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MILK INSULIN CONTENT OF EGYPTIAN LACTATING CAMELS

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The changes in the concentration of insulin in Egyptian dromedary camel milk were monitored during the first 5 months of lactation. The concentration of milk insulin was estimated in camels from different four locations in Matrouh governorate, Egypt. As well, the correlation between the content of milk insulin and the concentration of milk protein was evaluated. Results showed that the lactation period significantly affected the concentration of milk insulin. The maximum level (1856.8 ± 804.4 µU/ml) of insulin was recorded in colostrum at 0 hr (the first suckling after parturition). During the normal milk period, the mean concentration of insulin was 55.1 ± 33.2 µU/ml without significant difference due to the sampling time. The concentration of insulin in colostrum and milk of camels was considerably higher than blood-serum insulin. The level of milk insulin in both colostral and normal milk periods was not correlated with serum insulin. Significant differences were observed in the insulin content of camel milk samples, which collected from four locations. The highest content of insulin was in milk of camels that receiving concentrate diet, while the lowest one was in milk collected from camels grazing native pastures. The relationship between insulin and protein contents of camel milk was not significant.

Keywords: Camel milk, Insulin, Lactation period, Milk protein

INTRODUCTION

Camel milk is unique in terms of nutritional and therapeutic properties (Al haj and Al Kanhal, 2010; and Kaskous, 2016), as well camel milk was reported to have anti-diabetic properties. Several studies (Agrawal *et al.*, 2011; and Ejtahed *et al.*, 2015) found out that consumption of camel milk periodically helped to control diabetes and reduced the daily insulin dosage needed for diabetic patients. Other investigations emphasized a hypoglycemic effect of camel milk on diabetic experimental animals (Sboui *et al.*, 2010; and Khan *et al.*, 2013).

The precise reasons of the hypoglycemic effect of camel milk are indefinite. Some hypotheses have been suggested for this effect those need confirmation. Unlike other mammals, camel milk has a high concentration of insulin, which resists proteolytic degradation during passage through the gastrointestinal tract and can be absorbed in its intact form into bloodstream (Zagorski *et al.*, 1998; and Agrawal *et al.*, 2011). A second explanation is that insulin in camel milk is encapsulated in nano lipid vesicles that can enter into the circulation in the active form (Malik *et al.*, 2012). A third suggestion is that camel milk contains insulin-like protein/small molecular substances that imitate insulin interaction with its receptor (Agrawal *et al.*, 2011; and Malik *et al.*, 2012). The small-sized immunoglobulins naturally present in camel milk, may have a stimulative effect on the β-cell of the pancreas (Agrawal *et al.*, 2007).

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Insulin concentration of camel milk has been reported in few studies (Singh, 2001; Wernery *et al.*, 2006a and 2006b; and Hamad *et al.*, 2011), however studies regarding the changes in milk insulin during lactation are very scarce. To the best of our knowledge, only one published study that reported the changes in camel milk insulin content during lactation period (Wernery *et al.*, 2006a). Concerning the Egyptian dromedary camel, there is no cited study on this subject. Therefore, the current study aimed mainly to monitor changes in the concentration of insulin in milk of Egyptian lactating camel throughout the first 5 months of lactation period. As well aimed to estimate the insulin concentration in milk of camels in some locations of Matrouh governorate, Egypt and to detect any relationship between the content of milk insulin and the concentration of milk protein.

MATERIALS AND METHODS

Materials

Samples of dromedary camel milk were collected from four locations at the same region in Matrouh governorate, Egypt namely: 1) Camel Research Center, which belongs to the Ministry of Agriculture and Land Reclamation; 2) El Mathany village, k 40, El Saloum Road, West of Matrouh; 3) Ras Abu-Laho Bahary, k 35, Ageeba road, West of Matrouh; 4) K25, Siwa Road, South of Matrouh.

Samples of camel colostrum and milk were obtained from the first location to estimate and monitor the changes in milk insulin concentration during the first 5 months of lactation period. Samples were taken individually from 13 camels at 0 (the first suckling after parturition), 24, 48, 72 and 96 hrs, 7 and 14 days, 1, 2 and 5 months of lactation. After milking, samples were immediately frozen and stored at -24°C until analysis. From 0 hr until the 14th day of lactation, the concentration of milk insulin was only determined, and from the 1st until the 5th month, milk samples were analyzed for both insulin and protein. Instantly following milking camels, blood samples were taken randomly from lactating camels and withdrawn from the jugular vein of the animals for blood insulin estimation. Camels were fed twice daily on concentrate feed mixture in pelleted and rice straw, while fresh water was available all day times.

From the other three locations, normal milk samples were collected from Twenty-one dromedary camels, which belong to the nomads. In location 2, animals (n = 8) fed on the pastures of wheat and barley. In locations 3 (n = 5) and 4 (n = 8), animals were allowed to graze freely on natural pasture. Water was provided once every 48 hrs. Milk samples were transported to the laboratory in ice boxes within 3-4 hours and kept frozen at -24 °C until the determination of protein and insulin.

Methods

Insulin concentration of camel colostrum, milk and serum was determined by enzyme-linked immunosorbent assay (ELISA) using a commercially human insulin kit (DRG Instruments GmbH, Germany). The assay was performed in accordance with the manufacturer's instructions.

Serum was prepared by the method of Goers (1993) and kept under -24 °C until analysis.

Total protein (N × 6.38) of milk samples was determined according to Ling (1963). All measurements were carried out in two replicates.

Statistical Analysis

The data were analyzed by a general linear model procedure of the Fisher's Protected Least-Significant Difference (PLSD) test using SAS, 2004 (SAS Institute, Inc., Cary, NC). This test combines ANOVA with comparison of differences between the means of the treatments at the significance level of $P \leq 0.05$. Correlations were calculated using Pearson's correlation coefficient.

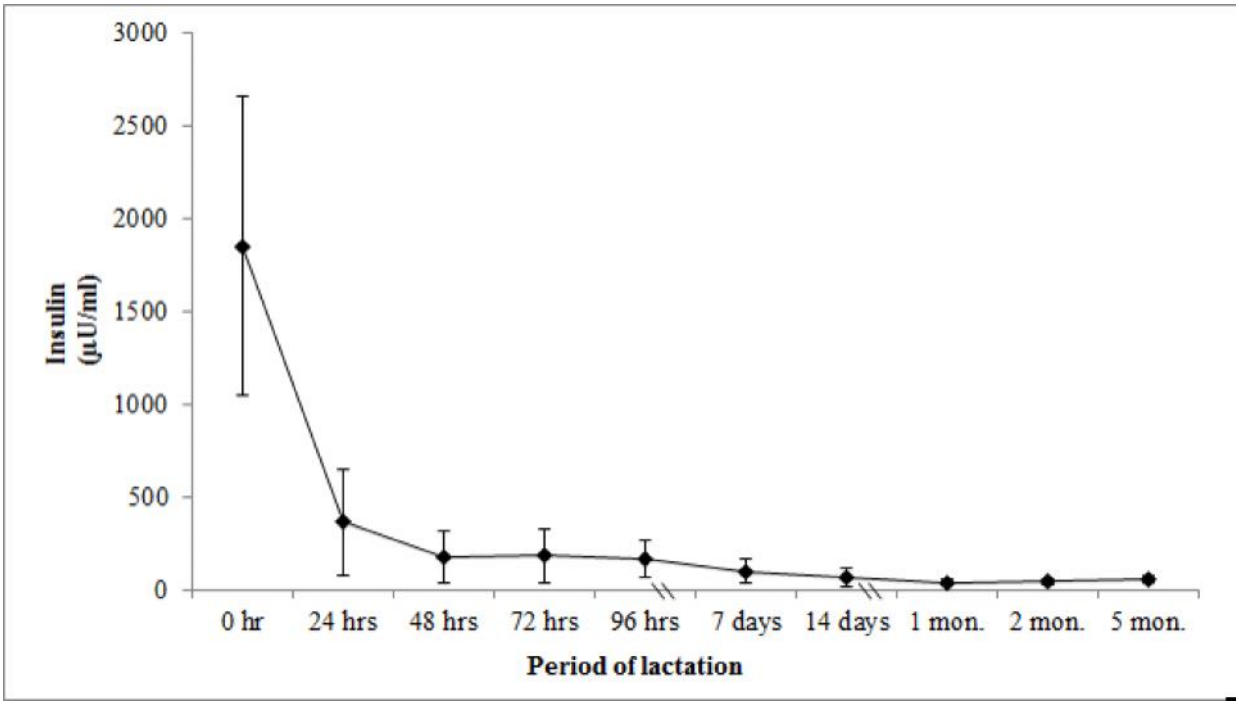
RESULTS AND DISCUSSION

Milk Insulin Concentration

The changes in insulin concentration of camel milk throughout 5 months of lactation are presented in Figure 1. The concentration of insulin in camel milk was significantly ($P \leq 0.05$) affected by the stage of lactation. Colossal concentration of insulin (1856.80 ± 804.40 µU/ml) was detected in colostrum at zero hr (the first suckling after parturition). This concentration declined dramatically ($P \leq 0.05$) to reach a value of 367.5 ± 286.1 µU/ml at 24 hr of lactation, which represents 20% of its initial value. On the contrary, after 24 hr of lactation the concentration of insulin decreased gradually to reach 101.3 ± 65.6 µU/ml on day 7 of lactation and stabilized to some extent between the 2nd week and the 5th month.

A similar trend for changes in insulin concentration in dromedary camel milk during the lactation period was reported by Wernery (2006a). However in the current study, the values of insulin concentration in the colostrum were much higher than the values that were obtained by Wernery (2006a), who reported the mean concentration of insulin

Figure 1: Changes in Insulin Content (Mean ± SD) of Camel Milk During the First 5 Months of Lactation



was 400 µU/ml at the 1st day after parturition. These quantitative differences could be owing to the sampling time, analytical procedure used, genetic variations between animals and environmental factors.

In normal milk period, from the 2nd week until the 5th month of lactation, the stage of lactation had no significant effect on the insulin content of camel milk. The mean concentration of insulin during this period was 55.1 ± 33.2 µU/ml. This value is in line with those reported by Singh R (2001) and Hamad *et al.* (2011) being 52.0 and 58.7 U/L, respectively but it was slightly higher than the mean value obtained by Wernery (2006a), i.e., 40.5 µU/ml.

The presence of insulin in colostrum and milk was also reported in other species, including cows (Ontsouka *et al.*, 2003; and Ollikainen and Muuronen, 2013), buffaloes (Hamad *et al.*, 2011) goats and sheep (Zagorski *et al.*, 1998; and Magistrelli *et al.*, 2005) and humans (Shehadeh *et al.*, 2003).

The high concentration of several proteins in colostrum is a characteristic feature in different milking animals (McGrath *et al.*, 2016). The high levels of insulin in colostrum, particularly during the first 24 hrs after parturition, may be related to its physiological role over this period of calf life. Zagorski *et al.* (1998) suggested that during first few days of calves' life, endocrine system functions of calves to be inferior to adults'. Therefore, high level of insulin in colostrum is considered as an external source of this hormone to regulate the cellular glucose level. During these days, insulin is absorbed through the intestine of newborn calves in its active form until the neonatal endocrine system becomes entirely operative. In addition, other studies declared that Insulin may be important in the development of mammary gland (Mackle *et al.*, 2000), the growth and differentiation of neonate tissues (Aranda *et al.*, 1991) and influences the small intestinal growth and development in neonates (Shulman, 1990).

Blood-Serum Insulin Concentration

The mean concentration of blood-serum insulin was determined during the periods of colostrum (0 hr) and normal milk (Table 1). No significant differences were observed between two periods. The mean value of serum insulin was 5.3 ± 1.2 and 5.3 ± 2.2 µU/ml at 0 hr of lactation and through period of normal milk, respectively. In current study, the mean value of camel serum insulin was lower than the mean value reported by Wernery *et al.* (2006 a) which was 12.8 ± 7.6 µU/ml. In comparison to serum, both colostrum and milk of camels had considerably higher

Table 1: Insulin Content of Colostrum, Milk and Blood Serum During the First 5 Month of Lactation

Sampling Time	Insulin Concentration (µU/ml)	
	Milk	Serum
0 hr	1856.8 ± 804.4^{aA}	5.3 ± 1.2^{aB}
$1^{st} - 5^{th}$ month	46.7 ± 14.4^{Ba}	5.3 ± 2.2^{aB}

Note: Means in the same column with different small superscripts are significantly different at ($P \leq 0.05$), means in the same raw with different capital superscripts are significantly different at ($P \leq 0.05$).

insulin contents. This finding is in line with that reported by Wernery *et al.* (2006a) for camel and in other species (Kinouchi *et al.*, 1998; Xu *et al.*, 2000; and Magistrelli *et al.*, 2005). This may be attributed to the local production of insulin in the mammary glands or to the selective permeation from the blood (Weström *et al.*, 1987). Moreover, Magistrelli *et al.* (2005) suggested that the maternal serum to be the most probable source of insulin in milk, because insulin is only synthesized in considerable amounts in the pancreas, and because of the presence of specific receptors for insulin in mammary gland. Therefore, insulin may be sequestered from maternal serum and transferred into milk. Likewise, Britton and Kastin (1991) proposed that serum insulin could be concentrated and transferred to milk by trans-and paracellular processes.

There was no significant correlation between levels of camel milk insulin in both colostral and normal milk periods and serum insulin. No results have been found concerning the relationship between insulin in camel blood serum and camel milk except those reported by Magistrelli *et al.* (2005) who mentioned that morning goat-milk insulin was not correlated to morning plasma insulin levels.

Insulin Content of Camel Milk from Different Locations

The insulin contents of camel milk samples collected from some locations in Matrouh governorate are shown in Table 2. Significant ($P \leq 0.05$) differences were found between the mean values of milk insulin among locations. Insulin contents of milk collected from locations 1 and 2 were significantly ($P \leq 0.05$) higher compared that from the other two locations. The highest content of insulin was 61.8 ± 16.1 µU/ml in milk from location 2, which did not differ significantly ($P \leq 0.05$) from the insulin of milk from location 1. While the lowest value was 15.7 ± 10.3 µU/ml in milk collected from location 4, which did not differ greatly ($P \leq 0.05$) from location 3. These differences in insulin content of camel milk among locations may be attributable to different factors, particularly the feeding type. In current study, camels from location 1, which belong to Camel Research Center were fed on concentrate feed mixture and animals in location 2 were fed on pasture of wheat and barley, which can be considered as concentrate feed. Whereas animals in the other two locations (3 and 4), were naturally grazing on poor grasses. Previously, Magistrelli *et al.* (2005) reported that feeding of lactating goats can influence the level of milk insulin through the maternal diet, whereas milk insulin was significantly increased by high starch diet. In addition, variations among animals in age, parity, stage of lactation and management may be additional factors for the differences in the content of milk insulin among animals of these locations.

Relationship Between the Concentration of Milk Insulin and Milk Protein

The concentration of insulin and protein in camel milk samples, which collected from four locations of Matrouh governorate, is presented in Table 2. Data showed a variation

Table 2: The Concentration (Mean ± SD) of Insulin and Total Protein in Camel Milk Samples Collected from Four Locations in Matrouh Governorate

Constituents	Locations (Mean ± SD)				Minimum	Maximum	
	1	2	3	4			
Insulin (µU/ml)	55.1 ± 33.2^{a}	61.8 ± 16.1^{a}	21.9 ± 10.5^{b}	15.7 ± 10.3^{b}	6.2	88.3	
Total protein (%)	2.7 ± 0.5^{a}	2.1 ± 0.3^{b}	2.4 ± 0.3^{ab}	2.1 ± 0.2^{ab}	1.8	3.5	
r (insulin/total protein)							0.06^{ns}

Note: Means in the same raw with different superscripts are significantly different at ($P \leq 0.05$); r = Pearson's correlation coefficient; ns: Non significant at $P > 0.05$.

among animals in the concentration of milk insulin and milk protein.

Some studies showed that elevating circulating insulin in dairy cows resulted in an increase in milk protein content (McGuire *et al.*, 1995; Mackle *et al.*, 2000; and Winkelman and Overton, 2013). This effect could be attributed to the important role of insulin in the maintenance of mammary gland's ability to synthesize milk components (Martin and Baldwin, 1971), or due to the temporal alterations in the insulin-like growth factor system during the lifting of the circulating insulin (McGuire *et al.*, 1995; Griinari *et al.*, 1997; and Mackle *et al.*, 1999 and 2000). In current study, the variation in the concentration of insulin of camel milk samples was used to detect any relationship between the level of milk insulin and the content of milk protein.

Estimating the correlation between milk insulin and milk protein revealed a non-significant correlation (r = 0.06), which means the insulin level had no impact on the content of milk protein. A research study by Mackle *et al.* (2000) showed that intramammary infusion of insulin had no effect on the concentration of milk protein in dairy cow.

CONCLUSION

From the preceding results it can be concluded that camel colostrum had considerably high content of insulin than camel milk. Camel milk may provide a valuable amount of insulin depending on the feeding system. More extensive and detailed studies should be carried out to investigate the possible effects of feeding conditions on the levels of camel milk insulin. There is no relationship between the insulin in both serum and milk. Further studies are needed to investigate the mechanism of transferring the insulin from camel blood to milk and the influence of genetic and environmental factors on the content of camel milk insulin.

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