R. Bras. Zootec., 52:e20210189, 2023
https://doi.org/10.37496/rbz5220210189

Animal production systems and agribusiness
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Effect of milking hygiene, herd size, water hardness and temperature-humidity index on milk quality of dairy farms

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Received: October 30, 2021
Accepted: January 25, 2023

How to cite: López-Carlos, M. A.; Hernández-Briano, P.; Aguilera-Soto, J. I.; Carrillo-Muro, O.; Medina-Flores, C. A.; Méndez-Llorente, F. and Aréchiga-Flores, C. F. 2023. Effect of milking hygiene, herd size, water hardness and temperature-humidity index on milk quality of dairy farms. Revista Brasileira de Zootecnia 52:e20210189.
https://doi.org/10.37496/rbz5220210189

ABSTRACT - The objective of this study was to evaluate the effect of milking hygiene practices, herd size, water hardness, and temperature-humidity index (THI) on the physicochemical and microbiological characteristics of raw milk, and standard plate count (SPC) in milking machines of dairy farms in the central region of Mexico. Data were collected from fifty-three dairy farms during one year. The evaluated effects included milking hygiene conditions (good, medium, poor), herd size (1-50, 51-100, 101-150, ≥151 heads), water hardness (soft or moderately hard), and THI (comfortable or stressful). The increase in milking hygiene produced greater milk yield (MY) and energy corrected milk (ECM) but lower protein content, and decreased the individual bacterial count (IBC) and somatic cell count (SCC). The MY, ECM, protein content, IBC, and SCC were higher on bigger farms. The use of soft water reduced MY, IBC, and SCC, but improved fat, lactose, total solids (TS), and non-fat solids (NFS). Heat stress negatively affected fat, protein, TS, NFS, acidity, freezing point (FP), SCC, and methylene blue dye reduction test. Poor milking hygiene contributes to higher SPC in milking machine parts. Water hardness and THI did not affect SPC in all milking machine parts. Proper milking hygiene practices, larger herd size, softer water, lower THI, and adequate cleaning and disinfection of the milking machine parts benefits the physicochemical and microbiological quality of the milk.

Keywords: dairy, heat stress, milking practices, water characteristics

1. Introduction

Around the world, there are approximately 150 million households engaged in milk production, most of which are small and family-operated. The majority of them are in developing countries (Douphrate et al., 2013; Lowder et al., 2016; FAO, 2021). With more than six billion consumers of milk and milk products, milk production contributes to household livelihoods, food security, and nutrition for people (Adesogan and Dahl, 2020). Milk provides relatively quick returns for small-scale producers and is an important source of cash income (Kapaj, 2018). In Mexico, 50% of dairy farmers are small, but they contribute with 37% of national production (Val-Arreola et al., 2006; Rapsomanikis, 2015). A small farm in Mexico is one with an average of 13 mature cows, with an average production of 14 L/d (Méndez y Cazarín et al., 2000).

The objective of dairy farms is to produce milk in sufficient quantity and quality to ensure its profitability, also guaranteeing quality and safety of the product to protect the health of consumers and promote its commercialization (Popescu and Angel, 2009; Kapaj and Deci, 2017; Berge and Baars, 2020). Milk and its derivatives provide essential micro- and macronutrients to the diet (Marangoni et al., 2019). The quality of raw milk is determined by its nutritional, organoleptic, hygienic, and sanitary attributes, which must be acceptable for agroindustry and human consumption (Murphy et al., 2016). The general criteria applied to evaluate the quality of raw milk are its physicochemical characteristics and a low content of microorganisms and somatic cells (Barbano and Lynch, 2006; Cincović et al., 2010). Many factors can affect milk quality, such as herd size and management practices (Wenz et al., 2007; Zucali et al., 2011), good hygiene practices during collection and processing of milk (Cempírková, 2007; Elmoslemany et al., 2010), and environmental factors as heat stress measured by temperature-humidity index (THI) (Bertocchi et al., 2014; Zeinhom et al., 2016).

Good-quality raw milk is essential for producing quality milk and derivate products. However, there is limited information on the influence of various management factors on bulk tank physicochemical and bacterial counts on dairy farms (Murphy and Boor, 2000). Therefore, this study aimed to evaluate the effect of milking hygiene practices, herd size, water hardness, and heat stress on the physicochemical characteristics and sanitary quality of raw milk on typical dairy farms from the central region of Mexico.

2. Material and Methods

2.1. Study site

The present study met the guidelines of the local Research Ethics Committee. The study was conducted in Jalisco Highlands region, located in the Mesa Central or southern plateau of Mexico (latitude 21°23'18.4" N and longitude 102°14'03.5" W, and average 1,902 m a.s.l.). The average annual rainfall is 658.5 mm, and the maximum temperature reaches 30 °C during the summer and the minimum of 7 °C during the winter (INEGI, 2017). The geology is complex, composed of fractured volcanic rocks, as well as conglomerates, sandstones, and continental sediments. The farm water sources include groundwater (depth >100 to 400 m) and surface waters from dams, streams, lagoons, and ponds (CEAJ, 2005).

This region produces 60% of the total milk in the state of Jalisco and 19% of Mexico's production (SIAP, 2021). The small dairy farm is the predominant system. Depending on the surface and conditions of the cultivation field, the productive system can be intensive or semi-intensive systems. The livestock diet includes grains, cut fodder, and crop residues (Soltero-Gardea and Negrete-Ramos, 1997). Holstein-Friesian is the predominant dairy breed. Producers carry out preventive medicine and modern reproductive practices, although hygienic milking practices are variable among producers. The milk is sold to dairy processors or used for the elaboration of cheese and other dairy products (Montiel-Olguín et al., 2019).

2.2. Data collection

Fifty-three farms were selected to participate following the criteria: acceptance to participate in the study, shipping of milk to a local dairy processor, previous records of the constancy and compliance in the daily delivery of milk to the processor, and use of milking parlors equipped with modern milking technology. The chosen dairy farms were visited prior to the beginning of the study to evaluate herd size, compliance with milking hygiene practices, and water hardness.

The classification of dairy farms by milking hygiene was in agreement with the Official Mexican Standard NMX-F-730-COFOCALEC-2015, Milk product system - Dairy foods, recommended hygiene practices for obtaining milk. Compliance with the official recommendations for hygienic milking practices included the revision of facilities and equipment, livestock management, milking process, personnel, storage, and conservation of milk. The coding used in farms classification was: Good = dairy farms that satisfactorily comply with all the recommended milking hygiene practices; Medium = dairy farms that satisfactorily comply with more than half of the recommended hygiene practices; and Poor = dairy farms that satisfactorily comply with less than half of the recommended practices.

Number of cows in milk at the beginning of the study served for farm size classification. Looking for farm size class values close to the quartiles (Ma et al., 2020), herds were divided into four categories as follows: 1-50, 51-100, 101-150, and ≥151 heads.

Water hardness was determined using an EDTA titration based on Method 2340C from Standard Methods (Clesceri et al., 1999). According to the criteria indicated by Bagley et al. (1997), water hardness was classified as soft water (0 to 60 mg L^{-1} $CaCO_3$) and moderately hard water (61-120 mg L^{-1} $CaCO_3$). Climatic values (temperature and relative humidity) were obtained from a meteorological station located in the vicinity of the study site. The THI was calculated according to the following equation (Mader et al., 2006):

$$THI = 0.8 \times \text{ambient temperature} + [(\%\ \text{relative humidity} \div 100) \times (\text{ambient temperature} - 14.4)] + 46.4$$

In agreement with Dikmen and Hansen (2009) and Kadzere et al. (2002), THI was classified as comfortable if THI ≤72 or stressful if THI >72.

On enrolled farms, cows were milked twice daily (at 07:00 and 17:00 h). Raw milk was stored at approximately 4 °C in the chilled on-farm bulk tank. The dairy processor collects the chilled milk daily (pooled milk from the morning and evening milking). The plant routinely performs physicochemical analysis on receipt of milk. Records of milk yield (MY, kg cow d^{-1}), fat (g L^{-1}), protein (g L^{-1}), lactose (g L^{-1}), total solids (TS, g L^{-1}), non-fat solids (NFS, g L^{-1}), acidity (g L^{-1}), freezing point (FP, °H), and density (g L^{-1}) were retrieved and stored in a database.

Fat, protein, lactose, TS, and NFS values were determined through infrared spectrometry (Milkoscan FT-120, Foss A/S, Hillerod, Denmark). Physicochemical analysis was determined on each sample according to AOAC methods (AOAC, 2016). Acidity was determined by titration (AOAC method 947.05). The freezing point was determined (AOAC method 990.22) with Gerber Cryoscope C1 equipment (Gerber Instruments AG, Effretikon, Switzerland). Density (specific gravity) was determined using a pycnometer (AOAC method 925.22). Energy-corrected milk (ECM, kg d^{-1}) adjusted to 3.5 percent fat and 3.2 percent protein was calculated using the following equation (Hutjens, 2010):

$$ECM = (0.323 \times \text{milk yield}) + (12.82 \times \text{fat yield}) + (7.13 \times \text{protein yield})$$

Analysis of individual bacterial counts (IBC; bacteria mL^{-1}) and somatic cell counts (SCC; cells mL^{-1}) were performed using an automatic analyzer (BacSomatic, FOSS Electric A/S, Hilleroed, Denmark). The methylene blue dye reduction test (MBRT) was performed in duplicate according to the method described by Atherton and Newlander (1977), considering 5 h as good-quality milk and less than 2 h as poor-quality milk.

Dairy farms were visited monthly before afternoon milking for the bacteriological sampling of milking machine parts. Milk pump, teat cup, milk claw, wash line, milk line, and milk receiver were sampled by swabbing the edges and internal surfaces of each with a sterile swab moistened in 1 g L^{-1} peptone water, which was then immersed in a tube with 1 mL of peptone water solution. Samples were transported to the laboratory under refrigeration (4 °C) no later than 12 h after collection and subjected to inoculation (in triplicate) on Petri dishes with 15 mL of plate count agar. Plates were incubated at 37 °C for 24 h before colony counting. The microbiological analysis procedure complied with ISO: 4833:2003 method and AOAC 966.23 method (AOAC, 2016).

2.3. Statistical analyses

The statistical analysis was performed using SAS software (Statistical Analysis System, University edition). Normality assumptions were previously tested using the Shapiro-Wilk test, and homogeneity of variance (homoscedasticity) using Bartlett's test. Data were analyzed as a repeated measures design using the PROC MIXED procedure of SAS statistical package. The farm was the experimental unit, and the collection day was the repeated measurement. The model included the effects of milking hygiene conditions, herd size, water hardness, and temperature-humidity index. The analysis was carried out using the Restricted Maximum Likelihood (REML method) with repeated measurements and the ID assigned to the farm as subject, to specify the variation within farms over time. The RANDOM instruction was used to adjust for variation due to the effect between farms. Analyses were conducted using multiple covariance structures to determine the most appropriate by the smallest Akaike and Schwarz's Bayesian criteria. An autoregressive structure was used for the physicochemical characteristics MY, ECM, fat, protein, lactose, and FP, and the standard plate count (SPC) in the milking machine parts. Moreover, a variance component structure was used for the physicochemical characteristics TS, NFS acidity, and density, and the bacteriological characteristics IBC, SCC, and MBRT. For analyses, the SCC and bacteria data were transferred to log10 base.

Differences between means were established using the PDIFF instruction. The option ADJUST=TUKEY was used to request a multiple comparison adjustment. Results are presented as least squares means ± SEM and considered significant if P<0.05.

3. Results

In the study, a total of fifty-three dairy farms were evaluated. According to their milking practices, 34 dairy farms presented good milking practices, 16 presented medium milking practices, and three presented poor milking practices. According to herd size, 14 farms had 1-50 cows, 14 farms 51-100 cows, 13 farms 101-150 cows, and 12 farms ≥150 cows. Regarding THI, of all the farms evaluated, 30 of them presented a comfortable THI and 23 presented stressful THI.

3.1. Physicochemical characteristics of raw milk

Dairy farms with good hygienic conditions produced greater (P<0.05) MY and ECM but lower protein content than those with medium or poor hygienic conditions (Table 1). Moreover, dairy farms with poor hygienic conditions produced the lowest (P<0.05) MY, ECM, and lactose content. However, independently of milking hygiene practices, the milk content of fat, TS, NFS, acidity, FP, or density were unaffected (P>0.05).

Herd size affected (P<0.001) MY, ECM, fat, protein, TS, NFS, and FP variables, but did not affect (P>0.05) lactose, acidity and density (Table 1). The largest farms (≥151 heads) produce higher (P<0.05) MY and ECM than farms with 101-150 heads, while farms below 100 heads produce the lowest (P<0.05) MY and ECM. Fat content and FP were similar (P>0.05) among farms greater than 51 heads, but reduced (P>0.05) on farms with the smallest herd size (1-50 heads). The protein content in milk increases (P<0.05) in larger herd sizes (≥151 heads), similar (P>0.05) between farms with 51-100 and 101-150 heads, and reduced on farms with smaller herds (1-50 heads). The TS and NFS content were greatest (P<0.05) in 101-150 and >150 head farms, but lowest (P<0.05) on farms with smaller herd sizes.

On dairy farms where soft water is available, MY was reduced (P<0.001) by 1.7%, although fat (2.0%), lactose (3.0%), TS (2.0%), and NFS (1.1%) increased (P<0.001) when compared with dairy farms where hard water is available. Water hardness did not affect (P>0.05) ECM, protein content, acidity, FP, density, or temperature measurements (Table 1).

Temperature-humidity index influenced all physicochemical characteristics of raw milk, except density. When THI >72, the MY, ECM, and lactose content increased 5.8, 5.7, and 1.4%, respectively. However, fat (0.09%), protein (1.6%), TS (1.5%), NFS (2.1%), acidity (0.8%), and FP (0.1%) were reduced.

3.2. Sanitary characteristics of raw milk

Better milking hygiene practices reduce (P<0.05) individual IBC and SCC in milk, and increase (P<0.05) time for MBRT. Lower (P<0.05) IBC and SCC were observed on dairy farms with softer

Table 1 - Effects of herd size, milking hygiene, water hardness and THI on physicochemical characteristics of raw milk of fifty-three dairy farms in the highlands of central Mexico[1]

Item	n	MY (kg cow d^{-1})	ECM (kg d^{-1})	Fat (g L^{-1})	Protein (g L^{-1})	Lactose (g L^{-1})	TS (g L^{-1})	NFS (g L^{-1})	Acidity (g L^{-1})	FP (°H)	Density (g L^{-1})
Milking hygiene											
Good	34	25.2a	25.3a	3.55	3.14b	5.08a	12.13	8.58	1.320	−0.5426	1.031
Medium	16	24.3b	24.4b	3.53	3.18a	5.05a	12.09	8.56	1.320	−0.5428	1.031
Poor	3	20.7c	20.6c	3.50	3.21a	4.93b	12.02	8.53	1.318	−0.5424	1.035
SEM		0.12	0.11	0.03	0.01	0.02	0.04	0.04	0.002	0.0002	0.001
P-value		<0.001	<0.001	0.133	0.015	0.002	0.209	0.312	0.830	0.458	0.804
Herd size											
1-50	14	22.4c	22.2d	3.48b	3.12c	5.02	11.95c	8.49c	1.318	−0.5419b	1.030
51-100	14	22.6c	22.6c	3.53a	3.18b	5.02	12.05b	8.54bc	1.318	−0.5427a	1.030
101-150	13	23.3b	23.4b	3.56a	3.18b	4.99	12.16a	8.58ab	1.320	−0.5427a	1.030
≥151	12	25.3a	25.5a	3.55a	3.23a	5.05	12.18a	8.64a	1.320	−0.5433a	1.031
SEM		0.11	0.11	0.001	0.02	0.02	0.04	0.03	0.003	0.0002	0.001
P-value		<0.001	<0.001	<0.001	<0.001	0.634	<0.001	<0.001	0.738	<0.001	0.348
Water hardness											
Soft	48	23.2b	23.4	3.56a	3.19	5.07a	12.20a	8.60a	1.321	−0.5426	1.031
Moderate	5	23.6a	23.5	3.49b	3.16	4.92b	11.96b	8.51b	1.32	−0.5423	1.030
SEM		0.10	0.10	0.01	0.01	0.02	0.03	0.02	0.002	0.0002	0.001
P-value		0.001	0.3743	<0.001	0.127	<0.001	<0.001	<0.001	0.579	0.964	0.630
THI											
Comfortable	30	22.8b	22.8b	3.53a	3.18a	4.99b	12.18a	8.62a	1.32a	−0.5422	1.030
Stressful	23	24.2a	24.1a	3.50b	3.13b	5.06a	11.94b	8.44b	1.31b	−0.5426	1.029
SEM		0.09	0.09	0.002	0.004	0.004	0.008	0.006	0.005	0.0001	0.001
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.213

[1] Daily data for fifty-three dairy farms through one year (19,345 observations).
THI - temperature-humidity index; MY - milk yield; ECM - energy corrected milk; TS - total solids; NFS - non-fat solids; FP - freezing point; SEM - standard error of the mean.
a-c - Means with different letters in the same column are statistically different (P<0.05).

water compared with farms with moderate water hardness, but MBRT was unaffected (P>0.05) by water hardness (Table 2).

The IBC and SCC increased (P<0.001) but MBRT decreased (P<0.001) with increase in herd size. Farms with larger herds (≥101 heads) showed the highest (P<0.05) IBC and SCC and the lowest MBRT values. However, IBC was similar (P>0.05) among farms ranged 1 to 150 heads. The lowest SCC and highest MBRT were observed (P<0.05) on smaller dairy farms (1-50 heads). The THI did not affect (P>0.05) IBC, but negatively affected (P<0.001) SCC and MBRT in raw milk.

3.3. Bacteriological count in milking machine parts

The SPC was higher (P<0.05) in all parts of the milking machine on dairy farms with poor milking hygiene compared with farms with good or regular milking hygiene (Table 3). In addition, the SPC in the milk pump and milk claw increased (P<0.05) on farms with 101-150 heads, decreased on the smallest farms with 1-50 and 51-100 heads, and was lower on the larger farms (≥151 heads). A lower SPC (P<0.05) was observed in teat cups of dairy farms greater than 151 heads compared with farms with a lower number of heads. In wash line and milk line parts, greater SPC was observed on medium-sized farms (51-100 and 100-150 heads), but was minor on the smaller (1-50 heads) and larger (≥151 heads) farms. The SPC in milk receiver was similar (P>0.05) among farms with 1-50, 51-100, and 101-150 heads, but reduced (P<0.05) on farms ≥151 heads. However, water hardness and THI did not affect (P>0.05) SPC in milking machine parts (Table 3).

Table 2 - Effects of herd size, milking hygiene, water hardness, and THI on bacteriological characteristics of raw milk of fifty-three dairy farms in the highlands of central Mexico[1]

Item	n	IBC	SCC	MBRT
Milking hygiene				
Good	34	4.85c	5.55c	625a
Medium	16	4.97b	5.63b	616b
Poor	3	5.46a	5.88a	611c
SEM		0.05	0.03	1.4
P-value		<0.001	<0.001	<0.001
Herd size				
1-50	14	5.01b	5.60c	622a
51-100	14	5.07b	5.69b	619b
101-150	13	5.12ab	5.72ab	616bc
≥151	12	5.19a	5.75a	612c
SEM		0.05	0.03	1.3
P-value		0.004	<0.001	<0.001
Water hardness				
Soft	48	4.90b	5.63b	618
Moderate	5	5.29a	5.74a	617
SEM		0.04	0.03	1.2
P-value		<0.001	<0.001	0.660
THI				
Comfortable	30	5.09	5.65b	632a
Stressful	23	5.10	5.73a	602b
SEM		0.04	0.02	1.1
P-value		0.664	<0.001	<0.001

[1] Milk samples were obtained monthly in fifty-three dairy farms through one year (636 observations).
THI - temperature-humidity index; IBC - individual bacterial count (log_{10} bacteria mL^{-1}); SCC - somatic cell count (log_{10} cells mL^{-1}); MBRT - methylene blue dye reduction test (min); SEM - standard error of the mean.
a-c - Means with different letters in the same column are statistically different (P<0.05).

4. Discussion

4.1. Physicochemical characteristics of raw milk

Suranindyah et al. (2015) reported that improving environmental and pre-milking sanitation increased milk quality, density, and non-fat solids. In addition, Moroni et al. (2018) stated that good hygiene and management practices that include pre-milking udder preparation (wet cleaning and massage) is reflected in a state of well-being of the cows and improvement in milk secretion. Therefore, it is to be expected that the stables categorized with better milking hygiene conditions will obtain an improvement in milk production.

In agreement with our results, previous studies carried out both in Mexico (Carranza-Trinidad et al. 2007; García-Muñiz et al., 2007; Romo-Bacco et al., 2014) and worldwide (Allore et al., 1997; Weersink and Tauer, 1991; Simensen et al., 2010; Dong et al., 2016; Gargiulo et al., 2018), suggest that milk production and farm productivity increase as the herd size increases associated with a higher technological level in larger companies. In addition, previous reports (Allore et al., 1997; Oleggini et al., 2001) described that larger size herds had not only higher MY, but higher fat and protein contents in milk.

Energy-corrected milk was developed to put all cows on an equal basis for comparative purposes by equating to a common term various outputs of milk having distinct chemical components such as fat, protein, and lactose (Tyrrell and Reid, 1965). This indicator can be used as a predictor of dry matter intake (Mazumder and Kumagai, 2006) and as a decision tool to make adjustments in the diet

Table 3 - Effects of herd size, milking hygiene, water hardness, and THI on standard plate count (log_{10} ufc $swab^{-1}$) in milking machine parts of fifty-three dairy farms in the highlands of central Mexico[1]

		Part of the milking machine					
	n	Milk pump	Teat cup	Claw	Wash line	Milk line	Receiver
Milking higiene							
Good	430	2.14b	0.47b	0.95b	0.67b	1.66b	1.57b
Regular	182	2.01b	0.57b	0.92b	0.75b	1.70b	1.66b
Poor	24	3.17a	1.84a	2.71a	1.80a	2.89a	3.04a
SEM		0.13	0.18	0.14	0.14	0.14	0.15
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Farm size							
1-50	164	2.54b	1.11a	1.56b	0.84b	2.06b	2.21a
51-100	181	2.60b	1.09a	1.58b	1.33a	2.32a	2.33a
101-150	157	2.96a	1.14a	1.90a	1.30a	2.41a	2.41a
≥151	134	1.80c	0.64b	0.96c	0.82b	1.54b	1.40b
SEM		0.12	0.18	0.14	0.14	0.14	0.14
P-value		<0.001	0.043	<0.001	<0.001	<0.001	<0.001
Water hardness							
Soft	576	2.43	0.82	1.51	1.01	2.07	2.07
Moderately hard	60	2.52	1.10	1.54	1.14	2.10	2.10
SEM		0.10	0.15	0.12	0.12	0.19	0.12
P-value		0.471	0.153	0.823	0.383	0.880	0.839
THI							
Comfortable	370	2.53	1.00	1.57	1.06	2.17	2.2
Stressful	266	2.42	0.93	1.48	1.09	2.00	2.0
SEM		0.10	0.14	0.11	0.11	0.11	0.1
P-value		0.289	0.635	0.417	0.793	0.123	0.1480

[1] Milk samples were obtained monthly in fifty-three dairy farms through one year (n = 636).
THI - temperature-humidity index; SEM - standard error of the mean.
a-c - Means with different letters in the same column are statistically different (P<0.05).

(Boerman et al., 2015), in addition to being a trait to consider for the genetic selection of dairy cattle (Li et al., 2018). In agreement with our results, Adamczyk et al. (2017) reported improvements in ECM as herd size increased (>100 cows) in Polish Holstein-Friesian cows.

Water is an essential nutrient to sustain life and optimize growth, lactation, and reproduction of dairy cattle (Beede, 2005; Golher et al., 2021). Water hardness is expressed as the sum of calcium and magnesium concentration reported in equivalent amounts of calcium carbonate (Clesceri et al., 1999). It is generally accepted that water hardness does not affect animal performance or water intake (Looper and Waldner, 2002). Crooks (2020) even mentioned that there may be health benefits if livestock drinks hard water, because dietary requirements for magnesium and calcium are more easily met.

However, there is limited research about the effects of water hardness on milk production or its physicochemical composition. In a recent study, Senevirathne et al. (2018) investigated the effects of *ad libitum* drinking reverse osmosis water (17 mg L^{-1}, considered soft water) versus municipal/city water (249 mg L^{-1}, considered very hard water) on growth, nutrient utilization, and health scores of calves. They observed that hard water consumption increased (P = 0.01) mean daily water intake by 5.2%; however, soft water consumption increased (P<0.01) nutrient intake (DMI, crude protein, ether extract, starch, and neutral detergent fiber) at the postweaning period in 5.1%. In addition, Solomon et al. (1995) reported that high-producing dairy cows managed under desert conditions supplied with desalinized drinking water instead of the natural salty water from wells consumed 9.4% more water and produced 2.1 kg d^{-1} more milk, that contains more fat and protein (P<0.05).

Furthermore, several studies (Arce-Cordero et al., 2021; Crawford et al., 2008; Schaefer et al., 1982) suggest that the controlled inclusion of mineral elements such as sodium bicarbonate, calcium carbonate, or magnesium carbonate in the diet of lactating dairy cows acts as dietary buffers, improving the gastrointestinal pH. Therefore, the mineral content in the water could have an effect at this level. Although in the present study, the water consumption and ruminal pH were not measured, the results suggested a change in water intake or ruminal buffering that may be associated with the water mineral content.

Dairy cattle suffer heat stress when the temperature is out of the thermoneutral zone (Allen et al., 2013; Hansen, 1990). Although the thermoneutral zone is between 5 and 25 °C, heat stress is not only related to temperature but also to air humidity, which in conjunction alters the cow's capacity to dissipate heat (Qi, et al., 2015; Rhoads et al., 2009). Heat stress occurs when the THI index is >72 (Kadzere et al., 2002; Zeinhom et al., 2016). However, it depends on factors such as breed, diet, milk production level, age, and housing conditions (Roenfeldt, 1998).

The negative effects of heat stress on milk production and composition have been widely studied in dairy cattle (Qi et al., 2015; Lambertz et al., 2014; Ji et al., 2020). High-producing cows are much more susceptible to heat stress than low-producing animals (Gantner et al., 2017) because of the increased metabolic heat, making it more difficult for cows to preserve their thermoregulatory mechanism and maintain the body temperature in a thermoneutral zone and physiological homeostasis (Kadzere et al., 2002). Other studies reported a lack of a significant relationship between MY and rectal temperature (Dikmen and Hansen, 2009), attributing it to the fact that cows have a greater capacity for adaptation and regulation of body temperature through physiological modifications (Bernabucci et al., 2010) or even genetic inheritance (Ravagnolo and Misztal, 2000).

Similar to our results, several studies (Bouraoui et al., 2002; Tao et al., 2020; Zeinhom et al., 2016) reported that milk components decreased ($P<0.05$) in cows exposed to heat stress conditions. Levit et al. (2021) and Ouellet et al. (2019) reported that heat stress conditions negatively affect ECM in dairy cows. In this regard, the reduction of fat content could be due to the lower dry matter intake and minor proportions of acetate in the rumen (Bandaranayaka and Holmes, 1976; Bernabucci et al., 2015). In addition, the reduction in protein and lactose content could be due to the direct effect of heat stress on mammary gland synthesis (Bernaucchi et al., 2010; Cowley et al., 2015).

Furthermore, the stressful conditions in the study area occurred from mid-spring to mid-summer in April to August, coinciding with the season in which forage plants are stimulated to grow by the effects of the increase in temperature and humidity in this region. Gorlier et al. (2012) reported that the nutritional composition of the pastures depends on variations in their botanical and phenological composition, thus affecting the quality of the milk. In agreement with our results, Dahl et al. (1998) reported that the percentages of fat, protein, and TS are higher during the winter and lower during the summer. This variation could be related to changes in the availability and quality of food and climatic conditions.

During the rainy season, the pastures are low in fiber; therefore, the levels of fat in the milk are decreased. In addition, with the high temperature and relative humidity, the intake levels decrease. However, during the autumn and winter (dry season), the availability and quality of the pasture decrease, providing hay or agricultural waste with higher fiber content, thus increasing the levels of fat but decreasing milk production. The same factors could have affected the FP in this study because their values depend on TS content in the milk. (Zagorska and Ciprovica, 2013).

4.2. Sanitary characteristics of raw milk

Low bacterial count in milk is an important parameter to guarantee a safe product for consumers and preserve sensory traits and shelf life of milk and milk derivatives (Murphy et al., 2016). The microbial contamination of raw milk can occur from a variety of sources like dirty udders and animals, facilities, personnel, and milking equipment (Elmoslemany et al., 2010; Kelly et al., 2009). As expected and in agreement with previous studies (Gibson et al., 2008; Ózsvári and Ivanyos, 2021; Erdem and Okuyucu, 2019) the present study determined that better milking hygiene procedures help to reduce the total bacterial count in raw milk. Corresponding with our results, Berry et al. (2006) observed a positive relationship (P<0.05) between SCC and IBC, whereas Álvarez-Fuentes et al. (2012) reported that better hygienic conditions produced lower SCC and higher reductase time (P<0.05).

Our results corroborate several studies (Barkema et al., 1998; Sadeghi-Sefidmazgi and Rayatdoost-Baghal, 2014; Wenz et al., 2007; Zucali et al., 2011) which stated that a low SCC is associated with better management practices in the herd (good bedding, free-stall barns, wearing gloves during milking and shade-providing) and pre-milking udder preparation (teat disinfection, and the use of washable towels for teat cleaning or a wet disposable tissue for udder cleaning).

The MBRT is a widely used milk quality test that measures bacterial contamination in milk. In this test, bacterial activity changes the blue color of methylene in milk to white as the oxygen level diminishes due to bacterial activity. The shorter time for milk to change color, the more contaminated the milk (Moran, 2012). The lower value for MBRT observed in milk from farms with poor milking hygiene (higher IBC and SCC) confirm the usefulness of this simple but effective technique to detect the quality of raw milk (Pérez-Lomas et al., 2020).

Our results corroborated those of De Silva et al. (2016), who reported a reduction (10.8 to 16.5%) in milk bacterial counts after implementing good milking practices, and a strong relationship ($r^2 = 0.91$) between MBRT and milk bacterial counts. In light of the results obtained in the present study, the need to implement a permanent evaluation and an improvement of the hygienic procedures in facilities, equipment, animals, and personnel on dairy farms becomes evident, to provide a higher-quality product to the consumer.

Around the world, dairy farmers are trying to increase their herd size to benefit from economies of scale derived from lower investments per cow, lower variable costs per unit of production, and higher labor efficiency (Bailey et al., 1997; Espinoza-Ortega et al., 2007; Fariña and Chilibroste, 2019). In the US, Norman et al. (2000) and Oleggini et al. (2001) reported that larger herds have a lower SCC compared with smaller herds, suggesting that with expansion comes an increased level of knowledge and better udder health. The improvement of SCC on larger herd size farms should be expected if we consider that a better economy should be accompanied by greater participation of consultants and veterinarians. However, studies carried out in Mexico (León-Galván et al., 2015; Manjarrez-Lopez et al., 2012) and other countries (Allore et al., 1997; Archer et al., 2013; Simensen et al., 2010; Whitaker et al., 2000) reported that an increase in herd size is generally associated with an increased SCC in raw milk. The results suggest that more attention is required to optimize udder health management as herds increase cow numbers on dairy farms in the studied region of Mexico.

There is limited research about the effects of water hardness on the bacterial count or SCC in milk. Elmoslemany et al. (2009) reported that herds with medium or high water hardness were 2.5 and 4.7 times, more likely than herds with lower hardness scores to have a high bacterial count in bulk tank milk. In another study, the same authors (Elmoslemany et al., 2010) observed that water quality influences bacterial counts in bulk tank milk, because on the farms that account with a water purification system (P<0.01) or water softener (P<0.1), the risk to have elevated bacterial count in the bulk tank is reduced. Authors explain that hard water can reduce the effectiveness of cleaning chemicals and may lead to the formation of biofilms or deposits on the milking system (Cords et al., 2001). Biofilms are self-aggregated, stratified microbial communities, constituted by one or several kinds of bacteria and a self-produced matrix of extracellular polymeric substances (Flemming et al., 2016). In this regard, Wang et al. (2019) stated that calcium and magnesium ions are important nutrients required by bacteria for growth and cell maintenance and play multifaceted roles both in the initial adhesion of bacteria and in the maturation of the biofilm. Therefore, the greater water hardness could also influence the environmental microorganisms on dairy farms.

Although the values of bacterial counts and SCC are generally related, this was not the case in the present study. Climatologic factors affect the incidence of various diseases in dairy cows, such as mastitis (Morse et al., 1988; Whitaker et al., 2000; Zeinhom et al., 2016), and therefore it is expected that milk SCC observed a seasonal pattern (Elmoslemany et al., 2010; Quintão et al., 2017; Olde Riekerink et al., 2007). The results obtained in the present study could be explained by the complexity of changes in milk microbiota influenced by the climatic conditions and the specific conditions of hygiene and farm management.

The microbiota of raw milk originates from multiple sources of contamination (udder, milking system, and farm environment), which initiate from the microbial load in milk from the udder and continuously increase as it flows to the bulk tank. Therefore, the final microbiota composition in the bulk tank is highly diverse (Parente et al., 2020). Furthermore, Porcellato et al. (2021) demonstrated that a persistent and farm-specific microbiota is observed in the bulk tank, but changes in composition within the same farm are mostly driven by bacterial genera associated with mastitis (e.g., *Staphylococcus* and *Streptococcus*), and correlated with the weather (temperature and humidity) but not with farm settings, such as milking system or herd size. On the other hand, although MBRT is a good general indicator of the level of bacterial contamination in milk, its results can be influenced by the composition of the microbiota and the variation in growth rate and the reducing action of different types of bacteria present in milk (Taponen et al., 2019; Karakashev et al., 2003; Rodrigues et al., 2017).

4.3. Bacteriological count in milking machine parts

The SPC is a useful indicator of the bacterial count. Although it does not measure the total bacterial count present, it does evaluate the number of aerobic and mesophilic bacteria present in the sample (Chambers, 2002). The microbial contamination of bulk tank milk will occur by bacterial contamination of teats and udder, contamination of milking equipment surfaces, or by the presence of mastitis-causing microorganisms from the udder (Murphy and Boor, 2000). Milking machine components are made of rubber, steel, or plastic, materials that easily form bacterial biofilms that can be a source of milk contamination, even if adequate hygiene and sanitation are applied (Teixeira et al., 2005). Thus, proper cleaning and disinfection of the milking machine parts will reduce bacterial cross-infection between cows, reducing bacterial counts and SCC in milk (Moroni et al., 2018).

Our results are in agreement with those of Bava et al. (2011), who conducted a study to describe the characteristics of cleaning procedures for milking equipment applied on intensive dairy farms in Italy. They reported that farms classified as high and low milk total bacteria count significantly differed both in terms of liners and receiver bacterial contamination of milking machine. The results of the present study demonstrate the importance of proper milking hygiene, as it will allow the reduction of bacterial counts and improve the quality of the milk produced.

As observed in the present study, different parts of the milking machine can vary in bacterial counts. In this regard, Richard (1981) indicated that most microorganisms are present in the joints and complex parts of the milking machine and not on the surface of the equipment. Therefore, the profound cleanliness of milking equipment is necessary to reduce the number of microorganisms present in the milking machine.

No research reports were found regarding the relationship between farm size and SPC on the milking machine. In the present study, the sanitary quality of the milk worsened as the herd size increased, and the same phenomenon was observed in the SPC of farms with 1 to 150 heads. However, the larger farms (≥151) obtained the lowest SCP in all milking machine parts. The above can be explained by better cleaning and disinfection of the milking machine on the larger dairy farms; however, better hygiene procedures were not necessarily carried out during the milking process on these farms.

Reinemann et al. (2013) stated that acid washing is necessary to dissolve inorganic mineral deposits, therefore improper washing allows mineral precipitation on the surface of the milking equipment, allowing bacterial adhesion and formation of biofilms. In addition, Ohnstad (2013) indicated that farm water hardness evaluation is necessary to wash the tank and the milking equipment efficiently. This procedure allows using the correct amount of detergent and frequency of acid wash. However, in this study, the water hardness did not alter the SPC on milking machine parts, probably because the water in the area was only soft or moderately hard, but hard water with values greater than 121 mg $CaCO_3$ L^{-1} (ppm) was never observed.

Elmoslemany et al. (2010) and Soler et al. (1995) reported that summer temperatures may allow microorganism growth on milking equipment, especially under improper sanitation of milking equipment. Our results match those of Bramley et al. (1984), who did not observe differences in the bacterial counts of rinsing of the milking machines obtained in summer and winter. The authors attribute the farm cleaning procedures as the main source of bacterial contamination and not to seasonal ambient conditions. In the present study, THI did not affect the bacterial counts in the different parts of the milking machine, which indicates that homogeneous cleaning and sanitation is generally carried out throughout the year in each particular dairy farm.

5. Conclusions

Milking hygiene practices, herd size, water hardness, and heat stress have a remarkable impact on milk quality and bacteriological count of the milking machine on dairy farms. Proper milking hygiene practices, softer water, and adequate cleaning and disinfection of the milking machine parts improve the milk quality. Although the larger herds showed better physicochemical characteristics of the milk, they also showed worse individual bacterial count and somatic cell count. Heat stress negatively affects the physicochemical and microbiological quality of the milk.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: P. Hernández-Briano. Formal analysis: P. Hernández-Briano. Funding acquisition: M.A. López-Carlos. Investigation: M.A. López-Carlos, J.I. Aguilera-Soto, F. Méndez-Llorente and C.F. Aréchiga-Flores. Methodology: M.A. López-Carlos, J.I. Aguilera-Soto and O. Carrillo-Muro. Visualization: C.A. Medina-Flores. Writing – original draft: M.A. López-Carlos. Writing – review & editing: P. Hernández-Briano.

Acknowledgments

The authors express their gratitude to the 53 small-scale dairy farmers involved in this study, to undergraduate student Angel Aguilar for his invaluable technical assistance during the experimental procedures, and to the staff at the milk processing plant that kindly provided the milk database. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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