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EFFECT OF AGE ON CERTAIN URINE PARAMETERS OF YOUNG CAMELS (*Camelus dromedarius*)

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Supporting Information

ABSTRACT: The objective of this study was to identify reference values for certain urine parameters in relation to the age in young dromedary camels. Twenty one healthy young female camels (age <3-5 months) of Arabi local breed were divided into three groups ($\leq 3 \text{ m}$: n=6, 3-4 m: n=8 and 4-5 m: n=7). Jugular venous blood samples were collected and used to determine electrolytes and creatinine concentration, whereas the urine samples were used for the determination of urine pH, urine osmolality, and for the calculation of fractional excretion of electrolytes. The mean value of urine pH was 7.9±0.5 (reference range: 7.2-8.8 and the percentile range: 7.3-8.2) and the urine osmolality in young camels was 978.9±468 (reference range 235-1819 mOsmol/kg) for all age groups. Fractional excretion of Na⁺ (FE _{Na+}%) showed lower mean values compared to the fractional excretion of K⁺ and Cl⁻. The age had a significant (P<0.05) effect only on urine osmolality in camels of 4-5 months. The identified reference values of certain urine parameters can be used for the clinical diagnosis of renal diseases in growing animals. The data could be utilised for the clinical monitoring of the physiological and pathological status of animals fed strong electrolytes such as Na⁺, K⁺ and Cl⁻.

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INTRODUCTION

The kidneys are well known as being the main organs in the body responsible for the regulation of the extracellular electrolyte concentration and acid-base status of mammals by adjusting urine electrolyte excretion to maintain constant systemic pH (Bevensee and Boron, 2008). Evaluation of the renal function has been accomplished with the analysis of blood and urine to compare the concentration of electrolytes and creatinine in blood and urine, which is known as Fractional excretion of certain electrolyte (FE electrolyte).

The FE of electrolytes is a simple, inexpensive method that has been shown to be a reasonable indicator for renal clearance of electrolytes in animals (Lefebvre et al. 2008; Braun and Lefebvre, 2008). Therefore, measurement of FE electrolyte can be used as a potential tool to assess the kidney function (Lefebvre et al., 2008) and as a diagnostic and prognostic application to renal dysfunction (Troia et al., 2018). In veterinary practice, the FE of electrolytes has been calculated by comparing the amount of the substance excreted in the final urine with the amount filtrated through the glomerulus (Fleming et al., 1992).

Under physiological conditions, a considerable variation on the FE _{electrolyte} in relation to the age has been reported previously in calves (Henniger et al., 2013), dogs (Laroute et al., 2005), cats (Hoskins et al., 1991) and horses (Edwards et al., 1990). Many researchers have stated that the FE _{electrolyte} has been used to investigate and validate the evaluation of dietary adequacy, the response to treatment and the impaired renal function in animals (Fleming et al., 1991; Fleming et al., 1992; Ulutas et al., 2003; Ulutas and Sahal, 2005). In clinical practice, FE _{electrolyte} has been applied to cattle before calving in order to reduce their susceptibility to metabolic diseases mainly milk fever (Ulutas et al., 2003; Mellau et al., 2002) and in dairy cows with left displaced abomasum (Perotta et al., 2018). In camels, FE _{electrolyte} has been applied to cattle before applied to camels with experimentally induced metabolic acidosis (Elkhair, 2008).

Measurement of urine pH has been used as a practical and inexpensive method for monitoring the effectiveness of dietary cation-anion difference (DCAD) in lactating cows (Hu and Murphy, 2004) and to assess renal function after acid-load with NH₄Cl (Elkhair and Hartmann, 2010). Lubetskaya and Melnichuk (1999) used urine pH as an index for calculating the amount of bicarbonate for the treatment of acidotic calves. On the other hand, Mellau et al. (2002)

used urine pH as an indicator to assess the influence of short-term anion salt exposure in cows with experimentally induced hypocalcaemia. A close relationship has been reported previously between urine pH and diet content in cattle (Hu and Murphy, 2004; Hu et al., 2007). However, to our knowledge no published data has been available regarding the monitoring of urine parameters in young camels of different ages. Consequently, the study was aimed to identify reference values for certain urine parameters in young dromedary camels in relation to their age.

MATERIALS AND METHODS

Ethical approval

The study was approved by The Sudan Veterinary Council (EA/0030/2018).

Animals and Management

Twenty one healthy young female camels of Arabi local breed (Aged <3-5 months) were divided into three groups (\leq 3 m: n=6, 3-4 m: n=8 and 4-5 m: n=7). The young female camels were housed in an outdoor environment in a shaded corral in Camel Research Centre farm (Faculty of Veterinary Medicine, University of Khartoum, Sudan). The animals were maintained on grazing and browsing trees and shrubs in the vicinity of the Camel Centre and occasionally received fresh grass (*Sorghum lactabiocolor*, Abu 70) and concentrate supplements, which was offered daily with free access to fresh water.

Sample collection and laboratory analysis

The experiment was conducted during the period January 2007 to January 2008. Jugular venous blood samples were collected in the morning before starting feeding by jugular venipuncture using plastic syringes (7.5 mL, Pirmvetta®, Laboratory Technique, GmbH, Germany). The blood samples were centrifuged and the serum was collected in sterile containers for further analysis of serum electrolytes and creatinine concentration using a biochemical analyser (Roche Hitachi Modular, Roche) (Table 1).

A volume of 100 ml urine samples were collected in sterile containers via free catch or by perineal or preputial stimulation of the camels at the same time of the blood collection. Ten ml of fresh urine samples were used to determine urine pH using a pH meter (InoLab, Scientific Technical Workshops, Weilheim, Germany) (Table 1). The pH-meter was calibrated using a two-point calibration with pH 10.0-5.0. Urine osmolality was determined by the Osmometer (OSMOMAT 030, gonotec, Germany) (Table 1). The rest urine samples were used for the determination of electrolytes and creatinine concentration using a biochemical analyser (Roche Hitachi Modular, Roche) (Table 1).

Table 1 - Blood and urine parameters and methodology of measurements						
Parameter	Methodology	Equipment	CV (%)			
Urine pH	Ion selective electrode	pH meter				
Osmolality	Freezing point-depression measurement	OSMOMAT 030	1.3			
Na ⁺		Roche Hitachi Modular, Fa. – Roche Diagnostics –	1.26			
K+	Ion-selective electrode measurement		1.77			
CI-			2.39			
Creatinine	Kinetic colour test after Jaffé		2.59			
CV: Coefficient of variation						

Electrolytes and creatinine concentrations were used for the calculation of fractional excretion of electrolytes as described by Fleming et al. (1991):

 $\mathsf{FE}_{\mathsf{electrolyte}} (\%) = \frac{\mathsf{Urine-[electrolyte]}}{\mathsf{Plasma-[electrolyte]}} \times \frac{\mathsf{Plasma-[creatinine]}}{\mathsf{Urine-[creatinine]}} \times 100$

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 20.0. The normal distribution of the individual data was determined using a One-Sample Kolmogorov-Smirnov adjustment test. The statistical analysis of certain urine parameters were estimated using descriptive statistics procedures of the same programme. ANOVA tests (Levine's Test and Post Hoc Test) were used to assess the possible significant differences between the age groups. The mean difference was considered significant at $P \leq 0.05$.

34

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RESULTS

The statistical data of certain urine parameters shown in table 2 demonstrated that the mean values of urine pH and urine osmolality of young camels were 7.9 \pm 0.5 (Reference range: 7.2 – 8.8) and 979 \pm 468 mOsmol/kg (Reference range: 235-1819 mOsmol/kg), respectively. The values mean values of FE _{electrolytes (%)} of young camels for Na⁺, K⁺ and Cl⁻ were 0.07 \pm 0.04, 13.3 \pm 5.5 and 1.4 \pm 0.8, respectively.

The detailed results shown in table 3 indicated that the age had no significant effect on the most of urine parameters investigated. Urine osmolality showed a gradual decrease with advancing age; however, urine osmolality of the young camels (4-5 months) showed higher (P<0.01) values of 1179 ± 244 mOsmol/kg compared to the other age groups (891±362 and 870±496 mOsmol/kg).

iems	Statistical values	Young camels
Number of animals		21
Age		3 – 5 m
	$\overline{x} \pm s$	7.9±0.5
	Reference range ¹	7.2-8.8
Urine pH	Median	7.96
	(13. Quartile)	7.3-8.2
	$\overline{x} \pm S$	979±468
Osmolality	Reference range ¹	235-1819
(mOsmol/kg)	Median	1112
	(13. Quartile)	559-1292
	$\overline{x} \pm S$	0.07±0.04
FF (9/)	Reference range ¹	0.03-0.2
FE _{Na+} (%)	Median	0.06
	(13. Quartile)	0.05-0.1
	$\overline{x} \pm S$	13.3±5.5
FE κ+ (%)	Reference range ¹	1.9-3.9
$\Gamma \sqsubset K + (70)$	Median	12
	(13. Quartile)	12-16
	$\overline{x} \pm S$	1.4±0.8
FE a. (9/)	Reference range ¹	0.2-3.2
FE ci- (%)	Median	1.3
	(13. Quartile)	0.8-1.9

Table 3- Effect of age on certain urine parameters of young dromedary camels (n= 21)					
Items	Exp	Experimental young camels			
Number of animals	6	8	7		
Age	≤ 3 m	3-4 m	4-5 m		
Urine pH	7.9 ª±0.5	8.1ª±0.6	7.7 ª±0.8		
Urine osmolality (mOsmol/kg)	891ª±362	870ª±496	1179 ^b ±514		
FE _{Na+} (%)	0.06 °±0.03	0.07 ^a ±0.02	0.08 a±0.06		
FE _{K+} (%)	10.8 ª±4.2	14.2 ª±5.2	14.3 °±6.9		
FE _{CI-} (%)	1.2 ª±0.8	1.2 ª±0.7	1.8 °±0.8		
m: month; Means within the same row bearing different superscripts are significantly different at P≤0.05					

DISCUSSION

To our knowledge no published data is available regarding the reference values of certain urine parameters in camels less than 6 months of age. Consequently, the present study was proposed to identify a reference range for certain urine parameters in young camels for subsequent use. The statistical data obtained in the present study for the urine pH of 7.9±0.5 demonstrated the significant impact of the nutritional status of the animal on urine pH because the young camels fed milk and hay. Therefore, the result can be considered as a physiological or renal response to the diet shift from milk to hay.

The results presented in table 2 showed that the values of FE _{electrolytes} of young camels higher than those that of young ruminants mainly calves (Elkhair, 2008). This pattern of response could be explained by the specific morphometric features of the camel's kidney such as a long loop of Henle, a well-developed medulla and lower glomerular filtration rate (El Bahri et al., 1999; Abdalla and Abdalla, 1979). Moreover, the camel's kidney is known to have a strong capacity of water reabsorption and high ability to eliminate very concentrated urine (Abdalla and Abdalla, 1979; Ouajd and Kamel, 2009). Therefore, the higher mean values of FE _{Na+} and FE _{K+} of young camels obtained in the present study could be explained by the effects of renin- angiotensin- aldosterone system on the renal secretion of these electrolytes (Brobst, 1986), which influenced urine osmolality. Furthermore, many investigators stated that urinary excretion of electrolytes is a complex process involving filtration, reabsorption and secretion in various areas of the nephron (Koeppen and Stanton, 2013). Each of these processes may be influenced by numerous factors such as age (Hartmann et al., 1987), dietary intake (Hu and Murphy, 2004), the status of body fluids and the activity of various hormones (Koeppen and Stanton, 2013). Therefore, critical attention of this situation should be considered in fluid therapy of these animals.

The detailed results shown in table 3 indicated that the age had no significant effect on the most of urine parameters investigated. Urine pH of the calves showed approximately similar mean values ranging from 7.7-8.1 for all age groups and appeared to be neutral or alkaline urine. In contrast, variations on urine parameters have been reported previously in relation to the age in other animal species (Henniger et al., 2013; Elkhair, 2008; Laroute et al., 2005; Hoskins et al., 1991; Edwards et al., 1990).

The results obtained in the present study indicated that urine osmolality decreased with advancing age. This pattern of response can be attributed to the fact that the renal function can be modified with advancing age to satisfy the demands of animal's growth and/or depend on their nutritional status.

CONCLUSION

Urine pH, urine osmolality and urinary FE electrolyte can be used for monitoring of renal function in camels. The nutritional status of the animals has an influence on the renal function of the young camels rather than their ages. Further investigations are needed to identify a reference range for quantitative renal parameters to each age group. The results can be utilised for monitoring the effectiveness of applied fluid therapy in camels.

DECLARATIONS

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Author's contribution

The author performed the data collection and analysis. The author reviewed information, wrote up and approved the final manuscript.

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Conflict of interest

The author declares that there was no conflict of interest

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