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Effects of Season and Hygienic Factors on Milk Microbiological and Physicochemical Characteristics in Livestock Communities of Ayacucho, Peru

Gloria Betti Adrianzen-Facundo[1]*, Julio Alberto Ruiz-Maquen[1], Arturo Rodríguez[2], Wilmer Moncada[3], Mardeli Meneses[1], and Krizzya Chávez[1]

[1]*Laboratorio Microbiología e Inmunología Veterinaria,Universidad Nacional de San Cristóbal de Huamanga, Ayacucho, Peru*
[2]*Laboratorio Reproducción y Biotecnología Animal, Universidad Nacional de San Cristóbal de Huamanga, Ayacucho, Peru*
[3]*Remote Sensing and Renewable Energy Laboratory, Universidad Nacional de San Cristóbal de Huamanga, Ayacucho 05000, Peru*

*Corresponding author's Email: gloria.adrianzen@unsch.edu.pe

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ABSTRACT

Climatic changes can influence milking practices and the dairy production chain, thereby influencing the microbiological safety and physicochemical quality of raw bovine milk (RBM), with potential implications for public health. The present study aimed to evaluate the effects of seasonal variability on the hygienic factors and characteristics of RBM produced in livestock communities in the Socos district, Ayacucho, Peru. For mesophilic aerobic bacteria (MAB) and total coliform bacteria (TCB), the compact dry plate method was used. Sixty random samples were collected during the rainy season (RS) and the dry season (DS). Samples were taken from milkers' hands (six in the RS and six in the DS), rags (six in the RS and six in the DS), buckets (six in the RS and six in the DS), receiving tanks (eight in the RS and eight in the DS), and distribution centers (four in the RS and four in the DS). To evaluate physicochemical characteristics, including density, fat, protein, total solids, lactose, pH, and mineral salts, eight samples of Brown Swiss cattle RBM were collected directly from the tanks (four in the RS and four in the DS) of two distribution point centers. The present results indicated that the MAB count for hygienic factors was lower in the DS than in the RS, except in the milker's hands and distribution centers. For the TCB count related to hygienic factors, the values were lower in the DS than in the RS, except in receiving tanks and distribution centers. In the RS, the physicochemical qualities complied with the Peruvian Technical Standard (PTS), except for protein content and mineral salt levels. In the DS, the physicochemical quality did not comply with the PTS; however, these properties were within acceptable limits in the receiving tanks for MAB and on the milker's hands for TCB. As a result, the bacterial load on inert surfaces and the characteristics of RBM were lower in the DS compared to the RS. Still, contamination during milking and at distribution centers was greater.

Keywords: Coliform, Hygienic safety, Mesophilic, Milk, Physicochemical quality, Season

INTRODUCTION

The livestock communities in the Socos district of the Ayacucho region in Peru are known for their milk production from Brown Swiss cattle. This activity represents a major economic effort that supports local traditions and culture while also contributing to regional food security. However, these raw bovine milk (RBM) production processes face significant limitations, such as the absence of appropriate technologies, limited access to livestock services, and a lack of technical and institutional support from local and regional authorities. These factors have negative effects on the management of livestock activities from milking to marketing, affecting the hygiene of RBM and compromising its microbiological and physicochemical quality (Nyokabi et al., 2021; Valdivia Avila et al., 2021). Likewise, changes in the diet of cattle during the rainy (RS) and dry seasons (DS) influence the nutritional quality of the RBM, since during the RS, access to fresh pasture improves the nutritional composition of RBM, which is reflected in enhanced physicochemical qualities of RBM (Páez et al., 2002; Petróczki et al., 2020). In both climatic stages, there is a variation in the bacterial count and physicochemical qualities of RBM. For production systems located in high-Andean areas of Peru, it was found that increased humidity during the RS is associated with significant increases in total coliform bacteria (TCB) and mesophilic aerobic bacteria (MAB) counts. At the same time, RBM sold in local markets exhibited low fat and lactose content, as well as a high pH during the RS, exceeding recommended limits and raising concerns regarding potential public health risks (Millogo et al., 2010).

Raw bovine milk consumption is a vital source of essential nutrients in the diets of consumers in rural communities, which is why it is necessary to maintain safety and nutritional quality standards from milking to marketing (Contero

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et al., 2021). However, raw milk is a highly perishable product that undergoes rapid changes, either during transport or when stored improperly, and can cause gastrointestinal diseases in consumers (Perin et al., 2019). These vulnerabilities can be mitigated through appropriate technical assistance (Mendez and Osuna, 2007).

From a regulatory perspective, Legislative Decree 1062, the Food Safety Law in Peru, requires national, regional, and local authorities, as well as consumers and economic agents involved in the food supply chain, to cooperate in ensuring the safety of food for human consumption. It is worth noting that, out of the 2.3 million agricultural units, only 64% are livestock farms in the Peruvian highlands, as indicated in the 2012 Fourth National Agricultural Census conducted by INEI-Peru. Peruvian technical standard (PTS; N° 202.001.2003) established specific microbiological limits for LCB, MAB $\leq 10^6$ CFU/mL, and TCB $\leq 10^3$ CFU/mL (MINAGRI, 2017), which are necessary for assessing the hygienic safety and nutritional quality of RBM before marketing and industrialization, requiring producers to meet a minimum quality standard (Delgado-Callisaya et al., 2016; Feyisa et al., 2023). Therefore, countries regulated these critical physicochemical parameters for collection, shipment, and analysis, with slight differences in performance limits and available methods to ensure that RBM meets appropriate nutritional quality standards (Perin et al., 2019; Contero et al., 2021).

On the other hand, improper handling and sale of RBM in informal channels without temperature control can affect the proliferation of lactic acid bacteria, which rapidly acidify RBM. This is worsened by poor hygiene and low productivity, as small producers often have minimal resources, resulting in underdeveloped production (Knight-Jones et al., 2016). Therefore, hygienic safety should comply with the requirements of the minimum number of bacteria in RBM, as regulated by PTS (N° 202.001.2003), confirmed by Supreme Decree N° 007-2017-MINAGRI, for consumption by the population. The evaluation was performed by counting MAB and TCB throughout the production and marketing process. Milking processes, equipment cleaning and disinfection, milking facility conditions, environmental management of the barn, transportation, nutritional status of cattle, and health management are determining factors in the hygienic safety (Freitas et al., 2009; Chamchoy et al., 2022).

The evaluation of the physicochemical qualities of the RBM encompasses the measurement of total solids, fat, and protein contents, which are vital for evaluating nutritional value and suitability as a raw material in dairy product manufacturing derivatives. This ensures that RBM is safe for consumption and meets the quality standards required for marketing (Solanki and Rizvi, 2001; Park et al., 2007; Renhe et al., 2019).

Among the factors that influence the hygienic safety of RBM include the milking routine, disinfection of hands and equipment, the designated location for milking, cleaning pens free of cow feces, cleaning and drying teats, and feeding cows fresh forage, especially considering that, in most cases, the cows are underfed and do not yield the expected amount of milk with a balanced daily diet (Flórez, 2001). In extreme cases, some RBM traders manipulate milk by adding water either at the barn or during transportation (Brousett-Minaya et al., 2015; Jurado-Gámez et al., 2020), while others maintain poor animal health management practices (Neave et al., 2024; Mendez and Osuna, 2007), in addition to the climatic factors (Brousett-Minaya et al., 2015). Therefore, the present study aimed to assess the seasonal changes in factors affecting the hygienic safety and nutritional quality of RBM in livestock communities in the Socos district, Ayacucho, Peru.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in accordance with the provisions of the Research Ethics Committee of the National University of San Cristóbal de Huamanga (RCU No. 068-2021-UNSCH-CU) and the Law on Animal Protection and Welfare in Peru (Law 30407) for samples that were collected directly from the milkers' hands, from the utensils, and from the collection tanks at distribution centers.

Study area

The Socos district is located in the Cordillera of the central Andes, in the province of Huamanga, Ayacucho Region, Peru, with an average altitude of 3,400 m above sea level (Figure 1). It covers an area of 81.75 km², which represents 0.18% of the Ayacucho region's total area. The high-Andean zone is characterized by three seasonal stages, including the RS with rainfall between December and February, gradually decreasing in March, the DS between May and August, and the intermediate season in April, September, October, and November. The ambient temperature in the high zone ranges from 2 to 13°C, whereas the low zone experiences temperatures between 18 and 21°C. The district is characterized as a grazing zone, with low to moderate vegetation, as indicated by a normalized difference vegetation index range of 0.2 to 0.6 (Moncada et al., 2022).

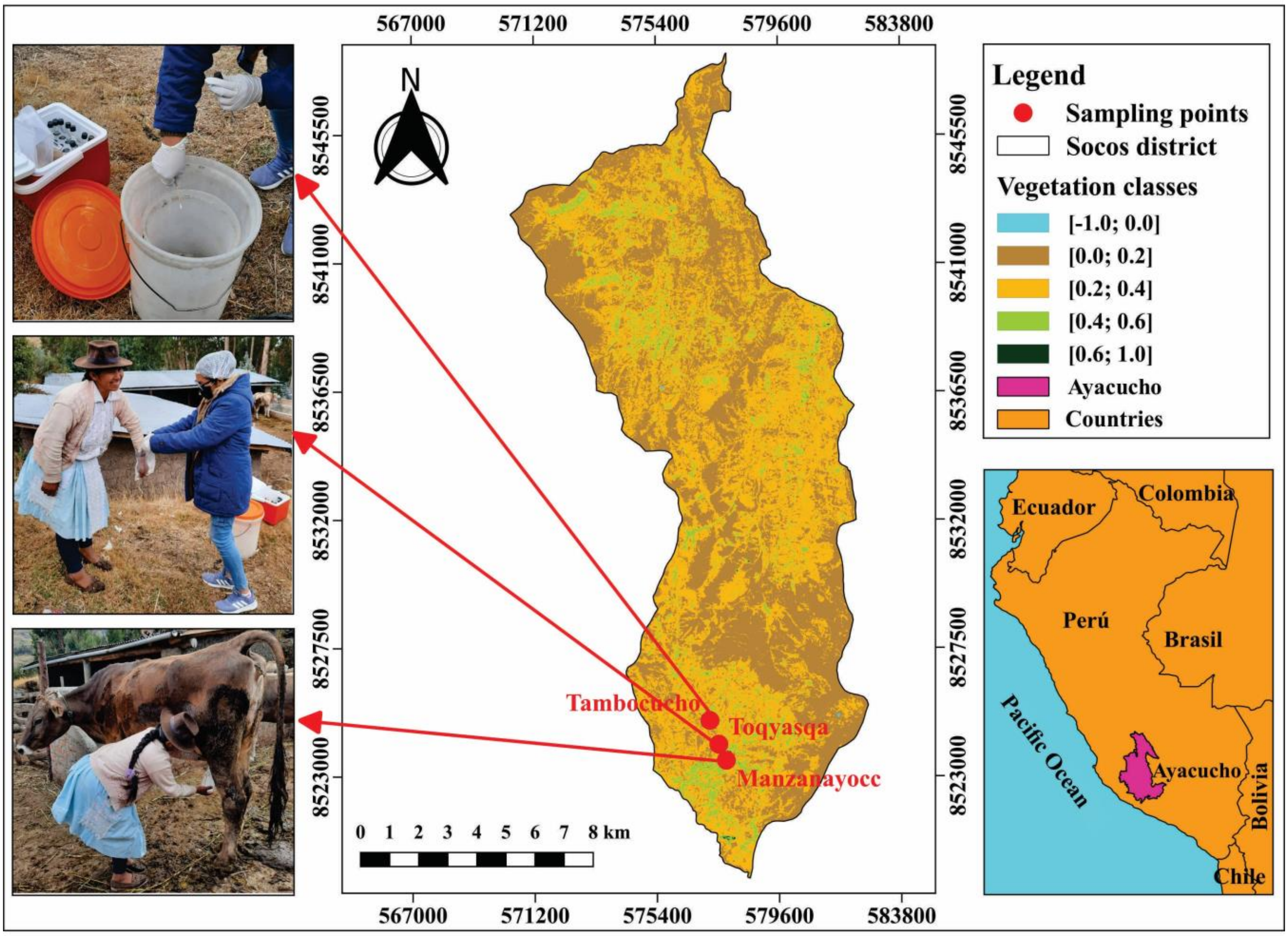

Figure 1. The geographical location of sampling for collecting raw bovine milk data in the towns of Tambocucho, Toqyasqa, and Manzanayocc in the district of Socos, Ayacucho, Peru

Raw bovine milk sampling

The sampling procedure during the RBM production chain, from milking to marketing, and the subsequent evaluation of the hygienic factors and physicochemical characteristics of RBM in the laboratory are illustrated in Figure 2. Initially, 30 RBM samples were collected in the RS (February 2021) and 30 RBM samples in the DS (August 2021) from farms located in the towns of Tambocucho, Toqyasqa, and Manzanayocc, in the district of Socos, Ayacucho, Peru. Samples were collected using the swab method from different sources, including the milker's hands (six in the RS and six in the DS), rags (six in the RS and six in the DS), buckets (six in the RS and six in the DS), receiving tanks (eight in the RS and eight in the DS), and distribution centers (four in the RS and four in the DS), for seasonal hygiene assessment. Additionally, eight RBM samples were obtained from the four teats of the Brown Swiss cow and stored in sterile 500 mL glass jars with lids for the seasonal evaluation of the nutritional quality of RBM.

The sampling method was random, with inclusion criteria for 30 cows in production and exclusion criteria for calves, heifers, dry cows, and cows older than eight years of age. The animals were fed a forage-based diet in extensive farming. The vaccination protocol was supervised by the National Animal Health Service (SENASA, Peru), which was responsible for conducting annual health checks on cattle in the Ayacucho region. Milking was performed manually, given that the average farm size was 50 head of cattle per farm (Gwandu et al., 2018).

The swabbing technique involved utilizing a moistened swab, dipped in 0.1% peptone water, which was systematically passed in a zigzag pattern over the surface of dry cloths arranged on the stones within the animal pen. This method was subsequently employed to sanitize the teats of the cow's udder. The swab was then placed back in the tube containing peptone broth, covered, and stored within an airtight container at 10°C. Additionally, swabs were collected from the inner surfaces of milking buckets and storage tanks at distribution centers (Knight-Jones et al., 2016).

In the lab, the swab inside the test tube was homogenized by rotating it with a vortex mixer. Subsequently, a micropipette was used to extract 1 mL of the homogenized peptone water sample, which was then placed on the compact dry plate for MAB and TCB, respectively. Both samples were incubated at 37°C for 48 hours for MAB and for 24 hours for TCB. Finally, the counts for MAB and TCB were determined utilizing the following formula.

$n \times f$ (CFU)   Formula 1

n represents the number of typical colonies, f signifies the dilution factor, and CFU means colony-forming units (Mizuochi and Kodaka, 2000; Wafula et al., 2016).

The method illustrated in Figure 2 enabled the efficient and systematic replication of sampling under different seasonal conditions, facilitating the comparison of hygienic and nutritional quality factors among different samples of RBM collected during the RS and DS. This method was particularly relevant in artisanal LCB production systems, where variations in management and environmental conditions can affect both the hygienic safety and nutritional quality of LCB, from the milking process to its sale in retail outlets (Chiarlone et al., 2025).

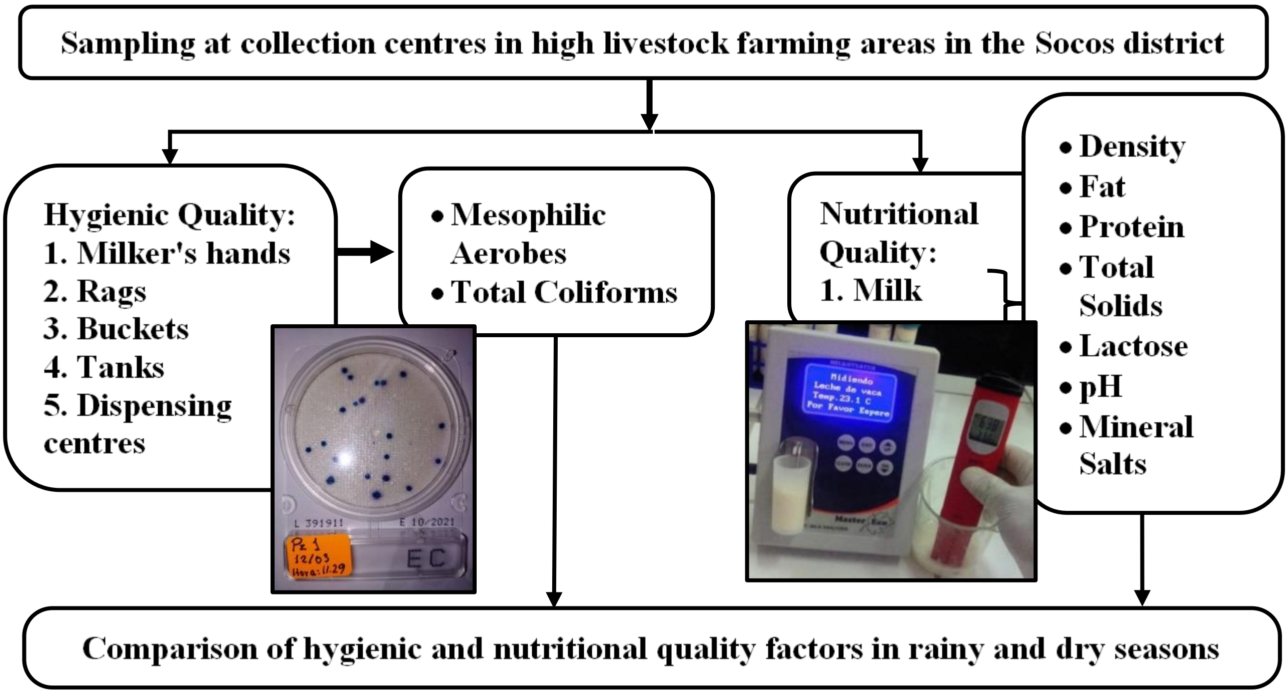

Figure 2. Sampling procedure based on the year and the factors affecting the hygienic safety and nutritional quality of raw bovine milk in the towns of Tambocucho, Toqyasqa, and Manzanayocc, located in Ayacucho, Peru

Hygienic safety

Milk performers were selected through targeted sampling, as the cow owners were responsible for milking their own cows. There was one milker per community, meaning that three milkers repeated or performed the same milking procedure twice in the RS and DS. The milkers resided in their own homes and managed their animals on a nearby farm (Deddefo et al., 2023). The processing of samples for assessing hygiene safety factors was conducted following the guidelines outlined in the technical guide for microbiological analysis of surfaces in contact with food and beverages, approved by resolution of the Ministry of Health (N° 461-2007-MINSA; PTS, 2016). Samples were collected from the hands of the three individuals responsible for milking, twice for each climatic stage, with six samples from the RS and six from the DS. The rinsing liquid was placed into a 150 mL bottle with a lid, then sealed and stored in an airtight container at a temperature not exceeding 10°C for storage, transportation, and laboratory analysis (Shehu et al., 2017).

The cloth rags (10 cm^2) used to clean the cow's udder teats belong to each person responsible for milking in a family group that owns a farm. Inappropriately, exposure of the rags to the environment in the animal pens causes them to become contaminated. After drying, cloth rags were reused to clean the teats of several cows on different occasions (Niboucha et al., 2022). Samples were taken from cloths belonging to different family groups before milking began (six in the RS and six in the DS), using a swab moistened with a diluting solution (0.1% peptone water), which was pressed lightly onto the inner surface of the test tube, pressing lightly with a rotating motion of the swab to remove excess solution. The swab was rubbed four times in a zigzag pattern on the surface of the dry cloth at a 30° angle, each time in the opposite direction to the previous one. The part of the swab containing the sample was deposited in a tube with the diluent solution, removing the part that had been in contact with the fingers. The samples were stored in an airtight container at a temperature not exceeding 10°C for preservation, transport, and analysis in the laboratory (Niboucha et al., 2022).

Plastic buckets, each with a capacity of 50 cm³ and a volume of 20 liters, were used to collect the RBM during the milking process. These buckets were assigned to each individual responsible for milking a family group that owned a farm. Plastic buckets were thoroughly cleaned with detergent and bleach, rinsed, and subsequently placed on stones within the animal pens to dry in the sun. Samples were taken from the buckets belonging to different family groups before milking began (six in the RS and six in the DS), in accordance with the procedure indicated in current regulations, using the swab method moistened in peptone water 0.1%, which was pressed lightly against the inner wall of the test tube with a rotating motion to remove excess solution. The swab was rubbed over the edges and internal surfaces of the bucket. The part of the swab containing the sample was placed in a tube with the diluent solution, removing the part that had been in contact with the fingers. The samples were stored in an airtight container at a temperature not exceeding 10°C for preservation, transport, and analysis in the laboratory (Adrianzen Facundo et al., 2024).

Samples were taken from the tanks before depositing the RBM inside them (six in the RS and six in the DS), using a swab moistened with peptone water 0.1 %, which was pressed lightly against the inner wall of the test tube with a rotating motion to remove excess solution. The swab was then rubbed on the inner surface of four tanks, from the base to the top of the neck, with circular movements, focusing more on the irregular surface with indentations. Two samples were taken from each tank for each climatic stage, resulting in a total of eight samples per stage. The part of the swab containing the sample was deposited in a tube with the diluent solution, removing the part that had been in contact with the fingers. The samples were stored in an airtight container at a temperature not exceeding 10°C for preservation, transport, and analysis in the laboratory (Adrianzen Facundo et al., 2024).

During the transportation of the RBM to the distribution centers, the intermediary or responsible individual for marketing the RBM positioned small frozen containers within the tanks to conserve the milk, which was transported approximately two hours after milking. The RBM was stored in 20-liter aluminum tanks, from which four samples from the RS and four from the DS, each measuring 250 mL, were extracted into sterile bottles at two ascertained points of sale supplied by the intermediary. The transfer of these samples to the laboratory took approximately 30 minutes. The bottles were properly labeled and stored within an airtight container containing refrigerant gel, ensuring a temperature not exceeding 10°C (Adrianzen Facundo et al., 2024).

Physicochemical quality of raw bovine milk

The physicochemical qualities of the RBM samples were determined using milk analysis equipment (Master Eco, Milkotester Ltd., Bulgaria). For this analysis, 6 mL of RBM was used to determine density (g/ml), fat (%), protein (%), lactose (%), total solids (%), and mineral salts (%). In addition, the pH of the RBM was determined using a digital pH meter (HI98107 Hanna, Mauritius). The results were evaluated using analysis of variance to identify potential differences between the two climatic periods under study (Geurts et al., 2017; Marini and Walczak, 2020).

Laboratory analysis

The samples were analyzed at the Microbiology Laboratory of the National University of San Cristobal of Huamanga, Peru. In the laboratory, the samples were stored at 2-4°C in a refrigerator for two hours. For processing, a stock solution of 90 mL of peptone broth 0.1% with 10 mL of RBM from each sample was prepared, then a dilution of 10^3 was obtained for the TCB count on compact dry EC plates (24 hours in an incubator at 37°C) and the dilution 10^4 for the MAB count on compact dry TC plates (48 hours in an incubator at 37°C). At the end of incubation, the microorganism colonies were counted using Formula 1. The results were expressed in CFU/ml and compared with the standard values of PTS: 202.001, using the rapid colony counting method with compact dry plates (Adrianzen et al., 2024). The regulation stated that the maximum allowable limits for MAB should not exceed 1 million CFU/mL, and for TCB, it should be 1,000 CFU/mL (PTS: N° 202.001.2003). In the absence of PTS, the Mexican Official Standard (MOS: N° 093-SSA1-1994) was applied to live and inert surfaces in contact with food (Pérez et al., 2016).

Statistical analysis

The measures of central tendency helped determine the mean and range of TCB and MAB counts. One-factor analysis of variance (ANOVA) was used to assess statistical differences between laboratory data and current regulations ($p < 0.05$). A descriptive comparison of changes between climatic periods (RS and DS) was made. All analyses used Excel 2019. Physicochemical results were evaluated using ANOVA ($p < 0.05$) to identify differences between the two climatic periods studied (Marini and Walczak, 2020).

RESULTS AND DISCUSSION

Hygienic safety

The MAB counts on the milker's hands indicated a significant decrease in the RS (20,517 CFU/ml) compared to the DS (1,067,933 CFU/surface; $p < 0.05$; Table 1). However, both values greatly exceeded the limit established by MOS-093 (< 3000 CFU/mL), indicating an apparent failure in milking hygiene. Applying a universal limit of 3000 CFU/mL without considering the contact surface area might underestimate the actual risk of contamination (Aliyo and Teklemariam, 2022). In rags, the MAB count was higher in the RS (533,333 CFU/mL) than in the DS (6,700 CFU/mL), with values higher than those established by NOM-093 (< 400 CFU/surface; $p < 0.05$). In milking buckets, the count was significantly higher in the RS (640,000 CFU/mL) compared to the DS (3,908 CFU/mL), with values well above those established by MOS-093 (< 400 CFU/mL; $p < 0.05$). The present results indicated that the sanitary conditions in the milking equipment were poor. Since the PTS (RM-461-2007/MINSA) lacked specific standards for food and beverage samples, these MOS guidelines were used as an alternative (Aliyo and Teklemariam, 2022). In contrast, for samples collected from RBM receiving tanks, the MAB count was higher in the RS (409,050 CFU/mL) than in the DS (188,813 CFU/mL); however, these values in the tanks were within the PTS range ($p < 0.05$). Similarly, for RBM samples at distribution centers, the MAB count in the RS (132,500 CFU/mL) complied with the PTS ($p > 0.05$). In contrast, the count in the DS (1,080,050 CFU/mL) significantly exceeded the PTS limit ($p < 0.05$). A significant difference in MAB counts was observed between the RS and DS, potentially attributable to the elevated ambient temperature during the RS ($p < 0.05$). In contrast, the influence of sanitary emergency measures related to the pandemic (SARS-CoV-2, February 2021) may have contributed to strict adherence to cleanliness in the utensils used for selling dairy products during the RS (Adrianzen-Facundo et al., 2024).

Table 1. Mesophilic aerobic bacteria counts and total coliforms in milking equipment and utensils as risk factors for milk safety in two seasons in the district of Socos, Ayacucho, Peru

Sampling zones	Mesophilic aerobic bacteria (MAB)					Total coliform bacteria (TCB)				
	PTS	RS	Complies	DS	Complies	PTS	RS	Complies	DS	Complies
Milker's hands	*< 3000	20,517	No	1,067,933	No	<100	10,375	No	82	Yes
Nipple cleaning cloths	*< 400	533,333	No	6,700	No	<10	12,850	No	1,303	No
Milking buckets	*< 400	640,000	No	3,908	No	<10	1,475	No	393	No
Receiving tank	<1,000,000	409,050	Yes	188,813	Yes	<1,000	5,323	No	7,711	No
Milk dispensing centre	<1,000,000	132,500	Yes	1,080,050	No	<1,000	2,688	No	199,050	No

Note: Six hand rinse samples and swabs from rags and buckets, eight samples from tanks, and four samples of milk were collected from the milk dispensing centres for each climatic season. Complies with regulations: Yes or No. PTS: Peruvian technical standard 202.001.2003 (CFU/ml). RS: Rainy season; DS: Dry season.

Regarding the TCB counts, the sampled zones indicated values above the standard criteria established by the PTS, highlighting that the results during the RS were higher than those of the DS ($p < 0.05$), except for the milker's hands during the DS (82 CFU/mL), which complied with the PTS standards ($p > 0.05$). A significant difference was observed in the majority of the results; the standards established by the PTS were not met ($p < 0.05$). The lack of compliance probably resulted from inadequate staff hygiene practices and exposure to heavily contaminated environments, such as pens soiled with cow feces. Therefore, these data revealed a concerning pattern of surface microbial contamination and related factors throughout the entire process from milking to marketing, posing a significant risk of foodborne illnesses to consumers.

Although the emergency health measures implemented during the SARS-CoV-2 pandemic promoted recommendations for frequent handwashing, evidence indicated that these practices should have improved, considering samples were collected in February 2021. Nonetheless, these practices were not effectively adopted in rural areas, highlighting ongoing deficiencies in hygiene habits at the primary production level (Chiarlone et al., 2025).

The variation in MAB and TCB counts between the DS and the RS is shown in Figure 3. In samples collected from the milker's hands, the yellow bars indicated a notable increase in MAB during the DS (1,047,417 CFU/mL) compared to the RS ($p < 0.05$). A similar pattern was observed for retail outlets, where BAM counts increased significantly (947,550 CFU/mL) during the DS compared to the RS ($p < 0.05$). These results suggested that direct handling of RBM and hygienic conditions at sales outlets were critical factors for bacterial proliferation, consistent with the findings of Lingathurai and Vellathurai (2013), who identified the milker's hands and utensils as primary sources of microbial contamination in RBM.

However, for samples collected from the surfaces of rags used during milking, there was a significant decrease in MAB (-526,633 CFU/mL) in the DS compared to the RS ($p < 0.05$). Similarly, for samples obtained from buckets, there was a notable decrease in MAB (-636,092 CFU/mL) in the DS compared to the RS ($p < 0.05$). The same trend was observed in samples taken from RBM receiving tanks, where the MAB count demonstrated significant reductions (-220,238 CFU/mL) in the DS compared to the RS ($p < 0.05$). This observation may result from frequent replacement of milking utensils and materials during the DS, which could lead to a decrease in MAB, as indicated by Brousett-Minaya et al. (2015) in RBM production systems in Andean regions, Peru. Regarding the variation in TCB counts between the

DS and the RS, there was a significant increase in TCB only in the distribution centers (196,363 CFU/mL) during the DS compared to the RS ($p < 0.05$), as indicated by the light blue bars. This pattern suggested that post-milking handling and exposure to poorly controlled environmental conditions at retail outlets were the major factors contributing to TCB contamination. Similar studies in Peru and Ecuador have demonstrated that the marketing phase was a critical point in the milk safety chain, where significant increases in BCT and *Escherichia coli* have been reported (Albuja Landi et al., 2021). In contrast, significant decreases in TCB counts were observed on the milkers' hands (-10,293 CFU/mL), rags (-11,547 CFU/mL), buckets (-1,082 CFU/mL), and RBM receiving tanks (-2,388 CFU/mL) during the DS compared to the RS ($p < 0.05$). The present results aligned with those reported by Bonfoh et al. (2003), who stated that seasonal variability and hygiene practices during milk handling are key factors influencing the presence of TCB, with lower contamination observed in seasons with reduced relative humidity.

Finally, it is important to note that in high-Andean communities, the hygiene practices of individuals responsible for milking were frequently inadequate, owing to the low temperatures that hinder the frequent washing of hands and utensils. This finding aligned with those of Hassen et al. (2022), who reported that the availability of warm water and environmental conditions serve as limiting factors for adherence to proper hygiene practices in traditional RBM production. These factors, along with a lack of infrastructure and training, contribute to the variability observed in bacterial counts between the DS and the RS.

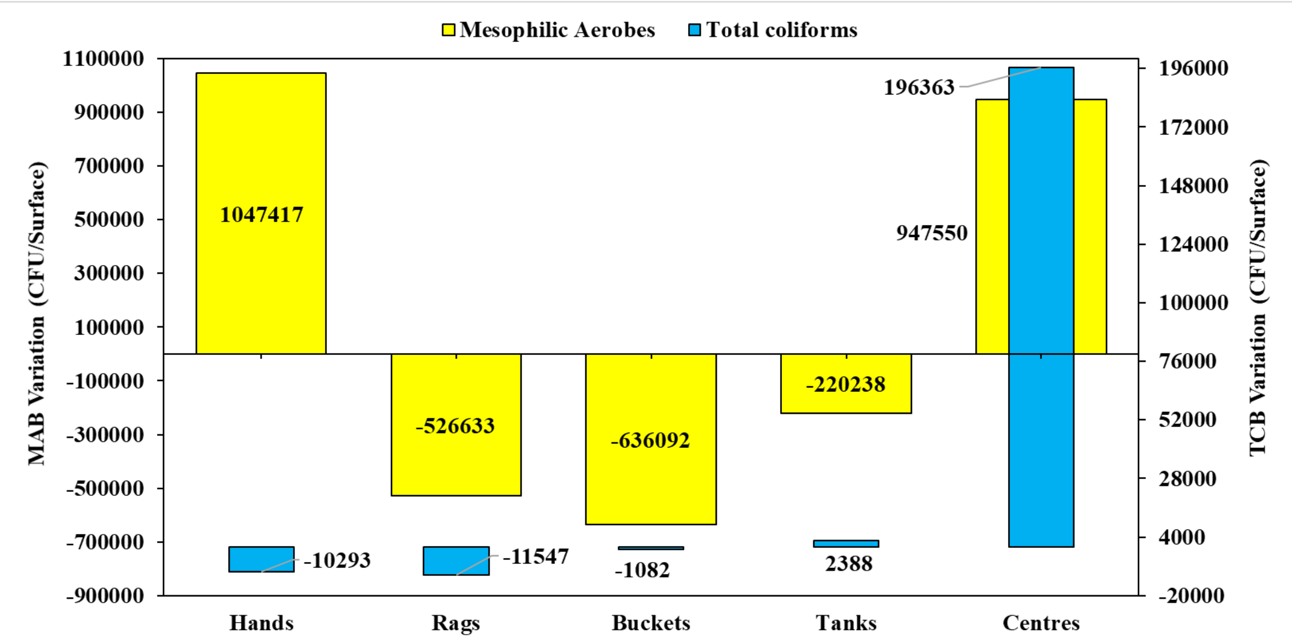

Figure 3. Variation of mesophilic aerobic bacteria and total coliform counts between the rainy season (RS: February 2021) and the dry season (DS: August 2021) in surface samples in the high livestock area of the Socos district, Ayacucho, Peru

Physicochemical characteristics

Table 2 presents a comparison of the mean values of the physicochemical characteristics of RBM (n=120 samples) concerning the parameters outlined by PTS (N° 202-001-2003) as well as additional parameters established in the studies conducted by Zavala (2005), Barbieri (2006), Viera (2013), Zevallos (2023), and Zevallos (2023) in accordance with climatic seasonality.

Regarding density, the mean RBM value during the DS (1.0290 g/mL) was marginally below the minimum threshold specified by the PTS (1.0296 g/mL); nonetheless, no statistically significant differences were observed between the two climatic periods ($p > 0.05$). The current findings are consistent with the values (1.0293 g/mL) reported in the Andean region of Junín, Peru, by Viera (2013). Still, the current results were lower than those documented by Brousett-Minaya et al. (2015) during the DS in Puno, Peru (1.030 g/mL) across eight sampled basins, as well as by Martínez and Díaz (2016) in Colombia (1.031 g/mL).

Regarding the percentage of raw bovine milk fat (RBMF), the average of RBMF in the RS (3.17%) complied with the PTS threshold (≥ 3.2%). In contrast, in the DS (3.03%), RBMF was below ≥ 3.2%, although without a statistically significant difference ($p > 0.05$). This decrease in the DS could potentially influence the technological quality of milk used in producing dairy products that require adequate fat content for optimal texture and flavor. Notably, the percentage of LCB fat in the RS, located in the high-Andean livestock areas of the Socos district, was slightly lower than the findings of Viera (2013), who recorded 3.73% in Junín, and by De la Sota (2016), who documented 3.72% in Junín. These RBMF results suggested that the reduced fat content may be attributable to a low-fiber pasture diet with limited energy intake, which aligned with the findings of Were et al. (2025) concerning the relationship between forage nutritional quality and milk lipid profile.

Table 2. Comparison of the physicochemical characteristics of the raw bovine milk in the district of Socos, Ayacucho, Peru, during the rainy and dry seasons

Parameters	PTS	Rainy season			Dry Season			
		Average	Complies	Limits LL - UL	Average	Complies	Limits LL - UL	p-value (0.05)
Density[1] (g/ml)	Min. 1.0296 Max. 1.0340	1.0297	Yes	1.0291 - 1.0303	1.0290	No	1.0284 - 1.0297	0.25
Fatty matter[1] (%)	Min. 3.2	3.17	Yes	3.12 - 3.22	3.03	No	3.03 - 3.05	0.27
Protein[2] (%)	3.48	3.46	No	3.45 - 3.47	3.44	No	3.43 - 3.44	0.44
Total solids[1] (%)	Min. 11.4	11.416	Yes	11.411 - 11.421	11.335	No	11.332 - 11.338	0.35
Lactose [2] (%)	5.08	5.09	Yes	5.08 - 5.10	5.04	No	5.03 - 5.05	0.27
pH[3]	6.60	6.60	Yes	6.60 - 6.60	6.56	No	6.56 - 6.56	0.00
Mineral salts[2] (%)	0.60	0.61	No	0.61 - 0.62	0.58	No	0.58 - 0.59	0.00

[1]PTS: Peruvian technical standard 202.001.2003 (CFU/ml, PTS-202.001.2003), [2]Zevallos (2023), [2]Viera (2013), [2]Barbieri (2006), [3]Zevallos (2023). Lower Limit: LL. Upper Limit: UL. Complies with regulations: Yes or No.

Regarding the protein content in RBM, the average values for both climatic seasons (3.44% and 3.46%) were below the reference values reported by Zevallos (2023). Although no statistically significant differences were observed ($p > 0.05$), a marginal advantage was apparent in the RS, likely due to the greater availability of green pastures, consistent with the findings of Evers et al. (2023). Studies in high-Andean regions emphasized that seasonality and nitrogen deficiency in forage are key factors affecting protein synthesis in RBM (Calsamiglia et al., 2010).

The total solids in the RBM indicated compliance with the PTS in the RS (11.416%) but not in the DS (11.335%), which were below these limits. Although there were no statistical differences ($p > 0.05$), a decrease was observed during the dry season, aligning with the findings of Altieri and Nicholls (2008), who linked the reduction in solids to intense frosts, lower precipitation, and low-quality forage. The current results were lower than those reported by Viera (2013), Brousett-Minaya et al. (2015), and De la Sota (2016), indicating a common pattern in high-Andean regions facing climatic stress, such as extreme cold or frost.

Regarding the percentage of lactose in RBM, the content during the RS (5.09%) slightly exceeded the expected value (5.08%) established by Zevallos (2023), but was lower during the DS (5.04%). Although the differences were not significant ($p > 0.05$), the decline in the DS could be related to acidification phenomena during transport and storage, where bacterial fermentation reduces lactose, possibly due to changes in ambient temperature or prolonged exposure to sunlight (O'Connell et al., 2016). The current values were higher than those reported by Viera (2013) in Junín, Peru, who obtained 4.17% during the DS, indicating that RBM may undergo an acidification process during transport and storage. It was estimated that the pH of RBM remained stable during the RS (6.60), which complied with the quality standards outlined by Barbieri (2006) and Zevallos (2023). In contrast, a significant decrease was observed during the DS (6.56; $p < 0.05$). This slight decrease was associated with fermentation processes and increased bacterial proliferation under conditions of lower forage availability and greater heat stress (Barbieri, 2006).

On the other hand, the percentage of mineral salts was significantly higher in the RS (0.61%) than in the DS (0.58%), consistent with the findings reported by Zevallos (2023) and possibly reflecting the influence of the mineralized diet and water availability during the RS. In both cases, significant differences were found between the two climatic periods ($p < 0.05$).

The values of physicochemical characteristics of RBM decreased from RS to DS, possibly due to the lower quality and availability of pasture in the DS (Figure 4; Mamani et al., 2011). Overall, seasonal climate variations in the high-Andean region of Ayacucho, Peru, had different effects on the physicochemical parameters of RBM, with noticeable decreases in fat, total solids, lactose, and pH during the DS. This pattern aligned with reports from other high-Andean ecosystems in Peru and Colombia, where variation in forage nutritional quality mainly explains seasonal changes in RBM (Viera, 2013; Calsamiglia et al., 2020).

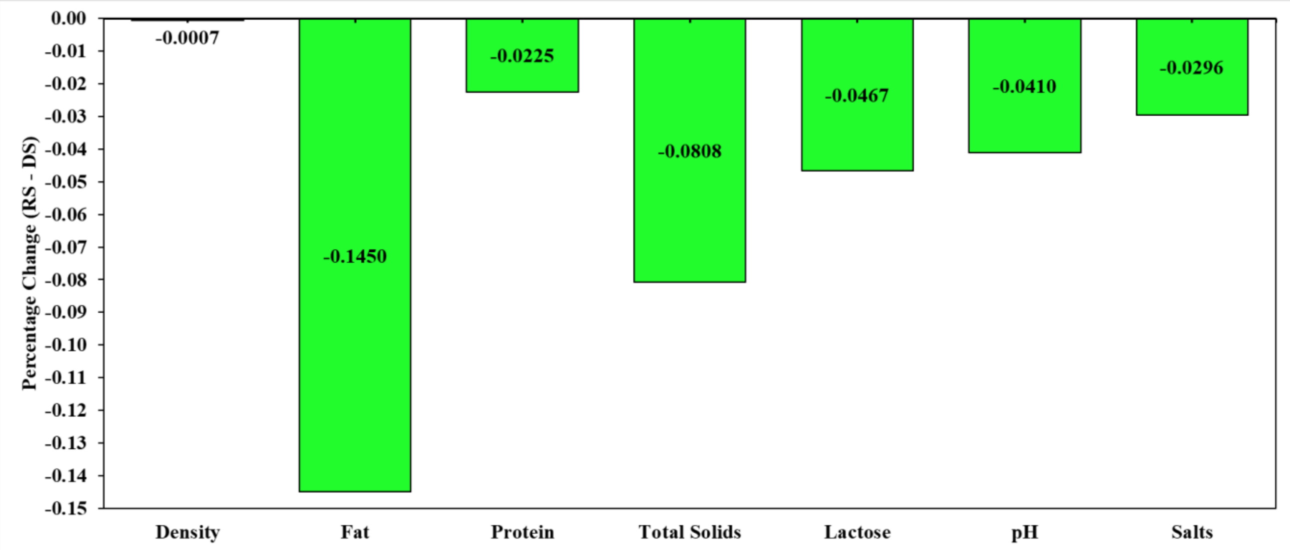

Figure 4. Percentage change of the physicochemical characteristics of raw bovine milk between the rainy (RS: February 2021) and dry (DS: August 2021) seasons in the surface samples in the high livestock area of the Socos district, Ayacucho, Peru

CONCLUSION

The count of mesophilic aerobic bacteria (MAB) on inert surfaces such as rags, buckets, and receiving tanks utilized in high-Andean livestock communities in Socos, Ayacucho, Peru, was significantly lower during the dry season (DS), indicating a possible inhibitory influence of diminished environmental humidity on bacterial growth. Conversely, samples from the milker's hands and raw bovine milk (RBM) at commercial outlets exhibited the highest bacterial counts during the DS, thereby highlighting human handling and post-milking procedures as critical sources of contamination. Regarding the counts of total coliform bacteria (TCB) within the sampled areas, reduced levels were observed during the DS, except in retail outlets. The failure of most results to conform to the thresholds established by the Peruvian Technical Standard (PTS; N° 202.001.2003) or the Mexican Official Standard for surfaces in contact with food (MOS N° 093-SSA1-1994) indicated inadequate hygiene practices during the production process, thereby posing a risk to public health. Concerning nutritional quality, the physicochemical qualities of the RBM were adversely affected during the DS. Key parameters such as density, fat content, total solids, lactose, and pH largely comply with regulatory standards, particularly during the rainy season (RS). Nonetheless, mineral salts exhibited significantly lower values during the DS compared to the RS, failing to meet the reference threshold. This decline in compositional quality was directly attributable to the reduced availability, quality, and nutritional value of forage typical of high-Andean ecosystems during the DS. Therefore, seasonality was a key modulator that interacted with management practices, microbiological risks, and the nutritional value of RBM, with direct implications for food security and the economy of high-Andean livestock communities. It is recommended that future studies use a multi-year (longitudinal) design and include other high-Andean communities. This would help confirm the observed patterns, assess the long-term effects of climate change, and develop predictive models for quality and safety.

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Availability of data and materials

Data from the present study is available upon reasonable request.

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Competing interests

The authors declared no conflict of interest.

Authors' contributions

Gloria Adrianzén and Julio Ruiz planned the experiment and conducted the fieldwork for sample collection. Wilmer Moncada and Arturo Rodríguez interpreted and statistically analyzed the data and results for the manuscript. Mardeli Meneses and Krizzya Chávez contributed to the field sampling and laboratory analysis. All authors of the revised articles read and approved the final edition of the manuscript.

Ethical considerations

All authors have thoroughly checked and confirmed ethical concerns regarding the originality of the data collected and analyzed before submitting it to the journal. The original article, after checking for plagiarism, was submitted to the journal.

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