Review Article

Strategies for Preventing Environmental Mastitis in Dairy Farming: A Review

ABSTRACT

The dairy industry must adhere to stringent international standards due to the growing demand for healthy, high-quality, and affordable dairy products worldwide. To ensure the quality of raw milk, key markers such as Bulk Milk Somatic Cell Count (BMSCC) and Total Bacterial Count (BMTBC) have become standard benchmarks. However, mastitis, the most common disease affecting dairy cows, poses a significant risk to both animal welfare and the long-term sustainability of the dairy sector. Mastitis leads to reduced milk production, increased treatment costs, milk withholding during treatment, higher labor requirements, and premature culling of affected cows. In India alone, mastitis costs the dairy industry 2.37 billion rupees annually, with subclinical mastitis accounting for approximately 70% of this loss. While contagious infections have been effectively controlled, environmental mastitis pathogens such as Streptococcus uberis, Escherichia coli, and Klebsiella spp now pose the primary concern for mastitis control. The management of cow bedding materials is crucial as they serve as a significant source of exposure to these environmental infections. This review study provides a detailed discussion of environmental mastitis pathogen control, emphasizing the critical role of bedding materials in reducing the risk of exposure to these pathogens.

*Keywords:*Environmental mastitis, Bedding material, Udder health, Dairy cows

1. INTRODUCTION

Consumers increasingly seek healthy, high-quality, and affordable dairy products produced through socially responsible practices, driving global demand (Sapp *et al*., 2009). To meet these demands, dairy processors now require milk that adheres to stringent international standards, with Bulk Milk Somatic Cell Count (BMSCC) and Total Bacterial Count (BMTBC) serving as key benchmarks (Costello *et al*., 2003; Rowbotham and Ruegg, 2015). However, mastitis, a prevalent ailment among dairy cows, remains a significant and costly challenge for the dairy industry (Sahu *et al*., 2023; Sahu *et al*., 2022). Mastitis, characterized by inflammation of the mammary glands, threatens both animal welfare and the financial stability of dairy operations (Kristula*et al*., 2008). Factors affecting incidence of mastitis has been illustrated in Fig 1. Impact of mastitis is evident through reduced milk production, treatment expenses, milk withholding post-treatment, increased labor requirements, and premature culling of affected cows. Of particular concern is subclinical mastitis, which is challenging to detect and manage due to its high prevalence, prolonged incubation period, and potential progression to clinical mastitis (Sahu *et al*., 2023; Seegers *et al* ., 2003). In India alone, mastitis inflicts an annual economic loss of approximately 2.37 billion rupees, with subclinical mastitis accounting for around 70% of this financial burden (Sahu *et al*., 2023). Additionally, subclinical mastitis results in substantial lactation losses ranging from INR 21,677 to 88,340 (Rathod *et al*., 2017). Furthermore, mastitis stands as the primary driver of antibiotic usage in dairy farming, contributing to the emergence of antibiotic-resistant pathogens and posing significant public health risks (Pol and Reugg, 2007). Addressing mastitis requires distinguishing between contagious and environmental sources of infection within dairy herds (Kristula*et al*., 2005). While contagious bacteria spread mainly during milking procedures, environmental pathogens, including *Streptococcus uberis, Escherichia coli,* and *Klebsiella spp*, thrive in the cow's surroundings, particularly in bedding materials (Kristula*et al*., 2008). Given the significant role of bedding materials in transmitting environmental mastitis pathogens, proper housing and management practices are critical for minimizing exposure to these bacteria (Smith and Hogan, 2001; Kristula*et al*., 2008). This underscores the importance of maintaining clean, well-drained dry cow areas and implementing routine cleaning protocols to mitigate the risk of mastitis transmission (Hogan & Smith, 2012).

Mastitis-causing bacteria in dairy herds are classified as contagious or environmental, depending on their primary source of transmission (Kristula*et al*., 2005). Contagious bacteria, like *Staphylococcus aureus* and *Streptococcus agalactiae*, are primarily spread during milking from infected udders to clean ones. Effective control measures such as teat dipping and dry cow therapy are used to manage these diseases (Smith and Hogan, 2001). Environmental mastitis can arise from various sources in the cow's environment, with the highest risk during the dry period, especially in the two weeks before and after calving. Due to advancements in managing contagious pathogens, environmental infections have become the main concern for mastitis control in modern dairy farming (Sharif *et al*., 2009; Ruegg, 2017). Environmental pathogens, including *Streptococcus uberis*, *Escherichia coli*, and *Klebsiella spp*, often lead to high rates of mastitis, particularly in herds with controlled infectious diseases (Kristula*et al*., 2005). Bedding materials, where cows spend much of their time lying down, serve as a significant reservoir for environmental mastitis bacteria (Krawczel and Grant, 2009). To prevent environmental mastitis, it's crucial to design housing and management systems that minimize exposure to environmental infections at the teat end (Smith and Hogan, 2001; Kristula*et al*., 2008). Clinical mastitis during lactation is often linked to infections during the dry period (Green *et al*., 2002). This underscores the importance of prioritizing cleanliness and effective management in maternity and dry cow housing. To reduce the risk of illness for both cows and calves, it's recommended to maintain well-drained, dung-free dry cow areas, regularly clean loose housing, and avoid manure packs (Hogan & Smith, 2012).This review paper delves into controlling environmental mastitis pathogens, which are increasingly causing intramammary infections in dairy cows. It emphasizes the critical role of bedding components in minimizing exposure to these pathogens. By enhancing mastitis management strategies and preserving udder health, these insights will assist dairy farmers in maintaining high-quality milk output and the long-term sustainability of the dairy industry.

2.1 Effects of Different Bedding Types on Mastitis Incidence in Dairy Cows

Mastitis in dairy cows can be influenced by various factors, including the type of bedding used. Studies have shown significant variations in bacterial counts not only between different bedding materials in dairy farming but also across seasons. Correlations between pathogen populations in bedding and teat skin have been observed, emphasizing the potential for teats to pick up bacteria from the environment (Zdanowicz *et al* ., 2004; DeVries *et al* ., 2012; Cook *et al* ., 2005). As teats are in direct contact with bedding for a substantial duration daily, contamination is likely to affect (Zdanowicz *et al*., 2004; DeVries *et al*., 2012; Cook *et al*., 2005).

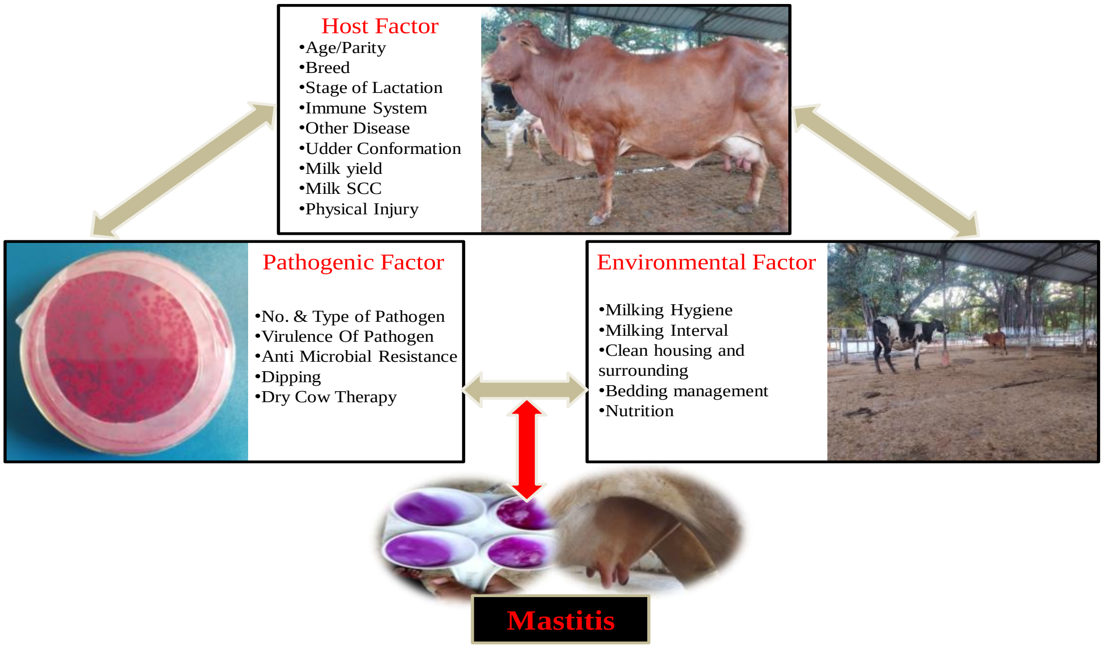


Fig. 1 Factors affecting Udder Health Status and Occurrence of Mastitis

Organic bedding materials, such as straw and sawdust, often have higher moisture content and are associated with elevated counts of gram-negative bacteria, including *Coliform spp*, *Klebsiella spp*, and *Streptococci spp*. Sawdust, a common organic bedding material, is preferred in confinement housing due to its availability and compatibility with manure treatment systems. However, the use of sawdust can lead to increased moisture and manure content, raising the pH of the bedding and promoting the growth of environmental organisms (Zehner *et al* ., 1986; Zdanowicz *et al* ., 2004). The escalating costs of traditional straw bedding have led to interest in alternative solutions, including recycled manure solids (RMS) bedding, which offers benefits such as improved cleanliness, reduced lesions, enhanced cow comfort, and economic advantages (Green *et al* ., 2014; Bradley *et al* ., 2018; Fournel *et al* ., 2019). However, some studies have highlighted concerns about increased undesirable bacterial populations with RMS bedding (Cole and Hogan, 2016; Rowbotham and Ruegg, 2016).An innovative bedding material called box compost, made from composted biodegradable household trash, has been introduced (Groot Antink, 2009). Subjected to a stringent heat treatment process, box compost contains added Lactobacillus species to inhibit the growth of harmful bacteria. Despite containing a higher concentration of gram-positive bacteria, deliberate inoculation with Lactobacillus species helps prevent the development of potentially harmful gram-negative environmental bacteria (Van Gastelen*et al* ., 2011).

It has been reported that BMSCC was lower on farms using inorganic bedding (compared to farms using organic bedding) when bedding was provided at intervals of more than 7 days and value of each unit of milk produced may rise if it were produced on farms utilizing inorganic bedding at a higher grade.Soft free-stall bases and foam mattresses have been shown to decrease the frequency of clinical mastitis and teat lesions. Rubber mats and multilayer mats also demonstrate lower hazard ratios for clinical mastitis compared to concrete, highlighting the importance of flooring in mastitis prevention (Ruud *et al* ., 2010; Fullwider and Palmer, 2004;Rowbotham and Ruegg, 2015). Sand bedding is often considered the best option for dairy cows due to its superior cushioning and traction. Sand bedding has been associated with lower bacterial growth and extended lying time, contributing to increased cow comfort (Lombard *et al* ., 2010). While challenges exist in handling sand in manure systems, advancements in separation technologies have made it more manageable (Kristula*et al* ., 2005). However, studies have indicated higher bacterial counts on teat ends for cows on sand bedding compared to sawdust, emphasizing the importance of proper management (Zdanowicz *et al* ., 2004; Godden *et al* ., 2008).

The cost of high-quality bedding often leads farmers to limit its use or seek alternatives, despite their awareness of the importance of adequate bedding (van Weyenberg *et al* ., 2015). Potential alternatives like Switchgrass, *Posidonia oceanica*, Miscanthus grass, and spelt husks should be considered for bedding in dairy cow systems (Ferraz *et al* ., 2020; Sanderson *et al* ., 2006; Wolfe *et al* ., 2018). These alternatives offer various advantages, including adaptability to temperate regions, resilience to diseases and pests, low fertilizer requirements, and economic viability (Frigon *et al* ., 2012; Wolfe *et al* ., 2018).

2.2 Optimizing Bedding Management for Improved Udder Health in Dairy Cows:

Ensuring the well-being of dairy cows is paramount for both animal welfare and economic efficiency, with the type and quality of bedding material playing a crucial role in providing a comfortable environment in free stalls. The chosen bedding material must possess several qualities, including thermal comfort, softness, durability, and sufficient friction to allow cows to stand and lie down without slipping, while also promoting cleanliness and overall health with minimal daily labor requirements (Chaplin *et al* ., 2000; Tucker *et al* ., 2009). Observations have indicated that cows exhibit a preference for spending more time in stalls filled with deep-bedded sawdust or sand compared to those with mattresses covered with only a thin layer of sawdust, underscoring the importance of bedding material in ensuring cow comfort (Manninen *et al* ., 2002; Tucker *et al* ., 2003). Notably, for every kilogram of sawdust or straw added to a mattress, cows increased their daily lying time by 12 minutes (Tucker and Weary, 2004).Monitoring lying behavior, with the goal of ensuring cows spend the recommended 12 to 13 hours per day lying down, serves as a useful criterion for assessing freestall comfort (Jensen *et al* ., 2005; Haley *et al* ., 2000).

Given that cows lie down for a significant portion of the day, the bedding surface serves as one of their primary and direct points of contact with the external environment. The moisture content of the bedding material significantly affects laying behaviors and cow health, with cows kept in stalls with higher dry matter percentages spending more time lying down compared to those in stalls with lower dry matter percentages. For instance, Fregonesi*et al* . (2007) found that cows kept in freestalls with bedding that had a dry matter (DM) percentage of 26.5% spent less time lying down than those kept in stalls with a DM of 86.4%. Similar to this, Reich *et al* .(2010) discovered that cows exposed to beddings with different DM percentages reduced their lying time on the wettest bedding surface, with the reduction in lying time only being moderately effected until the DM was dropped to

34% or below.The importance of bedding quality, particularly in terms of dry matter content, in influencing laying behaviors is evident from these findings.

The use of a propane-fueled flame moving across the surface of recycled sand bedding significantly reduces mastitis pathogens and moisture content on the surface layer, particularly within the top 25 mm of recycled sand bedding. Fresh recycled sand exhibits higher levels of flaming efficiency in suppressing bacterial populations compared to sand that has been in use for a longer period of time (Hogan *et al* ., 2012). Additionally, propane flames offer a useful and environmentally beneficial strategy for reducing disease populations in poultry litter material, enabling the sanitization of animal interaction areas without the need for potentially hazardous chemicals (Raffaelli *et al* ., 2010).

Environmental bacteria thrive best in a pH range of 4.4 to 8.7, and as the pH falls below this range, the optimal temperature for their growth rises, leading to a significant decline in their growth rate. It has been observed that the ability of bedding conditioners to be effective against mastitis bacteria in bedding for up to 48 hours, with acidic bedding additives proving more effective than alkaline conditioners at reducing bacterial burdens. Teat orifice integrity in dairy cows, crucial as the first line of defense against infections, did not show noticeable impacts from either alkaline or acidic bedding additions (O'May*et al* ., 2005; Hogan *et al* ., 1999). The addition of a clay-based acidic bedding conditioner led to a significant decrease in environmental counts of various bacterial species, including total gram-negative bacteria, *Streptococci spp, Coliforms spp,* and *Klebsiella spp*, both in the bedding material and on the teat ends, without affecting teat structural integrity (Proietto *et al* ., 2013).

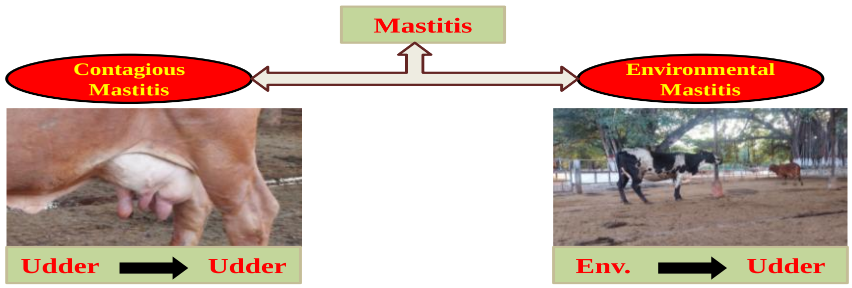
Lime treatment emerged as the sole effective approach, significantly reducing bacterial counts (Hogan *et al* ., 1997; Kristula*et al* ., 2008). The technique of sprinkling crushed limestone over bedding has been experimented with by some researchers and farmers to reduce bacterial presence, particularly potential mastitis pathogens. However, this method may not achieve thorough distribution of limestone throughout the bedding, limiting its maximum effect on bacterial flora. The habitual, long-term use of more than 0.5 kg of lime on mattresses may be linked to undesirable side effects, despite its benefits in suppressing bacterial growth. A commercial product comprised of 92.5% calcium carbonate, 5% sodium dichloroisocyanurate, and 2.5% aluminum sulfate, with a pH level of 3.7, proved effective in suppressing populations of *Coliform spp, Klebsiella spp.*, and *Streptococcus spp*, albeit with somewhat less potency compared to hydrated lime (Kristula*et al* ., 2008).

In order to enhance environmental mastitis control, it is imperative to gain a more comprehensive understanding of the interplay between bedding selection, bacterial bed count, and udder health. Additionally, there is a pressing need for evidence-based benchmarks to effectively monitor bedding hygiene. Patel *et al* . (2019) identified attainable benchmarks for bacterial counts using four different types of bedding for lactating cows [new sand, reclaimed sand, manure solids, and organic non-manure materials], indicating that for Coliforms, the recommended levels are ≤500 colony-forming units per cubic centimeter (cfu/cm³) for unused bedding and ≤10,000 cfu/cm³ for used bedding. For *Klebsiella spp*., both unused and used bedding should ideally register at 0 cfu/cm³. Similarly, *Staphylococcus spp*. should not exceed 0 cfu/cm³ for both unused and used bedding. As for *Streptococcus uberis* (SSLO), the suggested benchmarks are ≤500,000 cfu/cm³ for used bedding and 0 cfu/cm³ for unused bedding. Minor variations are proposed for SSLO in unused manure solids (MNS), where the recommended benchmark is ≤1,000 cfu/cm³. These benchmarks offer a reliable framework for monitoring bedding hygiene across a range of bedding materials.The findings indicate that maintaining bedding in a dry and loose condition leads to cleaner animals and reduces the risk of mastitis. Assessing cow cleanliness proves to be a valuable tool in guiding bedding management and evaluating the potential for subclinical mastitis (Fávero *et al* ., 2015). The experimental trials conducted by Ferraz *et al* . (2020) underscore the importance of conducting thorough physical, chemical, and biological analyses before selecting any material for use as bedding for dairy cattle. This is because the physical attributes of the bedding materials can have significant implications on their chemical and biological properties.

2.3 Alternative Approaches to Minimize the Occurrence of Mastitis:

Four key factors for preventing incidence of mastitis have been illustrated in Fig 2. Using the four key foundations defined by Klaas and Zadoks (2018), environmental mastitis control strategies are constructed. (1) Reducing the bacterial burden in the cow's surroundings. (2) Routinely removing bacterial contaminants from teats to prevent invasion. (3) Increasing the resilience and resistance of the host. (4) Improving dry-off processes as well as enhancing mastitis control techniques, including case detection and management. Sound husbandry practices, upholding proper udder hygiene, strict pre-milking sanitation procedures, using post-milking teat dip, performing effective milking machine cleaning, ensuring adequate cooling, storing milk within the temperature range of 0 to 4.4°C, treating mastitis during non-lactating periods, and culling of persistently infected animals are all essential elements in mastitis control (Sharif *et al* ., 2009; Reinemann, 2011).

Animal overcrowding ultimately increases the risk of disease transmission. In order to limit pathogen exposure to the mammary gland and thereby lower the incidence of mastitis, it is essential to maintain appropriate sanitation and proper ventilation in the farm building. Dung and urine should always be removed as soon as possible because they are common sources of illnesses on farms and should always be given with dry bedding. Ticks breed in the farm's cracks and crevices, and flies reproduce better in chilly, damp environments. For the purpose of preventing the development of lice, flies, and ticks, it is critical to seal any crevices and quickly dry any moist or humid places (Sharif *et al* ., 2009).



Milking Management	Immunity	Dry cow Management	Bedding Management
✓Pre-milking Sanitation procedure ✓Removal of dung and urine ✓Udder hygiene ✓Effective milking machine cleaning and application ✓Post-milking teat dipping ✓Overcrowding	✓Ration Balancing ✓Trace mineral and vitamin supplementation ✓Stress management	✓Nutrition (high fiber & low calorie diet) ✓Selective Dry Cow Therapy	✓Use of Bedding conditioner & Lime ✓Use of flames ✓Keeping the moisture content as low as possible ✓Thermal comfort, Softness, Friction, Durability of the bedding material

Fig. 2 Four Key Factors for Preventing the Occurrence of Mastitis

In particular, the first 1-2 weeks and the last 7–10 days before calving or early lactation are when there is a higher vulnerability to new environmental streptococci infections during the dry period. Notably, mastitis occurs twice as frequently at calving as it does at drying off. Dry animal antibiotic therapy can be used to treat infections acquired during the early dry period, but its effectiveness declines throughout the late dry period. But according to Jones (2006), 70% of environmental streptococcal infections could be cured with dry period therapy. While it may not be possible to completely eradicate environmental pathogen-induced mastitis from a dairy herd, it is possible to effectively manage it using precautions meant to lower exposure and strengthen the cow's immune system. In order to reduce the susceptibility to mastitis while keeping in mind Anti-Microbial Resistant (AMR), it is important to improve housing, nutrition, and immunity in nursing cows by the supplementation of nano minerals (Sahu *et al* ., 2023; Werven, 2018).

3. Conclusion:

The prevalence of mastitis may vary depending on the type of bedding utilized. Compared to organic bedding, inorganic bedding materials lessen exposure to mastitis pathogens. But it's also important to take into account practicality and compatibility with manure handling systems. A low-cost, readily available organic bedding material that inhibits the development of mastitis pathogens is required.

With regard to different manure management techniques, such a material would be useful and address the drawbacks of inorganic bedding. Mastitis is a complex condition that is impacted by a number of variables, including bacterial exposure in bedding, milking hygiene, diet, and more. A chain of potential circumstances that could encourage mastitis includes the type of bedding. Good management methods are ultimately the key to prevent mastitis. A comprehensive strategy for the health and cleanliness of dairy herds is needed to successfully avoid mastitis.

References-

Beauchemin J, Fréchette A, Thériault W, Dufour S, Fravalo P, Thibodeau A. Comparison of microbiota of recycled manure solids and straw bedding used in dairy farms in eastern Canada. J Dairy Sci. 2022;105(1):389-408.

Bradley AJ, Leach KA, Green MJ, Gibbons J, Ohnstad IC, Black DH, Payne B, Prout VE, Breen JE. The impact of dairy cows' bedding material and its microbial content on the quality and safety of milk–A cross sectional study of UK farms. Int J Food Microbiol. 2018;269:36-45.

Chaplin SJ, Tierney G, Stockwell C, Logue DN, Kelly M. An evaluation of mattresses and mats in two dairy units. Appl Anim Behav Sci. 2000;66(4):263-272.

Cole KJ, Hogan JS. Environmental mastitis pathogen counts in freestalls bedded with composted and fresh recycled manure solids. J Dairy Sci. 2016;99(2):1501-1505.

Cook NB, Bennett TB, Nordlund KV. Monitoring indices of cow comfort in free-stall-housed dairy herds. J Dairy Sci. 2005;88(11):3876-3885.
Costello M, Rhee MS, Bates MP, Clark S, Luedecke LO, Kang DH. Eleven-year trends of microbiological quality in bulk tank milk. Food Prot Trends. 2003;23(5).

DeVries TJ, Aarnoudse MG, Barkema HW, Leslie KE, Von Keyserlingk MAG. Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. J Dairy Sci. 2012;95(10):5730-5739.

Fávero S, Portilho FVR, Oliveira ACR, Langoni H, Pantoja JCF. Factors associated with mastitis epidemiologic indexes, animal hygiene, and bulk milk bacterial concentrations in dairy herds housed on compost bedding. Livest Sci. 2015;181:220-230.

Ferraz PFP, Leso L, Klopčič M, Barbari M, Rossi G. Properties of conventional and alternative bedding materials for dairy cattle. J Dairy Sci. 2020;103(9):8661-8674.

Fournel S, Godbout S, Ruel P, Fortin A, Généreux M, Côté C, Landry C, Pellerin D. Production of recycled manure solids for bedding in Canadian dairy farms: I. Solid–liquid separation. J Dairy Sci. 2019;102(2):1832-1846.
Fregonesi JA, Veira DM, Von Keyserlingk MAG, Weary DM. Effects of bedding quality on lying behavior of dairy cows. J Dairy Sci. 2007;90(12):5468-5472.

Frigon JC, Roy C, Guiot SR. Anaerobic co-digestion of dairy manure with mulched switchgrass for improvement of the methane yield. Bioprocess Biosyst Eng. 2012;35:341-349.

Fullwider WK, Palmer RW. Use of impact testing to predict softness, cow preference, and hardening over time of stall bases. J Dairy Sci. 2004;87(9):3080-3088.

Godden S, Bey R, Lorch K, Farnsworth R, Rapnicki P. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. J Dairy Sci. 2008;91(1):151-159.

Green MJ, Green LE, Medley GF, Schukken YH, Bradley AJ. Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. J Dairy Sci. 2002;85(10):2589-2599.

Green MJ, Leach KA, Breen JE, Ohnstad I, Tuer S, Archer SC, Bradley AJ. Recycled manure solids as bedding for dairy cattle: a scoping study. Cattle Pract. 2014;22(2):207-214.

Groot Antink M. Boxcompostvoorkoe in opmars. Veldpost. 2009;24:17.

Haley DB, Rushen J, Passillé AD. Behavioural indicators of cow comfort: activity and resting behaviour of dairy cows in two types of housing. Can J Anim Sci. 2000;80(2):257-263.

Hogan JS, Smith KL. Bacteria counts in sawdust bedding. J Dairy Sci. 1997;80(8):1600-1605.

Hogan JS, Raubenolt L, McCormick JL, Weiss WP. Evaluation of propane flaming for reducing bacterial counts in sand bedding. J Dairy Sci. 2012;95(10):6152-6159.

Hogan JS, Bogacz VL, Thompson LM, Romig S, Schoenberger PS, Weiss WP, Smith KL. Bacterial counts associated with sawdust and recycled manure bedding treated with commercial conditioners. J Dairy Sci. 1999;82(8):1690-1695.

Hogan J, Smith KL. Managing environmental mastitis. Vet Clin Food Anim Pract. 2012;28(2):217-224.

Husfeldt AW, Endres MI, Salfer JA, Janni KA. Management and characteristics of recycled manure solids used for bedding in Midwest freestall dairy herds. J Dairy Sci. 2012;95(4):2195-2203.

Jensen MB, Pedersen LJ, Munksgaard L. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. Appl Anim Behav Sci. 2005;90(3-4):207-217.

Jones GM, Bailey TL. Understanding the basics of mastitis. Virginia Cooperative Extension. 2006. Publication No. 404-233. Virginia State University: 1-7.

Klaas IC, Zadoks RN. Bedding management for better udder health and milk quality. In Proceedings, International Bovine Mastitis Conference. Università Degli Studi Di Milano. 2018. Milan, Italy: 45-57.

Krawczel P, Grant R. Effects of cow comfort on milk quality, productivity and behavior. In National Mastitis Council Annual Meeting Proceedings. 2009;48:15–24.

Kristula MA, Dou Z, Toth JD, Smith BI, Harvey N, Sabo M. Evaluation of free-stall mattress bedding treatments to reduce mastitis bacterial growth. J Dairy Sci. 2008;91(5):1885-1892.

Kristula MA, Rogers W, Hogan JS, Sabo M. Comparison of bacteria populations in clean and recycled sand used for bedding in dairy facilities. J Dairy Sci. 2005;88(12):4317-4325.

Lombard JE, Tucker CB, Von Keyserlingk MAG, Kopral CA, Weary DM. Associations between cow hygiene, hock injuries, and free stall usage on US dairy farms. J Dairy Sci. 2010;93(10):4668-4676.

Manninen E, de Passillé AM, Rushen J, Norring M, Saloniemi H. Preferences of dairy cows kept in unheated buildings for different kind of cubicle flooring. Appl Anim Behav Sci. 2002;75(4):281-292.

O'May GA, Reynolds N, Macfarlane GT. Effect of pH on an in vitro model of gastric microbiota in enteral nutrition patients. Appl Environ Microbiol. 2005;71(8):4777-4783.

Patel K, Godden SM, Royster E, Crooker BA, Timmerman J, Fox L. Relationships among bedding materials, bedding bacteria counts, udder hygiene, milk quality, and udder health in US dairy herds. J Dairy Sci. 2019;102(11):10213-10234.

Pol M, Ruegg PL. Relationship between antimicrobial drug usage and antimicrobial susceptibility of gram-positive mastitis pathogens. J Dairy Sci. 2007;90(1):262-273.

Proietto RL, Hinckley LS, Fox LK, Andrew SM. Evaluation of a clay-based acidic bedding conditioner for dairy cattle bedding. J Dairy Sci. 2013;96(2):1044-1053.

Raffaelli M, Fontanelli M, Fransconi C, Sorelli F, Peruzzi A. Thermal disinfection of poultry grow-out facilities in central northern Italy. In: Proceedings of XVIIth World Congress of the International Commission of Agricultural and Biosystems Engineering. 2010. Québec City, QC, Canada: 1-8.

Rathod P, Shivamurty V, Desai AR. Economic losses due to subclinical mastitis in dairy animals: A study in Bidar district of Karnataka. Indian J Vet Sci Biotechnol. 2017;13(1):37-41.

Reich LJ, Weary DM, Veira DM, Von Keyserlingk MAG. Effects of sawdust bedding dry matter on lying behavior of dairy cows: A dose-dependent response. J Dairy Sci. 2010;93(4):1561-1565.

Reinemann DJ. Troubleshooting high bacteria counts in bulk milk: What needs cleaning, the machine or the cows? Countdown Meet. 2011. Melbourne, Australia.

Rowbotham RF, Ruegg PL. Association of bedding types with management practices and indicators of milk quality on larger Wisconsin dairy farms. J Dairy Sci. 2015;98(11):7865-7885.

Rowbotham RF, Ruegg PL. Associations of selected bedding types with incidence rates of subclinical and clinical mastitis in primiparous Holstein dairy cows. J Dairy Sci. 2016;99:4707– 4717.

Ruegg PL. A 100-year review: Mastitis detection, management, and prevention. J Dairy Sci. 2017;100:10381–10397.

Ruud LE, Bøe KE, Østerås O. Associations of soft flooring materials in free stalls with milk yield, clinical mastitis, teat lesions, and removal of dairy cows. J Dairy Sci. 2010;93(4):1578-1586.

Sahu C, Manimaran A, Kumaresan A, Rajendran D, Ezhuthupurakkal PB, Sharma D, Dey DK, Sivaram M. Supplementation of trisodium citrate improves recovery rate of subclinical mastitis in crossbred cows. Pharma Innov J. 2022;11(11):1023-1030.

Sahu C, Manimaran A, Kumaresan A, Rajendran D, Sivaram M. Role of Trisodium Citrate and Nanominerals in Mastitis Management in Dairy Animals: A Review. Agric Rev. 2023;R-2612:1-8.

Sanderson MA, Adler PR, Boateng AA, Casler MD, Sarath G. Switchgrass as a biofuels feedstock in the USA. Can J Plant Sci. 2006;86(Special Issue):1315-1325.

Sapp SG, Arnot C, Fallon J, Fleck T, Soorholtz D, Sutton- Vermeulen M, Wilson JJ. Consumer trust in the US food system: an examination of the recreancy theorem. Rural Sociol. 2009;74(4):525-545.

Seegers H, Fourichon C, Beaudeau F. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res. 2003;34(5):475-491.

Sharif A, Umer MUHAMMAD, Muhammad GHULAM. Mastitis control in dairy production. J Agric Soc Sci. 2009;5(3):102-105.

Smith KL, Hogan JS. The world of mastitis. In Proceedings of the Second International Symposium on Mastitis and Milk Quality. Vancouver, Canada. 2001:13.

Tucker CB, Weary DM, Fraser D. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. J Dairy Sci. 2003;86(2):521-529.

Tucker CB, Weary DM, Von Keyserlingk MAG, Beauchemin KA. Cow comfort in tie-stalls: Increased depth of shavings or straw bedding increases lying time. J Dairy Sci. 2009;92(6):2684-2690.

Van Gastelen S, Westerlaan B, Houwers DJ, Van Eerdenburg FJCM. A study on cow comfort and risk for lameness and mastitis in relation to different types of bedding materials. J Dairy Sci. 2011;94(10):4878-4888.

Van Weyenberg S, Ulens T, De Reu K, Zwertvaegher I, Demeyer P, Pluym L. Feasibility of Miscanthus as alternative bedding for dairy cows. Vet Med. 2015;60(3).

Werven TV. New Developments in Mastitis Research. In proceedings of WCDS Advances in Dairy Technology. 2018;30:299-306.

Wolfe T, Vasseur E, DeVries TJ, Bergeron R. Effects of alternative deep bedding options on dairy cow preference, lying behavior, cleanliness, and teat end contamination. J Dairy Sci. 2018;101(1):530-536.

Zdanowicz M, Shelford JA, Tucker CB, Weary DM, Von Keyserlingk MAG. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J Dairy Sci. 2004;87(6):1694-1701.

Zehner MM, Farnsworth RJ, Appleman RD, Larntz K, Springer JA. Growth of environmental mastitis pathogens in various bedding materials. J Dairy Sci. 1986;69(7):1932-1941.