

**STUDY OF THE HYGIENISATION EFFECT OF SEPARATED  
COW LIQUID MANURE USED AS BEDDING****P. Novák, F. Treml, J. Vokralova, S. Vlaskova, S. Slegerova,  
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The study is aimed to determine the safety and feasibility of using separated manure as a bedding material for dairy cows. High bacterial populations in bedding material will influence the high level of bacteria counts on udder surfaces (particularly teat ends) and increase risk of mastitis. The infection pressure of ambient bacteria also influences overall health status of cow, mainly hoofs and reproductive organs. Reduction of humidity in manure material is the main consideration of separating dairy manure. But when they become mixed with manure and water (urine), rapid growth of environmental mastitis pathogens starts because of available nutrients.

Key words: hygienisation, separation, cow liquid manure, bedding

*Introduction*

Bedding material and its manage it strongly influence the quality of cow's environment. A comfortable stall bed encourages resting, minimizes injury and fatigue, and accommodates reasonable rates of heat loss. The type of chosen bedding and daily bedding management can have a major impact on the udder health and the incidence of mastitis infections and also reproductive ability and problems with hoofs. Bedding materials serve as a primary source of environmental bacteria exposure of teat ends as the teats and udders are in frequent direct contact with the bedding materials. Increased bacterial populations will result in increased bacteria counts on teat surfaces or at least

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exposure to a large number of bacteria potentially resulting in increased environmental mastitis pathogen associated mastitis. Environmental bacteria cannot be completely eliminated from a dairy herd. Unlike the contagious agents, environmental bacteria that cause mastitis in dairy herds can survive in the environment for long periods of time. But coliforms and streptococci cannot live on teat skin for long periods of time. If these bacteria are present in large numbers on teat skin, it is result of recent contamination from a source such as bedding. Therefore, the number of these bacteria on teat skin is a reflection of the cow's exposure to the contaminating environment.

Also particle size of bedding influences bacterial populations. Finely chopped materials support greater bacterial numbers than the same bedding with larger particle sizes. Finely chopped organic material has greater surface area for attachment and colonization by bacteria. In addition, these materials adhere more readily to teat skin than larger materials, thus increasing exposure of the teat end to mastitis pathogens.

Growth rates of coliforms and environmental streptococci are greatest during warm, wet weather. The effects of season on bacterial populations in bedding are quite dramatic in regions that experience a wide variation of temperatures within a year. In general, the impact of bedding on exposure of cows in confinement housing decreases during cold weather and increases as temperatures and humidity increase (Hogan et al, 1987). Therefore, proper ventilation of barns is essential to moderate the effects of heat and humidity in housing areas. Climatic factors affecting exposure in herds where cows are maintained on dry lots differ from those of traditional Central European herds.

Bedding materials fall into two basic categories, organic and inorganic. Each has its advantages and disadvantages. Organic bedding materials consist of straw, hay, saw dust, wood shavings, crop residue, shredded paper, paper pulp residue, composted or dried manure and similar materials. They are used as bedding because they absorb moisture, are compatible with manure handling systems and are readily available. A major disadvantage of these materials is that they will support rapid growth of environmental mastitis pathogens when they become mixed with manure and water (urine) because of available nutrients. Contaminating bacteria grow to large numbers within 24 hours. The major pathogens associated with bedding materials are the environmental Streptococci and coliform such as *Escherichia coli* and *Klebsiella* spp.

In general, all bacterial populations are lower in inorganic bedding materials, such as sand, compared to organic bedding materials (Hogan et al., 1989). The numbers of environmental streptococci in sand bedding will vary depending upon the amount of soil in the sand and the degree of fecal contamination once the sand is placed in the stalls. Washed sand is the bedding

of choice, and maintenance of stalls is critical in order to achieve low numbers of pathogens in the bedding.

Using dairy wastes is an attractive alternate as bedding material because it is readily available and cheap. It is believed that reducing the moisture and higher temperature will reduce bacteria counts to acceptable levels. Mote et al. (1988) showed that initially coliform bacteria decrease in number but then increased to levels found in fresh feces under normal composting conditions. Composted bedding material did not have decreased coliform or environmental streptococci numbers. When incubated, in the laboratory, composted bedding material frequently yields bacteria numbers approaching 1 billion cfu per cc of bedding. Initial numbers of bacteria per cc of bedding depend, to a degree, on the amount of moisture present. In low moisture conditions, levels are substantially reduced when compared to those of higher moisture level.

Additives have been used in an attempt to decrease the bacteria counts of bedding materials. Lime (hydrated) added to sawdust or shavings has been shown to increase bedding pH, and reduce its water content. Adding one to two pounds of hydrated lime per stall to bedding inhibited bacteria growth but may or may not reduce the incidence of clinical mastitis. The effect of added lime is short lived (24 hrs.) and requires that lime be added daily. Research indicate that adding the lime to bedding prior to application and mixing just prior to use is the most effective means of reducing the bacterial population of existing bedding materials. Alkalisising and acidifying agents have been used to lower bacterial counts in sawdust and recycled manure. The antibacterial effects of bedding treatments were related to the pH of the bedding materials.

#### *Material and methods*

The samples of separated manure, bedding material and proper cattle manure were obtained from two dairy farms. Cows were housed in free-stall barns with boxes and bedding material was added daily. In farm 2, there was separated manure treated with hydrated lime in one group and the second group of boxes was untreated negative control. Samples were a composite of bedding from the back one-third of boxes; samples of manure and separated manure composed each average representative sample. The microbial analysis was done by quantitative microbiological method as requested ISO and EN norms. Samples of solid and liquid manure were tested by current quantitative microbiological methods. The dynamics of processing (thermophilic, mesophilic and psychrophilic microorganisms) and hygienic-epizootologic (coliforms, coli-faecalis, enterococci and moulds) microbial indicators were observed.

### Results

The dynamics of the bacteria counts was similar in both farms. Bedding material contained the highest number of thermophilic bacteria, moulds and yeast fungi, in addition coliforms and enterococci in farm 1 and mesophilic bacteria and psychophilic bacteria in farm 2. The lowest counts of bacteria were found in manure (excluding coliforms) in farm 1, and in separated manure (excluding enterococci) in farm 2. Additionally, in farm 2, there were proved the decreased number of thermophilic bacteria, psychophilic bacteria, coliforms and enterococci in bedding treated with lime. Bacterial counts of partial group ranged between  $10^4$  and  $10^{12}$ . These data are mentioned in table 1 and table 2. There is also presented the percentage comparison between bacteria counts in bedding material and treated bedding material in farm 2. The number of selected groups of bacteria decline of 54,3 - 99,7% (very significant).

Generally, higher number of bacteria was found in samples from farm 1, but it was mainly influenced by the macroclimatic conditions of sampling (October vs. December).

Table 1. - COUNTS OF BACTERIA IN FARM 1

	Total count of microorganisms (MPA)			ENDO agar		CZAPEK-DOX agar	
	thermo	meso	psychro	coliform bacteria	enterococci	moulds	Others
Manure	$3,6 \cdot 10^5$	$9,8 \cdot 10^9$	$2,8 \cdot 10^{10}$	$1,2 \cdot 10^5$	$8,5 \cdot 10^4$	$1,5 \cdot 10^6$	$7,8 \cdot 10^8$
Separated manure	$6,8 \cdot 10^5$	$2,0 \cdot 10^{12}$	$3,6 \cdot 10^{11}$	$6,3 \cdot 10^4$	$4,8 \cdot 10^4$	$2,5 \cdot 10^7$	$6,8 \cdot 10^9$
Bedding	$1,6 \cdot 10^6$	$1,6 \cdot 10^{12}$	$2,6 \cdot 10^{11}$	$1,5 \cdot 10^5$	$1,5 \cdot 10^5$	$7,0 \cdot 10^8$	$3,0 \cdot 10^{10}$

Table 2. - COUNTS OF BACTERIA IN FARM 2

	Total count of microorganisms (MPA)			ENDO agar		CZAPEK-DOX agar	
	thermo	meso	psychro	coliform bacteria	enterococci	moulds	others
Manure	$1,4 \cdot 10^6$	$4,0 \cdot 10^8$	$7,2 \cdot 10^8$	$2,0 \cdot 10^6$	$4,0 \cdot 10^4$	$1,0 \cdot 10^5$	$2,0 \cdot 10^7$
Separated manure	$1,1 \cdot 10^6$	$3,2 \cdot 10^8$	$5,3 \cdot 10^8$	$1,7 \cdot 10^5$	$3,5 \cdot 10^4$	$5,0 \cdot 10^4$	$1,6 \cdot 10^6$
Bedding with lime	$9,6 \cdot 10^5$	$1,6 \cdot 10^9$	$3,2 \cdot 10^8$	$5,5 \cdot 10^4$	$5,0 \cdot 10^3$	$5,0 \cdot 10^4$	$1,0 \cdot 10^7$
Bedding	$2,1 \cdot 10^6$	$5,6 \cdot 10^{10}$	$2,2 \cdot 10^9$	$1,8 \cdot 10^5$	$1,3 \cdot 10^4$	$3,0 \cdot 10^6$	$3,0 \cdot 10^9$
Decrease of (%)	54,3	97,1	85,5	69,4	61,5	83,3	99,7

### *Discussion*

The general goal is to keep bacteria counts to less than one million colony forming units (cfu) per milliliter (ml) where bedding contacts the udder, because coliform numbers in excess of  $10^6$  cfu/cc or /gram in bedding increased udder infections (Peters, 2002). Counts of coliforms ranged between  $1,5 \cdot 10^5$  (Farm 1) and  $1,8 \cdot 10^5$  (Farm 2), but total bacteria counts/number (psychrophilic, mesophilic, thermophilic) obtained  $1,86 \cdot 10^{12}$  resp.  $5,82 \cdot 10^{10}$  cfu/ml. After treatment with lime, total bacteria counts declined on  $1,92 \cdot 10^9$  CFU/g. But there is an important impact of sampling time after treatment. Hogan et al. (1989) have shown that pretreatment mean concentrations of total Gram-negative bacteria, coliforms, *Klebsiella* spp. and streptococci in recycled manure were  $10^5$ . Treatment of recycled manure with an alkaline conditioner or hydrated lime lowered the total bacterial count 100-fold ( $10^3$ ) for approximately 24 hours. But antibacterial activity of hydrated lime in bedding was diminished on day 2. The antibacterial activities of bedding treatments were related to the pH of bedding materials. Addition of these conditioners to recycled manure initially elevated pH to an extreme alkaline range prior to use as bedding. As the pH of the recycled manure was neutralized during use, the antibacterial effects of the alkaline conditioner and hydrated lime diminished. Despite the extremes in pH of bedding after the addition of hydrated lime and commercial conditioners, no changes in teat skin were detected by gross observations during the trial.

Reduction of humidity in manure material is the main consideration of separating dairy manure. Moisture level is a key criterion for keeping bacteria counts low. Samples in which the moisture has been reduced to approximately 50-60% frequently yield bacteria numbers that are in the range of 500,000 cfu/cc of bedding. However, after being in the stalls for 24 hours those bacteria numbers approach 70 million to one billion cfu per cc of bedding (Mote, 1988).

### *Conclusion*

Composted or dried manure solids have also been advocated as bedding material. They have been used successfully on some farms in the southwest where weather conditions contribute to a very dry environment for most of the year. These materials are usually free of most major environmental mastitis pathogens when initially applied however dried manure is an excellent medium for bacterial growth once moisture from urine and fresh manure are added (Carrol, 1978). In the northeast ambient humidity and air temperatures are not conducive to the effective use of dried manure solids or composted dairy waste

as a bedding material in respect to reducing teat skin exposure to environmental pathogens. Separating offered little benefit toward net reduction in teat end contamination by bacterial numbers in dairy waste solids.

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### UČINAK HIGIJENIZACIJE SEPARIRANOG TEKUĆEG GNOJA KRAVA KORIŠTENOG ZA STELJENJE

#### Sažetak

U radu je istraživana mogućnost upotrebe separiranog krutog gnoja kao stelje pri smještaju mliječnih krava. Brojne bakterijske populacije u stelji utječu na visoku razinu broja bakterija na površini vimena (naročito na krajevima sisa) i povećani rizik od mastitisa. Općenito bakterije u staji imaju utjecaj na zdravstveni status krava poglavito papaka i reproduktivnih organa. Osnovni cilj pri separaciji gnoja mliječnih krava, koji bi se koristio za steljenje, je smanjenje postotka vlage u njemu. Međutim kada se on pomiješa odnosno dođe u dodir s gnojem i urinom, patogene bakterije, uzročnici mastitisa počinju se brzo razmnožavati zbog dostupnosti hranjivih tvari.

Ključne riječi: higijenzacija, separacija, tekući gnoj, stelja

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