

RESEARCH ARTICLE

Effect of LAB consortia-based feeding and teat dipping on blood profile, antioxidant activity, and total immunoglobulin in Sahiwal cows having subclinical mastitis

Nutan Chauhan, Sachin Kumar (✉), Sravani Balaga¹, Ojal Singh, Sumit Kundgir and Ashis Kumar Samanta

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Abstract: The current study of sixty days duration investigates the effects of a probiotic based feeding and teat dipping on blood profile, antioxidant levels, and serum immunoglobulin status in *Sahiwal* cows suffering from subclinical mastitis. Twenty-four mid-lactating *Sahiwal* cows with similar parity and days in milk were selected and randomly assigned to four treatment groups based on milk somatic cell count (SCC) as follows: Group I (H): healthy cows (milk SCC <200,000 cells/mL) fed basal diet + 50 g uninoculated naturally fermented soybean meal (UFSBM); Group II (M): cows with SCM (milk SCC between 200,000-500,000 cells/mL) fed basal diet + 50 g UFSBM; Group III (PRO): cows with SCM fed basal diet + 50 g inoculated and fermented with LAB consortia (@ 1.5×10^9 CFU/g) soybean meal (IFSBM); Group IV (PRO+T): cows with SCM fed basal diet + IFSBM + teat dipping. Teat dipping was performed daily after evening milking at a dose rate of 8.94 to $8.77 \log^{10}$ CFU/ml. Results indicated no significant differences in dry matter intake (DMI) and body weight across treatment groups. However, haemoglobin (Hb) levels showed significant variation, with the healthy group exhibiting the highest Hb concentrations. Total leukocyte count (TLC) was significantly ($p < 0.05$) elevated in the “M” group as compared to animals under other groups, while lymphocyte counts were highest in the “H” group and lowest in the “M” group. The SOD and catalase enzyme activity were significantly highest ($P < 0.05$) in the “H”

group, followed by PRO+T, PRO and lowest in the “M” group. The blood serum immunoglobulins in the PRO and PRO+T groups were significantly lower than the “M” group ($P < 0.05$). The study substantiates that probiotics can positively influence certain haematological and biochemical parameters in cows with subclinical mastitis, suggesting potential benefits for improving overall health in dairy cattle.

Keywords: Subclinical mastitis, Probiotics, Haemato biochemical profile, *Sahiwal* cattle

Introduction

Subclinical mastitis (SCM) is a non-symptomatic form of intramammary inflammation that affects 20–50% of cows in a herd and significantly affects both milk production and the overall health of dairy animals, posing a serious challenge for dairy farmers. The incidence of SCM can result in significant financial losses due to reduced milk production and lowering of quality (Yang et al. 2011). Although, the SCM frequently remains undiagnosed in animals with subclinical mastitis, it could seriously impair the immune system and general health of the animals. The condition disrupts the blood-milk barrier and impairs production and secretion from udder epithelial cells, resulting in alterations to both blood biochemistry and milk compositions. This is largely due to the release of harmful toxins from the udder during mastitis (Krishnappa et al. 2016). The current trend in the diagnosis of subclinical mastitis aims to find precise blood biomarkers of the inflammatory conditions in the mammary tissue (Benic et al. 2018) with the goal of developing rapid and accurate diagnostic methods.

Probiotics have emerged as a promising alternative to conventional antibiotics for the management and treatment of mastitis. These beneficial microorganisms have the potential to reduce the incidence of mastitis in dairy cows by improving gut health, boosting antioxidant levels, and supporting the immune system (Rainard and Foucras, 2018). Certain novel probiotic strains from the lactic acid bacteria (LAB) group have the potential to prevent bovine mastitis (Chauhan et al. 2024). These probiotics can improve the health of cows with mastitis by positively affecting various haematological and biochemical

Rumen Biotechnology Lab, Animal Nutrition Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India

Sachin Kumar (✉)
Email: arensachin@gmail.com
Tel: +91-1842259069

indicators. LAB have been shown to minimise the incidence of mastitis and sustain lower somatic cell counts in milk, indicating improved udder health and less inflammatory responses (Singh et al. 2014).

The effects of probiotics in preventing mastitis have been recently established by several workers, both through supplementation in animals' diet and by applying them as teat dips. However, to the best of our knowledge, there has been no study combining both approaches, i.e. teat dipping and feeding probiotics, simultaneously. Based on this observation, we hypothesized that this dual approach might have a more significant impact. Keeping in view the above perspectives, the research was aimed to assess the effect of LAB consortia-based feeding and teat dipping on the blood profile, antioxidant activity and total immunoglobulin in *Sahiwal* cows with subclinical mastitis.

Materials and Methods

Ethical approval

All procedures performed in study involving animals were in accordance with the ethical standards of the Institutional Animal Ethics Committee (IAEC) of ICAR-National Dairy Research Institute, Karnal, Haryana, India, under Reg No. 1705/GO/Re/SL/13/CCSEA; dated 24/08/2023.

Selection of probiotics and preparation of consortia

A total of 23 bacterial strains were previously isolated from the milk samples of healthy *Sahiwal* cows and characterized for probiotic attributes (Chauhan et al. 2024). Out of these, the most promising six probiotic LAB isolates (*Lactobacillus helveticus* SML41, *Lactobacillus helveticus* SML60, *Weissella confusa* SML61, *Lactocaseibacillus rhamnosus* SML63, *Weissella confusa* SML64, and *Pediococcus acidilactici* SML76) were taken and a consortium was prepared. This bacterial consortium was used for oral feeding as well as teat dipping in *Sahiwal* cows with subclinical mastitis. The teat dip was prepared at a concentration of 8.94 to 8.77 log¹⁰ CFU/mL, while the dietary probiotic was given as fermented feed at a rate of 50 g per animal per day at a dose rate of 1.5 x 10⁹ CFU/g for 60 days.

Experimental design, selection of animals, and feeding management

An experiment of 60 days duration was conducted at the Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal, India. Twenty-four mid-lactating *Sahiwal* cows with similar parity and days in milk were selected and randomly assigned to four treatment groups based on milk somatic cell count (SCC) as follows: Group I (H): healthy cows (milk SCC <200,000 cells/mL) fed basal diet + 50 g uninoculated naturally fermented soybean meal (UFSBM); Group II (M): cows with SCM (milk SCC between 200,000-500,000 cells/mL) fed basal diet + 50 g UFSBM; Group III (PRO): cows with SCM fed basal diet + 50 g

inoculated and fermented with LAB consortia soybean meal (IFSBM); Group IV (PRO+T): cows with SCM fed basal diet + IFSBM + teat dipping. Teat dipping was performed daily after evening milking. The basal diet comprising concentrate and roughage (Hybrid Napier) at 10:00 AM and 5:00 PM daily was provided to all the animals. The nutritional composition of concentrate and Hybrid Napier is presented in Supplementary Table 1.

Body weight changes and dry matter intake

The animals were weighed before feeding and watering in the morning for two consecutive days at the start of experimental feeding and thereafter at fortnightly intervals during the experimental period of 60 days. Voluntary intake of feed was recorded daily for individual animals by recording the weight of feeds offered and residue left. The dry matter (DM) of different feed ingredients and residue was estimated and the DM intake was calculated by subtracting the residual DM from the quantity of DM offered.

Blood biochemical indices

About 10 mL blood samples were collected in sterile heparinized vacutainer (BD Vacutainer TM, UK) tubes from jugular vein puncture on the 0th, 30th, and 60th day of the trial from the experimental animals. Immediately after collection, tubes were kept in an ice box and transported to the laboratory for further processing. General blood parameters viz. red blood cells (RBC) count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count were estimated following standard protocol by auto-hematology cell counter (Nihon kodhen, celltaca, Tokyo, Japan). For the estimation of serum biochemical parameters, blood samples were centrifuged at 3000 rpm for 15 minutes at 4°C, and serum was harvested for the estimation of various serum biochemical parameters. Estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, and globulin was performed using commercial kit (Recombigen Laboratories Pvt. Ltd., New Delhi). The estimation of calcium and phosphorus levels in the serum were performed using an auto-analyzer after following the standard protocol.

Estimation of antioxidant activity

For the estimation of antioxidant activity, the blood haemolysate was prepared from the blood samples collected at 0th, 30th, and 60th days of study (Kumar et al. 2016; Banakar et al. 2021). The antioxidant activity of superoxide dismutase (SOD) was estimated as per the method described by Madesh and Balasubramanian (1998). The catalase enzyme activity was estimated by spectrophotometer following the method of Aebi (1984). The

activity of glutathione peroxidase (GPx) was estimated spectrophotometrically using the method of Paglia and Valentine (1967). Total antioxidant activity was measured by FRAP assay according to the procedure given by Benzie and Strain (1999).

Analysis of total serum immunoglobulin

Total serum immunoglobulin estimation was performed by the protocol devised by McEwan et al. (1970). Briefly, 0.1 mL aliquot of serum was added to 6 mL of zinc sulphate solution in a test tube, and to a control tube containing 6 mL of sterile water. The solution was then gently shaken and allowed to stand for 60 min at room temperature. The relative turbidity of each tube was measured at 550 nm wavelength.

Statistical analysis

The experimental data were analyzed using the statistical tool SPSS 26.0 (SPSS Inc., version, Chicago, IL, USA). A two-way analysis of variance (ANOVA) with the general linear model (GLM) was performed to compare statistical differences across various treatments. Significant differences were determined at $P < 0.05$.

Results and Discussion

Body weight changes and dry matter intake

The body weight (BW) and dry matter intake (DMI kg/d) in lactating *Sahiwal* cows remained uninfluenced throughout the experimental period (Supplementary Table 2) across all treatments. Statistical analysis revealed no significant effects of the treatments on BW and DMI of the cows. Similarly, no significant differences were observed in BW and DMI over different periods and in the interaction between treatments and periods. This outcome is consistent with the findings of Stein et al. (2006), who reported that probiotic feeding did not affect the body weight of cows. These results are also in line with the findings of Philippeau et al. (2017), who reported no difference in DMI between animals in the probiotics and control groups. However, contrary to these findings, other researchers have shown that feeding probiotics to dairy cows can alter rumen bacterial fermentation, improve feed utilization, and increase DMI (Stella et al. 2007).

General blood profile

The general blood parameters (Table 1) viz. RBC count, PCV, MCV, MCH and MCHC did not show any significant differences among the various treatment groups. Whereas, the average Hb (g/dL) concentrations were highest ($P = 0.033$) in healthy (H) group, followed by PRO+T, PRO and lowest in animals under "M" group. These results align with previous research and all the findings fall within the normal physiological range (Kumar et al. 2012; Wood and Quiroz-Rocha, 2010). Ayyat et al. (2024) observed that feeding of multi-strain probiotics did not significantly affect general blood profile. The lower Hb levels in SCM group (M)

compared to other treatment groups aligns with the findings of Sadek et al. (2017) and Saleh et al. (2022). This observation can be explained by the fact that, during inflammatory conditions, the mononuclear phagocytic system increases iron trapping and storage under the influence of inflammatory cytokines. This response is thought to be a defensive mechanism that reduces iron availability to microorganisms, leading to reduced haemoglobin synthesis and the production of microcytic hypochromic RBCs (Stockham and Scott, 2002).

Total leukocyte count (TLC) and differential leukocyte count (DLC)

The data presented in Table 2 indicated that TLC was significantly lowest ($P = 0.002$) in the group I (H), followed by IV (PRO+T), and group III (PRO); with the highest value in group II (M). This suggests that probiotic supplementation, especially combined with teat dipping, effectively reduces TLC in subclinical mastitis cows over time. The healthy cows maintained a consistently low TLC, indicating no inflammation or infection. The subclinical mastitis cows without treatment showed an increase in TLC, indicative of ongoing inflammation. The reductions in TLC in the PRO and PRO+T groups suggest that these treatments help to mitigate the inflammatory response associated with subclinical mastitis. The significant increase in TLC observed in the "M" group may be attributed to various immunomodulatory effects, likely due to the increased demand for leukocytes in the infected udder. Saha et al. (2024) found that the feeding of probiotics balanced the TLC (WBC count) in cows with subclinical mastitis compared to the control group, possibly due to reduced inflammation and enhanced immunity in those animals.

The overall mean neutrophil count was significantly lowest in the "H" group and significantly highest in the "M" group, with intermediate levels in the PRO and PRO+T groups. While lymphocyte count was significantly highest in the "H" group and lowest in the "M" group. Whereas, eosinophil counts showed that the overall mean was significantly highest ($P = 0.012$) in the "M" group, followed by the PRO group and the PRO+T group. However, no significant effect was observed on monocyte and basophil counts with the supplementation of probiotic consortia, either orally as well as teat dip in the lactating *Sahiwal* cattle. Overall, probiotic supplementation, particularly, when combined with teat dipping, effectively modulates leukocyte counts in subclinical mastitis cows by reducing inflammation and improving immune function. The present study is consistent with previous research conducted by Saha et al. (2024) and Ayyat et al. (2024), which observed a decreasing trend in neutrophil counts and increase the lymphocytes in probiotic supplementation groups. Neutrophils are the first cells to respond as a defence against inflammation in the body, and a high neutrophil count indicates inflammation (Saha et al. 2024). Thus, a decrease in blood neutrophils in the probiotic-supplemented group may suggest diminished mastitis inflammation, enhancing the overall health

Table 1: Effect of LAB consortium based teat dipping and feeding on haematological parameters of lactating *Sahiwal* cows

Attributes	Day	Dietary groups			Mean	T	P value	
		H	M	PRO			PRO+T	P
RBC count ($\times 10^9/\mu\text{L}$)	0	6.36±0.34	6.03±0.59	6.00±0.62	6.04±0.61	0.272	0.596	0.972
	30	6.33±0.12	5.94±0.82	6.29±0.16	6.21±0.27			
	60	6.51±0.36	5.94±0.46	6.36±0.20	6.38±0.15			
	Mean	6.40±0.27	5.97±0.56	6.22±0.37	6.20±0.37			
Hb (g/dL)	0	9.92±0.12	9.39±0.49	9.38±0.57	9.37±0.15	0.033	0.790	0.982
	30	9.94±0.08	9.34±0.47	9.50±0.41	9.58±0.86			
	60	9.94±0.07	9.29±0.49	9.59±0.21	9.71±0.08			
	Mean	9.94±0.08 ^a	9.34±0.42 ^b	9.49±0.38 ^{ab}	9.55±0.46 ^{ab}			
PCV (%)	0	27.90±0.91	27.31±1.42	27.32±1.30	27.17±1.211	0.285	0.985	0.999
	30	28.37±0.33	27.11±1.50	27.26±1.68	27.32±1.41			
	60	28.37±0.36	26.96±1.711	27.26±1.35	27.33±1.41			
	Mean	28.21±0.57	27.12±1.35	27.28±1.26	27.27±1.17			
MCV (fL)	0	43.90±0.75	45.52±2.51	45.73±2.43	45.30±2.81	0.620	0.547	0.955
	30	44.85±0.80	45.95±2.15	43.32±1.44	43.98±0.90			
	60	43.69±1.70	45.45±0.49	42.86±1.45	42.91±1.85			
	Mean	44.15±0.61	45.64±0.97	43.97±1.02	44.06±1.07			
MCH (pg)	0	15.63±0.68	15.65±1.29	15.69±1.22	15.61±1.41	0.874	0.836	0.998
	30	15.71±0.30	15.94±2.61	15.10±0.28	15.44±1.66			
	60	15.30±0.84	15.73±1.83	15.08±0.57	15.23±0.41			
	Mean	15.55±0.58	15.77±1.72	15.28±0.75	15.43±1.12			
MCHC (%)	0	35.60±0.93	35.49±3.33	34.42±3.20	34.53±2.02	0.937	0.938	0.998
	30	35.03±0.63	34.58±3.33	34.92±1.80	35.05±2.63			
	60	35.05±0.57	34.59±3.78	35.20±0.99	35.58±1.791			
	Mean	35.22±0.69	34.55±3.02	34.84±1.93	35.05±1.94			

H=Healthy *Sahiwal* cow; M=Subclinical mastitis cow; PRO= Subclinical mastitis cow supplemented with probiotic consortia; PRO+T= Subclinical mastitis cow supplemented with probiotic consortia and teat dipped in probiotic consortia solution. T =treatment; P =time period; T*P =interaction between treatments and time period

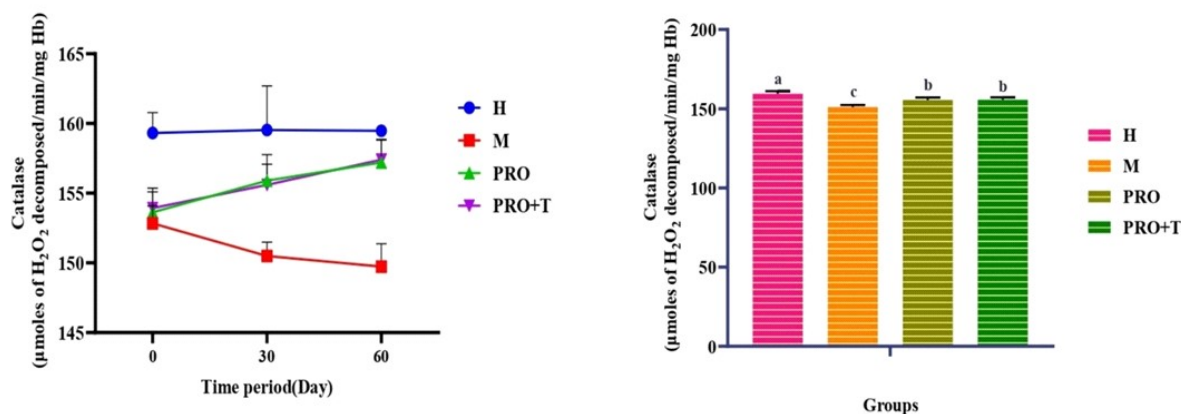


Fig. 1 Effect of LAB consortium supplementation and teat dipping on superoxide dismutase (SOD, U/mg Hb) activity

H=Healthy Sahiwal cow; M=Subclinical mastitis cow; PRO= Subclinical mastitis cow supplemented with probiotic consortia; PRO+T= Subclinical mastitis cow supplemented with probiotic consortia and teat dipped in probiotic consortia solution.

benefits. Our findings align with those of Mousa et al. (2019), who reported that probiotic supplementation in lambs significantly improved leukocyte and monocyte counts in the blood. Elevated lymphocyte and monocyte count play a crucial role in enhancing the body’s immune function by ultimately destroying the invading pathogens. Similarly, as observed in the present study, the significant increase in total neutrophil count and decrease the lymphocytes in the “M” group might be due to various immunomodulatory effects caused by the increased demand for leukocytes in the infected udder (Singh et al. 2014).

Serum biochemical parameters

Average total protein was highest ($p < 0.05$) in group “M” compared to the rest of the groups (Table 3). Albumin levels were similar in all the groups. The overall mean globulin levels were significantly higher ($p < 0.05$) in “M” group, followed by PRO and PRO+T groups with lowest in “H” group. The overall mean A/G ratio was significantly ($p < 0.05$) higher in the “H” group, followed by the PRO+T group, the PRO group, and the “M” group (Supplementary Fig.1).

All these mean values were within the normal physiological range, as reported by Zhybak et al. (2016). Similarly, Pandey et al. (2012) observed higher total protein levels in subclinical mastitis cows as compared to healthy animals. This increase in total protein could be attributed to the inflammatory response triggered by the infection in the udder, leading to a rise in acute phase proteins (APPs), such as haptoglobin, ceruloplasmin, and serum amyloid A, which are primarily produced by the liver and released into the bloodstream, thereby elevating the overall serum protein levels. Similarly, studies conducted by Pandey et al. (2012), and Singh et al. (2014) reported higher levels of globulin in the serum of mastitis cows. In contrast, Krishnappa et al. (2016), and Saleh et al. (2022) found decreased globulin levels in mastitis cows.

Cows with subclinical mastitis had a lower A/G ratio compared to healthy cows, highlighting the impact of infection on protein levels. Probiotic supplementation, particularly, when combined with teat dipping, improved the A/G ratio over time, suggesting a beneficial impact. These results align with previous research by Saleh et al. (2022) and Singh et al. (2014), who reported significantly lower A/G ratios in subclinical mastitis cows compared to healthy cows.

The overall mean of AST levels was significantly higher ($p < 0.05$) in the “M” group, followed by the PRO+T group, the PRO group and lowest in the “H” group (65.11 ± 0.53 U/L), respectively. Statistical analysis confirmed the significant effects of treatment ($p = 0.002$), time period ($p = 0.001$), and their interaction ($p = 0.002$). Whereas, no significant difference ($P > 0.05$) was observed in ALT levels across the treatment groups, with all values remaining within the normal physiological range (Oguri et al. 2013). These findings suggest that probiotic supplementation, particularly when combined with teat dipping, effectively managed liver enzyme levels in cows affected by subclinical mastitis. Cows with subclinical mastitis showed elevated AST levels indicative of hepatic stress, mitigated effectively by probiotic supplementation and teat dip interventions. Likewise, Saleh et al. (2022) reported higher levels of AST, a marker of oxidative stress, in subclinical mastitis cows compared to healthy cows. Similarly, Saleh et al. (2022) found no significant alterations in ALT levels in mastitis cows as compared to healthy cows, suggesting that subclinical mastitis does not affect ALT levels in cows. Consequently, neither the LAB consortia supplements nor the teat dip had any impact on ALT levels in this cohort.

Statistical analysis revealed no significant effect ($p > 0.05$) of treatment, time period, nor its interaction with treatment on serum “Ca” and “P” levels of lactating *Sahiwal* cows (Table 3), but numerically “Ca” level was highest in “M” group as compared to

Table 2: Effect of LAB consortium based teat dipping and feeding on TLC and DLC (%) of lactating *Sahiwal* cows

Attributes	Day	Dietary groups			Mean	T	P value	
		H	M	PRO			T	P
TLC (x 10 ³ /μL)	0	6.87±0.02	9.27±0.06	9.30±0.38	9.27±0.06	0.002	0.029	0.019
	30	6.86±0.02	9.44±0.05	8.93±0.15	8.57±1.62			
	60	6.87±0.02	9.80±0.16	8.19±0.90	7.20±0.54			
	Mean	6.86±0.02 ^c	9.50±0.25 ^a	8.81±0.70 ^b	8.34±1.25 ^{bc}			
Neutrophil	0	32.75±1.93	65.96±2.66	65.69±2.57	65.86±2.78	0.002	0.003	0.001
	30	32.79±1.97	67.08±1.86	58.26±3.12	47.75±5.90			
	60	32.76±1.50	68.24±2.07	48.86±2.39	42.29±2.60			
	Mean	32.77±1.57 ^d	67.09±2.16 ^a	57.60±7.67 ^b	51.97±11.25 ^c			
Lymphocyte	0	60.82±0.36	29.68±0.52	29.59±0.64	29.80±0.72	0.003	0.004	0.030
	30	60.81±1.42	30.04±0.53	41.54±3.36	46.63±1.40			
	60	60.86±2.38	29.48±0.85	48.05±1.85	54.28±0.65			
	Mean	60.83±1.40 ^a	29.73±0.61 ^d	39.73±8.33 ^c	43.57±10.87 ^b			
Eosinophil	0	1.34±0.02	2.30±0.03	2.32±0.04	2.34±0.04	0.012	0.034	0.030
	30	1.35±0.01	2.32±0.06	1.99±0.11	1.58±0.11			
	60	1.35±0.03	2.25±0.12	1.78±0.05	1.39±0.06			
	Mean	1.35±0.02 ^d	2.29±0.07 ^a	2.03±0.25 ^b	1.77±0.44 ^c			
Monocyte	0	1.43±0.06	1.38±0.07	1.39±0.06	1.40±0.05	0.219	0.809	0.866
	30	1.41±0.02	1.38±0.07	1.45±0.05	1.41±0.01			
	60	1.43±0.01	1.38±0.07	1.42±0.02	1.42±0.05			
	Mean	1.42±0.03	1.38±0.06	1.42±0.05	1.41±0.04			
Basophil	0	0.29±0.12	0.33±0.06	0.37±0.15	0.37±0.15	0.763	0.922	0.844
	30	0.30±0.10	0.37±0.06	0.33±0.06	0.37±0.06			
	60	0.37±0.06	0.37±0.15	0.37±0.06	0.30±0.10			
	Mean	0.31±0.09	0.36±0.09	0.36±0.08	0.34±0.10			

H=Healthy *Sahiwal* cow; M=Subclinical mastitis cow; PRO= Subclinical mastitis cow supplemented with probiotic consortia; PRO+T= Subclinical mastitis cow supplemented with probiotic consortia and teat dipped in probiotic consortia solution. T =treatment; P =time period; T*P =interaction between treatments and time period

Table 3: Effect of LAB consortia based teat dipping and feeding on biochemical parameters of lactating Sahiwal cow

Attributes	Day	Dietary groups				Mean	T	P	T*P
		H	M	PRO	PRO+T				
Total protein (g/dL)	0	6.99±0.01	7.35±0.31	7.32±0.16	7.31±0.16	7.24±0.22	0.002	0.130	0.084
	30	6.85±0.16	7.44±0.10	7.01±0.38	6.94±0.43	7.06±0.35			
	60	6.90±0.11	7.74±0.13	6.91±0.02	6.93±0.20	7.12±0.39			
	Mean	6.91±0.12 ^b	7.51±0.25 ^a	7.08±0.28 ^b	7.06±0.31 ^b				
Albumin (g/dL)	0	3.78±0.31	3.55±0.18	3.56±0.19	3.55±0.18	3.61±0.22	0.074	0.896	0.986
	30	3.77±0.09	3.52±0.18	3.57±0.17	3.59±0.17	3.61±0.17			
	60	3.75±0.09	3.51±0.19	3.64±0.13	3.67±0.29	3.64±0.19			
	Mean	3.77±0.17	3.53±0.16	3.59±0.20	3.60±0.15				
Globulin (g/dL)	0	3.52±0.06	4.18±0.06	4.13±0.11	4.26±0.14	4.02±0.32 ^A	0.002	0.004	0.001
	30	3.50±0.06	4.32±0.18	3.86±0.26	3.86±0.19	3.89±0.34 ^{AB}			
	60	3.47±0.02	4.36±0.06	3.88±0.11	3.53±0.20	3.81±0.38 ^B			
	Mean	3.49±0.05 ^c	4.29±0.13 ^a	3.96±0.20 ^b	3.88±0.35 ^b				
AST(U/L)	0	65.06±0.53	72.48±1.47	72.37±1.84	72.47±1.47	70.60±3.55 ^A	0.002	0.001	0.002
	30	65.19±0.67	72.61±2.06	68.67±2.12	65.37±0.45	67.96±3.42 ^B			
	60	65.08±0.62	75.75±2.20	67.96±2.18	65.37±0.57	68.53±4.70 ^B			
	Mean	65.11±0.53 ^c	73.61±2.31 ^a	67.67±2.72 ^b	67.74±3.65 ^b				
ALT(U/L)	0	26.67±1.55	26.65±1.58	26.66±1.55	26.65±1.52	26.66±1.32	1.000	0.995	1.000
	30	26.63±1.77	26.65±1.76	26.65±1.76	26.94±3.02	26.72±1.84			
	60	26.72±1.71	26.70±1.73	26.73±1.73	26.39±4.02	26.64±2.14			
	Mean	26.67±1.45	26.67±1.46	26.68±1.46	26.66±2.63				
Ca (mg/dL)	0	9.62±0.00	9.67±0.11	9.66±0.10	9.67±0.04	9.65±0.03	0.348	0.059	0.983
	30	9.67±0.07	9.82±0.01	9.76±0.07	9.78±0.23	9.76±0.06			
	60	9.73±0.06	9.91±0.03	9.79±0.02	9.78±0.03	9.80±0.02			
	Mean	9.67±0.03	9.80±0.05	9.73±0.04	9.74±0.07				
P (mg/dL)	0	4.72±0.07	4.74±0.22	4.71±0.21	4.73±0.22	4.73±0.02	0.980	0.655	1.000
	30	4.75±0.10	4.78±0.59	4.76±0.11	4.75±0.02	4.76±0.02			
	60	4.77±0.85	4.81±0.21	4.78±0.01	4.78±0.02	4.79±0.06			
	Mean	4.75±0.08	4.78±0.05	4.75±0.02	4.76±0.02				

H=Healthy Sahiwal cow, M=Subclinical mastitis cow, PRO=Subclinical mastitis cow supplemented with probiotic consortia; PRO+T=Subclinical mastitis cow supplemented with probiotic consortia and teat dipped in probiotic consortia solution. T =treatment; P =time period; T*P =interaction between treatments and time period

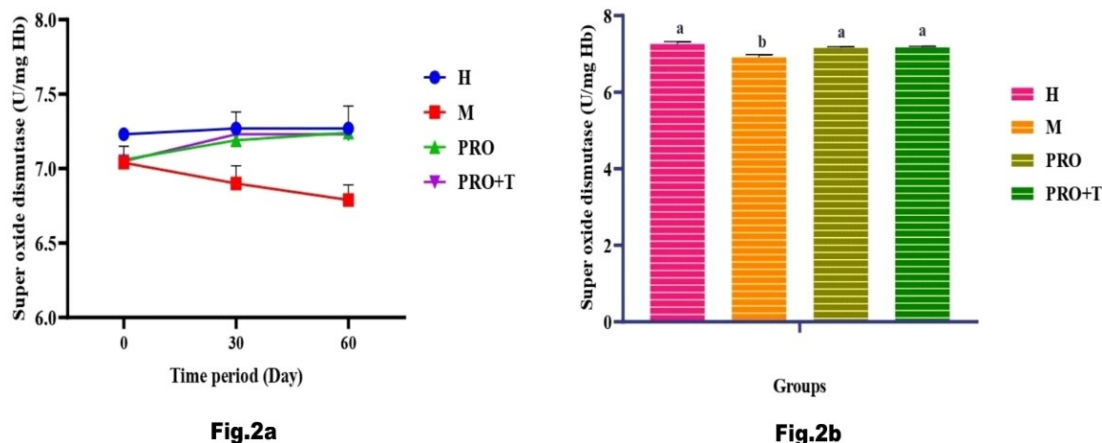


Fig. 2 Effect of LAB consortium supplementation and teat dipping on catalase activity in lactating *Sahiwal* cows

H=Healthy *Sahiwal* cow; M=Subclinical mastitis cow; PRO= Subclinical mastitis cow supplemented with probiotic consortia; PRO+T= Subclinical mastitis cow supplemented with probiotic consortia and teat dipped in probiotic consortia solution

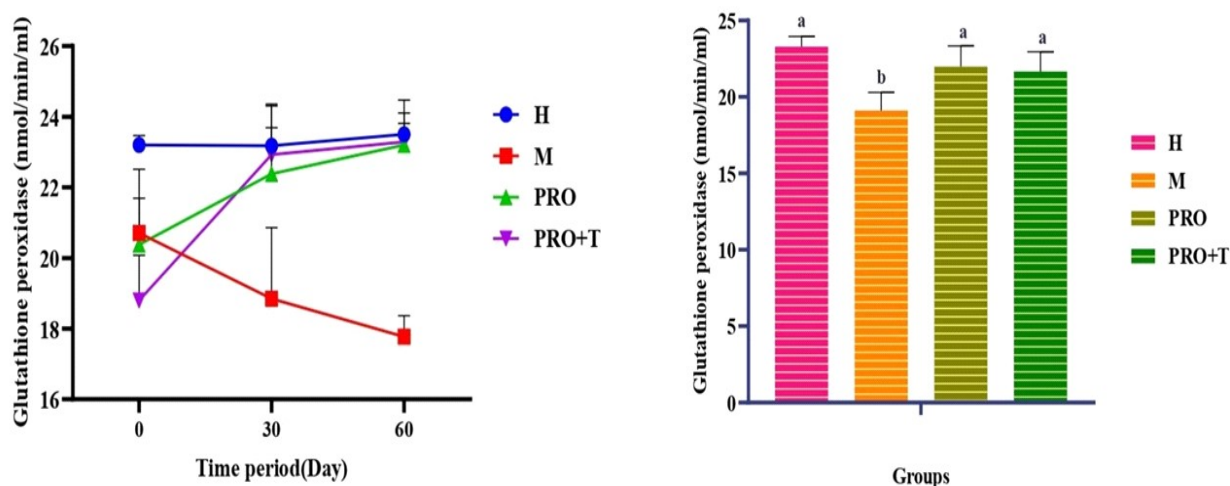


Fig. 3 Effect of LAB consortium supplementation and teat dipping on glutathione peroxidase (nmol/min/ml) enzyme activity in lactating *Sahiwal* cows

H=Healthy *Sahiwal* cow; M=Subclinical mastitis cow; PRO= Subclinical mastitis cow supplemented with probiotic consortia; PRO+T= Subclinical mastitis cow supplemented with probiotic consortia and teat dipped in probiotic consortia solution.

other groups. Similarly, Pegolo et al. (2023) found an increase in “Ca” levels in animals with subclinical mastitis as compared to healthy controls. In our study, serum “Ca” levels were reduced in the probiotics group as compared to the mastitis group as time progressed. El-Katcha et al. (2016) noted that bacterial probiotic supplementation had no clear effect on blood serum “Ca” and “P” levels. On the other hand, Qayyum et al. (2016) found a significant decrease in “Ca” levels in the serum of mastitis cows compared to healthy cows.

Antioxidant activity

The overall mean of SOD enzyme activity (Fig. 1) was higher in the “H”, PRO+T, PRO groups compared to “M” group. However, there was no effect of period and treatment × period interaction. The overall mean catalase activity (Fig. 2) was significantly highest ($p < 0.05$) in the “H” group, followed by the PRO+T group and the PRO group, and the lowest in the “M” group. Similar to SOD, the GPx (Fig. 3) level was higher in “H”, PRO+T, PRO

groups as compared to “M” group having the lowest value. Moreover, there was no effect of period and treatment \times period interaction in catalase and GPx activity. The total antioxidant levels (Supplementary Fig. 2) were reflected the same trend as observed in SOD levels.

In the same line, Rehman et al. (2017) found that the mean activities of SOD were significantly reduced in animals suffering from either subclinical or clinical mastitis as compared to healthy cows. Catalase activity was also significantly decreased in the mastitis group as reported by Jhambh et al. (2013). Sadek et al. (2017) and Saleh et al. (2022) also reported that GPx levels and total antioxidant activity were lower during subclinical mastitis, compared to healthy animals. The decreased antioxidant enzymatic activity could be attributed to the increased consumption needed to neutralize the ROS generated by the inflamed gland, indicating a compromised antioxidant defence mechanism (Jhambh et al. 2013). This reduction in activity could also be attributed to the depletion of the enzyme as it neutralizes the excessive ROS produced by the inflamed mammary gland (Novac et al. 2020).

Li et al. (2022) found that feeding probiotics to ruminants improved SOD, catalase, and GPx activity, supporting the potential role of probiotics in enhancing antioxidant defence mechanisms. Probiotics are recognized for their numerous health benefits, including strain-specific antioxidant activity and reduction of oxidative damage, the precise mechanisms underlying their oxidation-resistant properties remain to be fully elucidated. This research highlights the importance of understanding antioxidant dynamics in dairy cattle health management and milk quality enhancement strategies.

Total serum immunoglobulin

The total serum immunoglobulin level (Supplementary Fig. 3) was significantly lowest in the “H” group, followed by PRO, PRO+T, and highest in “M” group. The disruption of the milk-blood barrier allows gram-negative pathogens to enter systemic circulation, triggering an immune response that accounts for the increased immunoglobulin levels observed in the “M” group (Wall et al. 2016). Our findings suggest that supplementation with lactic acid bacteria (LAB) consortium or LAB consortium combined with teat dip resulted in decreased serum immunoglobulin levels; however, these values remained elevated compared to healthy cows, indicating an improved immune condition.

Conclusion

Probiotics can considerably improve the haematological and biochemical characteristics of *Sahiwal* cows suffering from subclinical mastitis. Probiotics may improve these animals' overall health, as seen by notable improvements in haemoglobin levels, antioxidant enzyme activity and reduced leukocyte count. The

potential of probiotics to help cows with subclinical mastitis regain their health was highlighted by the healthy control group, which showed ideal values across a number of metrics. Furthermore, differences in serum immunoglobulin levels across treatment groups suggest that probiotics might alter immunological function, which is important for the treatment of subclinical mastitis. All things considered, this study backs the use of probiotic therapies in dairy management procedures to improve animal productivity and health while lowering the need for antibiotics.

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