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Compositional, hygienic and sanitary quality of bovine milk in three regions of Ecuador

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ABSTRACT

Milk quality creates a scenario in which producers are required to meet standards established by regulations to ensure the production of a safe food product. This study aimed to characterize the compositional, hygienic, and sanitary quality of bovine milk in three regions of Ecuador: The Sierra (S), Coast (C), and Amazon (A). A total of 831 samples were analyzed following the LCL-INS-01 protocol to determine fat, protein, lactose, and total solids (TS) percentages, as well as total bacterial count (TBC), total and differentiated somatic cell counts (SCC and DSCC). The results indicated the following averages: **Compositional quality**: fat content—A: 4.05%, S: 3.87%, and C: 3.46%; protein content—C: 3.35%, A: 3.25%, and S: 3.22%; lactose content—S: 4.61%, A: 4.59%, and C: 4.29%; and TS—A: 12.61%, S: 12.47%, and C: 11.74%. **Hygienic quality**: TBC—A: 5.7 x 10^6, S: 5.5 x 10^6, and C: 2.4 x 10^5. **Sanitary quality**: SCC/ml—S: 354 x 10^3, C: 110 x 10^4, and A: 759 x 10^3; DSCC—S: 67.52%, C: 67.62%, and A: 78.21%. These findings indicate the presence of mastitis in all three regions.

In conclusion, all three regions met the compositional quality standards established by INEN. In terms of hygienic quality, compliance was observed in the Coast and Sierra regions, whereas sanitary quality requires improvement strategies across all regions. Addressing milk quality is a significant contribution to promoting better understanding and improvement in dairy production, consumer health, and the development of the dairy sector.

Keywords: milk, hygiene, somatic cells.

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Calidad composicional, higiénica y sanitaria de la leche bovina en tres regiones del Ecuador

RESUMEN

La calidad de leche forja un escenario donde los productores están obligados a cumplir estándares bajo normativas para generar un alimento salubre. El objetivo de este estudio fue caracterizar la calidad composicional, higiénica y sanitaria de la leche bovina en tres regiones del Ecuador: Sierra (S), Costa (C) y Amazonia (A). Se utilizaron 831 muestras bajo el protocolo LCL-INS-01 para determinar el porcentaje de grasa, proteína, lactosa y sólidos totales (ST), y el conteo de bacterias totales (CBT), conteo de células somáticas (CCS) totales y diferenciadas (CCSD). Los resultados indican los siguientes promedios: Calidad composicional, grasa, A: 4,05%, S: 3,87% y C: 3,46%. Proteína, C: 3,35%, A: 3,25%, y S: 3,22%. Lactosa, S: 4,61%, A: 4,59% y C: 4,29%. ST, A: 12,61%, S: 12,47% y C: 11,74%. Calidad higiénica, CBT, A: 5,7 x 10^6, S: 5,5 x 10^6 y C: 2,4 x 10^5. Calidad sanitaria: 354 x 10^3; 110 x 10^4; y 759 x 10^3 CCS/ml y 67,52, 67,62, y 78,21 %CCSD para la Sierra, Costa y Amazonia, para ambos parámetros, donde se determinó la existencia de mastitis. Se concluye que las tres regiones cumplieron con la calidad composicional según el Servicio Ecuatoriano de Normalización (INEN); en lo higiénico, la Costa y la Sierra, y en lo sanitario, las tres deben abordar estrategias en la producción. Estudiar la calidad de la leche resulta un aporte significativo para promover el entendimiento y la mejora en la producción lechera, así como la salud del consumidor y el desarrollo del sector lácteo.

Palabras clave: leche, higiene, células somáticas.

INTRODUCTION

In Ecuador, milk production systems (MPS) have significantly increased their yield, reaching a total of 6.1 million liters of milk per day at the national level by 2023 (Salguero *et al.* 2023). According to the Ecuadorian Dairy Observatory, milk production in 2022 amounted to 5,502,787 liters per day, highlighting its importance as a sector that generates both direct and indirect employment (Chanaluisa 2016). In this context, characterizing and understanding milk production and its compositional, hygienic, and sanitary quality is essential for implementing actions and developing strategies for decision-making regarding integral parameters that ensure a safe food supply for human consumption (Jurado *et al.* 2021; Valdivia *et al.* 2021). Raw milk must meet quality standards that require it to be homogeneous, free of contaminants, and possess a pleasant sensory profile (Vallejo *et al.* 2018), as stipulated by the Ecuadorian Standards Institute (INEN 2012).

As emphasized by Chuquín *et al.* (2016), it is evident that milk production without Good Livestock Practices (GLP) in Master Plan Production (MPP) leads to elevated levels of colony-forming units (CFU) and somatic cell counts (SCC), which pose risks to industrial processing and inclusion in the population's diet (Luigi *et al.* 2013). Compositional quality is evaluated based on nutritional content, including fat, protein, lactose, total solids (TS), and non-fat solids (NFS) (Vázquez *et al.* 2014; Moreira *et al.* 2020). Hygienic quality, as noted by Contero *et al.* (2019), involves total bacterial count (TBC) and CFU, both critical parameters for ensuring food safety. Meanwhile, sanitary quality involves analyzing somatic cell counts, both total (Contero *et al.* 2021) and differential (DSCC) (Orozco & Santana 2022), which are associated with inflammatory processes in the mammary gland, reflecting the internal health of the udder (Vallejo *et al.* 2018).

The primary causes of poor milk quality among producers are the lack of technical guidance and reliance on incorrect empirical practices from production to milk handling during storage and transportation, leading to increased microbial loads (Guevara *et al.* 2019). Additionally, the compositional quality of milk can be influenced by factors such as the number of calvings, lactation period, diseases, genetics, and climatic conditions (Ramírez *et al.* 2019).

This issue represents a global concern, particularly in Ecuador, where ensuring high-quality food and providing a foundation for the industrialization of raw materials to produce premium dairy derivatives remain priorities (Arrieta *et al.* 2019). Compositional, hygienic, and sanitary milk quality can lead to variations in production processes and impact the sensory and nutritional characteristics of the final product. As a result, the dairy industry has compelled primary producers to meet standards enforced by each nation (Jiménez *et al.* 2020).

Although studies on this topic have been conducted in Ecuador, they have been fragmented. Given this situation, the present study was conducted in three regions (Sierra, Coast, and Amazon), with the objectives to: 1. Analyze the compositional quality of bovine milk samples from production units using mid-infrared spectrophotometry. 2. Determine the hygienic quality of bovine milk samples through TBC using flow cytometry. 3. Assess the sanitary quality of bovine milk samples by analyzing SCC and DSCC using flow cytometry.

MATERIALS AND METHODS

Study area

The study was conducted in the main milk collection centers and dairy production units of Ecuador, considering small (1-5 ha), medium (5-20 ha), and large producers (> 20 ha) based on the percentages of milk production across the country's three regions. According to Márquez (2021), the distribution of milk production is as follows: 77.2% in the Sierra, 17.9% in the Coast, and 4.8% in the Amazon. Dairy farms in these regions primarily raise breeds such as Holstein, Jersey, Brown Swiss, F1 (H+J), (H+B), Girolando, Gyr, and Brangus, with most systems employing rotational grazing practices.

The initial sampling distribution comprised 750 milk samples (100%), determined by the budget allocated to the research and proportionally divided according to the regional milk production percentages. However, during characterization, a total of 831 samples were collected to obtain more comprehensive data on milk quality.

Milk sample collection

Milk samples were collected from various areas across ten provinces within the three regions of Ecuador (figure 1), which are the primary zones of bovine milk production. In the Sierra (Andean region), samples were obtained from Pichincha, Cotopaxi, Imbabura, Tungurahua, Carchi, Azuay, and Cañar (n = 570). In the Coast (Littoral region), samples were collected from Santo Domingo de los Tsáchilas and Manabí (n = 135). In the Amazon (Eastern region), samples were taken from Napo (n = 126).

Milk samples were collected from ten provinces across Ecuador's three primary bovine milk production regions (figure 1). In the Sierra (Andean region), samples were obtained from Pichincha, Cotopaxi, Imbabura, Tungurahua, Carchi, Azuay, and Cañar (n = 570). In the Coast (Littoral region), samples were collected from Santo Domingo de los Tsáchilas and Manabí (n = 135). In the Amazon (Eastern region), samples were taken from Napo (n = 126).

For the evaluation of milk quality across the three criteria (compositional, hygienic, and sanitary), each was assessed in two stages, described as follows:

Sampling procedure

Sampling followed the LCL-INS-01 protocol established by the Milk Quality Laboratory (LCL, by its initials in Spanish) at Universidad Politécnica Salesiana (UPS) (Alvear *et al.* 2021), with each sample coded and identified according to the producer or sampling area.

For compositional and sanitary quality evaluation, milk samples were collected from 40-liter milk cans using 40 ml plastic vials with white caps containing a preservative (8 ml bronopol and 0.3 mg natamycin tablets) provided by the laboratory. The samples were stored in a cooler maintained at 4 °C to 6 °C using gel refrigerants and transported to the LCL at the UPS Cayambe campus (Alvear *et al.* 2021).

For hygienic quality evaluation, sterile 40 ml vials with red caps were used. At the end of the collection process, 4 drops of azidiol bacteriostatic solution were added to preserve the samples. These were also transported in coolers maintained at 4 °C to 6 °C until they arrived at the laboratory (Alvear *et al.* 2021).

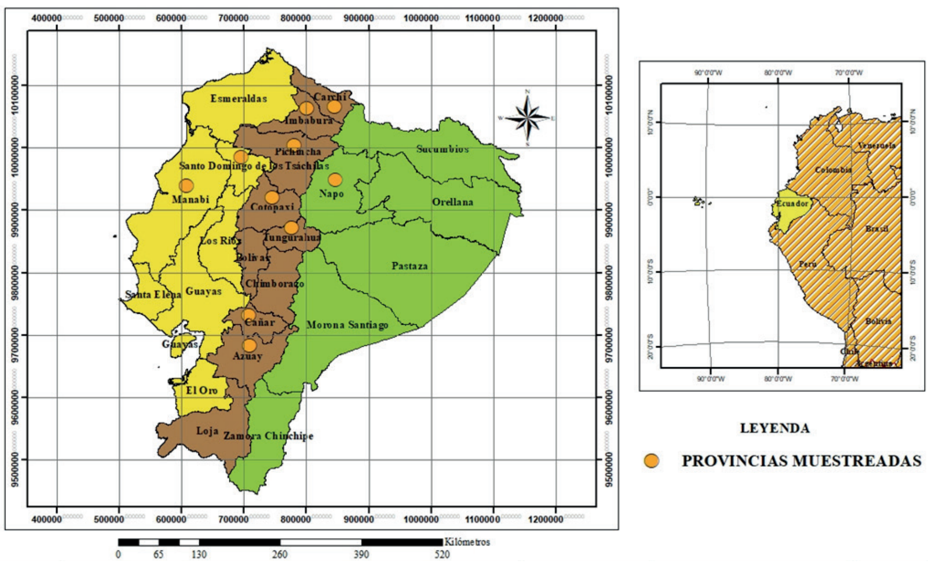

FIGURE 1. Milk sampling areas for characterizing milk quality in three regions of Ecuador.

Source: own elaboration.

Analytical procedures

Compositional analysis
The compositional analysis was conducted at the LCL using mid-infrared spectrophotometry (MILKOSCAN) in accordance with the ISO 9622-IDF 141/2013 method, Guideline for the Application of Mid-Infrared Spectrometry for Milk (LCL-PE-01) at UPS Cayambe (Alvear *et al.* 2021).

Total bacterial count (TBC)
TBC was analyzed using the BACTOSCAN FC equipment following the protocol Bacterial Count Protocol for the Evaluation of Alternative Methods, ISO 16297 IDF 161 / LCL-PE03 through flow cytometry. Results were initially expressed as TBC and subsequently converted to CFU/ml.

Sanitary analysis
Sanitary analysis was conducted using the FOSSOMATIC 7 DC equipment, based on the method Enumeration of Somatic Cells-Part 2: Guidance on the Operation of Fluoro-Opto-Electronic Counters, ISO 13366-2:2006(E) IDF 148-2:2006 / LCL-PE02 through flow cytometry (Orozco & Santana 2022). This analysis provided total somatic cell counts (SCC) and differentiated somatic cell counts (DSCC). Samples with SCC >700,000 cells/ml were classified as positive for mastitis, while samples with SCC <200,000 cells/ml were classified as originating from healthy cows (Quevedo 2018).

Statistical analysis
Microsoft Excel 2023 (Microsoft 365) was used to calculate averages for all analyzed components. Descriptive analysis was performed using Minitab 20.3 (64-bit, 2021), where the data dispersion of DSCC% relative to SCC/ml in the milk samples was examined to determine the presence of mastitis.

Regulatory review
The results were compared with the Ecuadorian Technical Standard INEN 9:2012 (INEN 2012). TBC analysis was further benchmarked against Ministerial Agreement 394 MAG, issued on September 4, 2013 (MAGAP 2013).

Ethical considerations
This project did not involve animal handling. Milk samples were collected from 40-liter milk cans at production units after producers from the three regions had gathered the milk. Sampling adhered to the internal guidelines of the UPS Milk Quality Laboratory (INS-01) and followed the regulations established by the Agency for Phytosanitary and Zoosanitary Regulation and Control (Agrocalidad) in the Instruction Manual for Sampling of Raw Milk and Whey (INT/CL/01).

RESULTS AND DISCUSSION

Compositional quality
The compositional quality of milk can vary depending on multiple factors and appropriate management practices, which are essential for ensuring the nutritional quality of the final product (Juárez *et al.* 2015; Bustamante *et al.* 2023). Regular analyses of milk composition are therefore crucial to preserving its nutritional value and suitability for human consumption.

Fat content

The average fat percentage in the Sierra region was 3.87% g/100 g (table 1), exceeding the standards for raw milk established by INEN 9:2012. These findings are consistent with those reported by Alvear *et al.* (2021), who recorded a fat percentage of 3.9% in Ecuador's Andean region in 2021. Similarly, the results align with data from two cantons in the province of Pichincha, where Salguero *et al.* (2023) found fat percentages of 4.0% in milk collection centers and 3.8% on dairy farms.

In the Coast region, the average fat percentage was 3.46% (table 1), which is also above the parameters established by INEN 9:2012 for raw milk. These values are comparable to those reported by Alvear *et al.* (2021), who evaluated the concentration of fatty acids nationwide and recorded a fat percentage of 3.55% for the Ecuadorian Coast. Vallejo *et al.* (2018) observed fat percentages ranging from 3.97% to 4.03% in Flavio Alfaro canton, 2.67% in Pedernales, and 2.71% in El Carmen. The variation in these results can be attributed to the type of cattle sampled. In this study, milk was predominantly sourced from single-purpose dairy breeds such as Holstein, Jersey, and Brown Swiss, whereas in Manabí, the samples came from dual-purpose breeds such as Girolando, Gyr, and Brangus (Vallejo *et al.* 2018).

In the Amazon region, the average fat content was 4.05% g/100 g, making it the region with the highest percentage of this physicochemical component among the three regions analyzed (table 1). These findings are consistent with those of Alvear *et al.* (2021), who reported a fat content of 4.13%. Similarly, Culqui (2022) observed fat percentages ranging from 3.79% to 4.16% across various milk collection centers in the Amazon region of Peru, which showed minimal differences from the present study.

The differences in fat percentages can be attributed to individual management practices within production units, which include increased production and improved

TABLE 1. Average values of compositional quality parameters in three regions of Ecuador

	Components			
Region	Fat (% g/100 g)	Protein (% g/100 g)	Lactose (% g/100 g)	Total solids (% g/100 g)
Highland	3.87	3.22	4.61	12.47
Coast	3.46	3.35	4.29	11.74
Amazon	4.05	3.25	4.59	12.61
Average				
Ecuador	3.79	3.27	4.50	12.27
Reference value a				
INEN	3.0*	2.9*	-	11.2*

Note: * VMP = Minimum permitted value. Norma Técnica Ecuatoriana INEN 9:2012. [a] (INEN 2012; Laboratorio Calidad de Leche-UPS 2023) There is no reference value.

Source: own elaboration.

handling of high-quality forage (Bernal *et al.* 2007; Requelme & Bonifaz 2012) and enhanced genetics through artificial insemination (Huanca 2001).

Calderón *et al.* (2006) indicated that fat content can be influenced by diet composition, specifically higher fiber concentrations, which lead to increased fat content in milk due to the compensatory effect of volatile fatty acids produced in the rumen. Fat quality is also affected by factors such as lactation stage, genetics, age, health, milking integrity, and seasonality (Harvatine *et al.* 2009; Belage *et al.* 2017).

Protein

Table 1 presents an average protein percentage of 3.22% in the Sierra region, the lowest among the analyzed regions. Nevertheless, this value exceeds the minimum standard established by INEN 9:2012. These averages are comparable to those reported by Alvear *et al.* (2021) for the Sierra region (3.22%) and by Chuquín *et al.* (2016) in the province of Carchi (3.2%).

According to regional averages (table 1), the Coast region exhibited a protein content of 3.35%, surpassing the INEN 9:2012 standard and standing out among the regions of Ecuador. These findings align with data from Suárez (2023), who reported protein percentages ranging from 3.2% to 3.4% in raw milk sold by five vendors in Chone. Similarly, Roncallo *et al.* (2012) observed protein percentages between 3.31% and 3.46% in dry Caribbean production systems, comparable to Suárez's (2023) results, which show minimal differences from this study.

However, the findings differ from the 3.15% protein reported by Alvear *et al.* (2021) for the Coast in 2021. In contrast, Vallejo *et al.* (2018) reported a higher protein percentage of 3.76% in Flavio Alfaro canton, likely due to superior nutritional management practices that enhance amino acid availability for protein synthesis, resulting in increased production.

In the Amazon region, the average protein percentage was 3.25% (table 1). This value exceeds the minimum threshold established by INEN 9:2012, classifying the milk as having good protein quality. These findings are consistent with Alvear *et al.* (2021), who reported a protein content of 3.2% for the region. Similarly, Culqui (2022) found comparable protein percentages in the Amazon region of Peru, ranging from 2.84% to 3.98% across 11 milk collection plants.

The differences in protein percentages are primarily attributed to the genetic variability of the cattle breeds managed within production units. In this region, the majority of producers raise Holstein cows, which produce milk with a lower protein content (3.0%) compared to other breeds such as Jersey or Guernsey cows, which have an average protein content of 3.9% (Penn State Extension 2022; Cajamarca 2022).

Additionally, feed management and quality play a significant role in milk composition, particularly its protein content. Inadequate nutrition can result in deficiencies in both milk production and composition (Cahuascanco *et al.* 2019). Another influencing factor is the presence of diseases in the dairy herd, specifically mastitis, which can lead to a reduction in the protein percentages of milk composition (Campabadal 1999).

Lactose

The Sierra region exhibited the highest lactose percentage, averaging 4.61%

g/100 g (table 1). This result is close to the findings of Cajamarca (2022), who reported a value of 4.78%, representing a difference of 0.17% from this study. Similarly, in an evaluation of the influence of milking on cheese quality within the Inti Churi organization, which assessed bovine milk, lactose percentages ranging from 4.53% to 4.71% were reported (Vallejo 2020). These values align with those of Huillca (2020), who recorded averages of 4.56% and maximum values of 4.80% in milk samples from 162 producers in the Urinsaya region.

For the Coast region, the lactose percentage averaged 4.29% (table 1). This value aligns with one of the results reported by Roncallo *et al.* (2012), where the lowest lactose percentage was 4.44%. However, in the same study, other treatments yielded higher lactose percentages, ranging from 4.59% to 4.65% (Roncallo *et al.* 2012). These latter findings are consistent with those of Aveíga *et al.* (2021), who reported values of 4.61%, 4.68%, 4.60%, and 4.62% in milk from four vendors in the Chone market —values higher than those obtained in this study.

The Amazon region had a lactose percentage of 4.59%, as shown in table 1. Santillan and Frías (2023) reported an average lactose percentage of 5.01%, reflecting a difference of 0.42% compared to this study. Similarly, Culqui (2022) found values ranging from 4.95% (the lowest across different plants) to 5.47% (the highest across 11 milk collection plants), which are higher than those observed in this investigation. Conversely, Ambuludi *et al.* (2017) reported lower averages of 4.36%, 4.30%, and 4.38%. Ortiz *et al.* (2023) documented averages of 4.26% and 4.58%, which are also lower than the values found in this study.

One factor contributing to reduced lactose percentages is product adulteration through water addition to increase volume, which ultimately lowers quality. Additionally, the presence of mastitis (marked by high somatic cell counts) negatively impacts lactose production (Campabadal 1999). Milking frequency also affects milk composition and quality, particularly lactose content. For instance, with once-daily milking, production decreases, while fat and protein concentrations increase, and lactose levels decline (Nandan & Kumar 2022).

Moreover, lactose percentages are influenced by the nutrition of lactating cows. Adequate glycogen availability allows for the physiological processes that synthesize lactose by forming glucose and galactose molecules. The relationship between blood glucose absorption and milk production is fundamental for nutrient concentration (Kittivachra *et al.* 2007).

Total solids

The average total solids (TS) percentage in the Sierra region was 12.47% g/100 g (table 1), exceeding the standard established by INEN (2012), which is 11.2%. This result aligns with data from Vallejo (2020), who identified minimum and maximum TS values ranging from 10% to 15%, and with Huamaní and Morales (2022), who reported an average of 12.62%. Similarly, Padilla *et al.* (2020) highlighted total solids percentages of 12.9% and 12.8% in a district in Peru, observed in two treatments: one without supplementation and another using nutritional blocks.

For the Coast region, the average TS percentage was 11.74%, the lowest among the regions analyzed (table 1); however, this value still exceeds the regulatory standard. These results are comparable to those reported by Sandoval *et al.* (2023), who found an average TS percentage of 12.11%. Similar values were observed by Romero *et al.* (2018) in their evaluation of milk in the department of Sucre, Colombia, with TS averages of 12.23% in the Subregion Sabanas, 11.71% in San Jorge, and 11.95% in the Gulf of Morrosquillo.

In the Amazon region, the average TS percentage was 12.61% (table 1), the highest among the three regions, indicating milk with superior total solids content. These findings differ from those of Ortiz *et al.* (2023), who reported averages of 11.29% in a control treatment and 12.68% in a supplementation treatment. The consistency of these results may be attributed to the presence of *Bos taurus* and *Bos indicus* crosses (Martínez & Gómez 2013), which are commonly managed in the Amazon region of Ecuador.

Total percentages can vary depending on the diet provided to the animals. Higher levels of protein and energy intake lead to increased levels of milk's physicochemical components (Linn 1988). Additionally, seasonal variations can influence TS levels. During the dry season, pastures typically lack the quality observed in the rainy season when they are more abundant and nutritious (Campabadal 1999).

Total solids are a critical factor influencing milk's suitability for processing and its sensory properties, such as flavor and consistency. This is particularly important when the raw material is used for dairy product transformation. Consequently, total solids are a key determinant of milk's nutritional and economic value (Yang *et al.* 2020).

Hygienic quality

Globally, regulations established by authorities and control entities determine permissible limits for total bacterial count (TBC) or colony-forming units (CFU). These parameters vary depending on the country, type of milk (requirements), production process, and storage conditions (Bustamante et al. 2023). Generally, high-quality milk production exhibits a low total bacterial count, whereas elevated TBC levels indicate potential contamination within the milk value chain (Kabera *et al.* 2020).

TABLE 2. Average TBC values that evaluate the hygienic quality of raw milk in the three regions of Ecuador

Region	TBC/ml	CFU/ml
Highland	5.5×10^6	1.5×10^6
Coast	2.4×10^5	6.6×10^4
Amazon	5.7×10^6	1.6×10^6
Average		
	3.8×10^6	1.1×10^6
Reference value [a]		
Ministerial agreement 394 MAG [b]	$3,0 \times 10^5$ ***	$3,0 \times 10^5$ ***
INEN 2012 [a]		$1,5 \times 10^6$

Note: *** VMP = Maximum allowed value. [a] (INEN 2012; Laboratorio Calidad de Leche-UPS, 2023) [b] Ministerial agreement 394 MAG (MAGAP 2013).

Source: own elaboration.

Table 2 shows that the Sierra region has an average value of 5.5×1065.5×10^6 TBC/mL or 1.5×1061.5×10^6 CFU/mL. A comparison with the INEN (2012) standard reveals compliance with the established regulations in this region. However, this average exceeds the maximum permissible value set by Ministerial Agreement 394, suggesting that these samples are not eligible for quality-based payment.

This result aligns with data from the Sierra Centro region, where a CFU/mL value of 1.5 million was reported up until 2013. By 2018, the average had dropped to 200×10^3 CFU/mL, indicating improved milk quality, largely attributed to the involvement of formal producers obligated to meet the standards of nationally recognized companies (Contero *et al.* 2021).

In a collection center in Tungurahua Province, Ecuador, results showed 1.22×10^8 CFU/mL for mesophilic aerobic bacteria during the rainy season, while the dry season recorded values of 8.07×10^6 CFU/mL (Albuja *et al.* 2021). Similarly, Guevara *et al.* (2019) reported mesophilic aerobic counts of 758×10^5 and 802×10^5 CFU/mL for two companies. Such elevated counts may stem from improper milking practices, such as using non-disposable towels shared among cows, using calves near the cows to stimulate milk release, and other practices (Aguilera *et al.* 2014).

In the Costa region, the average TBC was 2.4×10^5 TBC/mL or 6.6×10^4 CFU/mL, as shown in table 2. These values comply with both INEN (2012) and Ministerial Agreement 394 MAG standards, remaining well below the maximum permissible limit and indicating hygienic milk quality.

These results are consistent with those of Contero *et al.* (2021), who reported variable CFU/mL values from 2009 to 2018, ranging from high to low bacterial counts, culminating in an average of 2×10^5 CFU/mL in 2018. In Manabí Province, Ecuador, Arteaga *et al.* (2021) identified an average of 6.25$\log_{10}$ 106.25$\log_{10}$ CFU/mL or 1,778,279.41 CFU/mL, exceeding regulatory limits and reflecting poor milk quality.

In the Amazonian region, the average TBC was 5.7×10^6 TBC/mL or 1.6×10^6 CFU/mL, representing the highest TBC and CFU values in this analysis (table 2). These values surpass the maximum permissible limits established by INEN 9:2012 and Ministerial Agreement 394 MAG, indicating that production systems in this region do not meet hygienic quality standards.

Similar findings were reported by Contero *et al.* (2021), who documented CFU/mL values of 1×10^6 in 2009, though these decreased significantly to an average of <400×10^3 CFU/mL by 2018. In contrast, values reported by Ambuludi *et al.* (2017) ranged from 3.93×10^4 to 3.43×10^4 CFU/mL, which are not directly comparable.

Several factors can contribute to variations in TBC levels during milk production, including inadequate cleaning and sanitation during milking (Bonifaz & Requelme 2011). Carrillo *et al.* (2004) emphasized that failure to adhere to proper hygiene protocols within production units can lead to milk contamination, as bacteria are ubiquitous (Orozco & Santana 2022).

Additional contributing factors include improper temperature management, such as failure to cool and store milk immediately after milking, animal health issues (e.g., mastitis), water quality, cleanliness of production units and equipment, and cross-contamination caused by operators (Aguilera *et al.* 2014; Motta *et al.* 2014).

Although these challenges persist, producers in the region are implementing strategies and forming associations to enhance production quality through

TABLE 3. Sanitary quality in the three regions of Ecuador

Region	SCC/ml	% DSCCx LYMPHOCYTES + PMN
Highland	354 x 10^3	67.52
Coast	110 x 10^4	67.62
Amazon	759 x 10^3	78.21
Average		
	734 x 10^3	71.12
Reference value [a]		
INEN 2012	700 x 10^3	

Note: *** VMP = Maximum allowed value. [a] (INEN 2012; Laboratorio Calidad de Leche-UPS 2023) [b] Ministerial agreement 394 MAG (MAGAP 2013).

Source: own elaboration.

training, advisory services, and regulatory compliance (Motta *et al.* 2014). Consequently, efforts are being made to promote milk production aligned with best practices for milking, handling, and management (Deddefo *et al.* 2023).

Sanitary quality

Elevated somatic cell counts (SCC) indicate udder health problems in cows, such as infections (e.g., mastitis), which compromise the sanitary quality of milk (Orozco & Santana 2022). Sanitary quality is closely linked to hygienic quality, both of which are regulated by the Ministry of Agriculture and Livestock as well as by the companies to which producers deliver their milk. These regulations are based on standards designed to control and monitor milk production (Bustamante *et al.* 2023). The findings from this characterization are presented in table 3.

Differential somatic cell count (DSCC) refers to the proportion of white blood cells, including polymorphonuclear leukocytes (PMN), lymphocytes, and macrophages. Using advanced technology, FOSS has developed a method that simultaneously

TABLE 4. Frequency of DSCC for the identification of the presence of mastitis in the three regions of Ecuador

Quadrant [c]	Parameter [c] DSCC %–SCC/ml	N	Percentage	Reference [c]
A	<65–< 200,000	118	14.2	Healthy
B	>65–< 200,000	165	19.9	Sensitive
C	>65–> 200,000	511	61.5	Subclinical mastitis
D	<65–> 200,000	37	4.5	Chronic–removal

Note: [c] (Orozco & Santana 2022).

Source: own elaboration.

determines SCC and DSCC (Schwarz 2017).

This parameter incorporates four quadrants, each based on the percentage of DSCC and the total SCC value (×1000/mL×1000/mL) to reflect disease status, enabling more precise diagnoses (Orozco & Santana 2022).

Somatic cell count has been pivotal in detecting mastitis. According to Contero *et al.* (2021), an increased SCC is associated with inflammation caused by the non-specific immune response of phagocytosis, a key component of the animal's immune system. Contributing factors include the number of lactations, irritants affecting the udder, postpartum periods, and other variables (Gonçalves *et al.* 2018).

In the Sierra region, an SCC of 354×10^3 cells/mL was recorded (table 3), which complies with the INEN 2012 standard. However, when this value is analyzed alongside the DSCC parameter, with values of 354×10^3 cells/mL and 67.52% DSCC (lymphocytes + PMNs), this region falls into quadrant C (figure 2), indicating the presence of subclinical mastitis.

These findings are consistent with previous studies in the Sierra region, where SCC values below 400×10^3 cells/mL were reported in 2018 (Contero *et al.* 2021). Similar results were reported by Orozco and Santana (2022) in studies of mastitis across various sectors of the Ecuadorian Sierra, with values ranging from 200×10^3 to 500×10^3 cells/mL. According to mastitis dispersion quadrants, these values were classified under quadrant C, corresponding to subclinical and clinical mastitis.

In the Costa region, the average SCC was 110×10^4 cells/mL (table 3), exceeding the maximum permissible value set by INEN standards. This indicates non-compliance with regulatory parameters. An interpretation of the quadrants shows that most samples fall within the >65% DSCC (lymphocytes + PMNs) and >200,000 SCC/mL range, corresponding to cases of subclinical mastitis.

These results align with data from Sucre, Colombia, where an SCC of 1,002,382 cells/mL was reported (Arrieta *et al.* 2019). Similarly, Jurado *et al.* (2019) documented a maximum SCC of 111×10^4 cells/mL,

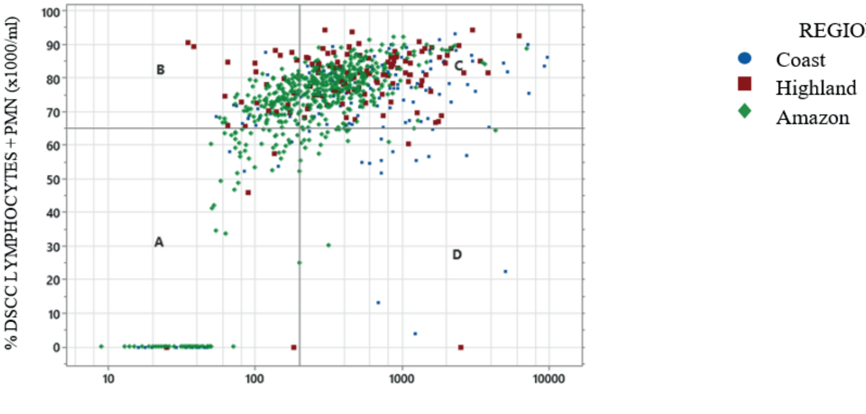

FIGURE 2. Dispersion of SCC and DSCC data in four quadrants for identification of mastitis type.

Source: own elaboration.

recommending that milk with such levels should neither be consumed directly nor used in industrial processes. In an assessment of milk quality among small- and medium-scale producers in Havana, Remón *et al.* (2019) reported an SCC of 1×10^6 cells/mL.

In the Amazonian region, the average SCC was 759×10^3 cells/mL (table 3), exceeding the INEN maximum permissible limit and failing to meet quality standards. Similarly, grouping cases into their respective quadrants showed that most samples fell into quadrant C (figure 2), corresponding to milk from cows with mastitis.

These results are consistent with those of Culqui (2022), who reported high SCC values of up to 705,823.5 cells/mL. Contero *et al.* (2021) noted that SCC values in the Amazonian and Sierra regions of Ecuador were similar, with values below 400×10^3 cells/mL in 2018. However, these values differ from those found in this study. Contrastingly, Santillan & Frías (2023) reported an average SCC of 210×10^3 cells/mL, associating this with milk of average quality suitable for various industrial processes.

In Ecuador, the majority of milk producers face issues with subclinical mastitis, as shown in table 4 and figure 2, with quadrant C classification (>65% DSCC lymphocytes + PMNs and >200,000 SCC/mL).

According to Vallejo *et al.* (2018), high SCC levels in raw milk affect the production of dairy products, primarily due to increased enzymatic activity, such as lipase and plasmin. These enzymes result in unstable curds, reduced cheese yields, bitterness in derivatives (Alhussien & Dang 2018), and decreased shelf life of final products (Krekelberg 2023).

Variations in SCC can be attributed to differences in plant-level regulations, many of which require producers to minimize somatic cell counts while maintaining sufficient production to sustain profitable systems (Ruegg & Pantoja 2013).

CONCLUSIONS

According to the Ecuadorian Technical Standard INEN 9:2012, Requirements for Raw Milk, all three regions of Ecuador meet the regulatory criteria for raw milk to be considered suitable for human consumption and nutritionally adequate.

In terms of hygienic quality, the Costa region complies with the parameters established by regulatory authorities, with a total bacterial count (TBC) of 2.4×10^5 CFU/mL or 6.6×10^4 CFU/mL. In contrast, the Sierra and Amazon regions showed average values of 5.5×10^6 TBC/mL or 1.5×10^6 CFU/mL and 5.7×10^6 TBC/mL or 1.6×10^6 CFU/mL, respectively, reflecting high bacterial content. This highlights the transitional state of production systems in these regions, which are adopting improved hygiene and management practices. The government and dairy industries have implemented stricter inspections, including periodic testing, to ensure milk meets quality and safety standards. Such analyses also inform milk pricing strategies, emphasizing quality-based payments to producers.

Regarding sanitary quality, the average somatic cell counts (SCC) of 354×10^3, 110×10^4, and 759×10^3 SCC/mL, along with differential somatic cell counts (DSCC, lymphocytes + PMNs) of 67.52%, 67.62%, and 78.21%, were recorded for the Sierra, Costa, and Amazon regions, respectively. Based on these values, the Sierra region meets the SCC regulatory standards. However, analyzing the relationship between

SCC and DSCC (lymphocytes + PMNs) reveals that milk samples from various provinces in Ecuador predominantly fall into quadrant C (>65% DSCC and >200,000 cells/mL). This classification indicates that most producers face issues with subclinical mastitis.

Subclinical mastitis is a common disease in production systems, leading to a decline in both the quality and quantity of milk produced. Severe cases may result in the culling of affected cows, causing significant economic losses for producers. These losses are further compounded by increased production costs associated with treating affected cows and by the broader impact on the value chain of this essential raw material.

CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this research.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

No artificial intelligence was used in the conduct of this research.

DATA AVAILABILITY

The data are available to the authors; however, all information remains the intellectual property of Universidad Politécnica Salesiana.

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