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## Chapter

# Microbiological Quality and Biochemical Characteristics of Lactic Acid Bacteria from Camel Milk as Affected by the Production System and Stage of Lactation

*Imen Fguiri, Manel Ziadi, Amel Sboui, Naziha Ayeb, Moufida Atigui, Samira Arroum, Mohamed Hammadi and Touhami Khorchani*

## Abstract

The aim of this work is to study the effect of lactation stage and camel farming system on microbiological, physicochemical parameters, and identification of lactic acid bacteria (LAB) of camel milk. Samples were collected from four camels in semi-intensive system and four camels in intensive system. Microbiological and physicochemical parameters were analyzed. Furthermore, to study the effect of lactation stage, samples were collected from three camels and followed during a period of 10 months of lactation from parturition. LAB were isolated from this sample and identified by biochemical methods. The difference between the physico-chemical characteristic basis of camel farming system are not statistically different except fat. The microbiological analysis showed a significant difference in total mesophilic bacteria, yeast, and molds and total coliform between intensive, semi-intensive, and extensive system. The difference between physicochemical and microbiological characteristics basis of lactation stage are statistically significant. In the intensive system, they were identified the same genre of bacteria: *Lactococcus lactis*, but in semi intensive system, we found different species of LAB. Eight of LAB identified as different *Lactococcus* or *Lactobacillus* was isolated in colostrums. The diversity of LAB was affected by lactation stage and farming system.

**Keywords:** camel milk, production system, lactation stage, lactic acid bacteria, milk proteins

## 1. Introduction

The dromedary camel (*Camelus dromedarius*) is the most important animal in the arid areas in the world. It is a multipurpose animal, used for its supply of milk, meat, hides, and transport [1].

Camel milk has a sweet and sharp taste normally, but at times it can taste salty and other times it tastes watery. The quality of milk is affected by the age of the animal, the stage of lactation, the quality and quantity of feed, as well as the amount of water available [2].

In Tunisia, camel breeding is conventionally extensive; a method perfectly suited to the biology of the specie and it is concentrated in the southern areas [3]. In addition to the extensive system; a new breeding method was developed in several places in the world that could be described as intensive system. This system is based on a set of techniques and ways to optimize production capacity of the animal [4].

Furthermore, there is the semi-intensive also called integrated system which was created due to the decrease in pasture and feed [5].

Camel milk contains inevitably microflora, the nature and significance are determined by the health status of the animal, milking conditions, temperature, etc. Even under rigorous collection conditions, the number of microflora does not exceed  $5 \times 10^3$  cells/ml [6] and this can be due to the inhibition of pathogens bacteria properties in camel milk [7].

Indeed, Al-Mohizea et al. [8] concluded that the hygienic quality of camel milk is satisfactory based on counts of four groups of microorganisms (total aerobic flora, psychrotrophic, coliform, and sporulating bacteria).

The dominant and beneficial microflora in camel milk represented by lactic acid bacteria (LAB) is a potential source of biological materials to be used in dairy technology [9]. LAB strain characterized by their ability to transform lactose and to improve the digestibility of fermented dairy products [10] as well as to preserve [11]. They were also employed for improvement of the taste, texture, and viscosity in the manufacture of dairy products [12]. The ability of LAB to produce probiotics [13] and stimulation of the immune system [14] render this group of microorganisms' essential importance dairy industry which gives added values for dairy product.

The effect of lactation stage and farming system on the physicochemical composition of milk has been the subject of some works [15, 16]. However the microbiological quality is not well studied.

The dominant and beneficial microflora in camel milk are mainly LAB. This group of bacteria is considered to be a potential source of biological agents for use in dairy technology [9]. This study aimed to determine the impacts of lactation stage, production system on physicochemical characteristics and microbiological quality especially the concentration of LAB in camel milk.

## **2. Material and methods**

### **2.1 Source of sampling**

#### *2.1.1 Effect of breeding system*

Eight camels (*C. dromedarius*) Maghrebi breed belonging to the herd of the Arid Lands Institute (Medenine) were studied: four camels in semi-intensive system (Medenine) and four camels in intensive system (Chenchou station, Gabès). Camels were followed during the sixth and ninth months of lactation. In addition to these two types samples were collected from four camels reared in extensive system on El Ouara (Ben Ghilouf, Tataouine) and brought to the laboratory of Livestock and Wildlife for physicochemical and microbiological analyses (**Table 1**).

Rearing method	Feeding
Intensive system	5 kg hay alfalfa
	5 kg of oat hay
	2 kg concentrate No. 7 (cow milk)
Semi-intensive system	6 kg green alfalfa
	6 hours of grazing
Extensive system	Daily supplementation of mixture (2.7 kg) of: barley (31%), olive pomace (54%), and wheat bran (15%)
	Halophytic plants

**Table 1.**  
*Different types of feeding depending on farming system.*

### 2.1.2 Effect of lactation stage

Three Maghrebi camels belonging to the herd of the Arid Lands Institute (Medenine) were monitored for a period of 12 months from parturition. The stage of lactation was subdivided in four periods:

- i. First period was colostrums phase: Samples were collected daily in the first week.
- ii. Second period (early lactation): Samples were collected weekly between second weeks and second month.
- iii. Third period (mid-lactation): Samples were collected between once a month from the third to eighth month.
- iv. Fourth period (end of lactation): Samples were collected once a month during the rest of lactation.

All the physicochemical and microbiological analysis was performed in the Laboratory of Livestock and Wildlife.

## 2.2 Physicochemical analyses

pH and acidity of milk were measured immediately after arrival at the laboratory. The viscosity (in cP) was determined by a Brookfield type viscometer (model DV-E, MA, USA). Dry matter, ash, and total nitrogen contents were determined by dry combustion in a furnace (550°C) that was purged with O<sub>2</sub> gas according to International standard methods [17]. The fat content was measured by an acid-butyrometric method using a “neusol solution.”

## 2.3 Microbiological analysis

Total aerobic mesophilic flora was carried out on plate count agar (PCA; Scharlau Chemie S.A.), incubated at 37°C for 72 h. Yeast and molds on Sabouraud Chloramphenicol (Pronadisa) and incubated at 25°C for 3–5 days. Total coliforms were grown in violet red bile agar (AppliChem) in double layer. LAB were plated on De Man-Rogosa-Sharpe (MRS) agar (Scharlau Chemie S.A.) and incubated at 30°C for 48 h.

## 2.4 Isolation and identification of LAB

LAB was isolated on MRS agar (Pronadisa) and incubated at 30°C for 24–48 h in order to apply the conventional tests for identification. All isolates were initially examined for Gram staining and catalase reaction. Only Gram-positive and catalase-negative isolates were considered. The biochemical identification was carried out using API systems. API 50 CH was used in conjunction with API 50 CHL medium for the identification of *Lactobacillus* and related genera strips according to the manufacturer's instructions (Biomérieux, Marcy-l'Etoile, France) [18].

## 2.5 Statistical analysis

SAS software (version 9) was used for statistical analysis of data. Production system and lactation stage were analyzed by ANOVA using general linear model (GLM) for determination their effect for physicochemical and microbiological characteristics. The means values were compared using SNK test.

## 3. Results and discussion

### 3.1 Effect of production system

#### 3.1.1 The physicochemical characteristics

The values of pH for the three production systems (i.e., intensive, semi-intensive, and extensive) averaged 6.40, which is relatively similar to the average pH value ( $6.43 \pm 0.07$ ) reported by [19] for Maghrebi Libyan camels kept different systems (intensive and extensive and slightly higher than that reported by [20] for raw milk (pH = 6.0). The minimum value of pH was observed in extensive system, and this is might be related to the high content of LAB in milk collected from camels under the same system (**Table 2**). The production system had no effect ( $P > 0.05$ ) on DM and ash contents of milk. Dry

Parameter	Production system			Significance
	Intensive	Semi-intensive	Extensive	
pH	$6.46 \pm 0.16^a$	$6.46 \pm 0.17^a$	$6.29 \pm 0.088^a$	NS
Acidity (°D)	$16.75 \pm 1.83^a$	$16.12 \pm 2.41^a$	$17.50 \pm 2.08^a$	NS
Viscosity (cP)	$3.65 \pm 0.54^a$	$3.85 \pm 0.87^a$	$4.62 \pm 1.06^a$	NS
Fat (g/L)	$21.37 \pm 6.90^b$	$26.00 \pm 8.78^{a,b}$	$34.75 \pm 10.68^a$	*
Dry matter (g/L)	$117.20 \pm 10.28^a$	$118.70 \pm 7.75^a$	$116.30 \pm 8.27^a$	NS
Ash (g/L)	$9.56 \pm 1.80^a$	$9.79 \pm 1.65^a$	$9.44 \pm 1.80^a$	NS
Protein (g/L)	$31.59 \pm 2.48^c$	$35.86 \pm 4.21^b$	$43.65 \pm 4.00^a$	**

<sup>a,b,c</sup>Means in the same line with the same letter are not statistically different ( $P > 0.05$ ).

NS, non-significant. \* $P < 0.05$ .

\*\* $P < 0.01$ .

**Table 2.**  
Effect of breeding system on physicochemical parameters of camel milk.

matter values were similar to those reported by [19] for milk representing intensive and extensive production systems (117.2 vs. 116.3 g/L), while the ash content was slightly higher (9.56 vs. 9.44 g/L). The extensive system presented a highest value of fat and protein contents. However, raising camels under intensive or extensive systems had no effect of fat content but milk from the extensive system had lower protein content (24.5 vs. 31.9 g/L) [19].

### 3.1.2 The microbiological characteristics

Significant differences were observed in the microbial load among the different production systems. The highest bacterial load was marked in extensive system, which can be due to the environment, processing condition and a transportation time from milking to analysis (**Table 3**). This result is similar for cow milk [21] who reported that the milk quality is affected by production system of livestock. The presence of LAB in camel milk was predictable because milk provides optimal natural environment for the growth of this group of bacteria whatever the source of milk is (sheep, goat, and cattle).

## 3.2 Effect of lactation stage

### 3.2.1 The physicochemical characteristics

It was observed that the pH value of camel milk was significantly affected during lactation (**Table 4**). Colostrum presented a low pH, due to high proteins contents [22]. Post-partum changes in gross chemical composition of camel milk showed an increase in fat. In late phase of lactation, the fat was significantly higher than in the early phase of lactation. The variation in protein content during the period of lactation was similar with result reported by [23].

### 3.2.2 The microbiological characteristics

The TPC fluctuated during lactation stages: increased in the early lactation stage followed by decrease in the mid-lactation before increasing again at the end, the values of TPC ranged between 2.64 and 2.30 log<sub>10</sub> CFU/ml (**Table 5**). The yeast and molds content in Moroccan camel's milk was found to be higher with an

	Production system			Significance
	Intensive	Semi-intensive	Extensive**	
TAPC (log <sub>10</sub> CFU/ml)	2.96 ± 1.24 <sup>b</sup>	4.13 ± 0.98 <sup>b</sup>	5.47 ± 0.64 <sup>a</sup>	**
Yeast/mold (log <sub>10</sub> CFU/ml)	1.36 ± 1.25 <sup>b</sup>	0.45 ± 0.64 <sup>b</sup>	6.36 ± 2.44 <sup>a</sup>	**
Total coliform (log <sub>10</sub> CFU/ml)	2.17 ± 1.68 <sup>b</sup>	2.53 ± 1.53 <sup>b</sup>	5.38 ± 0.26 <sup>a</sup>	**
LAB (log <sub>10</sub> CFU/ml)	0.13 ± 0.08 <sup>a</sup>	1.13 ± 0.20 <sup>a</sup>	1.43 ± 0.87 <sup>a</sup>	NS

<sup>a,b</sup>Means within the same line with different letter are statistically different ( $P < 0.05$ ).

NS, non-significant. \*\* $P < 0.01$ .

**Table 3.**  
 Effect of production system on microbiological parameters.

Parameters	Lactation stage				Significance
	First week	Early lactation	Mid-lactation	End last lactation	
pH	6.35 ± 0.18 <sup>b,c</sup>	6.45 ± 0.13 <sup>a,b</sup>	6.52 ± 0.13 <sup>b</sup>	6.64 ± 0.11 <sup>a</sup>	**
Viscosity (cP)	6.61 ± 2.55 <sup>a</sup>	5.82 ± 3.12 <sup>a,b</sup>	3.37 ± 0.45 <sup>c</sup>	4.17 ± 0.53 <sup>b,c</sup>	**
Fat content (g/L)	11.72 ± 9.78 <sup>c</sup>	20.30 ± 5.19 <sup>b</sup>	21.40 ± 4.77 <sup>b</sup>	28.44 ± 8.67 <sup>a</sup>	**
Dry matter (g/L)	127.70 ± 12.38 <sup>a</sup>	113.35 ± 8.16 <sup>b</sup>	106.09 ± 5.24 <sup>c</sup>	105.68 ± 4.01 <sup>c</sup>	**
Ash (g/L)	10.74 ± 2.42 <sup>a</sup>	7.95 ± 1.50 <sup>b</sup>	7.07 ± 1.23 <sup>b</sup>	6.96 ± 0.89 <sup>c</sup>	**
Acidity (°D)	24.78 ± 5.63 <sup>a</sup>	17.39 ± 4.04 <sup>b</sup>	15.40 ± 3.08 <sup>b</sup>	16.33 ± 2.64 <sup>b</sup>	**
Proteins (g/L)	43.07 ± 2.11 <sup>a</sup>	33.85 ± 2.26 <sup>b</sup>	27.93 ± 0.87 <sup>d</sup>	31.34 ± 1.61 <sup>c</sup>	**

<sup>a,b,c,d</sup>Means within a line with different letter are statistically different ( $P < 0.05$ ).

**Table 4.**  
Effect of lactation stage on physicochemical characteristics.

Parameters	First week	Early lactation	Mid-lactation	Late lactation	Significance
TAPC (log UFC/ml)	2.64 ± 0.80 <sup>a</sup>	2.30 ± 1.05 <sup>a</sup>	2.48 ± 0.99 <sup>a</sup>	2.41 ± 0.76 <sup>a</sup>	NS
Yeast and molds (log UFC/ml)	2.14 ± 0.99 <sup>a</sup>	2.17 ± 0.30 <sup>a</sup>	1.65 ± 0.82 <sup>a,b</sup>	1.28 ± 1.24 <sup>b</sup>	NS
LAB (log UFC/ml)	2.79 ± 0.53 <sup>a,b</sup>	2.00 ± 1.05 <sup>b,c</sup>	1.62 ± 1.23 <sup>c</sup>	2.48 ± 0.66 <sup>a</sup>	**

<sup>a,b,c</sup>Means within a line with different letter are statistically different ( $P < 0.05$ ).  
NS, non-significant. \*\* $P < 0.01$ .

**Table 5.**  
Effect of lactation stage on microbiological characteristics.

average count of 4.6 log<sub>10</sub> CFU/ml [12]. LAB counts ranged between 1.62 and 2.79 log<sub>10</sub> CFU/ml and the difference between lactation stages being significant with a maximum in colostrum stage. LAB was the predominant microflora in camel milk since it has been proved that they are capable of producing inhibitory substances other than organic acids (lactate and acetate) that are antagonistic toward other microorganisms [13].

### 3.3 Isolation and identification of LAB

Regarding the carbohydrates fermentations the strains were divided in two groups (Table 6). The first ones dominated by regular rods (SCC<sub>1,8</sub>, SCC<sub>1,7</sub>, SCC<sub>1,15</sub>, and SCC<sub>1,2</sub>) were tentatively identified as *Lactobacillus plantarum*, *Lactobacillus pentosus*, and *Lactobacillus brevis*. The second group was coccoid in shape (SLC<sub>ch14</sub>, SLC<sub>ch6</sub>, SCC<sub>1,13</sub>, SCC<sub>1,33</sub>, and SCC<sub>1,6</sub>). They were tentatively identified as *Lactococcus lactis* and *Pediococcus pentosaceus*. Earlier studies have been reported the presence of the *L. plantarum* and *L. brevis* in Sudanese fermented camel milk [24]. Sun et al. [25] isolated the *L. plantarum* and *L. lactis* from traditional fermented milk in Mongolia.

Strains	SLC <sub>ch6</sub>	SCC <sub>1,7</sub>	SLC <sub>ch14</sub>	SCC <sub>1,13</sub>	SCC <sub>1,33</sub>	SCC <sub>1,15</sub>	SCC <sub>1,24</sub>	SCC <sub>1,6</sub>	SCC <sub>1,8</sub>	SCC <sub>1,2</sub>
Glycerol	+	-	W	W	+	W	-	W	W	+
L-Sorbose	-	-	-	-	W	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	+	-	+	-	-
Amygdaline	-	+	+	W	W	+	-	+	-	-
Esculine	+	+	W	W	+	+	W	W	+	W
D-Melezitose	-	-	-	-	-	-	-	+	-	W
Amidon	-	W	+	W	+	-	W	-	W	W
Identification	<i>L. lactis</i> sp <i>lactis1</i>	<i>Lb</i> <i>plantarum</i>	<i>L. lactis</i> sp <i>lactis1</i>	<i>L. lactis</i> sp <i>lactis1</i>	<i>L. lactis</i> sp <i>lactis1</i>	<i>Lb</i> <i>pentosus</i>	<i>L. lactis</i> sp <i>lactis1</i>	<i>Pediococcus</i> <i>pentosaceus</i>	<i>Lb</i> <i>plantarum</i>	<i>Lb</i> <i>brevis</i>

+, positive; W, weakly positive; -, negative after 48 h of incubation at 37°C; SLC<sub>ch</sub>, Strain Milk Camel Chenchou; SCC, Strain colostrum camel.

**Table 6.**  
Fermentation profiles of LAB isolated for camel milk.



## **4. Conclusion**

The present study showed variations of physicochemical and microbiological characteristics in camel milk was affected by production systems and stages of lactation. Physicochemical characteristics of camel milk samples obtained from different production system revealed highly significant variations between these systems in the content of fat and protein. Additionally, stages of lactation showed variations in the physicochemical and microbiological characteristics of camel milk. LAB were also affected by production system and lactation stage.

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## **Conflict of interest**

The authors declare that there is no conflict of interests regarding the publication of this paper.

## **Author details**

Imen Fguiri<sup>1\*</sup>, Manel Ziadi<sup>2</sup>, Amel Sboui<sup>1</sup>, Naziha Ayeb<sup>3</sup>, Moufida Atigui<sup>4</sup>, Samira Arroum<sup>1</sup>, Mohamed Hammadi<sup>1</sup> and Touhami Khorchani<sup>1</sup>

1 Laboratory of Livestock and Wild Life, Institute of Arid Lands (IRA Medenine), Medenine, Tunisia


2 Laboratory of Microbial Technology and Ecology (LETMi), National Institute of Applied Sciences and Technology (INSAT), Tunis, Tunisia

3 Regional Center for Agricultural Research (CRRA) Sidi Bouzid, Tunisia

4 Higher School of Agriculture Mateur, University of Carthage, Tunisia

\*Address all correspondence to: imen.fguiri@yahoo.com

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