Research Article

Effect of Probiotic *Bacillus coagulans* on Performance and Blood Metabolites of Dairy Cows with Subclinical Mastitis

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Abstract | Subclinical mastitis is almost asymptomatic in nature, with no visible signs detected on the udder and most prevalent disease in dairy cows incuring a huge economic loss in the dairy industry. The treatment and prevention of this disease employing antibiotics are not always effective and have adverse effects in public health. The present study aims to measure the effectiveness of application of a probiotic (*Bacillus coagulans* MTCC 25250) feed additive in subclinical mastitis cows on improvement of body condition, blood parameters, milk yields and milk composition apart from mitigation of the disease. A total of 20 subclinical mastitis cows were randomly assigned into four groups with various probiotic doses comprising T1 (15 g/d/cow), T2 (30 g/d/cow), T3 (45 g/d/cow) and control (without probiotic) in a trial of 60 days. All four groups received a total mixed diet. Cows' body conditions and their bloods, milk yields and composition were investigated on the day of 0, 30 and 60. The results demonstrated that body conditions score, milk production, and compositions were improved in the T2 and T3 groups. Additionally, T2 and T3 groups showed a lower somatic cell and total bacterial counts in milk. The blood analyses showed that red blood cell, hemoglobin, lymphocyte, and monocyte counts were significantly higher for T1, T2 and T3 groups, however, white blood cell and neutrophil counts were decreased. The data suggest that application of a probiotic *Bacillus coagulans* MTCC 25250 as a feed supplement might be beneficial in milk yields and composition in subclinical mastitis cows. This potential probiotic strain would be useful for the mitigation of subclinical mastitis and improves the productivity of dairy cows.

Keywords | Probiotic, *Bacillus coagulans* MTCC 25250, Biochemical indices, Dairy industry, Blood parameters, Milk traits, Subclinical mastitis

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INTRODUCTION

Bovine mastitis is a widespread and critical problem in the dairy industry, negatively impacting the economic profitability and the well-being of dairy cattle (Cheng and Han, 2020; Kober *et al*., 2022). Mastitis is generally indicated as an inflammation of the mammary gland in either clinical or subclinical form. A devastating feature of mastitis is its recurrent nature (Jamali *et al*., 2018). The recurrent rate of mastitis is approximately 50%, causing poor milk quality, reducing milk yields, increasing the culling rate, and shrinking the animal's longevity in the farm (Wente *et al*., 2020). Mastitis is caused by various microorganisms such as bacteria, virus, protozoa, and fungi (Dalanezi *et al*., 2020). These pathogens most frequently affect the mammary gland of animals during the dry off and transition period and in the immunosuppression conditions of animals, resulting in progression of inflammation during early lactation phase (Cobirka *et al*., 2000). This infection starts in the subclinical form and can progress into clinical mastitis, which continues to the early stage of lactation (Bradley and Green, 2000). A study by Rajala-Schultz *et al*. (2011) revealed that the subclinical mastitis of animals in the dry period were progressed in to clinical mastitis in the early lactation in 50% cases.

The conventional treatment for controlling mastitis is based on antibiotic therapy (Cheng *et al*., 2008). However, suceess of the mastitis treatment relies on the pathogen specific therapy, and any irrational use of antibiotics can establish the development of antimicrobial resistance, resulting in a major threat to the well-being of cattle and public health (Dalton, 2006; Kober *et al*., 2022). In recent years, some countries have been using the vaccine for the control of bovine mastitis. However, the efficacy of vaccination is a questionable as well because multietiological microorganisms are involved to cause mastitis (Tashakkori *et al*., 2020; Urakawa *et al*., 2022). However, the low frequency of cure rate and high probability of antimicrobial resistance, and composition dependent of the target of vaccine application resulted in for exploration of an innovative and sustainable approach for treating mastitis in animals.

Recent studies suggested employment of probiotics seems to be a good choice for the treatment/control of mastitis (Shkromada *et al*., 2022; Spaniol *et al*., 2015). *Bacillus coagulans*, a gram-positive, spore-forming, lactic acid-producing bacteria, has been proved as a probiotic (Özüsağlam, 2010). After the administration, of the *Bacillus coagulans*, it can survive in gastrointestinal conditions and exert health benefits on the host. As a result, *Bacillus coagulans* have been widely used as a probiotic feed in animal husbandry worldwide (Zhou *et al*., 2020). Literature showed that supplementation of *Bacillus coagulans* can improve growth performance and reduce diarrhea in piglets (Zhang *et al*., 2018). Additionally, *Bacillus coagulans* exhibits a growth enhancing effect in broiler and in aquatics. e.g. shrimp (Zhou *et al*., 2020). However, so far, very limited numer of researches have been conducted to evaluate the effect of *Bacillus coagulans* on dairy farming. Thus, the present study was undertaken to evaluate the effects of *Bacillus coagulans* MTCC 25250 on the body reserves, blood metabolites, yield, and milk composition of cows with subclinical mastitis.

MATERIALS AND METHODS

The care and treatment of the experimental animals were performed by the guidelines and regulations of Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh. The animal experiment protocol was approved by the ethics committee, Sylhet Agricultural University, Bangladesh (SAU/Ethical committee/AUP/22/19).

Farms and animals

A positive test using the California Mastitis Test (CMT; DeLaval, Tumba, Sweden) to detect subclinical mastitis was used as the criteria to select cows for their the mastitis history during the previous lactation period. A total of 73 milking cows were present in a dairy farm located in the Sylhet region of Bangladesh. Machine milking was performed twice a day (08:00 h and 17:00 h). All milking cows were tested for California Mastitis test. Twenty four cows were showed positive results in California Mastitis test. We considered twenty (20) multiparous (2-4 lactations) crossbred cows (Local × Holstein) for this study, and the rest four cows were excluded due to different crossbred cows. The cows in this experiment had an average parity of 2.9 and days in milk (DIM) were 135 days, respectively. All animals were randomly allocated to four (4) treatment groups, and 5 cows were included in each treatment. Four dietary treatments were used in this trial and the description of the diet is shown as:

1. Control: Animals were fed a total mixed ration without probiotic supplementation;
2. T1: Animals were fed a total mixed ration with 15 g/d/cow probiotic supplementation;
3. T2: Animals were fed a total mixed ration with 30 g/d/cow probiotic supplementation;
4. T3: Animals were fed a total mixed ration with 45 g/d/cow probiotic supplementation. Probiotics (*Bacilllus coagulans* MTCC 25250 6.0 × 10^9 cfu/g) was supplied by the Square Pharmaceuticals, PLC., Bangladesh. The cows were fed adlibitum access to feed and water at 7.30 and 16.00 hours daily. Total mixed rations were prepared by mixing of concentrate mixture (38%), green grass (20.5%), and rice straw (41.5%). Diets were

prepared according to the NRC recommendations (NRC, 2001). The cows were housed in a tie-stall barn and remained in an identical manner through out the entire experimental period.

Sample collection and laboratory analysis

The total study period was 67 days with 7 days of the adjustment period. The following data were recorded, and sample collection was performed three times (start, 0 day; mid, 30 day; and end, 60 day) during the entire experiment period.

- Individual daily milk yields (MY, kg/d).
- Milk samples (100 ml per cow) were collected from individual cows at the evening milking and immediately transferred to the laboratory of Dairy Science, Sylhet Agricultural University, Bangladesh, and refrigerated at 4° C without preservative.
- Live body weight (BW), were measured using digital cattle weighing scale (Model: NVK-SCS-A12, NVK Electric Weighing Scale, China).
- Body Condition score (BCS), were measured by an experienced operator on the same day of milk sample collection based on the method described by Edmonson *et al.* (1989), using a 1 to 5 scale with 0.25 increments.

Detection of subclinical mastitis using California mastitis test

Milk samples were tested with the California Mastitis Test for showing the presence of subclinical mastitis, using the techniques described by Dingwell *et al.* (2003). This test was performed twice, at the start (0 d) and end (60 d) points.

Milk quality parameters

Milk samples were analysed to investigate the composition (fat, protein, lactose, and total solids) using an ultrasonic milk analyzer (MT-25, Wincom company Ltd, China). The somatic cell count (SCC) was determined using Eko-milk Scan (Somatic Cell Analyzer, Bulgaria) and the data were transformed into the logarithmic somatic cell score (SCS), according to Ali and Shook (1980) method.

Microbial analysis of milk samples

The standard plate count (SPC) and Coliform counts were determined in the collected milk samples, on the same test day of milk quality traits using the method of American Public Health Association (Saha and Ara, 2012), The plate count agar for SPC and EMB agar for the Coliform count were used and the methods described by Saha *et al.* (2022) were utilized for this microbial study.

Blood collection and analysis

Blood samples (50 ml) were collected at the start (0 days) and end (60 days) date from the jugular vein of cows into tubes containing sodium heparin. To determine blood parameters, blood samples were quickly shifted to the laboratory of Physiology, Sylhet Agricultural University, Bangladesh. Red blood cells (RBC) and White blood cells (WBC) were counted using Neubauer haemocytometer method. The Microhaematocrit technique were used to determine the packed cell volume according to Islam *et al.* (2014). Differential leukocyte test was performed using thin blood films stained with Giemsa stain by counting 100 white cells from each slide, and the relative abundance in percent of each white cell type was calculated.

Statistical analysis

The somatic cell score was calculated as SCS= $(\log)_2$ (SCC/100000) + 3. The statistical analyses were conducted using R software (R Core Team, 2016). Data were analyzed using a linear mixed model using the following equation:

$$y_{ijklm} = \mu + \beta_{1i} DIM_i + Parity_j + Period_k \times Group_l + Animal_m + \varepsilon_{ijklm}$$

Where y_{ijkl} is the response trait (BW, BCS, MY, Fat, Protein, Lactose, Total solids, Fat: Protein, SCC, SCS, SPC, Coliform, WBC, RBC, Hb, PCV, Neutrophil, Eosinophil, Lympocyte, Monocyte); μ is the general mean; β_{1i} is the partial regression coefficient on DIM_i; $Parity_i$ is the effect of parity number *j(j= 2,3,4)*; $(\text{Period})_k \times (\text{Group})_l$ is the effect of the interaction between the Period *k(k=start, middle, end)* and the Group *j(j=C, T1, T2, T3)*; $Animal_m$ is the random effect of the animal; and $\varepsilon_{ijklm} \sim NID(0,\sigma_e^2)$ is the residual. An analysis of variance of the linear mixed model was performed, and then multiple comparisons of means were made using a Tukey test. In addition, the estimated marginal means of the periods and groups were calculated, and their 95% confidence intervals were obtained for the data analysis.

RESULTS

Table 1 present the descriptive statistics for body weight (BW), body condition score (BCS), blood parameters, yields, traits, and microbial parameters of milk produced from treatment group cows during the experimental period. On average, the BW and BCS of treatment group animals were 420 kg and 3.17, respectively. The average milk yields was 9.6 kg/d, containing 4.18% fat, 3.57% protein, 4.97% lactose, and 13.0% total solids. The mean value of SCS was 5.06. The average amount of standard plate count (SPC) and coliform counts were 94.03 × 10^3 CFU/ml and 30.17 CFU/ml, respectively. Results from blood samples indicated that the mean value of White blood cell (WBC), Red blood cell (RBC), and hemoglobin (Hb) were 161.8 × 10^9/l, 6.26× 10^{12}/l, and 11.26 g/100 ml, respectively. The observed packed cell volume (PCV), neutrophil, eosinophil, lymphocyte, and monocyte were

23%, 35.98%, 0%, 33.39%, and 4.29%, respectively.

Table 1: Descriptive statistics for body weight (BW), body condition score (BCS), blood parameters, yield, traits, and microbial parameters of milk.

Traits[1]	n	Mean	SD	Median	Minimum	Maximum
BW, Kg	60	420	71.75	438	260	521
BCS	60	3.17	0.21	3.25	2.75	3.75
MY, Kg	60	9.57	2.11	9.45	5.6	15.5
Fat, %	60	4.18	0.47	4.12	3.47	5.51
Protein, %	60	3.57	0.22	3.58	3.03	4.08
Lactose, %	60	4.97	0.22	4.96	4.51	5.59
Total solids, %	60	13	0.37	13	12.23	14.21
Fat:Protein	60	1.17	0.14	1.19	0.91	1.46
SCC thous./ml	60	442.4	137.1	473	78	661
SCS	60	5.05	0.58	5.24	2.64	5.72
SPC, CFU/ml × 10^3	60	94.03	165.6	93.4	66.7	123.1
Coliform, CFU/ml	60	30.17	10.7	28.5	12	69
WBC, 10^9/L	60	161.76	28.28	156.7	121.6	231.9
RBC, 10^{12}/L	60	6.26	0.73	6.18	5.11	7.69
Hb, g/100ML	60	11.26	1.67	11.75	6.75	13.4
PCV, %	60	23	4.0	23	17	40
Neutrophil, %	60	35.98	11.19	36.17	18.75	57.31
Eosonophil, %	60	0	0	0	0	0
Lympocyte, %	60	33.39	8.15	33.33	17.67	47.29
Monocyte, %	60	4.29	0.82	4	3.23	6.21

[1]BW, Body weight; BCS, Body condition score; MY, Milk yield; WBC, White blood cell; RBC, Red blood cell; Hb, Hemoglobin; PCV, Packed cell volume; SPC, Standard plate count; SCC, Somatic cell count; SCS, Somatic cell score [SCS = 3 + log2 (SCC/100,000)].

The sources of variation in the statistical model are presented in Table 2. Days in milk (DIM) and parity exerted a smaller effect than the tested period, group, and period × group. DIM affected the milk-related traits (fat, fat: protein, SCS, and SPC), while no effects were found on blood parameters except RBC. Similarly, parity effect was significant for milk-related traits (fat, fat: protein, SCS, Total solids, and SPC), whereas a little effect observed on blood parameters (RBC, WBC, and hemoglobin). The variation included period, group, and period × group affected most of the studied traits are displayed in Table 2.

Effect of probiotic feeding on body and milk traits

The addition of *Bacillus coagulans* showed no effect on BW (Figure 1). On the other hand, the groups had received probiotic showed apparent beneficial effect on BCS and it is more pronounced for T2 and T3 groups. There was a trend towards increasing for the T2 group ($p < 0.05$) after 30 days of supplement and was better prominent after 60 days. The T3 group had little effect on BCS but did not significant different from the control and other treatment groups.

Table 2: Results from ANOVA (F-value and significance) for body weight (BW), body condition score (BCS), blood parameters, yield, traits, and microbial parameters of milk.

Traits[1]	DIM[2]	Parity	Period	Group	Period × Group
BW, Kg	0.20	4.04 *	0.00	0.04	0.02
BCS	1.36	2.50	0.46	3.98 *	4.57 **
MY, Kg	9.48 **	5.44 **	25.83***	2.78	0.95
Fat, %	5.25 *	12.66***	3.94 *	1.21	1.72
Protein, %	0.00	2.09	2.27	12.04***	2.71 *
Lactose, %	006	0.50	4.66 *	1.28	2.48 *
Total solids, %	1.23	2.75 †	1.46	1.81	1.76
Fat:Protein	3.06 †	7.78 **	5.57 **	1.84	0.61
SCC	3.01 †	0.89	41.21***	20.89***	6.22 ***
SCS	5.11†	1.72	29.9***	12.77***	6.48***
SPC, CFU/ml × 10^3	15.07***	14.99***	95.34***	40.39***	18.57***
Coliform, CFU/ml	0.16	1.36	14.67***	1.16	2.44 *
WBC, 10^9/L	0.4	5.83 **	2.58 †	18.61***	6.18 ***
RBC, 10^{12}/L	8.65 **	10.89***	22.20***	19.51***	6.04 ***
Hb, g/100ML	0.35	4.88 *	4.29 *	11.79***	3.51 **
PCV, %	1.07	1.45	0.15	2.26 †	0.78
Neutrophil, %	0.09	0.83	13.63***	12.81***	2.67 *
Eosonophil, %	1.60	1.93	2.56 †	2.30 †	2.56 *
Lympocyte, %	0.27	2.88 †	2.02	28.16***	8.18 ***
Monocyte, %	2.82	1.02	21.93***	8.65 ***	3.78 **

[1]BW, Body weight; BCS, Body condition score; MY, Milk yield; WBC, White blood cell; RBC, Red blood cell; Hb, Hemoglobin; PCV, Packed cell volume; SPC, Standard plate count. [2]DIM: Days in milk. Significant codes: †= 0.05; *= 0.01; **= 0.001; ***= 0; SCC, Somatic cell count; SCS, Somatic cell score [SCS = 3 + log2 (SCC/100,000)].

As expected, milk yield was decreased over the experimental period due to the advancement of lactation, whereas the decreasing rate was lower for the T3 group than other groups and indicated a significant difference ($p < 0.05$) after 60 days of supplementation (Figure 1). Fat percentage in milk did not differ among treatment groups, but protein percentage in milk was higher for T2 and T3 groups at the end of 60 days of supplementation. The supplementation of probiotic did not influence the other milk components (lactose, total solids, and fat: protein). Somatic cells are considered one of the major defense components of mammary gland found in milk. Our findings showed that supplementation of probiotic reduced the SCC on day 30 of this study ($p<0.05$)

and showed more pronounced reduction on day 60 (Figure 1). Regarding microbiological characteristics, probiotic supplementations decreased the total bacterial count in milk compared to the control (Figure 2). There was a decrease of bacterial count observed after 30 days of usage and more noticeable after 60 days of probitic usage.

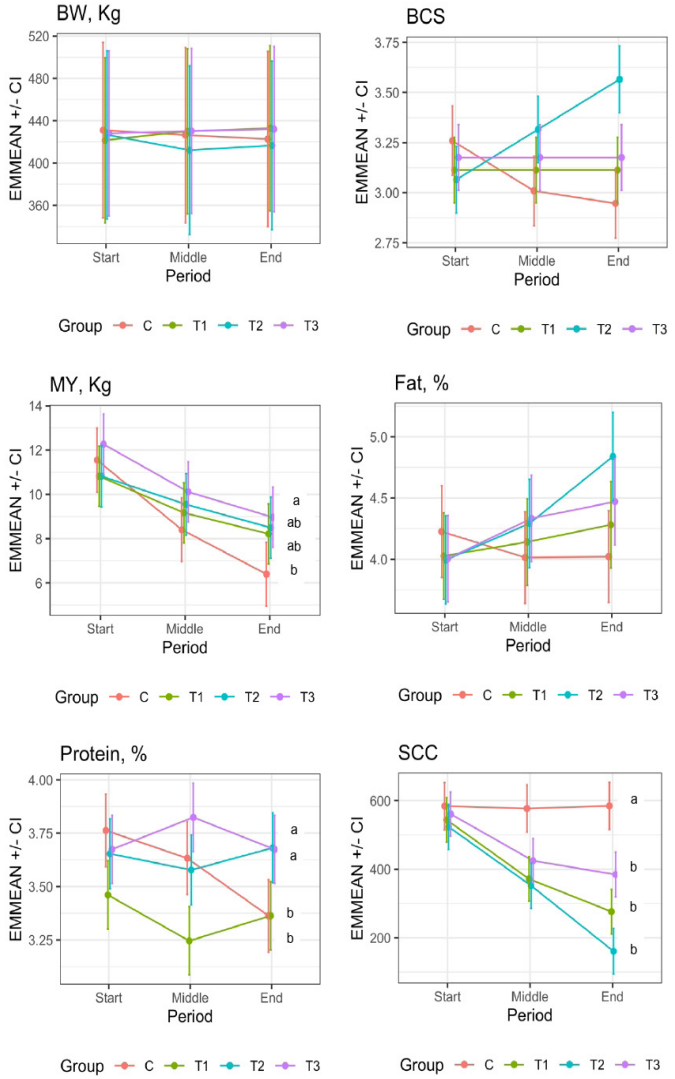

Figure 1: Least square mean ± standard error for body weight, body condition score (BCS), and milk traits (milk yield, fat, protein, somatic cell count) of cows with subclinical mastitis from the control and *B. coagulans* supplement groups.

Effect of probiotic feeding on blood metbolites

The results of blood metabolites are presented in Figure 3. Our study reveals a significant improvement of RBC and Hb in blood by adding probiotics in the diet. There was a tendency to increase RBC and Hb after 30 days of spplementation, as wll as the increasing trend was observed after 60 days of the study. However, the probiotic suplement groups showed decrease in WBC counts after 60 days of the trial. In the context of differential WBC count parameters (neutrophil, lymphocyte, and monocyte), a significant decrease in neutrophil count was observed after 60 days of supplementation for all probiotic-treated groups, whereas lymphocyte and monocyte were increased significantly in probiotic treated groups, which highlights that probiotic supplement improves the immune system. Thus, probiotic supplement in the subclinical mastitis of cow diet can be considered as a critical component for mitigating the mastitis.

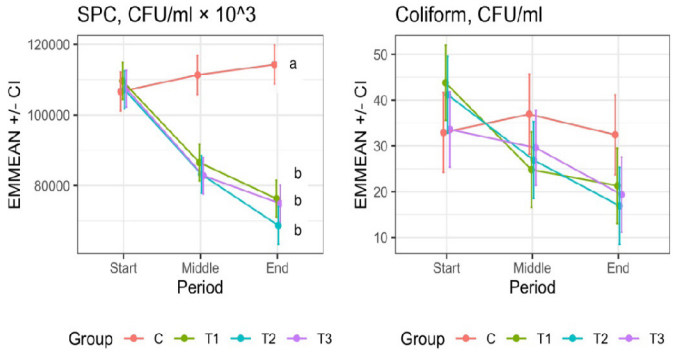

Figure 2: Least square mean ± standard error for microbial quality of milk (SPC: standard plate count; CFC: coliform count) from cows with subclinical mastitis from the control and *B. coagulans* supplement groups.

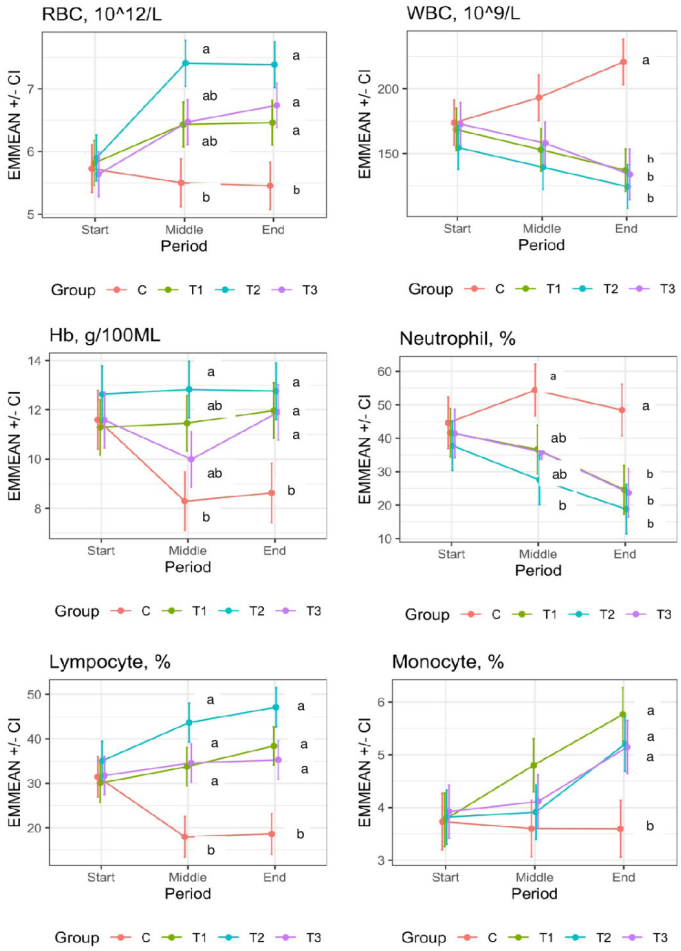

Figure 3: Least square mean ± standard error for blood metabolites (RBC: Red blood cell; WBC: White blood cell; Hb: Hemoglobin; Neutrophil; Lympocyte; Monocyte) of cows with subclinical mastitis from the control and *B. coagulans* supplement groups.

DISCUSSION

Dairy cattle health, milk yields, and quality are the significant factors for sustainable and profitable dairy farming. During the last two decades, several studies have focused on improving the cattle health, milk quality, and disease control by adding probiotics to the dairy ration (Lambo *et al*., 2021). However, studies related with the efficacy of probiotic against subclinical mastitis in cow are scarce. In the present study showed probiotic supplementation particularly use of *Bacillus coagulans* had a positive effect on the body condition score and similar to our study Fredebeul-Krein (2022) found that supplementation of some *Bacillus* spp. can improve the body condition, and assist in reducing the clinical mastitis in dairy cows. In addition, some researchers have observed improved body condition of dairy cows that have received *Bacillus* spp. as a single or multispecies probiotic (Choonkham *et al*., 2021; Merati and Tawhidi, 2022). Dietary probiotic supplement may help improve animal health status by improving nutrient digestion and absorption through the gastrointestinal tract (Mahesh *et al*., 2021).

The probiotic supplement has notable effects on daily milk yields and composition (Merati and Tawhidi, 2022). In the current study, with the advancement of lactation, the reduction of daily milk yields was as expected, but reduced the decreased rate for T3 probiotic supplement group as compared to the control. Souza *et al*. (2017) corroborated a positive response to milk yields treated with *Bacillus subtilis* spores. In order to illucidate this beneficial effect of probiotics on milk production, Spaniol *et al*. (2015) reported that probiotics may influence milk yields by improving the digestion, preventing ruminal acidosis, boosting the immune system and reducing the somatic cell count. Therefore, the supplementation with probiotics similarly influencing the protein content of milk from T2 and T3 group cows after 60 days of the study. On the other hand, the probiotic supplementation did not influence the fat and lactose percentage in milk. Like our study, Souza *et al*. (2017) reported that *B. subtilis* spores supplementation affected the protein metabolism in the rumen resulted an increased protein content in milk, whereas fat and lactose contents were not altered. Several literatures suggest that ruminal ammonia accumulation increased by the supplementation of *B. subtilis* in the diet which may contribute to the increase of protein content in milk (Qiao *et al*., 2010; Peng *et al*., 2012).

Generally, milk SCC (SCS) was increased in subclinical mastitis cows. This higher SCC not only detrimental to udder but also produces severe affects in the quality of raw milk which are prepared with the high SCC raw milk by enhanced protease and lipase enzyme concentrations, which are highly heat resistant and had a detrimental effect in the processing of milk and other milk products (Barbano *et al*., 2006). Milk SCC (SCS) was decreased in the current study by probiotic supplementation which indicating a beneficial effect. Sun *et al*. (2013) reported that supplementation of *Bacillus subtili* in natto reduced SCC in milk, but a crystal clear mechanism remained unclear, and their observation was supported by our findings.

The present study showed that the feeding of probiotic decreased the total bacterial count in milk, but coliform counts were comparable. There is no sufficient evidence in the literature on *Bacillus* spp. supplementation lowering the bacterial counts in milk in in subclinical studies. In the current investigation, a possible explanation of reduction numer of bacterial count in milk withthe probiotic suplement could be include the inhibitory effects of probiotics on the growth of pathogenic bacteria. The probiotics have exerted the inhibitory effects by the production of acetic, lactic and other acids, bacteriocin, nisin and other antimicrobial compounds for destroying pathogenic and spoilage bacteria present in raw milk.

In general, the health status of the animals could be reflected by blood parameters. The supplementation of probiotic may have the capacity to act on the blood parameters of animal (Choonkham *et al*., 2021). In this study, probiotic supplementation showed the increament of Hb concentration and RBC count, similar results were obtained by Kabir *et al*. (2022) and Ghazanfar *et al*. (2015) in fattening cattle and dairy heifers fed probiotic-supplemented diet, respectively. The probable reasons of elevated Hb concentration and RBC count in blood is that supplementation of probiotics can increase the absorption of iron salt and vitamins B from the small intestine which exerted a positive effect in the blood cells formation process (Ghazanfar *et al*., 2015). On the otherhand, control group recorded a higher leukocyte (WBC) count compared to probiotic supplement groups, however the presence of normal range of WBC in the blood reflecting absence of negative effects of immunity by WBC which also ensuring providing the regular immunity by WBC. Sarvesha *et al*. (2017) reported that circulating leukocytes content were increased in the blood and its value reached above the reference value in cows with clinical and subclinical mastitis. In this investigation, a decreased trend for the neutrophils counts was observed for probiotic supplementation groups. Neutrophils are the first cells to act as a defense against any inflammation in the body, and the high neutrophil count is an indication of inflammation. A decrease in blood neutrophils in probiotic supplemented group may be indicated by the diminished mastitis inflammation in the animals which enhancing the beneficiary list. Additionally, a probiotic supplementation causes an increased in the lymphocyte and monocyte counts in blood, which might indicate gut microbiota stimulating intestinal immune

system responses. Our findings corroborated with Mousa *et al.* (2019) results, who reported that supplement of *Bacillus* spp. to lamb significantly improved leukocytes and monocytes counts in the blood. Elevated lymphocyte and monocyte counts can play an essential role by improving body immune system with eventual destroying invading disease-producing agents.

CONCLUSIONS

In conclusion, our study reveals that addition of a probiotic (*Bacillus coagulans* MTCC 25250) in the diet of dairy cows had positive effects to mitigate subclinical mastitis in cows, influencing body condition score, daily milk yields, protein percentage and decrease the SCC and bacterial count in milk by the adding of 30 and 45 g of probiotic per day per cow. Moreover, blood parameters such as RBC, Hb, lymphocyte, and monocyte contents were improved in cows fed probiotics causing changes in local and systemic immunity for alleviating subclinical mastitis. The present study demonestrated that the probiotic (*Bacillus coagulans* MTCC 25250) supplementation to the diet can be a new alternative prophylaxis for control of subclinical mastitis in cow. Further studies with larger sample size and an establisment of mechanistic approach of probiotics will be important aspects in order to control subclinical mastitis precisely.

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NOVELTY STATEMENT

This is a novel study for evaluating the dietary supplement of probiotic Bacillus coagulans MTCC 25250 against subclinical mastitis in dairy cows. The research findings highlighted that application of Bacillus coagulans MTCC 25250 strain can be a new alternative to mitigate subclinical mastitis in dairy cows.

AUTHOR'S CONTRIBUTION

Conceptualization: SS, JPR and HK. Methodology: SS, AKM, MIA. Software: SS, MD, DA. Validation: SS, JPR, JDG, HK. Formal analysis: HOTA, SS, JPR, SSUA. Investigation: JDG, HK. Resources: HK, SS. Data curation; SS, AKM. Writing original draft preparation: SS. Writing review and editing: SS, JPR, MIA, MD, SSUA, HK. Visualization: MD, AKM, DA. Supervision: HK, JDG, SS. Project administration: SS, HK. Funding acquisition: HK, JPR, SS. Scientific corrections and editing for English: ND. All authors have read and agreed to the published version of the manuscript.

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Data availability

Data may be shared upon reasonable request to the corresponding author.

Conflicts of interest

The authors have declared no conflict of interest.

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