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Effect of nongenetic factors on milk compositional aspects and bacteriological quality in Tunisian Maghrebi dromedaries (Camelus dromedarius) reared under a traditional pastoral management system

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Abstract: This study was planned to evaluate the impact of nongenetic factors on physicochemical composition and microbial quality of milk from Maghrebi camels kept under traditional system in oasis areas of Tunisia. Milksamples from 69 animals were collected from lactating *Negga* over winter and summer. Animals belonging to private flocks were between 5 and 17.5 years of age, with parity numbers ranging from first to sixth. Samples were analyzed for basic composition and microbiological features according to standard methods. No significant association ($P > 0.05$) between physical characteristics and nongenetic factors has been observed. The maximum contents of total solids, protein, casein, and fat content were observed during winter. The third lactation was characterized with the highest content of total solids, protein, casein, and lactose; while the highest fat content was recorded in the second lactation. The highest levels of Ca, P, and K were recorded in the winter ($P<0.01$) whereas Na showed an opposite pattern ($P<0.01$). All major minerals were higher in milk from multiparous than primiparous camels, with maximum concentrations at the fourth lactation. The uppermost levels of mineral concentrations and chemical constituent were recorded in the age class of $7 \leq$ age ≤ 9 years. The microbial analysis of raw milk which is affected by season, parity, and age showed higher overall contamination levels in all studied bacterial counts. The highest levels were observed in winter, among the multiparous and oldest *Negga.* The results highlighted the complete absence of the two dangerous pathogens *Salmonella* and CSR in all analyzed samples.

***Keywords**: Camel milk, physicochemical composition, Microbiological quality, nongenetic factors, Variation, Oasis.*

Introduction

The dromedary is of particular interest in the Saharan regions because its breeding is possible in environments where the production of other animal species would be uncertain. It adapts better to desert climates and restrictive conditions because it can better value food resources characterized by their low availability and limited nutritional value. The camel is the most suitable animal anatomically and physiologically adapted to a harsh and painful drought environment, thus exhibiting a high production capacity during prolonged hot and dry periods (Al Haj & Al Kanhal, 2010).

In recent years, the desertification phenomenon is still increasing in Tunisia because of climate change causing degradation of soils and pastoral ranges, which severely impedes the development of breeding other species (sheep and goats). Because of this adaptation, the camel is an ideal means of valuing the desert areas and may contribute significantly to the economic and social improvement of the living conditions of the region's population and can contribute to the strengthening of food security through products enhancement. The camel is esteemed for its milk production recommended as functional food, hair, leather, and basically tasty diet meat. Given this state, raising camels is an excellent alternative for enhancing arid regions despite the meager fodder resources and very hostile eco-climatic conditions.

The population of Maghreb camels in Tunisia is estimated to be 100,000 animals (Chamekh et al., 2020). Animals are raised mainly within two management systems in the south of the country, a traditional pastoral system and a semi-intensive system created in response to the decline of pasture (Fguiri et al., 2018). Camels were traditionally used for meat production (Chamekh et al., 2020) and a substantial milking intended for local consumption (Ayadi et al., 2009). Over recent decades, in the oasis regions, a camel milk sector for marketing and human consumption has emerged throughout Tunisia.

Because of the rising market demand (Chamekh et al., 2020) and the potential health-promoting properties (Al Haj & Al Kanhal, 2010), camel milk have been given much attention and many studies on milk quantity and quality have been published in the world (Ahmed et al., 2012; Abdalla et al., 2015; Nagy et al., 2017; Ismaili et al., 2019; Karaman et al., 2021) and in Tunisia (Jemmali et al., 2016; Ayadi et al., 2018; Fguiri et al., 2018; Chamekh et al., 2020). Medium to large-scale variability in camel's milk Characteristics and quality aspects have been found in the literature showing that the main factors of variation were genetics (Aljumaah et al., 2012; Nagy et al., 2017), age (Al-Juboori et al., 2013; Abdalla et al., 2015; Singh et al., 2017), stage of lactation (Konuspayeva et al., 2010; Musaad et al., 2013), parity (Ahmed et al., 2012; Musaad et al., 2013; Chamekh et al., 2020), season (Abdalla et al., 2015; Nagy et al., 2017), calf sex (Nagy et al., 2017), geographical origin (Shuiep et al., 2008; Adugna et al., 2013), production system (Babiker & El-Zubeir, 2014; Aljumaah et al., 2012; Musaad et al., 2013; Ayadi et al., 2018), feed composition (Al-Saiady et al., 2012; Faye et al., 2013), milking practices (Ayadi et al., 2009; Jemmali et al., 2016) and health status (Konuspayeva et al., 2009; Pak et al., 2019).

In Tunisia, there is information paucity on the physicochemical composition and microbial quality of milk (Chamekh et al., 2020). Such literature work is extremely limited for camels raised in western oasis areas. The insufficient literature available on the dairy characteristics of camel milk refers only to the Maghrebi herds raised in the eastern region of Tunisia. In addition, most data are based on observations of particular research stations and rarely based on pastoral areas. However, there is much less information on physical characteristics, chemical composition, mineral content, bacteriological quality, the prevalence of pathogenic germs, and their variation according to genetics and environmental factors. Therefore, research into milk characteristics is needed to better characterize and provide critical information for the development of effective management plans to improve these genetic resources.

Thus, the main objective of this study was to evaluate the effect of some non-genetic factors on the quality of milk from local Maghrebi camels under a traditional pastoral management system.

Materials and methods

Animals and samples collection

Females (*Negga*) of one-humped camel (Camelus dromedaries) Maghrebi population from private flocks and reared in the continental oases' region of southern west Tunisia were used in this study, which was conducted between January and July 2019. Before the sample collection, basic data on the animals (health status, age, calving season, lactation stage, parity number…) were gathered from the breeders. Selected she-camel for this study included sixty nine lactating camels aged between 5 and 17.5 years, with parity numbers ranging from first till sixth and calved between December and May. All of the animals sampled were in their second and third months of lactation. Milk samples were taken at two different periods of the year. 35 samples during winter, collected from animals that calved between December and January, and 34 samples were obtained in summer from she-camel calves born in April and May.

The selected animals were maintained undera traditional system management where herds grazed on natural pastures mainly characterized by halophilic species around chotts and non-halophilic plants such as *Haloxylon salicornicum, Anabasis articulata, Atriplex mollis, Atriplex halimus, Retama Raetam, Haloxylan schmittianum, Panicum turgidum, Traganum nudatum, Calligonum comosum, Calligonum azel, Aristida Pungens, Limoniastrum guyonianum, Stipagrostis pungen, Zygophyllum album, Sueda fructosa, Tamarix aphylla, Tamarix articulata, Tamarix gallica*, *Rhus tripartitum, Ephedra alata,* and other endemic plants. In times of scarcity animals were given a supplementation based on barley (DM: 91%; CP: 12.7%; NDF: 26.3%; Ash: 3.9%; Net energy content: 1817.01Kcal. Kg^{-1}DM), wheat bran (DM: 86.92%; CP: 14.9%; NDF: 37.7%; Ash: 4.1%; Net energy content: 1624.97 Kcal. Kg^{-1}DM) throughout the year and dates (DM: 88.56%; CP: 3.11%; NDF: 17.81%; Ash: 2.69%; Net energy content: 1931.42 Kcal. Kg^{-1}DM) during the period from October to June when wasted dates are available. All feedstuffs were distributed to all animals without respect to their physiological stage.

Sixty nine random samples of fresh milk were collected in the earlier morning by direct manual milking from complete milking. A duplicate individual sample of 300 mL from healthy animals was collected by the breeder in two sterile tubes. Milk samples were immediately labeled and kept in an ice container during sampling and transportation to the laboratory. The first tube was brought to the animal production laboratory (CRRAO) for physicochemical and mineral analysis. The second tube was transported directly to the regional public health laboratory of Tozeur for microbiological analysis.

Physical and chemical analyses

Physical parameters (pH and density) were determined during the same sampling day. The pH was measured at 20 °C using a Consort C933 pH meter. To determine raw milk density, a Gerber thermolacto-densimeter was used and measurements were made at 20°C. The milk samples were analyzed using official AOAC International analytical methods for lactose (AOAC, 2005) and ash (AOAC, 2012). Following the IDF Standard Methods, fat (IDF, 2009), dry matter (IDF, 2010), and total protein (IDF, 2014) were determined. The casein content was determined by the difference between the total nitrogen and the non-casein nitrogen got by the Kjeldahl method (IDF, 2004). The casein/total nitrogenous matter ratio was determined to assess the cheese value of the studied milk.

Mineral element analysis

Milk samples were analyzed for macro-minerals, including calcium, sodium, potassium, and phosphorus. Calcium was measured according to IDF (2007) using an atomic absorption spectrophotometer (Analytikjena: nova 400). The determination of sodium and potassium was carried out through a Jenway flame emission spectroscopy according to AOAC, (1984). The colorimetric method involving the PhosphoVanado Molybdate complex (GB, 2010) was applied to quantify the phosphorus present in the milk sample.

Microbiological analyses

Samples were submitted for the microbial count of Total mesophilic aerobic bacteria (TMAB), Total coliforms count (TCC), Fecal coliforms count (FCC), Lactic acid bacteria (LAB), Sulphite-reducing clostridium (CSR), Yeast and molds (Y/M), Fecal streptococci (F. Strep), *Staphylococcus aureus* (*S. aureus*), as well as *Escherichia coli* (*E. coli*), and the occurrence of *Salmonella*.

After properly mixing the raw milk samples, 1 mL was taken and dilutions with 9 mL of peptone water were prepared for microbiological analyses. From this dilution, further decimal dilutions were prepared (ISO, 2001a) and plated on a suitable media. According to the International Organization for Standardization standards (ISO), TMAB (ISO, 2013), TCC and FCC (ISO, 2006), LAB (ISO, 1998), CSR (ISO, 2003a), yeast and mold (ISO, 2004), *S. aureus* (ISO, 2003b), *E. coli* (ISO, 2001b) and *Salmonella* (ISO, 2009) were done in duplicate samples with the results being averaged and the number of microorganisms is provided as a colony-forming unit per mL (CFU/mL). The bacterial counts were $\log_{10}$-transformed to normalize the distributions before performing statistical analysis.

Statistical analysis

Statistical analysis was performed using the SAS software (2004). The effect of season, parity, and age of the animals on physical characteristics, chemical composition, mineral concentrations, and microbiological quality were performed using the general linear model (GLM) procedure. The following model was applied:

$$Y_{ijk} = \mu + A_i + P_j + S_k + e_{ijk}$$

Where Y_{ijk} is the dependant variable (pH, density, dry matter, protein, fat, casein, lactose, ash, casein/protein, Ca, P, Na, K, FMAT, TCC, FCT, LAB, Y/M, F. Strep, *E. coli*, *S. aureus*, CSR and *Salmonella*); µ: is the overall mean; A_i is the fixed effect of age (age<7, 7 ≤age ≤9, 9<age≤12 and age >12); P_j is the fixed effect of parity number (j = 1, 2, 3, 4, 5 and 6); $S_k$is the fixed effect of season of lactation (Winter and summer); e_{ijk} is the residual error.

The differences between dependent variables according to age, parity, and the season were performed by comparing the least-square means using the Tukey's multiple comparisons test.

Results and discussion

Physical characteristics

The overall means of pH and density were 6.63±0.22 and 1030.6±2.54, respectively (Table 1). Many earlier findings on the physical parameters of camel milk are corroborating the results of the current investigation (Babiker & El-Zubeir, 2014; Al Haj & Al Kanhal, 2010) and differ from other research studies (Ismaili et al., 2019; Mohamed & Zoubeir, 2020).

The season, parity, and age-related factors had no effect (P>0.05) on the physical characteristics of the camel. Various scientists, including Ahmed et al., (2012) and Babiker and El-Zubeir, (2014), confirmed that pH, acidity, and density are not affected by season, parity, and age.

The pH and acidity levels are indicators of the health status of animals and hygienic quality milk. However, milk from healthy animals should have pH values from 6.4 to 6.7 (Singh et al., 2017). A slightly lower pH of 6.37 (Benmeziane-Derradji, 2021) and 6.0 (Al Haj & Al Kanhal, 2010) have also been recorded. The variation of pH value could be explained by animal's health status (Benmeziane-Derradji, 2021), milking practices, microbial flora (Al Haj & Al Kanhal, 2010), feed, and availability of water (Gorban & Izzeldin, 2001). High acidity indicates high numbers of microorganisms and consequent development of lactic flora, influenced by the temperature effect (Ismaili et al., 2019).

Table 1 - Effect of season, parity, and age on physical characteristics (mean ± standard deviation) of camel milk in Tunisian oasis region.

Variable	pH	Density
Overall	6.63 ± 0.22	1030.63 ± 2.54
Season	NS	NS
Winter	6.60 ± 0.19[a]	1031.20 ± 2.75[a]
summer	6.67 ± 0.25[a]	1030.04 ± 2.21[a]
Parity	NS	NS
First	6.76 ± 0.18[a]	1029.20 ± 1.78[a]
Second	6.60 ± 0.25[a]	1032.27 ± 2.57[a]
Third	6.69 ± 0.20[a]	1031 ± 2.07[a]
Fourth	6.58± 0.16[a]	1030.60 ± 2.95[a]
Fifth	5.65 ± 0.22[a]	1030.89 ± 1.96[a]
Sixth	6.56 ± 0.34[a]	1028 ± 1.41[a]
Age	NS	NS
Age < 7	6.69 ± 0.20[a]	1030.60 ± 2.95[a]
7≤age ≤9	6.66 ± 0.23[a]	1032.27 ± 2.57[a]
9<age≤12	6.61 ± 0.27[a]	1030.31 ± 2.09[a]
age >12	6.58 ± 0.16[a]	1029.73 ± 2.25[a]

a, b, c values with different superscripts within the same column are significantly different.
** P < 0,05; ** P < 0,01; *** P < 0,001; NS: P > 0,05.*

Chemical composition

The overall Averages value of the dry matter, protein, fat, casein, lactose, ash, and casein/protein ratio of camel milk samples have been mentioned in Table 2. Medium to large-scale variability in camel milk composition has been found in the literature. Several studies were conducted in Tunisia to study the chemical composition of milk from the Maghrebi camel population. Milk from she-camel gave in this study total solid, fat, protein, casein, lactose, and ash comparable to that reported by Jemmali et al., (2016), Sboui et al., (2016), Hamed et al., (2017) but was lower than findings by Ayadi et al., (2009) and higher than that founded by Chamekh et al., (2020) except for ash which is higher than in our study. The variation from the reported results could be attributed mainly to the difference in management conditions, including feeding, and environmental factors. Out of Tunisia, current results approached those of the Egyptian Maghrebi camel (Abdalla et al., 2015), Native Turkish breed (Karaman et al., 2021). Lower contents levels were noted in Ethiopian and Saudi camel (Al Haj & Al Kanhal, 2010) and Algerian breeds (Hadef et al., 2018). The variation in the results from different literature sources could be related to the region, the genetic potential of breeds, management conditions, environmental factors, feeding, and lactation stage at which samples were taken (Chamekh et al., 2020).

The overall mean of the Casein/protein ratio was 0.74 ± 0.06. Quantitatively, caseins are the most abundant proteins in camel milk and lay within the range of 52 and 87% of total proteins, as mentioned by Singh et al., (2017). Our finding is higher than that attained with the same Maghrebi dromedary population from the south and the center of Tunisia (Attia et al., 2000; Hamed et al., 2012) and similar to that in other breeds (Farah, 1993). Results from the current study inferred a higher concentration in whey proteins and a reduction in casein content regarding the milk from other ruminant species (Bernabucci et al., 2002; Raynal-Ljutovac et al., 2008), which has technological implications, such as a weaker texture of curd and lower cheese yield (Barlowska et al., 2020).

The season of lactation significantly influenced the total solids, protein, fat ($P < 0.001$), and casein ($P < 0.01$). The maximum contents of total solids, protein, casein, and fat content were observed during winter. These variations throughout the season could be related to seasonal changes in the quality of feed, environmental factors (Chamekh et al., 2020), and physiological stages (Musaad et al., 2013).

Several authors also reported this effect of seasonal variation on total solids, protein, casein, and fat. Results from the present study are congruent with those of Nagy et al., (2017) and Chamekh et al., (2020) who recorded maximum protein, fat, and total solids contents in December and January, while the minimum levels were observed during June and July. Similarly, Ahmed et al., (2012), Musaad et al., (2013), Hamed et al., (2017), and Ismaili et al., (2019) recorded the highest total solids, protein, fat, and casein content in winter than in summer. Several authors have stated an opposing trend. Bakheit et al., (2008) showed that protein and fat contents were higher during hot summer (May and July) and decreased during winter (November and January) and rainy (August and October) seasons. Similarly, Elbashir and Elhassan (2018) reported reduced Total solids, fat, and protein contents of camel milk in hot summer. In most of the above-mentioned studies, the variations were attributed to nutritional and environmental changes. However, Nagy et al., (2017) showed that seasonal changes were independent of nutrition factors and related mainly to environmental factors.

No significant differences between seasons of lactation were observed in the content of lactose, ash, and Casein-protein ratio ($P > 0.05$). These findings are in agreement with those of Hamed et al., (2017) and Ismaili et al., (2019). However, our results are in contrast with previous findings (Ahmed et al., 2012), which declared that season affects ash and lactose content with maximum levels in summer (Ahmed et al., 2012) or winter (Shuiep et al., 2008).

The parity of *Negga* imparted a significant effect on dry matter, fat ($P<0.001$), protein, casein ($P<0.01$), lactose, and casein/protein ratio ($P<0.05$), but not on the ash content ($P>0.05$) of camel's milk. After the first lactation, an Advance in lactation number was associated with a decline in most milk components. An increasing trend in total solids, protein, casein, lactose, and ash content was observed as the parity of camels advanced from the first to the third and started declining significantly from the fourth parity. Fat content showed an increasing tendency from the first to second parity with a decline at the third one. The third lactation was characterized with the highest content of total solids, protein, casein, and lactose, while the lowest levels were recorded in the seventh lactation. The impact of parity on camel milk production has been widely discussed in the literature. Several authors have confirmed the fact that the maximum potentiality of the camel is attained during the second and the third lactation and then decreases to reach its minimum at the sixth lactation (Ahmed et al., 2012; Nagy et al., 2017; Chamekh et al., 2020). Production of low values of their milk constituents during the first parity is evident that camels in the first parity are still growing and share nutrients between body building purpose and milk production (Zeleke, 2007).In contrast, various authors found that the milk of primiparous dromedaries was higher than that of multiparous dromedaries in terms of chemical composition (Nagy et al., 2017; Chamekh et al., 2020). However, other studies have stated a completely different impact of parity on chemical composition to our results and those mentioned above. As affected by animal parity, data advanced by Mostafa et al., (2017) showed an obvious superiority of chemical composition at the 7th and 8th parity. Other studies reported that the lactation number had no significant effect on milk composition (Ahmad et al., 2012; Al-Sultan & Mohammed, 2007; Musaad et al., 2013).

Analysis of variance of the animal's age factor showed a significant effect on the total solids, protein, casein ($P<0.001$), fat ($P<0.01$), lactose, and casein/protein ratio ($P<0.05$). Ash was not observed to be influenced by the age factor ($P>0.05$) (Table 2). In all chemical composition components, results showed an obvious superiority of the camels in the age class of $7\leq$ age ≤ 9 years compared to the *Negga* in the age class of $9<\text{age}\leq 12$, less than seven years, and over twelve years old. Chemical components gradually increased with advancing in age until reaching their peak during 7th, 8th, and 9th years. After that, they steadily decreased until they reached their minimum level after 12 years old. The ash content was relatively stable throughout the age class, with a slight fluctuation from 6.31 ±1.06 to 7.32±1.37 g/L. The variation of camel milk composition according to age factor was recognized by many authors (Shuiep et al., 2008; Al-Juboori et al., 2013; Singh et al., 2017; Karaman et al., 2021). However, reduction in milk composition from older camels as compared to intermediate age groups maybe due to wear of teeth resulting in poor feeding activity, reduction in the number and potency of milk secreting cells, and general weakness because of old age (Zeleke, 2007).

Table 2. Effect of season, parity, and age on chemical composition (g/L) of camel milk in Tunisian oasis areas.

Variable	Dry matter	Protein	Fat	Casein	Lactose	Ash	Casein/protein
Overall	115.24±15.67	30.98±6.40	32.84±4.88	22.77±4.27	37.21±4.64	6.87±1.59	0.74±0.06
Season	***	***	***	**	NS	NS	NS
Winter	117.36±16.03^{a}	32.30±7.07^{a}	34.65±4.25^{a}	23.83±4.92^{a}	37.83±4.50^{a}	7.27±1.55^{a}	0.74±0.06^{a}
summer	113.04±15.30^{b}	29.60±5.43^{b}	31.00±5.43^{b}	21.66±3.2^{b}	36.56±4.49^{a}	6.46±1.56^{a}	0.74±0.07^{a}
Parity	***	**	***	**	*	NS	*
First	104.18±2.70^{d}	29.19±4.16^{c}	30.62±3.71^{bc}	21.91±3.19^{bc}	33.45±6.86^{b}	6.31±1.06^{a}	0.75±0.02^{a}
Second	121.94±3.19^{b}	31.50±5.28^{b}	38.68±4.72^{a}	23.77±3.68^{b}	38.67±4.88^{a}	6.71±0.95^{a}	0.75±0.13^{a}
Third	137.87±7.24^{a}	35.14±9.11^{a}	33.48±4.23^{b}	25.32±6.30^{a}	39.27±3.31^{a}	7.55±1.60^{a}	0.72±0.08^{b}
Fourth	113.64±3.08^{c}	31.13±5.82^{b}	32.58±3.52^{bc}	22.64±3.76^{bc}	38.20±2.91^{ab}	7.02±2.30^{a}	0.72±0.02^{b}
Fifth	99.13±1.33^{e}	28.68±4.53^{c}	31.42±4.42^{bc}	20.59±1.86^{bc}	36.55±4.81^{ab}	6.96±0.94^{a}	0.71±0.2^{b}
Sixth	93.66±5.23^{f}	26.37±2.16^{d}	28.56±2.29^{c}	20.11±1.69^{c}	35.33±3.77^{ab}	6.81±2.14^{a}	0.76±0.01^{a}
Age	***	***	**	***	*	NS	*
Age <7	113.64±3.08^{b}	30.58±4.85^{b}	32.58±3.52^{b}	22.64±3.76^{b}	34.61±4.99^{c}	6.75±1.47^{a}	0.74±0.10^{a}
7≤age ≤9	137.87±7.24^{a}	34.9±8.11^{a}	36.60±5.10^{a}	25.32±6.30^{a}	38.84±3.09^{a}	7.32±1.37^{a}	0.72±0.03^{b}
9<age≤12	112.82±11.84^{b}	31.13±5.82^{b}	31.42±4.42^{b}	23.03±3.50^{b}	38.67±4.88^{a}	7.02±2.30^{a}	0.73±0.08^{b}
age >12	100.13±6.46^{c}	27.79±3.86^{c}	29.83±3.29^{b}	20.40±1.74^{c}	36.55±4.81^{b}	6.31±1.06^{a}	0.73±0.14^{b}

a, b, c, d, e, f values with different superscripts within the same column are significantly different.
** P < 0,05; ** P < 0,01; *** P < 0,001; NS: P > 0,05*

Mineral concentration

The overall mineral concentrations were 1.60±0.17g/L for calcium, 0.58 ± 0.18 g/L for phosphorus, 0.50± 0.13 g/L for sodium and 1.81 ± 0.33 g/L. The calcium content in the present milk samples was close to the literature's data (Mostafa et al., 2017) and higher than those cited by Faye et al., (2008), Konuspayeva et al., (2010), and Hamed et al., (2017). The phosphorus content in camel milk from Tunisian oasis areas appeared in similar levels to those of the literature (Konuspayeva et al., 2010; Singh et al., 2017) and in lower concentration than other ones (Faye et al., 2008; Mostafa et al., 2017). The results from the current study revealed that a high concentration of potassium and a low sodium level was detected. These results align with those of several authors (Mostafidi et al., 2016; Singh et al., 2017).

Camel milk is a rich source of minerals especially Ca and K (Benmeziane-Derradji, 2021) because of the forage eaten by camels such as Atriplex and Acacia, which usually have a high salt content and are possibly the reason for the salty taste of milk (Singh et al., 2017). Nevertheless, variations in mineral content were attributed to breed differences (Al Haj & Al Kanhal, 2010), feeding and production system (Singh et al., 2017), stage of lactation (Benmeziane-Derradji, 2021) analytical procedures (Attia et al., 2000), and water intake (Singh et al., 2017).

Season, parity, and age of animals exerted a significant effect on all minerals (Table 3). The highest levels of Ca, P, and K were recorded in winter ($P<0.01$). Na showed an opposite pattern and was higher in summer than in the winter ($P<0.01$). As suggested by Hamed et al., (2017), the variability in mineral concentrations between seasons in camel milk is due to a dilution effect, which is related to selective camel feeding behavior and changes in pasture composition. Mostafa et al., (2017) indicated that drought conditions, that characterize the southern west of Tunisia in the hot season, could generate a large variation in mineral contents from winter to summer.

The first parity recorded the lowest concentrations of macro minerals. By advancing in animal parity, all studied minerals were markedly increased up to the fourth parity after which the concentrations decline to attain lower levels at the sixth lactation. Similarly, previous reports showed variations of camel milk according to parity number. However, Aljumaah et al., (2012) reported that parity numbers showed variations on minerals content in camel milk. The highest mean of Ca, P, Na, and K were recorded during the fourth parity. Meanwhile, Elnour and Bakheit, (2012) and Elbashir and Elhassan, (2017) cited the highest amount of minerals from the fifth parity order. Otherwise, Mostafa et al., (2017) found that mineral concentrations increased by advancing in parity of animals to reach the maximum level in 7-8 parities.

A similar tendency to the parity factor was observed for the effect of age on mineral concentrations. The uppermost levels of mineral concentrations were recorded in the age class of $7 \leq$ age ≤ 9 years, followed by camels in the age class of $9<$age≤ 12. The lowest ones were those of animals over 12 years old, which comes behind young age animals (<7years). Production of milk with lower mineral concentrations by inferior animals is logical because at that age (<7 years) animals are being still in the growing stage and the supplied nutrients are partitioned for body building purposes and milk production (Zeleke, 2007). Likewise, older camels as compared to intermediate ages may suffer from a reduction in the number and efficiency of milk-secreting cells, wearing of teeth, and also a general weakness that may affect the mineral concentrations in camel milk (Zeleke, 2007; Elbashir & Elhassan, 2017).

Table 3. Effect of season, parity, and age on minerals content (g/L) of camel's milk in Tunisian oasis.

Variable	Ca	P	Na	K
Overall	1.60±0.17	0.58±0.18	0.50±0.13	1.81±0.33
Season	**	**	**	**
winter	$1.64{\pm}0.17^{a}$	$0.63{\pm}0.18^{a}$	$0.47{\pm}0.13^{b}$	$1.85{\pm}0.32^{a}$
summer	$1.57{\pm}0.18^{b}$	$0.53{\pm}0.17^{b}$	$0.53{\pm}0.14^{a}$	$1.77{\pm}0.33^{b}$
Parity	**	**	**	**
First	$1.50{\pm}0.07^{d}$	$0.48{\pm}0.12^{e}$	$0.44{\pm}0.13^{d}$	$1.74{\pm}0.25^{c}$
Second	$1.55{\pm}0.20^{c}$	$0.59{\pm}0.15^{c}$	$0.52{\pm}0.10^{b}$	$1.80{\pm}0.27^{b}$
Third	$1.61{\pm}0.22^{b}$	$0.68{\pm}0.19^{b}$	$0.56{\pm}0.20^{a}$	$1.82{\pm}0.37^{b}$
Fourth	$1.69{\pm}0.16^{a}$	$0.74{\pm}0.17^{a}$	$0.57{\pm}0.17^{a}$	$1.87{\pm}0.38^{a}$
Fifth	$1.62{\pm}0.20^{b}$	$0.53{\pm}0.21^{d}$	$0.48{\pm}0.11^{c}$	$1.86{\pm}0.38^{a}$
Sixth	$1.60{\pm}0.12^{b}$	$0.49{\pm}0.14^{e}$	$0.43{\pm}0.06^{d}$	$1.76{\pm}0.32^{c}$
Age	**	**	**	***
Age<7	$1.62{\pm}0.20^{b}$	$0.53{\pm}0.21^{c}$	$0.47{\pm}0.17^{c}$	$1.81{\pm}0.28^{b}$
7≤age ≤9	$1.66{\pm}0.58^{a}$	$0.71{\pm}0.17^{a}$	$0.56{\pm}0.10^{a}$	$1.87{\pm}0.38^{a}$
9<age≤12	$1.60{\pm}0.12^{b}$	$0.60{\pm}0.15^{b}$	$0.51{\pm}0.15^{b}$	$1.81{\pm}0.38^{b}$
age >12	$1.53{\pm}0.16^{c}$	$0.48{\pm}0.1^{d}$	$0.46{\pm}0.11^{c}$	$1.75{\pm}0.32^{c}$

a, b, c, d, e values with different superscripts within the same column are significantly different.
** P < 0,05; ** P < 0,01; *** P < 0,001; NS: P > 0,05.*

Bacteriological features

The overall bacteriological quality and the effect of different studied variation factors in camel milk are summarized in Table 4. As depicted in the results, raw milk exhibited a high rate of FAMT with slight variations between samples. These results are nearly similar to those reported by Adugna et al., (2013) and Wasie et al., (2015) and higher than those cited by Karaman et al., (2021), Abera et al., (2016). Extremely high Burdens of FMAT exceeding 8 $\log_{10}$CFU/mL were founded by Elhosseny et al., (2018) and Ismaili et al., (2019).

Total and fecal coliforms counts were 5.16 and 3.44 $\log_{10}$ CFU/mL, respectively. Our findings were closer to those advanced in literature by Wasie et al., (2015) and lower than the values of Benkerroun et al., (2003), Benyagoub and Ayat, (2015), and Ismaili et al., (2019).

The average count of LAB was 3.77 ± 0.65 $\log_{10}$CFU/mL at a low level. The count number was lower than those reported by Benkerroun et al., (2003) and Ismaili et al., (2019). The high levels of lysozyme and ascorbic acid in the camel's milk may explain the low level of LAB as mentioned previously by other researchers (Belkheir et al., 2016). The yeast and mold count of the camel's milk samples in this study was 4.22 ± 1.13 log_{10}CFU/mL. The average value is less than the values found in camel's milk samples in Sudan (Karaman et al., 2021) and Morocco (Ismaili et al., 2019). The lower yeast and mold counts could be because the natural milk pH favors bacterial growth and lowers yeast and mold content as detected in the samples of this study (Karaman et al., 2021).

In studies achieved in Tunisia on the same camel breed and focused on the enumeration of the mesophilic count, total LAB, and coliforms, lower levels were cited by Fguiri et al., (2012) and Jrad et al., (2013).

High total bacterial counts in raw milk mainly reflect the poor hygienic condition under which the milk was handled, storage temperature and time elapsed since milking, and the poor health of milking animals (Adugna et al., 2013). With the current study, the main source of contamination could be attributed to the contamination of the camels' udder by the hands of unhygienic milkers or unhygienic milking procedures. Microorganisms can be transferred from the environment, i.e., feces, bedding, and soil; from contaminated hands, clothing, and mouth of milk handling personnel (Alebie et al., 2021).

Streptococcus, S. aureus, and *E. coli* were prevalent in milk, and their incidences were 0.75, 0.53, and 0.93% from the 49 studied samples. The results of overall averages of the three pathogens mentioned above are in agreement with the findings of Benyagoub and Ayat, (2015) and Abera et al., (2016). Two primary sources caused S*treptococcus*, *Staphylococcus,* and *E. coli* in milk; the first one is the lack of proper hygienic measures and inappropriate manipulation during milking, whereas the second one is mastitis affecting animals (Benmeziane-Derradji, 2021). In the current study, the animals selected were healthy and milked respecting hygienic practices, thus *Streptococcus, Staphylococcus,* and *E. coli* prevalence in the studied milk samples may be linked to subclinical mastitis occurrence (Alebie et al., 2021).

The conducted study showed a complete absence of the two dangerous pathogens *Salmonella* and Sulphite-reducing clostridium, in all examined samples of camel's milk, suggesting that both pathogens are uncommon in camel milk in the sampled herds. Elhaj et al., (2014) and Benyagoub and Ayat, (2015) advanced a similar finding for the absence of *Salmonella* and Sulphite-reducing clostridium in Sudan and Algeria, respectively in the camel population.

Various studies have shown that several factors can affect the bacteriological quality of milk in camel species, including stage of lactation (Nagy et al., 2013; Fguiri et al., 2018), farm characteristics and practices (Abera et al., 2016), years and season (Nagy et al., 2013; Ismaili et al., 2019), animal health (Benkerroun et al., 2003), production systems and feeding practices (Fguiri et al., 2018), and hygiene of milking practice (El-Ziney & Al Turki, 2007).

The results presented in Table 4 showed a significant effect of season on bacterial counts, except for the *E. coli* and *S. aureus* ($P > 0.05$). Here, we noted that the counts of TMAB, TCC, FCC, Y/M, and *Streptococcus* ($P < 0.01$) had reached their peak during the winter. Based on Lactic acid bacteria (LAB), camel milk in summer was found to contain higher levels of contamination than in the winter ($P < 0.01$). Data risen from this study and connected to seasonal impact are in good agreement with those reported by Nagy et al., (2013).

The parity and age had a significant effect on all studied bacterial counts ($P < 0.01$ and $P < 0.05$). The levels of contamination increased with advancing in the number of parity and age of animals. The young and primiparous *Negga* produced milk with lower contamination levels compared to the primiparous and older ones. The same trend of variation according to parity and age has been observed in other ruminant species such as ovine (Sevi et al., 2000), caprine (Goetsch et al., 2011), and bovine (Osterås et al., 2006).

The current result showed that levels of microbial contamination of raw camel milk in the oasis regions of Tunisia were unsatisfactory and cannot comply with the standard requirements of Tunisian legislation on the hygiene of milk and dairy products (NT 14.141 (2004)). Over recent years, in the oasis regions, a camel milk sector for marketing and human consumption has emerged throughout Tunisia because of its potentially health-promoting properties. However, camel milk was commonly produced, conserved, and transported under unhygienic conditions. The bacteriological quality of raw milk should therefore be a major concern for farmers, the processors, and the general public because bacteria in milk can degrade milk components, decrease shelf life, and cause illnesses in human beings (Adugna et al., 2013).

These findings strongly advocate the necessity to practice adequate sanitary measures along the camel milk value chain to avoid the high risk of microbial contamination and transmission of pathogenic microorganisms.

Table 4. Effect of season, parity, and age on bacterial counts (log10 CFU/mL) in camel milk from the Tunisian oasis region.

Variable	TMAB	TCC	FCC	LAB	Y/M	F. Strep	E. Coli	S. Aureus	CSR	Salmonella
Overall	6.54±0.49	5.16±1.49	3.44±1.18	3.77±0.65	4.22±1.13	2.57±1.54	2.61±0.87	1.63±1.61	0	0
Season	**	**	**	**	**	**	NS	NS		
Winter	6.62±0.38 [a]	5.51±0.53[a]	3.69±0.55[a]	3.67±0.67[b]	4.40±1.08[a]	2.77±1.36[a]	2.76±0.78[a]	1.67±1.57[a]	0	0
Summer	6.47±0.58 [b]	4.81±1.98[b]	3.20±1.54[b]	3.89±0.63[a]	4.04±1.17[b]	2.37±1.71[b]	2.47±0.94[a]	1.59±1.69[a]	0	0
Parity	**	**	**	*	*	*	*	*		
First	6.22±1.11[b]	4.96±0.96[c]	3.01±1.54[d]	3.17±0.61[b]	3.39±1.76[b]	1.89±2.08[b]	2.28±1.38[b]	1.05±1.32[d]	0	0
Second	6.40±0.21[ab]	4.89±2.17[c]	3.22±1.28[c]	3.63±0.65[a]	3.48±1.35[ab]	1.99±1.83[b]	2.42±1.21[ab]	0.80±1.51[d]	0	0
Third	6.59±0.36[ab]	4.94±1.84[c]	3.41±1.59[c]	3.78±0.42[a]	4.12±1.25[ab]	2.21±1.79[b]	2.55±0.60[ab]	1.61±1.58[c]	0	0
Fourth	6.49±0.39[ab]	5.18±1.79[b]	3.50±1.26[b]	4.27±0.78[a]	4.24±0.99[ab]	2.81±1.73[b]	2.64±1.03[ab]	2.35±1.56[a]	0	0
Fifth	6.70±0.28[ab]	5.51±0.22[a]	3.69±0.62[b]	3.75±0.57[ab]	4.69±0.87[a]	3.03±0.75[a]	2.92±0.30[a]	1.92±1.65[b]	0	0
Sixth	6.90±0.20[a]	5.54±0.63[a]	3.85±0.22[a]	3.68±0.22[a]	4.66±0.56[a]	3.04±1.17[a]	2.66±0.68[ab]	1.51±2.07[c]	0	0
Age	**	*	*	*	*	**	**	**		
Age <7	6.01±0.39[b]	4.94±1.84[b]	3.22±1.28[b]	3.65±0.51[b]	3.84±1.44[b]	2.21±1.79[b]	2.10±1.22[c]	1.07±1.70[c]	0	0
7≤age ≤9	6.44±0.74[a]	5.13±1.69[a]	3.42±1.09[a]	3.75±0.57[b]	4.24±0.99[a]	2.49±1.74[b]	2.60±0.61[b]	1.39±1.46[b]	0	0
9<age≤12	6.59±0.32[a]	5.18±1.79[a]	3.50±1.26[a]	3.54±0.61[b]	4.66±0.56[a]	2.58±1.63[b]	2.63±1.03[b]	1.92±1.65[ab]	0	0
age>12	6.70±0.28[a]	5.31±0.80[a]	3.58±1.24[a]	4.27±0.78[a]	4.20±1.21[a]	3.03±0.75[a]	3.12±0.30[a]	2.35±1.56[a]	0	0

*a, b, c, d values with different superscripts within the same column are significantly different. * P < 0,05; ** P < 0,01; *** P < 0,001; NS: P > 0,05.*
TMAB: Total mesophilic aerobic bacteria; TCC: Total coliforms count; FCT: Fecal coliforms count; LAB: Lactic acid bacteria; Y/M: Yeast and mold; F. Strep: Fecal streptococci; E. coli: Escherichia coli; S. aureus: Staphylococcus aureus; CSR: Sulphite-reducing clostridium.

Conclusion

The current results contribute to the characterization of the local population of camels raised in Tunisian oasis regions regarding the physicochemical composition, mineral content, and bacteriological properties of milk. The analysis of the milk from Maghrebi camel reveals good physicochemical characteristics and an appreciable mineral profile compared to the standards encountered in the scientific literature. These properties make milk's camel a potentially valuable dietary food. However, our findings evinced that various factors, including the lactation season, parity, and age of the animals influenced the chemical and mineral composition of camel's milk. The highest values of chemical constituents and mineral concentrations were recorded in winter for the *Negga* aged between 7 and 9 years and in second, third, and fourth lactation. The Ash content was the most stable component.

Microbial analysis of raw camel milk, which is affected by season, parity, and age, revealed poor overall quality. Bacteriological results were above the standard criteria required by the Tunisian legislation on the hygiene of milk and dairy products and could be pathogenic. Therefore, strict hygienic controls should be implemented throughout the value chain to improve milk hygiene conditions from production to consumption and the work on the establishment of camel milk standards in Tunisia should be undertaken.

Finally, many variations factors such as diet composition, stage of lactation, health status, milking practices, and management should be the subject of subsequent investigations, trying to see over a sufficiently extended period, their involvement in the composition and quality of the milk produced.

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