**Original Research Article**

# Modulation of the standing and lying behavior for managing elevated somatic cell count (SCC) and reducing the risk of subclinical mastitis in dairy cows


## Abstract

**Aim**:Toexamine the effect of feed-offertime on standing and lying behaviors to manage Somatic Cell Count (SCC) and reduce risk of subclinical mastitis (SCM) in tie stall dairy cows.

**Study design**: Completely Randomized Design (5 treatments × 4 replications)

**Place and duration of the study**: National Cow Research Program, Chitwan, Nepal; from September, 2019 to March, 2020

**Methodology**: Twenty SCM-free dairy cows were recruitedon different feeding strategies (pre-milking, during milking, and post-milking feeding at varying intervals). Behavioral patterns (lying time, bout frequencies and duration, post-milking standing duration/PMSD) were obtained using pedometer and SCC by autoanalyzer fortnightly. The feeding strategyeffect on behavioral outlinesand SCC was analyzedfor univariate ANOVAby SPSS packages (version 25). The relationship between behavioral patterns and SCC were performed by Pearsons Correlation analysis.

**Results**: Immediate post-milking feeding (+iPMF)cows showed higher lying time ($P$=.003) and bout duration ($P$=.001) while the number of lying bout was higher ($P$=.001) in pre-milking feeding (-10PMF).The PMSD was logged higher ($P$=.001; CI: 101.53- 109.50) in +iPMF than in -10PMF and during-milking feeding cows but was similar with other post-milking feeding groups.Additionally, immediate and 15-minute post-milking feeding (+15PMF) resulted significantly lower SCC at both teat ($P$=.001, .005) and udder levels ($P$=.001) and are consistently associated with longer standing time after milking, suggesting decreased risk of SCM. Furthermore, post-milking standing duration showed significant but negative relationship with SCC of fore teats ($P$=.001; r=-0.26) and at udder level ($P$=.001; r=-0.298) while it was negative and non-significant with hind teats (r=-0.09 in LH and r=-0.06 in RH).

**Conclusion**: Post-milking feeding strategies, +iPMF and +15PMF, have the potential to improve dairy cow welfare and udder health by modulating behavioral patterns and reducing SCC. Further research is needed to optimize management practices for maximizing dairy cow welfare and udder health.



Keywords: Post-milking standing time, log10 SCC, feeding strategy, udder health, lying bout


## Introduction

The udder health is a prime focus of any dairy husbandry and maintaining its health describes the success or failure of such operation instantly or in long run. Among the udder health problems, the subclinical mastitis (SCM) is the common issue faced by dairy farmers and it may start right before parturition and is fairly common at all levels of parity, although the incidence is much higher in multiparous than in primiparous animals [1,2]. Among the diagnostic tools, milk somatic cell count (SCC) is extensively used as a measure to track udder healthand the milk quality [2,3] and up to 200,000 SCC/ml milk is the index figure for evaluating the udder health [4], though it may differ from region to region according to prevalence of udder infection and the governmental regulations. Leucocytes are the dominating cells during elevated SCC (>200, 000/ml milk) and comprises of macrophages, lymphocytes and neutrophils [5]. The increase in SCC is attributed to the excess migration of neutrophils into the milk from bloodstream during inflammation and during this period, more than 90% of the cells present may consist of polymorphonuclear leukocytes. Therefore, a higher SCC indicates a greater risk of intra-mammary infection and potential contamination of milk with [6]. That's why utilizing SCC is the widely adopted screening method as a diagnostic biomarker for udder infection, and the most specific test for diagnosing SCM [7]. The somatic cells in healthy cow milk consist of macrophages (66-88%) with neutrophils (1-11%), epithelial casts and mononuclear cells but in infected quarter,neutrophil level peaksand reaches up to 90% [8].

SCM is directly related with animals' standing and lying behavior and the poor husbandry practices so understanding these behaviors and implementing effective farm management, to cutdown odds of IMI, is crucial for both economic viability and animal welfare [9,10].It has long been understood that motivating dairy animals to stand longer after milking (PMSD) reduces the risk of mastitis [11]. Research had indicated that providing fresh feed can keep cows on their feet for some period after milking and promotes longer standing time[11–13], which help close teat canals before the udder contacts the stall surface when the animal lies down, thus reducing the chance of bacterial penetration[12,14].DeVries et al. [15]reporteda decreased risk of environmental mastitis intie-stall cows lying down between 40 to 60 min post-milking compared to those lying within 40 min. In tie-stall housing, cows typically stand for an average of 79 minutes after milking, much longer than in free-stall housing, where cows often lie down relatively quickly after returning from milking [12,13,16,17]. This change in behavior in free-stall cows may be due to longer time cows spent on pre-milking preparing as well as waiting in queue for milking turn[18]. Further, longer lying time has been found during the night and early morning hours;so shorterPMSD in free-stall housed and parlor milked cows may thus be a consequence of attempting to compensate for lost lying time [19].In addition,milking three times a day may further disrupt animals' natural behavior patterns and encouraging cows to lie down quickly after milking in free-stall management. However, whether feeding time-based strategy for modulating standing and lying events, trend of SCC and the risk of SCM works or not in dairy cows is unexplored in Nepalese context. Dairying is facing a continuous challenge to maintain the quality of milk and the large part of this poor-quality milk is associated with the poor udder health and higher SCM, which might be linked to poor behavioral patterns of dairy cows. Therefore, this research was focused to explorethe feeding strategy-based modulation ofbehavioral patterns of dairy cows for managing SCC to reduce the risk of SCM in tie stall management.

## Materials and methods

### Animal selection and research design

A total of 20 SCM free HF and Jersey crossbred cows were randomly selected from the milking herd of National Cow Research Program, National Agriculture Research Council, Rampur, Chitwan, Nepal. Parity, lactation stage, initial SCC and body condition scoreof the cows were indifferent across treatments (Table 1). SCM free cows (with lower iSCC, 84- 109 × 1000 cells/ml) were selected after screening the milking herd at NCRP with Somaticell Kit (Intervet Schering Plough, Whitehouse, NJ). Somaticell is an on-farm rapid test and is conducted as detailed by [20]. The experiment was carried out from September, 2019 to March, 2020 for the period of 180 days in Completely Randomized Design with 5 treatments each with 4 replications.

-10PMF: Feed offered 10 min before milking (10 min Pre- milking feeding)
MFD: Feed offered during milking (milking during feeding)
+iPMF: Feed offered right after milking (immediate PM feeding)

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+15PMF: Feed offered 15 min after milking (15 min PM feeding)
+30PMF: Feed offered 30 min after milking(30 min PM feeding)

The study cows, hand-milked at 05.00- 07.00morning and 16.00- 17.00 evening, were managed under conventional housing system in tail-to-tail fashion with assess to free grazing for 3- 4 hours daily at day time and standard housing space was provided according to national guidelines. The study cows were managed on similar husbandry routine, feeding management and feeding materials (dry roughages, green fodders and concentrates). Commercial concentrates on the basis of production level and *ad libitum* green fodders were offered to address the nutritional requirements of the study cows.

**Standing and lying behavior**

Data Loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Pocasset, MA, USA) that records leg orientation in 1 min intervals were used for measuring the standing and lying behaviors of dairy cows. Data loggers were placed on the hind limb (1 animal/treatment at each fitting) with bandaging tape for the first 5 days following the milk sampling at a month interval. Five-day logging period is used to improve the accuracy of behavioral data, following Ito et al. [21]whodemonstratedaccurate estimate lying behavior at herd with 3 days observation period.

Lying times (hr./day), frequency of lying bout (number/day) and lying bout duration (min/bout) were calculated as described byLedgerwood et al.[22]. Finally, the PMSD, the time elapsed between the end of milking and the first instance of lying down thereafter, was worked out.

Table 1. Description of experimental crossbred dairy cow in tie-stall management at NCRP, Rampur, Chitwan

| Treatments | # of animals | # of samplings | Mean parity | Mean lactation (mo.) | Mean iSCC (× 1000cells/ml) | BCS (1 to 5) |
|---|---|---|---|---|---|---|
| -10PMF | 4 | 12 | 3.25 | 2.5 | 87 | 2.75 |
| MFD | 4 | 12 | 2.50 | 3.0 | 102 | 2.5 |
| +iPMF | 4 | 12 | 2.25 | 1.75 | 95 | 2.5 |
| +15PMF | 4 | 12 | 3.0 | 2.5 | 109 | 3.0 |
| +30PMF | 4 | 12 | 2.0 | 2.25 | 84 | 2.5 |

[a] BCS evaluated on a 5-point scale (1- emaciated to 5- grossly over fat) [23]
-10PMF: 10 min Pre- milking feeding, MFD: milking during feeding, +iPMF: immediate PM feeding, +15PMF: 15 min PM feeding and +30PMF: 30 min PM feeding, iSCC: initial somatic cell count, BCS: body condition score

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**Milk SCC analysis**

For sampling of milk, teats and udders were cleaned and wiped off with towels soaked in antiseptic solution. Then, approximately 30 ml morning milk were sampled aseptically in sterilized plasticcontainers from each quarter at fortnightly interval for 180 days. The first 2-3 streaks of foremilk were discarded before collecting milk samples. SCC was performed using Lactoscan SCC (Milkotronic Ltd., Bulgaria; www.milkotronic.com) following the standard procedures; both the absolute (×1000 cells/ml) and $\log_{10}$SCC values were determined. The udder level SCC was calculated as a mean of four quarter SCC at each sampling.

**Statistical analysis**

The effect of feeding strategies on standing and lying behavior as well as SCC at teat and udder level was worked out by univariate analysis of variancewith the help of SPSS statistical packages (version 25) and stated as mean with standard error of the mean. The relationship of different events of standing and lying behavior (lying time, number of bouts, bout duration and PMSD) obtained due the treatment effects and SCC at teat and udder level in dairy cows were performed by Pearsons Correlation analysis. Mean differences were maintained at $p \leq 0.05$.

**Results**

**Standing and lying behavior of dairy cow**

The daily standing and lying events (mean and SEM), i. e. lying time (hrs./d), lying bouts (no./d), bout duration (min./d) and post- milking standing duration (min./d), in different feeding strategies in tie stall dairy cow at Chitwan, Nepal is presented in Table 2. The daily lying time of +iPMFcowswas higher ($P$=.003) than that of -10PMF, MFD, +15PMFand +30PMFgroup but the later fours were statistically similar. Likewise, the number of lying bout per day of -10PMF was higher ($P$=.001) compared to+iPMF, +15PMF and +30PMF cows but was statistically indifferent with MFD, while MFD was observed higher ($P$=.001) than that of +iPMF and +30PMF.Bout duration per day was recorded higher ($P$=.001) in +iPMF than that of -10PMF, MFD, +15PMF and +30PMF; among the later four treatments, +30PMF was higher ($P$=.001) than -10PMF but was similar with MFD and +15PMF. Lastly, the post- milking standing duration per day was logged significantly higher ($P$=.001; CI: 101.53- 109.50) in +iPMF than that at -10PMF and MFD but was statistically similar with +15PMF and +30PMF; -10PMF and MFD too showed the significantly difference ($P$=.001).

Table 2. Standing and lying behavior (mean and SEM) of tie stall dairy cow in different feeding strategies at Chitwan, Nepal

| Factors | Lying time (hours /d) | | Lying bouts (number/d) | | Bout duration (minutes/d) | | PMSD (minutes/d) | | 95% CI PMSD |
|---|---|---|---|---|---|---|---|---|---|
| -10PMF | $11.15^{b}$ | 0.22 | $11.75^{a}$ | 0.32 | $57.97^{c}$ | 1.70 | $62.65^{c}$ | 3.18 | 58.67- 66.64 |
| MFD | $11.13^{b}$ | 0.24 | $11.44^{ab}$ | 0.28 | $58.66^{bc}$ | 1.02 | $91.06^{b}$ | 2.36 | 87.07- 95.05 |
| +iPMF | $11.86^{a}$ | 0.18 | $10.59^{c}$ | 0.25 | $68.15^{a}$ | 1.67 | $105.52^{a}$ | 1.37 | 101.53- 109.5 |
| +15PMF | $11.08^{b}$ | 0.22 | $10.75^{bc}$ | 0.28 | $62.10^{b}$ | 0.89 | $101.36^{a}$ | 1.03 | 97.37- 105.35 |
| +30PMF | $10.67^{b}$ | 0.20 | $10.30^{c}$ | 0.21 | $62.54^{b}$ | 1.33 | $100.91^{a}$ | 1.34 | 96.92- 104.90 |
| F- value | 4.14 | | 5.61 | | 8.82 | | 74.32 | | |
| P-value | 0.003 | | 0.001 | | 0.001 | | 0.001 | | |

Mean within the respective column with dissimilar superscript letters are different at $P$<0.001 and $P$<0.01; -10PMF: animals with 10 min Pre- milking feeding, MFD: Animals with milking during feeding, +iPMF: Animals with immediate post-milking feeding, +15PMF: Animals with 15 min post-milking feeding and +30PMF: Animals with 30 min post-milking feeding; PMSD: post-milking standing duration; CI: confidence interval

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**Milk SCC in dairy cow**

Milk SCC at teat- and udder-level (mean ±SE) in different feeding strategies in tie stall dairy cow at Chitwan, Nepal is presented in Table 3. The +iPMF and +15PMF group cows experienced lowerSCC (*P*=.001, .005) at left quarters compared to +30PMF, -10PMF and MFD, though SCC of +iPMFcows was non-significantly lower than -10PMF and MFD in left hind teats. In right fore quarter,the +iPMF,+15PMF and +30PMF cows had statisticallylower (*P*=.001) milk SCC but SCC of +iPMF cows was similar to MFD, however a different trend was observed in right hind quarter with significantly lower (*P*=.001) milk SCC in +iPMF, +15PMF and MFD than that at +30PMF. Again, SCC of +iPMF cows was comparable with -10PMF. At udder level, +iPMF and +15PMF cows evident significantly lower (*P*=.001) SCC than -10PMF, MFD and +30PMF animals. The +iPMF and +15PMF groups consistently showed longer standing time after milking and experienced lower SCC at both teat and udder level (Figure 1).

Table 3. Milk log10 Somatic Cell Count (SCC) at quarter and udder level(mean and SEM) in different treatments in tie stall dairy cow at Chitwan, Nepal

| Factors | LF | LH | RF | RH | p SEM | Udder level | SEM |
|---|---|---|---|---|---|---|---|
| | Log10 SCC | | | | | | |
| -10PMF | 5.26[a] | 5.10[ab] | 5.36[a] | 5.14[ab] | 0.03 | 5.38[a] | 0.03 |
| MFD | 5.22[a] | 5.13[ab] | 5.24[ab] | 4.67[c] | 0.13 | 5.40[a] | 0.05 |
| +iPMF | 4.74[b] | 4.84[bc] | 4.97[bc] | 4.93[bc] | 0.10 | 4.97[b] | 0.07 |
| +15PMF | 4.51[b] | 4.75[c] | 4.75[c] | 4.78[c] | 0.12 | 4.96[b] | 0.08 |
| +30PMF | 5.31[a] | 5.27[a] | 4.92[c] | 5.33[a] | 0.09 | 5.37[a] | 0.04 |
| F-value | 11.89 | 3.83 | 6.36 | 5.08 | | 15.54 | |
| P-value | 0.001 | 0.005 | 0.001 | 0.001 | | 0.001 | |

Mean log10 SCC within each column with dissimilar superscript letters are significantly different at$P<0.001$ and $P<0.01$; -10PMF: animals with 10 min Pre- milking feeding, MFD: Animals with milking during feeding, +iPMF: Animals with immediate post-milking feeding, +15PMF: Animals with 15 min post-milking feeding and +30PMF: Animals with 30 min post-milking feeding; LF: left fore, LH: left hind, RF: right fore and RH: right hind quarter, Psem: pooled standard error of mean

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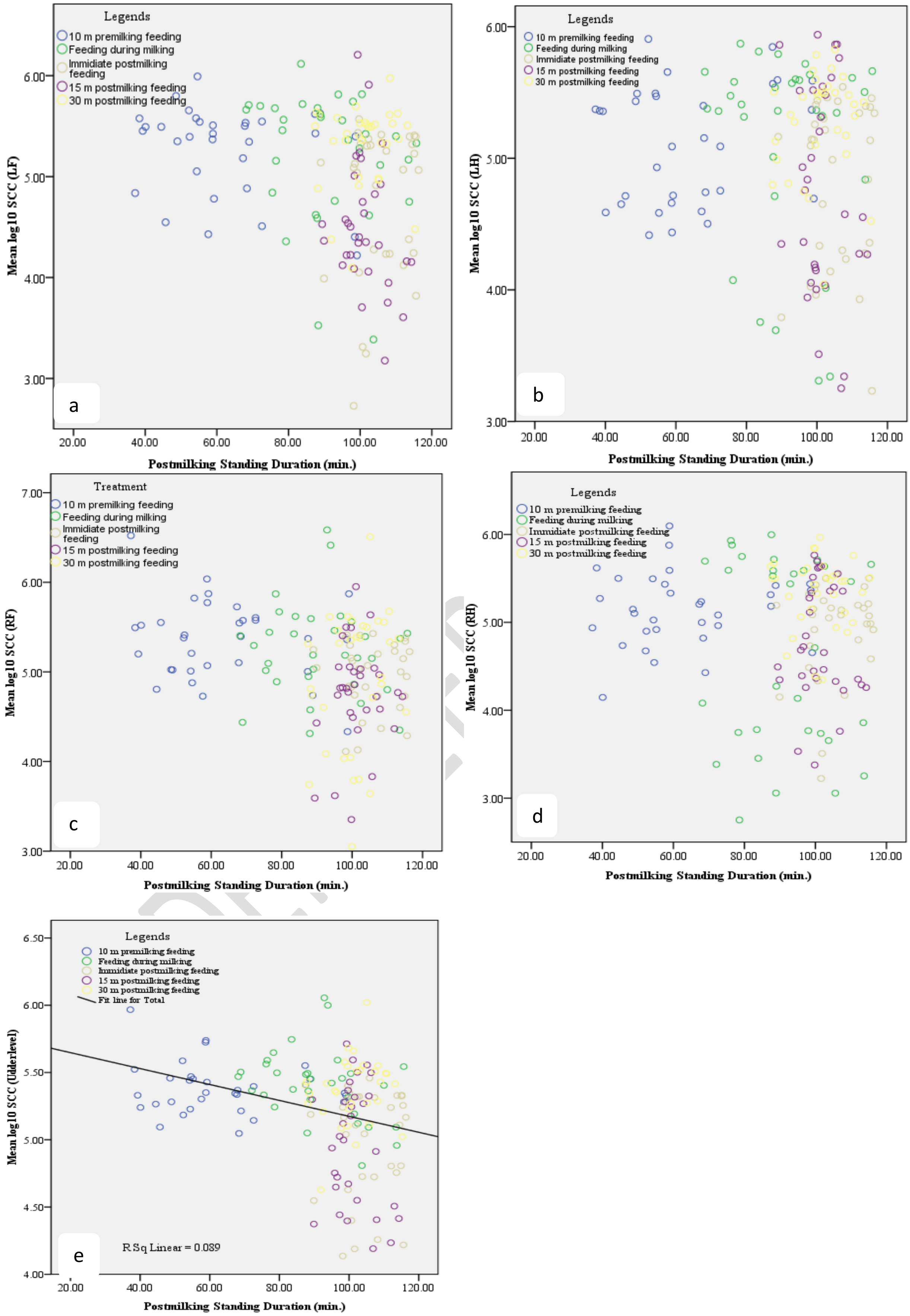
Legends
10 m premilking feeding
Feeding during milking
Immidiate postmilking feeding
15 m postmilking feeding
30 m postmilking feeding
Mean log10 SCC (LF)
Postmilking Standing Duration (min.)
a
Mean log10 SCC (LH)
b
Treatment
Mean log10 SCC (RF)
c
Mean log10 SCC (RH)
d
Fit line for Total
Mean log10 SCC (Udderlevel)
R Sq Linear = 0.089
e

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**Figure 1.** Relationship of post milking standing duration (PMSD) and mean log10 SCC in LF (a), LH (b), RF (c), RH (d) and at udder level (e) in crossbred dairy cow managed in different feeding time schedule. SCC: Somatic Cell Count, LF: left fore quarter, LH: left hind quarter, RF: right fore quarter and RH: right hind quarter

**Correlation of Standing and lying behavior and SCC in dairy cow**

The relationship of different events of standing and lying behaviorwithSCC at teat and udder level in tie stall dairy cow at Chitwan, Nepal was depicted in Table 4. Lying time had significant and positive (*P*=.001) relation with lying bouts (r=0.50) and bout duration (r=0.32) but was negative and non- significantly (*P*=.58) associated with post- milking standing duration (r=-0.05). Similarly, lying bouts showed negative and remarkable (*P*=.001) link with bout duration (r=-0.64) and post- milking standing duration (*P*=.001; r=0.30). Further, bout duration had a significant positive (*P*=0.002) correlation with post- milking standing duration (r=0.24). PMSD showed significant but negative relationship with SCC of fore teats (*P*=.001; r=-0.26) and at udder level (*P*=.001; r=-0.298) while it was negative and non-significant with hind teats (r=-0.09 in LH and r=-0.06 in RH). Finally, association of SCC of each teat was significant and positive with udder SCC.

Table 4. Correlation of different events of standing and lying behaviors with teat and udder level somatic cell count in tie stall dairy cow at Chitwan, Nepal

| | Lying Time | Lying Bouts | Bout Duration | PMSD | LF SCC | LH SCC | RF SCC | RH SCC |
|---|---|---|---|---|---|---|---|---|
| Lying Time (hrs./d) | | | | | | | | |
| Lying Bouts (no./d) | .502$^{**}$ | | | | | | | |
| Bout Duration (min./d) | .322$^{**}$ | -.641$^{**}$ | | | | | | |
| PMSD (min./d) | -.045 | -.301$^{**}$ | .238$^{**}$ | | | | | |
| LF SCC | - | - | - | -.263$^{**}$ | | | | |
| LH SCC | - | - | - | -.085 | .573$^{**}$ | | | |
| RF SCC | - | - | - | -.256$^{**}$ | .221$^{**}$ | .132 | | |
| RH SCC | - | - | - | -.056 | .161$^{*}$ | .145 | .186$^{*}$ | |
| Udder level SCC | - | - | - | -.298$^{**}$ | .725$^{**}$ | .629$^{**}$ | .520$^{**}$ | .458$^{**}$ |

| | Lying Time | Lying Bouts | Bout Duration | PMSD | LF SCC | LH SCC | RF SCC | RH SCC |
|---|---|---|---|---|---|---|---|---|
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| PMSD (min./d) | -.045 | -.301$^{**}$ | .238$^{**}$ | | | | | |
| LF SCC | - | - | - | -.263$^{**}$ | | | | |
| LH SCC | - | - | - | -.085 | .573$^{**}$ | | | |
| RF SCC | - | - | - | -.256$^{**}$ | .221$^{**}$ | .132 | | |
| RH SCC | - | - | - | -.056 | .161$^{*}$ | .145 | .186$^{*}$ | |
| Udder level SCC | - | - | - | -.298$^{**}$ | .725$^{**}$ | .629$^{**}$ | .520$^{**}$ | .458$^{**}$ |

**Significant at the 0.01 level (2-tailed) and *at the 0.05 level (2-tailed); LF: left fore, LH: left hind, RF: right fore and RH: right hind quarter, SCC: somatic cell count; PMSD: post-milking standing duration

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**Discussion**

The immediate post-milking feeding group (+iPMF) exhibited higher(*P*=.003) lying times (11.86 hrs./day) compared to other groups. This finding is in agreement with [24] and [25] who reported average lying time in dairy cows to range from 11.4 to 13.7 hrs./day. However, this study showed a longer lying duration than [26,27] who reported 10.5±2.26 and 10.9±2.26 hrs./day and a bit shorter lying time compared to the Canadian Code of Practice for Dairy Cow[28] which recommended a minimum of 12 h/d lying time for dairy cow. Similarly,Jensen e al.[29]describedat least 12–13 hrs./day lying time for heifers which is higher than the picture this work stated out.In contrast,DeVries et al.[30] described that changes in daily lying patterns of dairy cows coupled with decreased latency to lie down 20 min post- milking but without any alterations in lying duration on feeding green fodders 6 h post- milking.The +iPMFgroup showed thelowest lying bout frequencies(*P*=.001) than the other groups. This mightindicatethe more homogenous resting patterns or physiological balance in dairy cow. However, the -10PMF group showed significantly highest number of lying bouts (*P*=.001). The frequency of lying bouts in this study (10.30 to 11.75) was in comparable range to those discussed in earlierworksthat ranged between 10.7 and 11.9 bouts/day[25,31,32]. In contrast,Mattachini et al.[26] reported lower number of lying bouts (8.76±3.58) in primiparous Italian dairy cows. Therefore, immediate post-milking feeding promoted a greater comfort with pronounced physiological balance in dairy cows as evidenced by higher mean daily lying durationand expected to have better udder health with lower SCC.

The mean bout durationwas recorded to range from 57.97 to 68.15min./d (Table 2) and the +iPMF group experienced the significantly longer bout duration (*P*=.001) which could be understood as a deeper uninterrupted rest of dairy cows with a healthy udder condition yielding higher production. Moreover, the differences among +iPMF, +15PMF and +30PMF in bout duration, despite all being post-milking feeding strategies, suggest that feeding time alone may not be the only determinant. Both frequencies of lying bouts (r=0.50) and their duration (r=0.32)showed positive association (*P*=.001) with lying time and were greater in the morning compared to the evening.

The mean lying time recorded in this study were comparable and at par with [33] who reported anaverage lying time of 11.4 h/d with a frequency of 9.5 bouts/d but we observed the highernumber of lying bouts, might be associated withhigher parities of cows. In the same line,Mattachini et al.[26]described the longer lying time linked with higher parity, advanced lactation and higher BCS. Cows in their third or higher parity were observed lying down approximately 0.5 hours more daily compared to primiparous cows. Additionally, cows with a BCS 3.5 rested for about 1 hour more daily than those with a BCS 2.25[26].

Post-milking feeding strategies led to longer standing durations after milking (PMSD). The +iPMF, +15PMF and +30 PMF groups showed significantly higher PMSD (105.52, 101.36 and 100.91 min.)in comparison to pre- and during milking feeding. The delivery of fresh green fodders had promoted the cows to feed and might have stimulated the longer standing behavior after milking.Tyler et al. [34] described that the cows stood on an average for 48 min with freereach to feed after milking while standing duration was only 21 min when they did not have access to feed.Similarly,Shultz [35]reported shorter post-milking standing time in considerably more dairy cows (within 15 min) when feed was scarce after milking compared with when it was abundant.Johansson et al.[36] documented the correlation of time of feeding and lying behavior in dairy cows immediately after milking, who compared lying behavior of cows managed on three feeding strategies, i. e. 1.5 h pre-milking, right at milking, and 1.5 h post-milking feeding and found significantly lower lying episodes in cows within an hour of milking that were feed during milking. Surprisingly, cows that were fed 1.5 hours after milking spent the more time lying down during the initial hour post-milking. However, it is challenging to directly compare our results with [36] because we partitioned our cows in 5 groups based of feeding time and each group had much less time differences in terms of feed delivery.

The post-milking feeding strategies, especially the cows that were offered green fodders immediately after milking (+iPMF) and 15 min after milking (+15PMF)consistently experienced lower SCC at quarter and udder level in this study. This exciting lower milk SCC is probably associated to the post-milking feeding strategies we adopted in the study which motivated the study animals to pay more time for eating and thus resulted in longer standing durations after milking (Figure 1; $P$=.001, r=-0.26and r=-0.298 at teat and udder level in Table 4). The earlier studies well described that the presence and delivery of fresh green fodders/foragesencourage the cow's feeding behavior and promotes the longer standing time after milking[11,34,35,37–39].These findings lighting the lower SCC with immediate or 15 min post milking feeding (yielding higher PMSD) is in line withBharti and Bhakat[39] who reported that cows showing higher PMSD associated with post milking feeding practice experienced lower SCC, however they compared 15 min vs 90 min post milking feeding strategy for working out on SCC and intramammary infections. In line to the results of this study,Watters et al.[11] reported the association between standing period after milking and incidence of elevated SCC (eSCC) and stated that cows which rested more than 90 minutes after milking had a reduced chance of developing a new eSCC compared to those lying down earlier. Additionally, another study byDeVries et al.[38] found that cows standing for 40 to 60 minutes after milking were less likely to get a new intra-mammary infection. The Lower SCC observed in +iPMF and +15PMF cows in this study could be linked to the phenomenon where teat canals stay open for a period after milking. If the animals stand longer, it allows sufficient time for the teat and associated structures to undergo involution, reducing their degree of contact with the stall surface and significantly lowering the risk of infection [35].As obvious, the higher SCC in any teats in study animals posed the greater risk of increased SCC and intra-mammary infections in other teats (LH; r=0.57, RF; r=0.13- 0.22, RH; r=0.15- 0.19)

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and udder (r=0.46- 0.72) as a whole. The lower SCC in during milking feeding group (MDF) and 30 min post-milking feeding group (+30PMF) in right hind and fore teats in this study were difficult to describe. Overall, the post-milking feeding strategies, particularly +iPMF and +15PMF consistently yielded in lower SCC, likely attributed to longer standing durations after milking and decreased the risk of intramammary infections.

## Conclusion

The immediate and 15 min post-milking feeding group showed significantly higher lying times,lower number of bouts and extended bout durations compared to other groups, indicating that these feeding strategieshad promoted greater comfort with more homogenous resting patterns or physiological balance yielding substantial welfare and potentially healthier udder conditions in dairy cows. Post-milking feeding strategies also motivated the cows to lie down considerably late after milking for the first time and resulted a longer standing durations after milking compared to pre- and during-milking feeding groups. Furthermore, the post-milking feeding strategies, particularly immediate and 15-minute post-milking feeding, resulted in consistently lower somatic cell counts (SCC) at both teat and udder levels, indicating a potential reduction in the risk of IMI. Therefore, these findings underscore the importance of post-milking feeding strategies in promoting dairy cow welfare and udder health. However, further research is needed to explore the complex relationships between feeding times, lying behavior, and SCC to optimize dairy cow management.

## Ethical approval

The study did not involve approaches that challenge well-being and welfare animals; we, authors, hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

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