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Evaluation of the physicochemical and microbiological quality of raw milk in the dairy industry of Napo, Ecuador

Evaluación de la calidad fisicoquímica y microbiológica de la leche cruda en la industria láctea de Napo, Ecuador

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ABSTRACT

This document focuses on raw milk's physicochemical and microbiological attributes in Napo Province, Ecuador. They explained the problem of insufficient monitoring of quality control systems for raw dairy, emphasizing microbiological parameters that influenced its safety, compositional factors, and the degree of microbial activity. These parameters are necessary to improve Ecuador's dairy industry and international competitiveness. The study's objective focused on determining the value of the physicochemical and microbiological parameters of fat, protein, total solids, non–fat solids, SCC, and TBC to resolve the differences between regions and years. This study gathered 238 samples from collection centers and agricultural production units from Tena, Quijos, and El Chaco between 2020 and 2024. Physicochemical composition was determined using infrared spectrophotometry, while CCS and CBT were analyzed using flow cytometry. Statistical tests included the Kruskal–Wallis test, suitable for non–normal data. The results showed significant differences between cantons and years for all analyzed parameters. The average fat content was 4.03 g·100 mL^{-1}, and the protein was 3.28 g·100 mL^{-1}. SCC and TBC levels exceeded the legal limits, reflecting challenges in hygiene and management practices. Climate, nutrition, and how herds are managed influence milk quality. There is an urgent need to implement better hygiene and management practices to optimize raw milk's microbiological and compositional quality.

Key words: Microbiological quality; total bacterial count; raw milk; physicochemical composition; regional variability

RESUMEN

El presente trabajo analizó la calidad fisicoquímica y microbiológica de la leche cruda en la provincia de Napo, Ecuador. La investigación busca destacar la importancia de garantizar la inocuidad y el valor nutricional de la leche cruda, dado que factores como la composición y los niveles microbiológicos afectan su calidad y seguridad. Estos aspectos son críticos para el fortalecimiento del sector lácteo ecuatoriano y su competitividad en mercados internacionales. El objetivo del estudio fue evaluar parámetros fisicoquímicos y microbiológicos, como grasa, proteína, sólidos totales, sólidos no grasos, conteo de células somáticas (SCC) y conteo bacteriano total (TBC), con el fin de identificar variaciones por región y año. Se analizaron 238 muestras recolectadas entre 2020 y 2024 en centros de acopio y unidades productivas agropecuarias de los cantones Tena, Quijos y El Chaco. Los métodos incluyeron espectrofotometría infrarroja para composición fisicoquímica y citometría de flujo para CCS y CBT. Las pruebas estadísticas incluyeron la prueba de Kruskal–Wallis, adecuado para datos no normales. Los resultados evidenciaron diferencias significativas entre cantones y años en todos los parámetros analizados. Destaca un promedio de grasa de 4,03 g·100 mL^{-1} y proteína de 3,28 g·100 mL^{-1}. Los niveles de CCS y CBT superaron los límites normativos, esto refleja desafíos en las prácticas de higiene y manejo. Factores como el clima, la dieta y el manejo del ganado influyen en la calidad de la leche. Es urgente implementar mejores prácticas de higiene y manejo para optimizar la calidad microbiológica y composicional de la leche cruda.

Palabras clave: Calidad microbiológica; conteo bacteriano total; leche cruda; composición fisicoquímica; variabilidad regional

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INTRODUCTION

Milk is recognized worldwide as a fundamental food due to its high protein, vitamins, minerals, and lipids content. However, in recent years, its consumption has declined in various populations due to concerns about product quality [1]. Quality is also a key demand in key markets such as Asia, where quality certification and traceability significantly influence consumer purchasing decisions, reflecting the demand for transparency and safety in food products [2].

Parameters such as acidity, somatic cell content, and the presence of pathogenic microorganisms define the hygienic–sanitary quality of milk. In addition, chemical residues, heavy metals, and other contaminants may enter the milk during milking, transport, or storage. For instance, De la Cueva *et al*. [3] reported the presence of lead, arsenic, and mercury in raw milk collected in dairy areas close to industrial hotspots in Ecuador. Some of these contaminants may pose serious health risks since they are known to be hepatotoxic, immunotoxin, or even carcinogenic [4]. Dairy producers must follow sanitary and hygienic regulations to preserve the quality and safety of milk. Stricter Veterinary and sanitary control and adequate cleaning and sanitation in the processing plants significantly prevent microbial contamination. Monitoring somatic cell counts regularly is essential since they reflect milk's health status and general condition [5]. In addition, detecting chemical residues and adulterants underscores the importance of rigorous testing to ensure product quality [6].

Several factors determine milk composition and can be classified as natural (intrinsic) or management–related (extrinsic). Natural factors include genetics, which varies by species and breed; the number of lactations, as production and composition change with age; and physiological status, where the stage of pregnancy or type of calving (single or double) can significantly influence milk quality [7].

In Ecuador, at the end of 2022, 815,065 dairy cows were registered in more than 100,000 agricultural units, with a higher concentration in the Sierra region (79.5%), followed by the coast (16.3%) and the Amazon (4.2%) [8]. The Ecuadorian Technical Standard INEN 9 establishes minimum physic–chemical requirements for raw milk, such as fat content (≥3.0%), non–fat dry matter (≥8.2%), and limits for acidity, protein, and microorganisms, among other parameters. These standards aim to ensure quality that not only complies with local regulations but is also in line with international standards, thus strengthening the reputation and competitiveness of the Ecuadorian dairy industry in the global market [9].

In this context, this research aimed to evaluate the physic–chemical and microbiological quality of raw milk received by the dairy industries of Napo, Ecuador, a region with unique geographical and climatic characteristics that could influence the quality of the final product. This study evaluated milk quality by using sanitation and composite analysis parameters. It seeks to assist in enhancing the competitiveness and food safety of the dairy sector in Ecuador.

MATERIALS AND METHODS

Bioethical aspects

The raw milk samples analyzed were obtained by Agrocalidad during routine controls from 20 to 2024, in accordance with national food safety regulations. The company authorized their use in this research, which guarantees the traceability and anonymity of sensitive data

Study area

The research was conducted from 2020 to 2024 in dairy industries in the province of Napo, located in north–central Ecuador, a region characterized by diverse geography and rich biodiversity. This area is part of the Ecuadorian Amazon and covers an area of approximately 12,500 km^2. Its relief varies, combining the presence of tropical forests, mountains, and the influence of the Andes, creating a transitional ecosystem between the Andean foothills and the Amazon rainforest.

Experimental data

The data comes from the Milk Quality Laboratory of the Regulatory and Phytosanitary Control Agency (Agrocalidad) database. Between 2020 and 2024, 238 (N) analyses were carried out in the dairy industries of the province of Napo. The data corresponds to 3 cantons of the province of Napo: Tena 16.39% (n=39), Quijos 47.90% (n=114), and El Chaco 35.71% (n=85).

To determine the values of fat, protein, total solids, and solids–non–fat, researchers used the infrared spectrophotometry method with the MilkoScan™ FT+ (FOSS, Denmark), and the values were expressed in (g·100 mL^{-1}). To establish the somatic cell count (SCC) in raw milk, the Fossomatic™ FC (FOSS, Denmark) was used, and the total bacterial count was determined using the BactoScan™ FC+ (FOSS, Denmark), and the results were expressed in (× 1000·mL^{-1}). SCC and total bacterial count (TBC) were performed using the Flow Cytometry technique, recognized by the International Dairy Federation (IDF). This technique is based on fluorescent staining of the cells, followed by chemical treatment to disperse the milk components. Subsequently, a thin sample layer is conducted through a cell flow under a fluorescent detector, which captures the light emitted by the stained cells, generating electronic pulses that are quantified to determine the number of somatic cells or bacteria present per milliliter of milk [10].

Statistical analysis

The Shapiro–Wilk test showed that all the variables, except for protein, showed significant deviations from normality (P<0.05), indicating the need to use non–parametric tests for further analysis (TABLE I).

TABLE I
Descriptive statistics and Shapiro–Wilk normality test for physicochemical and microbiological parameters of raw milk (n = 238)

Variable	N	Half	SD	W*	*P* (Unilateral D)
Fat	238	4.04	0.58	0.73	<0.0001
Protein	238	3.28	0.15	0.97	0.0001
Total Solids	238	12.80	0.63	0.82	<0.0001
Non–Fat Solids	238	8.76	0.20	0.93	<0.0001
Somatic cell count	238	606.53	561.47	0.62	<0.0001
Total bacterial count	238	8027.54	13546.89	0.62	<0.0001

N: Number of observations, SD: Standard deviation, W*: Shapiro–Wilk test statistics for normality. D= One–tailed *P*–value for deviation from normality

The Kruskal–Wallis test identified significant differences ($P<0.05$) in the values of fat, protein, total solids, non–fat solids, somatic cell count (SCC), and total bacterial count (TBC), according to year and sampling location (TABLE II).

TABLE II
Summary with Kruskal–Wallis test values for each variable

Variable	H (K–W statistic)	Df	*P*–value	Significance
Fat	19.87	2	0.0108	Yes
Protein	42.01	3	<0.0001	Yes
Total Solids	21.99	3	0.0049	Yes
Non–Fat Solids	18.56	2	0.0173	Yes
Somatic cell count	27.66	3	0.0005	Yes
Total bacterial count	31.24	3	<0.0001	Yes

H: Kruskal–Wallis test statistic, Df: Degrees of freedom, *P*–value: Probability value, Significance: Indicates whether the test result was statistically significant (*Yes* if $P<0.05$)

RESULTS AND DISCUSSION

Physic–chemical parameters

Fat

Fat is a compound present in milk and is mainly composed of triglycerides, di – and mono–glycerides, fatty acids, sterols, carotenoids, vitamins (A, D, E, and K), and other trace elements [11] and is also a source of energy in the daily diet. This is the component of milk with the most significant variability, and this variation can also be found between cows of the same breed that receive different types of feed. One factor that most affects the fat percentage in milk is based on the amount of fiber in the diet or the ratio of roughage to concentrate. Therefore, as fiber concentration increases, the proportion of fatty acids is higher than that of milk fat [12]. The results on fat content in raw milk, which averaged 4.03 $g \cdot 100 mL^{-1}$, agree with research by Fernández and Tarazona [13], suggesting that factors such as crossbreeding and diet raise the fat percentage. In El Chaco canton, fat content was exceptionally high, with an average value of 4.17 $g \cdot 100 mL^{-1}$. This may be attributed to local dietary management and supplementation practices specific to the region.

Fat content varied significantly across the years, reaching a peak in 2021 followed by a declining trend thereafter (FIG. 1). This reduction could be linked to several factors, including changes in cattle diet composition, forage quality, and thermal stress. The type of feed and season have a significant impact on milk fat composition. For example, cows grazing on pasture during summer exhibit greater variability in fatty acid content compared to those fed total mixed rations (TMR) [14]. Moreover, forage availability and quality are seasonally adjusted to meet the nutritional needs of lactating animals, directly influencing milk quality [15]. Diets low in effective fiber or high in fermentable carbohydrates can alter rumen fermentation, while heat stress may reduce feed intake and impair fat synthesis. Investigating these aspects could provide valuable insights and enhance the scientific relevance of this study. According to Contero *et al.* [16], he states that from a total of n= 6872 analyzed in the laboratories of the Salesian Polytechnic University from the province of Napo in the period 2009–2018, average data were obtained in the Amazon region of 3. 82 ± 0.10 %, which differs from the general average obtained in this research, in which of the total number of samples analyzed during the period 2020–2024 a general average of 4.17 was obtained, which indicates that there is an increase in the fat content of raw milk produced in livestock farms in the province of Napo.

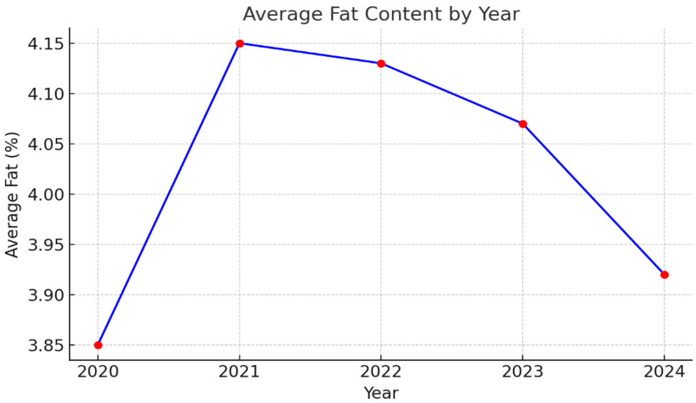

FIGURE 1. Average percentage of fat in raw milk by year, from 2020 to 2024. An increase in fat content is observed up to 2021, followed by a progressive decrease in the following years. Red dots represent the annual average values, connected by a blue line to show the trend more clearly

In the province of Napo, the areas of Quijos and El Chaco tend to have a colder climate compared to Archidona and Tena, which have warmer temperatures throughout the year; this climatic difference is mainly due to the altitude and geographical location of each area, Salazar–Cobo [17] reports that rising temperatures in the cantons of Quijos and El Chaco are associated with a decrease in the fat percentage of raw milk. In contrast, temperature increases in the cantons of Tena and Archidona do not appear to significantly affect milk fat content. These findings are consistent with the observations of Gunn *et al.* [18], who attribute variations in milk fat levels to several factors, including heat stress.

The Kruskal–Wallis test evaluated significant differences in the fat variable between different years and locations (Tena, El Chaco, and Quijos). The results indicated a statistically significant difference in the medians ($H = 19.87$, $P=0.0108$), reflecting a substantial influence of year and location on the fat values in the samples analyzed in this study. Reviewing the means and medians for each group revealed quite a difference between the groups studied, especially with the tremendous fat values in Canton El Chaco in 2021 (Table III).

The elevated milk fat levels in El Chaco canton in 2021 could be explained by region–specific environmental factors and herd management strategies. The climatic conditions at that time, specifically the occurrence of droughts and heavy rains, could have impacted the physiology of the animals and, consequently, the composition of milk; this agrees with Alvear *et al.* [19] who note that the fat component is more significant during some times, especially in the wet season. In this context, it is essential to consider that management practices, such as frequency and duration of milking, type of supplementary feeding, and stress level of the animals,

TABLE III

Descriptive Statistics of Milk Fat content (g·100 mL^{-1}) by Year and Canton

Year	Location	N	Mean	SD	Median	H	*P*
2020	Tena	39	3.84	0.36	3.87		
2021	El Chaco	12	4.32	1.29	3.96		
2021	Quijos	41	4.09	0.41	4.03		
2022	El Chaco	27	4.25	0.96	4.06		
2022	Quijos	29	3.99	0.47	4	19.87	0.0108
2023	El Chaco	31	4.11	0.29	4.15		
2023	Quijos	28	4.02	0.49	4.1		
2024	El Chaco	15	4.03	0.3	4.02		
2024	Quijos	16	3.81	0.52	3.83		

N: Number of observations, SD: Standard deviation, H: Kruskal–Wallis test statistic, *P*–value: Probability value

can also impact milk quality [20]. This interaction between environment, diet, and management has also been documented by Marini and Di Masso [21], who proposed that productive efficiency should be analyzed by incorporating additional variables such as longevity, reproductive behavior, and adaptability to the production system so that the implementation of management practices that reduce stress is essential to maintain animal welfare and optimize milk production [22].

Protein

Milk has traditionally been considered a concentrated source of nutrients, with a high biological value protein content about its energy content [23]. Milk provides easily digestible proteins of high biological value, given that it allows for the amino acids needed to cover requirements. The protein fraction is divided into whey proteins and caseins. The serum proteins are α–lactoalbumin, β–lactoglobulin, other albumins, immunoglobulins, lactoferrin, lactoperoxidase, protease–peptone, lysozyme, and transferrin 2 [24].

A key factor in milk protein content is the type of feed and nutrition they receive, primarily through proper dietary management regarding the forage/concentrate ratio and the source of protein included [25]. Optimal nutritional management can not only improve protein levels in milk but also enhance the immune function of the animals, decreasing their susceptibility to diseases, which impacts favorably on milk production [26], another critical factor according to Jurkovich *et al.* [27], social dynamics is a significant factor within herds as this can influence the stress levels of the cows, such as the genetic variability of the animal [28].

Proteins present in milk, such as casein and whey proteins, are highly valued in the food industry due to their distinctive functional properties, which are essential in various industrial processes and applications [29]; the protein content found showed an average of 3.28 g·100 mL^{-1}, higher than the minimum required by Ecuadorian regulations. According to Weber *et al.* [30], seasonal changes significantly affect the protein content of milk; this correlates with higher temperature and humidity indexes and lower fat and protein content in milk, especially when cows are outdoors [31]. Statistical analysis showed significant differences in protein according to year and location. The results showed a substantial difference in protein content between groups (H = 42.01, $P<0.0001$) (FIG. 2), indicating that the level of protein in raw milk varies significantly depending on the site and year of sampling.

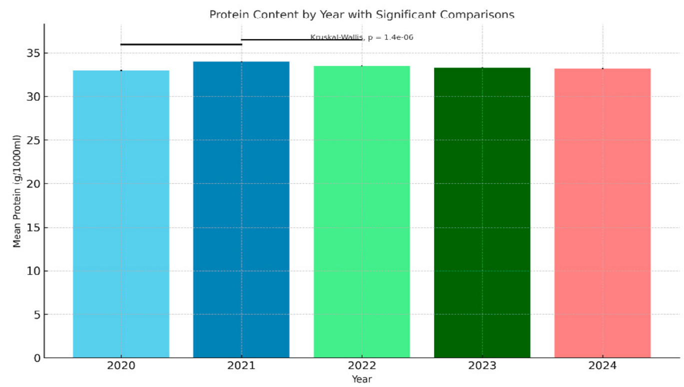

FIGURE 2. Mean protein content in raw milk (g·100 mL^{-1}) by year from 2020 to 2024. A Kruskal–Wallis test indicated statistically significant differences among years (*P*=1.4 × 10^{-6}). Bars show annual means with standard error, and black brackets denote significant pairwise comparisons

These findings indicate that some local regions or the time of year may influence the protein content in raw milk obtained from various locations over different years. What can also result from genetic factors play an essential role, as heritability estimates for milk protein range from 0.284 to 0.596, suggesting the possibility that genetic selection may increase protein content without negatively affecting milk production. Also, Movedi *et al.* [32] have indicated that climatic events or shifts in forage growth can also strongly influence milk composition; severe weather can lower the gross protein content of forage, which is essential for compositional quality. Such adaptive approaches as implementing drought–tolerant forage species and improving pasture control can lessen these impacts and increase resilience in dairy production systems.

Total solids

Milk's total solids (TS) are crucial for assessing its quality and nutritional value. TS generally includes fat, protein, lactose, and minerals, and its concentration can vary depending on several factors, including feeding practices and environmental conditions. Higher total solids content correlates with better nutritional profiles, including higher protein and fat content, improving dairy products' sensory attributes [33]. Physicochemical properties, such as pH and acidity, are influenced by total solids, affecting milk's overall quality and safety [34].

TS concentrations in raw milk vary significantly according to species, processing methods and geographical location. These factors influence the physicochemical composition of the milk. Milk from different species (cow, goat, buffalo) has natural differences in fat, protein and lactose content, which directly affect TS levels. Similarly, processing methods such as dilution, skimming or heat treatment can alter TS concentrations. In addition, geographical variations related to climate, altitude, forage availability and feeding systems also significantly influence the variability of this parameter.

A factor to consider is also the presence of microbial growth as high bacterial counts are often associated with the degradation of specific components of total solids, such as lactose, proteins and fats, due to the metabolic activity of bacteria, so that total solids levels will tend to decrease (FIG. 3), especially mesophilic bacteria (*E. coli* and *Salmonella*), can use lactose as an energy source, reducing its concentration and thus affecting the percentage of TS in raw milk [35], this correlates with Duarte *et al.* [36] that raw milk with high levels of TS can withstand longer storage periods without significant degradation.

Although an increase in total bacterial count (TBC) is generally expected to lead to a decrease in total solids (TS) due to the degradation of components such as lactose, protein and fat by bacterial activity, a positive correlation between TBC and TS was observed in our study. This unexpected trend could be attributed to factors such as accumulation of bacterial metabolites, evaporative water loss during handling or initial variations in milk composition. These findings suggest that, under certain conditions, higher CBT does not necessarily translate into a reduction of ST, highlighting the complexity of microbial interactions in raw milk.

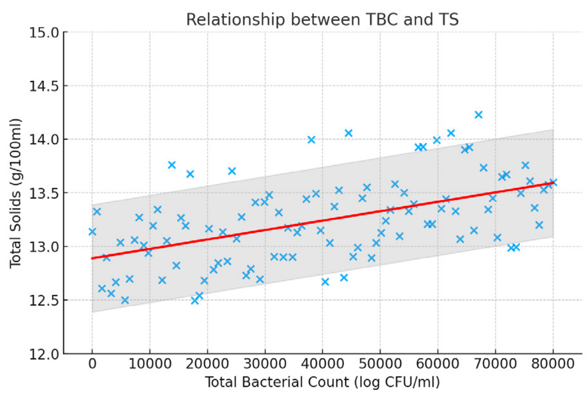

FIGURE 3. Scatter plot showing the relationship between TBC (× 1000·mL^{-1}) and TS (g·100mL^{-1}) in raw milk. The red line represents the linear regression model, and the shaded area indicates the 95% confidence interval

The average total solids (12.80 g·100 mL^{-1}) in this study are within the acceptable range for raw milk and comparable to those reported in other studies in the Amazon. Velez–Terranova *et al.* [37] suggest that seasonal and temperature variations directly affect total milk solids. Due to the amount of rainfall and the type of forage available, Amazonian conditions increase the lactose and protein content, contributing to high levels of total solids.

The figures obtained from the statistical analysis of the total solid's variable showed a stark difference in the values evaluated in the Kruskal–Wallis test (H = 21.99; P=0.0049). This denotes that the total solids content in milk differs in this study concerning the year and the collection location. In Tena, in 2020, the mean Total Solids content was 12.62 ± 0.36, whereas in other years and locations, the respective means ranged around 12.48 to 13.03 and 12.40 to 13.03 as medians.

As temperature and humidity affect stress levels for the animals, there is an impact on feed intake and, ultimately, milk composition, making seasonal changes particularly potent Kostovska *et al.* [38]. During warmer months, cows experience heat stress, reducing milk production and altering milk composition, including total solids and protein content. As Murphy *et al.* [39] noted, the number of total solids, the density, and other milk composition characteristics oscillated with the seasons. More milk solids were noted in colder seasons when cows had less heat stress. Hence, seasonality has more impact than other factors on the composition of raw milk.

Non–fat solids

The non–fat solids (NFS) composition of raw cow's milk, which includes protein, lactose, and minerals [40], usually ranges between 8% and 9%. The cow's diet significantly influences the nutritional index of milk, and variations in dietary components affect the levels of specific nutrients, including NFS levels (without short–chain glycerol). Research indicates that different feed supplements and nutritional practices can cause marked changes in milk composition and quality. It should be emphasized that different breeds and stages of lactation can cause variations in NFS content, and older cows generally produce milk with higher levels of NFS [41]. The health status of cows due to conditions such as subclinical ketosis can alter the nutritional quality of milk, negatively modifying the composition of NFS [42], so it is essential to consider that the nutritional quality of milk can be altered using different feed supplements and dietary practices, so it is of utmost importance to closely monitor the factors that can modify it to ensure that raw milk maintains its nutritional qualities. The NFS content was 8.76g·100 mL^{-1}, a value that meets the quality expectations of the Ecuadorian standard NTE INEN 9:2012.

The variability of NFS in raw cow's milk from the Amazon reveals significant variability influenced by regional and seasonal factors; this variability can be explained by various factors such as diet, cattle management, and environmental conditions since during periods of higher temperature, cows may experience heat stress, which reduces feed intake and therefore alters the concentration of specific components in the milk so that the composition of non–fat solids is higher in the winter months due to the lower presence of heat stress.

This type of variability in NFS, total solids, fat, and protein (FIG. 4) has implications for the dairy industry, as it affects the quality and functionality of milk in derived products such as cheese and yogurt. The results of this study highlight the importance of considering environmental and management factors in maintaining consistency in milk quality across regions and seasons. Variability in non–fat solids content may arise from changes in feeding strategies, forage quality, lactation stage, and climatic conditions such as temperature and humidity, which affect nutrient intake and metabolic efficiency in dairy animals.

Microbiological parameters

Somatic cell count

Somatic cell count (SCC) in raw milk is a critical indicator of milk quality and udder health, particularly mastitis, so high levels are generally associated with microbial contamination; they can negatively affect the chemical composition of milk [43]. International regulations control the amount of SCC in raw milk,

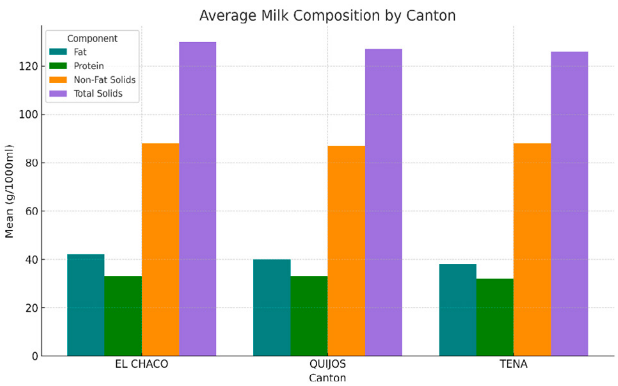

FIGURE 4. Average values (g·100 mL^{-1}) of fat, protein, non–fat solids, and total solids in raw milk from the cantons of El Chaco, Quijos, and Tena. Bars represent mean values by component and canton

such as the case of The European Union, which has established a maximum SCC threshold of 400,000 cells·mL^{-1} to ensure the safety and quality of milk [44].

The SCC values exceeded the limits established in Ecuadorian regulations. The Kruskal–Wallis test (H = 27.66, P=0.0005) reveals statistically significant differences in SCC values. It is important to emphasize that the Amazon region has very particular climatic, geographic, and cultural conditions, so dairy production management differs from other areas of Ecuador. A study conducted in the Amazon region of Peru, where SCC was evaluated in several collection centers, revealed significant differences in the Jumbilla basin, which exhibited the lowest SCC. In contrast, other basins showed higher counts [45], suggesting different types of dairy herd management within the Amazon region, depending on the place of production. Thus, the high mean SCC in El Chaco, in contrast to Tena and Quijos (FIG. 5), could be influenced by geographic and environmental factors specific to the region, such as altitude, climate, and other ecological elements. These factors affect the health and physiology of cows. They can influence milk production and SCC, which is related to what Guliński and Kroszka [46] state: high temperatures and higher humidity, as in hot climate zones, increase the risk of mammary infections, thus increasing SCC in milk.

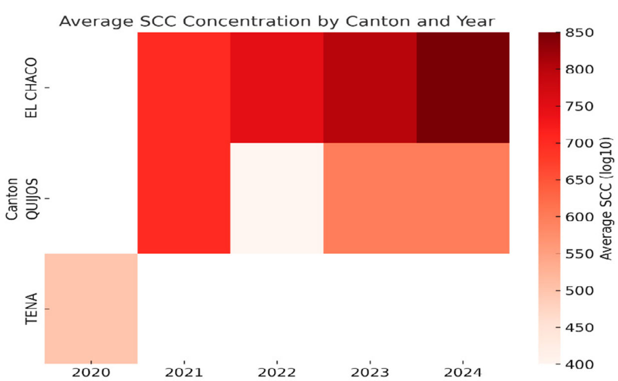

FIGURE 5. Heatmap showing raw milk's average somatic cell count (SCC, log$_{10}$ scale) by canton and year. Darker red tones represent higher values

The reduction of SCC in milk requires an integrated approach that combines good management practices with the control of environmental factors. Put hygiene protocols for milking and udder dipping; these practices should constitute Good Agricultural Practices (GAP) to prevent mastitis [47]. Moreover, the effect of heat stress on herd health, milk quality, and other factors must be considered. Ventilation systems can enhance welfare while, at the same time, reducing CSS through the control of environmental bacterial load [48].

Total bacterial count

Total Bacterial Count (TBC) is one of the basic parameters of quality raw milk and cows' health, especially when checking for mastitis. Elevated levels of CBT indicate that farm hygienic practices are low, which can affect an increase in CCS count and finally impact milk production and profitability [49]. In regions with a tropical climate like the Amazon, TBC control becomes complicated because of environmental conditions that support the growth of pathogens and impede proper milk preservation.

The mean TBC in raw milk (8027.54 × 1000) was much higher than the cutoff set by the regulations and was similar to Arauco–Villar *et al*. [50] findings of high bacterial burdens in the raw milk of Peruvian Andean herds, which was primarily due to poor hygienic milking and product handling practices. The P values relative to the Kruskal–Wallis (H) analysis of variance at the various sites are significant (P<0.05), validating that the differences in CBT between locations and years are indeed statistically significant. The most pronounced difference between 2020 and 2024 (FIG. 6) could be related to changes in health policies, management practices, or even climatic events that affected milk production conditions.

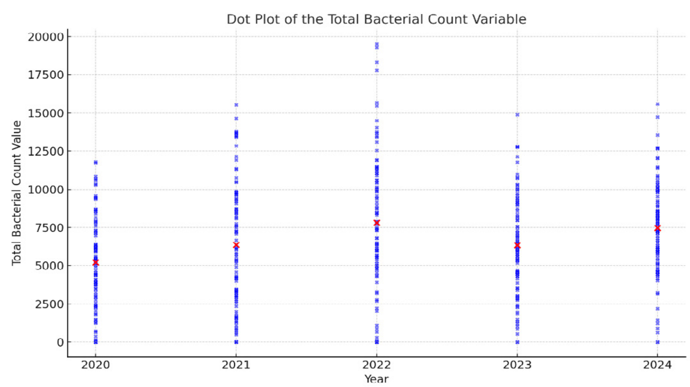

FIGURE 6. Dot plot shows the distribution of TBC values by year. Each blue dot represents an individual sample, while red dots indicate yearly meaning

Poor cleaning protocols for transport equipment can raise the bacterial load, as seen in an email where transported samples had higher counts than farm samples [51]. Poor storage practices can also influence the proliferation of spoilage microorganisms on dairy products, thus affecting shelf life and safety [52]. Also, tropical regions like the Amazon have difficulties because of high humidity combined with high temperatures that aid the growth of microorganisms.

The variability in mean values of TBC between Cantons, such as El Chaco (6814.84 × 1000 mL) and Quijos,(10633.84 × 1000 mL) over the years could suggest that certain areas face more significant difficulties in implementing effective hygiene and management controls, suggesting poor barn hygiene and inadequate cleaning of milking equipment contribute to elevated levels of bacteria in milk [53]; specific management practices, such as the use of appropriate drying materials and mastitis control programs, can substantially improve milk quality by reducing bacterial contamination counts.

Seasonal comparison of milk quality parameters

To determine whether precipitation intensity influences milk quality in the Amazonian region, samples were grouped into 'more rainy' (November to June) and 'less rainy' (July to October) seasons based on INAMHI criteria for the Amazon region. TABLE IV summarizes the results of the Mann–Whitney U test comparing milk parameters between both periods.

TABLE IV
Comparison of raw milk quality parameters between rainy and dry seasons in Napo, Ecuador (2020–2024)

Variable	Mean Rainy Season	Mean Dry Season	*P*–value	Sig.
Fat	4.05 ± 0.53	4.02 ± 0.64	0.057	No
Protein	3.28 ± 0.15	3.27 ± 0.15	0.7083	No
Total Solids	12.82 ± 0.61	12.78 ± 0.67	0.1366	No
Non–fat solids	8.76 ± 0.22	8.77 ± 0.22	0.9367	No
Somatic cell count	623.46 ± 514.48	582.36 ± 624.45	0.3686	No
Total bacterial count	9062.19 ± 14486.80	6549.48 ± 11955.84	0.0156	Yes

P–value: Probability value, Sig.: Significance, indicates whether the test result was statistically significant (*Yes* if $P<0.05$)

The seasonal comparison of raw milk parameters between the rainy and dry periods in Napo revealed that total bacterial count (TBC) was significantly higher during the rainy season ($P=0.0156$), while fat, protein, total solids, non–fat solids, and somatic cell count did not show significant differences ($P>0.05$) (TABLE IV). This result aligns with findings from other tropical regions, where increased rainfall and humidity contribute to greater microbial proliferation during milking, storage, and transportation processes [54]. Elevated TBC values during the rainy season may be linked to environmental contamination, inadequate sanitation during collection, and slower cooling times in high–moisture conditions [55]**.** Although no significant variation was observed in the physicochemical composition, the slight reduction in fat content during the dry season could reflect changes in pasture availability or animal hydration status, consistent with observations in similar Amazonian studies [56]. The lack of significant variation in somatic cell count suggests that udder health is not substantially affected by rainfall intensity in this region, likely due to consistent management practices across the year [57]**.** These findings underscore the importance of reinforcing hygienic handling and cold chain protocols during high–rainfall months to mitigate microbial risks, particularly in tropical dairy systems [58].

CONCLUSIONS

The high levels of TBC and SCC reflect the urgent need to improve hygiene practices during milking, storage, and transport in Napo province; training and adopting technologies are essential to reducing these values.

The physicochemical composition of milk (fat, protein, and total solids) varies significantly according to altitude, environmental conditions, and management practices in each canton, highlighting the importance of optimizing feeding and reducing heat stress in cattle.

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Conflict of interests

The authors declare no conflict of interest regarding the publication of this manuscript.

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