Vol 17 No 2, p 241-244

A COMPARATIVE STUDY ON SERUM VITAMIN K_1, A AND E LEVELS IN CAMELS (*Camelus dromedarius*) AND OTHER ANIMALS

A.M. Homeida[1], M.F. Hussein[2], R.S. AL-Jumaah[2], A.I. AL-Haidary[2], M.A. AL-Shaikh[2], I. Albokhadaim[1] and A. Gar elnabi[2]

[1]Camel Research Centre, King Faisal University, P O. Box 1757, Al-Hasa 31982, Saudi Arabia
[2]Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

ABSTRACT

Serum levels of vitamins K_1, A and E were determined in 22 camels (*Camelus dromedarius*). Samples from cows, sheep, goats and sand gazelles (*Gazella subgutturosa marica*) in the same area were included for comparison. Concentration of vitamin K_1 in adult camels (0.923±0.234 nmol/l) was significantly higher than in <1-year old camels (0.409±0.0.063 nmol/l). Vitamin K_1 concentration in adult camels was also significantly higher than that recorded in bovines, sheep and goats. Vitamin A and vitamin E levels were comparable in adult and young camels.

Key words: Camels, serum, vitamin A, E, K

Vitamin K is a fat soluble vitamin occurring naturally in two forms, as vitamin K_1 (phylloquinone) in plants and vitamin K_2 (menaquinone) produced by intestinal bacteria (Furie *et al*, 1999). Vitamin K_2 is not a single compound but a series of substances with side chains of varying lengths (Furie and Furie, 1992). Vitamin K is required for the liver production of clotting factors II, VII, IX and X (Gibbs, 1995). These factors are all glycoproteins with several 6-carboxyglutamic (GLa) acid residues clustered at N-terminal end peptide chain (Suttie, 1980). Other vitamin K-dependent GLa proteins include proteins C, S and Z which also function in the regulation of coagulation (Clouse and Comp, 1987). In addition to blood coagulation (Mann, 1999), vitamin K plays a key role in vascular biology (Berkner and Runge, 2004) and bone metabolism via synthesis of GLa-proteins (osteocalcin) and matrix GLa-proteins (Price, 1988).

Acquired clotting defects are more common than hereditary in domestic animals (Gentry and Downie, 1993). These include liver disease, vitamin K deficiency and excessive oral anticoagulant therapy, each of which requires treatment with vitamin K (Vermeer and Hamulyak, 1991).

There is a lack of published information on vitamin K in the camel's blood and of studies dealing with comparative aspects of vitamin K in domestic animals. Fat soluble vitamin requirements for all domestic animals except for the camel are published by National Academy of Sciences (Bishop, 2004). Therefore, vitamins A and E were assayed along with vitamin K in camels. Few other studies have been reported on vitamins A and E (Snow *et al*, 1992; Abbas and Ali, 2001; Mohamed, 2004; Mohamed, 2006) and more recently vitamin D (Mohamed, 2008) in Arabian dromedary camels. In view of this a comparative study on Vitamin K_1, A and E levels in camels and other animals was undertaken.

Materials and Methods

Blood Samples

Blood samples were collected by jugular venipuncture into plain vacuotainer tubes from 22 healthy camels (*Camelus dromedarius*), comprising 11 adult female and 11 young (< 1 yr) male camels, in addition to 8 adult cows (Friesian-Holstein), 10 adult female goats (*Aradi* breed), 8 adult male sheep (*Awassi* breed) and 4 female Arabian sand gazelles (*Gazella subgutturosa marica*), randomly sampled from Riyadh area in central Saudi Arabia. Sera were separated by clotting and stored at -30°C until analysis.

Vitamin Analysis

Vitamins A, E and K were estimated by HPLC method (Paixao and Campos, 2003). Briefly, 100

SEND REPRINT REQUEST TO A.M. HOMEIDA email: Homeida52@hotmail.com

µl samples of serum were extracted with 500 µl acetonitril, and the extracts were centrifuged at 2000 g for 5 minutes, evaporated under a stream of N_2 gas and re-dissolved in 2 ml hexane. Quantitative analysis was performed in a reverse phase HPLC system (SHIMADZU Corp., Kyoto, Japan) consisting of system controller (SCL-10AVP), column oven (CTO-10ACVP) and solvent delivery module (LC-10-ADVP). The mobile phase consisted of 95% methanol and 5% ethanol and the flow rate was 0.8 ml per minute. The detection was performed by an UV detector at three wave-lengths 325 nm for vitamin A, 300 nm for vitamin E and 245 nm for vitamin K. The quantification was performed with delivered standard solution (Sigma, UK). The vitamin concentrations were calculated via integration of the peak areas in the external standard calibration mode.

Data were analysed by ANOVA, using GLM procedure in SAS (Goodnight *et al*, 1986) and Duncan's multiple range test was used to detect significant differences between the means.

Results

Results of vitamin K_1 in serum of camels are given in table 1. Mean vitamin K concentrations were significantly (P> 0.05) higher in camels as compared to cattle, sheep and goats. Mean values of the vitamin in camels and gazelles were nearly double the values in other domestic animals. Values of vitamin K_1 in adult were also significantly higher than in young camels. Indeed mean vitamin K_1 values in young camels were comparable to those in other domestic animals.

Table 1. Mean (±SD) and ranges of vitamin K concentrations in serum of camels, cattle, sheep, goats and gazelles.

Species	(No.)	Mean (nmol/l)	±SD	Range
Camels				
Adult (3-4 years)	(11)	0.923[a]	0.234	0.717 - 1.329
Young (>1 year)	(11)	0.409[b]	0.063	0.268 - 0.559
Cattle	(8)	0.699[b]	0.072	0.556 - 0.776
Sheep	(8)	0.432[b]	0.109	0.271 - 0.464
Goats	(10)	0.446[b]	0.086	0.322 - 0.56
Gazelles	(4)	1.023[a]	0.114	0.90 - 1.125

a-b = Means bearing different superscripts are statistically different (P<0.05).

Table 2. Mean ±SD and ranges of vitamins A and E concentration in serum of camels.

Animals	(No.)	Mean	±SD	Ranges
Adult	(11)			
	Vit A (nmol/l)	0.156	0.063	0.105 – 0.276
	Vit E (mmol/L)	0.293	0.172	0.081 – 0.293
Young	(11)			
	Vit A (nmol/l)	0.132	0.044	0.085 – 0.181
	Vit E (mmol/L)	0.259	0.104	0.133 – 0.374

The mean values of vitamins A and E are summarised in table 2. These values were not significantly different between adult and young camels. The ranges for both vitamins in adult were also comparable to those in young camels.

Discussion

In the current study phylloquinone (vit K_1) but not menaquinone (vit K_2) was measured due to the fact that utilisation of nutritional phylloquinone for prothrombin synthesis is better than that of menaquinone (Groenen-van *et al*, 1995; Groenen-van *et al*, 1993). In addition, the intestinal micro-flora produces substantial amount of vit K_2, but the extent to which these products are absorbed has remained a matter of debate (Suttie, 1980).

Significantly higher levels of vitamin K_1 were observed in camels and gazelles compared to other domestic animals. It is established that factors determining body pool size of a vitamin are type of diet, rate of absorption, utilisation and metabolism (McDowell, 1989). In this study higher levels of vitamin in sera of camels may be due to high uptake of vitamin K_1 from plants. An explanation for this high uptake may come from the fact that the phylloquinone originates from pasture. The predominant pasture plants of camels in this area contain high amount of oil in g/kg dry matter basis, required to dissolve the vitamin. These plants included *Haloxylon saliconium* (31.5 g/kg), *Centenium elegant* (24 g/kg) *and Trifolium alexandrnum* (18 g/kg) (Gaili *et al*, 2000). In human subjects the uptake of vitamin K from green vegetables was 5 – 15 % depending on the fat content of the meal (Gijsbers *et al*, 1996). A marked difference was observed in the absorption of phylloquinone dissolved in oil and the slightly more hydrophilic menaquinone (Koivu-Tikkanen *et al*, 2000).

Lower levels of vitamin K_1 in serum of young camels compared to adults may further substantiate the observation that these young animals were still largely dependant on milk which may contain lower amount of the vitamin compared to pasture. Measurement of vitamin K in milk and supplementing vitamin K to pre-ruminants deserve further research.

Regarding serum vitamin A and E, the values were almost similar in adult and young camels

especially when ranges of concentration of vitamins are considered, suggesting that milk may also contain an appreciable levels of vitamins (Stahl *et al*, 2006). An insignificant age effect on these vitamins was previously observed in the llama (Smith *et al*, 1998). The present vitamin A values are comparable to those reported in Sudanese camels (Mohamed, 2004) whereas vitamin E values are lower than those reported in the latter as well as in racing camels in the United Arab Emirates (Snow *et al*, 1992). Both vitamins may interact in lipophilic areas in the body to protect cellular and liquid biological system against oxidative damages (Guo and Packer, 2000). Values of vitamin A were always higher (Snow *et al*, 1992) and vitamin E were always lower (Snow and Frigg, 1990; Al-Senaidy, 1996) in camels than those in other species. However, Hidiroglou and Williams (Hidiroglou and Williams, 1986) observed the reverse trend for plasma a-tocopherol depending on the diet. Therefore, the type of diet and other ecological factors should be considered when comparing results from different authors.

References

Abbas TA and Ali BH (2001). s Retinol values in the plasma of the Arabian camel (*Camelus dromedarius*) and the influence of aphlatoxicosis. Veterinary Research Communication 25:517-522.

Al-Senaidy AM (1996). Tocopherols in camel's plasma and tissues. Internationl Journal of Vitamin and Nutrition Research 66:210-216.

Berkner KL and Runge KW (2004). The physiology of vitamin K nutriture and vitamin K-dependent protein function in atherosclerosis. Journal of Thromb. Haemost. 2:2118-2132.

Bishop Y (2004). The Veterinary Formulary. 6th Revised edition. Pharmaceutical Press, London. pp 430-435.

Clouse LH and Comp PC (1987). The regulation of hemostasis: the protein C system. New England Journal of Medicine 314:1298-1304.

Furie B and Furie BC (1992). Molecular and cellular biology of blood coagulation. New England Journal of Medicine 326:800-806.

Furie B, Bouchard BA and Furie BC (1999). Vitamin K-dependent biosynthesis of gamma-carboxyglutamic acid. Blood 93(6):1798-1808.

Gaili ESE, Al-Eknah MM and Sadek MH (2000). Comparative milking performance of three types of Saudi camels (*Camelus dromedarius*). Journal of Camel Practice and Research 7:73-76.

Gentry PA and Downie HG (1993). Blood Coagulation and Hemostasis In: Swenson, M. J. and Reece, Wo (eds) Dukes, Physiology and Domestic Animals 11th ed. Ithaca, Cornell University Press N.Y. pp 49-63.

Gibbs CS (1995). Conversion of thrombin into an anticoagulant by protein engineering. Nature 378:413–416.

Gijsbers BLMG, Jie K-SG and Vermeer C (1996). Effect of food composition on vitamin K absorption in human volunteers. British Journal of Nutrition 76:223–229.

Goodnight JH, Sall JP and Sarle WS (1986). The GLM Procedure. In: SAS User's Guide. Statistics. NC, USA.

Groenen-van Dooren MMCL, Soute BAM, Jie K-SG, Thijssen HHW and Vermeer C (1993). The relative effects of phylloquinone and menaquinone-4 on the blood coagulation factor synthesis in vitamin K-deficient rats. Biochem. Pharmacology 46:433–437.

Groenen-van Dooren, Ronden MM, Soute BA and Vermeer C (1995). Bioavailability of phylloquinone and menaquinone after oral and colorectal administration in the vitamin K-deficient rat. Biochem. Pharmacology 50:797-801.

Guo Q and Packer L (2000). Ascorbate-dependent recycling of the vitamin E homologue Trolox by dihydrolipoate and glutathione in murine skin homogenates. Free Rad Biol. Med. 29:368-374.

Hidiroglou, M. and Williams, C. J. (1986). Interrelationship among liposoluble in ruminants. American Journal of Veterinary Research 47:1767-1771.

Koivu-Tikkanen TJ, Schurgers LJ, Thijssen HHW and Vermeer C (2000). Intestinal, hepatic and circulating vitamin K levels at low and high intakes of vitamin K in rats. British Journal of Nutrition 83:185-190.

Mann KG (1999). Biochemistry and physiology of blood coagulation. Thromb. Haemost. 82:165-174.

McDowell LR (1989). In: Mc Dowell, L.R. (Ed.). Vitamins in Animal Nutrition – Comparative Aspects to Human Nutrition. Vitamin A and E. Academic Press, London. pp 10–52:93–131.

Mohamed HE (2004). A note on vitamin A, C and E status in healthy and infected camel calves. Journal of Camel Practice and Research 11:63-64

Mohamed HE (2006). Factors affecting the Plasma contents of retinol and alpha tocopherol in camels (*Camelus dromedarius*). Journal of Animal Vet. Adv. 5(4):301-303.

Mohamed HE (2008). Vitamin D status in camels (*Camelus dromedarius*). Research Journal of Biological Science 3(4):446-447.

Paixao JA and Campos JM (2003). Determination of fat soluble vitamins by reversed-phase HPLC coupled with UV detection: a guide to the explanation of intrinsic variability. Journal of Liquid Chromatography 28(4):641-663.

Price PA (1988). Role of vitamin-K-dependent proteins in bone metabolism. Annual Review of Nutrition 8:565–583.

Smith BB, Van Saun RJ, Reed PJ, Craig AM and Youngberg A (1998). Blood mineral and vitamin E concentrations in llamas. American Journal of Veterinary Research 59(8):1063-1070.

Snow DH, Billah AM, Ridha A and Frigg M (1992). Plasma concentrations of some vitamins in camels. Proceeding of 1st. International Camel Conference Dubai, Allen, W. R.; Higgins, A. I; Mayhew, D. H; Snow, D. H. and

Wale, I.F. (Eds). R&W publications Ltd., Newmarket, U.K. pp 335-338.

Snow DH and Frigg M (1990). Plasma concentration of some vitamins in greyhounds. The Journal of Small Animal Practice 31:605-609.

Stahl T, Salliman HP, Duehlmeirer R and Wernery U (2006). Selected vitamins and fatty acid patterns in dromedary milk and colostrum. Journal of Camel Practice and Research 13(1):53-57.

Suttie JW (1980). Vitamin K-dependent carboxylation. Trends in Biochemical Sciences 5(11):302-304.

Vermeer C and Hamulyak K (1991). Pathophysiology of vitamin K-deficiency and oral anticoagulants. Thromb. Haemost. 66:153-159.

Camel αS1-Casein: Thermal and Chaperone Behaviors of Phosphorylated and Dephosphorylated States

Badraghi J.[1,2], Moosavi-Movahedi A.A.[2], Saboury A.A.[2], Niasari-Naslaji A.[3]
Sharifizadeh A.[1,2] and Banaei A.[1,2]

[1]Applied Science Institute (ACECR), University of ShahidBeheshti, Tehran, Iran

[2]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

[3]Dept. Clinical Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Protein aggregation is the leading cause of more than 20 different of so called protein conformational diseases such as Alzheimer's, Parkinson, Huntington's and cataract. The aim of this study was the extraction, purification and dephosphorylation of camel αS1 casein in order to study the thermal behavior and chaperone activity of αS1 casein on the anti aggregation of insulin and catalase. Camel αS1-CN was purified, using diethyl amino ethyl (DEAE) cellulose as the anion exchanger matrix and the purity of the protein sample was analyzed using SDS-PAGE and urea PAGE. Potato acid phosphates was used to remove the phosphate groups of the casein and degree of dephosphoryalation was assessed by using different techniques, including SDS-PAGE, Urea-PAGE and an spectroscopic methods. The thermal profile of the protein in phosphorylated and totally dephosphorylated states was monitored by following the change of intrinsic fluorescence intensity under forward- and backward temperatures between 10 to 90°C. On the base of this thermal stability of phosphorylated camel αS1 caseins were higher than dephosphoryated αS1-CN. Phosphorylated camel αS1 casein inhibits the aggregation of insulin and catalase. Our results indicate that dephosphorylated camel αS1 caseins reduce chaperone activity of casein but still show chaperone ability to some extent. The chaperone ability of the caseins depend mainly on the hydrophobic parts of the protein. Decreasing of the phosphate concentrations in the casein due to the dephosphorylation caused reduction in repulsive electrostatic interactions between negative charged αS1-CN, and the net negative charge of insulin molecules, resulting in the lower exposure of hydrophobic patches of casein and its reduced chaperone ability. In contrast, at high phosphate concentrations (phosphorylated casein), the higher repulsive electrostatic interactions between negative charged αS1-CN, and the net negative charge of insulin molecules, resulting in the higher exposure of hydrophobic patches of casein and its enhanced, chaperone ability.

(Presented in Scientific Symposium: Camel and Biomolecular Sciences; Organisers: Centre of Excellence for Veterinary Research on Iranian Indigenous Animals; Iran Society of Biophysical Chemistry; Centre of Excellence for Biothermodynamics; held on 13 December 2010; Institute of Biochemistry and Biophysics, University of Tehran, Tehran, IRAN; Courtesy: A. Niasari-Naslaji)