Influence of Season on Total and Differential Bulk Cow Milk Somatic Cell Counts in Dromedary (*Camelus dromedaries*) and Bovine Milk

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Abstract: The influence of season on Somatic Cell Counts (SCCs), differential leukocytes (Macrophage (MAC), Lymphocyte (LYM) and Poly Morphonuclear Neutrophils (PMNs)) in camel's and cow's milk was compared. Determining the Somatic Cell Count (SCC) in the cow's milk samples, we established that during the Winter season the somatic cell count was significantly higher in comparison to the dry season ($p < 0.05$). However, our results did not determine significant effect of season on SCC in dromedary milk. In wet season, macrophages were the predominant cell type in camel's milk and PMN in cow's milk. In camel's milk, MAC were the dominant cell type at the wet season and tended to maintain high levels as season changed. In cow, PMN numbers were higher in wet season and declined gradually with advanced season while LYM number increased in dry season ($p < 0.05$). Our results suggest that camels are more resistant to season variation in comparison to cows.

Key words: Somatic cells, leukocyte populations, camel's milk, season, dry, suggest

INTRODUCTION

Dromedary milk (*Camelus dromedaries*) can serve and produce considerable amount of milk in recurrent and prolonged hot and dry environment (Bekele *et al.*, 2011). Thus, camel milk is considered one the most valuable food sources of nomadic people in arid and semi-arid areas and had been consumed for centuries due to its nutritional values and medicinal properties (Kenzhebulat *et al.*, 2000; El Zubeir and Nour, 2006; Farah *et al.*, 2007; Lorenzen *et al.*, 2011). However, most camel milk is produced in traditional farming or pastoral systems by hand milking that cannot provide consistent quantity and quality of raw milk for urban markets (Abeiderrahmane, 2005). The Somatic Cell Count (SCC) in bovine milk is an indicator of udder health and milk quality and can be related to the cellular immune response after an inflammatory stimulus (Leitner *et al.*, 2000a, b, 2006, 2008; Ostensson *et al.*, 1988; Silanikove *et al.*, 2006; Vangroenweghe *et al.*, 2002).

All milk variety contains a certain level of somatic cells. These are Neutrophils (PMN cells), Lymphocytes (LYM) and Macrophages (MAC). The increase in SCC results from a transfer of white blood cells (immune cells) from the blood to the mammary gland (Sladek *et al.*, 2006; Verdi and Barbano, 1988). The increase in SCC in milk from infected animals is accompanied by a change in the leukocytes (Krrchen, 1981; Paape and Tucker, 1966). A

typical Intra Mammary Infection (IMI) with coliforms is associated with a dramatic increase of the PMN level up to 90%. Thus, the number of PMNs may be a more useful indicator in the evaluation of udder health than the SCC (Vangroenweghe *et al.*, 2002). In addition to IMI, stage of lactation, season, milk yield and number of lactations also influence SCC (Brolund, 1985; Harmon, 1994; Kennedy *et al.*, 1982; Verdi and Barbano, 1991; Silanikove *et al.*, 2010). Somatic cell counts have been determined in camel milk in the past but mainly from samples of individual animals to diagnose clinical or subclinical mastitis (Abdurahman, 1995, 1996; Chaffer *et al.*, 2000; Guliye *et al.*, 2002). To our knowledge, the interaction between season and cellular counting has not yet been fully considered in camel species. Therefore, the purpose of the present study was to examine variation in SCC and their subtypes in camel's milk between seasons and to compare them with cow's milk in similar situation.

MATERIALS AND METHODS

Milk sampling: The study was carried out using individual milk samples from 36 dromedary animals (*Camelus dromedarius*) of Maghrabi breed from the South and the Center of Tunisia and from 52 cows (Tunisian Holstein from one herd in the region of Sfax). The dromedaries were fed throughout the year exclusively

by grazing. In the case of cows, there is no grazing area and the herd's nutrition is based on roughages and concentrates (aboveground system). For both species, individual samples during early morning milking were collected into sterile bottles between May 2008 and March 2009 in 2 different days. The first few streams of milk from each quarter were discarded. Bovine samples were obtained by automated milking systems but dromedary samples were obtained by manual milking.

Cow and dromedary milk samples were collected and transferred immediately to the laboratory without preservative. Upon arrival each individual milk sample (500 mL) was divided in two portions into sterile bottles. The first sub-sample was stored at 4°C until SCC analyses. The second was stored at -18°C until the rest of analyses (protein fractions, casein fractions) were performed.

Somatic cell count: Somatic cells were counted using a Fossomatic 5000 (Foss Electric, Hillerød, Denmark) according to International Dairy Federation Standard (IDF, 1995).

Differential somatic cell counts: Differential SCC was determined microscopically in smears stained with May-Grunwald and Giemsa reagents. In addition from each milk sample, two preparations were used for differential cell staining and determination of the cell type (Gargouri *et al.*, 2008; adapted for cow's milk). In each slide, the SCC was enumerated in 16 subsections. The working factor was 393.1 in all cases and the average values were used for statistical analyses. The working factor was obtained taking into account the field diameter (0.18 mm), the observed total area (100 mm²) and the milk volume (0.01 mL).

Statistics: Statistical evaluations were performed using SPSS Software (Version 13). Firstly, the frequency distributions for SCC were skewed and thus log₁₀ transformation was applied prior to the analyses. Data were arranged according to two seasons; dry season (Aug-Oct) wet (Nov-Jan). Data were analyzed by one-way Analyses of Variance (ANOVA). The differences among the means of the analyses data were compared at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

The data presented in Table 1 reveals the SCC was significantly higher in wet season compared to dry season in cow milk. According to our findings most researchers

Table 1: Total somatic cell count in camel's milk and cow's milk (mean±SE)

Parameters	Wet season	Dry season	p-values
Camel milk			
SCC (10 ³ cell/mL)	171.44±83.86	47.1±18.29	0.13
Log ₁₀ SCC	2.23±1.92	1.67±1.21	0.97
Cow milk			
SCC (10 ³ cell/mL)	260.30±76.67	38.77±11.80	0.00
Log ₁₀ SCC	2.41±0.88	1.58±0.52	0.03

reported on higher SCC in milk in winter (Dobranic *et al.*, 2008; Rajcevic *et al.*, 2003). Contrary to the reports by Hanuo and Gabriel (1991) and Agabriel *et al.* (1995) who found that somatic cell counts are generally lowest during the Winter and highest during the summer which coincides with an increased incidence of clinical mastitis during the summer months (Smith and Hogan, 1995; Smith *et al.*, 1985; Todhunter *et al.*, 1991, 1995). Our results suggested that the cold stress could have increased the susceptibility to infection as well as increased the numbers of pathogens to which cows were exposed. Additional data support the association of rates of clinical mastitis with bacterial counts in bedding (Smith and Hogan, 1995; Todhunter *et al.*, 1995). These findings support the concept that the cold stress per se is not the cause of increased SCC but the increased SCC is a result of greater exposure of teat ends to pathogens, resulting in more new infections and clinical cases during the wet months. Precipitation also poses a series of problems and ingestion of pathogens resulting in environmental mastitis (Bramley, 1992; Hogan and Smith, 2003).

In this study, SCC in camel milk did not change with season. These results are consistent with Coleman and Moss (1989) who found that the season does not affect the SCC.

We could not correlate this seasonal change in SCC to any previous data in camels. However, Nagy *et al.* (2013) showed for log SCC, the mean was lowest in summer and highest in autumn. However, such a seasonal pattern has been also demonstrated in both dairy cows and sheep. Berry *et al.* (2006) reported a highly seasonal change of SCC in Irish dairy herds with low values from spring to summer and high values during the autumn to winter period. Others also described seasonal change in bulk milk SCC but the variation of the geometric mean was lower ($<100 \times 10^3$ cell/mL) and the peak occurred from July to September (Olde Riekerink *et al.*, 2007; Elmoslemany *et al.*, 2009). In dairy sheep month and month within herd were significant variation factors and maximum SCC values were observed from July to September (Gonzalo *et al.*, 2005). We concluded that the season has significant influence on the frequency of IMI cases was larger in the rainy season than in the dry

Fig. 1: Variation of Macrophage (MAC), Lymphocyte (LYM) and Polynuclear Neutrophil (PMN) with season of camel's milk

Fig. 2: Variation of Macrophage (MAC), Lymphocyte (LYM) and Polynuclear Neutrophil (PMN) with season of cow milk

season for cows (Nobrega and Langoli, 2011). There seems to be differences between dromedary camels and cows in some aspects of immune response.

As shown in Fig 1, the proportion MAC, LYM and PMN in wet season averaged ~57, ~24 and ~18% respectively in camel milk. These results are consistent with the role of MAC as the first immunological responder against invading pathogens (Hamed *et al.*, 2010; Sordillo and Streicher, 2002). During dry season, the relative proportion of MAC increased significantly, while LYM and PMN level decreased (Fig. 1).

Thus in healthy quarters, MAC was the predominant cell type in consistent with (Lee *et al.*, 1980; Riollot *et al.*, 2001). Particularly in camels where such changes were associated with significant increase in MAC, it may represent physiological differences in the innate immunity response to mammary gland infection.

In cows milk, the dominant cell type was PMN (64%), followed by LYM (38%) and MAC (22%) throughout wet season. Cow's milk on dry season, had higher proportion of LYM and low level of PMN (Fig. 2). Our results suggest an increased incidence of clinical mastitis during the rainy months. Thus, in uninfected cows, the predominant cell

type is MAC; however, during chronic mastitis, the predominant cell type switches to PMNs (Lee *et al.*, 1976; Riollot *et al.*, 2001).

Poly Morpho Nuclear cell percentage (PMN %) tended ($p < 0.1$) to be greater in cows during the wet season when compared to those during the hot season, there was an effect on the PMN percentage on positively diagnosed animals between the hot season and the rainy season. In addition, the proportion of MAC in camel's milk particularly at wet and dry was notably greater ($p < 0.05$) than in cow's milk (Fig. 1 and 2). As MAC belongs to the acquired immune system it is additional indication that the immune system in camels may be different from the one in cow's one and may relate the famous camel's resistance to stress and disease.

The lowest incidence of clinical mastitis in winter and in dry season in dromedary has been attributed to anatomical structure of mammary gland which renders mammary gland more resistant to cold and heat stress.

CONCLUSION

On the basis of this investigation, it is concluded that the seasonal variations in this part of subcontinent influences the total and differential somatic cell count. It was concluded that wet season significantly increases mastitis incidence in cows. The cows were more vulnerable to mastitis while camels can withstand rigors of adverse dry and wet season. However, the higher proportion of LYM in camel's than cow's milk at different season suggest that the innate and acquired immune system of camels is potentially different from that of cows and may relate to their famous capacity to resist stress and infections.

REFERENCES

- Abdurahman, O.A., 1995. Milk N-acetyl-B-D-glucosaminidase and serum albumin as indicators of subclinical mastitis in the camel. *Transboundary Emerging Dis.*, 42: 643-647.
- Abdurahman, O.A.S., 1996. The detection of subclinical mastitis in the bacterian camel (*Camelus bactrians*) using somatic cell count and California mastitis test. *Vet. Res. Commun.*, 20: 9-14.
- Abeiderrahmane, N., 2005. Modern Dairy Products from Traditional Camel Herding, an Experience in Mauritania. In: *Desertification Combat and Food Safety: The Added Value of Camel Producers*, Faye, B. and P. Esenov (Eds.). IOS Press, Amsterdam, Netherlands ISBN:1-58603-473-1, pp: 152-157.

- Agabriel, C., J.B. Coulon, G. Brunschwig, C. Sibra and C. Nafidi, 1995. A survey on milk quality variation on farms. *INRA. Prod. Ani.*, 8: 251-258.
- Bekele, T., N. Lundeheim and K. Dahlborn, 2011. Milk production and feeding behavior in the camel (*Camelus dromedaries*) during 4 watering regimens. *J. Dairy Sci.*, 94: 1310-1317.
- Berry, D.P., B. O'Brien, E.J. O'Callaghan, K.O. Sullivan and W.J. Meaney, 2006. Temporal trends in bulk tank somatic cell count and total bacterial count in Irish dairy herds during the past decade. *J. Dairy Sci.*, 89: 4083-4093.
- Bramley, A.J., 1992. Mastitis. In: *Bovine Medicine-Diseases and Husbandry of Cattle* Andrews, A.H., R.W. Blowey, H. Boyd and R.G. Eddy (Eds.). Blackwell Scientific Publications, Oxford, England, pp: 289-300.
- Brolund, L., 1985. Cell counts in bovine milk: Causes of variation and applicability for diagnosis of subclinical mastitis. *Acta Veterinaria Scand. Supplementum*, 80: 1-123.
- Chaffer, M., G. Leitner, A. Glickman, C.V. Crevel and M. Winkler *et al.*, 2000. Determination of udder health in camels (*Camelus dromedaries*). *J. Camel Pract. Res.*, 7: 171-174.
- Coleman, D.A. and B.R. Moss, 1989. Effects of several factors on quantification of fat, protein and somatic cells in milk. *J. Dairy Sci.*, 72: 3295-3303.
- Dobranic, V., B. Njari, M. Samardzija, B. Miokovic and R. Resanovic, 2008. The influence of the season on the chemical composition and the somatic cell count of bulk tank cow's milk. *Vet. Arch.*, 78: 235-242.
- El Zubeir, I.E.M. and E.M. Nour, 2006. Studies on some camel management practices and constraints in pre-urban areas of Khartoum state, Sudan. *Int. J. Dairy Sci.*, 1: 104-112.
- Elmoslemany, A.M., G.P. Keefe, I.R. Dohoo and R.T. Dingwell, 2009. Microbiological quality of bulk tank raw milk in Prince Edward Island dairy herds. *J. Dairy Sci.*, 92: 4239-4248.
- Farah, Z., M. Mollet, M. Younan and R. Dahir, 2007. Camel dairy in Somalia: Limiting factors and development potential. *Livest. Sci.*, 110: 187-191.
- Gargouri, A., H. Hamed and A. ElFeki, 2008. Total and differential bulk cow milk somatic cell counts and their relation with lipolysis. *Livestock Sci.*, 113: 274-279.
- Gonzalo, C., J.A. Carriedo, M.A. Blanco, E. Beneitez, M.T. Juarez, L.F. De La Fuente and F. San Primitivo, 2005. Factors of variation influencing bulk tank somatic cell count in dairy sheep. *J. Dairy Sci.*, 88: 969-974.
- Guliyev, A.Y., C.V. Crevel and R. Yagil, 2002. Detection of subclinical mastitis in dromedary camels (*Camelus dromedaries*) using somatic cell counts and the N-acetyl- β -D-glucosaminidase test. *Trop. Anim. Health Prod.*, 34: 95-104.
- Hamed, H., A. Gargouri, Y. Hachana and A.E. Feki, 2010. Comparison between somatic cell and leukocyte variations throughout lactation in camel (*Camelus dromedaries*) and cow's milk. *Small Ruminant Res.*, 94: 94.
- Hanuo, O. and B. Gabriel, 1991. The effect of lactation stage and order on lactose content in milk with respect to the health of mammary gland. *Evol. Manuf.*, 36: 817-823.
- Harmon, R.J., 1994. Physiology of mastitis and factors affecting somatic cell counts. *J. Dairy Sci.*, 77: 2103-2112.
- Hogan, J. and K.L. Smith, 2003. Coliform mastitis. *Vet. Res.*, 34: 507-519.
- IDF., 1995. Enumeration of somatic cells, FIL-IDF standard no. 148A. International Dairy Federation, Brussels, Belgium.
- Kennedy, B.W., M.S. Sethar, A.K.W. Tong, J.E. Moxley and B.R. Downey, 1982. Environmental factors influencing test-day somatic cell counts in Holsteins. *J. Dairy Sci.*, 65: 275-280.
- Kenzhebulat, S., B. Ernuhan and A. Tleuov, 2000. Composition of camel milk and its use in the treatment of infectious diseases in human. *Proceedings of the 2nd Camelid Conference on Agro-economics of Camelid Farming*, September 8-12, 2000, AgroMerkur Publ., pp: 101-101.
- Kirichen, B.J., 1981. Review of the progress of dairy science: Bovine mastitis; Milk compositional changes and related diagnostic test. *J. Dairy Res.*, 48: 167-188.
- Lee, C.S., F.P. Wooding and P. Kemp, 1980. Identification, properties and differential counts of cell populations using electron microscopy of dry cows secretions, colostrum and milk from normal cows. *J. Dairy Res.*, 47: 39-50.
- Lee, J.A., J.D. Roussel and J.F. Beatty, 1976. Effect of temperature-season on bovine adrenal cortical function, blood cell profile and milk production. *J. Dairy Sci.*, 59: 104-108.
- Leitner, G., B. Yadlin, A. Glickman, M. Chaffer and A. Saran, 2000a. Systemic and local immune response of cows to intramammary infection with *Staphylococcus aureus*. *Res. Vet. Sci.*, 69: 181-184.

- Leitner, G., E. Shoshani, O. Krifucks, M. Chaffer and A. Saran, 2000b. Milk leucocyte population patterns in bovine udder infection of aetiology. *J. Vet. Med.*, 47: 581-589.
- Leitner, G., N. Silanikove and U. Merin, 2008. Estimate of milk and curd yield loss of sheep and goats with intramammary infection and its relation to somatic cell count. *Small Ruminant Res.*, 74: 221-225.
- Leitner, G., O. Krifucks, U. Merin, Y. Lavi and N. Silanikove, 2006. Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. *Int. Dairy J.*, 16: 648-654.
- Lorenzen, P., R. Wernery, B. Johnson, S. Jose and U. Wernery, 2011. Evaluation of indigenous enzyme activities in raw and pasteurized camel milk. *Small Ruminant Res.*, 97: 79-82.
- Nagy, P., B. Faye, O. Marko, S. Thomas and U. Wernery *et al.*, 2013. Microbiological quality and somatic cell count in bulk milk of dromedary camels (*Camelus dromedaries*): Descriptive statistics, correlations and factors of variation. *J. Dairy Sci.*, 96: 5625-5640.
- Nobrega, D.B. and H. Langoni, 2011. Breed and season influence on milk quality parameters and in mastitis occurrence. *Braz. Vet. Res.*, 31: 1045-1052.
- Olde-Riekerink, R.G.M., H.W. Barkema and H. Stryhn, 2007. The effect of season on somatic cell count and the incidence of clinical mastitis. *J. Dairy Sci.*, 90: 1704-1715.
- Ostensson, K., M. Hageltorn and G. Astrom, 1988. Differential cell counting in fraction-collected milk from dairy cows. *Acta Vet. Scand.*, 29: 493-500.
- Paape, M.J. and H.A. Tucker, 1966. Somatic cell content variation in fraction-collected milk. *J. Dairy Sci.*, 49: 265-267.
- Rajcevic, M., K. Potocnik and J. Levstek, 2003. Correlations between somatic cells count and milk composition with regard to the season. *Agric. Conspectus Sci.*, 68: 221-226.
- Riollet, C., P. Rainard and B. Poutrel, 2001. Cell subpopulations and cytokine expression in cow milk in response to chronic *Staphylococcus aureus* infection. *J. Dairy Sci.*, 84: 1077-1084.
- Silanikove, N., G. Leitner, U. Merin and C.G. Prosser, 2010. Recent advances in exploiting goat's milk: Quality, safety and production aspects. *Small Rumin. Res.*, 89: 110-124.
- Silanikove, N., U. Merin and G. Leitner, 2006. Physiological role of indigenous milk enzymes: An overview of an evolving picture. *Intl. Dairy J.*, 16: 533-545.
- Sladek, Z., H. Ryznarova and D. Rysanek, 2006. Macrophages of the bovine heifer mammary gland: Morphological features during initiation and resolution of the inflammatory response. *Anat. Histol. Embryol.*, 35: 116-124.
- Smith, K.L. and J.S. Hogan, 1995. Epidemiology of mastitis. Proceedings of the 3rd International Conference on Mastitis Seminar, May 28-June 1, 1995, EAAP Publication, Tel Aviv israel, pp: 3-10.
- Smith, K.L., D.A. Todhunter and P.S. Schoenberger, 1985. Environmental mastitis: Cause, prevalence, prevention1, 2. *J. Dairy Sci.*, 68: 1531-1553.
- Sordillo, L.M. and K.L. Streicher, 2002. Mammary gland immunity and mastitis susceptibility. *J. Mammary Gland Biol. Neoplasia*, 7: 135-146.
- Todhunter, D.A., K.L. Smith and J.S. Hogan, 1995. Environmental streptococcal intramammary infections of the bovine mammary gland. *J. Dairy Sci.*, 78: 2366-2374.
- Todhunter, D.A., K.L. Smith, J.S. Hogan and P.S. Schoenberger, 1991. Gram-negative bacterial infections of the mammary gland in cows. *Am. J. Vet. Res.*, 52: 184-188.
- Vangroenweghe, F., H. Dosogne and C. Burvenich, 2002. Composition and milk cell characteristics in quarter milk fractions of dairy cows with low cell count. *Vet. J.*, 164: 254-260.
- Verdi, R.J. and D.M. Barbano, 1988. Preliminary investigation of the properties of somatic cell proteases. *J. Dairy Sci.*, 71: 534-538.
- Verdi, R.J. and D.M. Barbano, 1991. Properties of proteases from milk somatic cells and blood leukocytes. *J. Dairy Sci.*, 74: 2077-2081.