



## Low quality and presence of antibiotic residuals in raw milk sold in informal markets from Cañar, Azuay, and Loja-Ecuador

Puga-Torres Byron<sup>1\*</sup>, Aragón Eduardo<sup>1</sup>, Martínez Evelyn Pamela<sup>1</sup>, Ayala Valeria<sup>1</sup>, Caiza María Belén<sup>2</sup>, Luzón Michael<sup>1</sup>, Maiza Marlon<sup>1</sup>, Moncayo Génesis<sup>1</sup>, Pilaguano Lizbeth<sup>1</sup>, Sotomayor Patricia<sup>3</sup>, Vaca Verónica<sup>1</sup>, De la Torre David<sup>4</sup>, Navarrete Hugo<sup>5\*</sup> and Núñez Luis<sup>6,7</sup>

<sup>1</sup>Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador, Jerónimo Leyton s/n and Gatto Sobral, <sup>2</sup>James Brown Pharma, Quito, Ecuador, <sup>3</sup>Gobierno Autónomo Descentralizado de la Provincia de Pichincha, Quito, Ecuador <sup>4</sup>LABIGEN, Laboratorio de Biología y Genética Molecular, De las Higueras, 118, Quito-Ecuador, <sup>5</sup>Centro de Estudios Aplicados en Química y Carrera de Biología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador, <sup>6</sup>Facultad de Ciencias de la Salud, One Health Research Group, Universidad de las Américas, Quito, Ecuador, <sup>7</sup>Facultad de Ciencias de la Salud, Carrera de Medicina Veterinaria, Universidad de las Américas (UDLA), Quito, Ecuador, Antigua Vía a Nayón s/n, EC

**\*Corresponding Authors:** Byron Puga, PhD, Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador, Jerónimo Leyton s/n and Gatto Sobral, Av. Universitaria, Quito 170129, Ecuador; Hugo Navarrete, PhD, Centro de Estudios Aplicados en Química y Carrera de Biología, Pontificia Universidad Católica del Ecuador (PUCE) en Quito es Avenida 12 de Octubre 1076 y Vicente Ramón Roca, código postal 170525, Pichincha, Ecuador

**Submission Date:** January 27th, 2026; **Acceptance Date:** March 6th, 2026; **Publication date:** March 13th, 2026

**Please cite this article as:** Byron P. T., Eduardo A., Pamela M. E., Valeria A., Belén C. M., Michael L., Marlon M., Génesis M., Lizbeth P., Patricia S., Verónica V., David D. L. T., Hugo N., Luis N. Low quality and presence of antibiotic residuals in raw milk sold in informal markets from Cañar, Azuay and Loja-Ecuador. *Functional Food Science*. 2026; 6(3): 160 - 176.

DOI: <https://doi.org/10.31989/ffs.v6i3.1907>

### ABSTRACT

**Background:** Bovine milk is a dietary staple in Ecuador and a major source of income for smallholder farmers. However, milk distributed through informal markets often lacks adequate sanitary control and regulatory oversight, potentially compromising its physicochemical quality and safety.

**Objectives:** This study aimed to evaluate the physicochemical quality, nutritional composition, somatic cell counts, and presence of antibiotic residues in raw bovine milk sold through informal markets in the southern Ecuadorian provinces of Azuay, Cañar, and Loja.

**Methods:** A total of 358 raw milk samples were collected from informal retail points and analyzed for physicochemical parameters, nutritional composition, somatic cell counts, and antibiotic residues using standardized laboratory methods. Results were compared with Ecuadorian regulatory quality standards.

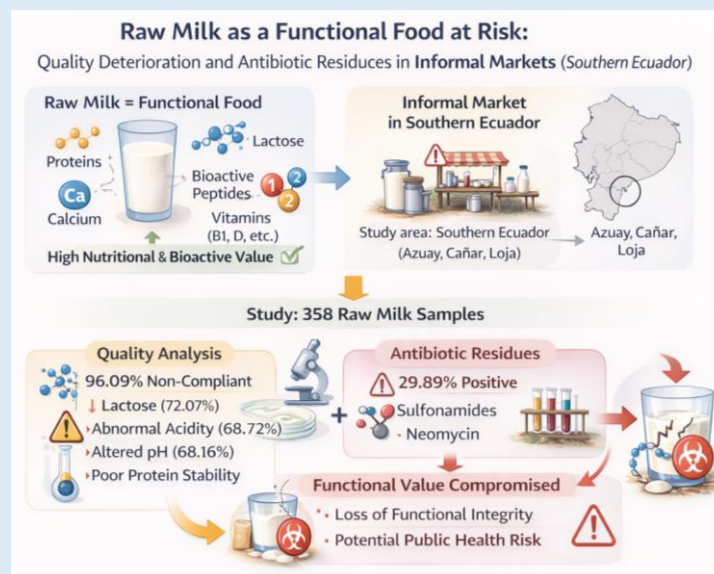
**Results:** Overall, 96.09% of the samples failed to comply with national quality standards. The highest non-compliance rates were observed for lactose content (72.07%), titratable acidity (68.72%), pH (68.16%), and protein stability (43.3%), suggesting inadequate hygienic practices and probable bacterial contamination. Additionally, 29.89% of samples contained antibiotic residues above permissible limits, with sulfonamides and neomycin being the most frequently detected compounds.

**Novelty:** This study provides comprehensive and up-to-date evidence on quality deficiencies and antibiotic contamination in raw milk sold through informal markets in southern Ecuador, a sector with limited systematic surveillance data.

**Conclusion:** The high prevalence of physicochemical non-compliance and antibiotic residues indicates that most raw milk marketed informally in Azuay, Cañar, and Loja is unsuitable for human consumption. These findings highlight the urgent need to strengthen regulatory enforcement, improve hygienic practices at the production and distribution levels, and promote responsible antimicrobial use in dairy systems.

**Keywords:** raw bovine milk; informal dairy markets; antibiotic residues; sulfonamides; neomycin; somatic cell count; physicochemical quality; Ecuador (Azuay, Cañar, Loja)

**Graphical Abstract:** Raw Milk as a Functional Food at Risk: Quality Deterioration and Antibiotic Residues in Informal Markets of Southern Ecuador



**Graphical Abstract:** Low quality and presence of antibiotic residuals in raw milk sold in informal markets

©FFC 2026. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0>)

## INTRODUCTION

Cow's milk is one of the most widely consumed foods worldwide and is valued for its high nutritional density, providing substantial amounts of high-quality proteins, lipids, carbohydrates, vitamins, and essential minerals. It also constitutes a foundational raw material for the dairy industry, underpinning the manufacture of products such as cheese, butter, and yogurt. Accordingly, ensuring that milk meets stringent quality specifications and safety standards is imperative for both public health and industrial processing [1–3].

However, ensuring the quality and safety of milk is not always feasible, particularly in developing regions (e.g., Latin America, Asia, Africa), where the informal sale of raw milk is widespread. This practice presents both health and economic risks [4], as the lack of appropriate oversight can lead to the sale of adulterated products (e.g., diluted with water), contaminated with biological agents (e.g., pathogenic microorganisms), and chemical residues (e.g., antibiotics, pesticides, heavy metals), rendering the milk unsafe for human consumption and posing a significant public health concern [5–10].

Culturally, many individuals continue to prefer milk that has not undergone industrial processing, believing it is more affordable, nutritious, natural, and of superior quality compared to pasteurized milk [11].

In Ecuador, the dairy sector represents a vital component of the national economy, contributing approximately 0.34% to the national GDP and generating around 10% of employment nationwide. Daily milk production is estimated at 5.3 million liters, of which 17% originates from the southern provinces of Azuay, Cañar, and Loja. Specifically, Azuay produces approximately 455,885 liters/day (8.58% of national production), Cañar 353,643 liters/day (6.66%), and Loja 102,609 liters/day (1.93%) [12].

Despite efforts to regulate the dairy industry, over 50% of milk in Ecuador is sold through informal channels,

bypassing standard quality controls and treatments, thereby significantly compromising its safety and nutritional quality, posing substantial public health risks [13–16].

The minimum quality requirements for raw milk intended for further processing are established by the Ecuadorian Technical Standard (NTE) INEN 9 (INEN, 2012). This standard has been utilized in various local studies to assess the quality of raw milk sold informally across different provinces, revealing high variability in compliance with quality parameters. Commonly, a large percentage of samples fail to meet these standards, indicating poor nutritional and hygienic quality [17-18].

In contrast, milk legally sold and processed by the formal dairy industry consistently meets nutritional and hygienic standards in accordance with national regulations [19-20].

Given the importance of milk safety and the widespread prevalence of informal markets in southern Ecuador (Azuay, Cañar, and Loja), this study aimed to evaluate the quality of raw bovine milk sold in these markets. The analysis focused on physicochemical properties, nutritional composition, hygienic quality (somatic cell count), and the presence of antibiotic residues in this widely consumed product.

## MATERIALS AND METHODS

**Study Design and Sampling:** This cross-sectional study was conducted between January 2024 and January 2025. Raw milk samples sold informally were collected from the provinces of Azuay, Cañar, and Loja in southern Ecuador. To obtain a representative sample for each province, a stratified sampling method was applied by canton. Accordingly, samples were collected from the cantons of Chordeleg, Cuenca, Gualaceo, Paute, and Sigsig in Azuay Province; Azogues, Biblián, Cañar, and El Tambo in Cañar Province; and Gonzanamá, Loja, and Saraguro in Loja Province.

The following establishments were designated as informal raw milk sale points: open-air markets, municipal markets, butcher shops, fruit and vegetable stores, restaurants, retail shops, motorized vehicles, street vendors, and private households. This approach resulted in the selection of 15 informal sale points in Azuay, 12 in Cañar, and 11 in Loja, with a 15-day interval between collection rounds. In total, 358 raw milk samples were collected for analysis.

**Laboratory Analysis:** Sample collection was conducted in accordance with the Ecuadorian Technical Standard NTE INEN ISO 707 [21]. Each milk sample was divided into two containers: one without preservatives and the other with bronopol (2-bromo-2-nitropropane-1,3-diol) as a preservative. A volume of 100 mL of raw milk was placed in each container. Both containers were labeled with alphanumeric codes and stored at 2–6 °C before being transported to the Milk Quality Control Laboratory located at the Uyumbicho Experimental Center of the Central University of Ecuador. Samples were analyzed within 72 hours of collection.

Milk composition parameters, including fat, protein, lactose, total solids (TS), solids-not-fat (SNF), and somatic cell count (SCC), were determined using the automated COMBIFOSS™ system (FOSS, Denmark), which integrates Fourier-transform infrared spectroscopy (FTIR) for physicochemical components and flow cytometry for SCC quantification. The FTIR method is based on the absorption of infrared radiation at specific wavelengths corresponding to molecular bonds (C–H, N–H, O–H), allowing simultaneous multi-component quantification through previously calibrated prediction models. Calibration was performed using reference standards traceable to ISO-compliant methods, and instrument performance was verified daily using control milk samples with known composition. SCC was determined by flow cytometry after fluorescent staining of cellular DNA, enabling discrimination and enumeration

of somatic cells based on light-scattering and fluorescence-intensity parameters. Internal quality control included duplicate analysis and routine verification with control materials to ensure repeatability and accuracy.

Relative density, freezing point depression, and detection of added water were analyzed using a LACTOSCAN automatic milk analyzer (Boeckel + Co GmbH + Co. KG, series SAP-CC-028608). Freezing-point determination is based on cryoscopic analysis, which measures the depression of the freezing temperature relative to pure water. This parameter is a recognized indicator of milk adulteration by water addition. Calibration was performed according to the manufacturer's specifications using certified reference solutions, and the results were interpreted in accordance with national regulatory limits.

Titrateable acidity was determined by volumetric titration with standardized NaOH solution and expressed as % lactic acid, following official dairy analytical procedures. pH was measured using a calibrated digital potentiometer (EON TRADING, Model PHT 004TA, Serial SN H00601659) with automatic temperature compensation. The pH meter was calibrated daily using buffer solutions (pH 4.00 and 7.00), ensuring analytical reliability.

Protein stability was evaluated using the alcohol stability test at 68% ethanol concentration, following the procedure established in Ecuadorian Standard INEN 9:2011 for raw milk quality assessment. This qualitative assay assesses the physicochemical stability of casein micelles under ethanol-induced stress and is commonly used as an indicator of milk freshness and mineral balance.

The presence of antibiotic residues was screened using commercial rapid immunochromatographic test strips (AMINO 3IN1 and 3IN1 BTS, Shenzhen Bioeasy Biotechnology Inc., Guangdong, China). These tests are

based on competitive lateral flow immunoassay principles for the qualitative detection of  $\beta$ -lactams, tetracyclines, and sulfonamides. Assays were conducted according to the manufacturer's instructions, including the use of positive and negative control samples. Detection limits are equal to or below the maximum residue limits (MRLs) established by international standards.

**Analytical Limitations and Validation Strategy:** All analytical procedures were conducted in accordance with the Ecuadorian Technical Standards, including NTE INEN ISO 707 for milk sampling and INEN (2011) for alcohol stability testing, which are harmonized with internationally recognized ISO methodologies. The analytical platforms used (COMBIFOSS®, LACTOSCAN®, calibrated pH meter, and Bioeasy rapid test kits) are widely applied in routine dairy quality control and regulatory monitoring. While we did not use more specific confirmatory methods to ensure reliability, all instruments were operated according to manufacturer specifications, routine calibration procedures were applied, and analyses were conducted under standardized laboratory conditions at an accredited institutional facility. The use of officially recognized regulatory methods strengthens comparability with national monitoring programs and international dairy quality assessment frameworks. Despite the inherent limitations of screen-based methodologies and cross-sectional sampling, the analytical approach employed provides strong evidence for regulatory compliance evaluation and public health risk assessment.

Statistical analysis: Laboratory results were organized in a Microsoft Excel spreadsheet, version 16.95.1-2025. Statistical analysis was performed using R

statistical software (RCran, version 1.2.5019) and the RStudio platform (RStudio Inc., Boston, MA, USA, version 1.2.5019).

To determine compliance with the quality parameters established by the NTE INEN 9 standard, the number of milk samples within the reference values was divided by the total number of samples analyzed in each province. This calculation was expressed as a percentage and applied to all physicochemical, nutritional, hygienic quality, and antibiotic-residue parameters. In addition, the mean, minimum, and maximum values were calculated for numerical parameters, including added water, cryoscopic point, pH, titratable acidity, relative density, SCC, protein percentage, fat, SNF, TS, and lactose. For protein stability and antibiotic residues, which are qualitative in nature, absolute and relative frequencies were calculated.

To determine whether milk quality differed across provinces of origin, two statistical tests were applied depending on the data type, using a significance level of 5%. For numerical parameters, normality was assessed using the Kolmogorov-Smirnov test ( $p$ -value < 0.000 for all parameters). Since the data did not follow normal distribution, the non-parametric Kruskal-Wallis test was used, followed by Dunn's post hoc test with Bonferroni correction. For qualitative parameters, the Chi-square test was used to assess whether there was an association between protein stability and the presence of antibiotic residues across different provinces.

## RESULTS

In total, 358 raw milk samples were analyzed from the provinces of Azuay, Cañar, and Loja, of which 96.09% did not comply with at least one of the analyzed milk quality parameters (Table 1).

**Table 1:** Descriptive statistics and regulatory compliance of raw milk quality parameters based on NTE INEN 9 in southern Ecuador (Azuay, Cañar, and Loja; N = 358).

Parameter	Reference values NTE INEN 9	Minimum Value	Maximum Value	Mean	Comply N (%)	Does not comply N (%)
Relative density (g/ml)	1.028 – 1.033	1.017	1,55	1.029	255 (71.23)	103 (28.77)
Titration acidity (°D)	13 – 17	3.80	63.00	18.89	112 (31.28)	246 (68.72)
pH	6.6 – 6.8	4.47	7.32	6.33	114 (31.84)	244 (68.16)
Fat (%)	Mín. 3.2	0.02	17.54	3.41	247 (68.99)	111 (31.01)
Crude protein (%)	Mín. 2.9	2.02	6.41	3.18	284 (79.33)	74 (20.67)
Total solids (%)	Mín. 11.2	7.98	25.05	11.96	263 (73.46)	95 (26.54)
Non-fat solids (%)	Mín. 8.2	5.67	13.69	8.56	289 (80.73)	69 (19.27)
Lactose (%)	Mín 4.8	2.76	6.37	4.62	100 (27.93)	258 (72.07)
Added water (%)	0.00	0.00	34.61	2.18	254 (70.95)	104 (29.05)
Freezing point (°C)	-0.536 a -0.512	-0.7590	-0.3400	-0.532	123 (34.36)	235 (65.64)
Somatic cells (CS/ml)	Máx. 700000	2000	8900000	386466	321 (89.66)	37 (10.34)
Protein stability	Negativo	-	-	-	203 (56.70)	155 (43.30)
Antibiotic residues	MRL*	-	-	-	250 (70.11)	107 (29.89)

\*MRL Maximum Residue Limit

The parameters with the highest percentage of non-compliance were lactose content at 72.07% (258/358), titration acidity at 68.72% (246/358), pH at 68.16% (244/358), freezing point at 65.64% (235/358), and protein stability at 43.3% (155/358). Additionally, 29.89% (107/358) of the samples tested positive for antibiotic residues, exceeding the Codex Alimentarius maximum limits (Table 1).

Regarding compliance with raw milk quality parameters by province, generally low percentages were observed: 5.21% in Loja (5/84), 4.49% in Azuay (8/178), and 1.04% in Cañar (1/96). Table 2 presents the percentage of compliance and non-compliance for each evaluated parameter according to the NTE INEN 9 standard, disaggregated by province.

**Table 2:** Descriptive statistics, regulatory compliance, and statistical comparison of raw milk quality parameters among provinces of southern Ecuador: Azuay (N = 78), Cañar (N = 96), and Loja (N = 84).

Parameter	Referencial value NTE INEN 9	Province	Minimum Value	Maximum Value	Mean	Comply N (%)	Does not comply N (%)	p value
Relative density (g/ml)	1.028 – 1.033	Cañar	1.019	1.033	1.029	72/96 (75.00)	24/96 (25.00)	<0.0001
		Azuay	1.017	1.040	1.030	127/178 (71.35)	51/178 (28.65)	
		Loja	1.019	1.055	1.029	56/84 (66.67)	28/84 (33.33)	
Titration acidity (°D)	13 - 17	Cañar	7.8	62	18.78	20/96 (20.83)	76/96 (79.17)	0.41
		Azuay	3.8	63	19.58	59/178 (33.15)	119/178 (66.85)	
		Loja	5.1	38	17.59	51/84 (60.71)	33/84 (39.29)	
pH	6.6 – 6.8	Cañar	4.65	7.32	6.38	20/96 (20.83)	76/96 (79.17)	0.016
		Azuay	4.47	7.18	6.20	61/178 (34.27)	117/178 (65.73)	
		Loja	5.4	7.31	6.52	52/84 (61.90)	32/84 (38.10)	
Fat (%)	Mín. 3.2	Cañar	2.32	5.37	3.52	84/96 (87.50)	12/96 (12.50)	0.11
		Azuay	0.20	17.54	3.38	113/178 (63.48)	65/178 (36.52)	

Parameter	Referencial value NTE INEN 9	Province	Minimum Value	Maximum Value	Mean	Comply N (%)	Does not comply N (%)	p value
		Loja	1.07	7.25	3.35	50/84 (59.52)	34/84 (40,48)	
Crude protein (%)	Mín. 2.9	Cañar	2.02	4.41	3.17	82/96 (85.42)	14/96 (14,58)	0.56
		Azuay	2.12	6.41	3.19	143/178 (80.34)	35/178 (19,66)	
		Loja	2.16	5.18	3.18	59/84 (70.24)	25/84 (29,76)	
Total solids (%)	Mín. 11.2	Cañar	7.98	14.06	12.02	80/96 (83.33)	16/96 (16.67)	0.34
		Azuay	8.9	25.05	11.99	126/178 (70.79)	52/178 (29.21)	
		Loja	8.86	16.58	11.82	57/84 (67.86)	27/84 (32.14)	
Non-fat solids (%)	Mín. 8.2	Cañar	6.56	9.92	8.50	74/96 (77.08)	22/96 (22.92)	0.07
		Azuay	6.87	13.6	8.63	154/178 (86.52)	24/178 (13.48)	
		Loja	6.31	10.13	8.47	61/84 (72.62)	23/84 (27.38)	
Lactose (%)	Mín 4.8	Cañar	3.00	5.09	4.58	15/96 (15.63)	81/96 (84.38)	0.003
		Azuay	3.62	6.37	4.68	60/178 (33.71)	118/178 (66.29)	
		Loja	2.76	5.28	4.53	25/84 (29.76)	59/84 (70.24)	
Added water (%)	0	Cañar	0.00	34.61	1.72	71/96 (73.96)	25/96 (26.04)	0.02
		Azuay	0.00	24.42	1.54	132/178 (74.16)	46/178 (25.84)	
		Loja	0.00	33.46	4.09	51/84 (60.71)	33/84 (39.29)	
Freezing point (°C)	-0.536 a -0.512	Cañar	-0.61	-0.34	-0.530	36/96 (37.50)	60/96 (62.50)	0.14
		Azuay	-0.759	-0.389	-0.536	54/178 (30.34)	124/178 (69.66)	
		Loja	-0.641	-0.346	-0.524	33/84 (39.29)	51/84 (60.71)	
Somatic cells (CS/ml)	Máx. 700000	Cañar	10000	1939000	268468.75	92/96 (95.83)	4/96 (4.17)	0.003
		Azuay	2000	8064000	368224.72	159/178 (89.33)	19/178 (10.67)	
		Loja	2000	8900000	559976.19	70/84 (83.33)	14/84 (16.67)	
Protein stability	Negativo	Cañar	-	-	-	50/96 (52.08)	46/96 (47.92)	<0.0001
		Azuay	-	-	-	102/178 (57.30)	76/178 (42.70)	
		Loja	-	-	-	51/84 (60.71)	33/84 (39.29)	
Antibiotic residues	MRL*	Cañar	-	-	-	68/96 (70.83%)	28/96 (29.17)	<0.0001
		Azuay	-	-	-	141/178 (79.21%)	37/178 (20.79)	
		Loja	-	-	-	42/84 (50.00%)	42/84 (50.00)	

\*MRL Maximum Residue Limit

Overall, the relative density of raw milk samples ranged from 1.017 to 1.055 g/ml, with a mean of 1.029 g/ml. A statistically significant difference was found between provinces ( $p < 0.0001$ ), where samples from Loja showed the highest density (1.055 g/ml) and those from Azuay the lowest (1.017 g/ml) (Table 2). For freezing point, 65.64% (235/358) of the samples did not meet NTE INEN 9 requirements, while 34.36% (123/358) were within the permissible range. Similarly, 29.05% (104/358) of samples comprised added water, with an average level

of 2.18%. Significant differences were found between provinces ( $p = 0.02211$ ), particularly between Azuay and Loja, while no significant differences were observed in freezing point ( $p = 0.1403$ ).

The pH of the samples ranged from 4.47 to 7.32, with a mean of 6.33, below the reference range of 6.6–6.8 (Table 1). Samples from Loja had the highest mean pH (6.52), significantly higher than those from Cañar and Azuay ( $p = 0.01566$ ) (Table 2). Titratable acidity ranged from 3.8 to 63 °D, with a mean of 18.89 °D. Overall,

68.72% (246/358) of samples failed to comply with the reference range of 13–17 °D (Table 1). Small differences in mean titratable acidity were observed among provinces, but these were not significant ( $p = 0.4112$ ). Protein stability testing showed that 43.30% (155/358) of samples curdled under the 68% alcohol test (Table 1). Significant provincial differences were found ( $p < 0.0001$ ), with Cañar showing the highest non-compliance rate at 47.92% (Table 2).

SCCs ranged from 2,000 to 8,900,000 cells/ml, with a mean of 386,466 cells/ml. Ecuadorian regulations allow up to 700,000 cells/ml, whereas international standards set stricter limits between 200,000 and 400,000 cells/ml. Results showed that 10.34% (37/358) of samples exceeded the national limit, suggesting the presence of mastitis in dairy herds. Statistically significant differences were observed ( $p = 0.002558$ ) between Azuay differing from Cañar and Loja.

Milk lipid content ranged from 0.02% to 17.54%, with a mean of 3.41%. According to NTE INEN 9, the minimum required content is 3%; 68.99% (247/358) of samples met this requirement, while 31.01% (111/358) did not, likely due to low-fat content, partial skimming, or dilution with water. No significant differences were found between provinces ( $p = 0.1095$ ) (Table 1). Crude protein content ranged from 2.02% to 6.41%, with a mean of 3.18%. The Ecuadorian regulation requires a minimum of 2.9%; only 79.33% (284/358) of samples complied, whereas 20.67% (74/358) did not. No significant differences were detected among provinces ( $p = 0.5642$ ).

For lactose, while NTE INEN 9 does not specify a regulatory range, international standards suggest a minimum of 4.8%. Results showed that 72.07% (258/358) of samples had lower lactose levels, potentially indicating bacterial degradation due to poor storage conditions, while 27.93% (100/358) met the expected threshold. Statistically significant differences were found ( $p =$

0.002578), with Azuay differing from Cañar and Loja (Table 1). TS ranged from 7.98% to 25.05%, with a mean of 11.96%. The Ecuadorian standard requires a minimum of 11.2%; 73.46% (263/358) of samples complied, while 26.54% (95/358) did not. SNF ranged from 5.67% to 13.69%, with a mean of 8.56%. Given the minimum requirement of 8.2%, 80.73% (289/358) of samples complied, while 19.27% (69/358) did not. Statistical analysis revealed no significant differences in TS ( $p = 0.3411$ ) or SNF ( $p = 0.07129$ ).

Antibiotic residues were detected in 29.89% (107/358) of samples, exceeding Codex Alimentarius limits, with significant differences among provinces ( $p < 0.0001$ ). Of the 107 non-compliant samples, 39.25% (42/107) were from Loja, 34.58% (37/107) from Azuay, and 26.17% (28/107) from Cañar. Regarding antibiotic families, sulfonamides alone were the most frequently detected (42.06%, 45/107), followed by neomycin (15.89%, 17/107), sulfonamides combined with neomycin (8.41%, 9/107), streptomycin alone (4.67%, 5/107), beta-lactams with sulfonamides, gentamicin, and neomycin (4.67%, 5/107), gentamicin alone (3.74%, 4/107), and sulfonamides with streptomycin, streptomycin with tetracyclines, beta-lactams with sulfonamides, beta-lactams with sulfonamides and gentamicin (2.80%, 3/107 each). Finally, 0.93% (1/107) of samples contained combinations of sulfonamides, tetracyclines, neomycin, and gentamicin.

## DISCUSSION

In this study, 358 raw milk samples were collected from informal markets in the provinces of Azuay, Cañar, and Loja between January 2024 and January 2025. The present study revealed a very high overall prevalence of non-compliance (96.09%), indicating widespread lack of quality in raw milk sold informally in southern Ecuador. Particularly concerning was the 29.89% prevalence of antibiotic residues exceeding Codex limits, alongside widespread physicochemical alterations (lactose 72.07%,

titratable acidity 68.72%, pH 68.16%, freezing point 65.64%). The prevalence of added water (29.05%) further suggests economically motivated adulteration or post-harvest handling deficiencies. Similar high prevalence rates of non-compliance in informal dairy chains have been reported in Latin America and Sub-Saharan Africa, where weak cold-chain infrastructure and limited veterinary oversight are common determinants [22-23]. From a public health perspective, the prevalence of antimicrobial residues observed in this study is substantially higher than that reported in regulated dairy supply chains in high-income countries (<5%), indicating significant gaps in compliance with withdrawal periods and surveillance systems [24].

The results of this study are consistent with findings from other investigations on informal raw milk in different Ecuadorian provinces. For example, in Pichincha, 94.31% of informally sold milk samples failed to meet at least one quality parameter [15]. Similarly, in Manabí and Santo Domingo de los Tsáchilas, 87.68% of samples were non-compliant [25]. In Imbabura and Carchi, 80.07% (217/271) of samples did not meet NTE INEN 9 standards [26-27]. Comparable issues have been reported in southeastern Mexico, where none of the informally sold raw milk samples were deemed suitable for consumption under local regulations [28].

In the present study, 28.77% of samples showed relative density values outside the acceptable quality range (1.028–1.033 g/ml); 29.05% were adulterated with added water, and 65.64% had freezing point values outside the established range (-0.536 to -0.512 °C). Similar findings were reported in Imbabura and Carchi, where 31% of samples were outside the density reference range, and 16.61% contained added water [26-27]. In Manabí and Santo Domingo de los Tsáchilas, 28.82% of samples were adulterated with water and 43.35% showed altered density values [25]. In Pichincha, 26% of samples failed to meet the density requirement

[15]. In Africa, similar problems have been observed, with water adulteration present in 25% of milk samples in Kenya [29], whereas in the Andean region of Peru, the prevalence was considerably lower, under 3% [30]. Water addition decreases milk density and is a common practice in informal markets to increase sales volume, thereby reducing nutritional value and posing health risks when contaminated water is used. Conversely, density may increase when milk is skimmed or adulterated with added solids [9,31-32].

Titratable acidity and pH showed the highest levels of non-compliance, with 68.72% and 68.16% of samples, respectively. These findings are similar to those in Manabí and Santo Domingo de los Tsáchilas, where 72.41% and 68.72% of samples failed pH and acidity requirements [25]. In Pichincha, 72.77% of samples exceeded the national limits for pH and acidity [15], while in Imbabura and Carchi, 70.48% and 58.30% of samples, respectively, were non-compliant [26-27]. Similar results have been documented in raw milk from Peru's inter-Andean region, where hygienic quality was notably poor [30]. High acidity and low pH are associated with bacterial proliferation due to inadequate milk handling, such as a lack of refrigeration and prolonged storage before consumption. Bacteria ferment lactose into lactic acid, lowering pH and increasing acidity, thereby altering sensory properties like taste and odor [33-34]. Such conditions pose consumer risks, as contaminated milk may harbor pathogenic bacteria such as *Escherichia coli*, *Salmonella spp.*, or *Listeria monocytogenes* [14,35–39].

This study also revealed the poor nutritional quality of raw milk sold informally in Azuay, Cañar, and Loja. Of all samples analyzed, 31.01% failed to meet fat content requirements, 20.67% protein, 26.54% TS, 19.27% SNF, and 72.07% lactose. These results suggest adulteration and poor nutritional quality, consistent with prior research in Manabí–Santo Domingo [25], Imbabura–Carchi [26-27], and Pichincha [15]. By contrast, higher

compliance with nutritional quality standards has been reported in dairy farms in the Peruvian Andes [30].

SCC is a key indicator of udder health, with values above 200,000 cells/ml suggesting subclinical or clinical mastitis [40]. Ecuadorian regulations, however, allow up to 700,000 cells/ml. In this study, 10.34% of samples exceeded the national limit. By comparison, in informal milk markets from São Paulo, Brazil, 77% of samples exceeded the local legal threshold of 600,000 cells/ml [41]. These results indicate that cows supplying informal markets are likely affected by mastitis, a disease that causes significant economic losses due to reduced milk yield, compromises animal welfare [42–44], and degrades milk nutritional quality [45].

One of the most concerning findings of this study was the high proportion of samples positive for antibiotic residues. In total, 29.89% of samples contained sulfonamides and aminoglycosides (e.g., neomycin). This figure is comparable to Pichincha (28.46%) [15] but much higher than Manabí–Santo Domingo (6.40%) [25] and Carchi–Imbabura (2.82%) [26–27]. These findings suggest inappropriate antibiotic use in Ecuadorian dairy production. In neighboring Peru, 37.5% of milk samples contained residues, mainly tetracyclines, beta-lactams, and cephalosporins [30], while in milk sold informally in São Paulo, Brazil, the prevalence was 59% [41]. This is also supported by surveys in rural Peru, where over 75% of producers reported selling milk from cows under antimicrobial treatment without discarding it [46]. Similarly, in Antioquia, Colombia, antibiotic-contaminated milk was fed to calves, used in dairy processing, or sold informally [47]. In Kenya, 18.4% of informal milk samples contained antibiotic residues [29].

The presence of antibiotic residues in milk leads to economic losses for the dairy industry, as contaminated milk must be discarded, and it inhibits the activity of cultures in fermented dairy products [48]. More importantly, it poses a major public health risk by causing

allergic reactions in sensitive individuals, disrupting gut microbiota in children and immunocompromised populations, and contributing to the emergence of antimicrobial resistance (AMR). AMR is a growing global health crisis, driven by the selection of resistant bacterial strains, making infections increasingly difficult to treat [49–53]. Implementing strict withdrawal periods for treated cows and routine testing of milk for residues would help mitigate these risks. This is especially critical in Ecuador, where sulfonamides are among the most widely used antibiotics in dairy herds, explaining their frequent detection in raw milk [15,18].

### Functional Food Perspective

#### Physicochemical Alterations and Loss of Functionality:

The high percentages of non-compliance in lactose (72.07%), titratable acidity (68.72%), pH (68.16%), and freezing point (65.64%) suggest microbial spoilage and adulteration practices, such as the addition of water (29.05%). From the perspective of milk as a functional food, these alterations compromise its biological value, since milk's functionality depends not only on its basic nutritional composition but also on the structural integrity of proteins, lipids, and carbohydrates, which can be affected by variations in pH and microbial activity [54–55]. Consequently, changes in the milk's titratable acidity can destabilize casein micelles and affect protein bioavailability [56–57].

#### Protein Bioavailability and Structural Stability:

Although 79.33% of the samples met the minimum crude protein requirement established by Ecuadorian regulations, 43.30% exhibited protein instability in the 68% alcohol test, indicating alterations in the casein fraction and possible enzymatic or bacterial activity. Protein destabilization affects digestibility and the release of bioactive peptides, reducing the functional potential associated with the antihypertensive, immunomodulatory, and antioxidant effects described

for milk proteins [54,58-59]. Furthermore, dilution with water reduces the nutrient density per unit volume, directly impacting the supply of essential amino acids, particularly in vulnerable populations where milk is a primary source of high-biological-value protein [60–62]

**Lactose Metabolism and Digestive Consequences:** The average pH (6.33), lower than the normal physiological range (6.6–6.8), and the elevated titratable acidity values (mean 18.89 °D) suggest partial fermentation. Bacterial hydrolysis of lactose produces lactic acid, altering the food's sensory and digestive profile. Although controlled fermentation can improve tolerance in individuals with hypolactasia, in contaminated raw milk, this process is uncontrolled and can lead to pathogen proliferation [63–65]. Furthermore, the reduction in lactose content can impair calcium absorption, as this disaccharide facilitates its intestinal bioavailability, compromising one of the main functional benefits of milk [57,66].

**Antibiotic Residues and Functional Risk:** Detection of antibiotic residues in 29.89% of the samples, exceeding Codex Alimentarius limits, is of high public health relevance. The presence of sulfonamides, aminoglycosides, and  $\beta$ -lactams not only poses a risk of hypersensitivity reactions but also contributes to the development of antimicrobial resistance, a global health threat [13,24,67]. From a functional perspective, antimicrobial residues can alter the consumer's gut microbiota and negatively affect digestive and metabolic processes [68–70]. Bacteria such as *Lactobacillus* are known to play a fundamental role in the public health of consumers and therefore in food safety, due to their ability to grow and thrive in diverse environments; research on their role in antibiotic resistance is necessary [71]. Furthermore, they interfere with industrial fermentation, inhibiting starter cultures in products such as yogurt and cheese, thus compromising the viability of functional dairy foods with probiotics [57,69].

**Relevance to Functional Food Safety:** While parameters such as fat, TS, and SNF did not show relatively high compliance rates, the high prevalence of alterations in other key indicators demonstrates that informal milk does not guarantee the minimum safety and quality standards required to maintain its functional character, since modern food safety is not limited to caloric availability but also incorporates dimensions of nutritional quality and chemical and microbiological safety [22]. However, the changes that may have occurred in the biochemical substances (alkaline phosphatase, lactoperoxidase, furosine and lactoferrin) which are considered important biochemical substances as markers for proper milk pasteurization are unknown [72]. In this context, the high level of non-compliance observed compromises not only the immediate health of the consumer but also the metabolic and preventive benefits traditionally attributed to milk as a functional food [73–75].

**Identification of Associated Risk Factors:** Based on the observed data and existing scientific evidence, several potential risk factors may explain the high prevalence of non-compliance: Inadequate mastitis treatment, because the presence of antibiotic residues, particularly sulfonamides and aminoglycosides, suggests improper therapeutic use and failure to respect withdrawal periods. Elevated SCCs in 10.34% of samples indicate mastitis as a probable driver of antimicrobial usage [76]. Deficient cold-chain and storage practices, since low lactose levels and elevated acidity are consistent with uncontrolled bacterial fermentation during transport and storage; in informal systems, milk is frequently marketed without refrigeration, increasing microbial proliferation and acidification [72]. Economically motivated adulteration, where freezing point depression and reduced density values are typical indicators of water addition. Adulteration reduces nutritional density and may mask spoilage [77]. Limited regulatory oversight in

informal markets, as informal marketing channels often operate outside systematic quality monitoring frameworks, increasing the likelihood of chemical and microbiological hazards [22].

**Potential Consumer Health Risks:** The findings suggest multiple levels of risk. For example, in the case of antimicrobial resistance (AMR), chronic exposure to subtherapeutic antibiotic residues can contribute to its development, which is recognized worldwide as a major health threat [13,24]. Microbiological risks are also present, as altered pH and high acidity indicate microbial activity, which could include pathogenic organisms such as *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* O157:H7, commonly associated with raw milk consumption [14,37-38,72]. Functional and metabolic consequences are another concern, as alterations in protein stability and lactose levels can reduce digestibility and affect calcium bioavailability, compromising milk's functional properties as a nutrient-rich food [78]. Allergic and hypersensitivity reactions are a concern, as  $\beta$ -lactam residues, even at low concentrations, can trigger hypersensitivity reactions in susceptible individuals [70]. Together, these risks go beyond acute foodborne illnesses, affecting long-term metabolic health and undermining milk's role as a functional food. This is even true in certain informally produced fermented milks, where hygiene and temperature are not controlled during product manufacturing, thus increasing the risk of microbial contamination and compromising product safety [79].

## CONCLUSION

Altogether, the results suggest that the informal trade of raw milk in southern Ecuador (Azuay, Cañar, and Loja) represents a significant risk to functional food security by compromising the nutritional integrity, bioavailability of its components, and the chemical safety of the product. In this study, 96.09% of the samples analyzed failed to

comply with at least one of the reference values established in NTE INEN 9, particularly for titratable acidity, lactose content, and protein stability. Additionally, 29.89% of the samples contained antibiotic residues, representing a potential source for the dissemination of antimicrobial-resistant pathogens and a serious threat to consumer health. Non-compliance with the standard suggests inadequate hygiene practices, insufficient refrigeration, prolonged storage, and inappropriate antibiotic use in dairy production. Addressing this issue requires One Health-based strategies, including stricter regulatory policies, routine testing for antibiotic residues, and comprehensive educational programs for dairy producers.

**Abbreviations:** Ecuadorian Technical Standard, NTE; Fourier-transform infrared spectroscopy, FTIR; Solids-non-fat, SNF; Somatic cell count, SCC; Total solids, TS; MRL, Maximum Residue Limit

**Conflict of Interest:** The authors declare no conflicts of interest.

**Consent to Publish declaration missing:** Not applicable.

**Consent to Participate declaration:** Not applicable.

**Funding Statement:** The research was funded by the Facultad de Medicina Veterinaria y Zootecnia – Universidad Central del Ecuador, through Research Project Code FMVZ-COIF-EXT-2024-04.

**Clinical trial number:** Not applicable.

**Ethical & Transparency Statements:** Not applicable. No animals or people were manipulated, nor were any surveys conducted on milk producers or sellers. The database is available whenever it is needed.

**Authors' Contributions:** Conceptualization: B.P.T. and E.A. Data curation: B.P.T. and D.D.T. Formal analysis:

B.P.T., P.S., V.A., M.L., M.M., G.M., L.P., V.V.  
 Methodology: B.P.T., P.S. and L.N. Software: B.P.T.  
 Validation: E.P.M., D.D.T., H.N. y L.N. Investigation: E.A.,  
 E.P.M., M.B.C., D.D.T., H.N. y L.N. Writing - original draft:  
 B.P.T., E.A., D.D.T., H.N. and L.N. Writing - review &  
 editing: E.P.M., V.A., M.L., M.M., G.M., L.P., V.V., M.B.C.  
 and P.S.

## REFERENCES

- Fusco V, Chieffi D, Fanelli F, Logrieco AF, Cho G, Kabisch J, et al. Microbial quality and safety of milk and milk products in the 21st century. *Compr Rev Food Sci Food Saf.* 2020; 19:2013–49. DOI: <https://doi.org/10.1111/1541-4337.12568>
- Magan JB, O'Callaghan TF, Kelly AL, McCarthy NA. Compositional and functional properties of milk and dairy products derived from cows fed pasture or concentrate-based diets. *Compr Rev Food Sci Food Saf.* 2021; 20:2769–800. DOI: <https://doi.org/10.1111/1541-4337.12751>
- Scholz-Ahrens KE, Ahrens F, Barth CA. Nutritional and health attributes of milk and milk imitations. *Eur J Nutr.* 2020; 59:19–34. DOI: <https://doi.org/10.1007/s00394-019-01936-3>
- Kanire E, Msuya E, Alphonse R. Drivers of dairy farmers' engagement in informal milk markets: Policy implications for developing countries. *J Agric Food Res.* 2024;16. DOI: <https://doi.org/10.1016/j.jafr.2024.101128>
- Boudebou A, Boudalia S, Bousbia A, Habila S, Boussadia MI, Gueroui Y. Heavy metals levels in raw cow milk and health risk assessment across the globe: A systematic review. *Science of the Total Environment.* 2021;751. DOI: <https://doi.org/10.1016/j.scitotenv.2020.141830>
- Boudebou A, Boudalia S, Boussadia MI, Gueroui Y, Habila S, Bousbia A, et al. Pesticide residues levels in raw cow's milk and health risk assessment across the globe: A systematic review. *Environmental Advances.* 2022;9(3):100266. DOI: <https://doi.org/10.1016/j.envadv.2022.100266>
- Castro-Bedriñana J, Chirinos-Peinado D, Ríos-Ríos E, Castro-Chirinos G, Chagua-Rodríguez P, De La Cruz-Calderón G. Lead, cadmium, and arsenic in raw cow's milk in a Central Andean Area and risks for the Peruvian populations. *Toxics* 2023;11(10):809. DOI: <https://doi.org/10.3390/toxics11100809>
- Santacroce L, Di Domenico M, Montagnani M, Jirillo E. Antibiotic Resistance and Microbiota Response. *Curr Pharm Des.* 2023; 29:356–64. DOI: <https://doi.org/10.2174/1381612829666221219093450>
- Techane T. Effect of adulterants on quality and safety of cow milk: a review. *Int J Diabetes Metab Disord.* 2023; 8:277–87. DOI: <https://doi.org/10.33140/IJDM>
- Zhang J, Wang J, Jin J, Li X, Zhang H, Shi X, et al. Prevalence, antibiotic resistance, and enterotoxin genes of *Staphylococcus aureus* isolated from milk and dairy products worldwide: a systematic review and meta-analysis. *Food Research International.* 2022; 162:111969. DOI: <https://doi.org/10.1016/j.foodres.2022.111969>
- Vidal-Martins AMC, Bürger KP, Gonçalves ACS, Grisolio APR, Aguilar CEG, Rossi GAM. Avaliação do consumo de leite e produtos lácteos informais e do conhecimento da população sobre os seus agravos à saúde pública, em um município do Estado de São Paulo, Brasil. *Boletim de Indústria Animal.* 2013; 70:221–7. DOI: <https://doi.org/10.17523/bia.v70n3p221>
- INEC: Encuesta de Superficie y Producción Agropecuaria Continua (ESPAC). [[https://www.ecuadorencifras.gob.ec/documentos/web-inec/Estadisticas\\_agropecuarias/espac/2024/Boletin\\_tecnico\\_ESPAC\\_2024.pdf](https://www.ecuadorencifras.gob.ec/documentos/web-inec/Estadisticas_agropecuarias/espac/2024/Boletin_tecnico_ESPAC_2024.pdf)] Retrieved February 19, 2026.
- Loor-Giler A, Sanchez-Castro C, Puga-Torres B, Santander-Parra S, Nuñez L. mecA and mecC positive strains of *Staphylococcus aureus* detected and isolated from raw milk of Ecuador. *Antibiotics.* 2025; 14:1255. DOI: <https://doi.org/10.3390/antibiotics14121255>
- Loor-Giler A, Sanchez-Castro C, Robayo-Chico M, Puga-Torres B, Santander-Parra S, Nuñez L. High contamination of *Salmonella* spp. in raw milk in Ecuador: molecular identification of *Salmonella enterica* serovars Typhi, Paratyphi, Enteritidis and Typhimurium. *Front Sustain Food Syst.* 2025; 9:1593266. DOI: <https://doi.org/10.3389/fsufs.2025.1593266>
- Puga-Torres B, Aragón E, Contreras A, Escobar D, Guevara K, Herrera L, et al. Analysis of quality and antibiotic residues in raw milk marketed informally in the Province of Pichincha – Ecuador. *Food Agric Immunol.* 2024;35. DOI: <https://doi.org/10.1080/09540105.2023.2291321>
- Terán J. Análisis del mercado de la leche en Ecuador: factores determinantes y desafíos. Tesis de maestría. Universitat Politècnica de València, 2019.
- Puga-Torres B, Aragón Vásquez E, Ron L, Álvarez V, Bonilla S, Guzmán A, et al. Milk quality parameters of raw milk in Ecuador between 2010 and 2020: a systematic literature review and meta-analysis. *Foods.* 2022; 11:3351. DOI: <https://doi.org/10.3390/foods11213351>

18. Vera MD, Puga-Torres B. Factors associated with the presence of antibiotic residues in raw milk from cows in the canton of El Carmen, Manabí, Ecuador. *Revista de La Facultad de Medicina Veterinaria y de Zootecnia*. 2025;72. DOI: <https://doi.org/https://doi.org/10.15446/rfmvz.v72n1.113703>
19. Arias-Sandoval CA, Bonifaz-García NF, Simbaña-Díaz PE, Argüello-Argüello AF. Compositional, hygienic and sanitary quality of bovine milk in three regions of Ecuador. *Rev Med Vet Zoot*. 2025;72(1). DOI: <https://doi.org/10.15446/rfmvz.v72n1.114348>
20. Salguero A, De la Torre D, Puga-Torres B. Calidad de leche cruda de pequeños productores de los cantones Cayambe y Pedro Moncayo, Ecuador, mediante análisis fisicoquímicos y ensayos cualitativos. *Revista de Investigaciones Veterinarias Del Perú*. 2023;34: e24611. DOI: <https://doi.org/10.15381/rivep.v34i1.24611>
21. INEN. Leche y productos lácteos. Directrices para la toma de muestras (ISO 707:2008, IDT) 2014:1–49.
22. FAO: The State of Food Security and Nutrition in the World 2023 (SOFI). FAO; 2023. [<https://openknowledge.fao.org/items/445c9d27-b396-4126-96c9-50b335364d01>] Retrieved February 18, 2026.
23. Grace D. Food safety in low- and middle-income countries. *Int J Environ Res Public Health*. 2015; 12:10490–507. DOI: <https://doi.org/10.3390/ijerph120910490>
24. WHO: Global antimicrobial resistance and use surveillance system (GLASS) report 2023 2023. [<https://www.who.int/initiatives/glass>] Retrieved February 18, 2026.
25. Puga-Torres B, Aragón E, Martínez EP, Buitrón F, Caizaluiza MJ, Gaibor E, et al. Quality and antibiotic residues in raw milk sold informally in Manabí and Santo Domingo de los Tsáchilas, Ecuador. *Ital J Food Saf*. 2025;15. DOI: <https://doi.org/10.4081/ijfs.2025.13885>
26. Calderón Pallo J, Puga-Torres B: Determinación de la calidad y detección de residuos antibióticos en leche bovina cruda comercializada informalmente en los cantones Montúfar, San Pedro de Huaca y Tulcán de la provincia del Carchi. Tesis de grado para optar por el título de Médico Veterinario. Universidad Central del Ecuador; 2023.
27. Cevallos del Castillo K, Puga-Torres B: Determinación de la calidad y detección de residuos antibióticos en leche cruda comercializada informalmente en los cantones Cotacachi, Otavalo y Antonio Ante de la provincia de Imbabura. Tesis de grado para optar por el título de Médica Veterinaria. Universidad Central del Ecuador; 2023.
28. Peralta-Torres J, Hernández-Hernández M, López-Segovia N, Boldo-León X, Trujillo-Castillo L, Quiñonez-Díaz L, et al. Estudio comparativo de calidad higiénicosanitaria, fisicoquímica y microbiológica de leche bovina en el sureste mexicano. *Rev MVZ Cordoba*. 2021; 26:1–8. DOI: <https://doi.org/10.21897/rmvz.2106>
29. Ondieki GK, Ombui JN, Obonyo M, Gura Z, Githuku J, Orinde AB, et al. Antimicrobial residues and compositional quality of informally marketed raw cow milk, Lamu West Sub-County, Kenya, 2015. *Pan Afr Med J*. 2017; 28:5. DOI: <https://doi.org/10.11604/pami.suppl.2017.28.1.9279>
30. Arauco Villar F, Guzmán Estremadoyro L, Pantoja Esquivel R, Mayorga Sánchez N, Unchupaico Payano I, De La Cruz ARH. Assessment of physicochemical, microbial, and hygienic quality of raw cow milk produced in dairy herds from the Peruvian Andes. *Granja*. 2025; 41:127–39. DOI: <https://doi.org/10.17163/lgr.n41.2025.08>
31. Condé VA, Valente G de FS, Minighin EC. Milk fraud by the addition of whey using an artificial neural network. *Ciência Rural*. 2020;50. DOI: <https://doi.org/10.1590/0103-8478cr20190312>
32. Ionescu A-D, Cîrci AI, Begea M. A review of milk frauds and adulterations from a technological perspective. *Applied Sciences*. 2023; 13:9821. DOI: <https://doi.org/10.3390/app13179821>
33. Bettera L, Levante A, Bancalari E, Bottari B, Gatti M. Lactic acid bacteria in cow raw milk for cheese production: which and how many? *Front Microbiol*. 2023; 13:1–14. DOI: <https://doi.org/10.3389/fmicb.2022.1092224>
34. Metz M, Sheehan J, Feng PCH. Use of indicator bacteria for monitoring sanitary quality of raw milk cheeses – a literature review. *Food Microbiol*. 2020; 85:103283. DOI: <https://doi.org/10.1016/j.fm.2019.103283>
35. Bedassa A, Nahusenay H, Asefa Z, Sisay T, Girmay G, Kovac J, et al. Prevalence and associated risk factors for Salmonella enterica contamination of cow milk and cottage cheese in Ethiopia. *Food Safety and Risk*. 2023; 10:2. DOI: <https://doi.org/10.1186/s40550-023-00101-3>
36. Castañeda-Salazar R, Pulido-Villamarín A del P, Ángel-Rodríguez GL, Zafra-Alba CA, Oliver-Espinosa OJ. Isolation and identification of Salmonella spp. in raw milk from dairy herds in Colombia. *Braz J Vet Res Anim Sci*. 2021;58: e172805. DOI: <https://doi.org/10.11606/issn.1678-4456.bjvras.2021.172805>

37. Padilla-Cerda A, Loor-Giler A, Puga-Torres B, Santander-Parra S, Núñez L. Raw milk as a source of campylobacter infection: isolation and molecular identification of campylobacter coli and campylobacter jejuni in Ecuador. *Pathogens*. 2025; 14:1155.  
DOI: <https://doi.org/10.3390/pathogens14111155>
38. Loor-Giler A, Robayo-Chico M, Puga-Torres B, Hernandez-Alomia F, Santander-Parra S, Piantino Ferreira A, et al. Escherichia coli O157:H7, a common contaminant of raw milk from Ecuador: isolation and molecular identification. *Foods*. 2025; 14:410.  
DOI: <https://doi.org/10.3390/foods14030410>
39. Sepahvand F, Rashidian E, Jaydari A, Rahimi H. Short communication: prevalence of Listeria monocytogenes in raw milk of healthy sheep and goats. *Vet Med Int*. 2022; 2022:1–5. DOI: <https://doi.org/10.1155/2022/3206172>
40. Hossain D, Singha S, Thof CM Van, Gough S, Uddin AM. Effect of somatic cells on milk quality and human health. *Veterinary Sciences: Research and Reviews*. 2021;7(1):31-34.  
DOI: <https://doi.org/10.17582/journal.vsr/2021/7.1.31.34>
41. Motta RG, Silva AV, Giuffrida R, Siqueira AK, Paes AC, Motta IG, et al. Indicadores de qualidade e composição de leite informal comercializado na região Sudeste do Estado de São Paulo. *Pesquisa Veterinaria Brasileira*. 2015; 35:417–23.  
DOI: <https://doi.org/10.1590/S0100-736X2015000500005>
42. Fagnani R, Nero LA, Rosolem CP. Why knowledge is the best way to reduce the risks associated with raw milk and raw milk products. *Journal of Dairy Research*. 2021; 88:238–43.  
DOI: <https://doi.org/10.1017/S002202992100039X>
43. Kaskous S, Farschtschi S, Pfaffl MW. Physiological aspects of milk somatic cell count in small ruminants—a review. *Dairy*. 2022; 4:26–42.  
DOI: <https://doi.org/10.3390/dairy4010002>
44. Pace RM, Pace CDW, Fehrenkamp BD, Price WJ, Lewis M, Williams JE, et al. Sodium and potassium concentrations and somatic cell count of human milk produced in the first six weeks postpartum and their suitability as biomarkers of clinical and subclinical mastitis. *Nutrients*. 2022; 14:4708.  
DOI: <https://doi.org/10.3390/nu14224708>
45. Jurado-Gómez H, Muñoz-Domínguez L, Quitiaquez-Montenegro D, Fajardo-Argotí C, Insuasty-Santacruz E. Evaluación de la calidad composicional, microbiológica y sanitaria de la leche cruda en el segundo tercio de lactancia en vacas lecheras. *Revista de La Facultad de Medicina Veterinaria y de Zootecnia*. 2019;66(1):53-66.  
DOI: <https://doi.org/10.15446/rfmvz.v66n1.79402>
46. Redding LE, Cubas-Delgado F, Sammel MD, Smith G, Galligan DT, Levy MZ, et al. Antibiotic residues in milk from small dairy farms in rural Peru. *Food Additives and Contaminants - Part A*. 2014; 31:1001–8.  
DOI: <https://doi.org/10.1080/19440049.2014.905877>.
47. Zapata-Salas R, Guarín JF, Ríos-Osorio LA. Consumption and informal trade of milk in the North of Antioquia (Colombia). *Vet Med Int*. 2024; 2024:6644328.  
DOI: <https://doi.org/10.1155/2024/6644328>
48. Omairi R, Krayem M, Khaled S, Salla M, El Khatib S. Antibiotic residues in milk and milk products: a momentous challenge for the pharmaceutical industry and medicine. *World J Pharmacol*. 2022; 11:48–55.  
DOI: <https://doi.org/10.5497/wjpv.v11.i4.48>
49. Alves JS, de Moura Souza R, Lima Moreira JP de, Gonzalez AGM. Antimicrobial resistance of Enterobacteriaceae and Staphylococcus spp. isolated from raw cow's milk from healthy, clinical and subclinical mastitis udders. *Prev Vet Med*. 2024; 227:106205.  
DOI: <https://doi.org/10.1016/j.prevetmed.2024.106205>
50. Dankar I, Hassan H, Serhan M. Knowledge, attitudes, and perceptions of dairy farmers regarding antibiotic use: lessons from a developing country. *J Dairy Sci*. 2022; 105:1519–32.  
DOI: <https://doi.org/10.3168/jds.2021-20951>
51. Hervin V, Roy V, Agrofoglio LA. Antibiotics and antibiotic resistance—Mur Ligases as an antibacterial target. *Molecules*. 2023; 28:8076.  
DOI: <https://doi.org/10.3390/molecules28248076>
52. Jeimy S, Wong T, Ben-Shoshan M, Copaescu AM, Isabwe GAC, Ellis AK. Drug allergy. *Allergy, Asthma & Clinical Immunology*. 2025;20(Suppl 3):78.  
DOI: <https://doi.org/10.1186/s13223-024-00936-1>
53. Süßmuth RD, Kulike-Koczula M, Gao P, Kosol S. Fighting antimicrobial resistance: innovative drugs in antibacterial research. *Angewandte Chemie International Edition*. 2025;64(10): e202414325.  
DOI: <https://doi.org/10.1002/anie.202414325>
54. Nielsen SD-H, Liang N, Rathish H, Kim BJ, Lueangsakulthai J, Koh J, et al. Bioactive milk peptides: an updated comprehensive overview and database. *Crit Rev Food Sci Nutr*. 2024; 64:11510–29.  
DOI: <https://doi.org/10.1080/10408398.2023.2240396>
55. Aydogdu T, O'Mahony JA, McCarthy NA. pH, the fundamentals for milk and dairy processing: a review. *Dairy*. 2023; 4:395–409.  
DOI: <https://doi.org/10.3390/dairy4030026>

56. Horne DS: Casein micelle structure and stability. In Milk Proteins. 3<sup>rd</sup> edition. New York: Academic Press (Elsevier); 2020:213-250.  
DOI: <https://doi.org/10.1016/B978-0-12-815251-5.00006-2>
57. Fox PF, Uniacke-Lowe T, McSweeney PLH, O'Mahony JA: Dairy Chemistry and Biochemistry. 2<sup>nd</sup> edition. Cham: Springer International Publishing; 2015.  
DOI: <https://doi.org/10.1007/978-3-319-14892-2>
58. Dalgleish DG. On the structural models of bovine casein micelles—review and possible improvements. *Soft Matter*. 2011; 7:2265–72.  
DOI: <https://doi.org/10.1039/C0SM00806K>
59. Korhonen H, Pihlanto A. Bioactive peptides: production and functionality. *Int Dairy J*. 2006; 16:945–60.  
DOI: <https://doi.org/10.1016/j.idairyj.2005.10.012>
60. Carbonaro M, Nucara A. Secondary structure of food proteins by Fourier transform spectroscopy in the mid-infrared region. *Amino Acids*. 2010; 38:679–90.  
DOI: <https://doi.org/10.1007/s00726-009-0274-3>
61. FAO: Guide to good dairy farming practice. FAO Animal Production and Health Guidelines 2011.  
[\[https://www.fao.org/4/ba0027e/ba0027e00.htm\]](https://www.fao.org/4/ba0027e/ba0027e00.htm)  
Retrieved February 17, 2026.
62. Mohanty DP, Mohapatra S, Misra S, Sahu PS. Milk derived bioactive peptides and their impact on human health – a review. *Saudi J Biol Sci*. 2016; 23:577–83.  
DOI: <https://doi.org/10.1016/j.sjbs.2015.06.005>
63. Walstra P, Wouters JTM, Geurts TJ: Dairy science and technology. 2<sup>nd</sup> edition. Boca Raton: CRC Press; 2005.  
DOI: <https://doi.org/10.1201/9781420028010>
64. Misselwitz B, Butter M, Verbeke K, Fox MR. Update on lactose malabsorption and intolerance: pathogenesis, diagnosis and clinical management. *Gut*. 2019; 68:2080–91.  
DOI: <https://doi.org/10.1136/gutjnl-2019-318404>
65. He T, Priebe MG, Harmsen HJ, Stellaard F, Sun X, Welling GW, et al. Colonic fermentation may play a role in lactose intolerance in humans. *J Nutr*. 2006; 136:58–63.  
DOI: <https://doi.org/10.1093/jn/136.1.58>
66. Gaucheron F. Milk and dairy products: a unique micronutrient combination. *J Am Coll Nutr*. 2011; 30:400S-409S.  
DOI: <https://doi.org/10.1080/07315724.2011.10719983>
67. Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules*. 2018; 23:795.  
DOI: <https://doi.org/10.3390/molecules23040795>
68. Maier L, Goemans CV, Wirbel J, Kuhn M, Eberl C, Pruteanu M, et al. Unravelling the collateral damage of antibiotics on gut bacteria. *Nature*. 2021; 599:120–4.  
DOI: <https://doi.org/10.1038/s41586-021-03986-2>
69. Virto M, Santamarina-García G, Amores G, Hernández I. Antibiotics in dairy production: where is the problem? *Dairy*. 2022; 3:541–64.  
DOI: <https://doi.org/10.3390/dairy3030039>
70. Beyene T. Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. *J Vet Sci Technol*. 2015;07.  
DOI: <https://doi.org/10.4172/2157-7579.1000285>
71. Alkharabsheh A. E. A., Taher E. D., Nadhum Abdul-Fatah B., Aboud HHussein AALHudairi A. AbAbouyounes M. Abdul Hafed Abdul Kader M., Saddam FFadhil O. Balas H. Antibiotic resistance profiling and histological characterization of Lactobacillus isolated from traditional dairy products. *Functional Foods in Health and Disease*. 2026; 16(1): 62–73.  
DOI: <https://doi.org/10.31989/ffhd.v16i1.1856>
72. Marmaryan G., Grigoryan H., Navasardyan D., Hambardzumyan G., Kovacic A., Sargsyan T., Qarimyan R., Hakhoyan G., Marmaryan Y. The impact of heat treatment conditions on the stability of selected biochemical parameters of small ruminant's milk in Armenia. *Functional Food Science*. 2025; 5(10): 552 – 556.  
DOI: <https://doi.org/10.31989/ffs.v5i10.1759>
73. Claeys WL, Cardoen S, Daube G, De Block J, Dewettinck K, Dierick K, et al. Raw or heated cow milk consumption: review of risks and benefits. *Food Control*. 2013; 31:251–62.  
DOI: <https://doi.org/10.1016/j.foodcont.2012.09.035>
74. Thorning TK, Raben A, Tholstrup T, Soedamah-Muthu SS, Givens I, Astrup A. Milk and dairy products: good or bad for human health? an assessment of the totality of scientific evidence. *Food Nutr Res*. 2016; 60:32527.  
DOI: <https://doi.org/10.3402/fnr.v60.32527>
75. Soedamah-Muthu SS, de Goede J. Dairy consumption and cardiometabolic diseases: systematic review and updated meta-analyses of prospective cohort studies. *Curr Nutr Rep*. 2018; 7:171–82.  
DOI: <https://doi.org/10.1007/s13668-018-0253-y>

76. Ruegg PL. A 100-year review: mastitis detection, management, and prevention. *J Dairy Sci.* 2017; 100:10381–97.  
DOI: <https://doi.org/10.3168/jds.2017-13023>
77. Everstine K, Spink J, Kennedy S. Economically motivated adulteration (EMA) of food: common characteristics of EMA incidents. *J Food Prot.* 2013; 76:723–35.  
DOI: <https://doi.org/10.4315/0362-028X.JFP-12-399>
78. Gaucheron F. Milk and dairy products: a unique micronutrient combination. *J Am Coll Nutr.* 2011; 30:400S-409S.  
DOI: <https://doi.org/10.1080/07315724.2011.10719983>
79. Sulieman A. M. E., Omer H., Abdelrhman M., Ibrahim S. M. Microbiological Quality of Zabady (Yogurt) Produced by Small-Scale Industries and Households in Khartoum and Gezira State, Sudan. *Dietary Supplements and Nutraceuticals.* 2026; 5(2):22 – 30.  
DOI: <https://doi.org/10.31989/dsn.v5i2.1900>