·6	1·3 ± 0·4	1·6 ± 0·4	1·0 ± 0·3	1·3 ± 0·4	1·5 ± 0·5	1·4 ± 0·2
Esch. coli	alk	0·6 ± 0·3	0·2 ± 0·2	0·9 ± 0·3	0·0 ± 0·0	0·3 ± 0·2	0·0 ± 0·0	0·3 ± 0·1
	unt	1·0 ± 0·4	0·7 ± 0·4	0·7 ± 0·3	0·4 ± 0·2	0·4 ± 0·3	0·9 ± 0·3	0·7 ± 0·1
coliforms	alk	0·9 ± 0·5	1·0 ± 0·5	0·1 ± 0·1	0·1 ± 0·1	0·3 ± 0·2	0·4 ± 0·4	0·5 ± 0·1
	unt	2·2 ± 0·7	1·3 ± 0·4	1·5 ± 0·4	0·9 ± 0·3	1·4 ± 0·4	1·2 ± 0·6	1·4 ± 0·2
Teat canal								
Staph. aureus	alk	1·4 ± 0·4	1·8 ± 0·8	2·8 ± 0·4	3·4 ± 0·3	2·2 ± 0·4	0·9 ± 0·5	2·1 ± 0·2
	unt	2·3 ± 0·4	1·6 ± 0·5	2·1 ± 0·3	3·0 ± 0·1	2·1 ± 0·4	1·2 ± 0·4	2·0 ± 0·2
Str. uberis	alk	2·0 ± 0·5	2·4 ± 0·7	1·7 ± 0·3	1·1 ± 0·3	2·4 ± 0·4	1·4 ± 0·6	1·8 ± 0·2
	unt	1·7 ± 0·5	3·0 ± 0·6	2·5 ± 0·5	1·2 ± 0·3	2·4 ± 0·4	2·9 ± 0·7	2·3 ± 0·2
Esch. coli	alk	1·3 ± 0·5	2·3 ± 0·7	1·4 ± 0·3	0·9 ± 0·3	0·9 ± 0·4	0·9 ± 0·6	1·3 ± 0·2
	unt	0·8 ± 0·5	2·0 ± 0·7	1·5 ± 0·5	0·4 ± 0·2	0·8 ± 0·3	2·0 ± 0·7	1·2 ± 0·2
coliforms	alk	2·3 ± 0·5	2·2 ± 0·8	0·6 ± 0·4	1·2 ± 0·4	2·6 ± 0·2	0·6 ± 0·6	1·6 ± 0·2
	unt	3·1 ± 0·5	2·6 ± 0·8	2·2 ± 0·5	1·2 ± 0·3	2·1 ± 0·5	3·2 ± 0·9	2·4 ± 0·3

count was 0·5 log units lower than in the samples from animals that were housed on untreated sawdust.

Escherichia coli

For cows that were housed on sawdust treated with the alkaline conditioner, a mean bacterial count of 0·3 ± 0·1 log_{10} cfu/ml was found for the teat skin swab samples (Table 1). In contrast, in the untreated sawdust group, the mean *Esch. coli* count was 0·4 log units higher. *Esch. coli* was not isolated from the teat skin of cows that were bedded on the sawdust treated with the conditioner at the end of weeks 4 or 6. Mean *Esch. coli* counts for the teat canal swab samples were 1·3 ± 0·2 log_{10} cfu/ml (sawdust + alkaline conditioner) and 1·2 ± 0·2 log_{10} cfu/ml (untreated sawdust).

Coliform bacteria other than Esch. coli

For the teat skin swab samples, the mean counts of coliform bacteria other than *Esch. coli* were 0·5 ± 0·1 log_{10} cfu/ml (sawdust + alkaline conditioner) and 1·4 ± 0·2 log_{10} cfu/ml (untreated sawdust) (Table 1). For the teat canal swab samples, the mean bacterial counts of 1·6 ± 0·2 log_{10} cfu/ml (sawdust + alkaline conditioner) and 2·4 ± 0·3 log_{10} cfu/ml (untreated sawdust) were recorded.

Mixed models

The bedding material was associated with the teat skin bacterial counts of *Str. uberis, Esch. coli* and other coliform bacteria and with the teat canal bacterial counts of coliform bacteria other than *Esch. coli* (Table 2). The week was associated with the teat skin and teat canal bacterial counts of *Staph. aureus*, the teat skin bacterial counts of *Esch. coli* and the teat canal bacterial counts of *Str. uberis* and coliforms other than *Esch. coli*. Teat skin and teat canal bacterial counts were not associated with the animal ($P > 0·05$).

Pearson correlations between teat skin and teat canal bacterial counts

For cows housed on sawdust bedding treated with the alkaline conditioner, the teat skin bacterial counts were significantly correlated with teat canal bacterial counts for *Staph. aureus* ($r = 0·43$, $P < 0·001$), for *Str. uberis* ($r = 0·32$, $P = 0·007$), and for coliforms other than *Esch. coli* ($r = 0·60$, $P < 0·001$), but not for *Esch. coli* ($r = 0·16$, $P = 0·106$). For cows housed on untreated sawdust, correlation coefficients were $r = 0·20$ ($P = 0·064$) for *Staph. aureus;* $r = 0·49$ ($P < 0·001$) for *Str. uberis;* $r = 0·33$ ($P = 0·005$) for *Esch. coli*; and $r = 0·54$ ($P < 0·001$) for other coliform bacteria.

Discussion

In the present study, the mastitis-causing pathogens *Staph. aureus, Str. uberis, Esch. coli* and other coliform bacteria were isolated from teat skin and teat canal swab samples obtained from lactating dairy cattle. The bovine teat skin can act as a reservoir of *Staph. aureus, Str. uberis* and coliform bacteria (Bramley, 1984; Kagkli et al. 2007; Piccinini et al. 2009). As demonstrated by Du Preez (1985), *Staph. aureus* is able to persist in teat canals for more than 3 months. However, it remains unclear whether the environmental mastitis-causing pathogens *Str. uberis* and *Esch. coli* colonize the bovine teat canal epithelium (Bramley et al. 1979; Bramley, 1984; Pryor, 2008).

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Table 2. *P* values of the linear mixed models (dependent variables: log_{10}-transformed teat skin and teat canal bacterial counts, fixed factors: bedding material, sampling week, interaction between bedding material and week). The subject was the teat

Pathogen	Bedding†		Week‡		Bedding × week§	
Teat skin	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value
Staph. aureus	0·725	0·329	12·273	<0·001	2·928	0·199
Str. uberis	6·118	0·014	1·760	0·222	0·803	0·497
Esch. coli	5·009	0·015	2·579	0·043	0·973	0·520
Coliforms	15·684	<0·001	1·609	0·189	0·611	0·712
Teat canal						
Staph. aureus	0·070	0·764	9·076	<0·001	1·089	0·499
Str. uberis	0·446	0·070	2·656	0·043	1·262	0·468
Esch. coli	0·259	0·902	2·218	0·079	0·717	0·557
Coliforms	7·526	0·008	4·445	0·005	2·300	0·067

†Bedding material: sawdust + alkaline conditioner, untreated sawdust
‡Sampling week: 1, 2, 3, 4, 5, 6
§Interaction between bedding material and sampling week

All of the animals included in this study were free of mastitis at the beginning of the trial period and remained free of clinical signs of mastitis throughout the trial. At the end of weeks 3 and 6, no intramammary infections were observed. However, it was not investigated whether the detection of mastitis-causing pathogens in the teat skin and teat canal swab samples could result from transient populations or long-term colonization of teat epithelia (Paduch et al. 2012).

In the present study, the bedding material was associated with the teat skin bacterial counts of the environmental mastitis-causing pathogens *Str. uberis, Esch. coli* and other coliform bacteria ($P<0·05$). Teat canal bacterial counts of coliform bacteria other than *Esch. coli* were associated with both the bedding material and the week ($P<0·05$). To our knowledge, the present study is the first work indicating that associations between bedding material and teat canal bacterial counts exist.

Bacterial growth in bedding materials is promoted by moisture and the availability of organic nutrients (Fairchild et al. 1982; Bey et al. 2002). In general, bacterial counts are higher in organic bedding materials than in inorganic materials (Hogan et al. 1989). As observed by Hogan & Smith (1997) the bacterial populations of environmental mastitis-causing pathogens in sawdust and on teat skin are affected by the addition of hydrated lime for 1 d. In contrast to the present study, the authors did not add fresh sawdust treated with an alkaline material once a day. Hogan & Smith (1997) and Hogan et al. (1999) attributed the reduction of bacterial growth to the addition of lime or commercial conditioners to bedding materials, as well as the resulting change in the pH value. As suggested by the authors, the reduction of bacterial populations in bedding materials may be associated with the reduction of the teat skin bacterial counts. The implications of this finding suggest that the teat skin and teat canal loads of environmental mastitis-causing pathogens may be decreased by the addition of an alkaline conditioner to sawdust. In this study, the pH value of unused sawdust treated with the alkaline conditioner was 9·8. As stated by Hughes (1999), both *Esch. coli* and *Str. uberis* are able to grow at pH values up to 9·5. It can be concluded that the addition of a hydrated lime-based conditioner to sawdust bedding may decrease the growth of bacteria in the bedding, the contamination of teat skin and teat canal with environmental pathogens and the risk of environmental mastitis. Smith et al. (1985) recommended reducing the environmental pathogen contamination of the teat end as a method for controlling environmental mastitis.

It is feasible that the trial period was too short to reveal longer term effects of bedding material on the *Str. uberis* and *Esch. coli* populations in teat canals, given that an association between bedding material and teat canal microbial bacterial counts was found only for coliform bacteria other than *Esch. coli*. Interestingly, the teat skin and teat canal bacterial counts did not vary by animal. This could be explained by the selective inclusion of animals that had normal teat skin, a lack of excessively callous rings around the teat orifices, clean udders and teat skin without traces of manure.

Teat canal swab samples were taken after detachment of the milking cluster. By this sampling procedure the risk of damage to the teat canal epithelium is reduced, because milk residues and teat canal laxity facilitate the insertion of swabs into teat canals (Paduch & Krömker 2011; Paduch et al. 2012). However, during milk flow part of the teat canal keratin with adhered microorganisms is removed from the teat canal (Capuco et al. 1994; Paulrud, 2005), which may affect teat canal bacterial counts.

Associations between the bedding material and the teat skin and teat canal bacterial counts of *Staph. aureus* were not observed. Both the teat skin counts and the teat canal counts of *Staph. aureus* were associated with the week ($P<0·001$). Important reservoirs of *Staph. aureus* – in contrast with environmental pathogens – include the milk from infected quarters, skin lesions, milking liners and milkers' hands (Smith et al. 1985; Haveri et al. 2008). The rationale underlying the relationship between the length of bedding exposure (in weeks) and the teat skin and teat canal bacterial counts of *Staph. aureus* could not be ascertained in this

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study. However, the present study indicates that, for the contagious pathogen *Staph. aureus* and the environmental pathogens *Str. uberis, Esch. coli* and other coliform bacteria, different risk factors affecting the teat skin and teat canal bacterial counts may exist. Paduch et al. (2012) found that teat-end hyperkeratosis scores are associated with the environmental pathogen loads of teat canals, but not with teat canal *Staph. aureus* loads.

In general, the teat skin bacterial counts of environmental pathogens and the teat canal bacterial counts of coliform bacteria other than *Esch. coli* are affected by the treatment of the bedding material with an alkaline conditioner. This may result in a lower number of intramammary infections caused by the mentioned pathogens while using the treatment of bedding materials. Further research is needed to characterize the long-term effects of the bedding material on microbial populations in bovine teat canals, the stability and dynamics of microbial populations on the teat epithelia of lactating dairy cattle, as well as methods that can be used to estimate the risk of mastitis.

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