



EFFECT OF MILKING FREQUENCY ON THE HYGIENIC AND NUTRITIONALLY QUALITY OF RAW MILK IN THE MID-NORTHERN REGION OF ALGERIA

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Abstract

The objective of this study was to determine the effects of reduced milking frequency on nutritional and microbiological cow's milk quality. Once-daily milking on a standard diet (ODM); 3-times daily milking on a standard diet (TDM) cows were assigned from 06 February to 30 May. Cows were on average 218 d into lactation at the start of the trial, and all cows were managed similarly throughout the trial. Milk yields and gross milk composition of cows on all treatments were measured. Results of this study showed a significant decrease in the average milk nutritional quality in TDM as compared to ODM as fat, lactose, protein, casein rates by 15, 8, 3.9 and 5.5% respectively. In addition, TDM decreased the minerals and vitamins levels by 5.8% as compared to milk obtained by ODM. In addition, the application of TDM in the milking affects the milk physical properties by the decrease of the milk conductivity, density and freezing point by 3.29, 1.07 and 8.1% as compared to ODM milk. Moreover, the practice of TDM affects the milk bacteriological quality by the increase of total bacteria count (TBC) (from 11.6 to 13.8), total aerobic mesophilic flora (FMAT) (from 6,4 to 7,8); total coliforms (TCol) (from 4,6 to 5.7); fecal Coliforms (FCol) (from 3,2 to 4.2). Additionally, TDM increase significantly the Sulphite reducing Clostridium (SRC), molds and yeasts counts in milk as compared to ODM. In fact, the improvement of hygienic milking conditions through applying good hygiene practices and new techniques has made it possible to reduce the microbial load of the raw milk samples analyzed. Therefore, the reduction in milk production in ODM was completely compensated by an increase in the concentration of milk components as fat, lactose, protein, casein, minerals and vitamins rates as compared to TDM.

Keywords: bacterial flora; improved conditions; raw milk. wholesomeness.



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1. Introduction

Frequency of daily milkings is of great importance in determining milk yield in dairy animals. Once daily milking of dairy cows is practiced in some countries either in early lactation to reduce metabolic stress or in late lactation to improve quality of farming life (Salama et al., 2003). 3-times daily milking implies that cows are milked automatically according to their diurnal routine. Previous studies reported that more frequent milking requires more variable costs (labor, utilities, milking supplies, and additional feed costs). In Spain, and other countries where the goat production systems are extensive or semi-extensive, high milking frequency is a major cost for dairy goat farms. Under these conditions, lower milking frequency increases labor productivity and reduces milk storage risks. However, for infrequent milking to be a practical strategy, it should have no long-term deleterious effects on milk yield or milk quality. Additionally, the system offers more flexible working hours for farmers and, thereby, a possibility of a better social life; but, the effect on the nutritional and microbiological quality of milk and the sustainability of production remains unanswered. In fact, the treatment of the milk can influence the quality leading to microbiological and compositional changes and consequent yield loss and lower quality of dairy products. Low-quality milk is associated to the proceedings applied to milking, mammary gland health and herd management in addition to the inadequate use of milking equipment. Availability of labour and time spent at milking are major issues on dairy farms. Reducing milking frequency from twice daily (TDM) to once daily (ODM) may offer a way of reducing labour. The food industry is under scrutiny from all sections of society Consumers care and require information about the quality and safety of products and how they are made (Flores-Miyamoto et al., 2014); the consumption of raw milk has become the main actor in the transmission of diseases and germs. In fact, Microbiological contamination of milk is an important issue because pathogens can affect food safety, and spoilage microorganisms can limit shelf life and affect quality or yield of milk products.(Flores-Miyamoto et al., 2014). The other common causative agents in food-borne disease outbreaks are bacterial toxins produced by *Bacillus*, *Clostridium* and *Staphylococcus* spp.(Zastempowska et al., 2016); The prevalence of foodborne pathogens in raw cow milk varies, but their presence has been demonstrated in many surveys and foodborne infections have been repeatedly reported for *Campylobacter*, *Salmonella* spp. and human pathogenic verocytotoxin-producing *Escherichia coli*. In industrialized countries, milk-borne and milk product-borne outbreaks represent 2–6% of the bacterial foodborne outbreaks (Claeys et al., 2013),

The policy of increasing milk production by the Algerian states has gone through the improvement of the quality of milk which has been obtained by the establishment of payment for milk according to its composition (butyrous and protein content) as well as its hygienic and sanitary quality (bacteriological and sanitary quality of raw milk, somatic cells, freezing point, inhibiting substances). Farmers whose milk does not meet all of these requirements may be rejected by the dairy processor, depending on company or government regulations; Raw milk is promoted as 'health food' despite the fact that it poses a realistic microbiological hazard for the consumers' health or life. Milk and dairy products significantly contribute to intake of calcium, phosphorus, riboflavin, vitamin B12, potassium, vitamin A, zinc, magnesium and proteins(Vranješ et al., 2015).

Samples preparation and dilutions for microbiological analysis were performed according to the recommendations of the International Dairy Federation (IDF, 1991).

Each sample of milk taken during the investigation period was subject to the following microbiological analysis. Microbiological analyzes performed are Total aerobic mesophilic flora (FMAT), total coliforms and faecal coliforms (CT,CF), staphylococcus aureus, Clostridium reducing sulphite, Salmonella, Yeasts and molds. The total aerobic mesophilic flora (FMAT) is counted on the PCA agar after inoculation in depth and incubation for 24 hours at 30°C. Coliforms are sought by seeding in depth on lactose bile agar with neutral red and crystal violet (VRBL)(Tir et al., 2015), incubated for 24 hours at 37°C for total coliforms and at 44°C for faecal coliforms. Enumeration of Staphylococcus aureus is carried out by surface seeding on solid selective medium (Baird Parker); Incubation takes place at 37°C for 24 to 48 hours(Hamiroune et al., 2016).Sulfite-reducing Clostridium spores are sought on appropriate media (meat and yeast extract medium) in the presence of sodium sulphite and iron alum, incubation takes place at 37°C for 48 hours(Tir et al., 2015). Heat treatment preliminary 10 min at 80°C is necessary in order to activate the spores of the clostridia the search for salmonella has been carried out in three steps, pre-enrichment in buffered peptone water (EPT) 24 hours at 37°C, followed by enrichment on Bouillon Selinity-cysteine (SFB) 24 hours at 37°C, then counting and isolation were carried out on Hecktoen medium after incubation for 24 hours at 37°C. Yeasts and molds are counted on OGA medium agar and incubated 48 to 72 hours at 25-30°C. The analyses were carried out in triplicate, and microbiological data were transformed by logarithm base 10 (log) and expressed in the descriptive statistic as mean and standard deviation (Std).

2.5. Data handling and analysis

The results of TBC, coliform count, , aerobic spore count (mesophilic), were expressed as CFU/ml and Cell/ml respectively, in addition, trans- formed into base-10 logarithm. Data collected were entered and managed in an Epi Info database (CDC, version 6.04). Descriptive statistics were then computed for different variables. Continuous and the proportions of categorical variables were computed and compared for statistical significance by Chi-square test at a critical probability of $P < 0.05$. Statistical analyses were carried out using SPSS software, version 22.

3. Result and duscution

3.1. Milk yield in ODM and TDM systems

Results of this study showed that ODM system caused a significant decrease in cow production milk by 25% as compared to TDM system.

3.2. Physicochemical and nutritional qualities of raw milk in ODM and TDM systems

This study showed that TDM system affects the milk indices as pH value, the average of the freezing point, the density and the conductivity as compared to ODM system. In addition, increasing the frequency of milking cows decreases the nutritional quality of milk, evidenced by a significant decrease in milk fat, lactose, protein, casein and minerals-vitamins rates by 13, 15, 3.9, 5.3 and 14.4% in TDM as compared to ODM.

3.3. Microbiological quality of raw milk in ODM and TDM systems

Results of this study showed that the practice of ODM reduced the bacteria pathogens count in milk such as TBC, FMAT, TCol and FCol by 15.9, 17.9, 19.2 and 23.8 respectively as compared to the practice of TDM. In addition, TDM increase significantly the Sulphite reducing Clostridium (SRC), mods and yeasts counts in milk as compared to ODM.

Discussion

Several studies have shown that increases milking frequency, favoring an increase in milk yield in the order of 5 to 10% compared with the fixed-frequency regimen of daily milkings (Bogucki et al., 2017; Hogenboom et al., 2019). Results of this study demonstrate that the practice of TDM increase significantly the milk yield by 25% as compared to ODM. Similarly, Knight et al. (1992) have reported that milk production increased by 14% after milking 4 times by compared with twice daily milking. In addition, Hillerton et al. (1990) have demonstrated that increase in milking frequency caused an increase in milk production by 10.4%. In a long-term study, Klei et al. (1997) reported that fat yield produced by cows milked three times daily was 4.7% greater than that from cows milked once daily. This study evaluated the effect of ODM and TDM on milk physicochemical indices. Results of this study revealed that the acidity of ODM or TDM raw milk is 6.69 and 6.75 respectively. These values samples were moderately acceptable with an average pH of 6, 7. In fact, the samples have a pH outside the fresh milk standards which are prescribed between 6.6 to 6.8. This acidity can be natural due to the stage of lactation, the casein content, mineral salts and ion content, or it may be due to hygienic conditions during the milking, the total microbial flora and its metabolic activity (Bousbia et al., 2018; Hameed et al., 2021; Kailasapathy, 2015). The decrease in pH for milk obtained by ODM is probably due to the richness of this milk in fatty acids, vitamins and minerals compared to milk obtained by TDM.

The average of the freezing point of ODM milk is -0.53°C , which is significantly lower than that of milk obtained by TDM (-0.74°C). This shows that milk obtained by ODM is rich in soluble substances such as vitamins, proteins, minerals...compared to milk obtained by TDM and therefore better nutritionally (Ahmad et al., 2013; Dantas et al., 2021; Kailasapathy, 2015).

In this study, the ODM milk density is 1036, while that of TDM is 1025. this decrease is due to the reduction in milk solids content as protein and fat (Ayadi et al., 2009).

The conductivity values ranged from 3.45 to 3.34 $\mu\text{S cm}^{-1}$; this says that the frequency of milking decreases the rate of calcium and other minerals as showed by our study. Accordingly, Hernández-Castellano et al., (2023) and Loiselle et al., (2009) reported that milking 4 times a day in early lactation reduced the decreases the nutritional quality of milk by reducing the rate of nutrients. Lacy-Hulbert et al., (1999) reported that increasing milking to 4 times a day during the first 3 weeks of lactation increases milk production and decreases significantly the Ca, Na and K levels in milk.

Results of this study showed that the increases of the milking frequency reduced significantly the milk composition as proteins, lipid, casein, vitamins and minerals... reflected in findings similar to those described previously. In ruminants, plasma glucose concentrations decrease because of the high energy demands associated with milking and the harnessing of glucose for the production of lactose. Low glucose levels can also be a consequence of decreased appetite and consequently

decrease in protein, casein, lipid, vitamin biosynthesis and secretion (O'Brien et al., 2002). The present study clearly showed that the nutritional quality of milk decreased in TDM as compared to ODM; which is in agreement with the findings of previous studies (Hernández-Castellano et al., 2023; Lacy-Hulbert et al., 1999; Loiselle et al., 2009; O'Brien et al., 2002).

Collection and frequency times, Storing during and temperature of raw milk from collection until reception in dairy industries are definitely one of the key factors helping to preserve milk quality. The total bacterial count is used as an important indicator of microbial quality of raw milk. The result of microbial quality of raw milk as indicated in Table 2 shows that total bacterial counts in the ODM were significantly different from TDM. In fact, we showed that increasing the frequency of milking increases the risk of contamination of the milk with pathogenic bacteria such as TBC, yeasts, molds, FMAT counts. In fact, reducing milking frequency minimize milk temperature fluctuation from milking (37°C) to storage (8°C) and risk of milk contamination (Reguillo et al., 2018). As previously observed, once-daily milking led to more time spent feeding, compared with cows milked twice daily and consequently milk of good microbiological quality (Jensen et al., 2023). This was in agreement with the finding of other previous reported whose reports comparatively that increases in milking frequency reduced feeding level reduces the amount of nutrients available to the udder which, in turn, decreases milk synthesis and quality (Hernández-Castellano et al., 2023). This finding agrees with results from where showed that milk quality have been affected by milking frequency. In dairy cows, as in other animals, stressful conditions by increases in milking frequency affect the milk quality (Hernández-Castellano et al., 2023). Whereas milking frequency affect milk production and quality (van den Borne et al., 2022). Therefore, milk quality and production, to a certain extent, is negatively affected by milking frequency. In fact, Once-daily milking partially alleviates the negative energy balance that occurs immediately postpartum or during periods of energy deficits and can improve milk quality.

References

- Ahmad, S., Anjum, F. M., Huma, N., Sameen, A., & Zahoor, T. (2013). Composition and physico-chemical characteristics of buffalo milk with particular emphasis on lipids, proteins, minerals, enzymes and vitamins. *J Anim Plant Sci*, 23(Suppl 1), 62–74.
- Ayadi, M., Hammadi, M., Khorchani, T., Barmat, A., Atigui, M., & Caja, G. (2009). Effects of milking interval and cisternal udder evaluation in Tunisian Maghrebi dairy dromedaries (*Camelus dromedarius* L.). *Journal of Dairy Science*, 92(4), 1452–1459.
- Bogucki, M., Sawa, A., & Kuropatwińska, I. (2017). Association of automatic milking systems milking frequency in primiparous and multiparous cows with their yield and milkability. *Acta Agriculturae Scandinavica, Section A—Animal Science*, 67(1–2), 66–70.
- Bousbia, A., Boudalia, S., Gueroui, Y., Belaize, B., Meguelati, S., Amrouchi, M., Ghebache, R., Belkheir, B., & Benidir, M. (2018). Nutritional and hygienic quality of raw milk intended for consumption in the region of Guelma, Algeria. *Asian Journal of Dairy and Food Research*, 37(3), 192–196.
- Dantas, A., Quinteros, G. J., Darvishvand, S. Y., Blanco, M., Hernandez, E., Prudencio, E. S., & Samsuri, S. (2021). The combined use of progressive and block freeze concentration in

- lactose-free milk: effect of process parameters and influence on the content of carbohydrates and proteins. *Journal of Food Process Engineering*, 44(11), e13867.
- Flores-Miyamoto, A., Reij, M. W., & Velthuis, A. G. J. (2014). Do farm audits improve milk quality? *Journal of Dairy Science*, 97(1), 1–9. <https://doi.org/10.3168/jds.2012-6228>
- Hameed, A., Anjum, F. M., Rehman, Z., Akhtar, S., Faraz, A., Hussain, M., & Ismail, A. (2021). Compositional and mineral profile of Sahiwal cow milk at various lactation stages as influenced by oxytocin administration. *Pakistan J. Zool*, 2021, 1–7.
- Hamiroune, M., Berber, A., & Boubekour, S. (2016). Évaluation de la qualité bactériologique du lait cru bovin à divers stades de la chaîne de production laitière dans des fermes en Algérie. *OIE Revue Scientifique et Technique*, 35(3), 925–946. <https://doi.org/10.20506/rst.35.3.2580>
- Hernández-Castellano, L. E., Sørensen, M. T., Foldager, L., Herskin, M. S., Gross, J. J., Bruckmaier, R. M., & Larsen, M. (2023). Effects of feeding level, milking frequency, and single injection of cabergoline on blood metabolites, hormones, and minerals around dry-off in dairy cows. *Journal of Dairy Science*, 106(4), 2919–2932.
- Hogenboom, J. A., Pellegrino, L., Sandrucci, A., Rosi, V., & D’Incecco, P. (2019). Invited review: Hygienic quality, composition, and technological performance of raw milk obtained by robotic milking of cows. *Journal of Dairy Science*, 102(9), 7640–7654.
- Jensen, M. B., Franchi, G. A., Larsen, M., & Herskin, M. S. (2023). Effects of feeding level and milking frequency on behavior of dairy cows before dry-off. *Journal of Dairy Science*, 106(4), 2739–2749. <https://doi.org/https://doi.org/10.3168/jds.2022-22284>
- Kailasapathy, K. (2015). Chemical composition, physical, and functional properties of milk and milk ingredients. *Dairy Processing and Quality Assurance*, 77–105.
- Lacy-Hulbert, S. J., Woolford, M. W., Nicholas, G. D., Prosser, C. G., & Stelwagen, K. (1999). Effect of milking frequency and pasture intake on milk yield and composition of late lactation cows. *Journal of Dairy Science*, 82(6), 1232–1239.
- Loiselle, M. C., Ster, C., Talbot, B. G., Zhao, X., Wagner, G. F., Boisclair, Y. R., & Lacasse, P. (2009). Impact of postpartum milking frequency on the immune system and the blood metabolite concentration of dairy cows. *Journal of Dairy Science*, 92(5), 1900–1912.
- O’BRIEN, B., Ryan, G., Meaney, W. J., McDONAGH, D., & Kelly, A. (2002). Effect of frequency of milking on yield, composition and processing quality of milk. *Journal of Dairy Research*, 69(3), 367–374.
- Reguillo, L., Hernández, M., Barrientos, E., Perez-Rodriguez, F., & Valero, A. (2018). Evaluation of the Influence of Frequency of Milk Collection and Milking Dayshift on the Microbiological Quality of Raw Milk. *Journal of Food Quality*, 2018, 1306107. <https://doi.org/10.1155/2018/1306107>
- Salama, A. A. K., Such, X., Caja, G., Rovai, M., Casals, R., Albanell, E., Marín, M. P., & Martí, A. (2003). Effects of Once Versus Twice Daily Milking Throughout Lactation on Milk Yield and Milk Composition in Dairy Goats. *Journal of Dairy Science*, 86(5), 1673–1680. [https://doi.org/https://doi.org/10.3168/jds.S0022-0302\(03\)73753-9](https://doi.org/https://doi.org/10.3168/jds.S0022-0302(03)73753-9)
- Tir, E., Heddar, M., Bouklila, N., & Bounoua, S. (2015). Etude De La Qualité Physico-Chimique

- Et Microbiologique De Laites Crus De Vache Dans Deux Fermes De La Wilaya De Tissemsilt “Algérie.” *January 2015*, 26. <https://doi.org/10.54246/1548-008-002-030>
- van den Borne, B. H. P., Di Giacinto Villalobos, A. M., & Hogeveen, H. (2022). Disentangling the relationships between lameness, milking frequency and milk production in Dutch dairy herds using an automatic milking system. *Preventive Veterinary Medicine*, 208, 105733. <https://doi.org/https://doi.org/10.1016/j.prevetmed.2022.105733>
- Zastempowska, E., Grajewski, J., & Twarużek, M. (2016). Food-Borne Pathogens and Contaminants in Raw Milk – A Review. *Annals of Animal Science*, 16(3), 623–639. <https://doi.org/10.1515/aoas-2015-0089>

Table 1:

| | N | ODM | TDM |
|------------------------|----|-------|-------|
| milk yield | 40 | 13.1 | 9.7 |
| Freezing point (°C) | 40 | -0.53 | -0.74 |
| Density | 40 | 1036 | 1025 |
| Conductivity (µS cm-1) | 40 | 3.45 | 3.34 |
| pH | 40 | 6.69 | 6.75 |
| Fat | 40 | 3.31 | 2.87 |
| Lactose | 40 | 4.17 | 3.51 |
| Protein | 40 | 2.79 | 2.68 |
| Casein | 40 | 3.97 | 3.76 |
| Minerals and Vitamins | 40 | 6.53 | 5.59 |

Table 2:

Row milk microbiological quality in ODM and TDM system. Results expressed in log 10cfu/mL. Results expressed by Mean \pm SD

| | ODM | | | TDM | | | P value |
|-------------------------------|------|------|------------------|------|------|------------------|---------|
| | Min | Max | Mean \pm SD | Min | Max | Mean \pm SD | |
| TBC | 4.2 | 27.3 | 11.69 \pm 0,43 | 5.9 | 31.7 | 13.80 \pm 0,74 | |
| FMAT (10⁵) | 2.7 | 12.4 | 6,42 \pm 0,43 | 3.1 | 14.4 | 7,81 \pm 0,43 | |
| T.Col (10⁵) | 0 | 9.7 | 4,6 \pm 0,41 | 0 | 11.3 | 5.7 \pm 0,41 | |
| F.Col (10⁵) | 1.63 | 7.1 | 3,29 \pm 0,47 | 1.91 | 8.7 | 4.29 \pm 0,47 | |
| S.R.C | 0 | 73.4 | 17.1 \pm 1.3 | 0 | 74.7 | 19.3 \pm 1.3 | |
| Molds | 0 | 6.9 | 3,23 \pm 0,33 | 0 | 8.1 | 4.1 \pm 0,33 | |
| Yeasts | 2.1 | 9.3 | 4,58 \pm 0,29 | 2.7 | 10.4 | 5.22 \pm 0,29 | |