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ORIGINAL ARTICLE

Comparison of the Contents of Calcium, Magnesium and some Environmental Microorganisms in Cow Feed and Raw Milk Produced in Cameroon

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Abstract

A chemical and microbiological study was conducted on twelve dairy farms in the West Region of Cameroon (Central Africa) during the period 2019-2020. Two hundred and sixteen samples of drinking water, green fodder consumed by dairy cows and raw milk produced were analyzed. Calcium and Magnesium contents were assessed by volumetry and atomic absorption spectrometry methods. Lactobacilli, yeasts and molds were isolated on solid culture media by surface spreading techniques. The average Ca^{2+} concentrations were 42 mg/l, 1674 mg/kg and 1215 mg/l, in the drinking water, green fodder and milk produced respectively. That of Mg^{2+} was 26 mg/l, 1913 mg/kg and 160 mg/l respectively. The Ca^{2+} content of the raw milk produced was not significantly different ($p>0.05$) from that of the green fodder consumed by the cows. However, the drinking water had a significantly lower Ca^{2+} and Mg^{2+} content than the green fodder consumed and the raw milk produced ($p<0.05$). The average concentration of microorganisms in drinking water, green fodder and produced milk was $7.3x10^6$ CFU/ml, $3.7x10^7$ CFU/g and $1.6x10^7$ CFU/ml for lactobacilli, $7.4x10^6$ CFU/ml, $6.3x10^7$ CFU/g and $1x10^7$ CFU/ml for yeasts, and $1.7x10^4$ CFU/ml, $5.7x10^5$ CFU/g and $1.3x10^4$ CFU/ml for molds respectively. The cell abundance dynamics of lactobacilli in green fodder consumed by the cows was not significantly different from that in the milk produced ($p>0.05$). Similarly, the yeasts and molds abundance dynamics in the drinking water of the cows were not significantly different from those in the milk produced ($p>0.05$).

Practical application

The mineral content of milk depends mainly on the mineral concentration of the feed consumed by the cows. Green fodder and drinking water are potential sources of microorganisms that can have an impact on the microbiota of the raw milk produced. It is therefore essential to treat the water before the animals are drunk and to improve fodder production in the region.

Key words: Drinking water, Green fodder, Calcium and Magnesium content, Microbial abundance, Raw milk quality

1. Introduction

Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) are alkaline minerals that play an important role in the human system. Milk and milk products are rich sources of both macronutrients, especially Ca^{2+}. The concentrations of Ca^{2+} and Mg^{2+} are about 4 times higher in cow milk compared to human milk.

The concentrations of Ca^{2+} and Mg^{2+} in cow milk are closely related (Nogalska *et al.,* 2017). These ions play an important role in the structure and stability of casein micelles in milk (Zwierzchowski & Ametaj, 2019). Furthermore, due to its high water activity and the presence of key nutrients, cow's milk is an excellent nutritional matrix for the growth of a large number of microorganisms

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(Hassan & Frank, 2011). Raw milk can be contaminated with so-called environmental microorganisms such as lactobacilli, yeasts and molds (Ndahetuye *et al.,* 2020). These microorganisms are present in a wide variety of environments, including soil (most often associated with the rhizosphere), plants (especially decaying plant material) and animals (especially the oral cavity, intestinal tract and vagina) (Kaouche-Adjlane & Mati, 2017). Furthermore, the dairy sector depends on natural resources such as land, water. The production of raw cow's milk requires large amounts of fodder and water. The latter could have a significant influence on the mineral and microbiological qualities of the milk produced. Green fodder such as grass and crop residues constitute the basis of the diet of dairy cattle. Water is a vital nutrient involved in many essential physiological functions such as digestion, absorption, thermoregulation and waste disposal. Its quality is a key parameter in the health and productivity of livestock. On dairy farms, water is even more important as cows need to have good milk production and water is the basis of milk. To produce 1 kg of milk, a cow needs to drink about 0.8 liter of water (Giri *et al.,* 2020).

In Cameroon, in some regions, milk is a popular commodity. However, its production faces many problems. Breeders feed the herd with collected fodder and water them with any water (Kouamo *et al.,* 2017; Maïworé *et al.,* 2018). These feeding practices can result in milk of questionable quality. It is within this framework that this work aim as at making a comparative approach of the mineral and microbiological characteristics of water and green fodder consumed by cows and of raw milk from some dairy farms in the West Region of Cameroon (Central Africa).

2. Materials and Methods

2.1 Selection and mapping of study dairy farms

The selected sample sites are defined as geographically different study locations in terms of latitude, longitude, altitude and soil type. Vegetation is largely determined by soil type and water quality is influenced by the nature of the soil through which it flows. Twelve dairy farms were selected (F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12) (Figure 1).

2.2 Sampling

Sampling was carried out in a random and simple manner and consisted of taking samples of drinking water, green fodder and raw milk produced during the months of December 2019 and February, April, June, August, October 2020. The choice of this period made it possible to complete the two seasons of the year (the rainy season and the dry season). The choice of this period allowed the seasonal cycle of the year to be completed. A total of 72 water samples, 72 green fodder samples and 72 raw milk samples were collected.

The water resource consisted of well water (7 farms) and river water (5 farms). The water samples were collected in 500 ml sterile glass bottles. These bottles were filled 3/4 with water to allow homogenization before plating. These vials were first rinsed in the field with the water to be analyzed, then filled to the brim and capped to limit outgassing (Rodier *et al.,* 2016). All samples were then transported to the laboratory kept in a clod room for analysis.

The basic ration for dairy cattle in the region was green fodder of grasses and legumes harvested from fallow land and natural or cultivated pastures. This was served to the animals in its entirety for some and cut into small fragments for others. Single samples were combined into a bulk sample.

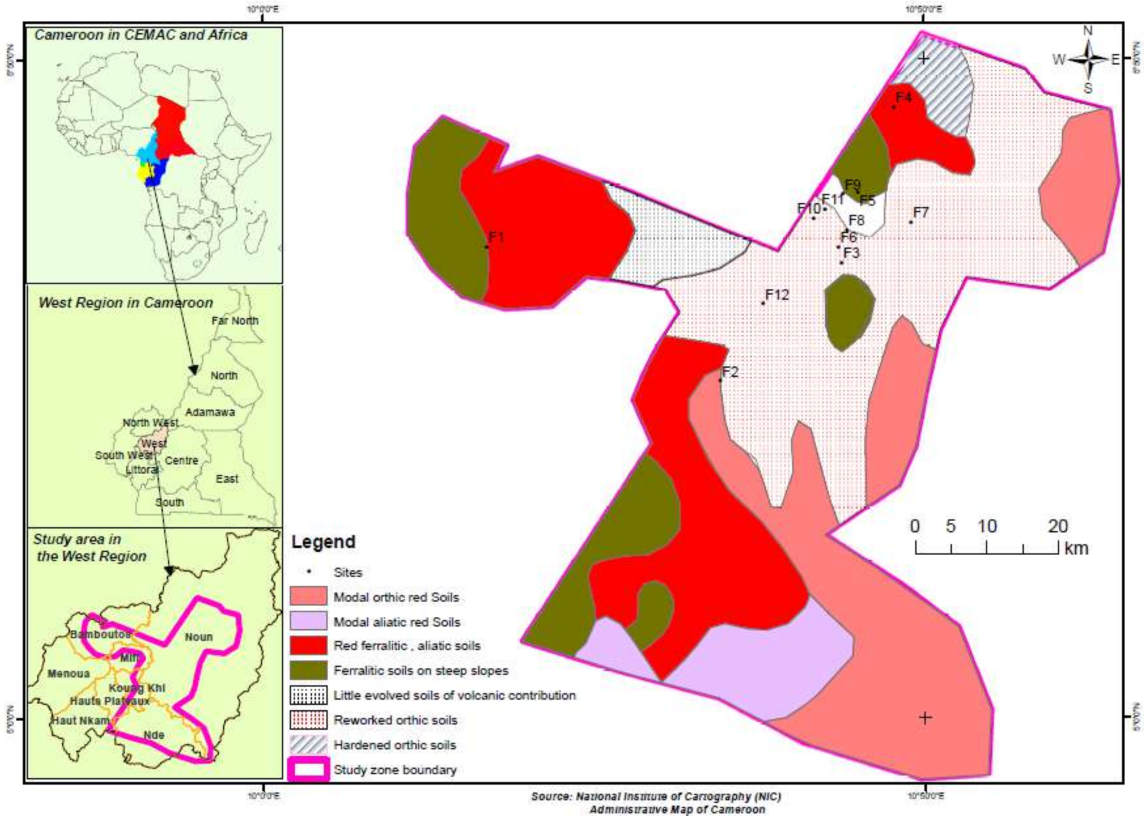

Figure 1: Soil map of the sampling sites

This was then mixed as homogeneously as possible and spread uniformly on a clean surface. The fodder was shortened to a maximum stem length of 5 cm. The representative sample was taken from the bulk sample by re-sampling approximately 3 single samples uniformly distributed. Each sample was placed in tightly sealed plastic bags, from which the air was previously evacuated to protect the sample from air, light and moisture (Glauser, 2007). All labeled samples were transported to the laboratory in a refrigerated box for analysis.

Aseptic collection of milk samples from the farm was carried out in accordance with the National Mammalian Council guidelines (NMC, 2017). Milk samples were collected after washing and drying of the teat end. The first milk stream (approximately 250 ml) was discarded and a tube was filled with the milk that was subsequently milked. All milk samples were collected in the morning. At each arrival, approximately 250 ml of raw milk was collected in sterile glass bottles and transported at temperatures of 4-8°C in coolers for analysis within a maximum of 12 hours after collection (Rodier *et al.,* 2016).

2.3 Chemical analysis

The Calcium content of the drinking water and green fodder consumed by the cows was evaluated by complexometry according to the AFNOR NFT 90- 003 standards using the Complexon III (Rodier *et al.,* 2016). 50 ml of drinking water sample and 1g of ground green fodder suspended in 50 ml of distilled water. 1 ml

Potassium Cyanide (KCN) 1 %, 1 ml Sodium Hydroxide (NaOH 2N) and a pinch of Pahern's reagent, color indicator H-H-S-N-N [2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthyl-azo)-3-naphthenic acid] were added respectively. The pink colored mixture obtained was then titrated with Idranal III until the appearance of a clear blue color. The results expressed in mg/l for drinking water and in mg/kg green fodder were calculated by the formula:

$$Ca^{2+} = (\text{burette descent (ml)}) \times 20$$

Magnesium contents of drinking water and green fodder consumed by the cows were evaluated by volumetric (Rodier *et al.,* 2016). To 50 ml of drinking water sample and 1g of ground green fodder suspended in 50 ml of distilled water, 1 ml of Potassium Cyanide (KCN 1 %), 1 ml of ammonia buffer and a pinch of Eriochrome T black were respectively. A violet colored solution was obtained which, when assayed with Idranal III, turned solid blue. The results expressed in mg/l for drinking water and in mg/kg green fodder were obtained by the formula:

$$Mg^{2+} = (\text{burette descent (ml)}) \times 12$$

The determination of Calcium and Magnesium contents of the raw milk produced was carried out by atomic absorption spectrometry on a Techtron 1200 instrument (AFNOR, 2007). Approximately 10 ml of milk sample was diluted with approximately 100 ml of distilled water acidified with 10 ml of hydrochloric acid (HCl) in order to promote the solubilization of minerals. The solution was made up to 500 ml with distilled water. This stock solution was diluted 10 times with a solution of a Lanthanum salt (500 ppm La). The reading was taken at the following wavelengths: Ca^{2+} (422.7 nm), Mg^{2+} (285.1 nm), and the results were expressed in mg/l. For each element, the corresponding calibration curve was plotted.

2.4 Microbiological analyses

The microorganisms isolated were lactobacilli, yeasts and molds. For each water and milk sample, 1 ml was added to a sterile test tube containing 9 ml of sterile physiological water (NaCl 8.5 %). For the green fodder collected, three gram of stems and leaves was centrifuged for 1 h at 150 rpm in 100 ml of phosphate-buffered saline (PBS) in order to remove the epiphytic bacteria. The resulting stock solutions were diluted decimally from 10^{-1} to 10^{-7} to determine the dilution factor resulting in a well counted number of colonies. One ml of each dilution was taken, inoculated in duplicate on agar culture media and incubated at specific temperatures according to the microorganism sought (AFNOR, 1999).

Lactobacilli were counted after plating 100 µl of each diluted sample on MRS agar (Man, Rogosa and Sharpe) supplemented with cycloheximide (0.1 mg/l). After 48 hours of incubation at 37 °C, white colonies of uniform size were counted (Anas & Mebrouk, 2019).

Yeasts and molds were tested on PDA (Potatoes Dextrose Agar) supplemented with chloramphenicol and incubated for 3 to 5 days at 25 °C. Each yeast and mold colony was observed under a light microscope to avoid confusion with resistant bacteria. The presence of yeast was indicated by the formation of soft round colonies similar to bacterial colonies, but larger, opaque and sometimes pigmented. Molds form thick, fluffy colonies, pigmented or not, sometimes invasive (AFNOR, 2002). The results were expressed in colony forming units per ml or per gram (CFU/ml or CFU/g).

2.5 Data analysis

The computer processing of all the data collected in the field was done using the EXCEL-2010 spreadsheet. Statistical analysis was carried out using SPSS Version 20. Statistical differences between the mean values were determined using Student's t-test, at $p < 0.05$ for a statistically significant difference.

3. Results and Discussion

3.1 Mineral characteristics of drinking water and green fodder consumed, and in raw milk produced by the cows

3.1.1 Calcium and Magnesium concentrations in cow's drinking water

In the drinking water, Ca^{2+} varied from 14 to 66 mg/l with an average of 42 mg/l. Mg^{2+} varied between 7 and 34 mg/l with an average of 26 mg/l (Figure 2A). The highest Ca^{2+} values were obtained in the rainy season (82 and 80 mg/l). The lowest average Ca^{2+} values (9 mg/l) were obtained in the dry season. The dry season drinking water was the most loaded with Mg^{2+} (47 and 37 mg/l), the least loaded being the rainy season water (4 mg/l) (Figure 2B).

The Calcium and Magnesium content of water corresponds to its hardness. The low concentrations of Calcium and Magnesium in the drinking water indicate that we are in the presence of soft water. These results showed low mineralization of the studied waters. In fact, under the action of the meteorization of calcareous rocks, calcium is introduced into the water system by entrainment from the soil into the infiltration water, by leaching and by runoff (Akhtar *et al.,* 2021).

The relatively higher Magnesium levels during the dry season suggest an anthropogenic source (soil fertilization habits). Indeed, most farmers in the region use large amounts of animal waste from livestock and chemical fertilizers during the dry season to improve their production. Soil fertility is therefore increased by using mineral fertilizers enriched with microelements (Djoukeng, 2016). These ions move from the soil into the aquifer by mass and diffusion movements (Whitehead, 2000).

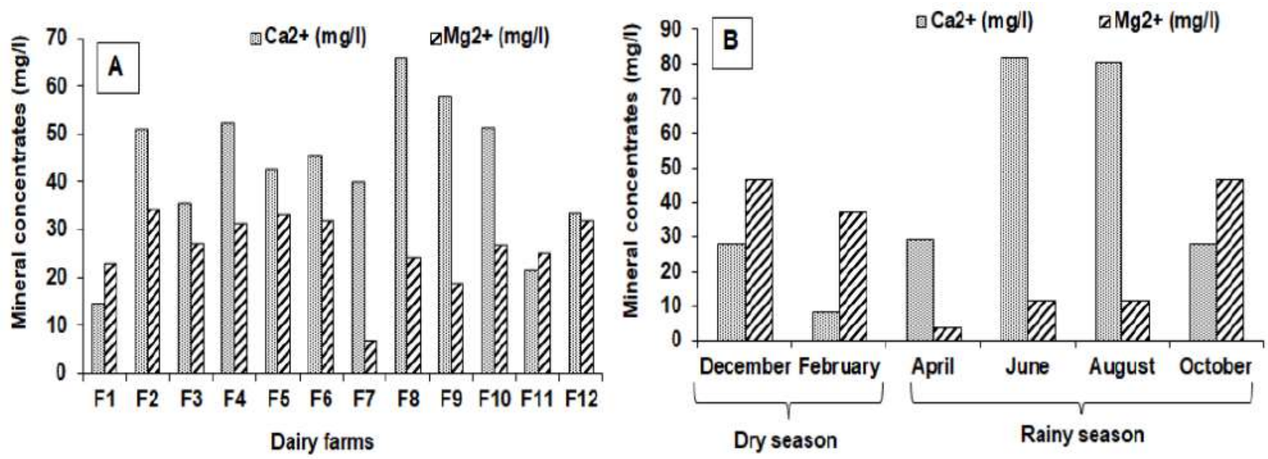

Figure 2: Spatial (A) and temporal (B) variation of Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) contents in cow drinking water

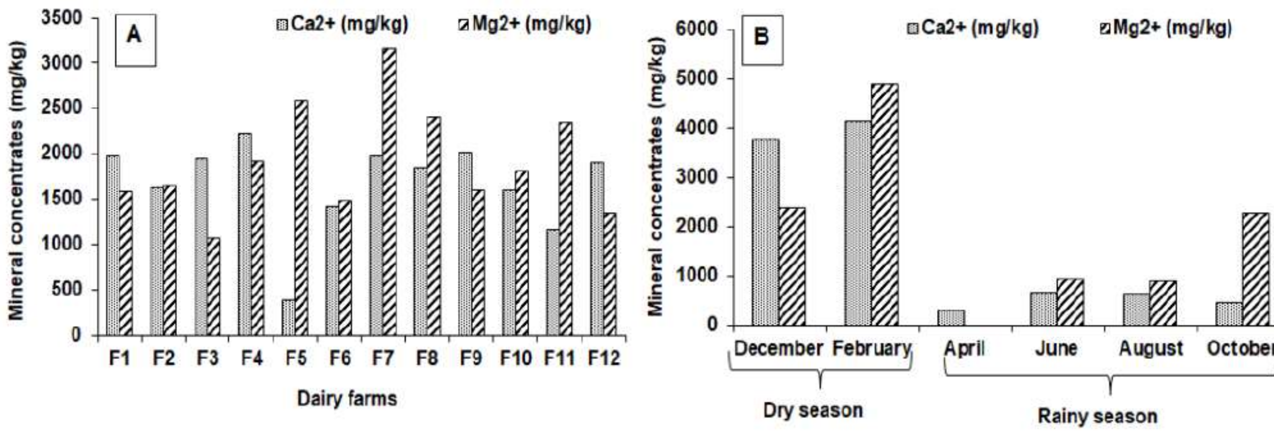

Figure 3: Spatial (A) and temporal (B) variation of Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) contents in green fodder consumed by cows

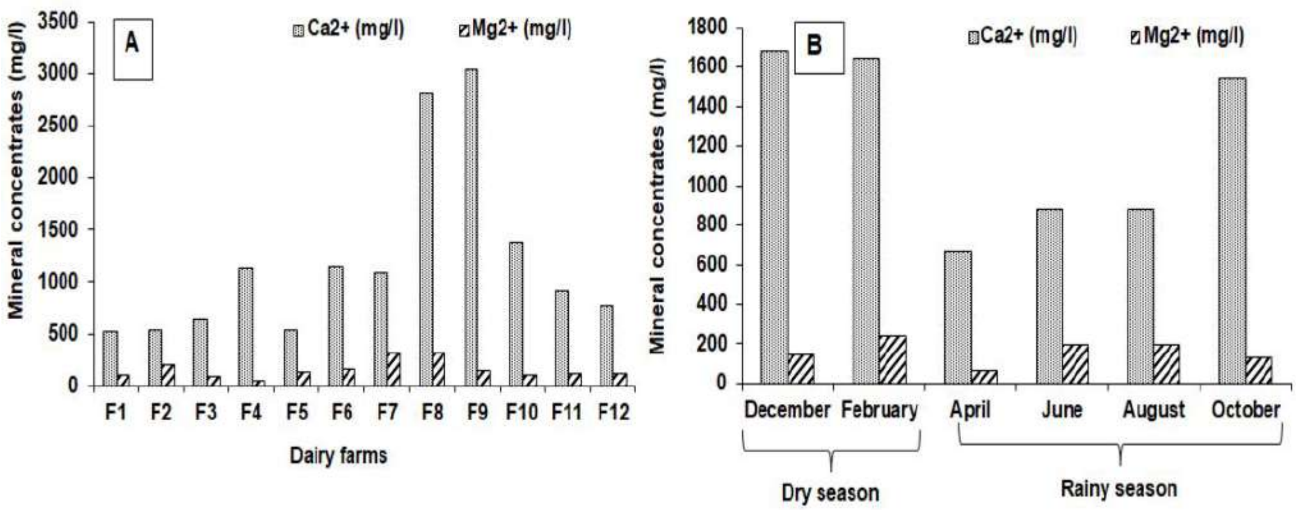

Figure 4: Spatial (A) and temporal (B) variation of Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) contents in raw milk produced

3.1.2 Calcium and Magnesium concentrations in green fodder consumed

In the green fodder, the average Ca^{2+} content was 1674 mg/kg with a minimum of 383 mg/kg and a maximum of 2230 mg/kg. The average Mg^{2+} content showed fluctuations from 1062 mg/kg to 3151 mg/kg with an average of 1913 mg/kg (Figure 3A). The highest values of Ca^{2+} and Mg^{2+} in the green fodder (4161 and 4916 mg/kg respectively) were obtained during the dry season. The lowest mean values (322 and 33 mg/kg for Ca^{2+} and Mg^{2+} respectively) were obtained during the rainy season (Figure 3B).

These values are in line with INRA (2010) standards for cattle fodder quality (Ca^{2+}: 1000-3000 mg/kg and Mg^{2+}: 1000-4000 mg/kg). Indeed, pastoral resources used for cattle feeding in the West Cameroon Region are mainly made up of grasses and legumes. Green fodder from grasses and legumes have high dry matter contents and are important sources of protein, lipids, carbohydrates and mineral elements (Brodziak *et al.,* 2021). In addition, the relatively high levels observed on some farms suggest an anthropogenic source.

In the West Cameroon Region, the combined effect of cultivation practices, high rainfall, relief and soil type, favors various forms of water erosion, leading to soil degradation and a drop in agricultural production potential, hence the heavy use of fertilizers. The majority of farmers use large amount of animal waste from livestock, chemical fertilizers and plant protection products to improve their production. Soil fertility is therefore increased by using mineral fertilizers enriched with microelements (Djoukeng, 2016). The mineral fraction in the soil composition is immediately available to the plant (Whitehead, 2000). Furthermore, detailed studies on cation uptake have shown that the rate of uptake by plants depends on the concentration of these elements in the first few centimetres of soil (Whitehead, 2000).

Seasonal variations suggest a relatively higher mineral content during the dry season. Indeed, during the dry season, natural pasture richer in grasses is scared, so farmers resort to cultivated pasture richer in legumes. And legumes contain more minerals than grasses (Delaby & Horan, 2021). Grasses and legumes differ in their root development and differences in their mineral concentration are, for most elements, influenced by soil supply. Grasses have a fibrous root system and legumes have mostly tap roots (Whitehead, 2000). Thus, legumes tend to be more efficient at absorbing minerals from the soil than grasses (Delaby & Horan, 2021). The mineral contents of green fodder are often poor when harvested under rainy conditions (Cuvelier & Dufrasne, 2015).

3.1.3. Calcium and Magnesium concentrations in raw milk produced

In the raw milk produced, the Calcium level showed fluctuations ranging from 523 to 3045 mg/l with an average of 1215 mg/l. The average Magnesium level was 160 mg/l with a minimum of 57 mg/l and a maximum of 325.15 mg/l (Figure 4A). The highest Ca^{2+} values in milk were recorded during the dry season (1682 and 1642 mg/l). The highest Mg^{2+} values in milk (238 mg/l) were also recorded during the dry season. Milk was lowest in Ca^{2+} and Mg^{2+} during the months of April (rainy season) (667 and 63 mg/l respectively) (Figure 4B).

The average Ca^{2+} concentration in raw milk was within the normal physiological range (1000-1400 mg/l) (Litwinczuk *et al.,* 2004). The average Mg^{2+} content of milk was slightly above the lower reference limits (100-150 mg/l) (Litwinczuk *et al.,* 2004). Cow's milk from the Western Region is relatively rich in Ca^{2+} and Mg^{2+}. This could be attributed to the feeding practice including the nature of the fodder (Król *et al.,* 2016; Nogalska *et al.,* 2020). In addition, the seasonal variation observed would be due to climatic conditions and ration changes. This variation would also be linked to the photoperiod and the hormonal changes (prolactinemia in particular) that it causes in the animal. Indeed, cows exposed to high temperatures reduce their milk production and, consequently, milk components increase (Blanco-Penedo *et al.,* 2020).

Stress due to heat alters mammary cell function. In response, the mammary gland strives to maintain the integrity of the cell-cell junction by synthesizing more protein to compensate the heat stress-induced protein losses (Blanco-Penedo *et* *al.,* 2020). Calcium and magnesium concentrations fluctuate in the same direction in lactation as protein (Blanco-Penedo *et al.,* 2020). The season would also act through the diet (characteristics and food availability) (Bouamra *et al.,* 2019). Some authors showed that, the composition of milk is related to the mineral composition and availability of the basic fodder distributed (Milis *et al.,* 2018). It has been shown that fodder feeding is richer in minerals during the dry season in the West Cameroon Region. These results are similar to those reported by Matallah *et al.* (2015).

3.2 Microbial characteristics of drinking water, green fodder and raw milk produced by the cows

3.2.1 Microorganism load in cows drinking water

In the drinking water, lactobacilli load ranged from 6.7x10^5 to 1.5x10^7 CFU/ml with an average of 7.3x10^6 CFU/ml. Yeasts showed an average concentration of 7.4x10^6 CFU/ml with a minimum value of 3x10^6 CFU/ml and a maximum value of 1.2x10^7 CFU/ml. Molds fluctuated from 0 to 5.6x10^4 CFU/ml with an overall average of 1.7x10^4 CFU/ml (Figure 5A).

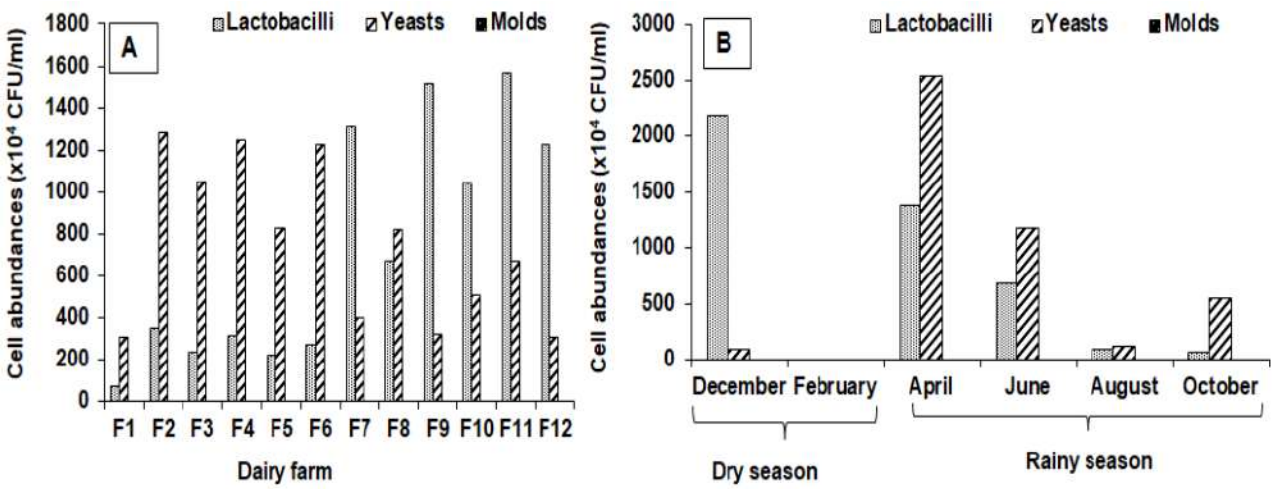

Figure 5: Spatial (A) and temporal (B) variation of lactobacilli, molds and yeasts in cow drinking water

These authors reported that grazing is accompanied by significant changes in milk composition. This phenomenon was also reported by Kaouche-Adjlane & Mati (2017) in the study conducted in the mid-northern region of Algeria on 144 samples of raw milk of small mix.

The highest microbial abundances (1.3x10^7 CFU/ml) were observed during the rainy season. The water samples during the dry season harbored the lowest cell abundance (1x10^4 CFU/ml) (Figure 5B).

The number of colony forming units (CFU) of yeasts and fungi can reach up to 10^6 CFU/ml in groundwater and 10^7 CFU/ml in surface water.

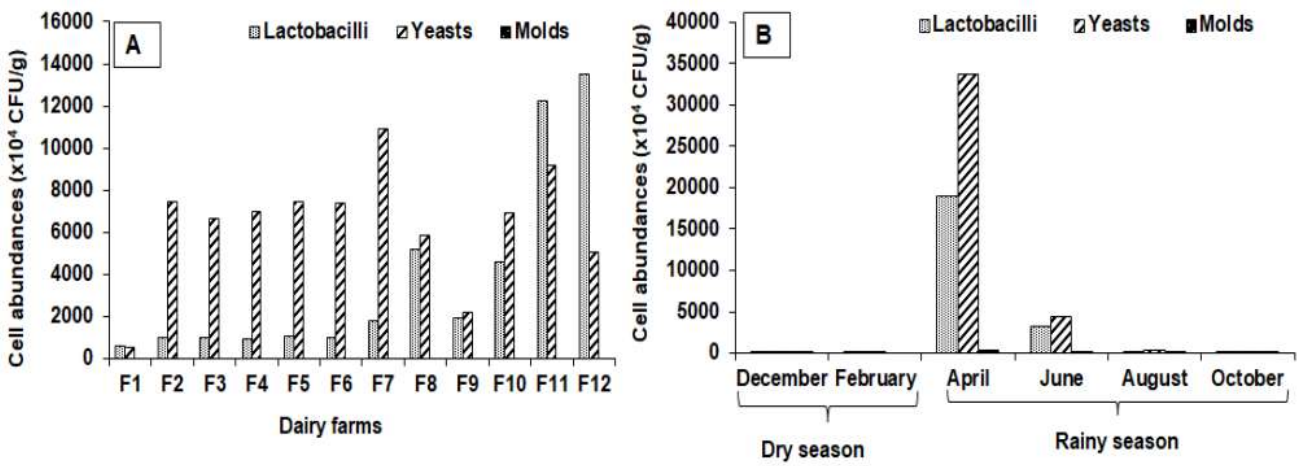

Figure 6: Spatial (A) and temporal (B) variation of lactobacilli, molds and yeasts in green fodder consumed by cows

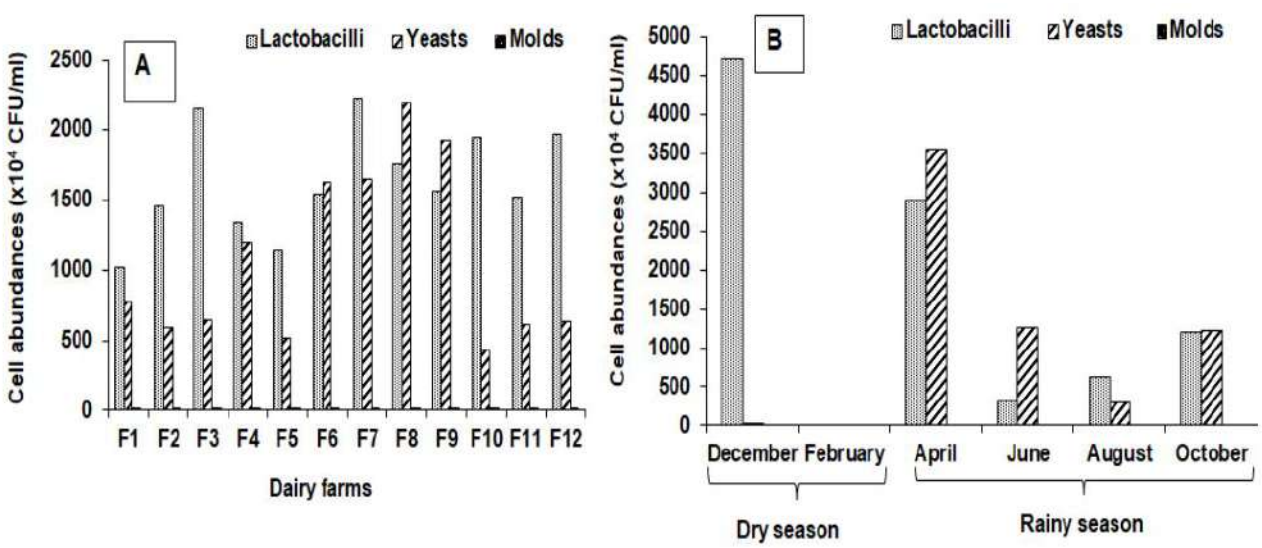

Figure 7: Spatial (A) and temporal (B) variation of lactobacilli, molds and yeasts in raw milk produced

Their growth is influenced by a range of conditions including temperature, pH, water activity and the presence of nutrients (Dalcanton *et al.,* 2018). Moreover, the relatively high cell abundances during the rainy season suggest that rainfall or an increase in the thickness of the water column could favors a significant increase in cell abundances in cow drinking water.

In fact, during rainy season, runoff water is loaded with soil microorganisms and wastewater in areas with little or no sanitation, and infiltrates into the soil to recharge the underlying groundwater. This infiltration can increase the bacterial load of groundwater and surface water by a factor of 100 during rainfall (Curriero *et al.,* 2001).

3.2.2 Microorganism load in the green fodder consumed

In the green fodder, lactobacilli ranged from $5.9x10^6$ to $1.3x10^8$ CFU/g with an average concentration of $3.7x10^7$ CFU/g. Yeasts counted ranged from $5.1x10^6$ to $1x10^8$ CFU/g with an average of $6.3x10^7$ CFU/g. The average molds density was $5.7x10^5$ CFU/g with a minimum value of $5.5x10^4$ CFU/g and a maximum value of $9.1x10^5$ CFU/g (Figure 6A). The green fodder exhibited the richest microbial load during the rainy season while the lowest abundance was recorded with the dry season ($5x10^4$ CFU/g) (Figure 6B).

The green fodder contained relatively higher microbial load. Indeed, the phyllosphere is home to many bacteria, filamentous fungi and yeasts. On the leaves, about 10^6-10^7 bacteria are reported to be present per cm^2 (Bringel & Couée, 2015; Yaseen Mir *et al.,* 2022). Several works have been carried-out on pastures contamination. According to them, microorganisms present in animal feces, livestock effluents and wastewater can contaminate soils and pastures, and survive for several months (ANSES, 2020). The environment is therefore a source of danger and contributes to bacterial and fungal contamination of the phyllosphere. Moreover, rainfall is known to favor microbial growth in the phyllosphere. The soil plays a role in the establishment of microbial communities in the phyllosphere, at the level of the rhizosphere, by recruiting the microorganisms available in the free soil fraction (Gal *et al.,* 2003).

Drought leads to abiotic stress which has important consequences on the soil microbiota. In addition to osmotic stress, soil drought will increase soil heterogeneity, limit nutrient mobility, access by microorganisms, and increase soil oxygen, all of which lead to a decrease in microbial biomass and function (Naylor & Coleman-Derr, 2018; Jansson & Hofmockel, 2020). Thus, during the dry season, the microorganisms of the phyllosphere find themselves in an oligotrophic environment and are therefore very dependent on their ability to exploit the carbon (C) and nitrogen (N) resources provided by the plant through its relatively impermeable cuticle for biochemical exchanges. With increased rainfall (or flooding), the soil pores will fill with water and become anaerobic (without oxygen), thus changing the composition of the microbial communities. Furthermore, studies have shown that after rainfall, the concentration of pathogens in the air and over the fields is higher, enriching the phyllosphere with microorganisms (Codex Alimentarius, 2021).

3.2.3 Microorganism load in the raw milk produced

In the milk produced, lactobacilli ranged from $1x10^7$ to $2.2x10^7$ CFU/ml with an average of $1.6x10^7$ CFU/ml. Yeasts ranged from $4.3x10^6$ CFU/ml to $2.1x10^7$ CFU/ml with an average concentration of $1x10^7$ CFU/ml. The average abundance of molds was $1.3x10^4$ CFU/ml with a minimum value of $2.2x10^3$ CFU/ml and a maximum value of $5.4x10^4$ CFU/ml (Figure 7A). On the other hand, the highest microbial loads were observed during the months of April (rainy season) ($2.1x10^7$ CFU/ml). The lowest microbial abundances were found in February (rainy season) ($8x10^4$ CFU/ml) (Figure 7B).

The milks studied were quite high in lactobacilli, yeasts and molds. These are permanent elements of the environment. They may reflect that during handling, the milk has the milk was contaminated (López *et al.,* 2003).

The microorganisms found could also come from the diet. This relationship has been described by many authors who have reported an increase in the number of yeasts and molds in the milk produced, linked to a higher concentration of mycotoxins in the feed used. These microorganisms according to these authors are very often transferred from feed to milk (Kaouche-Adjlane & Mati, 2017). Furthermore, the results obtained showed relatively higher microbial load during the months of April (beginning of the rainy season). This could be related to the increased number of microorganisms in the water and green fodder consumed by the cows during this period. These microorganisms might be transferred from the feed to the milk.

Temperature seems to have an indirect effect on the microbiological quality of milk. Significant increase in cell counts is frequently observed during the summer period (Agabriel *et al.,* 1995; Bony *et al.,* 2005). Summer conditions are indicators of sub-clinical infection situations (Bony *et al.,* 2005). The results obtained by Sraïri *et al.* (2009), showed lower average microorganism loads during the coldest or driest months (October-November-December-January and June). Kaouche-Adjlane *et al.* (2014) indicated that the most contaminated milks were those collected during the warmer months of the year.

3.3 Comparison of Calcium and Magnesium contents in raw milk produced, green fodder and water consumed by cows

Table 1 shows the comparison of Calcium and Magnesium contents of milk, green fodder and drinking water. The mean Ca^{2+} content of the milk produced was not significantly different (p=0.06) from that of the green fodder. Statistically significant differences (p<0.05) in Ca^{2+} content were observed between green fodder and drinking water on the one side and between produced milk and drinking water on the other. Mg^{2+} levels were statistically different (p<0.05) in the three biotopes (drinking water, green fodder and milk produced).

Table 1. "*p-value*" from the comparison of the Calcium and Magnesium contents amongst raw milk produced, green fodder and drinking water of cows

Variables	Milk/ Green fodder	Green fodder/ Water	Milk/ Water
Ca^{2+}	0.0601	0.0001*	0.0001*
Mg^{2+}	0.0001*	0.0001*	0.0001*

*: $p < 0.05$ (significantly different)

The Ca^{2+} content of the raw milk produced seems to be related to that of the green fodder consumed by the cows. Thus, the diet affects the mineral content of the milk. Indeed, the most important variation in the mineral content of milk is determined by environmental factors, notably the feeding system of the cows (INRA, 2010). Mineral concentrations in fodder are associated with local soil and climatic conditions (Brodziak *et al.,* 2021). Factors influencing the mineral composition of soil and pastures include fertilizers, the amount of sewage sludge generated, the soil type or the proximity to mining and industrial areas (Schwendel *et al.,* 2014). Green fodder from grasses and legumes provides large amounts of Calcium and Magnesium. These minerals are thought to pass directly from the blood to the lumen of the acini through the lactogenic cells of the mammary epithelium (Brodziak *et al.,* 2018).

3.4 Comparison of microbial load in raw milk produced, green fodder and water consumed by cows

In Table 2, the mean abundances of lactobacilli in the milk produced were statistically comparable (p=0.14) to those in the green fodder consumed by the cows. However, the mean abundances of yeasts and molds in the milk were significantly (p<0.05) lower than in the green fodder. The average lactobacilli, yeasts and molds in the green fodder were significantly ($p < 0.05$) higher than those the drinking water. Yeasts and molds in the raw milk produced and in the drinking water did not show a statistically significant difference (p= 0.13 and p = 0.44). However, the lactobacilli in the milk were significantly (P<0.05) higher than those in the drinking water.

The dynamics of cell abundance of lactobacilli in the green fodder consumed by the cows varies in the same direction as that of lactobacilli in the milk produced. Similarly, the yeasts and molds in the drinking water of the cows follow the same variation curve as the yeasts and molds in the milk produced. These lactobacilli, yeasts and molds would probably be transferred from the feed to the milk produced. Traditionally, it was thought that bacteria in milk come from contamination of the external environment, the skin of the mammary gland or the oral cavity of the offspring (Addis *et al.,* 2016). However, several studies suggest that bacteria in milk do not originate solely from external colonization and an endogenous route of bacterial transmission has been proposed (Young *et al.,* 2015; Addis *et al.,* 2016). Therefore, microorganisms from different anatomical locations in the udder may somehow enter the mammary gland. Thus, in the entero-mammary pathway, it has been hypothesized that bacteria can leave the intestinal lumen, migrate through the mesenteric lymph nodes to the mammary gland, probably via immune cells such as dendritic cells.

Table 2. "*p-value*" from the comparison of Lactobacilli, Yeasts and Molds abundances amongst raw milk produced, green fodder and drinking water of cows

Variables	Milk/ Green fodder	Green fodder/ Water	Milk/ Water
Lactobacilli	0.1461	0.0426*	0.0001*
Yeasts	0.0013*	0.0006*	0.1383
Molds	0.0020*	0.0021*	0.4481

*: $p < 0.05$ (significantly different)

The transfer of gut bacteria to the mammary gland of cows was reported by Young *et al.* (2015), who studied the composition and diversity of fecal microbiota, milk leukocytes and blood leukocytes in lactating cows. This entero-mammary pathway involves a highly regulated interference between bacterial cells, immune cells (dendritic cells and macrophages) and epithelial cells (Perez *et al.,* 2007). Another hypothesis is that the consumption of contaminated water alters the gut microbiota as well as the dynamics of microorganism abundance in the udder, and/or may directly or indirectly alter the microbiota of the udder skin (Zhang *et al.,* 2015). Water and green fodder consumed by cows are potential sources of microorganisms that can impact the digestive microbiota and contaminate the milk via the entero-mammary route.

4. Conclusion

A comparative approach to the mineral and microbiological characteristics of water and green fodder consumed by cows, and of raw milk from some dairy farms in the Western Region of Cameroon was made. The mineral content of the green fodder consumed by the cows influences the mineral content of the raw milk produced. The diet thus affects the mineral content of the milk. The dynamics of cell abundance of lactobacilli in the green fodder consumed by the cows varies in the same direction as that of lactobacilli in the milk produced. Similarly, the yeasts and molds in the drinking water of the cows follow the same variation curve as the yeasts and molds in the milk produced. These lactobacilli, yeasts and molds would probably be transferred from the feed to the milk produced.

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Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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