Elevated levels of blood urea nitrogen and creatinine in the last trimester of pregnancy of dromedary camels (*Camelus dromedarius*)

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Key words:
blood parameters, dromedary camel, heavy pregnancy, metabolites

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Received: 20 May 2015
Accepted: 19 August 2015

Abstract:

BACKGROUND: The knowledge of blood parameters is important for assessing the physiological status and health of animals. **OBJECTIVES:** This study was conducted to determine the effect of heavy pregnancy on some serum indices of dromedary camels. **METHODS:** Twenty clinically healthy female dromedary camels aged between 4-5 years were divided into two equal groups: I: pregnant camels in their last trimester; and II: non-pregnant age-matched controls. The concentration of glucose, calcium, phosphorus, albumin, total protein, uric acid, blood urea nitrogen (BUN), creatinine and the activity of aspartate amino transferase (AST), alanine amino transferase (ALT), and gamma glutamyl transferase (GGT), was measured. **RESULTS:** The results of this study show that the concentrations of glucose, calcium and phosphorus remained unchanged in pregnant camels compared to non-pregnant ones. The concentrations of serum BUN and creatinine in pregnant camels were higher, and these aforementioned differences were significant (p=0. 02 and 0.003 respectively). GGT activity was lower in pregnant than in non-pregnant camels (p=0. 04). **CONCLUSIONS:** The increase in BUN and creatinine levels might be part of the homeorhetic mechanisms for adaptation of camels during heavy pregnancy. The high urea-recycling rate in camels may transfer urea to the gastrointestinal tract as a source of "non-protein nitrogen" instead of being excreted as urine. The reduction of GGT as at the time of heavy pregnancy, may be attributed to its conversion to glutathione, as an antioxidant.

Introduction

Camel (*Camelus dromedarius*) is a unique animal, in adapting to the harsh climatic conditions of the desert. Camels can tolerate prolong water deprivation, high heat load, and poor quality feeds. The crucial role of the camel in the economic and social life of pastoralists in arid and semi-arid regions of several places in the world has been noted by researchers (Ouajd and Kamel, 2009; Gaughan, 2011). There is a wide range of mechanisms for coping against nutritional deficiencies in animals. Camels have more efficient fermentation in pre-stomach, high intestinal absorption of nutrients, high neoglucogenesis, low ketogenesis and a high lipomobilization, along with great urea recycling for proteins (Ouajd and Kamel, 2009). Pregnancy is a dynamic process characterized by dramatic physiological changes. The increase in metabolic functions during pregnancy results in alteration of the biochemical profile. The alteration in routine serum clinical biochemistry is yet to be properly studied, during the different stages of pregnancy in camels (Omidi et al., 2014). The liver metabolizes carbohydrates and lipids and also plays an essential role in amino acid metabolism (Ouajd and Kamel, 2009). To evaluate the health of the liver, some blood constituents and enzymes including aspartate amino transferase (AST), alanine amino transferase (ALT), and gamma-glutamyl transferaseare (GGT) were measured routinely. When the liver is injured, the enzymes within the hepatocytes enter the bloodstream (Woreta and Alqahtani, 2014). Total protein and albumin are the proteins made by the liver. Creatinine and blood urea nitrogen (BUN) are waste products removed from the blood by the kidneys. Creatinine is a breakdown product of creatine phosphate in the muscle. Serum creatinine is a marker used for renal function assessment (Kamili et al., 2013; Patel et al., 2013). High BUN usually means that kidney function is less than normal, but other factors may affect the BUN level. The aim of this study was to investigate the effect of heavy pregnancy on the indices of the liver and kidney health in the serum of dromedary camels.

Materials and Methods

This study was conducted on female Iranian single-humped camels in February 2013. The camels were reared at the camel breeding station (52 km away from the city of Zabol, 31°1′47″North latitude, 61°29′52″east longitude, and 478 m above the sea level) in Sistan and Baluchestan Province, Southeastern Iran. Twenty female camels, aged between four and five years were used for this study. The pregnant camels were selected in consultation with a cameleer who is conversant with their mating history. Ten non-pregnant and ten camels in their last three months of pregnancy were selected for this study. Blood samples (10 ml) were obtained from the jugular vein of camels and placed in vacuum containers at 6 p.m. for two consecutive days. The serum was prepared by removing the clot from the whole blood sample and subsequently centrifuged at 750 × g for 15 min. The sera were stored at −21°C awaiting analysis. The samples with hemolysis were discarded. The biochemical parameters were measured using a standard autoanalyser (Hitachi 717, Boehringer. Mannheim, Germany) by commercial kits (Parsazmoon, Co, Iran). The levels of total protein and albumin were determined by biuret reaction (Meyer et al., 1992) and bromocresol green dye binding method (Ueno et al., 2013), respectively. The activity of GGT was measured using the SZASZ method (Szasz, 1976), and those of AST and ALT were measured using the colorimetric method of Reitman and Frankel (1957). Serum enzyme activities were measured according to the specific reaction of each enzyme using basic standard techniques. Thereafter, the glucose level was determined after enzymatic oxidation in the presence of glucose oxidase as described by Barham and Trinder (1972). All data were expressed as SI units. In order to compare the two groups (pregnant and non- pregnant), the non-parametric Mann-Whitney U test was performed. The correlation between two parameters was analyzed using Spearman's correlation test. All statistical calculations were performed using SPSS version 20 software. The experiment was approved by the animal welfare committee of the Agriculture Faculty of Birjand University.

Results

The concentrations of glucose, calcium, phosphorus, albumin, total protein, BUN, creatinine and uric acid, as well as the enzyme

Table 1. Serum levels of some variables in pregnant and non-pregnant camels (n=20). (1)Blood urea nitrogen, (2)Aspartate aminotransferase, (3)Alanine aminotransferase, (4) γGlutamyl transferase. (*) significant at the 0.05 level. (**) significant at the 0.01 level.

variable	Pregnant (n=10)			Non-pregnant (n=10)			
Percentiles	25th	50th (medium)	75th	25th	50th (medium)	75th	p-value
Glucose (mmol/l)	3.39	3.85	4.52	3.20	3.88	4.59	0.91
Calcium (mmol/l)	2.12	2.23	2.25	1.96	2.11	2.25	0.28
Phosphorus (mmol/l)	1.42	1.58	1.61	1.52	1.58	1.64	0.52
Albumin (g/l)	30.02	30.35	30.80	20.60	30.05	30.25	0.07
Total protein (g/l)	50.95	60.70	70.70	50.55	60.10	70.12	0.24
Uric acid (µmol/L)	0.47	0.50	0.50	0.40	0.50	0.50	0.73
BUN1 (mmol/l)	4.28	5.17	6.60	3.48	3.92	4.82	0.02*
Creatinine (µmol/l)	80.44	89.28	97.24	62.76	64.53	78.67	0.003**
AST2 (IU/l)	39.50	45.00	50.00	39.50	42.00	46.25	0.30
ALT3 (IU /l)	2.00	5.00	6.00	3.75	5.00	7.00	0.64
GGT4 (IU /l)	5.10	5.85	6.32	6.05	6.70	7.40	0.043*

Table 2. Correlation coefficients between the variables measured in pregnant and non-pregnant camels. (1)Alanine aminotransferase, (2)Aspartate aminotransferase, (3)Blood urea nitrogen, NS: Non-significant- (*)Correlation is significant at the 0.05 level (two-tailed)- (**) Correlation is significant at the 0.01 level (two-tailed).

Variable A	Variable B	Spearman's correlation (r)	
		Pregnant camel (n=10)	Non-pregnant camel (n=10)
ALT1	Glucose	0.77**	NS
ALT	Albumin	0.78**	NS
AST2	Albumin	0.68*	NS
AST	BUN3	NS	0.66*
Albumin	Total protein	NS	0.79**
Albumin	Creatinine	NS	0.71*
Creatinine	Total protein	NS	0.81**
Creatinine	BUN	NS	0.72*
Total protein	BUN	NS	0.63*

activity of AST, ALT and GGT in the blood serum of pregnant and nonpregnant camels are shown in Table 1. Physiological status and pregnancy period had a significant effect on the levels of BUN and creatinine. Lower serum levels of GGT were observed in pregnant camels. The Spearman rank correlation coefficients are shown in Table 2 for only those health indicators that significantly correlated with the concentration of at least one constituent. Eventually, all pregnant camels carried pregnancy to term and each delivered a calf.

Discussion

In late pregnancy, the nutrient requirements increases to meet the growth requirements of the fetus. In the present study, glucose concentration remained without difference in pregnant camels. Thus, this finding is in line with that of Zvorc et al. (2006) in sows during pregnancy. Contrary to these findings, some researchers reported lower serum glucose levels in pregnant camels (Saeed et al., 2009), pregnant goats (Khan and Ludri, 2002), and pregnant sheep (Moallem et al., 2012). A wide variation in the blood glucose concentration of camels was reported. The glucose level of the blood ranged from 1.5 mmol/l in adult camels to 12 mmol/l in 7 day old calves (Yadav and Bissa, 1998). This wide range of glucose level may be attributed to the difference in age, sex, physiological conditions and seasonal differences (Barakat and Abdel-Fattah, 1971; Nazifi et al., 1999; Amin et al., 2007). The camels considered for the present study were of the same age and there was no seasonal variation in sampling time. Approximately, the low serum glucose concentration of camels in this study (3.85 mmol/l) may be attributed to protein and energy deficiency in the diet during winter (Nazifi et al., 1999). Therefore, unchanged blood glucose concentrations in pregnant camels need to be considered as a result of sufficient homeostasis, in maintaining the concentration of blood glucose in a constant range. In this study, calcium and phosphorus concentration remained unchanged in pregnant camels. Contrary to the results of this study, in various investigations on pregnant camels (Saeed et al., 2009), Holstein cows (Nazifi and Sami, 1997), and mares (Filipović et al., 2010), calcium and phosphorus levels were reported to be lower than that of the non-pregnant animals. The similar levels of calcium and phosphorus in pregnant and non-pregnant camels might be due to a balance between the increase in absorption of these minerals from the intestine and their sufficient supply to the fetus (Fudge and Kovacs, 2010). The range of total protein concentration reported by various researchers is 50.5 to 80.0 g/l in camels (Yadav and Bissa, 1998). The higher serum levels of total protein and albumin observed in pregnant camels in comparison with nonpregnant camels were not significant (60.70 vs. 60.10 g/l and 30.35 vs. 30.05 g/l respectively). The range of serum total protein in this study is in accordance with the study of Dowelmadina et al. (2012). During heavy pregnancy, there is abundant synthesis of proteins in the liver, and this is as a result of the higher energy requirement for fetal growth. Glucocorticoids improve the mobilization of extra hepatic proteins and transport amino acids to liver cells. The mobilized amino acids in liver cells are utilized during gluconeogenesis, which is the primal source of energy for the fetus (Satué and Montesinos, 2013). The slight increase in total protein concentrations in late pregnancy, is as a result of hormonal changes in the organism. The higher levels of total protein concentration in the blood plasma of pregnant mares compared to nonpregnant ones were recorded by Milinković-Tur et al. (2005). The interpretation of variations in liver and serum enzyme activities is complicated because the activity is affected by changes in the levels of cofactors, activators and inhibitors, as well as by changes in the concentration of the enzyme itself. ALT and AST activities showed significant correlation with serum albumin, corresponding to the higher activity of the liver along with natural mechanisms, which combat oxidative stress during heavy pregnancy. In the serum, albumin represents the major plasma antioxidant component (Lin et al., 2009). In pregnant camels, the positive correlations between ALT activity and glucose were seen. This finding revealed that the higher activity of the liver had a significant effect on glucose metabolism. Some researchers suggested that liver enzymes, especially ALT, were significantly associated with insulin resistance in human diabetic patients (Zhang et al., 2010). Insulin resistance in camels differs most likely with sheep and ponies. The high plasma glucose concentrations together with low insulin levels may be of advantage to camels with extremely poor quality feeds (Elmahdiwt al., 1997). The correlations between some parameters in non-pregnant camels were positive. On the contrary, these correlations differed in pregnant camels, indicating homeostatic mechanisms for each component. By comparing the obtained results, a statistically significant decrease in the GGT activity was recorded in the pregnant camels relating to the non-pregnant (5.85 vs. 6.70 IU/l). Similar finding in pregnant mares have been found by Milinković et al. (2005). They found that GGT activity reached the lowest value in the final third of the pregnancy period. GGT is involved in the metabolism of glutathione (the most abundant cellular thiol antioxidant), which plays an important role in maintaining the antioxidative status of the entire body (Chen et al., 2013). The most striking result of the present study is that, BUN and creatinine in pregnant camels were higher than non-pregnant ones and this difference was significant (p=0.02 and p=0.003, respectively). The quantity of creatinine formed each day depends on the total body content of creatine, which in turn depends on the dietary intake, rate of synthesis of creatine, and muscle mass (Patel et al., 2013). Camelids have very powerful mechanisms in urea recycling. Camels can recycle up to 90% of BUN, in contrast to ruminants, which present a value of 10 to 30%. The nitrogen recycling in camels increases in the case of lower proteins in diet and/or dehydration (Ouajd and Kamel, 2009). Camel has very particular anatomical structures in the kidney (Monjezi et al., 2014). Only a small volume of water is lost during the elimination of urea by the production of concentrated urine (Abere and Oguzor, 2011). Significantly higher BUN was recorded in pregnant camels (5.17µmol/l) compared with the nonpregnant ones (3.92 µmol/l). The creatinine values observed in pregnant and non pregnant camels were similar to those previously published by other researchers (Kamili et al., 2013). Furthermore, the creatinine of pregnant camels (89.28 µmol/l) was significantly higher than nonpregnant camels (64.53 µmol/l). In the present study, the camels were not dehydrated or deprived of water but their access to feed supplies was limited. It is known that kidney function in camels is less sensitive to dehydration compared to other species (Kamili et al., 2013). Changes in the concentration of plasma creatinine depend not only on the renal excretion of creatinine, but also on its production and volume of distribution. The exceptionally high level of BUN in camels, in comparison to other livestock, is of interest in view of the camel's ability to utilize urinary nitrogen at times of poor grazing or water deprivation. The highest value of BUN in the late pregnancy period was reported by Durak and Altinek (2006) in ewes. The high requirement for energy by pregnant ewes led to an increase in BUN level. In summary, the present study revealed that protein catabolism and high need for energy by pregnant camels, during the late trimester of the pregnancy period, may affect the catabolism of protein in the body resulting to an increase in BUN and creatinine levels. On the other hand, the high urea-recycling rate in camels may transfer urea to the gastrointestinal tract as a source of "non-protein nitrogen" instead of being excreted as urine. Kidney health indices must be cautiously interpreted especially in heavy pregnancy period. Further research should be done to investigate the physiologic state of the liver and kidneys of dromedary camels experiencing a heavy pregnancy.

Ackvowledgments

This article was extracted from the thesis prepared by Mahsa Asiaban to fulfill the requirements required for earning the Master of Science degree. The authors gratefully acknowledge the financial support for this work that was provided by the Vice Chancellor for Research at Birjand University and the kind help of the animal research center of Zabol district and Shiraz University.

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مجله طب دامی ایران، ۱۳۹۴، دوره ۹، شماره ۴، ۲۵۵-۲۴۹

سطوح بالای ازت اوره خون و کراتینین در سه ماهه آخر آبستنی شتر تک‌کوهانه (*Camelus dromedarius*)

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(دریافت مقاله: ۳۰ اردیبهشت ماه ۱۳۹۴، پذیرش نهایی: ۲۸ مرداد ماه ۱۳۹۴)

چکیده

زمینه مطالعه: برای ارزیابی وضعیت فیزیولوژیکی و سلامت حیوانات، دانش در مورد ترکیبات خونی نقش مهمی دارد. **هدف:** این مطالعه با هدف تعیین اثر آبستنی سنگین بر برخی شاخص‌های سرمی شتر تک‌کوهانه انجام شد. **روش کار:** بیست شتر تک کوهانه ماده سالم بین ۴-۵ سال به دو گروه مساوی تقسیم شدند. یك: شترهای آبستن در سه ماهه آخر آبستنی و دو : شترهای شاهد همسن و غیرآبستن . غلظت گلوکز، کلسیم، فسفر، آلبومین، پروتئین تام، اسید اوریك، نیتروژن اوره خون (BUN) ، کراتینین و فعالیت آسپارتات آمینو ترانسفراز (AST)، آلانین آمینو ترانسفراز (ALT) و گاماگلوتامیل ترانسفراز (GGT) اندازه‌گیری شد. **نتایج:** غلظت گلوکز، کلسیم و فسفر در شترهای آبستن نسبت به شترهای غیر آبستن بدون تغییر باقی ماند. غلظت BUN و کراتینین سرم در شترهای آبستن بالاتر از شترهای غیر آبستن بود و این اختلاف معنی‌دار بود، به ترتیب ($p=۰/۰۰۳$ and ۰/۰۲). میزان فعالیت GGT در شترهای آبستن نسبت به غیر آبستن پائین‌تر بود ($p=۰/۰۴$). **نتیجه‌گیری‌نهایی:** افزایش میزانBUN و کراتینین ممکن است بخشی از مکانیسم‌های هومئورتیک در آداپته شدن شترها به شرایط آبستنی سنگین باشد. میزان بالای چرخش مجدد اوره در شترها ممکن است اوره را به جای دفع از طریق ادرار به دستگاه گوارش به عنوان یک منبع نیتروژن غیر پروتئینی منتقل کند. کاهش GGT در زمان آبستنی سنگین، ممکن است به دلیل تبدیل به گلوتاتیون به عنوان یک آنتی اکسیدان باشد.

واژه‌های کلیدی: پارامترهای خونی، شتر تك کوهانه، آبستنی سنگین، متابولیت‌ها

*) نویسنده مسؤول: تلفن: ۳۶۱۳۸۷۴۵ (۷۱)۹۸+ نمابر: ۳۲۲۸۶۹۴۰ (۷۱)۹۸+ Email: aomidi@shirazu.ac.ir