

ARTICLE

Effect of subclinical mastitis on milk yield and milk composition parameters in dairy camels

Leyla Hade^{1,2*}, Brahim Hamad^{1,2}, Hebib Aggad²

¹University of El Oued, Faculty of Life and Natural Sciences, Department of Agronomy, 39000 El Oued, Algeria

²University of Tiaret, Institute of Veterinary Sciences, Laboratory of Hygiene and Animal Pathology, 14000 Tiaret, Algeria

ABSTRACT This study investigated the effects of subclinical mastitis on milk yield and milk composition parameters in dairy camels. A total of 140 camel milk samples were collected from multiparous she-camels (7-10 years old) and were subjected to bacteriological culture studies; 76 samples displayed subclinical mastitis and 64 samples were healthy. Furthermore, the samples were analyzed via standard procedures to determine the yield and composition parameters of the milk such as pH, electrical conductivity, protein, fat, lactose, and ash content. The results showed that the milk yield was significantly low ($p < 0.05$) in infected animals compared to healthy animals (3.80 vs 4.32 L·day⁻¹). Moreover, compared to the values observed in healthy milk samples, milk from animals with subclinical mastitis showed significantly decreased ($p < 0.05$) protein (3.33 vs 3.40%) and fat (3.67 vs 3.74%) contents. However, no significant changes ($p > 0.05$) were observed in pH, electrical conductivity, lactose, or ash content. The results of the uninfected milk samples revealed that there was significant correlation between the protein and fat content ($r = 0.781$; $p < 0.01$) and between the lactose and ash content ($r = 0.701$; $p < 0.01$). Conversely, the infected ones showed that there was higher significant correlation between the protein and fat contents ($r = 0.807$; $p < 0.01$) and a medium correlation between the lactose and ash contents ($r = 0.603$; $p < 0.01$). In conclusion, the results suggest that subclinical mastitis is negatively associated with a reduced milk yield and lower protein and fat content. Thus, these parameters can be used to diagnose mastitis in dairy camels.

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*Corresponding author

E-mail: leila.hade¹@yahoo.fr

hade¹-leyla@univ-eloued.dz

Introduction

Dromedary camels are considered the best livestock animals in arid, semiarid, and desert areas; camel milk is a valuable source of both nutrition and income in these areas (Saleh and Faye 2011; Ali et al. 2016; Legesse et al. 2017; Elbashir and Elhassan 2018). Camels can produce more milk for a longer period of time in arid zones and harsh environments than any other domestic livestock species (Seifu and Tafesse 2010; Eissa et al. 2011; Alluwaimi et al. 2017). Nevertheless, similar to other dairy animals, camels are affected by udder infections such as mastitis, which is a complex disease occurring worldwide among such animals. Heavy economic losses can occur as a result of clinical and subclinical mastitis (Toroitich et al. 2017; Gramay and Ftiwi 2018; Ali et al. 2019).

Mastitis, particularly the subclinical type, is one of the most persistent and widely spread disease condition that affect milk hygiene and quality among dairy camels (Husein et al. 2013; Hade¹ et al. 2016). The prevalence of

subclinical mastitis in this species varies among different studies (15-67.4%; Bhatt et al. 2004; Abera et al. 2010; Seifu and Tafesse 2010; Alamin et al. 2013). Subclinical mastitis causes the animal to suffer, reduces the milk yield, alters milk properties, impairs preservation and processing, and represents a public health concern for consumers of camel milk (Saleh and Faye 2011). In the same context, according to Fazlani et al. (2011), mastitis is known to cause a loss of approximately 70% of the camel milk production. Moreover, Ali et al. (2016) reported that subclinical mastitis modifies the composition of camel milk by decreasing the protein, fat, and lactose content; increasing enzymatic activity; and creating a higher electrical conductivity.

Therefore, many studies have been performed to investigate the effects of subclinical mastitis on milk yield and composition in other animal species. However, there are no reports in the literature regarding this association in camels. Thus, this study aimed to determine the impact of subclinical mastitis on milk yield and milk composition parameters in dairy camels.

Materials and methods

Study area

This study was performed in a dairy farm located in Bir Naam, Southeast Algeria. This area is characterized by an arid climate with an average annual temperature of 21.8 °C and an average precipitation of 11.75 mm. The rainy period begins at the start of September and lasts until May with a maximum of 19 mm of precipitation in November. The remainder of the year, pastures in the region are considered arid with an average maximum temperature of 33.6 °C and a very low precipitation of 2 mm in July. The vegetation commonly consists of steppe plants such as *Stipa tenacissima* and *Ampelodesmos tenax*.

Animals

The present study evaluated 140 milk samples obtained from multiparous healthy dairy she-camels. The animals were between 7 and 10 years old, with an average weight of 300 to 350 kg and an average milk yield of $4.04 \pm 0.10 \text{ L} \cdot \text{day}^{-1}$. All animals were maintained under the same conditions (grazing and supplementary farming systems). The animals were submitted to the same diet, which was primarily based on pasture (grass pasture system). The animals were supplemented with some barley concentrate and dry hay straw, with a higher intake during the dry season. The animals were hydrated regularly and hand-milked twice daily at 12-hour intervals. Calving generally occurs during the winter season and starts as early as November.

Sample collection

At the beginning of the experiment, all the camels were subjected to clinical examinations. The camels' udders were examined visually and by palpitation for the presence of any lesions by assessing redness, pain, heat, and swelling. Moreover, milk samples from each animal were obtained and assessed for any change in color and consistency. In this study, the 140 milk samples were collected just before the morning milking during the late stages of lactation. The teats were washed thoroughly and dried with a single-use paper towel. The first three streams of milk from each teat were discarded. The teat end and orifice were sanitized with cotton swabs soaked in 70% alcohol. The samples were collected from each animal in a sterile bottle. Furthermore, the milk samples were labeled, stored in an ice box, and transported immediately within 2-4 hours after collection to the laboratory for the bacteriological and physicochemical analyses.

Milk analysis

Measurement of milk production

Upon arrival to the laboratory, the quantity of the collected milk from each animal was measured with a graduated cylinder. The daily milk yield of the animals was the sum of the morning and evening milking.

Bacteriological analysis

The microbiological cultures were established according to the standard procedures of the National Mastitis Council (NMC 1987). All milk samples were used for the detection of specific bacteria that cause subclinical mastitis. For this purpose, a loopful of each milk sample was cultured on defibrinated sheep blood agar (7%), nutrient agar, BCP (Bromcresol Purple Lactose) agar, and Chapman agar. Moreover, the plates were incubated at 37 °C for 24-48 hours. A sample was considered positive for subclinical mastitis when the growth of five or more identical colonies was detected on the streaks (Contreras et al. 1997; Pradié et al. 2012). However, the growth of two or more different bacterial colonies (> 5 CFUs per type) was considered contamination, and the result was removed from the analysis (Gonzalo et al. 2002; Pradié et al. 2012). The resulting growth was identified based on morphology, colony characteristics, Gram reaction, hemolytic characteristics, catalase test, and classic biochemical tests. Staphylococci and micrococci were identified based on their growth characteristics on triple sugar iron agar, mannitol salt agar, the nitrate reductase test, the urease test, the coagulase test, and the catalase test (Forbes et al. 2002; Quinn et al. 2011). Isolates that were tentatively identified as streptococci were evaluated according to their growth characteristics on the sheep blood agar, catalase production, and sugar fermentation tests. Gram-negative isolates were sub-cultured on BCP agar and were further tested using triple sugar iron agar, motility assessments, the IMViC test (Indole, Methyl red, Voges-Proskauer, and citrate utilization tests), and the urease test (Forbes et al. 2002; Quinn et al. 2011).

Physical analysis

The pH of the milk samples was determined using a pH-meter (Hanna HI 99161, Romania) equipped with a combined glass electrode after calibration in buffer solutions of pH 4 and 7. Furthermore, the electrical conductivity was estimated with an electrical conductivity meter (Hanna EC 215) after the device was calibrated using standard buffer solutions.

Chemical analysis

The protein content was determined through quantification of the nitrogen content by the Kjeldahl method

following the AOAC (2000). Nitrogen was then multiplied by a standard factor (6.38) so that the results were expressed as total protein content. The fat content was determined by the Gerber method according to the AOAC (2000). The lactose content was determined by Bertrand's method (AFNOR 1993). The ash content was determined by an AOAC (2000) technique using carbonization of the samples in a direct flame and subsequent calcination in a muffle at 550 °C for 4-6 hours.

Statistical analysis

The data were analyzed using the Statistical Analysis System Program SPSS v.16.0. The model applied included the fixed effect of subclinical mastitis. Data are displayed in the tables as the mean ± SEM. Differences in the mean values were tested using the independent-samples T test or the Mann-Whitney U test after evaluating the normal distribution by the Shapiro-Wilk and Kolmogorov-Smirnov tests. Pearson's correlation test was used to interpret the relationship between the various studied parameters. A probability level (*p*) of 0.05 was chosen as the limit for statistical significance in all tests.

Results

The effect of subclinical mastitis on milk yield and composition parameters is shown in Table 1. Among the 140 samples analyzed, 76 were positive and 64 were negative. In addition, our study indicated that subclinical mastitis had a significant (*p* < 0.05) effect on milk yield (3.80 vs

Table 1. Effect of subclinical mastitis on milk yield and composition parameters (n =140).

| Parameters | Means ± SEM | | p-value |
|-----------------------------------|-------------|-------------|---------|
| | Healthy | Infected | |
| Number of samples | 64 | 76 | |
| Milk yield (L·day ⁻¹) | 4.32 ± 0.14 | 3.80 ± 0.14 | 0.023 |
| pH | 6.56 ± 0.01 | 6.53 ± 0.01 | 0.220 |
| EC (mS·cm ⁻¹) | 6.84 ± 0.14 | 6.98 ± 0.13 | 0.459 |
| Protein (%) | 3.40 ± 0.02 | 3.33 ± 0.02 | 0.039 |
| Fat (%) | 3.74 ± 0.01 | 3.67 ± 0.01 | 0.006 |
| Lactose (%) | 4.20 ± 0.04 | 4.10 ± 0.03 | 0.066 |
| Ash (%) | 1.02 ± 0.02 | 0.94 ± 0.02 | 0.064 |

EC: Electrical conductivity; SEM: standard error of mean. Means in the same row are significantly different (*p* < 0.05).

4.32 L·day⁻¹), protein content (3.33 vs 3.40%), and fat content (3.67 vs 3.74%), with these parameters being significantly lower in the positive samples than in the negative samples (*p* < 0.05). However, no significant (*p* > 0.05) effect of subclinical mastitis was found on pH, electrical conductivity, lactose, or ash contents.

The correlations between all of the studied parameters are reported in Table 2. In the healthy samples, the results showed that protein had a highly significant positive correlation with fat content (*r* = 0.781; *p* < 0.01) and moderate positive correlation with ash (*r* = 0.264; *p* < 0.05). In comparison with the uninfected samples, the protein was significantly correlated with fat content (*r* = 0.807; *p* < 0.01), lactose (*r* = 0.343; *p* < 0.01) and ash (*r*

Table 2. Relationship between the different studied parameters (n =140).

| Healthy (64) | | | | | | |
|---------------|---------|---------|---------|---------|---------|---------|
| | pH | EC | Protein | Fat | Lactose | Ash |
| Milk yield | - 0.200 | - 0.173 | - 0.108 | - 0.075 | - 0.139 | - 0.205 |
| pH | | - 0.147 | 0.032 | 0.147 | - 0.067 | - 0.011 |
| EC | | | - 0.084 | - 0.176 | 0.275* | 0.227 |
| Protein | | | | 0.781** | 0.196 | 0.264* |
| Fat | | | | | 0.068 | 0.187 |
| Lactose | | | | | | 0.701** |
| Infected (76) | | | | | | |
| | pH | EC | Protein | Fat | Lactose | Ash |
| Milk yield | 0.188 | 0.070 | 0.006 | - 0.035 | 0.035 | 0.068 |
| pH | | - 0.049 | - 0.077 | - 0.061 | 0.044 | 0.005 |
| EC | | | - 0.020 | - 0.126 | 0.162 | 0.295** |
| Protein | | | | 0.807** | 0.343** | 0.390** |
| Fat | | | | | 0.348** | 0.299** |
| Lactose | | | | | | 0.603** |

*: *p* < 0.05. **: *p* < 0.01. All Pearson correlation coefficients are significant at *P* < 0.05.

= 0.390; $p < 0.01$) in the infected samples. Moreover, a medium significant positive correlation was found in the samples with subclinical mastitis between fat and lactose ($r = 0.348$; $p < 0.01$) and between fat and ash ($r = 0.299$; $p < 0.01$). Likewise, lactose was positively correlated with ash in the healthy samples ($r = 0.701$; $p < 0.01$) and in the infected samples ($r = 0.603$; $p < 0.01$). Similarly, the electrical conductivity showed significant positive correlations with lactose ($r = 0.275$; $p < 0.05$) in the uninfected milk samples and with ash ($r = 0.295$; $p < 0.01$) in the infected ones. Furthermore, in the healthy samples the milk yield was negatively correlated with all studied parameters; however, these relationships were not significant ($p > 0.05$). Conversely, in the samples with subclinical mastitis the milk yield was negatively correlated with fat ($r = -0.035$; $p > 0.05$) only.

Discussion

Unlike clinical mastitis, which is responsible for high economic losses but easier to eliminate, subclinical mastitis is quite problematic because of its chronicity and relative incurability in dairy herds. Moreover, microbial infections alter the milk composition and render milk less suitable for consumption and processing.

In the present investigation, subclinical mastitis had a significant negative effect on milk yield. Such findings concur with previous reports in dairy cows (Tesfaye et al. 2010; Gonçalves et al. 2018), dairy goats (Pleguezuelos et al. 2015; Gelasakis et al. 2016; Hanuš et al. 2017), and dairy sheep (Marti De Olives et al. 2013). In these studies, a significantly lower milk yield was reported in mastitic animals than in non-mastitic animals. Nevertheless, no reports are available about the changes in the milk yield of mastitic she-camels. The decrease in the milk yield is attributed to the fact that mastitis is associated with mammary tissue damage, which reduces the number and activity of epithelial cells and consequently contributes to the decreased milk production (Zhao and Lacasse 2008).

The milk pH in our study was not affected by subclinical mastitis; this result differs from that of Ali et al. (2016), who mentioned that the pH of infected milk significantly increases in relation to the severity of the CMT reaction. According to Hadef et al. (2016), determining the pH of camel milk is not a suitable method for detecting subclinical mastitis in camels. The pH of milk may depend on other factors such as milk yield, lactation stage, milk composition, and the nature of fodder. In addition, the insignificant change in pH might also be due to the increased levels of citrates and bicarbonates during subclinical mastitis (Ogola et al. 2007).

Similarly, the electrical conductivity of the milk sam-

ples did not differ significantly between healthy and infected milk in our study. This observation is in agreement with the findings of Eberlein (2007), who suggested that the electrical conductivity values in his study seem to be correlated in some camels with a positive CMT reaction but show no correlation to CMT and total bacterial counts or pathogenic bacteria in other camels or in the same camels on other days. Furthermore, several studies have demonstrated that electrical conductivity was not considered adequate as a method of diagnosing mastitis in camels (Younan et al. 2001; Bhatt et al. 2004; Eberlein 2007; Hadef et al. 2016). Contrary to the results of this study, Ali et al. (2016) found that the electrical conductivity of mastitic milk was significantly higher than that of milk obtained from healthy animals; they attributed their finding to the increased milk somatic cells. Moreover, the augmentation of electrical conductivity is due to the increased leakage of various ions and salts as a result of the increased permeability of vascular membranes following inflammatory reactions.

These contradictory results regarding the variation of electrical conductivity in relation to subclinical mastitis may be caused by breed differences, feeding, stage of lactation, parity number, and season. Furthermore, cell membrane permeability is altered during mastitis, which leads to the increased leakage of blood components into the udder and changes the milk composition (Sharif and Muhammad 2008).

In this study, the protein concentration decreased in the milk of she-camels affected with subclinical mastitis. Similarly, Eman Fathi et al. (2012) and Ali et al. (2016) reported that healthy she-camels produce milk with a higher protein percentage relative to mastitic camels (3.87 vs. 2.90%, respectively). The change in protein may be explained by epithelial cell damage (decrease in synthesis) and an increase in vascular permeability with the passage of immunoglobulins, serum protein, and enzymes from the blood, which may lead to increased proteolysis (Forsbäck et al. 2010). Moreover, subclinical mastitis was associated with a decrease in milk fat percentage. These results are in agreement with other studies (Eman Fathi et al. 2012; Ali et al. 2016). The decrease in fat percentage seems to be a result of a decrease in the synthesis and secretion activities of mammary glands (Le Maréchal et al. 2011). It is also important to note that variations in the fat percentage can be affected by the stage of lactation (Abdalla et al. 2015; Hadef et al. 2018), genetics, management, season (Shuiep et al. 2008), and geographic location (Konuspayeva et al. 2009).

Data in the literature regarding the effect of subclinical mastitis on the total concentration of milk protein and fat in other animal species (cows, goats, and sheep) are very conflicting. Some authors sustain that there is a decrease

of these parameters in mastitis cases (Yarabbi et al. 2014; Barrón-Bravo et al. 2013; Bianchi et al. 2004), whereas others report an increase of these parameters (Guariglia et al. 2015; Pleguezuelos et al. 2015; Olechnowicz et al. 2010). By contrast, other studies have reported that subclinical mastitis has no significant influence on protein and fat contents in milk (Hachana et al. 2018; Ying et al. 2002; Summer et al. 2012).

The current study showed that there is an inverse but non-significant relationship between the values of lactose and subclinical mastitis ($p > 0.05$). Our result is consistent with previous reports on camel milk (Eman Fathi et al. 2012). Moreover, Ali et al. (2016) agree that there is a reduction in the concentration of lactose in milk obtained from animals with subclinical mastitis; however, their results were significant. According to Leitner et al. (2011), the lactose concentration in milk closely reflects both the reduction in milk yield and the deterioration of milk's ability to curdle. Furthermore, the decrease in lactose content can be explained by a decreased capacity for synthesis of the disaccharide by the mammary gland or an increase in the NaCl concentration in the milk, resulting in osmotic disruption in the gland and sugar deprivation by bacteria (Rysanek and Babak 2005).

In addition, according to the literature, factors other than mastitis can also influence the milk lactose content. In the same context, Aljumaah et al. (2012) found that the lactose percentage in camel milk is significantly influenced by the management system, breed parity, and stage of lactation. The present results indicate that the concentrations of ash obtained from infected and healthy she-camels were not significantly different ($p > 0.05$). This finding is in agreement with the results reported by Alemu et al. (2013) who indicated that there was no significant difference in the concentrations of ash in cattle. This finding can be attributed to the relationship between the ash content and the electrical conductivity, which are intimately linked. Consequently, these two parameters did not display significant variation in our study (Kaikci et al. 2012).

The significant positive correlation observed in the present study in the uninfected and infected milk samples between protein and fat contents agreed with the results of previous studies (Musaad et al. 2013; Elobied et al. 2015; Nagy et al. 2017). Similarly, Konuspayeva et al. (2010) sustain that the correlation between protein and fat is widely described in other dairy animals. Moreover, the significant relationship found in the two groups between the other parameters included in this study was in agreement with the results of Musaad et al. (2013) and Elobeid et al. (2015), except for the positive significant correlations observed between electrical conductivity and lactose in the uninfected samples and ash in the infected ones. In

the same context, Musaad et al. (2013) reported a significant negative correlation of electrical conductivity with lactose and ash, whereas Elobeid et al. (2015) declared that there was no significant correlation between the previous parameters. According to Eberlein (2007), the relationship observed between electrical conductivity and ash in the samples with subclinical mastitis in this study can be explained by damage caused to the udder parenchyma cell membranes in mastitis cases. The increase of the permeability of the barrier between blood and milk occurred due to an increase in the contents of chloride (Cl^-) and sodium (Na^+), which leads to a higher electrical conductivity of milk. In addition, unlike our study, Nagy et al. (2017) found a negative correlation between fat and lactose concentrations.

Moreover, the current study revealed that the correlations between the all studied parameters and milk yield in the healthy samples were negative, which was in agreement with previous reports (Musaad et al. 2013; Elobied et al. 2015; Nagy et al. 2017). The observed negative correlation between milk production and other milk components can be attributed to the dilution effect due to the changing milk quantity that had been described earlier. In contrast, in the infected samples the milk yield was negatively correlated only with fat content. This finding can be explained by the significant decrease of the quantity of milk produced by the infected animals; thus, the dilution effect was attenuated in this case.

In conclusion, this study showed that the subclinical infection of mammary glands had a statistically significant negative effect on the milk yield, protein, and fat contents in dairy camels. Therefore, monitoring changes in these parameters is a suitable method for the diagnosis of subclinical mastitis in this species. However, no significant influence of subclinical mastitis was found on pH, electrical conductivity, lactose, and ash content. Therefore, this study indicates that subclinical mastitis is a significant cause of deterioration of important milk components, which has an economic impact on the productivity of this animal species.

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