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Assessment of Physicochemical, Microbial, and Hygienic Quality of Raw Cow Milk Produced in Dairy Herds from the Peruvian Andes

Evaluación de la calidad fisicoquímica, microbiana e higiénica de la leche de vaca producida por rebaños en los Andes peruanos

Fernando Arauco Villar*[1], Leonor Guzmán Estremadoyro[1], Rafael Pantoja Esquivel[1] †, Noemí Mayorga Sánchez[1], Ide Unchupaico Payano[1] and Alex Rubén Huamán de la Cruz[2,3]

[1] *Universidad Nacional del Centro del Perú, Facultad de Zootecnia, Av. Mariscal. Castilla N. 39 El Tambo, Huancayo, Perú.*
[2] *Universidad Tecnológica del Perú.*
[3] *Universidad Peruana Unión, Unidad de Posgrado.*

*Corresponding author: faraucov@gmail.com

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Abstract

The study was performed in Mantaro Valley, Junín, Perú, with the aim to evaluate the physicochemical, microbial, and hygienical quality of 40 raw cows' milk collected from dairy herds from four provinces: Huancayo (n = 13), Concepción (n = 11), Jauja (n = 9), and Chupaca (n = 7). Physicochemical properties were quantified by evaluating the fat content, density, non-fat-solids, protein, water add, freezing point, salts, total solids, lactose, and pH using the milk analyzer Lactoscan SP. Microbial quality was determined through viable mesophilic bacteria (VMB), total coliforms (TC), fecal coliforms (FC), and yeast and mold (YMC). In addition, antibiotic presence was measured by SNAPduo*ST plus test kit and Reduction time by Methylene Blue Dye Reduction (MBRT). The results found in this work indicate that physicochemical features of raw cow milk were adequate compared to standard levels. In microbial quality, only Chupaca showed higher values (6.28 log cfu/mL) than recommended (5.3 log cfu/mL). Likewise, total bacterial/mL in Huancayo (H, 19.12×10^5) and Concepcion (C, 1.18×10^5) were relatively high concerning the acceptable level (1×10^5 bacteria/mL of raw milk). Antibiotic presence was found in 37.5% (n = 15) from the total of samples (n = 40). MBRT analysis reported 32.5%, 45.0%, and 22.5%, as of excellent, good, and acceptable quality, respectively. Thus, it was concluded that physicochemical properties presented an appropriate level whereas microbial quality in the areas was good but is recommendable for enriched hygienic practices, personal hygiene in milk handling due to microbial presence, and educating the public on safety issues.

Keywords: milk quality, physicochemical characteristics, bacteriological quality, Andean dairy farming.

Resumen

El estudio se llevó a cabo en el Valle del Mantaro, Región Junín, Perú, con el objetivo de evaluar propiedades fisicoquímicas, calidad microbiana e higiénica de 40 muestras de leche cruda recolectada de rebaños bovinos lecheros de cuatro provincias: Huancayo (n = 13), Concepción (n = 11), Jauja (n = 9) y Chupaca (n = 7). Las propiedades fisicoquímicas se cuantificaron mediante la evaluación del contenido de grasa, densidad, sólidos no grasos, proteína, adición de agua, punto de congelación, sales, sólidos totales, lactosa y pH utilizando el analizador de leche Lactoscan SP. La calidad microbiana se determinó a través de bacterias mesófilas viables (VMB), coliformes totales (TC), coliformes fecales (FC) y levaduras y mohos (YMC). Además, la presencia de antibióticos se midió mediante el kit de prueba SNAPduo * ST plus y el tiempo de reducción mediante la reducción de colorante azul de metileno (MBRT). Los resultados reportados en este estudio indican que las propiedades fisicoquímicas de la leche cruda de vaca fueron adecuadas en comparación con los niveles estándar. En calidad microbiana, solo Chupaca mostró valores superiores (6,28 log ufc/mL) a los recomendados (5,3 log ufc/mL). Asimismo, las bacterias totales por mL en Huancayo (H, $19{,}12 \times 10^5$) y Concepción (C, $1{,}18 \times 10^5$) fueron relativamente altas en comparación con el nivel aceptable (1×10^5 bacterias por mL de leche cruda). La presencia de antibiótico se encontró en el 37,5% (n = 15) del total de muestras (n = 40). El análisis MBRT informó 32,5%, 45,0% y 22,5%, como de calidad excelente, buena y aceptable, respectivamente. Así, se concluyó que las propiedades fisicoquímicas presentaron nivel adecuado mientras que la calidad microbiana en las zonas fue buena pero recomendable para enriquecer las prácticas higiénicas, la higiene personal en el manejo de la leche por presencia de microbios y concienciar al público en temas de seguridad.

Palabras clave: calidad de la leche, características fisicoquímicas, calidad bacteriológica, ganadería lechera andina.

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Orcid IDs:

Fernando Arauco Villar: https://orcid.org/0000-0002-1768-8388
Leonor Guzmán Estremadoyro: https://orcid.org/0000-0002-1817-1356
Rafael Pantoja Esquivel: https://orcid.org/0000-0001-7080-1426
Noemí Mayorga Sánchez: https://orcid.org/0000-0001-6157-468X
Ide Unchupaico Payano: https://orcid.org/0000-0001-6922-9311
Alex Huamán De La Cruz: https://orcid.org/0000-0003-4583-9136

1 Introduction

Cow´s milk has a complex biochemical composition and is by far the main type of milk consumed worldwide (Boudalia et al., 2016). Due to their characteristics of containing an important source of nutrients, proteins, vitamins, carbohydrates, and energy-containing fats is highly recommendable for immunological protection and the human diet (MINAGRI, 2005). Milk offers an excellent environment for microbial growth and zoonotic agents, which accelerated the degradation of milk quality and shelf-life (Gemechu and Amene, 2016; Kra et al., 2013). Milk freshly obtained from a healthy animal theoretically is safe for human consumption (Thorning et al., 2016). However, milk can be easily contaminated during or after being secreted from the udder by food-borne pathogens or spoilage microorganisms such as feed, soil, air, water, animal feces, equipment, and people (Elrahman et al., 2009; Owusu-Kwarteng et al., 2020).

Besides, the prevalence of spoilage microorganisms and pathogenic agents in milk and dairy products may be impacted by a great number of factors and their possible combinations. Among these factors are the hygiene level, the health condition of the cow, dairy herd and environment, prestorage and milking conditions, farm management practices, technologies, microbial hazards, animal feedstuffs and husbandry, season, and geographic location (Alhussien and Dang, 2018; Hnini et al., 2018; Owusu-Kwarteng et al., 2020). Food-safety risks linked with dairy products and cow milk consumption vary between developing (smallholder dairy farmers) and developed (industrialized with pasteurization technologies) countries. Thus, a high bacterial presence signifies poor production hygiene or ineffective milk pasteurization (Owusu-Kwarteng et al., 2020).

Peru produced about 2.8 million metric tons (MMT) of fluid milk per year, and its per capita milk consumption is 84 liters (USDA, 2019). Cajamarca (18%), Arequipa (18%), and Lima (13%) are the regions that concentrate on milk production with some modern dairy farms and technologies (Bernet et al., 2001). However, most milk production is by small herds (USDA, 2019). The Mantaro Valley produces about 80,000 liters of milk by day, with 7% intended for the production of cheese, butter, yogurt, and other derived products (Correo, 2019).

Thus, milk production in the Mantaro Valley is a key piece for the livelihood of the population. However, there is no published data on the composition and hygienic quality of raw cow's milk produced by small producers. Therefore, this investigation aims to evaluate the quality of milk through its physicochemical properties and its microbiological analysis. Previously, a survey was carried out on the owners and managers of dairy herds, and on the milk collectors to establish potential risk factors that may be influencing the quality of the milk.

2 Materials and Methods

2.1 Study area and population

The study was performed in the Mantaro Valley (MV) located in Junin region, Peru, at 3 200 masl, and constituted by Jauja (J), Concepción (C), Huancayo (H), and Chupaca (CH) provinces. The MV is a fluvial inter-Andean valley with a diversified agricultural activity that produces several crops (maize, potatoes, and vegetables), and it is not a very developed dairy farming; it has small herds of less than three cows; medium herds, between four and ten cows, and the largest, more than ten and up to a hundred cows.

2.2 Protocol of sampling

The study was performed between October 2019 - February 2020. Forty milk samples from dairy herds and collection centers (processing plant and food markets) were collected from four provinces: Huancayo (n =13), Concepción (n =11), Jauja (n =9), and Chupaca (n =7) located at the Mantaro Valley, Junín Region, Perú. Criterion selection was made through discretionary sampling, taking into consideration only herds with a cows' population greater than 5 cows.

The milk samples were collected aseptically with a sterile bucket directly from the milk can, randomly selected according to the production volume of the dairy herd, in sterile glass jars with a capacity of 500 mL, and transported to the laboratory inside a Styrofoam box with refrigerant (3°C- 9°C) (Brousett-Minaya et al., 2015), for the corresponding analyses; all equipment used for milk samples was sterilized and clean to avoid contamination or influence on the properties or composition (Brousett-Minaya et al., 2015).

2.3 Physicochemical analysis of raw cow milk samples

The milk constituents (protein, fat, solid and solids-non-fat (SNF), and lactose) and physical features (percentage water, freezing point, salts, and density) were quantified through a milk analyzer Lactoscan SP (Apple Industries services-La Roche Sur Foron, France). An amount of 25 mL of each milk sample was taken in the sample holder, placing the analyzer in the recess position and starting the measurement. After the measurement (45 s), the digital indicator (IED display) shows the obtained results; the procedure was performed in duplicate (Juárez-Barrientos et al., 2016).

Antibiotic residues (beta-lactam, tetracycline, and cephalexin) were measured using SNAP-duo*ST plus test kit, following the methodology described by Cardoso et al. (2019). The AOAC method was employed to measure the acidity in the samples (AOAC International, 2000). pH was quantified through an Orion pH – meter after calibrating (7.02 to 4.00) by soaking in a small volume of milk taken from a beaker.

2.4 Microbiological analysis

Microbial analysis of cow milk samples includes the quantification of colony-forming units per mL (CFUs) of viable mesophilic bacteria (VMB - ISO 4833-1:2013 reference method), fecal (FC) and total coliforms (TC), and yeast and fungi through adequate average (ISO, 2013). The determination of hygienic quality included the count of Viable Aerobic Mesophilic Microorganisms expressed in colony-forming units per mL, preparing and homogenizing the sample with successive decimal dilutions and using plate count agar (APC) and incubating for 24-48 hours at 37°C.

For the determination of Total Coliforms and Fecal Coliforms, indicative of product fecal contamination, the method consists of culturing milk samples according to protocol to determine the presence of total coliforms and the most probable number (MPN) which consisted of making serial tenfold dilutions, as mentioned above, reaching a 10^{-8} dilution. 1 mL of each dilution was added to each of the tubes, containing 10 ml of simple lactose broth with an inverted Durham tube to determine the presence of gas and were incubated for 24 hours at 37°C and, after this time, the tubes that showed gas formation were reviewed, i.e., those in which the presence of bubbles was observed in the Durham tubes and those that were negative to the presence of gas were incubated for a further 24 hours. After this time, the results were read, the highest dilution in which the presence of gas was observed in the three tubes with simple lactose broth was selected, and a roast was taken from the tubes with gas to seed in a MacConkey agar plate to determine if the gas was due to the presence of fecal coliform bacteria; this plate was incubated for 24 hours at 37°C and the presence of colonies of fecal coliform bacteria, which are characterized by a pink color, was determined. For determining Fungi and Yeasts, Sabouraud agar was used, with incubation at 25°C for 24 to 72 hours and with daily examination of the culture. All equipment used for analysis was previously sterilized and used according to the manufacturer's guidelines.

2.5 Hygienic quality of raw milk

The hygienic quality of milk refers to the quantity and type of bacteria present as a consequence of its handling during the milking process, storage and transport (12-24 hours) and for this purpose it was used the reductase assay in milk (Methylene Blue Reduction Time -MBRT) according to the peruvian technical standard NTP 202.014:2004 (MINAGRI, 2018), making the first reading at half an hour of incubation (37° C) and subsequent readings at one-hour intervals.

The Methylene blue reduction test is based on the color transmitted to the milk sample when a dye is added (1 % methylene blue solution in methanol) which disappears more or less quickly (Yadav et al., 2018). The color disappears because of the removal of oxygen and substances reduced by bacterial metabolism. Samples were evaluated as: Excellent (decolorized $>$ 4 hours), Good (decolorized between 3 to 4 hours), acceptable (decolorized of 0.5 to <3 hours), and unacceptable (decolorized $<$ 0.5 h). Reduction time for each cow milk sample was annotated in an excel format and processed statistically.

2.6 Statistical analysis

Data obtained were processed by the CRAN R free software, version 3.3.6 (R Team Core, 2019). Differences in physicochemical and microbial features for each province were performed by one-way variance analysis (ANOVA). After ANOVA, the Tukey test was applied with a significance of 95%.

3 Results and discussions

3.1 Physicochemical characteristics of raw cow milk

Results of the physicochemical characteristics of raw cow milk obtained in Huancayo, Jauja, Concepción and Chupaca are shown in Table 1, where the fat content (%), ranged from 3.29- to 3.77, and the average for four provinces were 3.58 ± 0.50. Similar results were reported by Montes de Oca-Flores et al. (2019), Kra et al. (2013), and Asefa and Teshome (2019) with 3.46%, 3.26 ± 1.18%, 3.89 ± 0.58%, respectively. In contrast, a greater average fat content of 6.02 ± 0.76% was reported by Gemechu and Amene (2016) in Ethiopia.

There was an observed significant difference ($p < 0.05$) in fat content (%) among the four provinces, besides, there is no significant difference between Jauja and Chupaca. The fat content of milk can be affected by the time of year that affects the diet, as well as by management practices and the racial component (Desyibelew and Wondifraw, 2019). The European Union and the Food and Drug Administration (FDA) establish that the fat content in total fluid and unprocessed milk should not be less than 3.5% and 3.25%, respectively. The results obtained in this work are within the recommended standards.

Specific gravity (average 1.03 ± 0.01 g/dm^3) was under those revealed by Gwandu et al. (2018) and Gemechu and Amene (2016). Likewise, differences were not observed ($p > 0.05$) among the provinces studied.

The non-fat solids content of the milk ranged between 8.47 ± 0.51% and 8.99 ± 0.72%, with an overall mean of 8.82 ± 0.62%, with significant differences ($p < 0.05$) between provinces (except Huancayo and Jauja). These results are higher than those reported by Gemechu and Amene (2016) in Ethiopia (8.08 ± 0.13%) and by Mahmoudi and Norian (2015) in Iran (8.50%). The European Union considers that the standard quality content of lean solids should not be lower than 8.59% for raw milk (Tamime, 2008). The results found in this paper showed values higher than 8.59% for three provinces (Huancayo, Jauja and Concepción), and 8.47 ± 0.51% for Chupaca. These values, slightly lower than the EU quality standard, may be related to seasonality, feeding practices, lactation period and milking method.

The protein contents in milk were 3.28 ± 0.31% in Huancayo, 3.27 ± 0.26% in Jauja, 3.19 ± 0.15% in Concepción and 3.28 ± 0.31% in Chupaca, with a general average of 3.21 ± 0.25%. There was a significant difference ($p < 0.05$) between Huancayo, Concepción and Chupaca but not between Huancayo and Jauja (p 0.05). The results found were similar to those reported by Asefa and Teshome (2019) and Mahmoudi and Norian (2015) with 3.16 ± 0.31% and 3.40%, respectively. The quality standard of the European Union (EU) and the Food and Drug Administration suggested values of not less than 2.73% and 2.9% for raw whole milk samples, respectively. In all cow's milk samples collected in the four provinces, values were found within the recommended standards.

In the milk samples from the provinces of Huancayo and Chupaca, the presence of water was detected (0.56 ± 2.03% and 1.59 ± 2.98%, respectively). According to Rasheed et al. (2018) and Tripathy et al. (2019), natural milk, being a food rich in nutrients, proteins and vitamins, should not suffer any type of adulteration, which could cause serious risks for the health. According to our results, the incorporation of water into milk is an adulteration practice that reflects one of the negative behaviors of some producers in Huancayo and Chupaca in order to increase the total volume of the product to be offered.

Total solid (TS) concentration ranged from 12.35%- 12.91% with an overall 12.52 ± 1.11%, and Jauja province presented a significant difference ($p < 0.05$) concerning other provinces (p 0.05). It was higher than the reported by Desyibelew and Wondifraw (2019) (11.89 ± 0.40 %) and Hnini et al. (2018) (11.84 ± 0.28 %) in Ethiopia and Morocco, respectively. The European Union (EU) established that the total solid content should not have a value lower than 12.5 %. Thus, the average TS content in milk samples is slightly inside the recommended standard. The slightly lower values than those of the EU, found in Huancayo, Chupaca and Concepción, can be due to management practices and poor feeding that tend to affect milk quality, although this is not statistically significant (Picinin et al., 2019).

Table 1. Mean ± standard deviation (S.D) and Tukey test comparison of physicochemical characteristics of raw cow's milk samples obtained from four Provinces at Mantaro Valley (n= 40).

Variables	Huancayo (H) (n = 13)	Jauja (J) (n = 9)	Concepción (C) (n = 11)	Chupaca (CH) (n = 7)	Media global (n = 40)
Fat content (%)	3.29 ± 0.32^a	3.77 ± 0.50^b	3.63 ± 0.47^c	3.77 ± 0.67^b	3.58 ± 0.50
Specific gravity (g/dm^3)	1.03 ± 0.02^a	1.03 ± 0.01^a	1.03 ± 0.01^a	1.03 ± 0.02^a	1.03 ± 0.01
Non-fat solids (%)	8.96 ± 0.73^a	8.99 ± 0.72^a	8.75 ± 0.42^b	8.47 ± 0.51^c	8.82 ± 0.62
Protein (%)	3.28 ± 0.31^a	3.27 ± 0.26^a	3.19 ± 0.15^b	3.07 ± 0.18^c	3.21 ± 0.25
Water adds (%)	0.56 ± 2.03^a	0.00 ± 0.00^b	0.00 ± 0.00^b	1.59 ± 2.98^c	0.46 ± 1.72
Freezing point	560 ± 52^a	570 ± 32^a	561 ± 27^b	535 ± 51^c	558 ± 42
Salts (%)	0.72 ± 0.07^a	0.72 ± 0.07^a	0.71 ± 0.03^a	0.69 ± 0.04^a	0.71 ± 0.05
Total solids (%)	12.37 ± 1.24^a	12.91 ± 1.21^b	12.48 ± 0.85^a	12.35 ± 1.21^a	12.52 ± 1.11
Lactose (%)	4.97 ± 0.55^a	4.95 ± 0.41^a	4.83 ± 0.22^b	4.67 ± 0.28^c	4.88 ± 0.41
pH	6.67 ± 0.27^a	6.62 ± 0.29^a	6.78 ± 0.23^b	6.76 ± 0.11^b	6.70 ± 0.24

Values on each horizontal line followed by the same letter do not differ significantly at $p < 0.05$, n= number of milk samples.

The lactose content ranged between 4.67 % and 4.97 % with an overall average of 4.88 ± 0.41 %. There were significant differences ($p < 0.05$) between Huancayo, Concepción and Chupaca, but not (p 0.05) between Huancayo and Jauja. Elrahman et al. (2009) revealed lower lactose values (4.33 ± 0.02 %) in raw milk from cows in Sudan. However, Asefa and Teshome (2019) found similar results to those found in the present work in Ethiopian cows (4.77 ± 0.42 %). Unlike the fat concentration in milk, the lactose concentration is similar in all dairy breeds and cannot be easily altered by feeding practices. Lactose is very important as it influences the absorption of minerals such as copper, zinc and calcium, especially during breastfeeding.

The pH values of the milk samples from Jauja and Huancayo did not present significant differences ($p > 0.05$), the same as between Concepción and Chupaca, with the general average pH being 6.70 ± 0.24. By measuring the pH of milk, impurities, deterioration and signs of mastitis infection can be detected, which can help understand the causes of some changes in its composition.

Fresh milk has a pH value of 6.7; a lower value may be indicative of deterioration due to bacterial degradation, when lactose is broken down and lactic acid is formed due to the presence of lactic acid bacteria (LAB), which ends up producing coagulation or curds with a characteristic smell and flavor ("sour" milk). Milk with pH values higher than 6.7 may indicate the presence of cows with mastitis, so measuring pH can offer a quick way to detect this disease. Small variations in pH 6.7 can affect the time required for pasteurization and the stability of the milk after treatment.

3.2 Microbial quality of raw cow milk

Table 2 presents the microbial quality in raw cow milk samples collected from Mantaro Valley.

The total of viable mesophilic bacteria (VMB) counts expressed in logarithm showed an overall mean of Log 4.82 ± 6.49 CFU/100 mL (0.66 ± 30.76 × 10^5 CFU/mL). The results found in milk samples showed that in Huancayo the BMV exceeded the permissible level of Log 5.3 CFU/mL (5 × 10^5 CFU/mL) according to official standards (MINAGRI, 2017), since Log 6.28 ± 6.74 CFU/mL was determined (19.12 ± 54.78 × 10^5 CFU/mL). Microbial contamination is related to the handling chain and to the animal itself when it is infected (Gwandu et al., 2018); therefore, the milking process, the milking environment, the handling of the milk and its storage would be carried out in unhygienic conditions to a greater degree in Huancayo due to the presence of a high microbial count in the milk. Similar findings were reported by Gwandu et al. (2018) in Tanzania and Ogot et al. (2015) in Kenya.

The mean fecal coliform count (FC) from four provinces showed significant differences ($p < 0.05$) among them (Table 2). The FC showed an overall mean Log 2.06 ± 2.52 CFU/mL, ordered as follow: Chupaca (2.50 ± 2.73) Concepción (1.49 ± 1.81) Jauja (1.34 ± 2.69) Huancayo (0.84 ± 1.34). Similar results were revealed by Abdalla and Elhagaz (2011) who determined coliform counts of Log 2.23 ± 0.14 CFU/mL from cow milk samples obtained on farms in Sudan. In contrast, higher FC was reported by Gemechu and Amene (2016) with an overall mean of Log 5.10 ± 0.29 CFU/mL. In the present study, some cows are kept in a muddy barn, and under hygienic poor conditions. These conditions probably have influenced the milk sample contamination, which increases the microbial count.

Table 2. Mean ± standard deviation (S.D) and Tukey test comparison of microbial counts (log10 CFU/mL) belongings to milk samples studied from four Provinces at Mantaro Valley (n= 40).

Variables	Huancayo (H) (n = 13)	Jauja (J) (n = 9)	Concepción (C) (n = 11)	Chupaca (CH) (n = 7)	Media global (n = 32)
VMB (CFU/mL × 10^5)	19.12 ± 54.78^a	0.95 ± 1.24^b	1.18 ± 1.64^c	0.31 ± 0.42^d	0.66 ± 30.76
VMB Log	6.28 ± 6.74^a	4.98 ± 5.10^b	5.07 ± 5.21^b	4.48 ± 4.63^c	4.82 ± 6.49
TC (CFU/mL × 10^3)	0.25 ± 0.46	0.34 ± 0.47	0.27 ± 0.44	0.50 ± 0.56	0.33 ± 0.47
TC Log	2.39 ± 2.66^a	2.53 ± 2.67^b	2.44 ± 2.65^a	2.70 ± 2.75^c	2.52 ± 2.67
FC (CFU/mL × 10^3)	0.01 ± 0.02	0.22 ± 0.49	0.03 ± 0.06	0.32 ± 0.53	0.12 ± 0.32
FC Log	0.84 ± 1.34^a	1.34 ± 2.69^b	1.49 ± 1.81^c	2.50 ± 2.73^d	2.06 ± 2.52
YMC (CFU/mL × 10^5)	1.87 ± 5.81	0.67 ± 1.33	0.06 ± 0.06	0.47 ± 0.78	0.81 ± 3.27
YMC Log	2.76 ± 5.76^a	4.83 ± 5.12^b	3.77 ± 3.82^c	4.67 ± 4.89^d	4.01 ± 4.90

VMB = viable mesophilic bacteria, TC = total coliforms, FC = fecal coliforms, YMC = yeast and mould count. Means ± S.D followed by different superscript letters in the same row specify significant difference ($p < 0.05$), CFU= colony forming unit per mL, n = number of milk samples.

It is important to determine the total number of bacteria as well as the type of microorganisms present. Thus, coliform bacteria can grow at temperatures of 4°C to 7°C, resisting pasteurization and reducing the shelf life of milk and altering the quality of derived products such as cheese and yogurt. Therefore, it is important to cool the milk to maintain the hygienic quality of the product. Sources of contamination can come from bacteria inside the mammary gland, important in dairy herds with a high presence of mastitis and from bacteria coming from outside the animal, the main source of contamination of milk. The final number of microorganisms in the milk has to do with the environment (pastures, corrals, etc.), the level of bacterial contamination, favorable conditions for the development of bacteria in milk storage and hygiene practices.

The mean total coliform count presented a significant difference ($p < 0.05$) among cow milk samples obtained from dairy farms in each province, and overall mean was Log 4.82 ± 6.49 CFU/mL (ranging from 4.48– 6.28 CFU/mL). Thus, the overall mean count of total coliform of raw cow milk from four provinces was lower than the reported by Gemechu and Amene (2016) who reported an elevated presence of total bacterial Log count of 7.09 ± 0.34 CFU/mL in milk samples collected from dairy farms of Bench Maji-Zone, Ethiopia. On the other hand, total bacteria obtained in Huancayo (19.12×10^5) and Concepción (1.18×10^5) were relatively higher than the acceptable level (1×10^5 bacteria per mL of raw milk).

The higher count of microbial observed in Huancayo may be due to a lack of suitable preparation and knowledge about the use of clean milking utensils and material (plastic containers), maintaining clean the milk production area, correct udder treatment of the cow by milkers handling, and poor hygienic quality. Likewise, most milk production is carried out in small herds. The presence of fecal coliform bacteria indicates unsanitary conditions and non-well hygienic practices in storage or production (Martin et al., 2016). Through prevention programs in milking, the possibility of contamination with total coliforms and particularly fecal coliforms, which constitutes a risk to public health, can be reduced.

The yeast and mold count (YMC) mean were Log 2.76 ± 5.76, Log 4.83 ± 5.12, Log 3.77 ± 3.82, and Log 4.67 ± 4.89 CFU/mL for cow milk samples analyzed from H, J, C, and CH, respectively with an overall mean Log 4.01 ± 4.90 CFU/mL. Significant differences were observed between mold and yeast counts ($p > 0.05$) (Table 2). Among provinces, J was higher in YMC than CH, C, and H. Similar YMC values were reported by Gemechu and Amene (2016) based on three cities examined from Ethiopia with an overall mean of Log 3.90 ± 0.48. However, in other cities of Ethiopia, Habtamu et al. (2018) reported higher YMC values, with an overall mean of Log 7.21 ± 0.21 CFU/mL.

Ortiz-Durán et al. (2017) in Colombia, mention that the presence of fungi in milk can be an indicator of poor hygiene or disease in the mammary gland, evidencing the presence of *Candida* spp., and to a lesser percentage *Aspergillus* spp. in all milk samples evaluated, which suggests a factor that puts the safety and quality of milk and its derivatives at risk. The higher YMC values found in cow's milk analyzed in provinces J and CH could be related to poor personal hygiene, air contamination by organisms, uncleaned containers, and poor practices of milk handlers.

3.3 Hygienic quality of raw cow milk

The presence of antibiotic residues (betal-lactams, tetracycline and cephalexin) was found in 37.5% (n= 15) of the total samples (n= 40). The provinces of Huancayo (n= 13), Jauja (n= 9), Concepción (n= 11) and Chupaca (n= 7) showed the presence of antibiotics in 30.7%, 44.4%, 54.5%. 14.3%, respectively (Table 3). The cause of a high aerobic mesophilic count is due to the presence of bacteria in the milk residue left on the surface of the materials used for collecting or storing milk, dirty or unfurnished udders before milking and rapid non-cooling of the milk (Calderón et al., 2006).

The methylene blue reduction test is suitable for inferring the number of organisms contained in milk samples (Nandy and Venkatesh, 2010). As the bacterial load in milk increases, the oxidation-reduction indicator passes more quickly to its leucobase, thus representing an indirect metabolic count. However, if the number of microorganisms present in raw milk with a reducing effect is low, the test would not agree with the bacterial count obtained in plates, not reflecting, as already mentioned, the real microbial contamination of the milk, which makes it lose value as a tool within rapid tests (Luigi et al., 2013).

Of the 40 milk samples processed for MBRT (Table 2), 13 (32.5%) had excellent quality, 18 (45.0%) had good quality, 9 (22.5%) had acceptable quality, and no sample (0%) had poor quality. By province, Huancayo (n= 13) presented excellent, good and acceptable quality in 38.4% (H2, H3, H5, H6 and H7), 38.4% (H1, H4, H8, H9 and H11) and 23.2%. (H10, H12 and H13), respectively.

In Jauja (n= 9), 55.6% (n=5, J1, J2, J4, J7 and J8) of the samples had excellent quality, and 22.2% (n= 2) of the samples had good (J3 and J9) and acceptable quality (J5 and J6). For Concepción (n= 11), 27.3%, 54.6% and 18.1% had excellent (C2, C4 and C5), good (C1, C3, C6, C7, C8 and C10) and acceptable (C9 and C11) quality.

In Chupaca (n= 7), 71.4% had good quality (CH2, CH3, CH4, CH5 and CH7) and 28.6% acceptable quality (CH1 and CH6). In total, the quality of the Mantaro Valley was considered to have good quality (3.50 ± 1.26 hours).

Table 3. Presence of antibiotic residues, MBRT test (reduction time of methylene blue reduction time), and quality classification based on each collection point and its average.

Site	Point	Antibiotic residues presence	Reduction time (hours)	Reduction time average (hours)	Quality
	H1	No (-)	3.0 (Good)		
	H2	No (-)	4.5 (Excellent)		
	H3	No (-)	4.5 (Excellent)		
	H4	Yes (+)	4.0 (Good)		
	H5	Yes (+)	5.0 (Excellent)		
	H6	Yes (+)	4.5 (Excellent)		
Huancayo (H) (n = 13)	H7	No (-)	5.5 (Excellent)	3.54 ± 1.43	Good
	H8	No (-)	3.5 (Good)		
	H9	No (-)	4.0 (Good)		
	H10	Yes (+)	1.0 (Acceptable)		
	H11	No (-)	4.0 (Good)		
	H12	No (-)	1.5 (Acceptable)		
	H13	No (-)	1.0 (Acceptable)		
	J1	No (-)	4.5 (Excellent)		
	J2	No (-)	5.0 (Excellent)		
	J3	No (-)	3.5 (Good)		
	J4	Yes (+)	5.0 (Excellent)		
Jauja (J) (n = 9)	J5	No (-)	1.5 (Acceptable)	3.33 ± 1.31	Good
	J6	Yes (+)	1.5 (Acceptable)		
	J7	Yes (+)	3.5 (Excellent)		
	J8	Yes (+)	2.0 (Excellent)		
	J9	No (-)	3.5 (Good)		
	C1	Yes (+)	3.5 (Good)		
	C2	Yes (+)	1.5 (Excellent)		
	C3	No (-)	3.0 (Good)		
	C4	No (-)	4.5 (Excellent)		
	C5	No (-)	4.0 (Excellent)		
Concepcion (C) (n = 11)	C6	Yes (+)	5.5 (Good)	3.54 ± 1.29	Good
	C7	No (-)	5.0 (Good)		
	C8	Yes (+)	3.5 (Good)		
	C9	No (-)	4.0 (Acceptable)		
	C10	Yes (+)	3.5 (Good)		
	C11	Yes (+)	1.0 (Acceptable)		
	CH1	No (-)	2.5 (Acceptable)		
	CH2	No (-)	4.0 (Good)		
	CH3	No (-)	4.0 (Good)		
Chupaca (CH) (n = 7)	CH4	No (-)	4.0 (Good)	3.57 ± 0.68	Good
	CH5	No (-)	4.0 (Good)		
	CH6	Yes (+)	2.5 (Acceptable)		
	CH7	No (-)	4.0 (Good)		
Total (n = 40)				3.50 ± 1.26	Good

Table 3 shows the results of hygienic quality through antibiotic presence, methylene blue reduction time (MBRT), and quality classification of raw cow milk samples collected in four provinces from Mantaro Valley.

The recognition of the risk factors present throughout the biological and production process of poor quality milk should allow the different actors involved (producers, collectors and processors of milk) to rethink their respective work schemes in order to adopt corrective measures to improve the hygienic quality of milk.

4 Conclusions

As for the physicochemical properties of raw cow's milk samples from the four provinces of the Mantaro Valley, the values found were within national and international standards.

The determination of the hygienic quality of the milk sampled from the four provinces showed an acceptable level to the methylene blue reduction test (MBRT). The microbiological analysis detected the presence of viable mesophilic bacteria, total coliforms, fecal coliforms and yeast and mold counts, which may be related to poor sanitary conditions, dirty collection materials, bad milking environment, among others.

Recommendations to produce milk of good hygienic quality based on the Code of Hygienic Practice for Milk and Dairy Products CAC/RCP 57, published in 2004 by the Codex Alimentarius (MIDAGRI, 2004), are to improve general hygiene practices, both in the environment and milking, as well as in post-milking handling and hygienic storage of milk. Although it is true that the composition of milk varies due to a multiplicity of factors, genetics and nutrition play a determining role in its compositional quality and it is on them that dairy producers in the Mantaro Valley should focus more attention.

Declaration of interest

The authors declare not to have a conflict of interest.

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Authors' contribution

F.A.V.: conceptualized and designed the manuscript, L.G.E.: wrote the manuscript, N.M.S.; I.U.P.; ARHDLC: statistical analysis, interpretation, and edited the manuscript.

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