IJAS Iranian Journal of Applied Animal Science

Influence of Wet and Dry Season on Milk Composition of Dromedary Camels (*Camelus dromedarius*) from Tunisia

Research Article

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Received on: 11 Jul 2016
Revised on: 19 Sep 2016
Accepted on: 15 Oct 2016
Online Published on: Mar 2017

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Online version is available on: www.ijas.ir

ABSTRACT

This study investigated the effect of season (wet and dry) on milk composition of the dromedary camel (*Camelus dromedarius*). Milk samples representing the wet and dry seasons were analyzed for total solids (TS), fat (F), solids-non-fat (SNF), ash, crude protein (CP), nitrogen soluble at pH 4.6 (SN), mineral content (MC) and casein fraction (CnF). Camel milk had higher ($P<0.05$) fat content in the wet season. Crude protein, TS, SNF and SN contents (%) were also higher ($P<0.01$) during the wet season. Ash content was similar ($P>0.05$) in the two seasons. The concentrations of Mg, Na and K were affected by season while the concentrations of Ca and Cl were not. The results from the present study indicate that the increase of milk CnF observed in the wet season was not due to the increase in β-casein and α1-casein contents. Among caseins, the κ-casein and α2-casein fractions did not differ between the two seasons. Conversely, milk from summer camels showed higher contents of αs1-casein and β-casein. It was concluded that the effects of season on the chemical composition of camel milk may impact the processing characteristics of camel milk.

KEY WORDS camel milk, casein fractions, chemical composition, season.

INTRODUCTION

Dromedary camel (*Camelus dromedarius*) can survive and produce considerable amount of milk in hot and dry environments (Bekele *et al*. 2011). Thus, camel milk is considered one the most valuable food sources for nomadic people in the arid and semi-arid areas and has been consumed for centuries due to its nutritional values (Kenzhebulat *et al*. 2000; Mal *et al*. 2006; Lorenzen *et al*. 2011). Many factors influence the concentration of major constituents (fat, protein, lactose and minerals) in milk within species. Previous finding pointed out that the variation in camel milk composition could be attributed mainly to geographical origin and seasonal variations (Nagy *et al*. 2013; Konuspayeva *et al*. 2009; Sallam *et al*. 2008).

Some authors have reported seasonal changes (Todorova, 1998; Bertoni *et al*. 2005) on milk protein fractions, but these data are not conclusive in camel milk. The relative proportions of individual components of casein are subject to considerable variation, which can have an effect on to properties of milk during technical processe (Pabst, 1994; Remeuf, 1994). However, limited information is available on camel milk composition under pastoral systems in Tunisia. The objective of this study was to determine the effects of season (wet and dry) on camel milk composition.

MATERIALS AND METHODS

Milk sampling

The study was carried out using individual milk samples

from 36 dromedary camels (*Camelus dromedarius*) of Maghrabi breed from the south and the center of Tunisia. The dromedaries were fed throughout the year exclusively by grazing. Individual samples during early morning milking were collected into sterile bottles between May 2008 and March 2009. The first few streams of milk from each quarter were discarded. Milk samples were obtained by hand milking. Milk samples (about 1000 mL each) were collected and chilled (4 ˚C) before transferred to the laboratory. At the laboratory, each milk sample was sub-sampled and aliquots were taken for analyses. All physicochemical parameters were determined on the day of sampling. In addition, another aliquot of about 100 mL of milk sample was taken and stored at -20 ˚C for further analysis on protein fraction. For each milk sample, all of the analytical assessments were carried out in duplicate.

Chemical composition determinations

Milk fat (F) content was determined using Gerber method (International Dairy Federation, 1981). Total solid (TS) contents were determined using the forced draft oven method (Marshal, 1993). Ash content was determined burning away all the organic matter at 550 ˚C in a muffle furnace (Marshall, 1993). SNF % was calculated by subtracting the fat % from TS % and calculated according to the following equation:

SNF %= TS % - fat %

The mineral content was determined in an autoanalyzer (SYNCHRON CX9 ALX system, Beckman Coulter Inc (ref:442790).

Separation of milk nitrogen and protein fractions

The pH 4.6-insoluble fraction containing the isoelectric caseins was prepared by precipitation of milk with 1 M sodium acetate buffer (pH 4.6) followed by centrifugation at 6000 rpm and 5 ˚C for 15 min. The casein pellet recovered was first washed three times with 1 M sodium acetate buffer (pH 4.6) and then, to remove the remaining fat and other low density components, they were washed twice with a mixture of sodium acetate buffer and dichloromethane (1:1, v/v). The final protein precipitate was then lyophilized before analysis.

The pH 4.6-soluble nitrogen fraction (SN) was filtered through filter paper (Whatman No. 1) and kept frozen until used. TN and SN fractions were determined in triplicate by the Dumas method (International Dairy Federation, 2002). Casein nitrogen (CnN), crude protein (CP) and Casein (Cn), expressed as g per 100 mL of milk, were calculated as follows according to Ribadeau-Dumas and Grappin (1989):

CnN= TN − SN
CP= TN × 6.38
Cn= CnN × 6.36

Casein as percentage of CP was calculated as: (Cn/CP) × 100. The proportion Cn/CP was used as an index of proteolysis (Ma *et al*. 2003).

Reversed phase-HPLC casein analysis

Whole casein from individual dromedary milks was separated in duplicate by reverse phase HPLC according to the procedure developed by Alim *et al*. (2005) with a Summit × 2 dual gradient HPLC system (Dionex, Indstein, Germany). Casein samples were reduced for 1 h at room temperature in a sample buffer containing 1 ml of 8 M urea, 0.1 M Bis-Tris, 0.3% mercaptoethanol and 1.3% sodium citrate. Reduced samples were diluted (1:5, v/v) with 6 M urea and 0.1% trifluoroacetic acid. Samples (20 µL) previously filtered through a 0.45 µm filter were injected into a C18-bonded silica gel (250 mm×4.6 mm) with a particle diameter of 5 µm and pore width of 300 nm (Europa Protein, Teknokroma, St Cugat, Spain), at a constant temperature of 46 ˚C. The mobile phase consisted in 0.1% trifluoroacetic acid in ultrapure water (solvent A) and 0.1% trifluoroacetic acid in acetonitrile (solvent B). For casein separation, elution was achieved with a linear gradient from 33% to 49% of solvent B in 35 min at a flow rate of 1 mL/min, and the eluted peaks were detected by UV absorbance at 220 nm. Data were processed with the chromatographic system‟s software ChromoLion (Dionex) and the percentage of each casein fraction was determined.

Hydrolysis of dromedary casein by chymosin

Solutions of dromedary casein (1%, w/v) in 50 m*M* sodium acetate buffer at pH 6.6 containing 0.02% thimerosal to prevent microbial activity were treated with chymosin (180 International Milk Clotting Units mL−1, Maxiren 180, DSM Food Specialties, Seclin Cedex, France) at a level of 0.1% (v/v). The solutions were rotated (13 rpm) at 30 ˚C during 30 and 60 min. At the end of each period, chymosin was inactivated by heating (90 ˚C, 5 min) and the pH was lowered to 4.6. After centrifugation (4500 g, 15 min), the pellets were redissolved in the chromatographic sample buffer and aliquots were taken for chromatographic separation.

Statistical analysis

Statistical treatments of data were performed using SPSS software (version 13). Data were arranged according to two seasons; dry season (Aug-Oct) and wet season (Nov-Jan). Data were analyzed by one-way analysis of variance

(ANOVA). The differences among the means of the analysis data were compared at a significance level of (P<0.05).

RESULTS AND DISCUSSION

The fat content in camel milk was lower (P<0.05) in the dry season (Table 1).

Table 1 Composition of camel milk in the wet and dry seasons (Mean±SE)

Milk constituents	Wet season (n=16)	Dry season (n=20)	P-value
Fat (%)	4.64±0.31	3.25±0.20	0.029
TS (%)	12.52±0.64	10.24±0.32	0.002
Ash (%)	0.50±0.05	0.60±0.24	0.213
SNF (%)	7.88±0.33	6.99±0.12	0.011
CP (g/100 mL)	2.61±0.06	2.36±0.03	0.004
TN (g/100 mL)	0.41±0.01	0.37±0.006	0.721
SN (g/100 mL)	0.14±0.01	0.11±0.002	0.003
CnN (g/100 mL)	0.27±0.00	0.26±0.004	0.045

TS: total solids; SNF: solids-non-fat; CP: crude protein (N×6.38); TN: total nitrogen; SN: nitrogen soluble at pH 4.6 and CnN: casein nitrogen.
SE: standard error.

This might be due to nutritional status of the animals during the wet season, where feeds are more easily available and are richer in crude protein, carbohydrates, minerals and vitamins. Our results are consistent with those reported by Sevi *et al.* (2004), suggesting that reduction in fat content of milk, probably is a consequence of a greater secretion of prolactin whose concentration in plasma is higher in the summer than in the winter.

Total solid content was highest (P<0.05) in the wet season and decreased significantly during the dry season, which is in agreement with Elvan and Sebnem (2008). This might be attributed to the reason that camels during hot seasons provides milk with lower total solid because the calves needs more fluids (Shuiep *et al.* 2008). The SNF content in camel milk was lower (P<0.05) in the dry season. Sharma *et al.* (2002) confirmed the effect of seasons on SNF content, who found that SNF content varied among seasons being highest in winter (8.98%) followed by summer (8.84%).

There was no significant correlation between ash content and season (Table 1). Similar results were expressed by Biye *et al.* (2014) and Rao and Mishra (2010), who reported that ash content was not significantly influenced by season. The mean CP content of the camel milk was 2.93 (g/100 mL) for the wet season and 2.36 (g/100 mL) for the dry season. Similarly, Haddadin *et al.* (2008) found that the CP content is lowest in August (2.48%) and highest in December and January (2.9%). The variation in fat and protein correspond to the data given in the literature. Seasonal patterns in the fat and protein have been described in different countries and under different management practices. In the present study, number of important camel milk constituents (i.e. F, CP, TS, SNF, SN and CnN) showed the highest mean values in wet season and the lowest in dry season. It is attributed to the fact that the green fodder is available in the south and the center of Tunisia during rainy season. Higher Mg was obtained during dry season compared to the wet season (4.92 *vs.* 2.26 mmol/L, Table 2).

Table 2 Variation in mineral content (mmol/L) of camel milk during the wet and dry seasons (Mean±SE)

Mineral	Wet season (n=16)	Dry season (n=20)	P-value
Mg	2.26±0.33	4.92±0.41	0.000
Cl	61.12±0.09	61.93±3.82	0.891
K	63.59±2.99	52.4±2.55	0.010
Na	30.57±0.66	33.53±1.18	0.028
Ca	10.47±0.20	10.15±0.65	0.125

Mg: magnesium; Cl: chloride; K: potassium; Na: sodium and Ca: calcium.
SE: standard error.

During the dry season, the average of Na content was found to be significantly higher (P<0.05) than that of the wet season while K was the opposite being significantly lower (P<0.01) in the dry season. The difference in milk mineral concentrations between seasons is due to a "dilution effect" as reported by Guler (2007), which is related to animal feeding behaviour and changes in pasture composition.

In the present study, the reduction in CP contents of camel milk during the dry season was mainly due to the reduction in casein content (Table 1). The reduction of casein content in summer milk has also been reported by other authors (Hermansen *et al.* 1999; Mackle *et al.* l999). β-casein and α1-casein content were lower (P<0.05) in the wet season, while no difference was found for κ-Cn and α2-Cn between seasons (Figure 1).

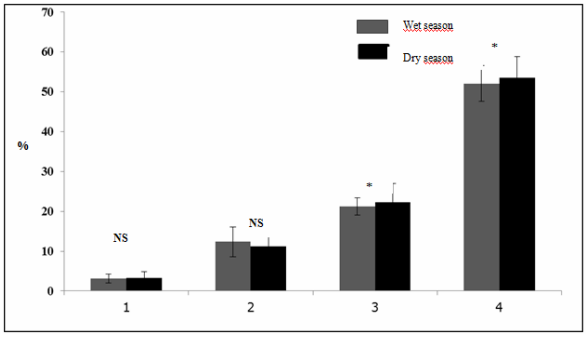

Figure 1 Mean±SD of casein fraction in the wet and dry season in camel milk (1: κ-Cn; 2: α2-Cn; 3: α1-Cn and 4: β-Cn)
Values are the mean ± SE
NS: non significant
* (P<0.05)

However, Kroeker *et al.* (1985) observed no definitive seasonal trend for the relative percentage of casein fractions.

However others authors (Bernabucci *et al*. 2002) showed that β-CN and α1-CN decreased during summer in cow's milk. Our results suggest that decrease in αS1 and β-casein contents may cause the poor cheese making properties of wet season. The results of the present study clearly demonstrated that chemical composition of camel milk is affected by the season. It could, therefore, be concluded that camel milk composition is a reflection of seasonal changes in quality and availability of feed as well as parity differences. However, more work is needed to verify these effects and also to study the effects of management and breed differences on milk composition. Our study showed that the casein fractions were affected by season. For this reason, during the rainy season, it is necessary to prevent deterioration of the quality of milk that can affect the yield and quality of cheese.

CONCLUSION

The results of the present study clearly demonstrated that chemical composition of camel milk is affected by the season. It could, therefore, be concluded that camel milk composition is a reflection of seasonal changes in quality and availability of feed as well as parity differences. However, more work is needed to verify these effects and also to study the effects of management and breed differences on milk composition. Our study showed that the casein fractions were affected by season. For this reason, during the rainy season, it is necessary to prevent deterioration of the quality of milk that can affect the yield and quality of cheese.

ACKNOWLEDGEMENT

This work was supported by Grants from the Tunisian Ministry of Higher Education, Scientific Research and Technology.

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