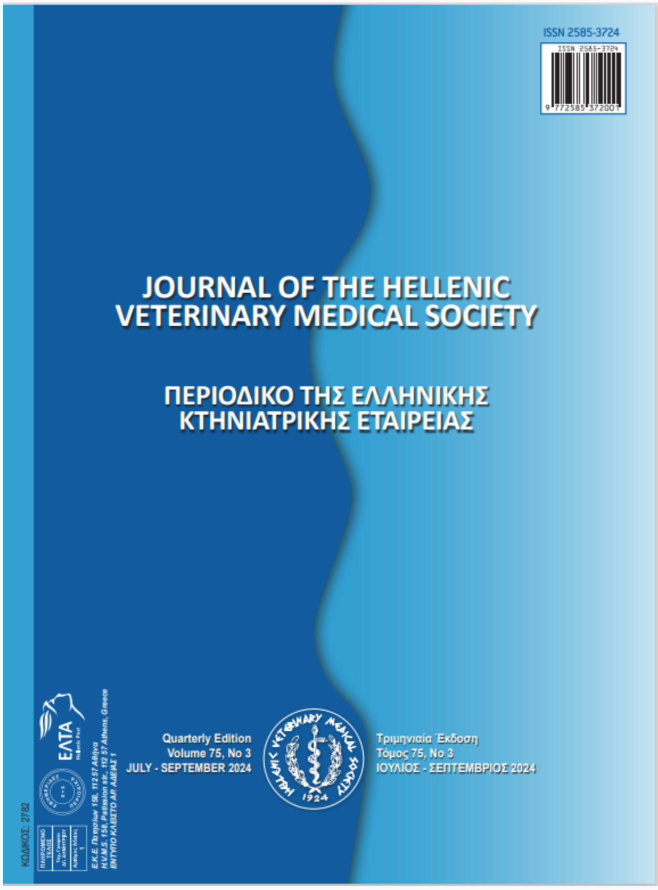
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 3 (2024)



The impact of bedding materials and disinfectants on udder health and mastitis control in dairy cows

MS Shahedani, F Ahmadi, Hİ Tosun

doi: [10.12681/jhvms.37052](https://doi.org/10.12681/jhvms.37052)

J HELLENIC VET MED SOC 2024, 75 (3): 8083-8098
ΠΕΚΕ 2024, 75 (3): 8083-8098

Research article
Ερευνητικό άρθρο

The impact of bedding materials and disinfectants on udder health and mastitis control in dairy cows

M.S. Shahdani[1], F. Ahmadi[2], H.İ. Tosun*[3]

[1]*Department of Animal Science, Shahrekord University, Shahrekord, Iran*

[2]*Department of Animal Science, University of Zanjan, Zanjan, Iran*

[3]*Rumico Livestock Management and Nutrition, Aydın, Türkiye*

ABSTRACT: This study investigates the impact of bedding materials and disinfectants on mastitis prevalence in dairy farming, focusing on key parameters such as herd size, housing conditions, bedding types, disinfectants, and bedding pH. Conducted as an observational study spanning 31 commercial dairy farms situated in diverse cities across Iran, our research encompassed a comprehensive dataset gathered from a total of 77,032 cows. The study was conducted over a continuous six-month period, during which we collected and analyzed data on a range of 740 to 5,280 cows across the participating farms. Bedding materials included manure, manure & soil, pumice, sand, and bagasse trash, with disinfectants like Calcium carbonate, Calcium hydroxide, Formalin, and lime applied. Teat and bedding samples were analyzed for mastitis prevalence, somatic cell count (SCC), bacterial load, and pH levels. Our findings reveal significant associations between farm factors and health indicators. Larger herd sizes were negatively associated with mastitis prevalence, while housing, bedding, and pH displayed significant negative associations. Disinfectants exhibited a positive association with mastitis prevalence. SCC levels were significantly negatively associated with bedding, indicating its influence on udder health. Bedding types and disinfectants demonstrated significant variations in mastitis prevalence, SCC, teat total count, and bed total count. Notably, manure bedding displayed the highest mastitis prevalence, while bagasse showed significant differences compared to other materials. In conclusion, this underscores the critical importance of bedding materials and disinfectants in ensuring the efficient management of dairy farms. Practical implications suggest considering alternative bedding materials, monitoring herd size, and selecting appropriate disinfectants to optimize udder health. This study contributes valuable insights into mastitis control, emphasizing the need for tailored interventions in dairy farm practices.

Keywords: Bedding materials; Dairy farming; Mastitis; Somatic cell count; Udder health

Corresponding Author:
Halil İbrahim Tosun, Rumico Livestock Management and Nutrition, Efeler, 09020, Aydın, Türkiye
E-mail address: hitosun@hotmail.com

Date of initial submission: 22-02-2024
Date of acceptance: 16-03-2024

INTRODUCTION

Dairy farming stands as a critical component of the agricultural sector, ensuring a consistent and high-quality global milk supply by prioritizing the well-being of cattle (Evans et al., 2022; Dayoub et al., 2024). Udder health is crucial both for consumers and for dairy farmers due to its' impact on the production of high-quality milk, on cows' welfare and on the duration of their productive life (Ruegg et al., 2017; Themistokleous et al., 2019). However, the effective management of mastitis, a prevalent and economically burdensome disease in dairy cattle, presents a formidable challenge, impacting the financial returns for farmers (Kovačević et al., 2023; Tomanić et al., 2023). Mastitis not only leads to decreased milk prices and potential milk confiscation but also results in an overall decline in milk production. The economic implications of mastitis, encompassing treatment costs, market withdrawal, and additional labor, highlight its greater threat to the dairy farmer's economic interests than to the individual animal's health (Cvetnić et al., 2016).

Hygiene practices, particularly those related to bedding materials and disinfectants, play a pivotal role in maintaining the health and productivity of dairy cattle, both in conventional and automatic milking farms (Singh et al., 2020; Ventura et al., 2021; Zigo et al., 2021; Themistokleous et al., 2022). Disinfectants serve diverse functions, including disease prevention, mastitis control, biosecurity enhancement, bedding material sterilization, mitigation of environmental pathogens, optimization of reproductive health, reduction of somatic cell counts, mastitis prevalence, and overall farm hygiene maintenance (Klaas and Zadoks, 2018; Cobirka et al., 2020; Alanis et al., 2021; Zigo et al., 2021). Additionally, antimicrobials play a significant role in influencing animal health and production performance (Kovačević et al., 2022). This study holds scientific importance by providing insights into the optimization of dairy farm management. Through an examination of factors such as housing conditions, bedding materials, disinfectants, pH levels, and total bacteria count on bedding, the research investigates their impact on mastitis prevalence and somatic cell count (SCC). Understanding these factors can guide targeted interventions, ultimately improving milk quality, animal health, and overall farm productivity, thereby benefiting both the dairy industry and public health.

Detecting subclinical mastitis, where clinical signs are absent, poses a challenge. Identifying reliable biomarkers in milk that indicate pathogen-specific changes during early subclinical disease stages is crucial for timely diagnosis (Kovačić et al., 2019). The association between oxidative stress and inflammation during intramammary infections underscores their role in mastitis pathogenesis (Turk et al., 2017). Advancements in reducing contagious mastitis pathogens, particularly Staphylococcus aureus, have shifted attention to environmental pathogens, a major concern on US dairy farms (Ruegg, 2017). Literature consistently emphasizes the link between Staphylococcus aureus intramammary infections and the risk of enterotoxin presence in milk and dairy products (Benić et al., 2018). Staphylococcus aureus is a prevalent cause of mastitis, with variable infection prevalence ranging from 2% to over 50%, contributing to 10-12% of clinical mastitis cases (Cvetnić et al., 2021). Sporadic mycobacteria-induced mammary gland infections underscore the need for ongoing monitoring of mammary gland health (Cvetnić et al., 2022). Environmental mastitis, primarily instigated by environmental streptococci or streptococci-like organisms (SSLO; e.g., Streptococcus uberis, Lactococcus lactis), coliform bacteria (e.g., Escherichia coli, Klebsiella spp.), and NAS (e.g., Staphylococcus chromogenes) (Piepers et al., 2007; Oliveira et al., 2015; Patel et al., 2019; Tibebu et al., 2021), prompts strategies centered around four fundamental pillars. These include reducing bacterial load in the cow's environment, frequent removal of bacterial load from teats to prevent intrusion, enhancing host resistance and resilience, and improving mastitis control practices (e.g., case detection and management, dry-off procedures), as proposed by Klaas and Zadoks (2018).

Given that cows spend 12 to 14 hours daily lying down (Krawczel et al., 2012; Tucker et al., 2021), bedding emerges as a crucial source of teat end exposure to environmental mastitis pathogens, with numerous studies indicating a correlation between bedding bacteria counts (BBC) and bacterial load on the teat end (Andrews et al., 2019; Patel et al., 2019; Singh, 2022; Haider et al., 2023). Additionally, mounting evidence suggests a positive association between bedding characteristics and intramammary infections (IMI), particularly highlighting the risk posed by high coliform counts in bedding (Cheng and Han, 2020; Ndahetuye et al., 2020; Robles et al., 2020; Frechette et al., 2021).

The ability of bedding to support bacterial growth varies based on type, with inorganic materials like sand proving inhibitory to bacterial growth, potentially reducing mastitis risk. However, organic bedding materials may elevate mastitis risk, albeit offering advantages in manure handling and positively influencing soil fertility due to higher organic matter content (Hohmann et al., 2020; Ahmed et al., 2022; Haxhiaj et al., 2022). In alternative housing systems, such as Compost Bedded Pack (CBP), maintaining an appropriate chemical substrate in bedding is essential to support aerobic microbial activity integral to the composting process (Fávero et al., 2015; Ferraz et al., 2020; Varma et al., 2021; Oliveira et al., 2023). Regardless of the housing system, the compostability of bedding materials is deemed desirable, as demonstrated by composted manure's potential to improve soil fertility and reduce the environmental impact of dairy systems (Leso et al., 2020; Rayne and Aula, 2020).

Predominantly used bedding materials include sawdust, wood shavings, and sand, while others such as bagasse, peanut shells, and woodchips are also commonly employed (Werther et al., 2000; Janni et al., 2007; Kjaer et al., 2007; Damasceno et al., 2022). The escalating demand for conventional bedding materials such as sawdust and bagasse (Diarra et al., 2021) has resulted in increased prices, prompting farmers to seek cost-effective alternatives for bedding options. This exploration may involve considering materials like different wood shavings, straw, or hay to maintain the comfort and hygiene of dairy cows while managing economic constraints.

In examining the application of disinfectants for beddings in dairy farms, our study explores the diverse effects of various disinfectant types on the prevalence of mastitis, somatic cell count, overall microbial load of bedding material, and the total bacterial quantity on the teats. The research aims to offer comprehensive insights by assessing the impact of various bedding materials, including manure, manure & soil, pumice, sand, and bagasse, on mastitis prevalence and somatic cell count in dairy cows. Exploring the impact of beddings, disinfectants, and the correlation between bedding pH level and microbial load are essential components, alongside examining how disinfectants like calcium carbonate, calcium hydroxide, formalin, and lime affect hygiene, microbial load, and milk quality. The hypothesis suggests that variations in bedding materials and hygiene practices, particularly those affecting pH and microbial growth, play a crucial role in influencing the prevalence of mastitis in dairy farms.

MATERIALS AND METHODS

In a comprehensive observational study encompassing 31 commercial dairy farms situated across various cities in Iran, we gathered data from a total of 77,032 cows. This extensive research spanned six consecutive months, capturing insights from herds ranging in size from 740 to 5,280 individuals. A veterinarian conducted sample collection and data recording during regular visits, with each farm being visited twice a month. The criteria used to record a case as "mastitis" were based on a thorough examination conducted by experienced veterinarians during regular farm visits. Diagnostic assessments included clinical symptoms such as swelling, redness, and abnormal milk appearance, coupled with bacterial analyses of teat swab samples.

Data collection

Bedding materials on these farms were categorized into five distinct types: manure, manure & soil, pumice, sand, and bagasse trash. Additionally, a variety of disinfectants, namely Calcium carbonate, Calcium hydroxide, Formalin, and lime, were employed for bed disinfection, with some farms not utilizing any disinfectants (referred to as NON). Before applying disinfectants, the pH of each was measured to determine optimal options. This involved combining sterile water with samples at a 1:4 ratio, using a pH meter (EZ-101 PermaCheck™) for measurement. Calcium hydroxide exhibited the highest pH value among the disinfectants. Wearing clean disposable gloves, the sampler collected bedding samples from stalls by obtaining grab samples from the top 5 cm at 15 randomly selected locations. After mixing in a clean bucket, the sample was divided into two Ziploc bags. One sample was immediately sent to the on-farm laboratory for pH and moisture content measurement, while the other was frozen at -20°C. The frozen samples were shipped on ice to the laboratory for total bed count analysis. Following the method outlined by Godden et al. (2008), each bedding sample was combined with deionized water at a 1:9 ratio and thoroughly mixed every 10 minutes. After 30 minutes at room temperature, the pH value was measured using a pH meter (EZ-101 PermaCheck™). Moisture content percentage was calculated by drying two 2-g sub-samples at 100°C for 24 hours. To determine the total bed count, samples were thawed at room temperature, and a 50-mL sub-sample was weighed before transferring it to a sterile plastic bag (Whirl-Pak, Nasco, Fort Atkinson, WI), combined with 250 mL of sterile water for a dilution factor of 1:5. The bedding-water mixture was allowed to rest at room temperature for 10 minutes, shaken, and 200 µL of the resulting bedding suspension were inoculated onto CNA and MacConkey agars at four dilutions (1:5, 1:50, 1:500, and 1:5,000). Cultures were incubated aerobically at 37 ± 2°C for 42 to 48 hours. A microbiologist visually inspected and identified bacteria groups (Bacillus spp., Staphylococcus spp., SSLO, coliforms, Klebsiella spp., non-coliform gram-negatives, or Prototheca spp), counting colonies on the dilution plate within an optimal range of 25 to 250 per plate. Representative colonies were confirmed using MALDI-TOF. The total count of bacteria was determined by combining counts from all bacterial groups, measuring colony forming units per milliliter of bedding, per gram of wet bedding, and per gram of dry bedding.

To obtain teat samples for bacterial analysis, a systematic approach was employed. Prior to milking preparation, teats from 50 cows within each farm were swabbed individually using sterile swabs (Cultiplast, Milan, Italy). Subsequently, these swabs were carefully placed into individual sterile tubes, each containing 3 cc of physiological serum, and subjected to autoclaving at 121 °C for 15 minutes (Oxoid - Product Detail, n.d.). The sterile tubes, housing the swabs, were then promptly frozen at -20 °C until further analysis for bacterial presence. For bacterial identification, the swabs were methodically streaked across selective agars. Following an incubation period at 37 °C for 24 hours, the total bacterial count was estimated through manual assessment. To assess somatic cell count (SCC), morning milk samples from individual cows were collected over a span of six consecutive months. The acquired data were recorded, and subsequently, the values were employed for statistical analysis.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics Version 20 (SPSS Inc., Chicago, IL, USA), employing a significance threshold of $p < 0.05$. Descriptive statistics encompassing mean, median, standard deviation, and range were computed for key variables, including mastitis prevalence, SCC, teat total count, microbial load in bedding material, and pH level in bedding. A multiple regression model was employed to examine our hypothesis and investigate the combined influence of various factors, including farm size (X1), housing conditions (X2), bedding materials (X3), type of disinfectant (X4), and pH level in bedding (X5) on mastitis prevalence (Y1), SCC (Y2), microbial load in bedding material (Y3), and teat total bacterail count (Y4). The model is represented as:

$$Y = \beta 0 + \beta 1\, X1 + \beta 2\, X2 + \beta 3\, X3 + \beta 4\, X4 + \beta 5\, X5 + \varepsilon$$

In the regression model, where Y denotes dependent variables, $\beta 0$ represents the intercept, and $\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$, $\beta 5$ are the regression coefficients corresponding to the independent variables $X1$, $X2$, $X3$, $X4$, $X5$, and ε signifies the error term. Analysis of Variance (ANOVA) was employed to elucidate statistically significant variations in mastitis prevalence among discrete categories of types of bedding and disinfectant. Subsequently, post-hoc tests, specifically Tukey's HSD, were conducted to discern and characterize specific pairwise differences between the identified groups.

In the correlation analysis, correlation coefficients (r) were calculated to investigate the associations among variables. The formula for the Pearson correlation coefficient (r) is expressed as follows:

$$r = \frac{\Sigma(Xi-X)\,(Yi-Y)}{\sqrt{\Sigma(Xi-\bar{X})^2 \,.\, \Sigma(Yi-\bar{Y})^2}}$$

In the model, where r is the correlation coefficient, Xi and Yi represent individual data points for the variables being correlated, $\bar{X}$ and $\bar{Y}$ denote the mean of the respective variables.

RESULTS

Overview of farm characteristics and descriptive statistics

The research findings of overview of farm characteristics and descriptive statistics are reported as mean ± standard deviation. Table 1 provides an overview of dairy farm characteristics for key parameters. The parameters include herd size (740 to 5,280, mean = 2,482±1,311), bed total bacterial count (800,000 to 83,000,000, mean = 22,049,174±20,972,228), bedding pH (6.60 to 9.50, mean = 8.46±0.73), teat total bacterial count (420,000 to 18,300,000, mean = 4,709,654±4,116,884), SCC (124,000 to 389,000, mean = 247,603±58,492), and mastitis prevalence (1.70% to 12.00%, mean = 4.62±2.49).

Table 2 presents descriptive analysis of various bedding materials used in dairy farms, focusing on their impact on mastitis prevalence, SCC, bacterial load in bedding, and teat total count. The findings shows that the mean mastitis prevalence is highest in

Table 1. Overview of dairy farms characteristics

	Min	Max	Mean	Std. Dev.
Herd size	740	5.280	2.482	1.311
Bed total count	800.000	83.000.000	22.049.174	20.972.228
Bedding pH	6.60	9.50	8.46	0.73
Teat total count	420.000	18.300.000	4.709.654	4.116.884
SCC	124.000	389.000	247.603	58.492
Mastitis (%)	1.70	12.00	4.62	2.49

Table 2. Descriptive analysis of various beddings in dairy farms

Variables	Beddings	Mean	Std. Dev.	95% CI		Min	Max
				Lower Bound	Upper Bound		
Mastitis (%)	Manure	5.59	2.51	5.11	6.07	3.00	10.30
	Manure & Soil	4.25	1.52	3.70	4.81	2.90	5.90
	Pumice	4.87	2.40	4.01	5.73	2.70	8.20
	Sand	4.17	2.62	3.72	4.62	1.70	12.00
	Bagasse	3.00	0.39	2.84	3.15	2.50	3.40
SCC (1000x, cells/mL)	Manure	291.38	37.607	284.21	298.55	237	389
	Manure & Soil	233.45	46.541	216.38	250.52	183	275
	Pumice	264.00	53.841	244.59	283.41	211	335
	Sand	216.69	55.233	207.22	226.17	124	323
	Bagasse	220.58	44.238	202.71	238.44	161	254
Bed total bacterial count (1000x)	Manure	31736	17835	26820	36652	1600	72000
	Manure & Soil	11179	12917	3721	18636	800	41800
	Pumice	36226	18759	28649	43803	1680	63000
	Sand	17809	22330	13050	22568	800	83000
	Bagasse	8167	7341	5202	11132	1200	36000
Teat total bacterial count (1000x)	Manure	6641	4079	5863	7419	480	18300
	Manure & Soil	4590	4442	2961	6220	440	14820
	Pumice	6330	4788	4603	8056	665	16000
	Sand	3288	3336	2716	3860	420	15320
	Bagasse	2109	1920	1333	2884	480	7740
Bed moisture content (%)	Manure	18.13	0.34	17.46	18.80	10.00	24.00
	Manure & Soil	12.97	0.23	10.40	15.53	5.00	21.00
	Pumice	6.54	0.21	6.12	6.97	3.00	9.00
	Sand	9.97	0.31	9.36	10.57	3.00	19.00
	Bagasse	21.58	1.04	19.44	23.71	14.00	32.00

the manure bedding at 5.59%, followed by pumice (4.87%), manure & soil (4.25%), sand (4.17%), and bagasse (3.00%). Similarly, for SCC (1000x, cells/mL), manure bedding has the highest mean at 291.38, while sand has the lowest mean at 216.99. In terms of bed total bacterial count (1000x), pumice exhibits the highest mean at 36,226, followed by manure (31,736), sand (17,809), manure & soil (11,179), and bagasse (8,167). Teat total bacterial count (1000x) follows a similar pattern, with manure having the highest mean at 6,641, followed by pumice (6,330), manure & soil (4,590), sand (3,288), and bagasse (2,109). Furthermore, Bed DM content (%) varies among disinfectants (Manure: 18.13%, Manure & soil: 12.97%, Pumice: 6.54%, Sand: 9.97%, Bagasse: 21.58%).

Table 3 provides overview of descriptive statistics for various variables associated with different disinfectants used in dairy farming. Noteworthy variations are observed across mastitis prevalence, ranging from 2.70% to 8.20% (Calcium carbonate: 4.14%, Calcium hydroxide: 2.70%, Formalin: 5.55%, Lime: 4.02%, NON: 8.20%). Somatic cell count (SCC 1000x) levels display diversity, spanning from 215 to 299 cells/mL (Calcium carbonate: 250, Calcium hydroxide: 215, Formalin: 287, Lime: 230, NON: 299). Teat total bacterial count (1000x) and Bed total bacterial count (1000x) also show variability, with ranges for Teat

Table 3. Descriptive analysis of various disinfectants on mastitis, SCC, teat total count, bed total count, bed DM content, and bedding pH in dairy farms

Variables	Disinfectants	Mean	Std. Dev.	95% CI Lower Bound	95% CI Upper Bound	Min	Max
Mastitis (%)	Calcium carbonate	4.14	0.21	4.08	4.20	3.80	4.40
	Calcium hydroxide	2.70	0.59	2.60	2.81	1.70	3.80
	Formalin	5.55	0.74	5.23	5.88	4.60	6.10
	Lime	4.02	1.30	3.64	4.41	2.70	5.90
	NON	8.20	2.15	7.72	8.68	4.50	12.00
SCC (1000x, cells/ mL)	Calcium carbonate	250	34	240	259	198	288
	Calcium hydroxide	215	45	207	223	124	275
	Formalin	287	6	285	290	283	295
	Lime	230	70	209	251	158	327
	NON	299	49	288	310	230	389
Teat total bacterial count (1000x)	Calcium carbonate	4262	3551	3273	5250	486	16300
	Calcium hydroxide	1774	1466	1521	2028	420	7930
	Formalin	8484	3554	6908	10060	2800	16100
	Lime	6032	3965	4855	7210	660	14820
	NON	8051	4029	7148	8953	1580	18300
Bed total bacterial count (1000x)	Calcium carbonate	16467	19141	6948	25985	1200	69000
	Calcium hydroxide	6397	6074	5153	7641	800	36000
	Formalin	48733	13425	34645	62822	32800	72000
	Lime	24958	15167	19580	30336	2600	61000
	NON	45971	15293	41837	50105	12900	83000
Bed moisture content (%)	Calcium carbonate	15.42	5.10	14.00	16.84	7.00	22.00
	Calcium hydroxide	12.66	6.74	11.49	13.82	4.00	32.00
	Formalin	18.73	1.03	18.27	19.18	17.00	22.00
	Lime	10.91	5.21	9.36	12.45	3.00	24.00
	NON	13.71	5.90	12.38	15.03	3.00	23.00
Bedding pH	Calcium carbonate	8.68	0.49	8.54	8.82	7.90	9.50
	Calcium hydroxide	9.03	0.33	8.97	9.08	7.80	9.50
	Formalin	7.32	0.26	7.21	7.44	6.80	7.80
	Lime	8.50	0.40	8.38	8.62	7.60	9.40
	NON	7.68	0.50	7.57	7.80	6.60	8.50

total count: Calcium carbonate (3273-5250), Calcium hydroxide (1521-2028), Formalin (6908-10060), Lime (4855-7210), NON (7148-8953), and Bed total count: Calcium carbonate (6948-25985), Calcium hydroxide (5153-7641), Formalin (34645-62822), Lime (19580-30336), NON (41837-50105). Additionally, Bed DM content (%) varies among disinfectants (Calcium carbonate: 15.42%, Calcium hydroxide: 12.66%, Formalin: 18.73%, Lime: 10.91%, NON: 13.71%). Bedding pH levels demonstrate distinct values (Calcium carbonate: 8.68, Calcium hydroxide: 9.03, Formalin: 7.32, Lime: 8.50, NON: 7.68).

Regression analysis and correlation findings

Table 4 presents the regression analysis results for mastitis prevalence, SCC (1000x), Bed Total Bacterial Count (1000x), and Teat Total Bacterial Count (1000x). Mastitis prevalence exhibited a statistically significant intercept of 13.609 (β), with herd size showing a significant negative association (-0.001, T = -5.765, P = 0.001, 95% CI: -0.001, 0.000). Housing, bedding, and pH also displayed significant negative associations, with coefficients of -0.812, -0.386, and -0.930, respectively (P = 0.004, P = 0.001, p = 0.001). Disinfectants showed a significant positive association (0.820, T = 11.227, P = 0.001, 95% CI: 0.676, 0.963). For SCC (1000x), the intercept was 378,517 (β), and herd size exhibited a significant negative association of -0.014 (T = -4.982, P = 0.001, 95% CI: -0.020, -0.009). Bedding displayed a highly significant negative association with SCC, as reflected by a coefficient of -22,512 (T = -10.527, P = 0.001, 95% CI: -26,719, -18,305). Disinfectants exhibited a positive association (10,916, T = 5.150, P = 0.000, 95%

Table 4. Regression analysis results for variables impacting mastitis prevalence, SCC, bed total count, and teat total count

Variables		Coefficients β	Coefficients Std. Err	T values	P values	R^2	95,0% CI Lower	95,0% CI Upper
Mastitis (%)	Intercept	13.609	1.417	9.604	0.000	0.650	10.821	16.397
	Herd size	-0.001	0.000	-5.765	0.000		-0.001	0.000
	Housing	-0.812	0.281	-2.886	0.004		-1.366	-0.259
	Bedding	-0.386	0.074	-5.235	0.000		-0.530	-0.241
	Disinfectants	0.820	0.073	11.227	0.000		0.676	0.963
	pH	-0.930	0.167	-5.564	0.000		-1.259	-0.601
SCC (1000x)	Intercept	378.517	41.150	9.198	0.000	0.470	297.562	459.471
	Herd size	-0.014	0.003	-4.982	0.000		-0.020	-0.009
	Housing	-11.026	8.171	-1.349	0.178		-27.100	5.048
	Bedding	-22.512	2.139	-10.527	0.000		-26.719	-18.305
	Disinfectants	10.916	2.120	5.150	0.000		6.746	15.087
	pH	-5.960	4.854	-1.228	0.220		-15.509	3.590
Bed Total Bacterial Count (1000x)	Intercept	170805.066	16429.032	10.397	0.000	0.710	138408.720	203201.413
	Herd size	-3.761	1.035	-3.633	0.000		-5.802	-1.720
	Housing	-13298.402	2887.305	-4.606	0.000		-18991.868	-7604.937
	Bedding	-1937.210	700.482	-2.766	0.006		-3318.488	-555.932
	Disinfectants	4549.916	771.677	5.896	0.000		3028.250	6071.583
	pH	-15292.259	1830.717	-8.353	0.000		-18902.242	-11682.275
Teat Total Bacterial Count (1000x)	Intercept	23908.424	2917.301	8.195	.000	0.460	18169.180	29647.669
	Herd size	-0.346	0.201	-1.720	.086		-0.742	0.050
	Housing	-713.233	579.253	-1.231	.219		-1852.806	426.339
	Bedding	-708.783	151.609	-4.675	.000		-1007.046	-410.520
	Disinfectants	693.418	150.286	4.614	.000		397.759	989.078
	pH	-2064.122	344.135	-5.998	.000		-2741.144	-1387.100

CI: 6,746, 15,087), while housing and pH did not demonstrate statistically significant associations ($p > 0.05$).

In the analysis of bed total bacterial count (1000x), the intercept was estimated at 170,805.066 (β), and herd size displayed a significant negative association of -3.761 (T = -3.633, P = 0.001, 95% CI: -5.802, -1.720), indicating that larger herd sizes are associated with lower bed total count. Housing exhibited a highly significant negative association with a coefficient of -13,298.402 (T = -4.606, P = 0.001, 95% CI: -18,991.868, -7,604.937). Bedding and pH also contributed significantly, with bedding showing a negative association (-1,937.210, T = -2.766, P = 0.006, 95% CI: -3,318.488, -555.932) and pH displaying a highly significant negative association (-15,292.259, T = -8.353, P = 0.000, 95% CI: -18,902.242, -11,682.275). Disinfectants exhibited a positive association (4,549.916, T = 5.896, $p < 0.000$, 95% CI: 3,028.250, 6,071.583). For Teat Total Bacterial Count (1000x), the intercept was estimated at 23,908.424 (β). While herd size exhibited a negative association of -0.346, it did not reach statistical significance (T = -1.720, P = 0.086, 95% CI: -0.742, 0.050). Housing showed non-significant associations, with coefficients of -713,233 (T = -1.231, P = 0.219, 95% CI: -1,852,806, 426,339) and pH displaying significant negative association -2,064,122 (T = -5.998, P = 0.001, 95% CI: -2,741,144, -1,387,100). Bedding demonstrated a highly significant negative association of -708,783 (T = -4.675, P = 0.001, 95% CI: -1,007,046, -410,520), suggesting that certain bedding conditions are associated with a decrease in teat total count. Disinfectants exhibited a significant positive association (693,418, T = 4.614, P = 0.001, 95% CI: 397,759, 989,078), indicating a decrease in teat total bacterial count with the use of disinfectants. These findings suggest that bedding and disinfectants significantly influence the observed variations in teat total count.

The correlation matrix presented in Table 5 examines the relationships between farm factors (Herd size, Housing, Bedding, Disinfectants, and pH) and key indicators associated with udder health, encompassing mastitis prevalence, SCC, and total bacterial load in bedding and teats. The correlation coefficients unveil significant associations between specific farm factors and health indicators. Noteworthy findings include a positive correlation between mastitis prevalence and specific disinfectants (r = 0.704), positive correlations between SCC and both disinfectants (r = 0.404) and Teat Total Bacterial Count (r = 0.517), a negative correlation between SCC and Bedding (r = -0.522), positive correlations between bed total bacterial count and specific disinfectants (r = 0.716), and positive correlations between teat total bacterial count and specific disinfectants (r = 0.517).

Multiple comparisons of type of beddings and disinfectants

Table 6 provides a comparative analysis of various bedding materials in relation to mastitis prevalence within dairy farms. The findings uncover statistically significant variations in mastitis prevalence across different beddings, offering crucial insights for effective mastitis management. Manure bedding exhibited a notably higher mastitis prevalence compared to Manure & Soil, Sand, and Bagasse (Table 2), with mean differences of 1.34%, 1.42%, and 2.60%, respectively (P = 0.01). Similarly, bagasse bedding displayed the most substantial and statistically significant mean difference in mastitis prevalence when compared to Manure (-2.60%, P = 0.01, 95% CI: -3.62 to -1.58), Manure & Soil (-1.26%, P = 0.05, 95% CI: -2.50 to -0.02), Pumice (-1.87%, P = 0.01, 95% CI: -3.11 to -0.64), and Sand (-1.18%, P = 0.02, 95% CI: -2.18 to -0.18).

Table 7 presents a comprehensive comparative analysis of the impact of various disinfectants on mastitis prevalence and SCC in dairy farming. The results of the multiple comparisons analysis unveil statistically significant distinctions in both mastitis prevalence and SCC across various disinfectants. Remarkable mean differences and confidence intervals underscore the significant impact of specific disin-

Table 5. Correlation matrix for mastitis prevalence, SCC, bed total count, and teat total bacterial count with farm factors

Variables	Herd size	Housing	Bedding	Disinfectants	pH
Mastitis prevalence	-0,176	-0,043	-0,289	0,704	-0,694
SCC	-0,119	0,105	-0,522	0,404	-0,483
Bed total count	-0,049	-0,24	-0,321	0,716	-0,785
Teat total count	-0,027	-0,067	-0,377	0,517	-0,627

Table 6. Comparison of beddings for mastitis prevalence (%) in dairy farms

Bedding (I)	Bedding (J)	Mean Diff. (I-J)	Std. Error	P Values	95% CI Lower Bound	95% CI Upper Bound
Manure	Manure & Soil	1.34*	0.48	0.01	0.39	2.29
	Pumice	0.72	0.48	0.13	-0.22	1.66
	Sand	1.42*	0.31	0.01	0.81	2.02
	Bagasse	2.60*	0.52	0.01	1.58	3.62
Manure & Soil	Manure	-1.34*	0.48	0.01	-2.29	-0.39
	Pumice	-0.61	0.60	0.31	-1.79	0.56
	Sand	0.08	0.47	0.87	-0.85	1.01
	Bagasse	1.26*	0.63	0.05	0.02	2.50
Pumice	Manure	-0.72	0.48	0.13	-1.66	0.22
	Manure & Soil	0.61	0.60	0.31	-0.56	1.79
	Sand	0.69	0.47	0.14	-0.23	1.61
	Bagasse	1.87*	0.63	0.01	0.64	3.11
Sand	Manure	-1.42*	0.31	0.01	-2.02	-0.81
	Manure & Soil	-0.08	0.47	0.87	-1.01	0.85
	Pumice	-0.69	0.47	0.14	-1.61	0.23
	Bagasse	1.18*	0.51	0.02	0.18	2.18
Bagasse	Manure	-2.60*	0.52	0.01	-3.62	-1.58
	Manure & Soil	-1.26*	0.63	0.05	-2.50	-0.02
	Pumice	-1.87*	0.63	0.01	-3.11	-0.64
	Sand	-1.18*	0.51	0.02	-2.18	-0.18

*The mean difference is significant at the 0.05 level.

Table 7. Comparison of disinfectants for mastitis prevalence and SCC in dairy farms

	Disinfectants (I)	Disinfectants (J)	Mean Diff. (I-J)	Std. Error	P Values	95% CI Lower Bound	95% CI Upper Bound
Mastitis (%)	Calcium carbonate	Calcium hydr	1.44*	0.20	0.01	1.04	1.84
		Formalin	-1.41*	0.31	0.01	-2.03	-0.79
		Lime	.12	0.25	0.63	-0.37	0.61
		NON	-4.06*	0.22	0.01	-4.49	-3.63
	Calcium hydroxide	Calcium carb	-1.44*	0.20	0.01	-1.84	-1.04
		Formalin	-2.85*	0.28	0.01	-3.41	-2.29
		Lime	-1.32*	0.21	0.01	-1.73	-0.90
		NON	-5.50*	0.18	0.01	-5.84	-5.15
	Formalin	Calcium carb	1.41*	0.31	0.01	0.79	2.03
		Calcium hydr	2.85*	0.28	0.01	2.29	3.41
		Lime	1.53*	0.32	0.01	0.90	2.16
		NON	-2.65*	0.30	0.01	-3.23	-2.06
	Lime	Calcium carb	-0.12	0.25	0.63	-0.61	0.37
		Calcium hydr	1.32*	0.21	0.01	0.90	1.73
		Formalin	-1.53*	0.32	0.01	-2.16	-0.90
		NON	-4.18*	0.23	0.01	-4.63	-3.73
SCC (1000x, cells/mL)	Calcium carbonate	Calcium hydr	34.57*	7.77	0.01	19.28	49.87
		Formalin	-37.58*	12.06	0.01	-61.30	-13.85
		Lime	19.61*	9.60	0.04	0.73	38.50
		NON	-49.16*	8.47	0.01	-65.82	-32.50
	Calcium hydroxide	Calcium carb	-34.57*	7.77	0.01	-49.87	-19.28
		Formalin	-72.15*	10.93	0.01	-93.65	-50.65
		Lime	-14.96	8.13	0.07	-30.95	1.03
		NON	-83.74*	6.76	0.01	-97.03	-70.45
	Formalin	Calcium carb	37.58*	12.06	0.01	13.85	61.30
		Calcium hydr	72.15*	10.93	0.01	50.65	93.65
		Lime	57.19*	12.29	0.01	33.01	81.37
		NON	-11.59	11.43	0.31	-34.08	10.90
	Lime	Calcium carb	-19.61*	9.60	0.04	-38.50	-0.73
		Calcium hydr	14.96	8.13	0.07	-1.03	30.95
		Formalin	-57.19*	12.29	0.01	-81.37	-33.01
		NON	-68.78*	8.80	0.01	-86.08	-51.47

*The mean difference is significant at the 0.05 level.

fectants, such as calcium carbonate, formalin, and lime, which exhibit notable effects on mastitis prevalence and SCC when compared to each other. Notably, calcium hydroxide demonstrates a significant mean difference of -1.44% (P = 0.01, 95% CI: -1.84 to -1.04) in mastitis prevalence, while lime exhibits a mean difference of -0.12% (P = 0.63, 95% CI: -0.61 to -0.37) compared to calcium carbonate. Furthermore, the comparison results indicate a significant decrease in SCC, with calcium hydroxide showing a reduction of 34,570 cells/mL (P = 0.01) and lime exhibiting a decrease of 19,614 cells/mL (P = 0.04) compared to calcium carbonate.

Table 8 provides detailed results of the impact of various disinfectants on teat total bacterial count (1000x) in dairy farming. Noteworthy mean differences and confidence intervals highlight specific disinfectants' substantial effects on teat total count. For instance, compared to calcium carbonate, formalin results in a significant mean decrease of 4,222.27 (P = 0.01, 95% CI: -5,782.75 to -2,661.79), while lime exhibit decreases of 1,770.80 (P = 0.01, 95% CI: -3,012.70 to -528.89). Additionally, calcium hydroxide shows a significant decrease of 2,487.26 (P = 0.01, 95% CI: -3,492.90 to -1,481.62) when compared to calcium carbonate. Further significant findings include a substantial mean decrease of 6,709.53 (P = 0.01, 95% CI: -8,123.22 to -5,295.84) for formalin compared to calcium hydroxide, and a notable de-

Table 8. Comparison of disinfectants for teat total bacterial count (1000x) in dairy farms

Disinfectants (I)	Disinfectants (J)	Mean Diff. (I-J)	Std. Error	P Values	95% CI Lower Bound	95% CI Upper Bound
Calcium carbonate	Calcium hydr	2487.26*	511.18	0.01	1481.62	3492.90
	Formalin	-4222.27*	793.21	0.01	-5782.75	-2661.79
	Lime	-1770.80*	631.28	0.01	-3012.70	-528.89
	NON	-3789.16*	556.94	0.01	-4884.82	-2693.50
Calcium hydroxide	Calcium carb	-2487.26*	511.18	0.01	-3492.90	-1481.62
	Formalin	-6709.53*	718.60	0.01	-8123.22	-5295.84
	Lime	-4258.06*	534.51	0.01	-5309.60	-3206.51
	NON	-6276.42*	444.27	0.01	-7150.43	-5402.41
Formalin	Calcium carb	4222.27*	793.21	0.01	2661.79	5782.75
	Calcium hydr	6709.53*	718.60	0.01	5295.84	8123.22
	Lime	2451.47*	808.45	0.01	861.02	4041.92
	NON	433.10	751.84	0.57	-1045.98	1912.18
Lime	Calcium carb	1770.80*	631.28	0.01	528.89	3012.70
	Calcium hydr	4258.06*	534.51	0.01	3206.51	5309.60
	Formalin	-2451.47*	808.45	0.01	-4041.92	-861.02
	NON	-2018.37*	578.43	0.01	-3156.31	-880.43

*The mean difference is significant at the 0.05 level.

Table 9. Comparison of disinfectants for bed total bacterial count and bedding pH in dairy farms

	Disinfectants (I)	Disinfectants (J)	Mean Diff. (I-J)	Std. Error	P Values	95% CI Lower Bound	95% CI Upper Bound
Bed total bacterial count (1000x)	Calcium carbonate	Calcium hydr	10.07*	3.17	0.01	3.81	16.32
		Formalin	-32.27*	5.81	0.01	-43.73	-20.81
		Lime	-8.49*	3.61	0.02	-15.61	-1.37
		NON	-29.50*	3.35	0.01	-36.11	-22.90
	Calcium hydroxide	Calcium carb	-10.07*	3.17	0.01	-16.32	-3.81
		Formalin	-42.34*	5.19	0.01	-52.57	-32.10
		Lime	-18.56*	2.49	0.01	-23.48	-13.64
		NON	-39.57*	2.09	0.01	-43.70	-35.45
	Formalin	Calcium carb	32.27*	5.81	0.01	20.81	43.73
		Calcium hydr	42.34*	5.19	0.01	32.10	52.57
		Lime	23.78*	5.47	0.01	12.99	34.56
		NON	2.76	5.30	0.60	-7.69	13.21
	Lime	Calcium carb	8.49*	3.61	0.02	1.37	15.61
		Calcium hydr	18.56*	2.49	0.01	13.64	23.48
		Formalin	-23.78*	5.47	0.01	-34.56	-12.99
		NON	-21.01*	2.71	0.01	-26.37	-15.66
Bedding pH	Calcium carbonate	Calcium hydr	-0.35*	.067	0.01	-0.48	-0.22
		Formalin	1.36*	.104	0.01	1.15	1.56
		Lime	0.18*	.082	0.03	0.02	0.34
		NON	0.99*	.073	0.01	0.85	1.14
	Calcium hydroxide	Calcium carb	0.35*	.067	0.01	0.22	0.48
		Formalin	1.7*	.094	0.01	1.52	1.89
		Lime	0.53*	.070	0.01	0.39	0.67
		NON	1.34*	.058	0.01	1.23	1.46
	Formalin	Calcium carb	-1.36*	.104	0.01	-1.56	-1.15
		Calcium hydr	-1.71*	.094	0.01	-1.89	-1.52
		Lime	-1.18*	.106	0.01	-1.38	-0.97
		NON	-0.36*	.098	0.01	-0.56	-0.17
	Lime	Calcium carb	-0.18*	.082	0.03	-0.34	-0.02
		Calcium hydr	-0.53*	.070	0.01	-0.67	-0.39
		Formalin	1.18*	.106	0.01	0.97	1.38
		NON	0.81*	.076	0.01	0.66	0.96

*The mean difference is significant at the 0.05 level.

crease of 2,451.47 (P = 0.01, 95% CI: -4,041.92 to 861,02) for lime compared to formalin.

Table 9 elucidates the comparison of various disinfectants in terms of bed total bacterial count (1000x) and bedding pH in dairy farms. A significant mean decrease is observed for bed total bacterial count with calcium hydroxide compared to calcium carbonate, indicating a difference of -10.07 (P = 0.01, 95% CI: -16.32 to -3.81). Similarly, a substantial mean increase of 42.34 is noted for bed total bacterial count with formalin compared to calcium hydroxide (P = 0.01, 95% CI: 32.10 to 52.57). Additionally, in the comparison between calcium hydroxide and lime, a significant mean increase of 18.56 is observed for bed total bacterial count with calcium hydroxide compared to lime (P = 0.01, 95% CI: 13.64 to 23.48). Regarding the effects of different disinfectants on bedding pH, the analysis indicates a significant mean increase with calcium hydroxide compared to calcium carbonate, showing a difference of 0.35 (P = 0.01, 95% CI: 0.22 to 0.48). Similarly, when comparing calcium hydroxide to formalin, a significant mean increase of 1.7 is identified for bedding pH with calcium hydroxide compared to formalin (P = 0.01, 95% CI: 1.52 to 1.89). In the comparison between calcium hydroxide and lime, a significant mean increase of 0.53 is observed for bedding pH with calcium hydroxide compared to lime (P = 0.01, 95% CI: 0.39 to 0.67).

DISCUSSION

Our research study investigated the impact of bedding materials and disinfectants on udder health and mastitis prevalence in dairy farming. The significance of this research stems from mastitis being a major concern for dairy farmers, influencing milk quality, animal welfare, and farm productivity. By exploring the relationships between bedding materials, disinfectants, and udder health indicators, this study aimed to provide evidence-based management strategies to reduce mastitis risk and improve udder health. The findings supports the theory that mastitis prevalence varies among different bedding materials, with the highest prevalence observed in manure bedding, followed by pumice, manure & soil, sand, and bagasse. Notably, bagasse bedding exhibits a statistically significant lower mastitis prevalence compared to other materials. The ability of bedding to support bacterial growth varies by type, with inorganic materials like sand proving inhibitory to bacterial growth, potentially reducing mastitis risk (Haxhiaj et al., 2022; Dziuba et al., 2023). Conversely, organic bedding materials may heighten mastitis risk, although they offer advantages in terms of manure handling and positively influencing soil fertility due to their higher organic matter content (Manning, 2024). Additionally, our results indicate that the selection of bedding material plays a crucial role in influencing mastitis prevalence in dairy farms. Moreover, effective management practices, including targeted interventions and the use of disinfectants, may contribute to reducing mastitis risk and improving udder health (Alawneh et al., 2020; Cheng and Han, 2020; Zigo et al., 2021). In our investigation, we explored the relationship between the utilization of disinfectants and various aspects of udder health, including pH levels, total counts of bacteria on beds and teats, mastitis prevalence, and somatic cell count. The study reveals noteworthy correlations between certain disinfectants and these udder health factors. Particularly noteworthy is the correlation observed between mastitis prevalence and specific disinfectants, suggesting that the application of these disinfectants may be associated with a decrease in mastitis rates. These findings are consistent with the conclusions drawn by Kovačević et al. (2022), underscoring the significant role of antimicrobials in influencing animal health. Furthermore, our research identified correlations between total bacterial counts on bedding and specific disinfectants, as well as between total bacterial counts on teats and specific disinfectants. This implies that the use of disinfectants may contribute to a reduction in bacterial load both in bedding and on teats, potentially enhancing udder health.

The moisture content plays a crucial role in the selection of appropriate bedding materials. Fregonesi et al. (2007) found that, regardless of the season, cows exhibit a preference for low-moisture bedding. Optimal microbial activity is sustained under conditions of adequate moisture; hence, materials with elevated moisture content are unsuitable (Sharun et al., 2021). In our study, both manure and bagasses exhibited the highest moisture levels. The results indicate that bagasse demonstrated superior performance, as evidenced by the data on total bed and teat counts. The total bed count for bagasses was the lowest, whereas pumice and manure recorded the highest levels. Bed materials were found to harbor prominent bacteria such as E. coli, Klebsiella spp, and coliforms. Manasa et al. (2019) associated the transmission of mastitis-causing pathogens with environmental factors, particularly involving Klebsiella spp and coliforms. Gram-negative bacteria, particularly E. coli and Klebsiella spp, contribute to over 40% of clinical mastitis cases (Oliveira et al., 2015). Gorden et al. (2018) highlighted the prevalence of Escherichia coli, and the severity of clinical mastitis attributed to Klebsiella spp., while Cvetnić et al. (2021, 2022) underscored Staphylococcus aureus as a common cause of mastitis. The prevalence of Staphylococcus aureus ranges widely, from 2% to over 50%, contributing to 10-12% of clinical mastitis cases. Furthermore, a previous study by Hogan and Smith (2003) emphasized that environmental mastitis pathogens pose a significant risk to bovine teats, primarily due to exposure to bedding materials. Economic losses associated with bovine mastitis include reduced milk yield, inferior milk quality, increased production expenses, medication costs, milk loss during and post-treatment, reduced milking days, decreased milk prices, heightened labor requirements, and increased recruitment expenses (Azooz et al., 2020; He et al., 2020; Puerto et al., 2021; Kovačević et al., 2023; Tomanić et al., 2023). Cvetnić et al. (2016) emphasized that the greater threat lies in its impact on the dairy farmer's economic interests rather than on the individual animal's health. The selection of bedding material should consider the presence of pathogens in the raw material. Hayes et al. (2001) suggested that total bacterial count could be valuable in assessing farm sanitation, overall udder health, and ensuring appropriate temperatures for milk handling and storage. Our findings align with Gleeson (2013), who reported a substantial reduction in Staphylococci and Streptococci on teat skin when using hydrated lime for bedding compared to ground limestone. Additionally, our results support Hogan et al. (2003), demonstrating the effectiveness of hydrated lime on organic cubicle bedding materials in significantly reducing bacterial counts on cubicle beds. Consistent with previous findings that lower bacterial counts in bedding materials correlate with a decrease in new infections (Hogan and Smith, 2003), our results demonstrate that calcium hydroxide lowered the total bed count and mastitis incidence. These findings are in line with Chettri (2006), who revealed a 45% reduction in mastitis incidence with daily application of hydrated lime in dairy cow free-stalls. Gleeson (2013) has also focused on reducing both Staphylococcus spp. and Streptococcus spp. through direct application of hydrated lime to cubicle beds.

Sand bedding is a popular choice for housing dairy cows due to its numerous advantages in improving cow comfort, health, and overall herd management. It provides a soft and comfortable surface for cows, is easy to level and maintain, ensuring a consistent and comfortable environment (Galama et al., 2020; Singh et al., 2020a). Additionally, the cooling properties of sand can be particularly beneficial in hot climates, reducing the risk of heat stress in dairy cows (Ji et al., 2020; Shephard and Maloney, 2023). Sand also facilitates the straightforward removal of manure, promoting a cleaner and healthier living environment for the cows (Herskin et al., 2020). Furthermore, considering that sand is a natural resource, its use is considered more environmentally friendly when compared to certain bedding alternatives. However, establishing a sand bedding system may require an initial investment in infrastructure, such as sand separators and proper manure handling equipment. Effective management, including regular cleaning and replenishing of sand, is crucial to ensure its continued efficacy. It is important to highlight that successful sand bedding management demands attention to detail and a commitment to regular maintenance. Based on our research findings, bagasse appears to be an optimal bedding material due to its favorable impact on bacterial loading and mastitis prevalence. The fibrous nature of bagasse provides a comfortable and absorbent bedding material for animals. When used as bedding, bagasse helps absorb moisture, provides a soft surface for animals to rest on, and contributes to maintaining a clean and dry environment in the animal housing. Additionally, the use of bagasse for bedding is a sustainable practice, involving the recycling of byproducts from sugarcane processing (Cabrera, 2021; Diarra et al., 2021; Mohammed et al., 2022). Farmers and agricultural operations often explore various options for bedding materials, and bagasse can be a viable choice for those seeking an eco-friendly solution. However, specific practices may vary depending on regional availability, local agricultural methods, and economic considerations.

The findings of our study, which are based on specific conditions, may not be universally applicable to all dairy farms. Variations in farm management practices, environmental factors, and herd characteristics could influence the relationships observed between bedding materials, disinfectants, and udder health indicators. While our investigation focused on the impact of bedding materials and disinfectants, it did not fully address other factors influencing mastitis prevalence and udder health, such as milking practices, nutrition, and cow hygiene. This highlights the need for a more comprehensive exploration to achieve a holistic understanding of udder health management. Caution is advised when generalizing our findings, as they may not easily extend to dairy farms with different management practices, geographical locations, or herd sizes. Challenges in establishing causal relationships between bedding materials, disinfectants, and udder health indicators stem from the study's design, underscoring the necessity for additional research, including longitudinal studies and controlled experiments, to provide more robust evidence of causality. However, the robustness of our findings may be impacted by the study's data collection methods and sample size. Variations in data collection techniques and sample representativeness could potentially introduce biases. Despite statistical analyses, the potential for confounding variables and unmeasured factors could affect result interpretation, emphasizing the importance of addressing potential confounders and controlling for relevant variables. It is important to note that, despite our efforts, reliance on published literature and existing data sources may introduce publication bias, potentially limiting the inclusivity of available evidence. Given these limitations, a critical approach is essential when interpreting our study's findings. Additional research and consideration of contextual factors are crucial when applying these findings to dairy farm management practices.

CONCLUSIONS

Our study illuminates crucial considerations for dairy farmers aiming to optimize udder health and mitigate mastitis risk. By employing multivariate regression and correlation analyses, we identified key variables that significantly influence somatic cell count (SCC) and mastitis prevalence. The choice of bedding type and disinfectant emerged as pivotal factors with substantial impacts. In particular, our findings underscore the importance of selecting appropriate bedding materials, such as pumice, sand, or bagasse, and effective disinfectants like calcium carbonate or calcium hydroxide. These variables demonstrated noteworthy associations with lower SCC and reduced mastitis prevalence, emphasizing their practical relevance in dairy farms. Therefore, dairy farmers should consider these specific factors when making decisions about bedding materials and disinfectants. Optimal choices in these areas can lead to improved udder health, decreased bacterial load, and ultimately contribute to sustainable and thriving outcomes in dairy farming practices.

Nevertheless, prudent interpretation is essential, given the study's limitations. Further research is imperative to address potential confounders and ensure the broader applicability of our findings across diverse dairy farming contexts. This ongoing exploration will fortify our understanding and support the continual improvement of udder health practices and mastitis control in the dairy industry.

ACKNOWLEDGEMENTS

We express our sincere appreciation to the dairy farms in Iran and their dedicated workers for their crucial contributions to this study. Their cooperation and commitment were integral to the success of our research, enriching our understanding of mastitis control in dairy farming. We extend our thanks for their pivotal role in advancing knowledge and fostering improvements in udder health management.

CONFLICT OF INTEREST

The author/s declared that there is no conflict of interest.

REFERENCES

Ahmed A S, Diab H M, Alkahtani M A, Alshehri M A, Saber H, Badr H, Dandrawy M K, El-Mansi A A, Shati A A, Ahmed A E (2022) Molecular epidemiology of virulent e. coli among rural small scale dairy herds and shops: efficacy of selected marine algal extracts and disinfectants. International journal of environmental health research, 32(1), 72-94. https://doi.org/10.1080/09603123.2020.1727422

Alanis V M, Zurakowski M, Pawloski D, Tomazi T, Nydam D V, Ospina P A (2021) Description of the characteristics of five bedding materials and association with bulk tank milk quality on five new york dairy herds. Frontiers in veterinary science, 8, 636833. https://doi.org/10.3389/fvets.2021.636833

Alawneh J I, James A S, Phillips N, Fraser B, Jury K, Soust M, Olchowy T W J (2020) Efficacy of a lactobacillus-based teat spray on udder health in lactating dairy cows. Frontiers in veterinary science, 7, 584436. https://doi.org/10.3389/fvets.2020.584436

Andrews T, Neher D A, Weicht T R, Barlow J W (2019) Mammary microbiome of lactating organic dairy cows varies by time, tissue site, and infection status. Plos one, 14(11), e0225001. https://doi.org/10.1371/journal.pone.0225001

Azooz M F, El-Wakeel S A, Yousef H M (2020) Financial and economic analyses of the impact of cattle mastitis on the profitability of Egyptian dairy farms. Veterinary world, 13(9), 1750-1759. https://doi.org/10.14202/vetworld.2020.1750-1759

Benić M, Maćešić N, Cvetnić L, Habrun B, Cvetnić Ž, Turk R, Đuričić D, Lojkić M, Dobranić V, Valpotić H, Grizelj J, Gračner D, Grbavac J, Samardžija M (2018) Bovine mastitis: a persistent and evolving problem requiring novel approaches for its control-a review. Veterinarski arhiv, 88(4), 535-557. https://doi.org/10.24099/vet.arhiv.0116

Cabrera F C (2021) Eco friendly polymer composites: A review of suitable methods for waste management. Polymer Composites, 42(6), 2653-2677.

Cheng W N, Han S G (2020) Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments - A review. Animal bioscience, 33(11), 1699-1713. https://doi.org/10.5713/ajas.20.0156

Chettri R (2006) Evaluation of hydrated lime treatment of free-stall bedding and efficacy of teat sealant on incidence of dairy cow mastitis (Doctoral dissertation). http://hdl.handle.net/10415/525

Cobirka M, Tancin V, Slama P (2020) Epidemiology and classification of mastitis. Animals, 10(12), 2212. https://doi.org/10.3390/ani10122212

Cvetnić L, Samardžija M, Habrun B, Kompes G, Benić M (2016) Microbiological monitoring of mastitis pathogens in the control of udder health in dairy cows. Slovenian Veterinary Research, 53(3), 131-40. Open acess.

Cvetnić L, Samardžija M, Duvnjak S, Habrun B, Cvetnić M, Jaki Tkalec V, Đuričić D, Benić M (2021) Multi Locus Sequence Typing and spa typing of Staphylococcus aureus isolated from the milk of cows with subclinical mastitis in Croatia. Microorganisms, 9(4), 725. https://doi.org/10.3390/microorganisms9040725

Cvetnić L, Špičić S, Kompes G, Habrun B, Katalinić-Janković V, Cvetnić M, Zdelar-Tuk M, Reil I, Duvnjak S, Cvetnić Z, Benić M (2022) Bovine mastitis caused by rapid-growth environmental mycobacteria. Vet. Stanica, 53, 493-501. https://doi.org/10.46419/vs.53.5.11

Damasceno F A, Day G B, Taraba J L, Barbari M, Oliveira C E A, Frigeri K D M, Vieira F M C, Bambi G (2022) Determination of thermal, chemical and physical properties of bedding materials for compost dairy barns. Animals (basel), 12(18), 2450. https://doi.org/10.3390/ani12182450

Dayoub M, Shnaigat S, Tarawneh R A, Al-Yacoub A N, Al-Barakeh F, Al-Najjar K (2024) Enhancing animal production through smart agriculture: Possibilities, hurdles, resolutions, and advantages. Ruminants, 4(1), 22-46. https://doi.org/https://doi.org/10.3390/ruminants4010003

Diarra S, Lameta S, Amosa F, Anand S (2021) Alternative bedding materials for poultry: Availability, efficacy, and major constraints. Frontiers in veterinary science, 8, 669504. https://doi.org/10.3389/fvets.2021.669504

Dziuba M, Caixeta L S, Boyum B, Godden S, Royster E, Rowe S (2023) Negatively controlled trial investigating the effects of dry cow therapy on clinical mastitis and culling in multiparous cows. Journal of dairy science, 106(8), 5687-5695. https://doi.org/10.3168/jds.2022-22845

Evans H C, Briggs E F, Burnett R H, Contreras-Correa Z E, Duvic M A, Dysart L M, Gilmore A A, Messman R D, Reid D, Rasit U, Kaya M A, Memili E (2022) Harnessing the value of reproductive hormones in cattle production with considerations to animal welfare and human health. Journal of animal science, 100(7), skac177. https://doi.org/10.1093/jas/skac177

Fávero S, Portilho F, Oliveira A, Langoni H, Pantoja J (2015) Factors associated with mastitis epidemiologic indexes, animal hygiene, and bulk milk bacterial concentrations in dairy herds housed on compost bedding. Livestock science, 181, 220-230. https://doi.org/https://doi.org/10.1016/j.livsci.2015.09.002

Ferraz P F P, Ferraz G, Leso L, Klopcic M, Barbari M, Rossi G (2020) Properties of conventional and alternative bedding materials for dairy cattle. Journal of dairy science, 103(9), 8661-8674. https://doi.org/10.3168/jds.2020-18318

Frechette A, Fecteau G, Cote C, Dufour S (2021) Clinical mastitis incidence in dairy cows housed on recycled manure solids bedding: A canadian cohort study. Frontier in veterinary science, 8, 742868. https://doi.org/10.3389/fvets.2021.742868

Fregonesi J A, Veira D M, Key-Serlingk M A G, Weary D M (2007) Effects of bedding quality on lying behavior of dairy cows. Journal of dairy science., 90: 5468-5472. https://doi.org/10.3168/jds.2007-0494

Galama P J, Ouweltjes W, Endres M I, Sprecher J R, Leso L, Kuipers A, Klopcic M (2020) Symposium review: Future of housing for dairy cattle. Journal of dairy science, 103(6), 5759-5772. https://doi.org/10.3168/jds.2019-17214

Gleeson D (2013) Evaluation of hydrated lime as a cubicle bedding material on the microbial count on teat skin and new intramammary infection. Irish journal of agricultural and food research, 159-171. http://www.jstor.org/stable/23631028

Godden S, Bey R, Lorch K, Farnsworth R, Rapnicki P (2008) Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. Journal of dairy science, 91(1), 151-159. https://doi.org/10.3168/jds.2007-0415

Gorden P J, Ydstie J A, Kleinhenz M D, Brick T A, Smith J S, Griffith R W, Larry W W, Suzanne M R, Min Z, Pritam K S, Jonathan P M, Coetzee, J. F. (2018) Comparative plasma and interstitial fluid pharmacokinetics and tissue residues of ceftiofur crystalline free acid in cattle with induced coliform mastitis. Journal of veterinary pharmacology and therapeutics, 41(6), 848-860. https://doi.org/10.1111/jvp.12688

Haider A, Ikram M, Shahzadi I, Asif R M (2023) Bovine mastitis. in polymeric nanoparticles for bovine mastitis treatment (pp. 49-80). Springer. https://doi.org/https://doi.org/10.1007/978-3-031-39947-3_4

Haxhiaj K, Wishart D S, Ametaj B N (2022) Mastitis: What it is, current diagnostics, and the potential of metabolomics to identify new predictive biomarkers. Dairy, 3(4), 722-746. https://doi.org/10.3390/dairy3040050

Hayes M C, Ralyea R D, Murphy S C, Carey N R, Scarlett J M, Boor K J (2001) Identification and characterization of elevated microbial counts in bulk tank raw milk. Journal of dairy science, 84(1), 292-298. https://doi.org/10.3168/jds.S0022-0302(01)74479-7

He W, Ma S, Lei L, He J, Li X, Tao J, Wang X, Song S, Wang Y, Wang Y, Shen J, Cai C, Wu C (2020) Prevalence, etiology, and economic impact of clinical mastitis on large dairy farms in China. Veterinary microbiology, 242, 108570. https://doi.org/10.1016/j.vetmic.2019.108570

Herskin M S, Fogsgaard K K, Thomsen P T, Houe H, Forkman B, Jensen M B (2020) Dairy cows with mild-moderate mastitis change lying behavior in hospital pens. Translational animal science, 4(2), txaa038.

https://doi.org/10.1093/tas/txaa038
Hogan J, Smith K L (2003) Coliform mastitis. Veterinary research, 34(5), 507-519. https://doi.org/10.1051/vetres:2003022
Hohmann M F, Wente N, Zhang Y, Krömker V (2020) Bacterial load of the teat apex skin and associated factors at herd level. Animals, 10(9), 1647. https://doi.org/10.3390/ani10091647
IBM Corporation (2011) IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY.
Janni K A, Endres M I, Reneau J K, Schoper W W (2007) Compost dairy barn layout and management recommendations. Applied engineering in agriculture, 23(1), 97-102. https://doi.org/10.13031/2013.22333
Ji B, Banhazi T, Perano K, Ghahramani A, Bowtell L, Wang C, Li B (2020) A review of measuring, assessing and mitigating heat stress in dairy cattle. Biosystems engineering, 199, 4-26. https://doi.org/10.1016/j.biosystemseng.2020.07.009
Kjaer J, Olsen P, Bach K, Barlebo H C, Ingerslev F, Hansen M, Sorensen B H (2007). Leaching of estrogenic hormones from manure-treated structured soils. Environmental science and technology, 41(11), 3911-3917. https://doi.org/10.1021/es0627747
Klaas I C, Zadoks R N (2018) An update on environmental mastitis: Challenging perceptions. Transboundary and emerg diseases, 65 Suppl 1, 166-185. https://doi.org/10.1111/tbed.12704
Krawczel P D, Klaiber L B, Butzler R E, Klaiber L M, Dann H M, Mooney C S, Grant R J (2012) Short-term increases in stocking density affect the lying and social behavior, but not the productivity, of lactating Holstein dairy cows. Journal of dairy science, 95(8), 4298-4308. https://doi.org/10.3168/jds.2011-4687
Kovačić M, Samardžija M, Đuričić D, Vince S, Flegar-Meštrić Z, Perkov S, Gračner D, Turk R. (2019) Paraoxonase-1 activity and lipid profile in dairy cows with subclinical and clinical mastitis. Journal of Applied Animal Research, 47(1), 1-4. https://doi.org/10.1080/09712119.2018.1555090
Kovačević Z, Samardžija M, Horvat O, Tomanić D, Radinović M, Bijelić K, Vukomanović A, Kladar N (2022) Is There a Relationship between Antimicrobial Use and Antibiotic Resistance of the Most Common Mastitis Pathogens in Dairy Cows?. Antibiotics, 12(1), 3. https://doi.org/10.3390/antibiotics12010003
Kovačević Z, Mihajlović J, Mugoša S, Horvat O, Tomanić D, Kladar N, Samardžija M (2023) Pharmacoeconomic Analysis of the Different Therapeutic Approaches in Control of Bovine Mastitis: Phytotherapy and Antimicrobial Treatment. Antibiotics, 12(1), 11. https://doi.org/10.3390/antibiotics12010011
Leso L, Barbari M, Lopes M A, Damasceno F A, Galama P, Taraba J L, Kuipers A (2020) Invited review: Compost-bedded pack barns for dairy cows. Journal of dairy science, 103(2), 1072-1099. https://doi.org/10.3168/jds.2019-16864
Manasa V, SaiKumar T V, Rao T P, Kumar K A (2019) Incidence of clinical and sub-clinical bovine mastitis caused by Staphylococcus aureus in Proddatur region of Andhra Pradesh. International Journal of Chemical Studies, 7(3), 788-792
Manning A (2024) Bedding material for dairy cows: effect on udder health and milk quality. Livestock, 29(1), 18-21. https://doi.org/10.12968/live.2024.29.1.18
Mohammed M, Rahman R, Mohammed A M, Adam T, Betar B O, Osman A F, Dahham O S (2022) Surface treatment to improve water repellence and compatibility of natural fiber with polymer matrix: Recent advancement. Polymer testing, 107707. https://doi.org/10.1016/j.polymertesting.2022.107707
Ndahetuye J B, Twambazimana J, Nyman A K, Karege C, Tukei M, Ongol M P, Persson Y, Bage R (2020) A cross sectional study of prevalence and risk factors associated with subclinical mastitis and intramammary infections, in dairy herds linked to milk collection centers in Rwanda. Preventive veterinary medicine, 179, 105007. https://doi.org/10.1016/j.prevetmed.2020.105007
Oliveira C E A, Tinoco I F F, De Oliveira V C, Rodrigues P H M, Silva L F D, Damasceno F A, Andrade R R, Sousa F C, Barbari M, Bambi G (2023) Spatial distribution of bedding attributes in an open compost-bedded pack barn system with positive pressure ventilation in brazilian winter conditions. Animals (basel), 13(5), 786. https://doi.org/10.3390/ani13050786
Oliveira C S, Hogeveen H, Botelho A M, Maia P V, Coelho S G, Haddad J P (2015) Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. Preventive veterinary medicine, 121(3-4), 297-305. https://doi.org/10.1016/j.prevetmed.2015.08.001
Patel K, Godden S, Royster E, Crooker B, Timmerman J, Fox L (2019) Relationships among bedding materials, bedding bacteria counts, udder hygiene, milk quality, and udder health in US dairy herds. Journal of dairy science, 102(11), 10213-10234. https://doi.org/https://doi.org/10.3168/jds.2019-16692
Piepers S, De Meulemeester L, De Kruif A, Opsomer G, Barkema H W, De Vliegher S (2007) Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. Journal of dairy research, 74(4), 478-483. https://doi.org/10.1017/S0022029907002841
Puerto M A, Shepley E, Cue R I, Warner D, Dubuc J, Vasseur E (2021) The hidden cost of disease: I. Impact of the first incidence of mastitis on production and economic indicators of primiparous dairy cows. Journal of dairy science, 104(7), 7932-7943. https://doi.org/10.3168/jds.2020-19584
Rayne N, Aula L (2020) Livestock manure and the impacts on soil health: A review. Soil systems, 4(4), 64. https://doi.org/https://doi.org/10.3390/soilsystems4040064
Robles I, Kelton D, Barkema H, Keefe G, Roy J, Von K M, De Vries T (2020) Bacterial concentrations in bedding and their association with dairy cow hygiene and milk quality. Animal, 14(5), 1052-1066. https://doi.org/doi:10.1017/S1751731119002787
Ruegg P L (2017) A 100-Year Review: Mastitis detection, management, and prevention. Journal of dairy science, 100(12), 10381-10397. https://doi.org/10.3168/jds.2017-13023
Sharun K, Dhama K, Tiwari R, Gugjoo M B, Iqbal Y M, Patel S K, Pathak M, Karthik K, Khurana S K, Singh R, Puvvala B, Amarpal S R, Singh K P, Chaicumpa W (2021) Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review. National library of medicine, veterinary, 41(1), 107-136. https://doi:10.1080/01652176.2021.1882713.
Shephard R W, Maloney S K (2023) A review of thermal stress in cattle. Australian veterinary journal, 101(11), 417-429. https://doi.org/10.1111/avj.13275
Singh A K (2022) A comprehensive review on subclinical mastitis in dairy animals: Pathogenesis, factors associated, prevalence, economic losses and management strategies. CaBi digital library. https://doi.org/https://doi.org/10.1079/cabireviews202217057
Singh A K, Kumari T, Rajput M S, Baishya A, Bhatt N, Roy S (2020) A review: Effect of bedding material on production, reproduction and health and behavior of dairy animals. International journal of livestock research, 10(7), 11-20. https://doi.org/10.5455/ijlr.20200207073618
Singh J, Singh B B, Tiwari H K, Josan H S, Jaswal N, Kaur M, Kostoulas P, Khatkar M S, Aulakh R S, Gill J P S, Dhand N K (2020) Using dairy value chains to identify production constraints and biosecurity risks. Animals (basel), 10(12), 2332. https://doi.org/10.3390/ani10122332
Themistokleous K. Karagiannis I, Boscos C, Panousis N, Kiossis E (2020) Epidemiological evaluation of subclinical mastitis of dairy cows in Greece. Journal of the Hellenic Veterinary medical society. 70, 4, 1865-1874. https://doi.org/10.12681/jhvms.22237
Themistokleous K, Sakellariou N, Kougioumtzis A, Kiossis E (2022) Automatic Milking in Greece: First Insight into Udder Health of Dairy Cows. Journal of the Hellenic Veterinary Medical Society, 73(2), 4253-4260. https://doi.org/10.12681/jhvms.29782
Tibebu L, Belete Y, Tigabu E, Tsegaye W (2021) Prevalence of Staphylococcus aureus, methicillin-resistant Staphylococcus aureus and potential risk factors in selected dairy farms at the interface of animal and human in Bishoftu, Ethiopia. Veterinary medicine: Research and reports, 241-251. https://doi.org/https://doi.org/10.2147/VMRR.S331968
Tomanić D, Samardžija M, Kovačević Z (2023) Alternatives to Antimicrobial Treatment in Bovine Mastitis Therapy: A Review. Antibiotics, 12(4), 683. https://doi.org/10.3390/antibiotics12040683
Tucker C B, Jensen M B, De Passille A M, Hanninen L, Rushen J (2021) Invited review: Lying time and the welfare of dairy cows. Journal of dairy science, 104(1), 20-46. https://doi.org/10.3168/jds.2019-18074
Turk R, Koledić M, Maćešić N, Benić M, Dobranić V, Đuričić D, Cvetnić L, Samardžija M (2017) The role of oxidative stress and inflammato-

ry response in the pathogenesis of mastitis in dairy cows. Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka, 67(2), 91-101. Open acces

Varma V S, Parajuli R, Scott E, Canter T, Lim T T, Popp J, Thoma G (2021) Dairy and swine manure management - Challenges and perspectives for sustainable treatment technology. Science of the total environment, 778, 146319. https://doi.org/10.1016/j.scitotenv.2021.146319

Ventura G, Lorenzi V, Mazza F, Clemente G A, Iacomino C, Bertocchi L, Fusi F (2021) Best farming practices for the welfare of dairy cows, heifers and calves. Animals, 11(9), 2645. https://doi.org/10.3390/ani11092645

Werther J, Saenger M, Hartge E U, Ogada T, Siagi Z (2000) Combustion of agricultural residues. Progress in energy and combustion science, 26(1), 1-27. https://doi.org/https://doi.org/10.1016/S0360-1285(99)00005-2

Zigo F, Vasil M, Ondrasovicova S, Vyrostkova J, Bujok J, Pecka-Kielb E (2021) Maintaining optimal mammary gland health and prevention of mastitis. Frontiers in veterinary science, 8, 607311. https://doi.org/10.3389/fvets.2021.607311