

Effects of Flooring Types on Teat End Bacteria Counts, Milk Quality, Hygiene and Behaviour of Dairy Cows Housed in Tie-stall Closed Barn

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Summary

This study aimed to investigate environmental mastitis causing bacteria counts in the teat end, somatic cell counts (SCC) of milk samples, cleanliness scores and behavior of cows kept on concrete and rubber mat floorings. For this purpose, 19 Holstein-Friesian dairy cows were allocated into concrete and rubber mat groups. Swab samples were taken from the teat ends to determine the bacterial counts causing environmental mastitis. Milk samples were collected from a composite of all four quarters to determine the SCC. Instantaneous sampling method was utilized to observe the behavioral activities of cows. Cows were visually evaluated to determine the udder cleanliness score. Independent samples t-test was utilized in the statistical analysis of the obtained data. Coliform ($P < 0.05$), *Escherichia coli*, and *Klebsiella* spp. ($P < 0.01$) counts of the swab samples taken from the cows housed on concrete flooring were significantly higher than rubber mat group. However, no statistically significant differences were found between groups in terms of total bacteria, *Streptococcus* spp., and *Enterobacteriaceae* counts. The SCC on samples taken from cows kept on concrete surface were significantly higher ($P < 0.05$) than that of animals housed on rubber mat. Furthermore, cows in the rubber mat group were determined to be significantly cleaner ($P < 0.05$) than those in concrete group. It was also determined that the cows housed on rubber mat spent significantly longer time for lying behavior ($P < 0.05$), which is a significant indicator of animal comfort. The time spent for standing without eating was considerably higher ($P < 0.01$) in concrete group. In addition, the times spent for eating was significantly lower ($P < 0.01$) in the concrete group. It was concluded that, using rubber mat instead of concrete for flooring in tie-stall barns decreases the contamination of environmental mastitis pathogens, increases milk quality and cow cleanliness score as well as animal comfort and welfare.

Introduction

Mastitis is the costliest disease in milk production worldwide, resulting in substantial economic losses and impairing the health and welfare of affected cows. Since sources of environmental pathogens include soil, feces, and bedding, all of which are found within dairy cattle housing systems, it is much more difficult to control the incidence of environmental

mastitis (Zdanowicz *et al.* 2004). Microorganisms causing environmental mastitis are usually transferred from environment to cows rather than other infected cows in the herd. Therefore, management practices that will reduce exposure of environmental pathogens to the teat end could result in reduced incidence of intramammary infections (Klass and Zadoks 2017).

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The cleanliness of the udder is considered to affect the type and quantity of microorganisms on teat skin, and dirty teats and udders are thought to be a significant source of environmental bacteria for intramammary infections. Factors contributing to udder and teat cleanliness (and hygiene of the udder as well as teat ends) are the type of housing systems such as tie-stall and free-stall dairy barns, high indoor humidity, type of animal (heifer, cow, bull or calf), and failure to clean the cows during the year (Neja *et al.* 2016). Especially, types of the bedding and flooring materials in the tie-stall barn may play important roles in the contamination of the microbes to the udder since teats are in close contact with flooring and bedding materials for a long time. In recent years, effects of the various flooring and bedding materials on the microbial population of teat ends, udder health and welfare of the cows housed in free-stall barns were studied by several researchers (Kristula *et al.* 2008; Norring *et al.* 2008; Calamari *et al.* 2009; Paduch 2013; Proietto *et al.* 2013). However limited information is available regarding the hygiene of cows kept in the tie-stall barns (Neja *et al.* 2016). Therefore, this study was undertaken to compare influences of the two types of the flooring materials (rubber mats vs concrete flooring) on the population of the environmental mastitis pathogens on the teat ends, somatic cell counts (SCC), cleanliness of cows, as well as behavioral activities of the dairy cows housed in the tie-stall barn.

Materials and methods

Animal housing and handling

Nineteen lactating Holstein Friesian cows were allocated into the treatment groups according to their parities, and average parity of the groups was balanced as 2 parities for each one. The trial lasted for 10 weeks. Stage of Holstein Friesian cows used in the study were between 100 and 125 days in milk. The animals were housed in a tie-stall barn during the trial, and it was divided into two sections. In the first unit of the barn, 10 cows were kept in tie-stalls whose floors were made of concrete. In the second unit, 9 cows were maintained in the tie-stalls whose floors were fitted with rubber mats. Each stall was 180 cm long and 110 cm wide. Throughout the trial, dry hay and concentrate feed were offered to the animals in the individual feeders that were available in their individual stalls. Drinking water was also provided by automatic cattle waterers in each stall. The animals were kept as tied during the duration of the experiment.

Microbiology

To determine bacterial populations on the teat end, teat swab samples were taken on the fifth and ninth

weeks of the trial. For this purpose, teat swabs were obtained from right front and rear teats of quarters free of infection, and the same teats were used at each sampling. They were collected about 2 h before evening milking, and 10 h after morning milking around 16.00 pm. Teats were not washed prior to swabbing. Visible bedding material adhered to the teats were removed by using dry paper towel. Sterile cotton swabs were dipped and moistened in a swab solution containing 0.85% sodium chloride and 0.1% proteose-peptone and 0.2% sodium thiosulfate. Excess liquid was squeezed from the swab on the side of the tube. The teat swab was taken from the tube, held approximately perpendicular to the teat axis, and rotated three times on the exterior of the teat orifice. Swabs were returned to the swab solution, and the tube was held in an ice bath until dilution and plating within 3 h. Teat swab samples in the tubes were prepared for plating by shaking of the tubes, excess broth from the swab was removed by pressure against the tube wall. Approximately 1.0 ml of broth was retained for using in the preparation of the dilution series and the samples were serially diluted until 10^{-5} level. The serial dilutions were surface plated on six selective media. The agar plates were inoculated in duplicate with either 0.1 ml of swab solution or dilutions (10^{-2} , 10^{-3} , 10^{-4} , 10^{-5}) prepared with 1/4 Ringer's solution. Plate Count Agar (PCA; Biokar Diagnostics, France) was used for estimation of total bacterial counts (Desmaures *et al.* 1997). Total *streptococci* count of the samples were determined by direct plate method using M17 agar (M17, Merck) (Pacini *et al.* 2006). Violet Red Bile Agar was used for estimation of total *Coliform* (Chen *et al.*, 1998). Violet Red Bile Dextrose Agar (VRBD, Merck) and selective Chromocult Agar were used respectively for determination of number of *Enterobacteria* and the number of *E. coli* respectively. The number of *Klebsiella* was determined on Simmons Citrat Agar (SCA, Merck) with myo-inositol. Inoculated plates were incubated 48 h at 37 °C. Results were reported as $\log_{10}$ cfu/ml of swab solution.

Somatic cell count

Milk samples were collected during the fifth and ninth weeks of the trial for the analysis of Somatic Cell Count (SCC). These samples were collected from a composite of all four quarters. Then, they were immediately refrigerated at 4 °C after collection until they were analysed for SCC. SCC (direct measurement) of the milk samples was determined by using DeLaval cell counter from DeLaval International AB, Tumba, Sweden. Results were expressed as $\log_{10}$ of cells/ μ l (thousand cells/ml).

Behavioral activities of cows

The Behavioral activities of cows under study were observed using the instantaneous sampling method

as used by Kartal and Yanar (2011). Observation of cow behaviors were conducted and recorded weekly by walking through the barn, at a distance from the stall at least 2.1 m, every 15 min from 9.00 until 12.00 h. Behaviors were recorded for each of the following activities 1: lying (cow's body contacted bedding and ground), 2: Standing (cow was inactive in upright position), 3: eating (cow's head was in feed bucket), 4: Drinking water (cow's head was in the waterer), 5: Ruminating at standing position, 6: Ruminating at lying position. The Percentage of time spent on each activity was calculated for each week.

Cow cleanliness score

The amount of any foreign material including dirt and fecal matter on the udder of the cows was evaluated by using hygiene scoring system. In this system, each cow was scored for cleanliness of the udder after visual inspection by an evaluator. A 5-point scale was used according to methods utilized by Reneau *et al.* (2005). The animals were compared with model animals in photographs and scored based on the following categories: 1 = clean; 2 = small spots of dirt; 3 = moderately dirty; 4 = mostly covered in dirt on legs and belly; 5 = very dirty, with caked-on dirt. The recording of the udder cleanliness scores was carried out for all experimental cows at fifth and ninth weeks of the experiment.

Statistical analysis

All dependent variables were assessed for normality, then SCC and bacterial counts data were normalized by $\log_{10}$ transformation. The data were statistically analyzed by using an independent samples *t*-test using cows as the observational unit (SPSS, 2011, ver. 20.0). Results are expressed as mean ($\pm$ SE).

Results

The means and standard errors for bacterial counts in swab samples from teat skin ($\log_{10}$ cfu/ml) of cows are presented in Table 1. There was no statistically significant difference between concrete and rubber mat groups in terms of total bacteria count ($P>0.05$). *Streptococcus* spp. counts were similar in the teat skin swab samples of cows in both bedding groups and the differences were not statistically significant ($P>0.05$). *Enterobacteriaceae* counts of the samples in the rubber mat group were slightly fewer than in the concrete group; however, this difference was not statistically significant. It was determined that

Coliform counts in teat skin swab samples of cows in the concrete group were significantly higher than the group housed on rubber mat ($P<0.05$). Similar results were observed for the *Escherichia coli* counts. Swaps taken from the cows housed on rubber mat had significantly fewer *Escherichia coli* counts compared to the concrete bedded group ($P<0.01$). Furthermore, *Klebsiella* spp. count was significantly greater ($P<0.01$) in the concrete group than that in the rubber mat group.

Figure 1 represents the means and standard errors for SCC of the milk samples taken from cows in concrete and rubber mat bedding materials. SCC of the milk samples taken from the cows bedded with rubber mat was significantly lower ($P<0.05$) than the samples obtained from the cows housed on concrete. Differences between cows in the two different flooring types, in terms of cleanliness scores, were also statistically significant ($P<0.05$) (Figure 2).

Table 1. Means and Standard Errors for Bacterial Counts in Teat Skin Swab Samples ($\log_{10}$ cfu/ml) of Cows

	Group	N	$\bar{X} \pm S_{\bar{X}}$		Sig.
Total Bacteria ($\log_{10}$)	Rubber Mat	18	5.88	0.09	ns
	Concrete	20	5.80	0.10	
<i>Streptococcus</i> spp ($\log_{10}$)	Rubber Mat	18	6.01	0.10	ns
	Concrete	20	5.99	0.11	
<i>Enterobacteria</i> ($\log_{10}$)	Rubber Mat	18	2.81	0.21	ns
	Concrete	20	3.12	0.15	
<i>Coliform</i> ($\log_{10}$)	Rubber Mat	18	2.71	0.19	*
	Concrete	20	3.10	0.13	
<i>Escherichia coli</i> ($\log_{10}$)	Rubber Mat	18	3.13	0.26	**
	Concrete	20	3.35	0.16	
<i>Klebsiella</i> ($\log_{10}$)	Rubber Mat	18	1.79	0.38	**
	Concrete	20	2.55	0.21	

*: $P<0.05$, **: $P<0.01$, ns: Nonsignificant

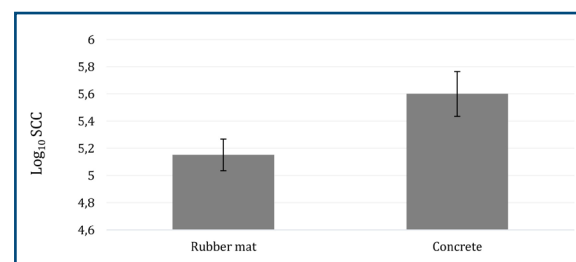


Figure 1. Means and Standard Errors for SCC of the Milk Samples of Cows in Concrete and Rubber Mat Flooring Types.

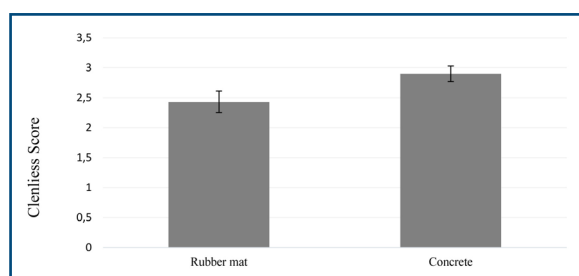


Figure 2. Means and Standard Errors for Cleanliness Scores of the Cows in Concrete and Rubber Mat Flooring Types.

Since the study was carried out in a tie-stall barn, cows spent their entire time in the stalls. Means and standard errors for percentage of time spent on different activities by cows are given in Table 2.

Table II. Means and Standard Errors for Percentage of Time Spent on Different Activities by Cows

Behaviors %	Concrete		Rubber Mat		Sig.
	$\bar{X} \pm S_{\bar{X}}$		$\bar{X} \pm S_{\bar{X}}$		
Lying	29.52	0.64	31.48	0.69	*
Standing	25.00	1.54	19.35	1.64	**
Eating	28.22	0.78	31.65	0.83	**
Drinking water	0.72	0.16	1.14	0.17	ns
Ruminating while standing	7.42	0.85	7.48	0.91	ns
Ruminating while lying	9.09	1.19	8.88	1.27	ns

*, $P < 0.05$, **, $P < 0.01$, ns: Nonsignificant

The effects of flooring types on the percentage of time spent on drinking water, ruminating while lying and standing behaviors of cows was not statistically significant. Cows in both groups spent most of their time for lying and eating behavior, and these behaviors were followed by standing. Cows housed on rubber mat spent significantly more time lying ($P < 0.05$) compared to cows housed on concrete floor. On the other hand, the percentage of time spent by standing was significantly higher ($P < 0.01$) in concrete group than the rubber mat group. The time spent for eating behavior was also affected significantly ($P < 0.01$) by different bedding types, cows in the rubber mat group spent more time for this activity.

Discussion

Since cows spend their entire time in stalls in tie-stall barns, a quality bedding is vital for welfare of cows as well as the quality of milk produced. An increase in the number of environmental bacteria in the bedding materials may result in mastitis and higher Somatic Cell Count (SCC) (Gautam *et al.* 2020). Hogan *et al.* (1989) reported that there was a correlation between the intramammary *Coliform* infections and

the number of bacteria in the teat end, and bedding was reported as the mean source for the teat end bacteria by many researchers (Natzke and LeClair 1976; Hogan *et al.* 1989; Zdanowicz *et al.* 2004; Kristula *et al.* 2008; Proietto *et al.* 2013). In the present study, there was no statistically significant difference between two flooring groups for the total bacteria count, *Streptococcus* spp. And *Enterobacteriaceae* counts of the teat end swap samples taken from the cows in both group. Similarly, differences in gram negative-bacteria and *Streptococcal* bacteria counts were reported as not statistically significant by Hogan *et al.* (1999) in three different bedding materials (copped newspaper, pelleted corn cobs and wood shavings).

Existence of *Coliform* species on the teat ends after udder preparation reported to be a source of pathogen exposure during milking (Munoz *et al.* 2008). Concrete flooring resulted in significantly higher *Coliform* counts compared to RM bedded group ($P < 0.05$). These findings are comparable with Kristula *et al.* (2008), who indicated that *Coliform* counts were significantly lower in mattress flooring when treated with lime.

Escherichia coli is reported to be the most common species, isolated in more than 80% of cases of coliform mastitis cases (Botrel *et al.* 2010; Suojala *et al.* 2013). *Escherichia coli* counts were significantly affected by the flooring type in this study ($P < 0.01$). Teat end swab samples had 7% less *Escherichia coli* counts compared to the samples taken from the cows housed on concrete.

Klebsiella is known as an opportunistic pathogen and cause environmental mastitis (Schukken *et al.* 2012). The isolation rate of this microorganism in the milk samples was reported between 2 to 9% in clinical mastitis cases (Levison *et al.* 2016; Masse *et al.* 2020). Samples taken from the cows housed on concrete had 42.5% more *Klebsiella* counts compared to rubber mat group. This difference was statistically significant ($P < 0.01$). Similarly, *Escherichia Coli* and *Klebsiella* counts were reported to be significantly lower in mattress flooring when treated with lime compared to different treatments by Kristula *et al.* (2008). Overall results of the present study are supported by Gautam *et al.* (2020) who indicated that clinical mastitis cases were 14% higher than rubber mats in concrete flooring. Additionally, Kumar *et al.* (2017) reported that teat and udder wounds as well as mastitis cases were considerably higher in cows housed on concrete.

The cleanliness score of cows can be utilized as a tool in the assessment of the level of bacterial exposure (Ward *et al.* 2002; Reneau *et al.* 2005; Munoz *et al.* 2008). Cows in the rubber mat group were determined to be significantly cleaner than that in concrete group ($P < 0.05$). The physical feature of

RM, that is easy to clean, and less moisture holding compared to concrete, can explain this result. Notably, lower cleanliness scores of the cows in the RM group ($P<0.05$) may be the explanation for the fewer counts of *Coliform*, *Escherichia coli*, *Klebsiella* spp. compared to concrete housing.

Several indicators are used for the assessment of mastitis in milk, Somatic Cell Count (SCC) is the most widely used procedure worldwide (Halasa and Kirkeby, 2020). SCC of the milk samples were also affected significantly ($P<0.05$) by different flooring types (Figure 1). Lower SCC could be attributed to the lower cleanliness scores and fewer environmental-mastitis causing bacteria counts of cows in the RM group. Calamari *et al.* (2009) reported similar SCC counts for the cows housed on rubber mat. The results of the current study are in harmony with the findings of Sant-Anna and Parandos da Costa (2011), and Erdem and Okuyucu, (2019) who noted that increase in the cow hygiene result in a decrease in the SCC count of milk.

Quality bedding that provides comfortable flooring for cows to lie down and take rest helps to improve health, welfare and productive performance in the herd (Singh *et al.* 2020). Physiological disorders show up in the cows that are deprived of lying (Thomsen *et al.* 2012). It was determined that the cows housed on rubber mat spent significantly ($P<0.05$) longer time for lying behavior compared to concrete group (Rubber Mat = 31.48%, Concrete = 29.52%). As expected, the time spent for standing without eating was considerably higher ($P<0.01$) in the cows housed on concrete. When the cows are housed on hard floor and bedding materials such as concrete the time spent for lying decreases significantly (Haley *et al.* 2001). It was noted by Büyükkök *et al.* (2019) that concrete was the least preferred bedding by the cows in free-stall barn type. We assume that cows in the RM group spent a longer period of their daily time lying, since rubber mats are more comfortable and desirable for cows. Results of the present study agree with the findings of Haley *et al.* (2000), Haley *et al.* (2001), Rushen *et al.* (2007), Norring *et al.* (2008), Graunke *et al.* (2011) who reported longer lying

bouts and shorter standing bouts for rubber mats compared to concrete surfaces.

Eating bout of cows in the rubber mat group was the longest compared to other behavioral activities. In addition, the times spent for eating was significantly lower ($P<0.01$) in the concrete group compared to rubber mat group.

There was no statistically significant difference between two groups for water drinking behavior. Similarly, Karakok *et al.* (2009) determined that when cows are provided with bedding the times spent for feeding behavior increases.

In contrast, no difference was reported between concrete and rubber mat groups for eating behavior by Haley *et al.* (2001). Resting and eating behaviors are two of the indicators of animal comfort (Anderson 2001).

One reason for the less amount of time spent for the feeding behavior in the concrete group could be explained by the stress and restlessness faced by the cows. There was no statistically significant difference between two groups in terms of ruminating times spent standing or lying. Our results are comparable with the findings of Karakok *et al.* (2009), who reported similar ruminating times for bedded and non-bedded cows.

Conclusions

The findings of this study provide evidence that using rubber mats instead of concrete for flooring type in tie-stall barns reduces the presence of environmental bacteria sources on the teat ends and increase milk quality by keeping the cows cleaner. Our results also highlight that behavioral indicators of animal comfort such as the time spent for eating and lying behaviors increases when cows are provided with rubber mat bedding instead of hard concrete surface. Cows that are forced to spend their entire time on a concrete surface appears to be reluctant to lie down and display signs of restlessness and discomfort which may negatively affect overall animal productivity.

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