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Housing conditions of dry cows: effects on teat contamination and somatic cells at the beginning of the subsequent lactation

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ABSTRACT

The study investigated the effects of housing conditions and temperature-humidity index (THI) during the dry period on total bacteria counts of bedding material, animal cleanliness, and teat bacterial contamination on 212 cows from three farms. Additionally, milk yield, quality, total somatic cells, and leucocyte fractions were evaluated in the subsequent lactation on a subgroup of 119 cows. The results showed a relationship between persistent high THI, teat bacterial contamination, and pathogens on teat skin. Somatic cell count (SCC) in milk at the beginning of the next lactation was not affected by THI during the dry period. Multiple correlation analysis revealed a relationship among high milk SCC, high percentage of neutrophils, high bacterial count of bedding during the dry period, and high milk production at dry-off. The study confirms that critical environmental conditions, such as high THI and poor bedding hygiene, during the dry period, can affect teat bacterial contamination and increase the risks for high SCC in the next lactation. High milk production at dry-off may be another risk factor.

HIGHLIGHTS

- Temperature and humidity in the barn during the dry period affect total bacterial and pathogen contamination on teats.
- Bedding bacterial count during dry period, milk production at dry-off, and post-calving somatic cell counts were positively associated.

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Introduction

Effective dry period management is essential to maintain cow productivity in future lactations and prevent udder health issues (McMullen et al. 2021). The success of the dry period and the future career of cows can be influenced by various factors among which the most important appear to be dry-off management, dry period length, housing systems, animal and bedding hygiene, and heat stress. According to Green et al. (2007), maintaining a clean housing environment for dry cows is closely related to reduced probabilities of udder health issues after calving. Regular pen sanitisation and frequent bedding material replacement can help reduce the risk of mastitis during and after the dry period (Nitz et al. 2021). Moreover, the type of bedding material can influence the bacterial contamination of cow teats (Wolfe et al. 2018). Prolonged high temperatures and elevate humidity can induce heat stress in lactating cows, resulting in reduced intake and milk production, impaired fertility, and increased incidences of diseases, such as mastitis and lameness (Dahl and McFadden 2022). This condition can also lead to elevated Somatic Cell Count (SCC) in bulk tank milk (Tao et al. 2018). Heat stress can also affect the physiology and behaviour of dry cows, particularly when the Temperature-Humidity Index (THI) exceeds 77 (Ouellet et al. 2021), leading to reduced feed intake (Tao et al. 2011) and decreased milk production in the next lactation (Tao et al. 2018; Seyed Almoosavi et al. 2021). Moreover, heat stress reduces the immune response of dry cows, leading to lymphocyte proliferation and suppression of the killing power of neutrophils in the blood (Gupta et al. 2023). Tao et al. (2011) reported a tendency towards increased milk SCC in the next lactation of cows exposed to high temperatures during the dry period. However, to the best of

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our knowledge, no studies have investigated the effect of THI during the dry period on the three fractions of differential somatic cell count (neutrophils, lymphocytes, and macrophages) in milk after calving.

The study aimed to investigate the effect of Temperature-Humidity Index, and housing conditions on animal cleanliness, bedding material, and teat bacterial contamination, along with the presence of pathogens on cow teat skin during the dry period. Additionally, milk production, quality, and SCC, including total count and different leucocyte fractions, in the next lactation were evaluated.

Materials and methods

The study took place over a two-year period (2020–2021) on three commercial dairy cattle farms (farms A, B, and C) in Northern Italy. Lactating cows were 204, 620, and 177, while the average daily milk production per cow was 32.4 ± 7.2, 32.2 ± 7.7, and 34.2 ± 7.7 kg, for farms A, B, and C, respectively. All three farms milked cows twice daily and housed them in free-stall barns with individual cubicles, without pasture access. Farm A and farm C housed dry cows in free-stall barns with cubicles with sawdust as bedding material, while farm B used permanent litter with straw. Ventilation fans in the dry cows' pens were only present at farms A and C. The dry-off protocol was abrupt on all farms, with an average dry period length of 64 ± 26 days. Selective dry cow therapy was applied on all three farms, following the protocol reported by Mondini et al. (2023) which involves the use of teat sealants with or without intramammary antimicrobials based on the mastitis risk for each animal.

A total of 212 cows from the three herds were monitored during the dry period, while a subgroup of 119 cows was also monitored during the last days of lactation (17 ± 10 days before dry-off) and the first days (26 ± 15 days) of the subsequent lactation.

The cleanliness of the 212 dry cows was evaluated using the Hygiene Scoring method (Cook and Reinemann 2007). This method involves observation without the need for physical contact with the animals. Evaluations were conducted once per cow during the dry period (20 ± 12 days after dry-off) by two trained evaluators.

Teat bacterial contamination was evaluated, once per cow, on 212 dry cows in the mid-dry period (20 ± 12 days after dry-off) using sterile culture swabs gently passed over the teats and immediately placed in sterile plastic zip-lock bags. All samples were transported to the laboratory under refrigeration (4 °C) within 12 h after collection for mesophilic bacterial count determination (CFU/swab at 30 °C) following UNI EN ISO 4833-1:2013. Real-Time PCR was used to identify four pathogens on the swabs: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*, and *Prototheca* spp.

A total of 28 bedding material samples from the dry cow pens were collected using sterile tissue shoe covers on the barn floor. All samples were transported to the laboratory under refrigeration (4 °C) within 12 h after collection, for mesophilic bacterial count determination (CFU/g at 30 °C) following UNI EN ISO horizontal method 4833-1:2013. Microbiological analyses on teat swabs and bedding as well as milk quality analyses were conducted in the laboratory of the Lombardy Breeders' Association. Data on bacterial contamination of bedding material was associated with the months of the dry period of each cow to obtain a single value per cow.

Temperature and relative humidity of the dry cow pens were continuously monitored at 2-min intervals using HOBO data loggers (Onset Computer Corporation, Pocasset, MA, USA) placed above the resting area, throughout the entire study. The THI was calculated using the formula proposed by Segnalini et al. (2013).

For each month of the two-year period, five variables were calculated: average THI, maximum THI, number of days with an average THI $>$ 72, average number of hours per day with an average THI $>$ 72, and maximum number of hours per day with average THI $>$ 72. These variables were associated with the months of the dry period of each cow to obtain the average values of the five variables for each cow.

Milk production and quality were recorded for the 119 cows. Milk production at the end of lactation (323 ± 54 days in milk, 16 ± 10 days before dry-off; Table 1) and at the beginning of the subsequent

Table 1. Main characteristics of the 119 cows from the three herds at the end of lactation.

	Farm A		Farm B		Farm C	
	Average	*SD*	Average	*SD*	Average	*SD*
N. of lactation	2.0	1.1	1.6	0.9	1.8	1.0
Lactation length, days	348	58	335	59	332	48
Dry period length, days	61	15	52	14	61	12
Milk yield, kg/day/cow	23.4	6.5	26.2	4.9	28.3	7.7
Somatic cell count (SCC), log10 cells/mL	4.81	0.42	4.60	0.35	4.89	0.53

lactation (25 ± 15 days in milk) was obtained from the milking equipment software. For milk quality assessment, samples were collected during the afternoon milking, stored at 4 °C, and analysed within 12 h after collection. Fat and protein milk content (%), SPC (CFU/ml), and SCC (cells/ml) were measured using MilkoScan FT6000, Bactoscan FC, and FOSSOMATIC TM 7 Electronic cell counter (Foss Analytical A/S, Hillerod, Denmark), respectively.

On the same milk samples, individual leucocyte fractions (neutrophils–NEU, macrophages–MAC, and lymphocytes–LYM) and Total Leucocyte Count (TLC), defined according to Mondini et al. (2023), were measured as cells/ml through fluorescence-imaging using the Vetscan DC–Q Milk Analyser (AAD-Advanced Animal Diagnostics, NC, USA).

Statistical analysis

Statistical analyses were performed using SAS (v 9.4, SAS Institute Inc., USA). Before the analyses, teat, bedding, and milk bacterial counts, and SCC were log10-transformed.

The description was carried out using PROC MEANS and PROC FREQ. Fisher's exact test was used to analyse contingency tables between positive and negative swabs for pathogen presence and THI classes (n. days with average THI > 72 equal to 0 or 1 *vs.* n. days with average THI > 72 higher than 1).

The correlation matrix (PROC CORR) was performed between teat bacterial contamination and the different THI indicators.

A General Linear Model (GLM) analysis was performed with the following model:

$$Y_{\text{ijklmno}} = M + \text{Farm}_i + \text{Parity}_j + \text{DPL}_k + \text{HSU}_l + \text{Heat}_m + \text{Swabs}_n + e_{\text{ijklmno}}$$

where:

- Y = milk fat, protein, SCC, TLC, NEU, MAC, LYM, and SPC from cows at the beginning of the lactation, teat bacterial contamination, and bedding bacterial contamination
- Farm_i = farms A, B, and C (i = 1–3) as random effect
- Parity_j = number of lactation at dry-off (primiparous or multiparous, j = 1–2)
- DPL_k = dry period length (<57 or ≥57 days, k = 1–2)
- HSU_l = hygiene score of the udder [clean (1–2) or not clean (3–4), n = 1–2]
- Heat_m = no. days of the months of dry period with THI > 72 (≤1 or >1 day, o = 1–2)
- Swabs_n = contamination of teat swabs with *S. aureus* or *Prototheca* spp. (positive or negative, q = 1–2)

The multiple correspondence analysis was performed with PROC CORRESP.

The categorical variables were:

- Milk TLC at the beginning of lactation (low <5 and high ≥5 $\log_{10}$ cells/ml)
- Milk SCC at the beginning of lactation (low <5 and high ≥5 $\log_{10}$ cells/ml)
- Milk NEU at the beginning of lactation (low <55% and high ≥55% of TLC)
- Milk fat at the beginning of lactation (low <3.6% and high ≥ 3.6%)
- Milk protein at the beginning of lactation (low <2.8% and high ≥2.8%)
- Milk SPC at the beginning of lactation (low <4 and high ≥4 $\log_{10}$ CFU/ml)
- Milk production at the beginning of lactation (low <35 and high ≥35 kg/day)
- Milk production at dry-off (low <18.5 and high ≥18.5 kg/day)
- Duration of dry period (short <57 and long ≥57 days)
- No. of days of the dry period with THI > 72 (low ≤1 and high >1)
- Bedding bacterial count (low ≤7 and high >7 $\log_{10}$ CFU/g)
- Udder hygiene score [clean (1–2) and not clean (3–4)]
- Teat bacterial contamination (low <6.2 and high ≥6.2 $\log_{10}$ CFU/swab)

For numeric variables, classes were defined based on the median value.

Results and discussion

The 212 cows had, on average, a lactation number at dry-off equal to 1.9 ± 1.1, with an average lactation length of 337.8 ± 55.9 days, and a duration of dry period of 58.9 ± 15.8 days. The average milk production at dry-off was 19.4 ± 7.0 kg/day.

Farm B had the dry cows in the poorest hygiene conditions for flanks, legs, and udder, along with significantly higher teat contamination compared to the other two farms (GLM, $p < 0.0001$; Figure 1). The average teat bacterial contamination for dry cows across

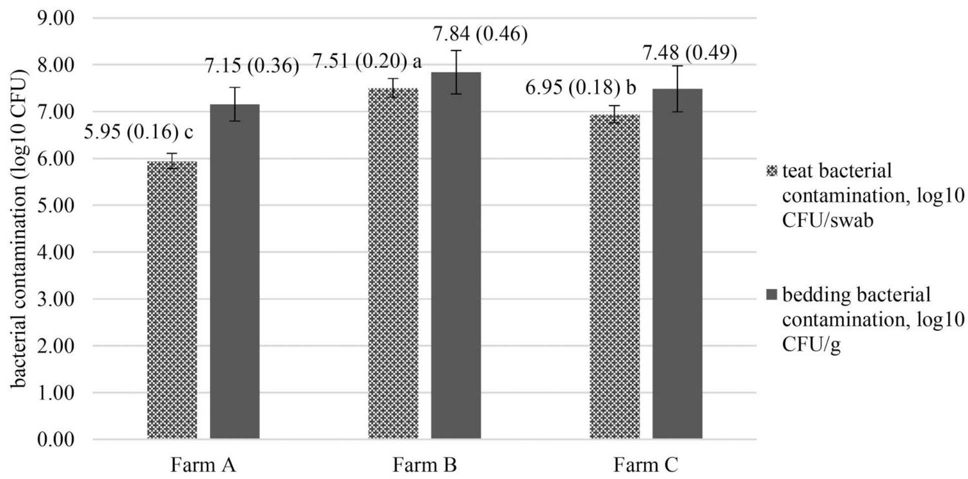

Figure 1. Teat and bedding bacterial contamination for each farm (mean with standard error). Different letters indicate statistically significant differences ($p < 0.05$).

Table 2. Temperature-humidity index (THI) during the dry periods of the 212 monitored cows for each farm.

	Farm A				Farm B				Farm C			
	Mean	*SD*	Min	Max	Mean	*SD*	Min	Max	Mean	*SD*	Min	Max
Average THI	59.4	8.17	47.3	73.3	62.8	9.80	44.7	73.6	62.0	11.0	45.4	77.9
Maximum THI	73.3	5.79	60.5	82.0	75.6	6.52	65.6	84.1	72.4	8.84	56.8	86.4
No. days with average THI >72	6.30	10.2	0	29.0	12.7	13.1	0	31.0	8.17	12.5	0	31.0
Average No. hours/day with average THI >72	2.85	5.06	0	15.0	5.61	6.42	0	15.6	5.32	6.70	0	16.2
Maximum No. hours/day with THI >72	5.90	8.29	0	23.0	10.8	10.9	0	24.0	8.79	10.3	0	24.0

all farms was 6.53 (SE 0.10) $\log_{10}$ CFU/swab while the bacterial count of bedding in dry cows' pens was 7.48 (SE 0.25) $\log_{10}$ CFU/g.

The average bacterial contamination of bedding material was higher than that reported by Patel et al. (2019) and lower than Hohmann et al. (2020). However, these studies focused on bedding for lactating cows, not dry cows.

Cows on farm B exhibited poor hygienic conditions likely due to difficulties in maintaining clean permanent straw bedding. The relationship between bedding type and cow hygiene remains unclear in the literature, partially due to the confounding effects of the resting area design (with or without cubicles) and bedding material renewal frequency (Patel et al. 2019; Robles et al. 2020).

Hohmann et al. (2020) reported that cleaner cubicles reduce teat bacterial contamination, a finding confirmed in this study: dry cows on farm B, housed on permanent straw litter with high bacterial count, showed the highest teat contamination. The average teat swab bacterial count was higher than that reported by Hohmann et al. (2020) for lactating cows. Generally lactating cow teats are cleaner than dry cow ones because of the daily sanitisation during milking and because they are generally kept on clean bedding due to the high attention of farmers for productive animals. For the same reason, the hygiene scores revealed a higher percentage of dry cows with unclean udders compared to studies on lactating cows (Sandrucci et al. 2014), particularly on farm B.

Teat swabs from 212 dry cows showed the absence of *Mycoplasma bovis* and *Streptococcus agalactiae* while 27% of swabs were positive for *Staphylococcus aureus* and 3% for *Prototheca* spp. *S. aureus* contamination was prevalent in the farms with dry cows housed in cubicles (farms A and C), while *Prototheca* spp. was more frequent in farm B with permanent litter.

Swabs positive for pathogens showed significantly higher SPC, 7.09 (SE 0.18) $\log_{10}$ CFU/swab, than negative samples, which registered an SPC value of 6.50 (SE 0.12) $\log_{10}$ CFU/swab (GLM, $p < 0.01$). The relationship between pathogen presence and high bacterial counts on teat swabs emphasises the importance of teat cleanliness during the dry period for udder health. The persistence of *S. aureus* could potentially impact the future prevalence of mastitis cases (Piccinini et al. 2009).

Table 2 presents THI data during the dry periods of the 212 monitored cows for each farm. In some cases, there have been prolonged durations of high THI values, both in terms of hours per day and the number of days, particularly in Farm B. The average THI of all

Table 3. Milk production and somatic cells of the 119 cows.

	At the end of lactation (last 30 days of lactation)		At the beginning of next lactation (first 60 days of lactation)	
	Average	*SD*	Average	*SD*
Milk production, kg/day/cow	25.5	6.6	41.2	7.9
Somatic cell count (SCC), $\log_{10}$ cells/mL	4.8	0.4	4.8	0.6
Total leucocyte count (TLC), $\log_{10}$ cells/mL	4.8	0.4	5.0	0.5
Neutrophils, % TLC	55.6	16.2	60.3	17.9
Lymphocytes, % TLC	25.9	15.8	16.9	13.0
Macrophages, % TLC	18.7	15.4	23.0	18.8

the three farms was 61.3 ± 9.63, while n. days with THI > 72 was 9.02 ± 12.1.

The study examined the correlation between teat bacterial contamination across the three farms and the THI indicators. The highest correlation was found between teat contamination and the persistence of heat stress conditions (n. days with THI > 72) during the dry period ($r = 0.51$) while the weakest correlation was observed between teat contamination and maximum THI ($r = 0.37$).

A significantly higher frequency of positive swabs for *S. aureus* and *Prototheca* spp. was noted when the number of days with THI > 72 was >1, compared to when it was ≤ 1 (Fisher's exact test $p < 0.01$).

Persistent high THI conditions promoted bacteria proliferation in bedding materials and increased teat bacterial count, as reported by Hohmann et al. (2020), facilitating the presence of *S. aureus* (Zecconi et al. 2006). Therefore, ensuring bedding cleanliness is crucial to reduce bacteria spread (Green et al. 2007), particularly under challenging environmental conditions.

Table 3 shows the data of the subgroup of 119 cows monitored from the end of the lactation to the beginning of the next one. Only internal teat sealants were applied in 50% of the cows, the other 50% was treated with both intramammary antimicrobials and teat sealant.

The average TLC value at the end of lactation was below 100,000 cells/ml, indicating good mammary conditions in the studied herds (Cobirka et al. 2020). Percentages of NEU both before dry-off and at the beginning of the subsequent lactation are much higher than that reported by Godden et al. (2017).

The results from GLM analysis showed a higher NEU percentage in the milk of cows with unclean udders during the dry period, 68.1% (SE 4.0%), compared to cows with clean udders, 60.5% (SE 2.0%) ($p = 0.07$). However, udder hygiene did not significantly affect milk SCC or TLC.

High THI values during the dry period correlated with an increase in milk SPC: mean counts were 4.8 (SE 0.08) $\log_{10}$ CFU/ml and 4.5 (SE 0.09) $\log_{10}$ CFU/ml in cows exposed and not exposed to high THI, respectively (GLM $p < 0.01$). This may be due to the persistence of high THI during milk sampling, considering the short interval (30 days) between THI evaluation and milk collection. The THI values did not show any effect on milk SCC, TLC, or leukocyte fractions.

The multiple correspondence analysis ($n = 119$) showed a close relationship between high milk production and elevated milk protein and fat content at the beginning of lactation (Figure 2). Moreover, high milk production at dry-off was associated with high milk SCC and TLC at the beginning of lactation, along with a high percentage of NEU, consistent with the findings of Vilar and Rajala-Schultz (2020), who noted an increased mastitis risk when milk production at dry-off exceeds 15 kg/day. The high NEU percentage at the beginning of lactation could potentially indicate future udder problems (Halasa and Kirkeby 2020).

The dry period length did not appear to be associated with an increase of milk SCC or single leucocyte fraction at the beginning of lactation. This suggests that extending the duration of lactation and/or shortening the dry period, to achieve lower production levels at dry-off, could not increase the risks of udder problems, in line with the findings of O'Hara et al. (2020). Additionally, a relationship was observed among high SPC, high SCC, and TLC in milk, and a high bacterial count of bedding during the dry period. High THI values during the dry period are confirmed to be associated with high bacterial contamination of teats and dirtier cows, while they do not seem to influence udder health after calving.

Conclusion

In conclusion, this study confirmed the impact of housing conditions and cleanliness of dry cows on somatic cell count and neutrophil percentage in milk at the beginning of the next lactation, suggesting implications for udder health.

Key risk factors during the dry period include bedding hygiene, udder cleanliness, and milk production at dry-off. While the direct impact of environmental conditions, such as temperature and humidity, during

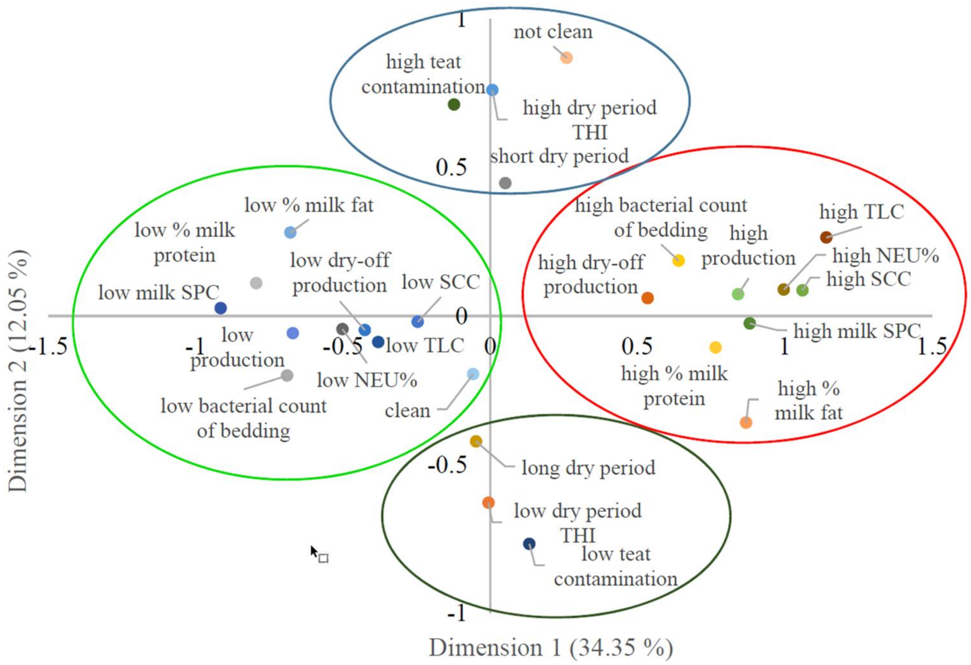

Figure 2. Multiple correspondence analysis (SCC: somatic cell count; TLC: total milk leucocyte count; NEU: neutrophils; SPC: standard plate count).

the dry period on milk somatic cell count in the subsequent lactation remains unclear, they do affect teat bacterial contamination and the presence of pathogens on teat skin.

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Ethical approval

Not required.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Data available on request from the corresponding author.

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