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Association of California Mastitis Test Scores with Somatic Cell Count in Bovine Colostrum and Passive Immunity Transfer in Dairy Calves: A Cohort Study

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Abstract

Colostrum quality is fundamental for adequate transfer of passive immunity (TPI) to calves. However, practical on-farm methods for assessing its microbiological quality remain limited. We investigated associations of the California Mastitis Test (CMT) with bovine colostrum microbiological quality, somatic cell count levels, mammary gland health, and TPI outcomes. We hypothesized that lower CMT scores would be associated with superior colostrum quality and TPI in calves. A cohort study evaluated colostrum provision to 93 Holstein calves. Dams were classified according to CMT scores as LOW (trace or score 1, N = 58) or HIGH (score 2 or 3, N = 35). Statistical significance was set at $p \leq 0.05$. Despite LOW dams producing higher colostrum volume ($p = 0.0045$) and higher total IgG mass ($p = 0.0098$), microbiological outcomes and colostrum IgG concentration did not differ between groups. A tendency for higher calf serum Brix was associated with lower CMT scores ($p = 0.0577$), but no associations were found for serum IgG concentration ($p = 0.1412$) or apparent efficiency of absorption ($p = 0.4223$). These findings suggest that despite CMT's association with SCC, it cannot be used as a tool to identify colostrum capable of providing better TPI to dairy calves.

Keywords: colostrum sanitary quality; somatic cell count; apparent efficiency of absorption

1. Introduction

The ruminants' placenta is classified as synepitheliochorial, which, although it protects the fetus from microorganisms originating from the dam, also does not allow the transfer of maternal antibodies during gestation [1]. Consequently, newborn calves depend primarily on the absorption of immunoglobulins present in maternal colostrum, a process known as transfer of passive immunity (TPI). The most abundant of these antibodies is immunoglobulin G (IgG), which enhances innate immune responses that are essential for the development of adaptive immunity [2]. Along with immunological proteins, colostrum provides a variety of bioactive compounds that support the development of the neonatal immune system [3]. Although providing high immunological quality colostrum is essential to achieve high serum IgG levels in calves, that is not the only parameter responsible for a successful transfer. Beyond IgG concentration, management practices, environmental conditions and pathogen exposure will have a major impact on calf health and TPI outcomes. Colostrum with poor microbiological quality represents a significant risk for pathogen transmission to calves. The "leaky" configuration of the small intestine epithelium in the first

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hours of life may allow not only bigger molecules like immunoglobulins to go through but also bacteria, which can impair the absorption of important bioactive components from the lumen to the bloodstream [4]. Furthermore, high bacterial loads can reduce IgG absorption due to prior binding, and because of that, calves fed high-bacteria colostrum tend to achieve lower serum IgG concentrations [5]. Therefore, colostrum microbiological quality poses a critical point to minimize challenges to neonatal calves and ensure efficient IgG absorption.

The dry period of cows represents a critical origin point for early lactation mastitis due to immunosuppression and increased teat canal susceptibility [6], and pathogens such as Staphylococcus aureus may impair secretory function and IgG transfer [7]. Additionally, colostrum from infected cows can act as a vehicle for pathogen transmission to calves, challenging the gut and influencing the microbiota establishment [8]. Thus, it would be highly valuable to identify colostrum that might contain pathogens and could pose a threat to calves right in their first meal. Despite its importance, microbiological evaluations cannot be performed immediately before colostrum feeding. Laboratory methods require incubation periods of 24 to 48 h for bacterial identification, limiting their use for immediate decision-making. Accordingly, there is a demand for accessible tools capable of rapidly identifying, under field-applied conditions, whether the colostrum intended for calf feeding meets acceptable microbiological quality standards.

The California Mastitis Test (CMT) is a rapid and practical test that indirectly and visually estimates the cellular content in milk, specifically the somatic cell count (SCC), which is strongly associated with the occurrence of subclinical mastitis. Regardless of its subjective nature, CMT remains one of the most widely used field tests to identify mammary quarters with elevated SCC, a parameter suggestive of intramammary infection. Although well established for milk, data on CMT usage for colostrum evaluation is limited. Previous studies have associated higher SCC levels in colostrum with pathogen presence and reduced immunological quality [9]. Sergeant et al. [10] tested the use of CMT to evaluate colostrum and reported high specificity (96.7 to 97.6%) but low sensitivity (12.3 to 10.7%) when using a score of 2 as the cutoff. The high specificity indicates that most non-infected quarters were correctly classified as negative, whereas the low sensitivity indicates that a substantial proportion of infected quarters could be missed. However, neither sensitivity nor specificity alone establishes the probability of infection following a positive or negative result, as these predictive values are also influenced by the prevalence of infection in the population. Thus, although these findings support further investigation of CMT as a practical and accessible tool for evaluating colostrum in relation to intramammary infection, they do not establish its diagnostic reliability or suitability as a screening tool. In particular, the low sensitivity reported suggests that a negative CMT result cannot reliably exclude infection.

We hypothesized that colostrum CMT scores would reflect differences in colostrum SCC and that stronger CMT reactions would be associated with poorer microbiological quality and potentially less favorable TPI outcomes in dairy calves. Specifically, stronger CMT reactions were expected to reflect increased cellular content potentially associated with intramammary infection, rather than physiological variation in colostrum SCC. Therefore, the primary aim of this study was to investigate if the CMT could be used as a quick screening on-farm tool to assist in the decision-making regarding colostrum's microbiological quality before providing it to calves. Specifically, we aimed to: (1) investigate the association between CMT scores and colostrum SCC, thereby contributing to the characterization of SCC variation in bovine colostrum; (2) evaluate whether CMT scores were associated with microbiological indicators of colostrum quality; and (3) investigate the associations between CMT scores and passive immunity outcomes in dairy calves, assessed through apparent efficiency of absorption (AEA) of IgG and TPI. Additionally, we evaluated whether colostrum SCC and its estimation through CMT were associated with early calf performance.

By integrating colostrum cellular and microbiological characteristics with calf TPI outcomes and early performance, this study aimed to provide further insight into the potential application of CMT in colostrum quality assessment and its practical relevance for on-farm decision-making.

2. Materials and Methods

All animal procedures were authorized by the Ethical Committee on the Use of Animals of the Escola Superior de Agricultura "Luiz de Queiroz" (CEUA/ESALQ), University of Sao Paulo, Piracicaba, SP, Brazil, protocol n° 64452602210.

2.1. Experimental Design

This study employed a prospective cohort design, in which 93 Holstein cows were monitored at calving, and their colostrum was evaluated using the CMT prior to feeding it to their calves. The field trial was conducted in a commercial farm located in Itatiba, São Paulo, Brazil, which was chosen because of its proximity and partnership with our laboratory. At the beginning of the trial, the farm had around 1020 lactating cows housed in free-stall facilities, with an average milk yield of 30 kg/d. Cows were milked twice a day in a 16-unit parallel rapid exit milking parlor. Calvings were followed between late November of 2024 and late January of 2025. The number of animals enrolled was determined based on time and resource constraints, rather than by an a priori sample size calculation. Consequently, the available sample size may have limited the precision of the estimates and the ability to detect associations between CMT scores and the evaluated outcomes, particularly TPI in calves.

2.2. Dam Management, Sample Collection and Colostrum Provision

Pregnant dams (cows and heifers) were transferred to a free-stall prepartum barn between 250 and 260 days of gestation, with sawdust bedding and an external grazing pasture, to which they had access during the day. With the start of labor, dams were moved to a calving pen within the same facility, with hay bedding for comfort. All calvings were watched, and interventions were performed only in case of mispositioning that would impair labor progression. Only two cows needed assistance during labor. Any dam that gave birth within the trial period was eligible for enrollment, as long as it did not present signs of clinical mastitis during colostrum milking, assessed through a black-bottom cup test. Three cows presenting clinical mastitis were excluded before enrollment. Of an initial number of 93 cows, three were subsequently excluded from the statistical analysis due to high bacterial counts that invalidated their assessment. Colostrum was collected within 30 min after calving and evaluated using the CMT and the Brix refractometer. Calves were separated from their dam and placed into individual pens with hay bedding and lamp-heating. The navel was disinfected using a 10% iodine solution.

Afterwards, the dam was safely restrained in a metal headlock to allow sample collection and testing. Pre-dipping was performed using chlorine diluted at a 1:3 ratio for each teat with a non-return dip cup, followed by individual teat drying with paper towels. In multiparous cows, stripping was applied to each teat to remove the bismuth subnitrate teat sealant from the teat canal. Subsequently, for both multiparous cows and heifers, the first three streams of colostrum were discarded. Another three streams from each teat were stripped into a black-bottom cup to detect clinical mastitis, based on the presence of macroscopic abnormalities in colostrum (e.g., clots or blood). Only those whose colostrum showed no macroscopic abnormalities were included in the study.

The CMT was then performed for each teat using a CMT reagent (CMT Solução®, Tadabras, Bragança Paulista, SP, Brazil), as described by previous authors and further de-

tailed in a technical report by the University of Missouri [11,12]. Equal volumes of colostrum and CMT reagent (2 mL each) were mixed in the test paddle. Reactions were assessed immediately after complete mixing. All tests were performed by the same researcher, who had received prior training in CMT application and score interpretation. In the present study, a wide variation in colostrum coloration was observed during CMT evaluation. However, scoring was based only on the degree of viscosity resulting from the reaction between colostrum and the reagent [13]. Absence of viscosity was classified as negative, although this pattern was observed in only one teat of one heifer. Slight viscosity that dispersed upon paddle movement was classified as "trace". Fluid viscosity upon agitation was classified as weak reaction (score 1). Persistent viscosity during agitation was classified as moderate reaction (score 2), whereas substantial viscosity adhering to the paddle was classified as strong reaction (score 3).

CMT scores were recorded for each quarter and cow during field assessments, without assigning animals to treatment or classification groups at that time. Only after completion of all field collections and data recording were animals categorized into two CMT score groups (Group LOW—including "trace" and score 1; and Group HIGH—including score 2 and score 3) for subsequent statistical analyses, based on the maximum CMT score observed among the four quarters. Although CMT provides quarter-level information, the presence of at least one highly reactive quarter (score 2 or 3) was used as the criterion for classification into Group HIGH. This approach was based on colostrum management practices, as all colostrum quality analyses were performed on the total colostrum yield rather than on individual quarters, since colostrum is provided to calves after complete milking and homogenization.

For SCC and microbiological culture analyses, equal-volume composite samples were collected from the four mammary quarters before complete milking. Teat ends were then disinfected with 70% alcohol, starting with forequarters and finishing with rear quarters. Two composite samples per cow were collected into sterile 15 mL Falcon tubes by harvesting approximately 3.5 mL of colostrum per quarter, beginning with the rear teats and followed by the fore teats. Samples were immediately frozen at −20 °C until subsequent microbiological culture and SCC analysis. Both microbiological culture and SCC analyses were performed two months after the last collection, and samples went through one thaw cycle in a bain-marie before being used for microbiological culture and SCC analysis.

Total milking was then performed using an individual milking unit equipped with a vacuum-line pulsator and bucket system (GEA Equipamentos e Soluções Ltda., Campinas, SP, Brazil). After total milking, total colostrum yield was recorded and homogenized. Immunological quality was assessed using a digital Brix refractometer (Milwaukee Instruments, MA871, Rocky Mount, NC, USA), calibrated with distilled water before each sample. An additional sample of the homogenized whole colostrum was collected and immediately frozen in a sterile 15 mL Falcon tube for bacterial plate counts and in three non-sterile 2 mL Eppendorf tubes for IgG concentration determination by RID. This last set of samples remained stored at −20 °C for three months before being analyzed.

Colostrum feeding followed the farm's established protocol, consisting of an initial feeding of 2.5 L of maternal colostrum within the first hour of life, followed by an additional 2 L within the subsequent 6 h, regardless of the calf body weight. Colostrum quality was assessed using a digital Brix Refractometer before being provided to calves, with a minimum acceptance threshold of 25% Brix. Colostrum samples below this threshold were considered unsuitable for feeding, and calves from the three cows producing colostrum below 25% Brix were excluded from the study. No alternative colostrum source was provided. When a dam produced less than the 4.5 L required by the farm protocol, the volume provided at the second feeding was restricted to the remaining available colostrum from the same dam. The

actual volume provided at the second feeding was recorded and used to calculate the total mass of IgG provided to the calf. The total mass of IgG provided to each calf was calculated based on the measured IgG concentration and the actual total volume of colostrum supplied across both feedings. Following the second colostrum feeding, calves were transitioned directly to milk replacer. No additional colostrum or transition milk was provided before blood sampling.

2.3. Calf Management and Evaluations

Calf blood was collected, and body measurements were performed to evaluate potential associations with the type of colostrum provided (based on CMT reactivity), passive transfer of immunity, and early-life performance. At 48 h after colostrum feeding, blood samples were collected via jugular venipuncture into tubes containing a clot activator to assess serum Brix as an estimate of TPI [14]. Following Brix assessment, serum samples were immediately frozen for subsequent IgG quantification.

Throughout the experiment period, calves were fed 6 L/day of a commercial milk replacer reconstituted at 12.5% dry matter (Sprayfo, Nutreco Brasil Nutricao Animal LTDA, Campinas, SP, Brazil), divided into two feedings (3 L in the morning and 3 L in the afternoon). A commercial starter concentrate (Maxxi Milk Baby Laminada, Alisul, RS, Brazil) and water were offered ad libitum starting on the second day of life.

Body weight was estimated using a weight tape (Fita de Pesagem JA—Bovinos e Equinos; JA Saúde Animal, Patrocínio Paulista, SP, Brazil) at birth, at 7 d of age (week 1), and fortnightly thereafter, with the final measurement at 63 d of age (week 9), to monitor growth performance without interfering with routine farm management. Additionally, withers height, hip width, and heart girth were measured to assess structural development, as described previously [15].

All procedures, including CMT evaluation, colostrum sampling, immunological quality assessment, blood collection, passive transfer evaluation, and calf measurements, were performed by the same researcher to ensure procedural consistency and minimize inter-observer variability. Laboratory analyses were conducted without access to the CMT classifications, which were linked to the corresponding laboratory data only after all analyses were completed. Nevertheless, the potential for observer bias represents a limitation of this study, particularly regarding the subjective nature of CMT scoring and calf body measurements requiring manual assessment.

2.4. Laboratory Analyses

Microbiological culture and identification were performed at the Qualileite Laboratory, located at the School of Animal Science and Food Engineering of the University of São Paulo (FZEA/USP), Pirassununga, SP, Brazil. Microbiological culture and microorganism identification were conducted by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Billerica, MA, USA), following the methodology described by previous work [16]. Briefly, samples were thawed at room temperature, homogenized, and streaked onto 5% defibrinated bovine blood agar plates using sterile cotton swabs. Plates were incubated at 37 °C for 24 h. Bacterial growth was considered positive when ≥3 similar colonies were observed, except for hemolytic colonies, for which a single colony was considered positive due to the potential presence of *Staphylococcus aureus*. Samples would be classified as contaminated when ≥3 distinct colony types were present with significant growth; however, no samples met this criterion. For the statistical analysis, pathogens were classified as major or minor agents according to their level of virulence, which is related to their ability to cause infection, intensity of damage caused, and capacity to evade immune responses. Major mastitis pathogens are

known for causing intense intramammary infections in contrast to minor agents, which are usually associated with self-limiting mastitis [17].

Positive samples were then subcultured onto a polished steel target plate (MSP 384; Bruker Daltonics, Bremen, Germany). Each spot received 1 µL of 70% formic acid and 1 µL of matrix solution (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and was air-dried for 10 min. A bacterial test standard was used for calibration, along with positive (*Escherichia coli*) and negative controls. Spectra were acquired in linear mode over a mass range of 2000–20,000 m/z using FlexControl 3.4 software and processed with MALDI Biotyper Compass 4.1.100 (Bruker Daltonics). Identification scores ≥ 2.0 were considered reliable for species-level identification, following the manufacturer's criteria. All positive samples yielded a single bacterial isolate; therefore, no samples required classification based on the simultaneous isolation of minor and major mastitis pathogens.

SCC was determined by direct microscopic counting according to the method of Prescott and Breed (1910) [18], with Rosenfeld staining. Flow cytometry could not be performed due to lack of local resources. Colostrum samples were thawed in an agitated bain-marie at 38 °C, homogenized, and diluted 1:1 in phosphate-buffered saline (PBS; pH 7.2). Aliquots were placed on two separate glass slides, performed consecutively under the same conditions. The duplicate slides were air-dried for 24 h, fixed in methanol, stained, and examined under oil immersion on a 100× objective (L1000T Microscope, Bioval, Chongqing, China). Cells were counted in 100 microscopic fields per area using a zigzag pattern for both slides, and the mean of the two counts was used as the final SCC value. Final SCC (cells/mL) was calculated accounting for the microscope field factor and sample dilution. All counting was performed by the same researcher. The pooled coefficient of variation between duplicate preparations was 10.4%, based on 87 samples with two quantifiable counts.

Additional colostrum samples were used to determine total plate count (TPC), total coliform count (TCC), and *Escherichia coli* count (ECC) using Petrifilm media (Petrifilm 3M, Neogen, Lansing, MI, USA) as described previously [19]. Samples were thawed in an agitated bain-marie at 38 °C, homogenized, and serially diluted. Serial dilutions (1:100,000 to TPC; 1:10,000 to TCC; and 1:1000 to ECC) were plated and incubated under recommended conditions for each microbial group. Colony counts were performed by three independent evaluators, and the average was used. Results were expressed as colony-forming units (CFU)/mL after correction for dilution. Plates classified as "too numerous to count" were excluded from statistical analyses.

Colostrum IgG concentration was determined by radial immunodiffusion (RID; Triple J Farms, Bellingham, WA, USA). Samples were thawed in an agitated water bath at 38 °C and diluted 1:10 in 0.9% saline solution. Aliquots of 5 µL were added to RID wells along with manufacturer-provided standards. Plates were incubated at 22 °C for 24 h, and precipitation ring diameters were measured using a digital caliper. IgG concentrations were calculated using standard curves and corrected for dilution. The same procedure was applied to calf serum samples, without prior dilution, for IgG quantification. Each sample was analyzed once; therefore, duplicate measurements were not available to assess within-assay variability. IgG concentrations were determined based on the calibration range established by the manufacturer-provided standards (280 to 2800 mg/dL).

Total IgG mass produced per cow was calculated as the product of colostrum IgG concentration and total colostrum yield. Provided IgG was defined as the total mass of IgG consumed by each calf across both feeding meals and was calculated as the sum of the IgG masses ingested in each meal.

AEA was calculated according to Quigley and Drewry (1998) [20] using the following equation:

$$\text{AEA (\%)} = (\text{serum IgG} \times \text{BW} \times 0.09/\text{provided IgG}) \times 100$$

where serum IgG (g/L) was measured at 48 h, and BW is the birth weight (kg). The plasma-volume coefficient of 0.09 represents an estimated plasma volume of 0.09 L/kg of body weight.

2.5. Statistical Analysis

Explanatory variables included CMT score, colostrum Brix, colostrum volume, colostrum IgG concentration, total IgG yield, total IgG fed to calves, parity, SCC, TPC, TCC, ECC, microbiological culture results, calf serum Brix, calf serum IgG and AEA. All analyses were conducted at the cow level rather than at the quarter level, considering that a composite sample would provide results more consistent with the colostrum that is provided to the calf after total milking.

Correspondence analysis was conducted to evaluate the association between categorical CMT score groups and bacteriological culture classification. Accordingly, associations among variables were assessed using Spearman's rank correlation. Principal component analysis (PCA) was performed to visualize relationships among variables and observations (individual cows). The PCA was based on a Spearman correlation matrix due to non-normality of the data. Results were presented in a biplot, along with eigenvalues, eigenvectors, and correlations between original variables and principal components. Associations between CMT score categories and colostrum microbiological culture results were evaluated using the chi-square test.

Colostrum-related variables were compared across CMT score categories using generalized linear models. Colostrum volume, brix, IgG, and total IgG1 were analyzed considering a normal distribution, whereas SCC and TPC were analyzed for a lognormal distribution, and ECC and TCC were analyzed based on a negative binomial distribution due to the occurrence of zero values. In general, all the models for the above-mentioned variables were similar, including the effect of parity order as a covariate, and differed only in the distribution of the response variable. Adjusted means, standard errors of the means, and 95% confidence intervals were back-transformed to the original scale of the variables when non-normal distributions were used. The model is as follows below:

$$y_{ijk} = \mu + CMT_i + Parity_j + \varepsilon_{ijk}$$

y_{ijk} = value obtained from the response variable for a given CMT score category, dams' parity order, and observation;
μ = constant common to all observations;
CMT_i = CMT score group, $i = 2$;
$Parity_j$ = continuous covariate effect of parity order of the dams;
ε_{ijk} = random error associated with observation y_{ijk}.

Calf colostrum management variables were also compared across CMT score categories using linear mixed models (variables: serum Brix, serum IgG, AEA, and provided IgG). Those models were similar to the above-mentioned one but also included the sex of the calves and birth body weight as covariates. The general model follows below:

$$y_{ijklm} = \mu + CMT_i + Parity_j + Sex_k + BBW_l + \varepsilon_{ijklm}$$

y_{ijklm} = value obtained from the response variable for a given CMT score category, dam's parity order, calf sex, birth body weight, and observation;
μ = constant common to all observations;

CMT_i = CMT score group effect, $i = 2$;
$Parity_j$ = continuous covariate effect of parity order of the dams;
Sex_k = calf sex effect, with $k = 2$;
BBW_l = continuous covariate effect of calf birth body weight;
ε_{ijklm} = random error associated with observation y_{ijklm}.

Calf growth outcomes (body weight, average daily gain, withers height, hip width, and heart girth) were analyzed using repeated measures ANOVA, including the fixed effects of CMT score groups, week of age, and their interaction, with calf included as a random effect. Different variance-covariance structures were tested, and the structure with the lowest Akaike Information Criterion was selected. Compound symmetry (CS) was selected for body weight and withers height, variance components (VC) for ADG, first-order autoregressive moving average [ARMA(1, 1)] for hip width, and unstructured (UN) covariance for heart girth. Model assumptions of normality and homoscedasticity were evaluated using residual plots and histograms. The general model follows below:

$$y_{ijkl} = \mu + CMT_i + Week_j + CMT \times Week_{(ij)} + \delta_k + \varepsilon_{ijkl}$$

y_{ijkl} = value obtained from the response variable for a given CMT score category, week, calf, and observation;
μ = constant common to all observations;
CMT_i = CMT score group, $i = 2$;
$Week_j$ = week effect, $j = 6$;
$CMT \times Week_{(ij)}$ = interaction effect between CMT score and week;
δ_k = random effect with repeated measures for calves, $k = 93$;
ε_{ijkl} = random error associated with observation y_{ijkl}.

All statistical analyses were performed using SAS OnDemand, version 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were obtained using PROC MEANS and PROC UNIVARIATE. Spearman correlations were obtained using PROC CORR; chi-square tests using PROC FREQ; PCA using PROC PRINQUAL and PROC PRINCOMP; ANOVA, and mixed models using PROC GLIMMIX. Associations were considered statistically significant at $p \leq 0.05$. All findings were interpreted within the context of the exploratory nature of the study and should therefore be considered preliminary rather than confirmatory.

3. Results

Samples were collected from 93 Holstein cows, primiparous and multiparous, and their respective 93 calves (67 females and 26 males) were followed up. Based on the CMT score results, cows were divided into two groups: LOW: cows with CMT score < 2 (traces and score 1; N = 58); and HIGH: cows with CMT score ≥ 2 (includes score 2 and score 3; N = 35). Among the 35 cows in the HIGH group, 18 (51.4%) presented elevated CMT scores in only one mammary quarter (score ≥ 2), whereas 17 (48.6%) presented elevated scores in two or more quarters.

Colostrum immunological quality showed median ± MAD (median absolute deviation) values of 30.3 ± 3.8% as measured by Brix refractometry and an IgG concentration of 179.6 ± 30.5 g/L by RID. Median volume of colostrum yield was 5.5 ± 1.75 L per cow. Median colostrum volume provided for calves was 4.15 L in the LOW group and 3.89 L in the HIGH group. Among calves receiving less than the target volume of 4.5 L, median colostrum volume was 2.97 L (n = 10) in the LOW group and 3.12 L (n = 11) in the HIGH group.

Figure 1 presents the relationship between CMT scores and microbiological culture results according to agent pathogenicity. The first two dimensions explain 100% of the total variation (Dimension 1: 86%; Dimension 2: 14%). Spatial proximity between categories

indicates stronger associations, with major pathogens clustering closer to higher CMT scores, and lower CMT scores clustering closer to minor pathogens and negative cultures.

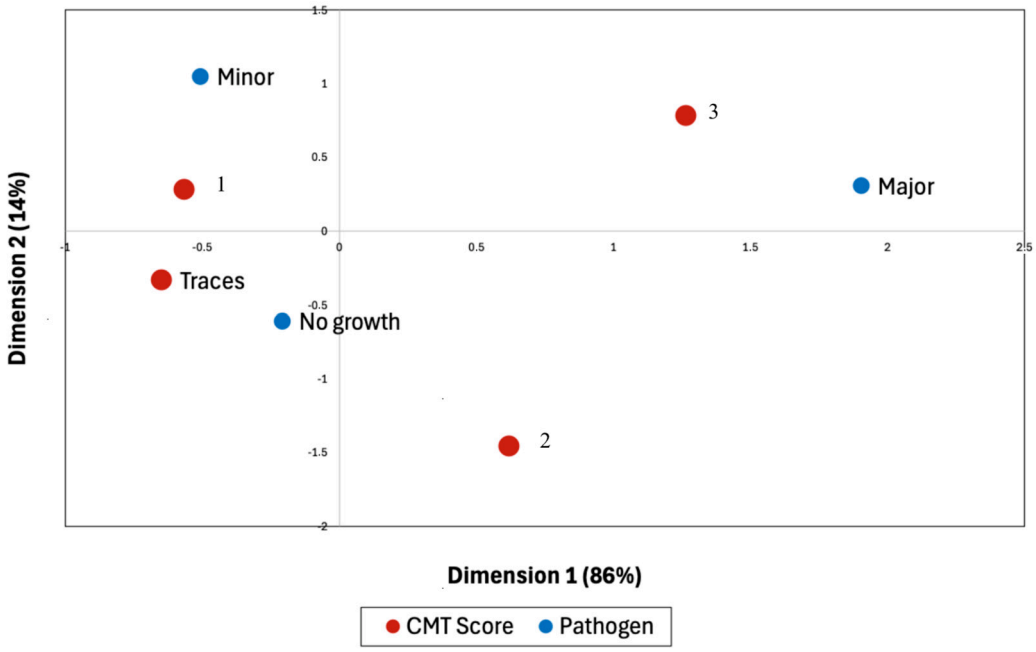


Figure 1. Correspondence analysis plot illustrates the relationship between CMT scores and bacteriological culture classification. Red points represent CMT scores, and blue points represent pathogen groups (major pathogens—*Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Enterobacter* spp., *Enterococcus* spp., *Escherichia coli*, *Serratia marcescens*, *Klebsiella* spp., and *Candida* spp.; minor pathogens—NAS; *Staphylococcus chromogenes*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, and *Staphylococcus xylosus*, and *Corynebacterium* spp.; and no growth); The relative proximity between points indicates the degree of association between categories.

Of the 93 analyzed samples, only 43% (N = 40) showed microbial growth on culture, as presented in Table 1.

Table 1. Distribution of microbiological culture results according to CMT score for colostrum samples (Qui-square test).

Culture Result	LOW [1]	HIGH [2]
Major pathogens	5 (8.6%)	8 (22.9%)
Minor pathogens	19 (32.7%)	8 (22.9%)
No growth	34 (58.6%)	19 (54.2%)
Total	58 (100%)	35 (100%)

[1] LOW: Traces or Score 1; [2] HIGH: Score 2 or Score 3; Qui-square = 3.97; *p*-value = 0.137; Major pathogens—*Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Enterobacter* spp., *Enterococcus* spp., *Escherichia coli*, *Serratia marcescens*, *Klebsiella* spp. and *Candida* spp.; Minor pathogens—NAS; *Staphylococcus chromogenes*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, and *Staphylococcus xylosus*, and *Corynebacterium* spp.; and no growth.

Among the cows in the LOW group, 24 had a pathogen isolated in colostrum between major and minor pathogens (41.4%), whereas in the HIGH group this proportion was 45.7% (N = 16) (Table 1). *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Enterobacter* spp., *Enterococcus* spp., *Escherichia coli*, *Serratia marcescens*, *Klebsiella* spp., and *Candida* spp. were classified and grouped as major mastitis agents, while non-aureus staphylococci (NAS; *Staphylococcus chromogenes*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, and *Staphylococcus xylosus*) and *Corynebacterium* spp. were classified and grouped as minor agents.

Among major pathogens detected in the colostrum of the LOW group cows were *Candida* spp. (3), *Staphylococcus aureus* (1), and *Streptococcus dysgalactiae* (1). In the HIGH group, the following major pathogens were identified: *Enterobacter ludwigii* (1), *Enterococcus faecalis* (1), *Serratia marcescens* (1), *Staphylococcus aureus* (1), *Escherichia coli* (1), *Klebsiella pneumoniae* (1), and *Candida* spp. (2). Although major pathogens were more prevalent among cows from the HIGH group (61.5%), this association was not statistically significant (Table 1; $p = 0.137$).

Staphylococcus chromogenes was the most prevalent species among colostrum from LOW cows. In contrast, cows with higher CMT reactivity exhibited a more diverse microbial profile, including pathogens of greater virulence (major pathogens). Overall, the second most prevalent species was *Staphylococcus xylosus*.

A moderate positive Spearman correlation was observed between the CMT score and colostrum SCC (r = 0.63), as well as with parity (r = 0.35; Figure 2). In contrast, colostrum volume, colostrum Brix, colostrum IgG concentration, ECC, TCC, CPP, serum Brix, serum IgG, and AEA were not strongly correlated with CMT score. Colostrum yield showed a strong positive correlation with total IgG mass produced by the cow (r = 0.68) and a moderate positive Spearman correlation with the amount of IgG fed to the calf (r = 0.38). A moderate positive correlation was found between colostrum IgG concentration and colostrum Brix (r = 0.41), as well as with the amount of IgG fed to the calf (r = 0.38). Colostrum IgG concentration was strongly and negatively correlated with AEA (r = −0.78) and positively correlated with total IgG mass produced (r = 0.56) and IgG mass fed to the calf (r = 0.86). A moderate positive correlation was observed between SCC and parity (r = 0.34), while a strong positive correlation was found between ECC and TCC (r = 0.72). Serum Brix was strongly positively correlated with serum IgG concentration (r = 0.80), and serum IgG showed a moderate positive correlation with AEA (r = 0.40). AEA showed moderate negative correlations with total IgG mass produced (r = −0.32) and parity (r = −0.31), as well as a strong negative correlation with IgG mass fed to the calf (r = −0.66). Total IgG mass fed was strongly correlated with IgG mass produced (r = 0.77). Finally, parity was moderately positively correlated with both total IgG mass produced (r = 0.32) and IgG mass fed to the calf (r = 0.30).

The PCA biplot (Figure 3) illustrates the heterogeneity of observations in the present study, with sampling units (cows) distributed within all four quadrants of the axes. The first two principal components (PC1 = 25% and PC2 = 18%) accounted for 43% of the total variance in the dataset, indicating that a substantial proportion of the variability was not captured by this two-dimensional representation. Therefore, the biplot should be interpreted primarily as a descriptive visualization of the correlation structure among variables and the distribution of observations, rather than as a comprehensive representation of the variability of the dataset. Variables positioned closer to the axes contributed more strongly to the principal components. The position of each point reflects the magnitude of the measured variables according to its distribution within the quadrants. Observations located closer to a given variable indicate higher values for that parameter. In addition, the proximity between variables reflects the potential strength of their associations. Accordingly, the pairs colostrum Brix and colostrum IgG, and serum IgG and serum Brix, showed stronger relationships. The variables ECC, TCC, SCC, and CMT score also presented proximity in the biplot, suggesting potential relationships among these parameters. The distribution of individual observations across the quadrants further highlights the heterogeneity among cows in the measured variables.

Correlations in Table 2 indicate the association degree between each variable and the principal component axes. The closer the magnitude of the correlation is to 1, the greater the contribution of the variable to the respective axis.

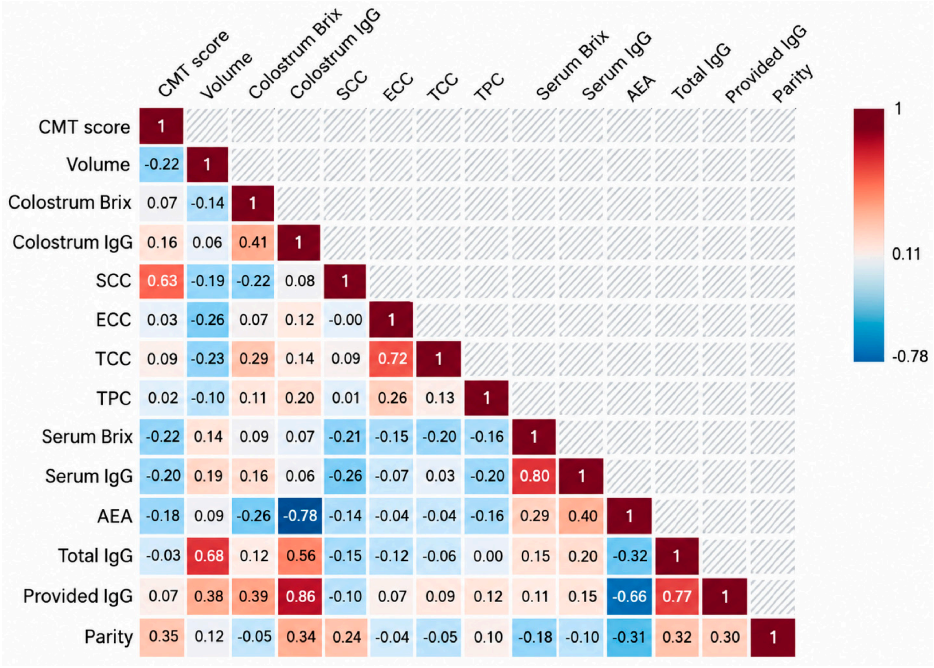


Figure 2. Heatmap of Spearman correlation coefficients between variables of cows, calves, and the CMT score. Volume: colostrum volume; Colostrum IgG: colostrum IgG concentration; ECC: Escherichia coli count; TCC: total coliform count; TPC: total plate count; Serum Brix: serum Brix of calves after 48 h; Serum IgG: serum IgG concentration in calves after 48 h; AEA: apparent efficiency of absorption of IgG; Total IgG: total IgG produced by the cow; Provided IgG: total IgG provided to the calf.

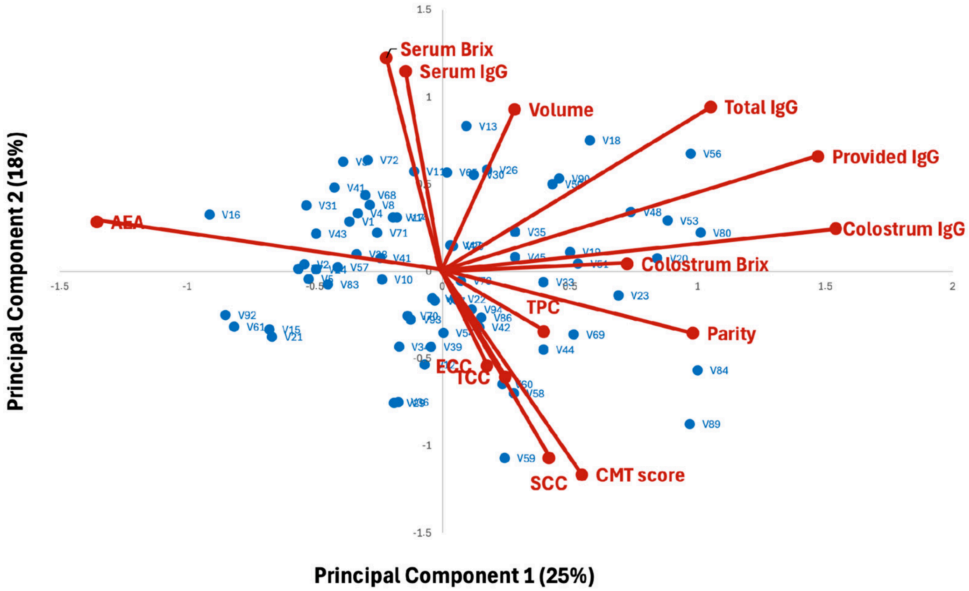


Figure 3. A biplot of Principal Component Analysis (PCA) representing animal distribution and variable correlation with components. Blue dots represent individual experimental units, whereas red lines and dots represent the vectors of the evaluated variables, indicating their direction and association with the principal components. Overlapping sample labels reflect the close proximity of distinct experimental units and do not represent duplicated observations. Volume: colostrum volume; Colostrum IgG: colostrum IgG concentration; SCC: somatic cell count; ECC: Escherichia coli count; TCC: total coliform count; TPC: total plate count; Serum Brix: serum Brix of calves after 48 h; serum IgG: serum IgG concentration in calves after 48 h; AEA: apparent efficiency of absorption; Total IgG: total IgG produced by the cow; Provided IgG: total IgG provided to the calf.

Table 2. Eigenvectors, eigenvalues and correlations of the variables with the principal components (PCA).

Variables	Eigenvectors		Correlations	
	PC1	PC2	PC1	PC2
CMT score	0.174	−0.399	0.322	−0.642
Volume	0.090	0.317	0.167	0.511
Colostrum Brix	0.231	0.014	0.427	0.023
Colostrum IgG	0.492	0.084	0.910	0.135
SCC	0.133	−0.366	0.246	−0.589
ECC	0.055	−0.186	0.103	−0.299
TCC	0.078	−0.208	0.145	−0.334
TPC	0.126	−0.118	0.234	−0.190
Serum Brix	−0.070	0.418	−0.130	0.673
Serum IgG	−0.046	0.392	−0.085	0.631
AEA	−0.432	0.097	−0.799	0.156
Total IgG	0.335	0.322	0.621	0.518
Provided IgG	0.335	0.322	0.621	0.518
Parity	0.469	0.225	0.869	0.362
Eigenvalues	3.42	2.58	Cumulative	6.00
Explained variance	25.0%	18.0%	Cumulative	43.0%

PC1: Principal Component 1; PC2: Principal Component 2; Volume: colostrum volume; SCC: somatic cell count; ECC: Escherichia coli count; TCC: total coliform count; TPC: total plate count; Serum Brix: serum Brix of calves after 48 h; Serum IgG: serum IgG concentration in calves after 48 h; AEA: apparent efficiency of absorption; Total IgG: total IgG produced by the cow; Provided IgG: total IgG provided to the calf.

The variables contributing most to PC1 were colostrum IgG (r = 0.910), provided IgG (r = 0.621) and parity (r = 0.869), along with the negative association of AEA (r = −0.799). Variables associated with PC2 included volume (r = 0.511), serum Brix (r = 0.673), serum IgG (r = 0.631), and provided IgG (0.518), as well as the negative associations of CMT score (r = −0.642) and SCC (r = −0.589). The PC1 axis reflects a variation related to IgG yield and AEA in calves, whereas PC2 is associated with indicators of IgG uptake by the calf.

Dams from the LOW group produced a higher volume than those in the HIGH group (7.3 vs. 4.9 L; $p = 0.0045$), as shown in Table 3. However, variables regarding colostrum immunological quality (colostrum brix and colostrum IgG) did not show any significant differences between groups ($p = 0.1710$ and $p = 0.5774$, respectively). Colostrum SCC was significantly higher in the HIGH group compared with the LOW group (887 vs. 279 × 10^4 cells/mL; $p < 0.0001$). No significant differences were observed between groups for bacterial load counts, including ECC ($p = 0.3495$), TCC ($p = 0.3700$), or TPC ($p = 0.8631$). Conversely, total IgG production was significantly higher in the LOW group compared with the HIGH group (1194 vs. 808 g; $p = 0.0098$).

Table 3. Adjusted means ± standard error (95% confidence interval) Median ± MAD of colostrum variables between groups of dams with different CMT scores.

Variables	N	Colostrum		*p*-Value
		LOW [1]	HIGH [2]	
		Adjusted Means ± SE (95%CI)		
Volume, L	90	7.3 ± 0.47 (6.3–8.2)	4.9 ± 0.63 (3.6–6.2)	0.0045
Brix, %	90	30.9 ± 0.61 (29.7–32.1)	32.4 ± 0.81 (30.8–34.0)	0.1710
IgG, g/L	90	162 ± 7.3 (147–176)	169 ± 9.8 (150–188)	0.5774
SCC, ×10^4 cells/mL	88	279 ± 27.8 (228–669)	887 ± 122.3 (339–1154)	<0.0001

Table 3. *Cont.*

Variables	N	Colostrum		*p*-Value
		LOW [1]	HIGH [2]	
		Adjusted Means ± SE (95%CI)		
ECC, ×10^3 CFU/mL	81	12.5 ± 5.9 (4.8–32.0)	26.7 ± 16.7 (7.7–92.7)	0.3495
TCC, ×10^3 CFU/mL	71	32.1 ± 22.4 (8.0–128.8)	100.2 ± 95.5 (15.0–671.0)	0.3700
TPC, ×10^6 CFU/mL	78	226 ± 66.0 (123–384)	257 ± 107.3 (106–527)	0.8631
Total IgG [3], g	90	1194 ± 85.0 (1025–1363)	808 ± 113.7 (582–1034)	0.0098

[1] LOW: Traces or Score 1; [2] HIGH: Score 2 or Score 3; ECC: Escherichia coli count; TCC: total coliform count; TPC: total plate count; [3] Total IgG: total IgG produced by the cow.

Table 4 shows that despite the tendency for higher serum Brix values for calves born from LOW cows ($p = 0.0577$), no statistically significant differences were detected between groups for serum IgG concentration ($p = 0.1412$), apparent efficiency of absorption ($p = 0.4223$), or total IgG mass provided to the calves ($p = 0.7015$). Although adjusted means were numerically higher in the LOW group across all variables, these differences were not statistically significant.

Table 4. Adjusted means ± standard error (95% confidence interval) of variables between groups of calves born from cows with different CMT scores.

Variables	N	CMT Scores		*p*-Value
		LOW [1]	HIGH [2]	
		Adjusted Means ± SE (95%CI)		
Serum Brix, %	93	11.2 ± 0.15 (10.9–11.5)	10.7 ± 0.20 (10.3–11.1)	0.0577
Serum IgG, g/L	93	24.9 ± 0.63 (23.6–26.1)	23.4 ± 0.83 (21.7–25.0)	0.1412
AEA [3], %	93	26.6 ± 1.37 (23.9–29.3)	24.8 ± 1.80 (21.2–28.4)	0.4223
Provided IgG [4], g	93	664 ± 34.2 (596–731)	642 ± 45.0 (553–731)	0.7015

[1] LOW: Traces or Score 1; [2] HIGH: Score 2 or Score 3; [3] AEA: apparent efficiency of absorption; [4] Provided IgG: total IgG mass provided to the calf.

Table 5 indicates calf body measurements over time. Differences were found across weeks for all performance variables (age effect; $p < 0.0001$), which is expected due to calf growth. However, no significant effect of dam CMT score was detected for body weight ($p = 0.1093$), ADG ($p = 0.4862$), withers height ($p = 0.1797$), hip width ($p = 0.6842$), or heart girth ($p = 0.0810$). Although adjusted means were numerically higher in calves born from LOW CMT score cows across all measurements, these differences were not statistically significant. Furthermore, no significant CMT score × week interactions were observed for any of the evaluated variables.

Table 5. Adjusted means ± standard error (95% confidence interval) for calf body measurements over time by dams' CMT score, including week and interaction effects.

Variables	N	CMT Score		*p*-Values		
		Adjusted Means ± SE (95%CI)				
		LOW [1]	HIGH [2]	CMT Score	Week	CMT × Week
Body weight, kg	482	51.8 ± 0.69 (50.5–23.2)	50.0 ± 0.86 (48.3–51.8)	0.1093	<0.0001	0.1976
ADG, kg/d	391	0.44 ± 0.02 (0.40–0.48)	0.41 ± 0.03 (0.37–0.46)	0.4862	<0.0001	0.8160
Withers height, cm	484	80.4 ± 0.42 (79.5–81.2)	79.4 ± 0.53 (78.4–80.5)	0.1797	<0.0001	0.7143
Hip width, cm	485	21.0 ± 0.21 (20.6–21.5)	20.9 ± 0.27 (20.4–21.4)	0.6842	<0.0001	0.2731
Hearth girth, cm	485	82.8 ± 0.53 (81.7–83.8)	81.3 ± 0.66 (79.9–82.6)	0.0810	<0.0001	0.8832

[1] LOW: Traces or Score 1; [2] HIGH: Score 2 or Score 3; N = number of observations; growth observations performed in 93 calves.

4. Discussion

The importance of providing colostrum of high immunological and microbiological quality for successful passive transfer in calves is well established in the literature. However, relatively few studies have specifically investigated the microbiological quality of colostrum fed to dairy calves and rapid means to assess it. Considering CMT as a fast on-farm tool for identifying intramammary infections, the present study aimed to evaluate its potential as a cow-side indicator of colostrum microbiological quality.

Out of the 93 cows evaluated, 58 exhibited lower CMT reactivity (LOW: traces or score 1), whereas 37.6% showed higher reactivity (HIGH: score 2 or score 3), which, in milk, would typically indicate subclinical mastitis. Because colostrum is a secretion that physiologically contains higher cellularity than milk [21], some degree of reactivity was expected. However, it was hypothesized that higher CMT scores would be associated with intramammary infections at calving, i.e., bacterial load, reflected by elevated SCC. A positive correlation between CMT and SCC was observed, supporting the use of CMT as an indicator of cellularity in colostrum. Furthermore, the significant differences in SCC between groups reinforce the ability of CMT to identify variations in SCC, consistent with its established application in milk.

An important methodological consideration is that SCC and microbiological culture were evaluated using equal-volume composite samples collected from the four mammary quarters before complete milking, whereas bacterial plate counts and IgG concentrations were determined using samples collected from the homogenized whole-colostrum by the end of milking. These sampling matrices may not be directly comparable. Because equal volumes from each quarter were combined, the composite samples did not account for differences in colostrum production among quarters and may not represent entirely the composition of the colostrum consumed by the calf. Furthermore, pooling samples from all quarters could dilute the contribution of a quarter with a particularly high SCC or bacterial load. Conversely, bacterial contamination introduced during milking, collection, or handling could influence bacterial counts in the composite samples. Therefore, differences in sampling procedures and quarter contribution should be considered when interpreting the agreement between SCC, culture results, bacterial counts, and IgG concentrations.

The median SCC observed in the present study was 3668×10^3 cells/mL, higher than that reported by Ferdowsi et al. [9] and within the range described for infected mammary glands [7]. Differences among studies may partially reflect methodological variation, as previous studies used flow cytometry. Cows in the LOW group presented lower levels of SCC (1410×10^3 to 3440×10^3 cells/mL) in comparison to cows from the high-reactivity group (5057×10^3 to $13{,}885 \times 10^3$ cells/mL). These two ranges are in accordance with the expected ranges for colostrum collected from healthy and infected mammary glands, respectively [7].

Initial concern was raised regarding the preservation and integrity of colostrum immune cells after sample thawing, since no preservative was added prior to freezing. However, upon processing the samples, cell structures were observed to remain intact. Although this observation does not establish that cell counts were quantitatively preserved after freezing and thawing, it indicates that cellular structures remained morphologically recognizable. The inherent preservative properties of colostrum have been discussed in previous studies [22]; however, whether these properties contribute to the preservation of bovine colostrum leukocytes during freezing remains to be determined.

The SCC was markedly higher in colostrum from cows in the HIGH group compared to those in the LOW group. Colostrum naturally contains higher SCC than milk, primarily due to the immune cell population. In cases of intramammary infection, there is a marked increase in leukocytes and epithelial cell shedding due to inflammation, leading the cell

count to rise above physiological ranges [23,24]. Considering the literature previously cited, it is reasonable to speculate that the elevated counts observed in colostrum with higher CMT scores were due to an increase in multiple cell types, rather than solely macrophages present in a healthy mammary gland from a fresh cow. Despite that, differential leukocyte counts were not performed in this study, preventing the identification and quantification of individual cell populations and limiting our ability to determine which cell types contributed most to the elevated SCC. Although duplicate preparations were performed, this method may be more susceptible to observer-dependent variability and provides limited information on the cellular composition of colostrum compared with automated approaches. Therefore, the results should be interpreted as estimates of total cellularity rather than precise characterization of individual cell populations. Nevertheless, the SCC reported here is consistent with those described by previous authors [7], in which groups were classified according to the presence of infection, and the highest median SCC values were observed in colostrum samples positive for microbiological growth.

Microorganism prevalence at calving was similar between groups, with isolates detected in 41.3% and 45.7% of samples from LOW and HIGH-reactivity groups, respectively. *Enterobacter ludwigii*, *Enterococcus faecalis*, *Serratia marcescens*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida* spp. were isolated from cows with higher CMT. Although no differences were found in microorganism prevalence in the present study, these major pathogens may act as mastitis agents known to induce greater increases in SCC, which could explain scenarios where higher CMT reactivity is observed [17].

A relevant consideration when interpreting the microbiological findings is the use of composite colostrum samples rather than individual quarter samples. Although the isolation of viable bacterial colonies meeting the established growth criteria provides bacteriological evidence consistent with intramammary infection, this sampling approach does not allow identification of the affected mammary quarter or definitive confirmation of infection at quarter level. No evidence of contamination was observed based on the established criteria, as none of the samples presented growth patterns consistent with contamination. Nevertheless, the possibility of contamination during sample collection or transient bacterial presence in the teat canal cannot be completely excluded. Therefore, microbiological results should be interpreted considering these limitations of composite milk sampling.

Another methodological consideration is the potential influence of sample storage on bacterial recovery. In the present study, colostrum samples were stored at −20 °C for three months before microbiological processing. Although freezing allows sample preservation, prolonged storage and subsequent thawing may affect bacterial viability, depending on the microorganism and its susceptibility to temperature-related stress. Consequently, reductions in viable bacterial populations during storage or thawing may have influenced bacterial recovery in culture, potentially contributing to an underestimation of microbiological contamination. However, as the effect of freezing and thawing on bacterial viability was not directly evaluated in this study, its potential contribution to the microbiological findings remains uncertain.

Non-Aureus Staphylococci, including *Staphylococcus capitis*, *S. xylosus*, and *S. chromogenes*, were frequently isolated, particularly in samples with lower CMT reactivity. In addition to being associated with subclinical infections, these microorganisms are also often isolated in postpartum cows without an active infection, causing a moderate increase in SCC [25].

Staphylococcus aureus and environmental pathogens such as *Escherichia coli* were detected in cows with higher SCC and more severe inflammatory responses. Further, five different *Candida* species were identified in the samples, indicating the presence of diverse yeast contaminants in the colostrum. Although yeast contamination may be associated with

environmental exposure and inadequate hygiene during collection and handling [26], the present findings alone do not establish the specific source of contamination or demonstrate failures in biosecurity practices. Their detection alongside the detection of high bacterial loads may point to challenges in maintaining adequate hygiene during colostrum collection and handling. Together, these findings highlight the importance of appropriate hygiene practices to minimize microbial contamination of colostrum.

Although infection prevalence did not differ between groups, a few factors must be considered. Intermittent shedding of pathogens and composite sampling represent limitations to bacterial growth evaluation, which can lower the odds of isolation due to dilution and the time point of collection, even in high-SCC samples. Previous studies have reported higher isolation rates for 3 days postpartum, suggesting that some infections may not be detected at calving [10]. Consequently, pathogens can still be present despite a lack of isolation, interfering in colostrogenesis and posing as an initial threat to neonate calves.

The overall median colostrum IgG concentration exceeded the recommended thresholds of 50 g of IgG/L for high-quality colostrum [27], as well as the total IgG mass provided to calves of 300 g established by Godden et al. [28]. At first, we hypothesized that cows with lower CMT scores would have higher IgG concentration. However, no difference in IgG concentration was observed between CMT groups, consistent with previous findings indicating that infection does not necessarily alter IgG concentration but may reduce colostrum volume [7]. In the present study, cows in LOW produced a higher volume of colostrum than cows in the HIGH group. Accordingly, total IgG yield differed between groups, reflecting the greater colostrum volume produced by cows in the LOW group rather than differences in IgG concentration.

Additionally, considering the low correlation between colostrum Brix and colostrum IgG concentration, it is reasonable to question other factors that may interfere with refractometer readings, including SCC and colostrum composition. Certain components of colostrum composition affect its refractive index, such as high fat content and casein micelles [29]. Another hypothesis is that increased SCC itself may contribute to higher Brix readings due to the increase in cellularity, since cells also add to the protein content of the sample. Although this potential effect has not been established, there could be a confounding factor caused by the mammary gland inflammatory status around parturition. Thus, even though refractometry is established as the most important tool for on-farm colostrum quality evaluation, further research is warranted to investigate whether elevated SCC may influence Brix readings and could interfere in the interpretation of colostrum quality.

Total plate count was high in both groups, with no association with ECC or TCC. However, the exclusion of plates classified as "too numerous to count" may have resulted in an underestimation of bacterial counts, particularly if these samples were not evenly distributed between CMT groups. McGuirk and Collins [30] reported counts ranging from 150×10^3 to $32{,}000 \times 10^3$ CFU/mL, and in the study by Kayasaki et al. [31] the mean TPC was 1224×10^3 CFU/mL. Milking hygiene equipment management and preservation of teat integrity should be considered when interpreting the relationship between mammary health indicators and the microbiological quality of harvested colostrum [32]. Additionally, bacterial contamination introduced during harvesting can increase the bacterial load of colostrum independently of its initial mammary origin, potentially weakening associations between CMT reactivity and postmilking bacterial counts [33].

Although calves receiving colostrum from cows in the LOW group exhibited higher serum Brix values, no differences were detected in serum IgG concentration or AEA between groups. It is important to note that serum Brix is an indirect estimate of serum protein concentration and is not specific to IgG, as its values may be influenced by other circulating proteins and physiological factors, including hydration status. Therefore, the

higher serum Brix values observed in calves from the LOW group should not necessarily be interpreted as evidence of improved IgG absorption or transfer of passive immunity. Considering the complexity of colostrum composition and the interactions among its immunomodulatory components in the neonatal gut, other colostrum constituents may have contributed to the observed differences in serum Brix values, although this hypothesis warrants further investigation.

Colostrum naturally has a higher proportion of macrophages compared to lymphocytes and neutrophils [34]; nonetheless, increases in the SCC due to inflammation may alter this cellular profile, particularly by an increase in the neutrophil population. In addition, inflammation of the mammary gland is associated with increased concentrations of immune mediators and inflammatory proteins, including cytokines, which may be secreted into colostrum and potentially influence intestinal permeability and IgG absorption [35]. Although these mechanisms provide a possible biological link between mammary gland inflammation and neonatal intestinal function, neither colostrum cytokine concentrations nor intestinal permeability or injury were evaluated in the present study. Therefore, their potential contribution to IgG absorption remains speculative and warrants further investigation.

Microorganisms present in colostrum may also represent a potential influence on neonatal IgG uptake. Previous studies have proposed that interactions between IgG and microorganisms may affect IgG availability for absorption [36], while microbial adhesion to intestinal epithelial cells may potentially compromise epithelial integrity and absorptive function [37]. These mechanisms combined could theoretically contribute to a reduction in the amount of IgG available for uptake and its subsequent absorption by the calf. Although no differences in serum IgG concentration or AEA were detected between groups in the present study, the potential interactions between microorganisms present in colostrum, IgG availability, and intestinal function remain biologically relevant. However, bacterial counts did not differ between groups in the present study, and neither IgG binding to microorganisms nor intestinal integrity was directly assessed. Therefore, the extent to which these mechanisms influence IgG uptake under the conditions of the present study remains unknown. Given the substantial potential of colostrum to act as a vehicle for microbial exposure to calves at their first feeding, further mechanistic investigations are warranted to elucidate the relationships between microorganisms present in colostrum, IgG availability and absorption, as well as calf responses.

An important consideration when interpreting the correlations involving AEA is the mathematical coupling among the variables. Because AEA is calculated using serum IgG concentration in the numerator and provided IgG in the denominator, while provided IgG itself is determined by colostrum IgG concentration and the volume consumed, correlations involving these variables are partially influenced by their mathematical relationship. Therefore, these associations should be interpreted cautiously, as they do not independently establish biological relationships or mechanisms influencing IgG absorption efficiency.

Among the calf body measurements evaluated, no significant differences between groups were detected. Similarly, no significant group-by-week interactions were observed, indicating that the trajectories of body measurements over time did not differ between CMT groups. Therefore, our findings provide no evidence that colostrum CMT results could be associated with differences in calf body size or growth patterns over the evaluated period, or that they could predict subsequent performance.

5. Conclusions

On-farm microbiological assessment of colostrum using rapid and accessible methods prior to feeding remains challenging. In our study, CMT reactivity was not associated with microbiological colostrum quality, and its potential as a screening tool for this purpose

was not supported by our findings. Furthermore, no significant differences between CMT groups were observed for the evaluated colostrum quality indicators or calf passive transfer outcomes, indicating that CMT reactivity did not reliably discriminate between these parameters under the conditions of this study. Although differences in colostrum yield and, consequently, in the total mass of IgG produced were observed between groups, these findings should be interpreted in the context of colostrum production rather than as evidence of differences in colostrum quality. Overall, this study contributes to the evaluation of CMT as a potential on-farm screening approach and highlights its limitations for assessing microbiological colostrum quality. Further research is needed to identify rapid, practical, and reliable on-farm methods that can support microbiological colostrum assessment and assist producers in making timely decisions regarding colostrum management.

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Institutional Review Board Statement: All animal procedures were authorized on March of 2021 by the Ethical Committee on the Use of Animals of the Escola Superior de Agricultura "Luiz de Queiroz" (CEUA/ESALQ), University of Sao Paulo, Piracicaba, SP, Brazil, protocol n° 64452602210.

Informed Consent Statement: Not applicable, since the male calves were donated by the commercial farm for the trial.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data presented in this study are not publicly available due to restrictions by the research group.

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Abbreviations

The following abbreviations are used in this manuscript:

AEA	Apparent efficiency of absorption
CFU	Colony-forming units
CMT	California Mastitis Test
ECC	*Escherichia coli* count
MAD	Median absolute deviation
RID	Radial immunodiffusion
TCC	Total coliform count
TPC	Total plate count
TPI	Transfer of passive immunity

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