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An Observational Study of the Microbiological Quality of Bovine Colostrum Fed to Calves on Three Dairy Farms

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Simple Summary

This study investigated the microbiological quality of bovine colostrum across three dairy farms employing different hygiene practices during colostrum management. The aim of this study was to assess the levels of various microbial populations and physicochemical properties (pH, water activity, and Brix) in relation to established quality guidelines. Colostrum quality was categorized as good or excellent based on specific thresholds for microbial counts and Brix values. The findings revealed that only a moderate proportion of samples met the defined quality criteria. The farm with automated cleaning of feeding equipment showed lower microbial loads. Furthermore, heat treatment appeared to be beneficial, as colostrum from the farms using this method had reduced levels of total bacteria, coliforms, and *E. coli* compared to the farm feeding raw colostrum. This study concludes that a considerable fraction of calves received colostrum of suboptimal microbiological quality, particularly when less rigorous hygiene protocols were in place, underscoring the importance of effective hygiene practices in colostrum management for calf health.

Abstract

This study aimed to evaluate the microbiological quality of colostrum on three dairy farms with different colostrum management hygiene practices and to compare it with the current colostrum quality guidelines. On farm A, colostrum was fed raw, while on farms B and C it was heat treated. On farms A and B, the feeding equipment was cleaned manually, while on farm C, an automated cleaning system was used. Samples were collected from the calf-feeding equipment and submitted for microbial culture: total plate count (TPC); total coliform count (TCC); and *E. coli*, enterobacteria (ENTB), staphylococci (STAP), and lactic acid bacteria counts. In addition, pH, water activity (a_W), and Brix were analyzed. Colostrum quality was defined as follows: good quality (GQ)—TPC < 100,000, TCC < 10,000, STAP < 50,000 cfu/mL, and Brix $\geq$ 22%; excellent quality (EQ)—TPC < 20,000, TCC < 100, STAP < 5000 cfu/mL, and Brix $\geq$ 25%. Mean concentrations were as follows: TPC was 3.99×10^5 cfu/mL (min: 40.00, max: 1.32×10^7 cfu/mL); TCC was 1.17×10^4 cfu/mL

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(min: <detection limit, max: 6.37×10^5 cfu/mL); and STAP was 1.77×10^4 cfu/mL (min: <detection limit, max: 3.50×10^5 cfu/mL). Approximately 54% (GQ) and 32% (EQ) of samples met the defined criteria. Farm C consistently showed lower microbial counts across all culture types. Colostrum from farm B had lower TCC, LAB, and *E. coli* counts than farm A but not TPC, STAP, and ENTB. These results showed that a considerable proportion of calves were fed colostrum with suboptimal quality, especially when less rigorous hygiene practices were implemented.

Keywords: colostrum management; brix; heat treatment; hygiene

1. Introduction

Colostrum contains both beneficial and pathogenic bacteria that can be derived from the dam by direct shedding from the mammary gland and via the entero-mammary pathway or contaminated from the environment [1–3]. While beneficial bacteria, such as lactic acid bacteria (LAB), promote gut health [4,5], pathogenic bacteria can hinder it, reducing immunoglobulin absorption [6]. Newborn calves are highly susceptible to infections caused by various pathogens, including bacteria (e.g., *Escherichia coli* and *Salmonella* spp.), protozoa (e.g., *Cryptosporidium parvum* and *Eimeria* spp.), and viruses (e.g., rotavirus and coronavirus) [3,7,8]. Furthermore, calves are born agammaglobulinemic and can only absorb immunoglobulins during the first 24 h of life; therefore, timely colostrum intake is essential to ensure the transfer of immunoglobulins and other immune factors that provide protection during early life [9].

Proper hygiene practices are essential to minimize bacterial contamination of colostrum [3]. The absence of well-established standards for bacterial counts in colostrum [10] or the low adoption of such standards by farmers due to costs and limited training makes it challenging to define specific hygiene protocols for colostrum management, a frequently overlooked aspect of calf care. On the other hand, immunoglobulin concentration can be easily estimated on-site using a Brix refractometer. Brix values $\geq$ 22% usually indicate a good quality colostrum (immunoglobulin G (IgG) $\geq$ 50 g/L) [11]. Current standards for colostrum bacterial contamination are often based on dairy industry milk standards [12] and the guidelines established by McGuirk and Collins, which typically involve a total plate count (TPC) < 100,000 cfu/mL and total coliform count (TCC) < 10,000 cfu/mL [13]. While McGuirk and Collins [13] also suggest targets for Gram-negative non-coliform count (NCC) < 50,000 cfu/mL, streptococci non-ag. < 50,000 cfu/mL, and coagulase negative staphylococci < 50,000 cfu/mL, other studies propose more strict limits, such as TPC < 20,000 cfu/mL, TCC < 100 cfu/mL [10,11], and NCC < 5000 cfu/mL [10,14].

To achieve these goals, dairy farms must implement rigorous colostrum management protocols, emphasizing strict and consistent hygiene practices [12,15]. Dairy cows should be negative for pathogenic microorganisms transmissible through lacteal secretions, such as *Mycobacterium paratuberculosis*, *Salmonella* spp., *Mycoplasma bovis*, *Staphylococcus aureus*, and bovine diarrhea and bovine leukemia viruses [13]. Feeding colostrum from the dam to the calf (one-to-one feeding) can minimize the risk of transmitting infectious agents compared to pooled colostrum [16]. Teats should be thoroughly cleaned before colostrum harvesting, and all materials and equipment used to collect, store, and feed colostrum must be adequately sanitized [13]. Heat treatment (HT) of colostrum (i.e., 60 °C for 60 min) is another commonly recommended practice that may reduce bacterial load [17]; however, its effect on beneficial bacteria, such as LAB, is not entirely clear [4]. Unfed colostrum should be promptly stored in clean, closed containers at 4 °C for up to two days or frozen for longer-term storage [3,11,18]. The addition of preservatives, like potassium sorbate, can extend the refrigeration period [3]. While there is a growing body of research on colostrum management, there remains a significant gap in knowledge regarding specific hygiene practices [8,19,20]. The study by Hyde et al. [21] on British dairy farms highlights the importance of improving hygiene practices during colostrum collection and feeding to reduce bacterial contamination. In addition, better hygiene practices were associated with higher calf serum IgG levels [20], different methods of colostrum administration affected bacterial contamination [21,22], and the type of disinfectant used to clean the feeding apparatus affected the odds of having a TPC < 100,000 cfu/mL [16]. To establish adequate colostrum management protocols, further information is needed regarding the impact of various hygiene practices on both the microbiological and immunological quality of colostrum. Therefore, the aim of this study was to compare the microbiological quality of colostrum fed to calves on three dairy farms with different hygiene practices and to compare the results with current guidelines.

2. Materials and Methods

2.1. Study Design

This study was approved by the Ethics Committee for Animal Welfare (ORBEA) at Universidade de Trás-os-Montes e Alto Douro (UTAD, Portugal) under reference 2664-e-DZ-2023. Three dairy farms from the Alentejo region of Portugal (Table 1) were included in this study, based on their colostrum management protocols, in particular, their colostrum hygiene practices.

Table 1. Herd size and production characteristics of the three dairy farms included in this study.

Farm	Cows in Milking	Average Lactation No	Milk Production 305 d	Milk Fat 305 d	Milk Protein 305 d
A	21	2.7	8813	4.46	3.43
B	484	2.6	13,634	4.2	3.31
C	783	2.1	12,118	4.11	3.22

The milking systems on all three farms were cleaned and sanitized similarly: post-partum cows were milked at the end of the milking session, followed by a mechanical cleaning process involving a warm-water rinse (38–50 °C), a hot-water wash (>60 °C) with sodium hypochlorite disinfectant, and a final cold-water rinse. Post-partum cows were milked within 8 h after calving. Prior to milking, the teats of the post-partum cows were disinfected and cleaned with individual papers. Colostrum-related hygiene practices (Table 2) were as follows:

Table 2. Description of the hygiene practices used during colostrum handling.

Farm	Cleaning of Feeding Apparatus					
	HT [1]	Hot Water	Detergent	Disinfectant	Automated Washing
A	No	40–50 °C	A [2]	Yes [4]	No
B	Yes	40–50 °C	B [3]	No	No
C	Yes	60 °C	No	Yes [5]	Yes

[1] Heat treatment of colostrum at 60 °C for 60 min. [2] Surfactant detergent containing non-ionic (<5%) and ionic (5–15%) constituents. [3] Surfactant detergent with benzenesulfonic acid, C10-13-alkyl derivs., and sodium salts. [4] Sodium hypochlorite solution (2–3% activated chlorine). [5] Sodium hypochlorite solution (≥13% of activated chlorine).

Farm A: Colostrum was collected in a bucket and then frozen (−24 °C) in cleaned 1.5 L polyethylene bottles until use. At calving, the colostrum was thawed at 40 °C and fed to the newborn calf by a nipple bottle. The materials used for collecting, storing, and feeding the calves were washed by hand with hot water (40–50 °C), a surfactant detergent containing non-ionic (<5%) and ionic (5–15%) constituents (Jodel—Hygiene Products Manufacturing, Azambuja, Portugal) and with sodium hypochlorite (NaOCl) solution (2–3% activated chlorine) (Clorosol—Comércio e Indústria de Detergentes, Lda, Vila Nova de Famalicão, Portugal) and left to dry at room temperature.

Farm B: Milked colostrum was heat treated at 60 °C for 60 min with a commercial batch pasteurizer system (Coloquick, Calvex A/S, Skive, Denmark) and frozen (−24 °C) until needed in 4 L Coloquick colostrum bags. At calving, the colostrum was thawed at 40 °C and fed to the newborn calf by a nipple bottle or an oroesophageal tube. The materials used to collect and feed the calves were washed by hand with hot water (40–50 °C) and a surfactant detergent with benzenesulfonic acid, C10-13-alkyl derivs., and sodium salts (MasterChef, Lisbon, Portugal) and left to dry at room temperature.

Farm C: Milked colostrum was heat treated at 60 °C for 60 min with a commercial batch pasteurizer system (Coloquick, Calvex A/S, Denmark) and frozen (−24 °C) until needed in 4 L Coloquick colostrum bags. At calving, the colostrum was thawed at 40 °C and fed to the newborn calf by an oroesophageal tube. The materials used to collect and feed the calves were washed with hot water (60 °C) and sodium hypochlorite (≥13% of activated chlorine; Ipoclorix® PWG, QuimiTécnica.com, S.A, Barreiro, Portugal) with an automated washing system and left to dry at room temperature.

2.2. Sample Collection

Samples of colostrum (n = 68) were collected from three dairy farms (22 from farm A, 13 from farm B, and 33 from farm C) in 100 mL sterile recipients directly from the feeding apparatus used to feed the calf (either a nipple bottle or an oroesophageal tube) just before feeding. To ensure consistency, colostrum was thoroughly mixed prior to sampling and collected directly into a sterile container, always before any contact with the calf's mouth. The fed colostrum was either collected from the farm's colostrum bank or from the dam. Colostrum samples were from both primiparous (n = 33) and multiparous (n = 35) Holstein–Friesian cows, milked within 12 h after birth. To analyze the effect of HT, a sub-sample (n = 6) was collected from farm C before (raw) and after HT. All samples were frozen immediately at −24 °C after collection. The samples were later transported in a cold atmosphere to the Microbiology Laboratory-MED, University of Évora and frozen for two months until analysis. The samples were defrosted at 4 °C overnight before laboratory analysis.

2.3. Laboratory Analysis

Brix values were measured using a digital refractometer (ORF-E, Kern, Albstadt, Germany) to estimate IgG concentrations, as an indicator of immunological quality. From each sample of colostrum, 1 mL was diluted into 9 mL of buffered peptone water (BDH chemicals, VWR, Radnor, PA, USA), and decimals solutions were prepared. A total of 1 mL was pour-plated with 9 mL of each culture medium. This procedure was performed in duplicates for each sample. Incubation for each plate was performed as follows: mesophilic bacteria in Plate Count Agar (PCA; BDH chemicals, VWR, Radnor, PA, USA) at 30 °C for 48 h for TPC enumeration; coliforms in Chromogenic Coliform Agar (BDH chemicals, VWR, Radnor, PA, USA) at 37 °C for 24 h for TCC enumeration; LAB in de Man, Rogosa, and Sharpe Agar (MRS; BDH chemicals, VWR, Radnor, PA, USA) at 30 °C for 48 h under anaerobic conditions in an AnaeroJar (Oxoid, Hampshire, UK) using an Anaerocult® A sachet (Sigma-Aldrich, St. Louis, MO, USA), and anaerobiosis condition was confirmed with an Anaerotest® stripe (Sigma-Aldrich, St. Louis, MO, USA); enterobacteria (ENTB) in Violet Red Bile Glucose Agar (VRBG; BDH chemicals, VWR, Radnor, PA, USA) at 30 °C for 48 h; staphylococci (STAP) in Mannitol Salt Agar (MSA; BDH chemicals, VWR, Radnor, PA, USA) at 37 °C for 48 h; yeasts and molds in Rose Bengal Chloramphenicol Agar (RBC; BDH chemicals, VWR, Radnor, PA, USA) at 25 °C for 48 h. *E. coli* colonies (blue and violet colonies) were confirmed from Chromogenic Coliform Agar plates by performing Gram-negative staining and an oxidase negative test. *Clostridium* spp. was detected by heating the samples at 80 °C for 15 min, followed by plating in Sulphite Polymyxin Sulfadiazine (SPS) Agar (MERCK, Darmstadt, Germany). *Salmonella* spp. presence was identified with the *Salmonella* Supp Tab kit (Biomérieux, Marcy l'Etoile, França), where 25 mL of the sample was diluted in a sterile bottle with 225 mL peptone water at 37 °C and with 25 g of *Salmonella* growth supplement, and then, the content was thoroughly mixed and incubated at 41.5 °C for 24 h; finally, the reading was made with an immunodetection kit (miniVIDAS, BioMérieux, Marcy l'Etoile, France) according to the manufacturer specifications. The presence of *Listeria monocytogenes* was tested on ALOA® plates (Biomérieux, Marcy l'Etoile, France), and colonies were counted after an incubation period of 24 h at 37 °C. The pH (pH 1100L, pHenomenal®, VWR, Radnor, PA, USA) and a_W (Hygrolab, Rotronic, Bassersdorf, Switzerland) were measured in duplicates.

Microbial enumeration was performed using the standard plate count method, and the results were expressed as colony-forming units per milliliter (cfu/mL). Serial decimal dilutions (10^{-1} to 10^{-6}) were prepared to ensure accurate quantification. Colony count ranges considered acceptable were as follows: 15–300 cfu/plate for mesophilic bacteria on PCA (ISO 4833-1:2013) and LAB on MRS agar (ISO 15214:1998); 15–150 cfu/plate for ENTB on VRBG agar (ISO 21528-2:2004); 20–200 cfu/plate for STAP on MSA (following guidance from ISO 6888-1); and 10–150 cfu/plate for yeasts and molds on RBC agar (ISO 21527-1:2008). Plates outside these ranges were not considered.

2.4. Statistical Analysis

Data was analyzed using the software IBM SPSS Statistics (v27, Armonk, NY, USA). The sample size was calculated considering differences between farms with an $\alpha = 0.05$ and an 85% power, setting TPC from raw and HT colostrum [23] as the main variable to perform the calculation.

The general linear model procedure was used to estimate the least square means and standard errors for TPC, TCC, LAB, STAP, Brix, pH, and a_W, with farm (A, B, and C) as a fixed factor and plate duplicate as a random factor [24]. Differences between farms were tested with Tukey HSD; the results are presented as LSM (SEM). The Kruskal–Wallis test was used to compare differences in ENTB and *E. coli* counts between treatments [25]. Adjusted *p*-values were calculated using Bonferroni correction for multiple comparisons; the results are presented as the median (95% CI). The effect of HT was analyzed with a general linear model for repeated measures, and the results are reported as LSM (SEM).

Microbial counts were $\log_{10}$ transformed. Homoscedasticity was assessed with Levene's test, and the normality of the residuals was analyzed by inspection of normal probability plots and the Kolmogorov–Smirnov test. Significant values were considered as $p < 0.05$.

Overall colostrum quality was defined according to the current guidelines in two criteria: good quality (GQ)—TPC < 100,000, TCC < 10,000, STAP < 50,000 cfu/mL, and Brix $\geq$ 22%; or excellent quality (EQ)—TPC < 20,000, TCC < 100, STAP < 5000 cfu/mL, and Brix $\geq$ 25%.

3. Results

The descriptive statistics of all samples are shown in Table 3 as well as the percentage of samples that met current goals for TPC, TCC, and STAP. The percentage of samples meeting the thresholds for TPC, TCC, and STAP was 69% (47/68), 91% (62/68), and 90% (61/68) for GQ colostrum and 53% (36/68), 54% (37/68), and 69% (47/68) for EQ, respectively. Nevertheless, samples with very low to non-existent counts (e.g., TCC < detection limit (dl) cfu/mL) and samples with very high counts were found (e.g., TPC = 13,200,000.00 cfu/mL). Samples tested negative for *Salmonella* spp., *Listeria monocytogenes*, and yeasts and molds. Brix measurements showed that 75% (51/68) and 54% (37/68) of the samples had a Brix $\geq$ 22 and 25%, respectively. As an overall quality, 54% (37/68) and 32% (22/68) of samples met the criteria for GQ and EQ, respectively.

Table 3. Descriptive statistics for microbiological counts in colostrum samples (n = 68) on three Portuguese dairy farms and proportions of samples meeting current standards (Met).

Counts (cfu/mL)	Mean	Median	SD	Minimum	First Q	Third Q	Maximum	Met (%)
TPC [1]	3.99×10^5	1.68×10^4	1.55×10^6	4.00×10^1	4.75×10^2	2.07×10^5	1.32×10^7	69.9 [a]; 53.7 [b]
TCC [2]	1.17×10^4	7.00×10^1	7.51×10^4	<dl [6]	<dl	2.13×10^3	6.37×10^5	91.21 [a]; 54.4 [b]
LAB [3]	6.46×10^4	580.00	2.37×10^5	<dl	3.50×10^1	1.91×10^4	1.58×10^6	
STAP [4]	1.77×10^4	1.46×10^3	5.09×10^4	<dl	3.50×10^2	1.48×10^4	3.50×10^5	89.7 [a]; 69.1 [b]
ENTB [5]	3.99×10^5	1.68×10^4	1.55×10^6	4.00×10^1	4.75×10^2	2.07×10^5	1.32×10^7	
E. coli	4.82×10^2	<dl	2.12×10^3	<dl	<dl	1.45×10^2	1.71×10^4	

[a] GQ – TPC < 100,000, TCC < 10,000, STAP < 50,000 cfu/mL [13]; [b] EQ – TPC < 20,000, TCC < 100, STAP < 5000 cfu/mL [10]. [1] TPC = total plate count, [2] TCC = total coliform count, [3] LAB = lactic acid bacteria, [4] STAP = staphylococci, [5] ENTB = enterobacteria, [6] dl = detection limit.

Microbiological counts, pH, a_W (Table 4), and Brix (Figure 1) were different among the farms. Significantly lower microbial counts were found at farm C compared with farms A and B ($p \leq 0.002$), except for *E. coli* counts, where higher counts were only observed at farm A [2.89 (1.60–5.40)]. Farm B had lower TCC, LAB, and *E. coli* counts than farm A ($p < 0.001$). Significant differences were observed in colostrum pH between farms A and C ($p < 0.001$), and colostrum a_W was higher at farm C compared to farms A and B ($p = 0.032$). Brix values were higher at farm C than at farms A and B ($p = 0.003$).

The percentage of samples that had a TPC < 100,000 cfu/mL was 46% (10/22), 39% (5/13), and 97% (32/33) on farms A, B, and C, respectively (Figure 2a). For a TCC < 10,000 cfu/mL, 77% (17/22), 92% (12/13), and 100% (33/33) of the samples met this threshold on farms A, B, and C, respectively. On farms A, B, and C, 82% (18/22), 85% (11/13), and 97% (32/33) of samples had a STAP < 50,000 cfu/mL, respectively. When considering lower thresholds (EQ), different results were obtained (Figure 2b). On farms A, B, and C, respectively, 18% (4/22), 8% (1/13), and 94% (31/33) of samples had a TPC < 10,000 cfu/mL; 9% (2/22), 46% (6/13), and 88% (29/33) of samples had a TCC < 100 cfu/mL; and 32% (7/22), 69% (9/13), and 94% (31/33) had a STAP < 5000 cfu/mL. The frequency of samples with Brix $\geq$ 22% was 50% (11/22), 69% (9/13), and 94% (31/33) on farms A, B, and C, respectively, and 23% (5/22), 54% (7/13), and 76% (25/33) on farms A, B, and C, respectively, for Brix $\geq$ 25%. When considering an overall GQ (i.e., TPC < 10,000, TCC < 10,000, STAP < 50,000 cfu/mL, and Brix $\geq$ 22%), 14% (3/22), 31% (4/13), and 91% (30/33) of the samples on farms A, B, and C, respectively, met these criteria. When considering an overall EQ (i.e., TPC < 20,000, TCC < 100, STAP < 5000 cfu/mL, and Brix $\geq$ 25%), 0% (0/22), 0% (0/13), and 67% (22/33) of the samples met these criteria on farms A, B, and C, respectively.

Table 4. Microbiological counts in colostrum (expressed in $\log_{10}$ cfu/mL) regarding treatment (farm A, n = 22; farm B, n = 13; farm C, n = 33).

Counts	Farm A	Farm B	Farm C	*p*-Value
TPC *	5.13 (0.11) ^{a}	5.26 (0.15) ^{a}	2.80 (0.09) ^{b}	<0.001
TCC *	3.47 (0.15) ^{a}	2.12 (0.19) ^{b}	0.71 (0.12) ^{c}	<0.001
LAB *	4.31 (0.17) ^{a}	3.18 (0.21) ^{b}	1.41 (1.14) ^{c}	<0.001
STAP *	4.02 (0.12) ^{a}	3.55 (0.16) ^{a}	2.46 (0.10) ^{b}	0.002
ENTB #	2.89 (1.60–5.40) ^{a}	2.20 (<dl–5.23) ^{a}	<dl (<dl–3.13) ^{b}	<0.001
E. coli #	2.18 (0.00–3.74) ^{a}	<dl (<dl–4.23) ^{b}	<dl (<dl–3.34) ^{b}	<0.001
pH *	6.29 (0.02) ^{a}	6.27 (0.03) ^{ab}	6.22 (0.01) ^{b}	<0.001
a_w *	92.76 (0.13) ^{a}	92.67 (0.17) ^{a}	93.34 (0.09) ^{c}	0.032

* Results reported as LSM (SEM). # Results reported as median (95% CI). Different superscript letters (a, b, c) represent significant differences ($p < 0.05$) between farms. TPC = total plate count, TCC = total coliform count, LAB = lactic acid bacteria, STAP = staphylococci, ENTB = enterobacteria, dl = detection limit.

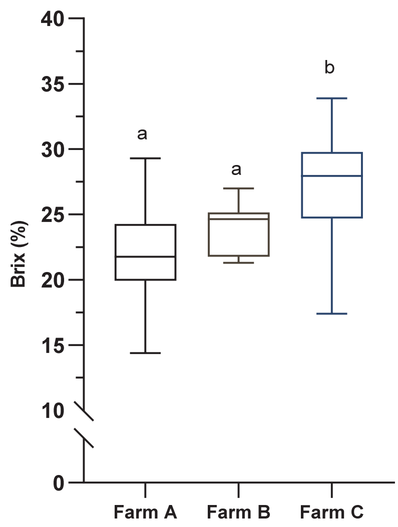

Figure 1. Boxplots of Brix (%) values across farms (farm A, n = 22; farm B, n = 13; farm C, n = 33). Different letters represent significant differences ($p = 0.003$).

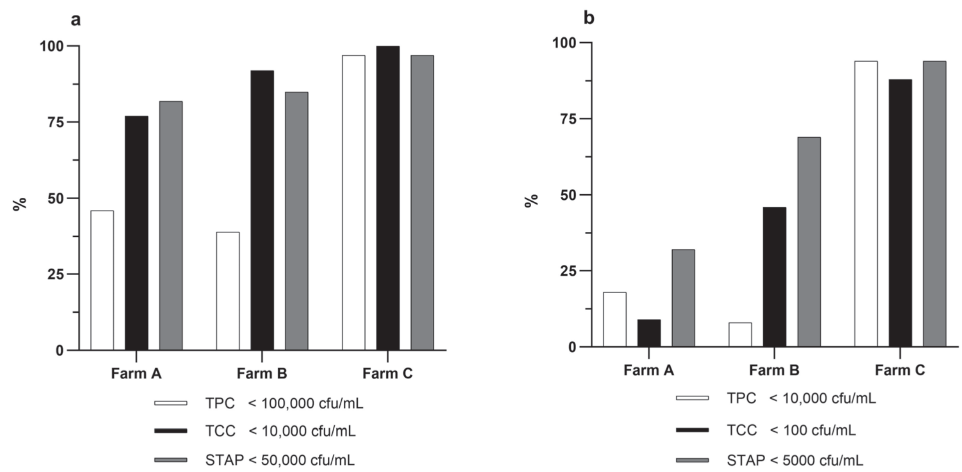

Figure 2. Percentages of colostrum samples meeting the goal for good quality (**a**) total plate counts (TPC) < 100,000 cfu/mL, for total coliform counts (TCC) < 10,000 cfu/mL, and for staphylococci (STAP) < 50,000 cfu/mL; and for excellent quality (**b**) total plate counts (TPC) < 10,000 cfu/mL, total coliform counts (TCC) < 100 cfu/mL, and staphylococci (STAP) < 5000 cfu/mL on each farm (farm A, n = 22; farm B, n = 13; farm C, n = 33).

A significant reduction in all microbial counts was observed after HT (Figure 3). However, a less pronounced effect was observed with STAP ($p = 0.016$) when compared with the remaining microbial counts ($p < 0.001$). Brix (21.5 and 21.5% SEM 1.09 for raw and HT, respectively) did not change with HT ($p = 0.500$). After HT, a decrease in the pH was observed (6.43 and 6.40 SEM 0.02 for raw and HT, respectively, $p = 0.017$).

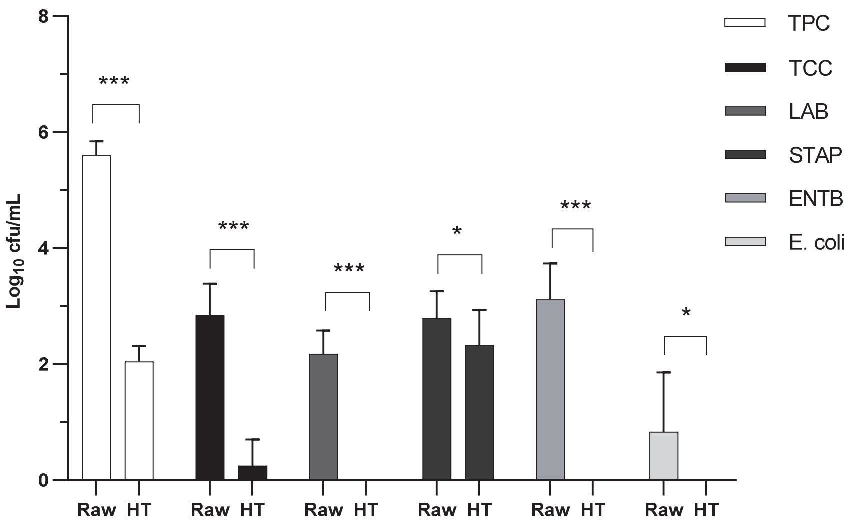

Figure 3. Microbiological counts before (raw) and after heat treatment (HT; 60 °C for 60 min) of farm C colostrum (n = 6). TPC, total plate counts; TCC, total coliform counts; LAB, lactic acid bacteria; STAP, staphylococci; ENTB, enterobacteria; E. coli, Escherichia coli. *** $p < 0.001$, * $p < 0.05$.

4. Discussion

While the important role of colostrum management in the transfer of passive immunity is well-established [26], empirical research examining the impact of on-farm hygiene management practices on colostrum microbiological content remains limited. In this study, we sought to compare the microbial counts in colostrum from three dairy farms with divergent hygiene practices during colostrum management. The results obtained revealed significant variations in microbial counts among the three farms, with lower counts being associated with higher levels of hygiene. However, colostrum samples were not collected at the time of harvesting due to the fact that the majority of the colostrum administered to the calves was sourced from the farms' colostrum banks. The aim was to analyze precisely what the calves were consuming. Consequently, comparisons between farms must be interpreted with caution. Previous research has primarily focused on microbial contamination during the harvesting process [14,27], but contamination can occur at any stage between milking and feeding [3]. Therefore, understanding the actual microbial content of colostrum that calves are consuming is essential for animal welfare [16,28].

The results of this study showed a wide range of values, with samples from only three farms (Table 3). A similar variability was found in other studies that sampled colostrum from a higher pool of farms [14,16,28]. To the authors' knowledge, no previous studies have evaluated the microbiological quality of colostrum on Portuguese dairy farms, limiting direct comparisons to the findings in other countries. Compared to Czech dairy farms, our study found lower TPC values, leading to a higher proportion of samples meeting quality standards, while TCC values were similar, with an identical proportion of samples below 10,000 cfu/mL [14]. However, the Czech study only analyzed raw colostrum samples collected directly from milking buckets, which may explain the higher TPC values. A study conducted on Colombian farms reported that 82% of fresh colostrum samples met the TPC < 100,000 cfu/mL standard and 76% met the TCC < 10,000 cfu/mL standard [22]. While the TPC was higher in the Colombian study, the TCC was lower compared to our findings. Additionally, Morril et al. [29] reported that, in the United States, only 54.8% of the samples had a TPC < 100,000 cfu/mL. This shows the great variability that exists in hygiene practices across farms. The stage at which samples are collected may account for the aforementioned variability between studies, but Stweart et al. [3] found no additional contamination from the milking bucket to the recipient where the calf would consume colostrum. This may not always be the case and highly depends on the hygiene practices. For example, Cummins et al. [30] reported very similar results with raw colostrum samples collected from the milking bucket; however, heat-treated colostrum had a much lower TPC.

There are many factors that can influence the microbial load of colostrum. Guzman-Carazo et al. [22] identified a lower microbiological quality in naturally fed calves compared to artificially fed calves, but Hyde et al. [21] observed that the feeding equipment was associated with higher TPC than the cow's teat. The main differences between these two farms are the HT of colostrum at farm B and the use of a disinfectant at farm A. Several studies have reported a decrease in bacteria after HT [17,31], but after HT, additional contamination can occur if proper hygiene conditions are not met. This may explain the lack of differences in TPC between farms A and B. Fecteau et al. [12] highlighted the importance of farm staff and the concept of hygiene, which can vary from person to person. In the same manner, the hygiene level during the milking process and the time between collection and storage or consumption can also vary within the same farm [3,13]. Another possibility is related to the fact that only detergent was used to clean the feeding materials at farm B, whereas at farm A, both detergent and disinfectant were used. It has been reported that cleaning the feeding materials with chlorhexidine gluconate or bleach increased the odds of meeting the recommended thresholds, but curiously, the cleaning frequency of the feeding materials did not [16]. In another study it was also found that using hot water to clean the feeding equipment and peracetic acid or hypochlorite to clean the feeding collection equipment was associated with lower TPC when compared to using only cold water [21]. Nevertheless, TCC, LAB, and *E. coli* were lower at farm B than farm A, which indicates that some of the coliform bacteria may have been reduced with HT. On the other hand, the results from farm C suggest that colostrum HT and cleaning feeding material with an automated system with hot water and disinfectant (i.e., sodium hypochlorite) effectively reduced the microbiological load. Coliforms were almost eliminated at farm C, showing that, with high hygiene standards, it is possible to meet more rigorous goals and have consistent results [10,11]. One of the most abundant genera in bovine colostrum is STAP [4], which seems to be more resistant to HT than other common bacteria found in colostrum [32]. Staphylococci are one of the most common isolates from bovine mastitis [33] and, therefore, may be harmful when ingested with colostrum. Nevertheless, according to Elizondo-Salazar et al. [31], STAP are not common calf pathogens, and HT reduced almost 1 $\log_{10}$, which did not occur in our study (Figure 3). Nonetheless, in the samples where HT was part of the protocol (i.e., farms B and C), the majority were <5000 cfu/mL, meeting the suggested goal; however, when considering a higher threshold (i.e., 50,000 cfu/mL), the three farms had more than 80% of the samples below the threshold. Although HT greatly reduced the microbial counts of all other cultures (Figure 3), Brix was not affected, in accordance with previous results [17,31]. Brix levels were significantly higher at farm C. However, this is unlikely to be related to the microbial counts, as other factors such as dam age, pre-partum diet, length of dry period, volume of colostrum, and vaccination may also affect the nutritional and immunological quality of colostrum [11]. A reduction in colostrum LAB with HT was reported by Trujillo et al. [34]. The decrease in LAB with HT and the lower values observed at farm C appear to be unfavorable outcomes. However, HT appears to promote the growth of beneficial bacteria at the intestinal level, as the calves' intestinal tract is already colonized by bacteria such as *Bifidobacterium* at birth [35]. Furthermore, HT colostrum has been shown to improve their growth, probably due to bioactive components, such as oligosaccharides, present in colostrum [35]. Santos et al. [27] reported counts for LAB in raw colostrum samples from Brazil, with levels higher than the ones from this study. The analysis of the effect of HT (Figure 3) showed that the raw samples from this farm already had lower LAB counts than the other farms, which may have influenced the counts after HT. We cannot explain this variation, but it has been shown that antimicrobials used during the dry period can affect the colostrum microbiota [36]. A cow's gut can be populated by LAB with antimicrobial activity against certain pathogens present in the same environment [37]. The relationship between the colostrum LAB and a cow's gut microbiome remains unclear. However, it was shown that some of the bacteria present in colostrum were detected in the cow's rectal content, including strictly anaerobic bacteria commonly present in the rumen and intestinal tract [38]. Colostrum has the capacity to modulate the newborn's calf immune system and gut microbiome [4], and the translocation of gut bacteria to colostrum can occur [1]; therefore, it would be plausible to think that these bacteria are important for the calf's defenses and gut colonization. Nevertheless, there is still a lack of information on the relationship between the colostrum microbiome and a calf's intestinal colonization.

Overall, only on farm C, optimal results regarding both microbiological (i.e., TPC and TCC) and immunological quality (Brix) were found, even when considering more restrictive thresholds. These findings align with those of Phipps et al. [16] and Morril et al. [29], who reported that only 23% and 39.4% of samples, respectively, met the established criteria. In Santos et al. [27], a similar approach was used, and 22.6% of the samples met the defined criteria (ENTB < 100,000 cfu/mL and IgG > 50 mg/mL). Therefore, a great proportion of calves from farms A and B received suboptimal colostrum. It is important to note that colostrum samples were frozen prior to culture, which may have influenced the bacterial counts. While calf health outcomes were beyond the scope of this study, the bacterial loads observed—particularly at farms A and B—raise concerns, as previous studies have linked poor colostrum hygiene to reduced passive immunity [39], increasing the risk of disease occurrence [40]. However, a direct association between colostrum microbial load and health outcomes warrants further investigation [20,41]. Another critical issue that also requires further research is the presence of multidrug-resistant bacteria in newborn calves, which can compromise not only calf health but also pose a risk to public health [42].

5. Conclusions

This study has demonstrated the variability in microbial composition among farms, highlighting the benefit of stricter hygiene practices to reduce microbial contamination of colostrum, such as the heat treatment of colostrum and the use of disinfectants and proper cleaning systems for feeding equipment. It also showed that, when more challenging thresholds were used, a considerable proportion of the colostrum samples were not considered to meet the criteria for high microbiological and immunological quality. This indicates that the identification of better hygiene practices and the establishment of strict hygiene protocols in colostrum management are necessary to ensure that high microbiological quality colostrum is administered to the calf.

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References

1. Addis, M.F.; Tanca, A.; Uzzau, S.; Oikonomou, G.; Bicalho, R.C.; Moroni, P. The Bovine Milk Microbiota: Insights and Perspectives from -Omics Studies. *Mol. Biosyst.* **2016**, *12*, 2359–2372. [CrossRef] [PubMed]
2. Lima, S.F.; Teixeira, A.G.V.; Lima, F.S.; Ganda, E.K.; Higgins, C.H.; Oikonomou, G.; Bicalho, R.C. The Bovine Colostrum Microbiome and Its Association with Clinical Mastitis. *J. Dairy Sci.* **2017**, *100*, 3031–3042. [CrossRef]
3. Stewart, S.; Godden, S.; Bey, R.; Rapnicki, P.; Fetrow, J.; Farnsworth, R.; Scanlon, M.; Arnold, Y.; Clow, L.; Mueller, K.; et al. Preventing Bacterial Contamination and Proliferation during the Harvest, Storage, and Feeding of Fresh Bovine Colostrum. *J. Dairy Sci.* **2005**, *88*, 2571–2578. [CrossRef]
4. Silva, F.G.; Silva, S.R.; Pereira, A.M.F.; Cerqueira, J.L.; Conceição, C. A Comprehensive Review of Bovine Colostrum Components and Selected Aspects Regarding Their Impact on Neonatal Calf Physiology. *Animals* **2024**, *14*, 1130. [CrossRef] [PubMed]
5. Otte, J.-M.; Podolsky, D.K. Functional Modulation of Enterocytes by Gram-Positive and Gram-Negative Microorganisms. *Am. J. Physiol. Liver Physiol.* **2004**, *286*, G613–G626. [CrossRef] [PubMed]
6. Malik, M.I.; Rashid, M.A.; Raboisson, D. Heat Treatment of Colostrum at 60 °C Decreases Colostrum Immunoglobulins but Increases Serum Immunoglobulins and Serum Total Protein: A Meta-Analysis. *J. Dairy Sci.* **2022**, *105*, 3453–3467. [CrossRef] [PubMed]
7. Barry, J.; Bokkers, E.A.M.; De Boer, I.J.M.; Kennedy, E. Pre-Weaning Management of Calves on Commercial Dairy Farms and Its Influence on Calf Welfare and Mortality. *Animal* **2020**, *14*, 2580–2587. [CrossRef] [PubMed]
8. Abuelo, A.; Havrlant, P.; Wood, N.; Hernandez-Jover, M. An Investigation of Dairy Calf Management Practices, Colostrum Quality, Failure of Transfer of Passive Immunity, and Occurrence of Enteropathogens among Australian Dairy Farms. *J. Dairy Sci.* **2019**, *102*, 8352–8366. [CrossRef]
9. Chase, C.C.L.; Hurley, D.J.; Reber, A.J. Neonatal Immune Development in the Calf and Its Impact on Vaccine Response. *Vet. Clin. N. Am. Food Anim. Pract.* **2008**, *24*, 87–104. [CrossRef]
10. Heinrichs, J.; Jones, C. Composition and Hygiene of Colostrum on Modern Pennsylvania Dairy Farms. Available online: https://extension.psu.edu/composition-and-hygiene-of-colostrum-on-modern-pennsylvania-dairy-farms (accessed on 21 July 2024).
11. Godden, S.M.; Lombard, J.E.; Woolums, A.R. Colostrum Management for Dairy Calves. *Vet. Clin. North Am. Food Anim. Pract.* **2019**, *35*, 535–556. [CrossRef]
12. Fecteau, G.; Baillargeon, P.; Higgins, R.; Paré, J.; Fortin, M. Bacterial Contamination of Colostrum Fed to Newborn Calves in Québec Dairy Herds. *Can. Vet. J.* **2002**, *43*, 523–527. [PubMed]
13. McGuirk, S.M.; Collins, M. Managing the Production, Storage, and Delivery of Colostrum. *Vet. Clin. N. Am. Food Anim. Pract.* **2004**, *20*, 593–603. [CrossRef]
14. Šlosárková, S.; Pechová, A.; Staněk, S.; Fleischer, P.; Zouharová, M.; Nejedlá, E. Microbial Contamination of Harvested Colostrum on Czech Dairy Farms. *J. Dairy Sci.* **2021**, *104*, 11047–11058. [CrossRef] [PubMed]
15. Palczynski, L.J.; Bleach, E.C.L.; Brennan, M.L.; Robinson, P.A. Giving Calves "the Best Start": Perceptions of Colostrum Management on Dairy Farms in England. *Anim. Welf.* **2020**, *29*, 45–58. [CrossRef]
16. Phipps, A.J.; Beggs, D.S.; Murray, A.J.; Mansell, P.D.; Stevenson, M.A.; Pyman, M.F. Survey of Bovine Colostrum Quality and Hygiene on Northern Victorian Dairy Farms. *J. Dairy Sci.* **2016**, *99*, 8981–8990. [CrossRef]

17. Godden, S.; McMartin, S.; Feirtag, J.; Stabel, J.; Bey, R.; Goyal, S.; Metzger, L.; Fetrow, J.; Wells, S.; Chester-Jones, H. Heat-Treatment of Bovine Colostrum. II: Effects of Heating Duration on Pathogen Viability and Immunoglobulin G. *J. Dairy Sci.* **2006**, *89*, 3476–3483. [CrossRef]
18. Robbers, L.; Bijkerk, H.J.C.; Koets, A.P.; Benedictus, L.; Nielen, M.; Jorritsma, R. Survey on Colostrum Management by Dairy Farmers in the Netherlands. *Front. Vet. Sci.* **2021**, *8*, 656391. [CrossRef]
19. Van Driessche, L.; Santschi, D.E.; Paquet, É.; Renaud, D.; Charbonneau, É.; Gauthier, M.L.; Chancy, A.; Barbeau-Grégoire, N.; Buczinski, S. Hygiene Management Practices and Adenosine Triphosphate Luminometry of Feeding Equipment in Preweaning Calves on Dairy Farms in Quebec, Canada. *J. Dairy Sci.* **2023**, *106*, 8885–8896. [CrossRef]
20. Barry, J.; Bokkers, E.A.M.; Berry, D.P.; de Boer, I.J.M.; McClure, J.; Kennedy, E. Associations between Colostrum Management, Passive Immunity, Calf-Related Hygiene Practices, and Rates of Mortality in Preweaning Dairy Calves. *J. Dairy Sci.* **2019**, *102*, 10266–10276. [CrossRef]
21. Hyde, R.M.; Green, M.J.; Hudson, C.; Down, P.M. Quantitative Analysis of Colostrum Bacteriology on British Dairy Farms. *Front. Vet. Sci.* **2020**, *7*, 601227. [CrossRef]
22. Guzman-Carazo, V.; Reyes-Vélez, J.; Elsohaby, I.; Olivera-Angel, M. Factors Associated with Microbiological Quality of Bovine Colostrum in Colombian Dairy Herds. *Int. Dairy J.* **2020**, *105*, 104670. [CrossRef]
23. Donahue, M.; Godden, S.M.; Bey, R.; Wells, S.; Oakes, J.M.; Sreevatsan, S.; Stabel, J.; Fetrow, J. Heat Treatment of Colostrum on Commercial Dairy Farms Decreases Colostrum Microbial Counts While Maintaining Colostrum Immunoglobulin G Concentrations. *J. Dairy Sci.* **2012**, *95*, 2697–2702. [CrossRef] [PubMed]
24. Herrera, M.; Galindo, J.; Padilla, C.; Guerra, C.W.; Medina, Y.; Sarduy, L.; Galingo, J. Proposal of a Mixed Linear and a Mixed Generalized Model for theanalysis of an Experiment in Rumen Microbiology. *Cuba. J. Agric. Sci.* **2020**, *54*, 149–156.
25. Raspa, F.; Chessa, S.; Bergero, D.; Sacchi, P.; Ferrocino, I.; Cocolin, L.; Corvaglia, M.R.; Moretti, R.; Cavallini, D.; Valle, E. Microbiota Characterization throughout the Digestive Tract of Horses Fed a High-Fiber vs. a High-Starch Diet. *Front. Vet. Sci.* **2024**, *11*, 1386135. [CrossRef]
26. Raboisson, D.; Trillat, P.; Cahuzac, C. Failure of Passive Immune Transfer in Calves: A Meta-Analysis on the Consequences and Assessment of the Economic Impact. *PLoS ONE* **2016**, *11*, e0150452. [CrossRef]
27. Santos, G.; Silva, J.T.; Santos, F.H.R.; Bittar, C.M.M. Nutritional and Microbiological Quality of Bovine Colostrum Samples in Brazil. *Rev. Bras. Zootec.* **2017**, *46*, 72–79. [CrossRef]
28. McAloon, C.G.; Doherty, M.L.; Donlon, J.; Lorenz, I.; Meade, J.; O'Grady, L.; Whyte, P. Microbiological Contamination of Colostrum on Irish Dairy Farms. *Vet. Rec.* **2016**, *178*, 474. [CrossRef] [PubMed]
29. Morrill, K.M.; Conrad, E.; Lago, A.; Campbell, J.; Quigley, J.; Tyler, H. Nationwide Evaluation of Quality and Composition of Colostrum on Dairy Farms in the United States. *J. Dairy Sci.* **2012**, *95*, 3997–4005. [CrossRef]
30. Cummins, C.; Berry, D.P.; Murphy, J.P.; Lorenz, I.; Kennedy, E. The Effect of Colostrum Storage Conditions on Dairy Heifer Calf Serum Immunoglobulin G Concentration and Preweaning Health and Growth Rate. *J. Dairy Sci.* **2017**, *100*, 525–535. [CrossRef]
31. Elizondo-Salazar, J.A.; Jayarao, B.M.; Heinrichs, A.J. Effect of Heat Treatment of Bovine Colostrum on Bacterial Counts, Viscosity, and Immunoglobulin G Concentration. *J. Dairy Sci.* **2010**, *93*, 961–967. [CrossRef]
32. Mann, S.; Curone, G.; Chandler, T.L.; Moroni, P.; Cha, J.; Bhawal, R.; Zhang, S. Heat Treatment of Bovine Colostrum: I. Effects on Bacterial and Somatic Cell Counts, Immunoglobulin, Insulin, and IGF-I Concentrations, as Well as the Colostrum Proteome. *J. Dairy Sci.* **2020**, *103*, 9368–9383. [CrossRef]
33. Pyörälä, S.; Taponen, S. Coagulase-Negative Staphylococci—Emerging Mastitis Pathogens. *Vet. Microbiol.* **2009**, *134*, 3–8. [CrossRef]
34. Trujillo, A.J.; Castro, N.; Quevedo, J.M.; Argüello, A.; Capote, J.; Guamis, B. Effect of Heat and High-Pressure Treatments on Microbiological Quality and Immunoglobulin G Stability of Caprine Colostrum. *J. Dairy Sci.* **2007**, *90*, 833–839. [CrossRef] [PubMed]
35. Malmuthuge, N.; Chen, Y.; Liang, G.; Goonewardene, L.A.; Guan, L.L. Heat-Treated Colostrum Feeding Promotes Beneficial Bacteria Colonization in the Small Intestine of Neonatal Calves. *J. Dairy Sci.* **2015**, *98*, 8044–8053. [CrossRef] [PubMed]
36. Patangia, D.V.; Grimaud, G.; Linehan, K.; Ross, R.P.; Stanton, C. Microbiota and Resistome Analysis of Colostrum and Milk from Dairy Cows Treated with and without Dry Cow Therapies. *Antibiotics* **2023**, *12*, 1315. [CrossRef] [PubMed]
37. Adeniyi, B.A.; Adetoye, A.; Ayeni, F.A. Antibacterial Activities of Lactic Acid Bacteria Isolated from Cow Faeces against Potential Enteric Pathogens. *Afr. Health Sci.* **2015**, *15*, 888–895. [CrossRef]
38. Chen, B.; Tang, G.; Guo, W.; Lei, J.; Yao, J.; Xu, X. Detection of the Core Bacteria in Colostrum and Their Association with the Rectal Microbiota and with Milk Composition in Two Dairy Cow Farms. *Animals* **2021**, *11*, 3363. [CrossRef]
39. Haggerty, A.; Mason, C.; Ellis, K.; Denholm, K. Risk Factors for Poor Colostrum Quality and Failure of Passive Transfer in Scottish Dairy Calves. *J. Dairy Res.* **2021**, *88*, 337–342. [CrossRef]

40. Pisello, L.; Sala, G.; Rueca, F.; Passamonti, F.; Pravettoni, D.; Ranciati, S.; Boccardo, A.; Bergero, D.; Forte, C. An Exploratory Cross-Sectional Study of the Impact of Farm Characteristics and Calf Management Practices on Morbidity and Passive Transfer of Immunity in 202 Chianina Beef-Suckler Calves. *Ital. J. Anim. Sci.* **2021**, *20*, 1085–1093. [CrossRef]
41. Back, P.; Hickson, R.; Sneddon, N.; Coleman, L.; Laven, R. Association of Bacterial Contamination of Colostrum with Passive Immunity and Growth Rates in Dairy Heifer Calves. *N. Z. J. Anim. Sci. Prod.* **2019**, *79*, 149–152.
42. Hang, B.P.T.; Wredle, E.; Börjesson, S.; Sjaunja, K.S.; Dicksved, J.; Duse, A. High Level of Multidrug-Resistant Escherichia Coli in Young Dairy Calves in Southern Vietnam. *Trop. Anim. Health Prod.* **2019**, *51*, 1405–1411. [CrossRef] [PubMed]