

Factors that influence the hygienic-sanitary health of milk in two dairy farms

Aymara Luisa Valdivia-Avila¹<https://orcid.org/0000-0001-7399-3638>, Yasmery Rubio-Fontanills¹<https://orcid.org/0000-0002-9396-4487>, Yonel Pérez-Hernández¹<https://orcid.org/0000-0002-7245-5632>, Ileana Sarmenteros-Bon²<https://orcid.org/0000-0002-7275-1618>, José Vega-Alfonso³<https://orcid.org/0000-0001-9453-2582>, Angelina Mendoza-Rodríguez⁴<https://orcid.org/0000-0001-7433-8109>

¹Centro de Estudios Biotecnológicos, Universidad de Matanzas. Autopista a Varadero, km 3 ½, Matanzas, Cuba. ²Facultad de Ciencias Empresariales, Universidad de Matanzas. Autopista a Varadero, km 3 ½, Matanzas, Cuba. ³Facultad de Ciencias Agropecuarias, Universidad de Matanzas. Autopista a Varadero, km 3 ½, Matanzas, Cuba. ⁴Laboratorio de Bromatología de la Leche. Triunvirato, Matanzas. E-mail: aymara.valdivia@umcc.cu

Abstract

Objective: To characterize some factors which influence the hygienic-sanitary quality of milk in two dairy farms with different production conditions.

Materials and Methods: A survey was done with the workers of one farm, belonging to an animal husbandry enterprise of Matanzas province, to determine their knowledge about the factors that affect milk quality and the fulfillment of the procedures for obtaining and preserving milk. In addition, the quality kinetics of the milk stored in two dairy farms of the enterprise, was evaluated.

Results: The fat percentage varied between 3,70-3,96 and 3,87-4,30 %; while acidity was 0,141-0,151 and 0,140-0,153 % for dairy farm 1 and 2, respectively. Density in both dairy farms remained in the established indicators. The reduction time of methylene blue was the indicator that showed higher variations. In dairy farm 1 it reached a time of 5 h and 30 min. during all the samplings. Nevertheless, in 2 it was 3,5 h in the initial sampling, and then it decreased until reaching 40 min. at 24 h.

Conclusions: The hygienic-sanitary quality of the produced milk depends on the existing conditions in the dairy farms. Acidity, density, fat and total solids remained within the established criteria, in spite of differences in the dairy farms. The reduction time of methylene blue was the most affected indicator in dairy entity 2, according to the hygienic conditions under which milking was carried out.

Keywords: quality, milk hygiene, milking

Introduction

The world milk production in 2019 reached 852 million metric tons, increasing by 1,4 % with regards to the previous year. In this period, the exports increased discreetly, which was related to the growth of the demand of this product in emerging countries, and with the population increase (FAO, 2020).

Milk is a complete and balanced feedstuff, which provides a high content of nutrients related to its caloric value, for which its consumption is necessary since childhood until old age (Fernández-Fernández *et al.*, 2015). The quality and stability of this product are conditioned by several factors, such as infrastructure, hygiene, production, conservation and integral management (Martínez-Vasallo *et al.*, 2017).

Milk quality is determined by its physico-chemical characteristics and its hygienic and nutritional qualities. Management during milking and bad hygienic practices in its processing could affect the qualities of this product (Gwandu *et al.*,

2018). Reaching optimum levels of milk quality, which guarantee its innocuousness, is a challenge for farmers and distributors. This is due to the numerous risks it is exposed to, since its secretion until its reception by the consumers, as well as to economic factors that limit the availability of efficient equipment which allows to preserve it for its processing.

Milk quality indicators can be affected by inadequate milking practices and long intervals between obtaining it and the distribution to the population, especially when it is not refrigerated or no preservatives are used to maintain its quality (Martínez-Vasallo *et al.*, 2015).

From these problems, the objective of this work was to characterize some factors that influence the hygienic-sanitary quality of milk in two dairy farms with different production conditions.

Materials and Methods

Location. The study was conducted in two dairy farms belonging to an animal husbandry enterprise,

Received: January 24, 2020

Accepted: September 17, 2020

How to cite this paper: Valdivia-Avila, Aymara L.; Rubio-Fontanills, Yasmery; Pérez-Hernández, Y.; Sarmenteros-Bon, Ileana; Vega-Alfonso, J. & Mendoza-Rodríguez, Angelina. Factors that influence the hygienic-sanitary health of milk in two dairy farms. *Pastos y Forrajes*. 43 (3):xx-xx, 2020.

This is an open access article distributed in Attribution NonCommercial 4.0 International (CC BY-NC4.0) <https://creativecommons.org/licenses/by-nc/4.0/> The use, distribution or reproduction is allowed citing the original source and authors.

located in Matanzas province, Cuba, with an altitude of 93 m.a.s.l.

Climate characteristics. The trial was developed in March, 2018, with average temperature of 23 °C. The dairy farms in which the study was conducted have a brown soil with carbonate and slightly undulated relief (Departamento de Suelo y Fertilizantes, 1984).

Management and feeding system. In the two units, the same feeding and management regime was applied. Milking takes place twice, at 4:00 a.m. and at 4:00 p.m. The animals were supplied star grass [*Cynodon nlemfuensis* (Vanderyst)], commercial concentrate feed for dairy cow, forage of king grass CT-115 (*Cenchrus purpureus* Schumach. Morrone), ground sugarcane (*Saccharum officinarum* L.) and mineral salts *ad libitum*.

Characterization of the animals. The breed, milking cows, daily production per milking cow (L), average lactation number and average lactation days, were considered (table 1).

Experimental procedure. A questionnaire was applied to fifteen milkers from one of the farms belonging to this entity, who represent 45 % of the population. This sample was determined upon considering the exploratory study. From the surveyed people, ten were men and five, women. Three of them have ninth grade of schooling, eleven reached twelfth, and one is a qualified worker. The sample is representative of the population.

The survey comprised the following aspects: 1) factors that influence the hygienic-sanitary quality of milk, 2) activities involved in the milking routine, 3) solutions that are used in the dairy farms to make the final antiseptics of the teat, 4) knowledge about the exploitation time, pulsations and vacuum pressure with which the milking equipment of the dairy farms work.

A characterization was made of the two dairy farms of the enterprise with different production conditions (table 2). The experience of the personnel who works in the units, the characterization of the mechanized milking equipment, the evaluation of the execution of the milking routine and the equipment hygiene, according to the procedures described by Nieto *et al.* (2012), were considered. The milking routine and equipment hygiene were evaluated at three different moments in March, 2018.

The execution of the milking routine was evaluated from the criteria described below:

1. The operations that compose the milking routine, carried out in the established order and with the required quality, were evaluated as good.
2. Those routines in which the order of the operations was altered, and were not developed with the required quality (the strip cup was not used or the udder was not properly washed), although the final antiseptics of the teats was done, were evaluated as regular.
3. The routines that omitted any of the operations comprised in milking, and those that were not

Table 1. Characterization of the animals from each dairy farm.

| Dairy farm | Breed | Milking cows | Daily production per milking cow, L | Average lactation number | Average lactation days |
|------------|-------|--------------|-------------------------------------|--------------------------|------------------------|
| 1 | Mambí | 43 | 4 | 5 | 175 |
| 2 | Mambí | 37 | 4 | 6 | 162 |

Table 2. Characterization of the dairy farms where the research was conducted.

| Aspects to be characterized | Dairy farm | |
|--|---|---|
| | 1 | 2 |
| Work experience of the workers (years) | 15-20 | Less than one |
| Model of the milking equipment | De Laval | Alfa-Laval |
| Years of exploitation of the milking equipment | 2 | More than 20 |
| Evaluation of the execution of the milking routine and hygiene of the milking equipment. | Good | Regular |
| Conditions of the milk storage tanks | Conservation between 4 and 5 °C with agitation and airtight closing | Conservation at 16 °C, with no agitation and without airtight closing |

executed with the required quality, were evaluated as bad.

Criteria that were used to evaluate the hygiene of the milking equipment:

- When no filth was observed on the equipment or in the milk storage tank, and when the established steps for their hygienization were followed, according to the description made by Nieto *et al.* (2012), it was evaluated as good.
- When slight traces of filth were observed on the equipment and in the milk refrigeration tank, it was qualified as regular. In these cases, although the protocol established for hygienization was followed, no hot water was used for cleaning.
- Hygiene was evaluated as bad when filth could be observed on the equipment and in the milk storage tank and the steps established for their cleaning were not followed. Neither detergent nor hot water was used.

After characterizing the dairy farms object of study, the quality kinetics of the milk stored in such facilities was evaluated.

Evaluation of the quality kinetics of the milk stored in two dairy farms under different production conditions. Sampling was performed thrice on different days of March. In the two dairy farms 500 mL of milk were collected from the storage tank of this product. Sampling was performed after carrying out the morning milking (4:00 a.m.) and at the following times: 8:00 a.m. (0 h), 10:30 a.m. (2,5 h), 4:45 p.m. (8,75 h) and 8:00 a.m. of the next day (24 h).

The samples were preserved in sterile flasks, with rubber lids, at a temperature between 4 and 6 °C for their transference to the laboratory, and they were analyzed before the four hours after their arrival. The established determinations were made according to the Cuban norms: acidity (ONN, 2000), density with the use of a milk densimeter (ONN, 2006a), total solids (ONN, 2001a), reduction time of methylene blue (MBRT) (ONN, 2006b) and presence of mastitis through the California test (CMT), according to ONN (2001b). Fat was determined by using the Gerber method (Nielsen, 2003). Each sample analysis was carried out in triplicate.

Statistical analysis. In the data corresponding to the questionnaire simple mathematical tools were used, such as frequency tables and means; while in milk quality the adjustment of the data to normal distribution was determined by Kolmogorov-Smirnov test. Variance homogeneity was done according to Bartlett's test (Sigarroat, 1985). The data were processed through simple classification ANOVA. The

Student Newman-Keuls test ($p \leq 0,05$) was carried out to determine whether there were significant differences among them. All the analyses were processed with the package SPSS[®], version 15.0 for Windows.

Results and Discussion

The processing of the survey allowed to determine that there were differences in the technological conditions of the dairy farms, because they had milking equipment with different exploitation times (between 2 and 20 years). In addition, it was shown that the workers' knowledge about the application of the procedures of the milking routine was not homogeneous.

The surveyed personnel acknowledged as factors that can affect the hygienic-sanitary quality of milk deficient water quality, not performing the final antiseptics of the teat (sealing), bad hygiene and cleaning of the milking equipment and incidence of mastitis.

According to Brouset-Minaya *et al.* (2015), the results of the microbiological indicators of milk quality are directly related to the quality of the water used in the dairy farms, milking hygiene and washing the equipment and utensils that are in contact with this product. In addition, mastitis reduces the shelf life of milk, situation that can have a high impact on the population's health (Hernández-Barrera *et al.*, 2015).

In general, from the total surveyed workers, 18 % showed deficiencies in the knowledge of the milking routine execution. Among the main detected problems are the changes in the execution order of the operations and the omission of the final antiseptics of the teat. With regards to the time of equipment utilization for performing this activity, 100 % of the surveyed people knew it. Lack of knowledge about the number of pulsations the equipment should make per minute could be observed in 24,8 % of the workers; while the ones who did not know the pressure at which it should work represented 60 % of the total (table 3).

These results are similar to the ones obtained in a questionnaire applied to 20 cattle ranchers in Peru, where it was noted that 100 % of the surveyed workers did not carry out the milking routine completely, and only 5 % sealed the teats in post-milking (Alvarado-Yacchi *et al.*, 2019).

Evaluation of the milk quality kinetics. In dairy farm 1, the acidity values of the milk preserved during 24 h remained in a normal range, between 0,14 and 0,15 %, without showing differences among the samplings performed (table 4).

Table 3. Results of the survey application.

| Evaluated indicator | Percentage of correct answers |
|---|-------------------------------|
| Correct order of the activities comprised in the milking routine | 82 |
| Naming the solutions that are used in the dairy farm to perform the final antiseptics of the teat | |
| • Propolis | 93,4 |
| • Iodine | 6,6 |
| Time of exploitation of the milking equipment | 100 |
| Number of pulsations of the milking equipment of the dairy farm | 75,2 |
| Vacuum pressure of the milking equipment of the dairy farm | 40 |

Table 4. Indicators of milk quality during 24 h of storage.

| Indicator | Collection time, h | | | | | | | | | |
|-----------------------------|--------------------|--------------------|--------------------|--------------------|--------|---------------------|----------------------|----------------------|---------------------|--------|
| | Dairy farm 1 | | | | | Dairy farm 2 | | | | |
| | 0 | 2,5 | 8,75 | 24 | SE ± | 0 | 2,5 | 8,75 | 24 | SE ± |
| Acidity, % | 0,141 | 0,146 | 0,151 | 0,148 | 0,0040 | 0,1400 ^b | 0,1483 ^{ab} | 0,1467 ^{ab} | 0,1533 ^a | 0,0013 |
| Density, g·mL ⁻¹ | 1,030 | 1,031 | 1,031 | 1,031 | 0,0001 | 1,0302 | 1,0302 | 1,0297 | 1,0300 | 0,0001 |
| Fat, % | 3,70 ^b | 3,70 ^b | 3,96 ^a | 3,76 ^b | 0,0075 | 3,87 | 3,87 | 4,27 | 4,30 | 0,0274 |
| Total solids, % | 12,30 ^b | 12,29 ^b | 12,65 ^a | 12,41 ^b | 0,0159 | 12,32 ^b | 12,32 ^b | 12,68 ^{ab} | 12,80 ^a | 0,0091 |

Different letters indicate significant differences for $p \leq 0,05$, according to *Student Newman-Keuls* test.

Romero *et al.* (2018) referred that between 0,13 and 0,17 % are normal values for this indicator. This result is related to the ideal refrigeration conditions existing in this dairy farm, where the temperature is stable (between 4 and 5 °C), and agitation remains with similar values in all the spots of the stored volume. In addition, the execution of the milking routine and cleaning of the unit equipment were evaluated as good, which contributed to obtaining milk with lower microbial content and higher hygienic-sanitary quality (Martínez-Vasallo *et al.*, 2017).

In dairy farm 2, acidity varied between 0,14 and 0,15 %, for which it also remained within the indicators established for this product. Nevertheless, significant differences were observed among the samplings conducted at 24 h compared the time of 0 h, with trend to the increase of this indicator (table 4).

This result could have been related to the proliferation of microorganisms that produce acid during their growth, because the temperature at which the milk is stored in that unit is 16 °C more than the recommendation (COFRICO, 2019). This is in addition to the lack of agitation inside the storage tank.

On the other hand, the executions of the milking routine and hygiene of the equipment present in this dairy farm were not the ideal ones. In general,

malpractices of manipulation and hygiene, refrigeration at inadequate temperatures and prolonged storage can increase the number of microorganisms in the milk from its obtainment until reaching the consumer (Martínez-Vasallo *et al.*, 2017).

Milk density in the two units remained in the range (1,029-1,031 g mL⁻¹) recommended by Martínez-Vasallo *et al.* (2017). In addition, no differences were appreciated among the samplings. This indicator decreased proportionally with the percentage of added water (Artica-Malliquia, 2016), for which the result proved that the milk was adulterated. The addition of water causes changes in the compositional quality (Martínez-Vasallo *et al.*, 2015) and decreases the nutritional value of this foodstuff.

The fat percentage of the samples from the two dairy farms (3,70-4,30 %) was in correspondence with the genetic characteristics of the animals present in the units (Herrera-Angulo *et al.*, 2017), and is typical from the genotypes that are exploited in the Cuban farmer sector and of the feed they receive, fundamentally based on pastures and forages (Martínez-Vasallo *et al.*, 2015).

In similar studies, with crossbred animals between the breeds Holstein and Creole, a value was also obtained over 3 % fat (Guevara *et al.*, 2019).

In dairy farm 1, this indicator was higher (3,96 %) in the sampling performed at 8,75 h and differed

from the others. This result could have been related to the entrance of fresh milk, from the afternoon milking, to the storage tank. Herrera-Angulo *et al.* (2017) proved that there are differences in the fat content between the milk obtained in the morning milking and the one from the afternoon milking, which is related, in turn, to the fact that in the latter there was lower milk production. In the sampling at 24 h decrease was observed in this indicator (3,76 %), which could be linked to the increase in the number and activity of psychrophilic microorganisms, which grow at lower temperatures than 7 °C. These microorganisms produce proteolytic enzymes, lipases and thermostable phospholipases, many of which degrade some components of milk and deteriorate its quality and the quality of its derivatives (López and Barriga, 2016; Velázquez-Ordoñez *et al.*, 2019). In the analysis conducted on the milk samples of this dairy farm, a cross of mastitis was detected. According to Aguilar-Gálvez and Álvarez-Díaz (2019), this disease generates modifications in milk protein and lipid composition.

In dairy farm 2 no statistical differences were found, when comparing the collection times established for this essay.

In both dairy farms, the fat content at 24 h was good (Gallego-Castro *et al.*, 2017), which can be due to the fact that the storage period of milk did not exceed 72 h, time from which its refrigerated conservation is not recommended (López and Barriga, 2016).

The content of total solids in the milk samples of both units (12,3-12,6 and 12,3-12,8 % for dairy farms 1 and 2, respectively) remained within the values established as normal (Brousett-Minaya *et al.*, 2015).

This indicator behaved similarly to what occurred with the fat content in both dairy farms, which is associated again to the incorporation of the milk from the second milking (Herrera-Angulo *et al.*, 2017); while the decrease of total solids at 24 h, in dairy farm, could be ascribed to the degradation of the majority components of total solids (fat, protein and lactose), due to the increase of the proteolytic and lipolytic activity of psychrophilic microorganisms (Aguilar-Gálvez and Álvarez-Díaz, 2019).

In the samplings carried out in dairy farm 1, no variations were observed in the MBRT results, which reached a time of 5 h and 30 min. (figure 1), higher than the 4 h reported by Artica (2016) for the milk to be classified as of good quality.

These results were in correspondence with the execution of the milking routine, cleaning of the equipment in this unit, experience of the personnel who works in it, good management and storage conditions they have available, aspects on which the conservation of milk quality depends (Delgado-Calisaya, 2016).

In dairy farm 2 there was significant decrease of the MBRT in the different samplings after the initial collection, with the lower time of 40 min. at 24 h (figure 2).

The value reached in the first sampling (210 min. o 3,5 h) indicated that milk was not of good microbiological quality. Afterwards, the deterioration of this indicator increased, favored by the prolonged storage in the tank, at temperature higher than 4 °C. In most countries preserving milk at 4 °C is recommended to control efficaciously bacterial growth (Jurado-Gómez *et al.*, 2019).

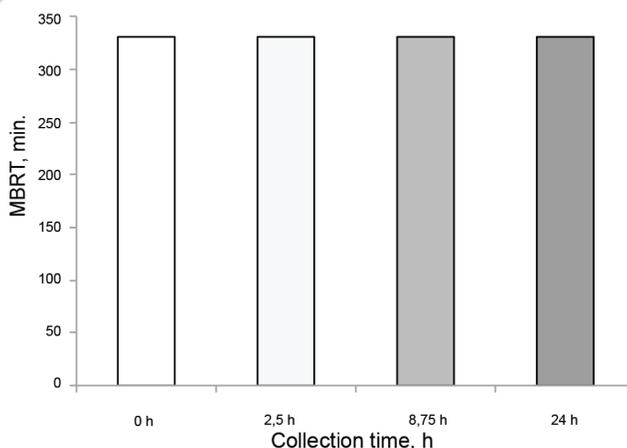


Figure 1. Methylene blue reduction time (MBRT) determined in milk samples from dairy farm 1.

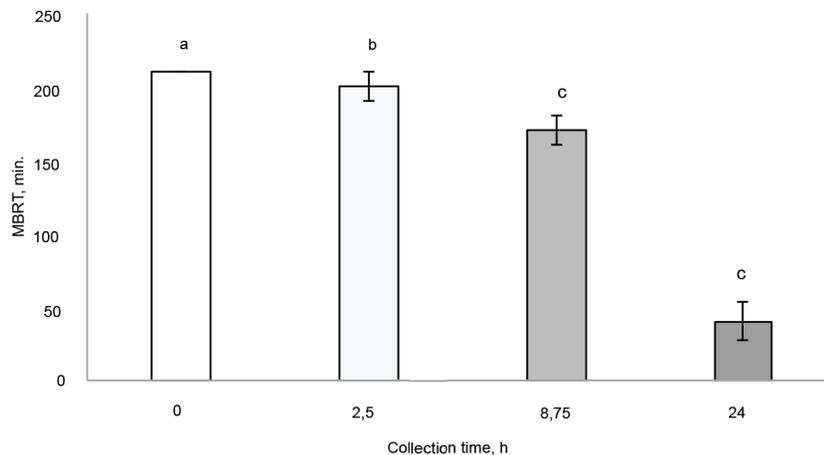


Figure 2. Methylene blue reduction time (MBRT) in milk samples from dairy farm 2. Different letters indicate differences among collection times for the same indicator, according to Student Newman-Keuls test ($p \leq 0,05$).

In Cuba, the payment system for milk quality takes as basis the MBRT. Bonus is applied for the increase of fat and penalization for low density and positivity to CMT (Martínez-Vasallo *et al.*, 2017). Nevertheless, in the MBRT it should be taken into consideration that psychrophilic and mastitis-causing microorganisms are very little reducers of methylene blue (Remón-Díaz *et al.*, 2019).

This negative result was influenced by the little experience of the workers who work in this dairy farm, which was observed in the visits made to the unit, where the execution of the milking routine and the equipment hygiene were evaluated as regular.

The study also showed the existence of mastitis in all the milk samplings carried out in dairy farm 1. Nevertheless, it was noticed that in it hygiene was fine and the execution of the milking routine was evaluated as good. In dairy farm 2, mastitis traces were found. These results are in correspondence with the deficiencies detected in milking management in this facility.

The results of the CMT tests, in both units, were within the permissible ones for the count of somatic cells, established by the International Dairy Federation in milk mixtures, which correspond to values from negative to weak positive (Remón-Díaz *et al.*, 2019).

Conclusions

The hygienic-sanitary quality of the produced milk depends on the existing conditions in each dairy farm, among which the human resources, milking equipment, management, hygiene and storage of this

product, stand out. Acidity, density, fat and total solids remained within the established values, in spite of the existing differences in the evaluated dairy farms. The MBRT was the most affected indicator in dairy farm 2, according to the hygienic conditions under which milking is carried out.

Acknowledgements

The authors thank the program of territorial CITMA projects for the contribution to funding this research.

Authors' contribution

- Aymara L. Valdivia-Avila. Design and setting up of the research, data analysis and interpretation, manuscript writing and revision.
- Yasmery Rubio-Fontanills. Data analysis and interpretation, manuscript writing and revision.
- Yunel Pérez-Hernández. Data analysis and interpretation.
- Ileana Sarmenteros-Bon. Survey design and result analysis, manuscript revision.
- José Vega-Alfonso. Research advisory and manuscript revision.
- Angelina Mendoza-Rodríguez. Analysis of the milk samples and data collection.

Conflict of interests

The authors declare that there are no conflicts of interests among them.

Bibliographic references

- Aguilar-Gálvez, F. & Álvarez-Díaz, C. A. *Mastitis bovina*. Machala, Ecuador: Ediciones UTMACH, 2019.

- Alvarado-Yacchi, Teresa H.; Vargas-Morán, J. R. & Vargas-Paredes, Ana C. Prácticas de manejo de ordeño, acopio y su importancia en la calidad de la leche, Matahuasi, Concepción y Apata, Junín (Perú). *Anales Científicos*. 80 (1):225-239, 2019.
- Artica-Malliquia, L. *Métodos para el análisis físico-químico de la leche y derivados lácteos*. Perú: Libros y Editoriales, TEIA, 2016.
- Brousett-Minaya, Magaly; Torres-Jiménez, Ana; Chambi-Rodríguez, A.; Mamani-Villalba, Bethy & Gutiérrez-Samata, H. Calidad físico-química, microbiológica y toxicológica de leche cruda en las cuencas ganaderas de la región Puno -Perú. *Sci. Agropecu.* 6 (3):165-176, 2015. DOI: <https://dx.doi.org/10.17268/sci.agropecu.2015.03.03>.
- COFRICO. *La refrigeración de los lácteos*. Newsletter. España. <https://www.cofrico.com/sectores/la-refrigeracion-de-los-lacteos/>, 2019.
- Delgado-Callisaya, P. A.; Parisaca, V.; Quispe, I.; Delgado, E. Jh. & Aduviri, M. Evaluación de la calidad de la leche cruda bovina (*Bos taurus*) en la Comunidad Mazo Cruz del Departamento de La Paz-Bolivia. *J. Selva Andina Anim. Sci.* 3 (1):43-48, 2016.
- Departamento de Suelo y Fertilizantes. *Mapa de suelo escala 1:25 000 del municipio de Matanzas*. La Habana: Departamento de Suelo y Fertilizantes, Ministerio de la Agricultura, 1984.
- FAO. *Dairy market review*. Rome: FAO. <http://www.fao.org/3/ca8341en/CA8341EN.pdf>, 2020.
- Fernández-Fernández, Elena; Martínez-Hernández, J. A.; Martínez-Suárez, V.; Moreno-Villares, J. M.; Collado-Yurrita, L. R.; Hernández-Cabria, Marta *et al.* Documento de Consenso: importancia nutricional y metabólica de la leche. *Nutr. Hosp.* 31 (1):92-101, 2015. DOI: <https://dx.doi.org/10.3305/nh.2015.31.1.8253>.
- Gallego-Castro, L. A.; Mahecha-Ledesma, Liliana & Angulo-Arizala, J. Producción, calidad de leche y beneficio-costo de suplementar vacas Holstein con *Tithonia diversifolia*. *Agron. Mesoam.* 28 (2):357-370, 2017. DOI: <https://doi.org/10.15517/ma.v28i2.25945>.
- Guevara, Deysi; Montero, Mayra; Rodríguez, Alinson; Valle, L. & Avilés, Diana. Calidad de la leche acopiada de pequeñas ganaderías de Cotopaxi, Ecuador. *Rev. investig. vet., Perú.* 30 (1):247-255, 2019. DOI: <http://dx.doi.org/10.15381/rivep.v30i1.15679>.
- Gwandu, S. H.; Nonga, H. E.; Mdegela, R. H.; Katakweba, A. S.; Suleiman, T. S. & Ryoba, R. Assessment of raw cow milk quality in small-holder dairy farms in Pemba Island Zanzibar, Tanzania. *Vet. Med. Int.*, 2018. DOI: <https://doi.org/10.1155/2018/1031726>.
- Hernández-Barrera, Jenny C.; Angarita-Merchán, Maritza; Benavides-Sánchez, D. A. & Prada-Quiroga, C. F. Agentes etiológicos de mastitis bovina en municipios con importante producción lechera del departamento de Boyacá. *Revista Investig. Salud Univ. Boyacá.* 2 (2):162-176, 2015.
- Herrera-Angulo, Ana M.; Mora-Luna, R. E.; Isea-Chávez, J. C. & Darghan, E. Producción y composición química de leches de vacas F1 x Cebú suplementadas con dos fuentes de nitrógeno no proteico. *Rev. Cient., FCV-LUZ.* 27 (2):119-130, 2017.
- Jurado-Gámez, H.; Muñoz-Domínguez, L.; Fajardo-Argoti, C.; Quitiaquez-Montenegro, D. & Insuasty-Santacruz, E. Evaluación de la calidad composicional, microbiológica y sanitaria de la leche cruda en el segundo tercio de lactancia en vacas lecheras. *Rev. Med. Vet. Zoot.* 66 (1):53-66. DOI: <http://dx.doi.org/10.15446/rfmvz.v66n1.79402>.
- López, A. L. & Barriga, D. *La leche composición y características*. Sevilla, España: Junta de Andalucía, 2016.
- Martínez-Vasallo, Ailin; Ribot-Enríquez, A.; Villoch-Cambas, Alejandra; Montes-de-Oca, Nivian ; Remón-Díaz, Dianys & Ponce-Ceballos, P. Calidad e inocuidad de la leche cruda en las condiciones actuales de Cuba. *Rev. Salud Anim.* 39 (1):51-61, 2017.
- Martínez-Vasallo, Ailin; Villoch-Cambas, Alejandra; Ribot-Enríquez, A.; Montes-de-Oca, Nivian; Riverón-Alemán, Yamilka & Ponce-Ceballos, P. Calidad e inocuidad en la leche cruda de una cadena de producción de una provincia occidental de Cuba. *Rev. Salud Anim.* 37 (2):79-85, 2015.
- Nielsen, Susanne, Ed. *Food analysis*. Berlin: Springer Science & Business Media, 2003.
- Nieto, D.; Berisso, R.; Demarchi, O. & Scala, E. *Manual de buenas prácticas de ganadería bovina para la agricultura familiar*. Buenos Aires: FAO, 2012.
- ONN. *Determinación de acidez*. NC 71. La Habana: Oficina Nacional de Normalización, 2000.
- ONN. *Determinación de la densidad o peso específico de la leche*. NC 119. La Habana: Oficina Nacional de Normalización, 2006a.
- ONN. *Leche, crema y leche evaporada. Determinación del contenido de sólidos totales*. NC 6731. La Habana: Oficina Nacional de Normalización, 2001a.
- ONN. *Prueba de California para el diagnóstico de mastitis*. NC 118. La Habana: Oficina Nacional de Normalización, 2001b.
- ONN. *Prueba de reducción del azul de metileno (TRAM)*. NC 282. La Habana: Oficina Nacional de Normalización, 2006b.

- Remón-Díaz, Dianys; González-Reyes, Dayaimi & Martínez-Vasallo, Ailin. Evaluación de la calidad higiénico-sanitaria de la leche cruda por métodos de flujo citométrico. *Rev. Salud Anim.* 41 (1):1-8, 2019.
- Romero, A.; Calderón, A. & Rodríguez, Virginia. Evaluación de la calidad de leches crudas en tres subregiones del departamento de Sucre, Colombia. *RECIA.* 10 (1):43-50, 2018. DOI: <https://doi.org/10.24188/recia.v10.n1.2018.630>.
- Sigarroa, A. *Biometría y diseño experimental*. La Habana: Editorial Pueblo y Educación, 1985.
- Velázquez-Ordoñez, V.; Valladares-Carranza, B.; Tenorio-Borroto, Esvieta; Talavera-Rojas, M.; Varela-Guerrero, J. A.; Acosta-Dibarrat, J. A. *et al.* Microbial contamination in milk quality and health risk of the consumers of raw milk and dairy products. In: G. Mozsik and M. Figler, eds. *Nutrition in health and disease. Our challenges now and forthcoming time*. London: IntechOpen, 2019. <https://doi.org/10.5772/intechopen.86182>.

PREPRINT