Vitamin C status in Sudanese camels

Vitamine C status van Sudanese kamelen

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit Utrecht
op gezag van de Rector Magnificus,
Prof. Dr. W.H. Gispen,
ingevolge het besluit van het College voor Promoties
in het openbaar te verdedigen
op maandag 3 juni 2002
des namiddags te 4.15 uur

door

Hassab Elrasoul Hussein Mohamed Elkhidir

geboren op 1 januari 1968 te Elgunied, Gezira, Sudan

Promotor: Prof. dr. ir. A.C. Beynen,
hoogleraar veterinaire diervoeding

CONTENTS

Scope of the thesis

Camels belong to the order of *Artiodactyla*, suborder *Tylopoda*, and family *Camelidae*. This family contains two genera; *Camelus* (Old World camel) and *Lama* (New World camel). Within the genus camel, there are the one-humped camels (*Camelus dromedarius*) and the two-humped camels (*Camelus bacterianus*). The term dromedary is derived from the Greek word "dromados" (run) and in the strict sense is used for riding camels. This thesis concerns vitamin C status of the one-humped camel, i.e. *Camelus dromedarius*.

There are different classification systems for camels (Blanc and Ennesser, 1989; Kohler, 1993). The Sudanese camels are classified according to their use as packing and riding camels. The former include Arabi camels with well-developed hindquarter, large hump, rigid body, relatively short neck and large head, and heavy bones and muscles. The riding camels include Bishari and Anafi camels, with relatively small head and ears, alert eyes, a fine and short neck, strong and fine shoulders, a very deep chest and well-sprung ribs right to the back. In the Sudan, one of the world's largest dromedary (*Camelus dromedarius*) population of about 2,500,000 heads is present, representing 14% and 18% of the total world and African population, respectively. Sudanese camels have more potential for meat, milk and hide production than imported cattle. Camels can play an important role in augmenting economically sustainable livestock production in arid and semi-arid zones of the Sudan (Osama and Idris, 2001). Camels also provide input into the village economy in relation to transportation.

Sudanese camels mainly occur in the central, Butana area, where this study has been conducted, and the western region, the Kordofan and Darfur states. Butana area consists of flat and semi-arid clay regions. Two vegetational zones are discerned: a semi-arid desert with Acacia shrubs and short grasslands in the northern part (annual rainfall up to 220 mm) and the low woodland savanna of central Sudan (annual rainfall from 210 to 620 mm). Trees commonly found in the study area are *Acacia mellifera*, *Acacia nubica* and *Acacia nilotica*. Grasses that dominate are *Cymogon nervatus*, which is a rather non-palatable grass, *Aristidia funiculata*, *Ipomoea cordofana* and *Blepharis persica*, the latter being a good forage plant with relatively high protein content.

Vitamin C (ascorbic acid) is a water-soluble vitamin that is synthesized by ruminant's liver (Hornig et al. 1984). Ascorbic acid assists in biosynthetic processes, particularly hydroxylation reactions by mixed-function oxygenase. Ascorbic acid thus participates in the biosynthesis of collagen, carnitin and catecholamines. Vitamin C has attracted the attention of scientists in the recent years as it relates to stress conditions (physical activity, high environmental temperature, disease, transportation) in different species of animals. Therefore, it was felt to be important to study vitamin C in camels and to describe factors affecting vitamin C status, especially as camels are kept under harsh conditions. Reports on vitamin C status in camels under natural grazing conditions were lacking. Previous studies only estimated the concentrations of vitamin C in plasma and tissues (Snow et al., 1992, Soliman et al., 1975), but did not relate vitamin C status to environmental factors, health and disease. Thus, in this study vitamin C status of camels was related to factors such as season, sex, breed, disease, and sexual activity and various infectious diseases. To assess vitamin C status, the concentrations in blood, urine, milk and tissues were measured.

The present studies mainly are concerned with ascorbic acid metabolism in Sudanese camels (*Camelus dromedarius*) browsing on natural vegetation. The thesis is

divided into nine chapters. The metabolic and physiological functions of ascorbic acid are briefly reviewed (Chapter 1a). Vitamin C metabolism in various stress conditions is reviewed (Chapter 1 b). In the first field study, the relation between season, breed, sex and sexual activity and ascorbic acid contents of blood, urine, and tissues is described (Chapter 2). The relation between reproductive status, including brucellosis, and vitamin C status was also examined (Chapter 3). In another field study, the content of ascorbic acid in milk and colostrum in relation to breed, udder condition, stage of lactation and parity was measured (Chapter 4). In chapter 5, the levels of plasma and leukocyte ascorbic acid in newborn calves and their dams are described. In a field study the relation between parasite infections, i.e. trypanosomiasis, sarcoptic mange and helminthiasis, and vitamin C status in Sudanese camels was investigated (Chapter 6). In a controlled experiment, the effect of habitual diet on vitamin C status was evaluated (Chapter 7). The pharmacokinetics of ascorbic acid administered through different routes has been determined (Chapter 8). From a comparative point of view, data on vitamin C status in Sudanese cattle and sheep are included in this thesis (Chapter 9).
Finally, the main findings are discussed and summarized leading to conclusions and recommendations for further research.

References

Blanc, C. P. and Ennesser, Y. (1989). A zoogeographical approach to subspecies differentiation of *Camelus dromedarius*. Rev. Elev. Med. Vet. Pays. Trop. 42, 573-587.

Hornig, D., Glatthaar, B. and Moser, U. (1984). General aspects of ascorbic acid function and metabolism. Workshop Ascorbic Acid in Domestic Animals. Royal Danish Agric. Soc., Copenhagen, pp. 3-24.

Kohler, R. I. (1993). About camels breeds. A re-evaluation of current classification systems. J. Anim. Breed Genet. 110, 66-73.

Osama, E. Y. and Idris, O. F. (2001). Camel potentials in the Sudan. International Conference on Reproduction and Production of Camelids. 6th Annual Conference on Animal Production Under Arid Conditions. College of Food Systems, University of Arab Emirates, Al-Ain, abstract p. 43.

Soliman, M.K., Toussef, G.W., mansour, S.A. (1975). Ascorbic acid content of erythrocytes, plasma, leukocytes and whole blood of healthy camels. Egypt. J. Vet. Sci. 12, 107-110.

Snow, D. H., Billah, A. M., Ridha, A. and Frigg, M. (1992). Plasma concentrations of some vitamins in camels. Proc. Ist. Int. Camel Confr. pp. 335-338. R.W. Publications, U. K.

Chapter 1a

Ascorbic acid metabolism in ruminants: a brief review

Introduction

Ascorbic acid (vitamin C) is a water-soluble, hexonic sugar, with a molecular weight of 176. It is known to play vital roles in numerous functions of the body, especially in hydroxylation reactions. It's requirement by animals may be increased when challenged in the form of immune and metabolic stress. The main objective of this brief review is to outline the catabolic and anabolic pathways of ascorbic acid as well as its metabolic functions.

Analytical Methods

The determination of ascorbic acid in various matrices can be carried out by several analytical techniques. Of these, high-pressure liquid chromatography (Behrens and Madere, 1987) and spectrophotometry (Ruiz et al., 1999) are the most common techniques applied. The former is preferable, as the spectrophotometric determination is very difficult and requires tedious pre-treatment to eliminate interfering substances. The "Chemistry of Ascorbic Acid" by Szent Gyorgi (1928) has reported the discovery of a carbohydrate derivative from the adrenal cortex of the ox, this derivative possessing strong reducing properties. The physiological activity appeared to be associated with the reducing power (Hay et al. 1967). Ascorbic acid is a fine crystalline, white or slightly yellow odorless powder with a tart taste. It is easily oxidized in air. Chemically, ascorbic acid is the enolic form of 3-oxo-L-gulofuranolactone, which is optically active in water and is highly labile as it is easily oxidized by the enzyme dehydroascorbate reductase to dehydroascorbic acid. Both the oxidized and reduced forms are physiologically active (Laggner and Goldenberg, 2000).

Blood samples for ascorbic acid determination should be deproteinized and stabilized immediately after collection. Lykkesfeldt et al. (1995) showed that a concentration of 5% metaphosphoric acid stabilizes ascorbic acid and dehydroascorbic acid during a two-month storage at 200 ºC. Unless reduced back to ascorbate, dehydroascorbic acid undergoes irreversible ring opening to 2,3-diketogulonic acid. According to Dhariwal et al. (1990), the two-electron oxidation product of ascorbate, dehydroascorbate, is labile at physiological pH and temperature (half life 5-7 min.). Dehydroascorbate can be reduced or recycled to ascorbate in blood by both erythrocytes (Christine et al., 1965) and neutrophils (Washko et al., 1993).

Ascorbic Acid Biosynthesis

L-ascorbic acid is biosynthetically formed in almost all mammals studied, except in man, several other primates and guinea pigs (Hornig, 1975). Ascorbic acid is a product of glucose metabolism in the glucuronate pathway (Touster, 1969). The anabolic pathway of ascorbic acid utilizes glucose as the initial substrate (Simpson and Ortwerth, 2000).

Ascorbic Acid Catabolism

The metabolic fate of ascorbic acid and its derivative in animals depends on a number of factors including animal species, route of ingestion, quantity and nutritional status. The catabolic pathways of ascorbic acid proceed through xylitol or through L-

ribulose to D-xylose as initial step for the conversion of L to D-sugars. Another pathway for the catabolism of ascorbate is by C_2 - C_3 carbon cleavage, to give rise to oxalate and a 4-carbon compound as intermediates. The major two ascorbic acid degradation pathways at physiological pH are the oxidative and the non-oxidative ones. The major pathway has erythrulose as is the major product of the non-oxidative degradation of dehydroascorbic acid (DHA), and also 2,3-diketogulonic acid (Simpson and Ortwerth, 2000). DHA rapidly hydrolyzes in solution at pH 7.0 to L-diketogulonate (2,3-DKG), which is very unstable and degrades further (Lykkesfeldt et al., 1995). Analysis showed that L-erythrulose (ERU) and oxalate were the primary degradation products of ascorbic acid regardless of which compound was used as the starting material. In the presence of high concentrations of H_2O_2, 2,3-DKG produces L-threonate, oxalate, and CO_2. In the absence of H_2O_2, 97 % of the products consist of ERU and oxalate (Simpson and Ortwerth, 2000).

Metabolic Functions of Ascorbic acid

Over the last few years, it has been recognized that ascorbic acid is involved in a great variety of biochemical processes beyond the scope of prevention of scurvy (Englar and Seifter, 1986). The metabolic functions of ascorbic acid in cattle have been repeatedly reviewed (Itze, 1984; Haag and Hofmann, 1987; McDowell, 1989). Apart from fermentation of dietary ascorbic acid in the rumen, fundamental differences between ruminants and monogastric animals with respect to ascorbic acid metabolism are not known.

Hydroxylation reactions

In most cases, ascorbic acid assists biosynthetic processes and regulatory mechanisms which comprise hydroxylation reactions according to the mixed function type (Hornig et al., 1984). Apart from its participation in collagen formation, ascorbic acid is a co-substrate for a variety of mono- and dioxygenases for redox reactions in biochemical processes, such as the conversion of dopamine to noradrenaline and for the metabolism of cholesterol and carnitin (Englar and Seifter, 1986). L- ascorbic acid participates in the biosynthesis of collagen, carnitin, catecholamines, cartilage, skin, skeletal and connective tissues (Jaffe, 1984). The synthesis of carnitin could be of special importance for cows in the postpartum phase, because at that time large amounts of stored fat are mobilized (Giesecke et al., 1987). Carnitin enables fatty acids to enter the mitochondria, where they are broken down to acetyl-coA by β-oxidation. Vitamin C also participates in the modulation of complex biochemical pathways, which are an essential part of the normal metabolism of immune cells (Ball et al., 1996). Ascorbic acid functions in cholesterol metabolism in that it is required for the transformation of cholesterol into bile acids which in turn facilitate fat absorption (Moore and Christie, 1984).

Anti-oxidant function

One of the major metabolic roles of ascorbic acid is its participation as anti-oxidant agent and free radical scavenger in numerous cellular oxidation processes (Jariwalla and Harakech, 1996). This activity is attributed to its properties as an electron

donor. Vitamin C is capable of protecting against oxidative injuries in the aqueous compartments and lipid bilayer of cell membranes (Halliwell and Gutteridge, 1985). It also scavenges aqueous-phase reactive oxygen radicals (ROS) by very rapid electron transfer and thus inhibits lipid peroxidation (Halliwell et al., 1987). Vitamin C plays an important role in the defence against oxidative damage, especially in leukocytes. It also protects the structural integrity of the cells of the immune system (Bendlich, 1993). Vitamin C was found to be effective against superoxide, hydroxyl radicals, hydrogen peroxide, peroxy radicals and singlet oxygen, thereby protecting phagocytes from oxygen radicals entering the cytoplasm from the phagosome (Sies et al., 1992). Vitamin C also functions in reducing the tocopheroxy radical, thereby restoring the radical scavenging activity of vitamin E (Niki, 1987). The ascorbate radical (semi-dehydroascorbate) is reduced to ascorbate by NADH-dependent semi-dehydroascorbate reductase (Green and O'Brein, 1973). Furthermore, vitamin C serves as a radical scavenger and general antioxidant for cellular metabolites including unsaturated fatty acids, vitamins A and E, and carotenoids (Gershoff, 1993).

The anti-oxidative function of ascorbic acid is evident when treating nitrate poisoning in cattle. Nitrate from the feed is reduced to nitrite in the rumen and leads to the formation of methaemoglobin in the blood (Mirvish et al., 1972). Due to its antioxidant potential, ascorbic acid was proved to be effective in curing post parturient haemoglobinurea in buffaloes (Chugh and Mata, 1997).

Role of Vitamin C in the metabolism of minerals

Vitamin C is necessary for iron metabolism, maintenance of normal tyrosine oxidation and acts as a hydrogen transport agent (Swenson, 1984). It plays a role in the maturation of erythrocytes, absorption and mobilisation of iron and in keeping constant the haemoglobin content (Natvig et al., 1963). Ascorbic acid keeps iron in the reduced, bivalent form and thus methaemoglobin is reduced (Buddeke, 1989). The relations between iron, as a generator of free radicals, and vitamin C have been studied (Herbert et al., 1996; Bearger et al., 1997). In addition to the influence of cortisol on the synthesis of ascorbic acid (Chatterjee et al., 1975), it has also been hypothesized that ascorbic acid itself could influence adrenal gland function. Ketotic cows injected subcutaneously/intravenously with ascorbic acid showed a transient elevation of the glucose level and a lowering of the ketone level in the blood (Imlah, 1961). An effect of ascorbic acid on the metabolism of calcium could also be relevant for dairy cows. By administering high doses of ascorbic acid, the hypocalcaemia of cows at an early stage of lactation has been successfully treated (Bizet, 1957).

References

Ball, S. S., Weindruch, R., Wolfrod, R. L., 1996. In: Johnson Jr., J. E., Wolford, R., Harman, D., Miquel, J. (Eds.) Free Radicals Aging and Degenerative Diseases. Allan Liss, New York, pp. 427-456.

Bearger, T. M., Polidori, M. C., Dabbagh, A., Evans, P. J., Halliwell, B., Morrow, J. D., Roberts, I. T. and L. J., Frei, B., 1997. Antioxidant activity of vitamin C in iron-overload human plasma. J. Biol. Chem. 272, 15656-15660.

Behrens, W. and Madere, R., 1987. A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic acid and dehydroascorbic acid in tissues, biological fluids and foods. Anal. Biochem. 165, 102-107

Bendlich, A., 1993. Physiological role of antioxidants in the immune system. J. Dairy Sci. 76, 2789-2794.

Bizet, E., 1957. Traitment des Paralegies rebelles des bovines par la vitamine C. Rec. Med. Vet. 133, 277-278.

Buddeke, E., 1989. Grunris der Biochemie. 5. Aufl. Verlag Walter der Gruyter. Berlin, New York.

Christine, L., Thomson, G., Igge, B., Brownie, A. C. and Stewart, C. P., 1965. The reduction of dehydroascorbic acid by human erythrocytes. Clin. Chim. Acta, 1, 557-569.

Chatterjee, I. B., Majumder, A. K., Banerjee, S. K., Roy, R. K., Ray, B. and Rudrapal, D., 1975. Relationships of protein and mineral intake to L-ascorbic acid metabolism including considerations of some dietary related hormones. Ann. N. Y. Acad. Sci. 258, 382-400.

Chugh, S. K. and Mata, M. M., 1997. Postparturient haemoglobinurea in buffaloes. An antioxidant responsiveness. Indian Vet. J. 74, 56-58.

Dhariwal, K. R., Wasko, P. W. and Levine, M., 1990. Determination of dehydroascorbic acid using high-performance liquid chromatography with colorometric electrochemical detection. Anal. Biochem. 189, 18-23.

Englar, S. and Seifter, S., 1986. The biochemical functions of ascorbic acid. Ann. Nutr. Rev. 6, 365-406.

Gershoff, S., 1993. Vitamin C (ascorbic acid): new roles, new requirements? Nutr. Rev. 51, 313-326.

Giesecke, D., Meyer, J., Graf, F. and Koak, F., 1987. Stoffwechselbelastung, freie Fettsauren und Ketogenese bei Kühen mit hoher Milchleistung. Adv. Anim. Physiol. Anim. Nutr. 18, 10-19.

Green, R. C. and O'Brein, P. J., 1973. The involvement of semi-dehydroascorbate reductase in the oxidation of NADH by lipid peroxides in mitochondria and micosomes. Biochim. Biophys. Acta. 293, 334-342.

Haag, W. and Hofmann, W., 1987. Untersuchungen zum Vitamin-C-Gehalt in Blutplasma und Leukocyten des Rindes. Tierärztl. Umschau. 42, 956-965.

Halliwell, B. and Gutteridge, J.M.C., 1985. Free Radicals in Biology and Medicine. Clarendon Press, Oxford, p. 346.

Halliwell, B., Wasil, M. and Grootveld, M., 1987. Biologically significant scavenging of the myeloperoxidase-derived oxidant hypochlorous acid by ascorbic acid

Hay, G.W., Lewis, B.A. and Smith, F. 1967. Ascorbic Acid. In: The Vitamins. Vol. 1. (Eds) E.D. Serbell, W.H. Jr. and Harris, R.S., Academic Press, New York, pp. 308-501.

Herbert, V., Shaw, S. and Jayatilleke, E., 1996. Vitamin C-derived free radical generation from iron. J. Nutr. 126, 1213-1220.

Hornig, D., 1975. Metabolism of ascorbic acid. World Rev. Nutr. Diet, 23, 225-228.

Hornig, D., Glatthaar, B. and Moser, O., 1984. General aspects of ascorbic acid function and metabolism. In: Ascorbic acid in Domestic Animals, eds. by I.Wegger, F. J. Tagwerk and J. Moustgaard. Copenhagen, Royal Danish Agricultural Society. pp. 3-24.

Imlah, P., 1961. A study of ascorbic acid in normal and ketotic cows. J. Comp. Path. 71, 28-43.

Itze, L., 1984. Ascorbic acid metabolism in ruminants. In: Ascorbic Acid in Domestic Animals, I. Wegger, F. J. Tagwerk and J. Moustgaard, (Eds). Copenhagen, Royal Danish Agricultural Society. pp. 20-30.

Jaffe, G. M., 1984. Vitamin C. In: Machlin, J. (ed.) Handbook of Vitamins Nutritional, Biochemical and Clinical Aspects. Marcel Dekker, Inc. New York and Basel, pp. 199-243.

Jariwalla, R. J. and Harakech, S., 1996. In: Harris, R.J. (Ed.) Ascorbic acid: Biochemistry and Biomedical Cell Biology, Plenum, New York, pp. 215-231.

Laggner, H. and Goldenberg, H. 2000. Biochem. J. 345, 195-200.

Lykkesfeldt, J., Loft, S., Poulsen, H. E., 1995. Anal. Biochem. 229, 329.

McDowell, L. R., 1989. Vitamins in Animal Nutrition. Academic Press, New York.

Mirvish, S. S., Wallcave, L., Eagan, M. and Shubik, P., 1972. Ascorbate-nitrite reaction: possible means of blocking the formation of carcinogenic N-nitroso compounds. Science, 177, 65-68.

Moore, J. H., Christie, W. W., 1984. Digestion, absorption and transports of fats in ruminant animals. In: J. Wiseman (Ed.): Fats in Animal Nutrition. Butterworth, pp. 123-149.

Natvig, H., Bjerkedal, T. and Johanssen, O. 1963. Acta. Med. Scand. 175, 3.

Niki, E., 1987. Interaction of ascorbic acid and α-tocopherol. Ascorbate regulation and its neuroprotective role in the brain. Trends Neurosci. 23, 209-216.

Ruiz Medina, A., Fernandez de Cordova, M. L. and Molina Diaz, A., 1999. A rapid and selective solid-phase UV spectrophotometric method for determination of ascorbic acid in pharmaceutical preparations and urine. J. Pharma. Biomed. Anal. 20, 247-254.

Sies, H., Stahl, W. and Sundquist, A. R., 1992. Antioxidant functions of vitamins: Vitamins E and C, β-carotene, and other carotenoids. Ann. N. Y. Acad. Sci. 669,7-20.

Simpson, G. L. W. and Ortwerth, B. J., 2000. The non-oxidative degradation of ascorbic acid at physiological conditions. Biochem. Biophys. Acta. 1501, 12-24.

Swenson, M. J., 1984. Dukes Physiology of Domestic Animals. 10th edition, Cornell University Press.

Szent-Goyrgi, A., 1928. Biochem. J. 22, 1387.

Touster, O., 1969. Aldonic and uronic acids. In: Comprehensive Biochemistry: Carbohydrate Metabolism, M. Florkin and E. Stotz, (Eds), Amesterdam, Elsevier, Vol. 17, pp. 219-242.

Washko, P. W., Wang, Y. and Levine, M., 1993. Ascorbic acid recycling in human neutrophils. J. Biol. Chem. 268,15531-15535.

Chapter 1b

Stressors and vitamin C metabolism in animals: a brief review

Summary

This brief review is an account of stressful conditions in relation to vitamin C meatbolism in animals. It considers the effects of various stressors on vitamin C blood status and consequently the immune response. It is aimed to describe the immune system-vitamin C interrelationship, different stressors and the beneficial effect of vitamin C supplements.

Introduction

Stress in animals results in a wide range of physiological changes in order to maintain their homeostatsis (34). The response to stressors comprises the activation of the sympathetic-adreno medullary system, which involves the immediate release of catecholamines, or the hypothalamic-pituitary-adrenocortical system, which involves a more gradual system release of glucocorticoids (34, 37).

One of the major roles of the water-soluble vitamin C (ascorbic acid), is its antioxidant property. This function is accomplished by inactivating harmful free radicals produced through normal cellular activity and mediated through various stressors (10). Mammals and poultry have evolved the ability to synthesize ascorbic acid in the liver and kidneys, respectively. Under normal conditions, the requirement of vitamin C is met enodogenously and there is no need for exogenous supplementation. However, under stress conditions, the status of vitamin C is greatly reduced. Therefore, this review was performed in an attempt to investigate the effect of different types of stress on vitamin C status.

Vitamin C and the Immune system

Ascorbic acid is the most important antioxidant extracelluar fluids (50). It is thought to be important in optimum functioning of the immune system through enhancement of neutrophil production and also through protection against free radical damage (6, 3). Vitamin C is found in high concentrations in blood leukocytes (35). The protective effect of vitamin C may in part be mediated through its ability to reduce circulating glucocorticoids (17). It has been reported that vitamin C stimulates either humoral or cell-mediated immunity of mice (32), guinea pigs and humans (40), rabbits (49) and calves (31). The favorable effect of ascorbic acid appears to occur only in the presence of sufficient quantities of the antioxidant, vitamin E (41).

Ascorbic acid acts as a scavenging or neutralizing substance of free radicals. The antioxidant function of ascorbic acid could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells. Nutrients involved in the antioxidant function are used at a greater rate in infected animals. Participation of vitamin C in augmenting the white blood cells function takes several ways. Indeed, ascorbic acid is involved in the immunological and antibacterial functions of white blood cells by several factors: increasing their mobility (42); stimulating the energy producing monophosphate shunt within the ell (2) and consequently is coupled with their phagocytic processes (42). Secondly, vitamin C protects leukocytes from auto-oxidation (2, 28). Thirdly, vitamin C increases serum immunoglobulins concentrations and antibody functions (28).

Vitamin C has been shown to be important in complement activity (46), antibody responses, and a variety of other immune functions in higher vertebrates (38). Vitamin C also stimulates the phagocytic capacity of neutrophils in the peripheral blood. It is suggested that vitamin C can be used with other immunomodualtors to adapt the defence by organisms (11).

Types of stressors affecting vitamin C concentrations

Vitamin C plays a potential role as a stress-relieving nutrient as it has recently been characterized as one of the anti-stress substances (4, 1). The synthesis of ascorbic by farm animals is reduced or may cease during stress caused by disease, vaccination, higher temperature, overcrowding or physical activity (22, 53). Fasting increased mean buffy coat ascorbic acid concentration and decreased mean plasma ascorbic acid concentration (30). It was proved that glutathione and ascorbic acid could significantly reduce ceftizoxime-sodium-induced lipid peroxidation, and they appear to be promising candidates for further investigation in this regard (45). Stress increases the demand for ascorbic acid (36)

Housing

The stress associated with confinement of calves decreased the immune response to a specific antigen and decreased concentrations of ascorbic in plasma (16). There was also a decrease of adrenal ascorbic in mice (47) and a drop in blood and adrenal levels in rats as well as increased urinary excretion (51). A correlation was found between temperature and the decrease in plasma ascorbic acid (43). Under these types of conditions, higher doses of ascorbic, amounting to 1.25-2.5 g/animal and day, have a positive effect on the immune system and health of calves (7, 26).

Under heat stress, supplementary vitamin C can support the bird's performance in modern poultry operations. The utilization of ascorbic acid usually is, under such conditions, elevated in individual organs and tissues, and high vitamin C applications can distinctly improve the immune response. Obviously, high producing poultry breeds under heat stress cannot synthesize sufficient vitamin C to cope with their requirements (19).

A marked decrease of L-ascorbic level was seen in purebred Friesian cattle as compared with 62.5% crossbred (Butana X Friesan) and Butana cattle during the summer season (33). The noticeable decrease in ascorbic acid levels may be attributed to the influence of high environmental temperature. This means that the metabolic need for ascorbic is increased under stressful conditions. It has been proved that conditions, such as extreme temperature, led to a higher metabolic requirement for vitamin C (18, 8, 15).

Vitamin C can reduce the negative effect of corticosterone by regulating their concentration. It is not yet fully understood whether this is achieved by reducing the synthesis and/or secretion of corticosterone, or by breaking it down. It was suggested that the administration of ascorbate could nullify the oxidative stress produced by exercise in thoroughbred racehorses, but it could not prevent muscular damage (55).

Transportation

Transportation is an acute stressor for ruminants and can elevate serum cortisol concentrations for 4 to 7 days post transportation. As a consequence of transportation, cortisol and other glucocorticoids suppress the immune response in cattle and other species (44). Ascorbic acid, pyridoxine and riboflavine when given to male Black Pied German cattle and before and after transportation helped them to adapt and increase their resistance to stress (54). These results may give a clue to the importance of supplementing transported sheep with vitamin C. However, it was found that plasma ascorbic acid in transported mares increased (5).

Weaning

Weaning is regarded as stress to young animals. To reduce the effect of post weaning in young guinea pigs it was considered necessary to add ascorbic acid before weaning (48). It is recommended that imported rhesus monkeys should be given ascorbic acid during the period of acclimatization. To study the effect of acute stress by dog barking, ewes were infused with oxytocin, prolactin and ascorbic acid with a resultant suppressed cortisol responsiveness to stress (12). For the horse, it was found that plasma ascorbate concentrations were low in weaned foals (24).

The beneficial effects of vitamin C supplementation

The vitamin C requirement of the calf is determined by the conditions under which it is kept. Ascorbic acid supplementation increased the concentrations of IgG in plasma of calves that were deprived of colostrum (7, 15). The levels of IgM in calves supplemented with vitamin E and C generally tended to be higher than those of control calves (23). However, dietary ascorbate wan not immunostiumlatory in dairy calves up to 56 days of age and appeared to inhibit antibody synthesis. At 14 day of age there was an interaction of ascorbic supplementation and colostrum feeding: plasma IgM concentrations were higher in colostrum-deprived calves fed ascorbate than in colostrums-deprived calves not fed ascorbate (15). However, attempts to stimulate immune function with dietary supplements of ascorbic acid had mixed success (14).

Dietary ascorbic acid was shown to increase antibody response to sheep red blood cells (34). Chickens fed an ascorbic acid supplemented-diet had lower heterophil to lymphocytes ratios than did untreated controls indicating that ascorbic acid may help chickens in coping with stress by improving humoral immune response to pathogens. Presently, the mechanism by which resistance occurs is not clear. Calves supplemented with vitamin C had a lower incidence of scouring (15). Similarly, vitamin C supplemented at 330 mg/kg reduced mortality and pericarditis in chicks infected with E. coli (21). The amount of vitamin C needed for this protective effect increased with a higher environmental stress level. In rabbits, the supplementation of ascorbic acid reduced the oxidative damage of erythrocytes, liver and kidney caused by T. brucei (52).

Under unfavorable conditions, dietary supplements of ascorbic acid often help to reduce the effects of stress (20) by alleviating the suppressive effect of corticoids on neutrophil function in cattle (44).

Adaptation

Vitamin C plays a central role in the bird's ability to cope with stress as its involved in the synthesis of adrenaline and corticosterone. These hormones are responsible for the mobilization of energy for the so-called essential functions such as blood flow, heat dissipation, maintenance of body temperature, respiration, etc. As long as vitamin C is not depleted, adrenaline, and later corticosterone, can be synthesized and released. This allows the bird to survive and remain productive. However, the greater the depletion of vitamin C, the smaller is the ability of the birds to synthesize these hormones. Vitamin C has yet another role to play in stress management. During stress, corticosterone is released in such large amounts that it became cytotoxic and suppress the immune functions (49). Further research is needed to explore this area.

Concluding remarks

Recent studies revealed that under stress conditions, supplementation of ascorbic acid could support the animal. The low ascorbic status resulted in depression of growth, increased susceptibility to infections and eventually death of the animal. The great advantage of vitamin C supplementation is that it is safe and easy to use. Furthermore, since vitamin C can be added to the feed or drinking water, it is a cost-effective tool to counteract heat stress in non-ruminants.

References

1. Afify, O. S. and Makled, M. N. 1995. Effect of ascorbic acid on productive and reproductive performance of Bouscat rabbits exposed to heat stress. First Egyptian-Hungarian Conference. pp. 313, 17-19 September, Alexandria, Egypt.
2. Anderson, R. 1981. In: Vitamin C (Ascorbic acid), J. N. Counseel and D. H. Hornig (Eds.), pp. 249, Applied Science Publishers, London.
3. Anderson, R. and Lukey, P. T. 1987. A biological role of ascorbate in the selective neutralization of extracellular phagocyte-derived oxidants. Ann. NY. Acad. Sci. 498, 229-233.
4. Axt J, Richter W, Ott W. 1968. Vitamin C content in the blood serum of various animal species under stress. 3. Effect of work stress on serum ascorbic acid and blood sugar in the horse. Arch Exp Veterinarmed. 22(6):1165-73
5. Baucus, K. L., Squires, E. L., Ralston, S. L., McKinnon, A. O. and Nett, T. M. 1990. Effect of transportation on the estrous cycle and concentrations of hormones in mares. J. Anim. Sci. 68(2), 419-426.

6. Bendlich, A., D apololito, P., Gabriel, E. and Machlin, L. J. 1984. Intercation of dietary vitamin C and vitamin E on quinea pig immune responses to mitogens. J. Nutr. 114, 1588-1593.
7. Blair, L. and Cummins, K. A. 1984. Effect of dietary ascorbic acid on blood immunoglobulins concentration in dairy calves. J. Dairy Sci. 67, Supp. 138-139.
8. Booker, W. M. 1960. Relation of ascorbic acid to adrenocortical function during cold stress. Fed. Proc. 19, 94-96.
9. Chatterjee, I. B., Majumder, A. K., Nandi, B. K.and Subramarias, N. 1975a. Synthesis and some major functions of vitamin C in animal. Ann. Acad. Sci. 258, 25-46.
10. Chew, B. P. 1995. Antioxidant vitamins affect food animal immunity and health. J. Nutr. 125(6), 1804-1808.
11. Coicoiu, M., Lupusoru, E. C., Colev, V., Badescu, M. and Parduraru, T. 1998. The evolvement of vitamin C and E in changing the immune response. Prev. Med. Soc. Med. Nat. Lasi. 102(1-2), 93-96.
12. Cook, C. J. 1995. Oxytocin and prolactin suppress cortisol response to acute stress in both lactating and non-lactating sheep. J. Dairy Sci. 64(3), 327-339.
13. Counseel, Blairm D. and Cummins, K. A. 1984. Effect of dietary ascorbic acid on blood immunoglobulin concentrations in dairy calves. J. Dairy Sci. 67(1), 138.
14. Cummins, K. A. 1989. Ascorbate in cattle. A review. Proc. Anim. Sci. 8, 22.
15. Cummins, K. A. and Brunner, C. J. 1989, Dietary ascorbic acid and immune response in dairy calves. J. Dairy Sci. 72, 129.
16. Cummins, K. A. and Brunner, C. J. 1991. Effect of calf housing on plasma ascorbate and endocrine and immune function. J. Dairy Sci. 74, 1582.
17. Degkwitz, E. 1987. Some effects of vitamin C may be indirect, since it affects the blood levels of cortisol and thyroid hormones. Ann. NY. Acad. Sci.
18. Desmarais, A. 1960. Ascorbic acid in cold acclimatization. Fed. Proc. 19, 88-93.
19. Fenster, R. 1989. Vitamin C and stress management in poultry production. Zootechnica. International. 16-21.
20. Gadient, M. and Wegger, I. 1985. Ascorbic acid in intensive animal husbandary. Nutr. Abstr. Rev. 5531.
21. Gross, W. B., Jones, D. and Cherry, J. 1988. Effect of ascorbic acid on the disease caused by Eschrichia coli challenge infections. Avian Dis. 32, 407-409.
22. Hidiroglou, M., Ivan, M. and Lessard, R. 1977. Effects of ration and inside versus outside housing on plasma levels of ascorbic acid, lactic acid, glucose and cholesterol in Hereford steers wintered under practical conditions. Can. J. Anim. Sci. 57, 519-529.
23. Hidiroglou, M., Batra, T. R. and Ivan, M. 1995. Effects of supplemental vitamins E and C on the immune responses of calves. J. Dairy Sci. 78, 1578-1583.
24. Hoffman,R.M., Kronfeld, D. S., Holland, J. L.and GrewenCrandell, K. M. 1995. Preweaning diet and stall weaning method influences on stress response in foals. J. Anim. Sci. 73(10), 2922-2930.

25. Hornig, D., Glatthar, B. and Moser, U. 1984. General aspects of ascorbic acid function and metabolism. In: Ascorbic acid metabolism in domestic animals. Proc. Royal Danish Agric. Soc. Copenhagen, Denmark, pp. 3-24.
26. Itze, L. 1984. Ascorbic acid metabolism in ruminants. In: Ascorbic acid metabolism in domestic animals. Proc. Royal Danish Agric. Soc. Copenhagen, Denmark, pp. 120-130.
27. J Anim Sci 1990 Feb;68(2):419-26
28. Jaffe, G. M. 1984. Vitamin C. In: Machlin, J. (ed.) Handbook of Vitamins Nutritional, Biochemical and Clinical Aspects. Marcel Dekker, Inc. New York and Basal, pp. 199-243.
29. Kobeisy, M. and Abdelall, Th.S. 1993. Effect of ascorbic acid on growth performance of some blood constituents of suckling buffalo calves. Assiut Vet. Med. J. 30(59), 52-59.
30. Lee, A. H., Spano, J. S., Swaim, S. F., McGuire, J. A. and Hughes KS. 1986 Evaluation of plasma and buffy coat ascorbic acid concentrations in dogs before and after a 24-hour fast. Am. J. Vet. Res. 47(9):2000-2003
31. Lundquist, N. S. and Philips, P. H. 1943. Certain dietary factors essential for the growing calf. J. Dairy Sci. 26, 1023.
32. Mafison, R. R. and Manwaring, M. H. 1937. Ascorbic acid stimulation of specific antibody production. Proc. Soc. Exp. Biol. Med.
33. Mohamed, H. E. 1996. Distribution of vitamin C in different tissues and plasma of camels, local and exotic breeds of ruminants. M.V.Sc. Thesis, University of Khartoum.
34. Morberg, G. P. 1985. Biological response to stress: Key to assessemtn of animal well-being? In: G. P. Morbeg (Ed.) Animal stress. P. 27. American Physiological Society, Bethesda, Mary Land.
35. Moser, V. 1987. Uptake of ascorbic acid by leukocytes. Ann. NY. Acad. Sci. 498, 200-205.
36. Newberne, P. M. and Conner, M. W. 1989. The vitamins. In: J.J. Kaneko (ED.). Clinical Biochemistry of Domestic Animals. (4^{th} Ed.). p. 796, Academic Press, San Diego, USA.
37. Oliverio, A. 1987. Endocrine aspects of stress: Central and peripheral mechanisms. In: P. R. Wiepkeme and P. W. M. Van Adrichem (Ed.). Biology of stress in farm animal. An integrative appraoch. P. 3, Martinus Njfhojj, Dordrecht, The Netherlands.
38. Panush, R. S. and Delafuente, J. C. 1985. Vitamins and immunopotence. World Rev. Nutr. Diet. 45, 97-132.
39. Pardue, S. L., Thaxton, J. P. and Brake, J. 1985. Role of ascorbic acid in chicks exposed to high environmental temperature. J. Appl. Physiol. 58, 1511-1516.
40. Prinz, W., Black, J., Gillich, G. and Mitchell, G. 1980. A systematic stuyd of the effect of vitamin C supplementation on the humoral immune response in ascorbate-dependent mammals. 1. The antibody response to sheep red blood cells in quinea pigs. Int. J. Vitamin Nutr. Res. 50, 294.
41. Pruiett, S. D., Morill, J. L., Bleche, F., Reddy, P. G., Higgins, J. and Anderson, N. V. 1989. Effect of supplemental vitamins C and E in milk replacer on lymphocytes and neutrophil function in bull calves. J. Anim. Sc. 67, 243.
42. Reece, W. O. 1991. Blood and its function. In: Physiology of Domestic Animals. Pp. 91, Lee and Fibiger, USA.

43. Riker, J. Y., Perry, T. W. and Mahorta, N. K. 1967. Influence of controlled temperature on growth rate and plasma ascorbic acid values in swine. J. Nutr. 92, 99.
44. Roth, J. A. and Kaeberele, M. L. 1985. In vivo effect of scorbic acid on neutrophil function in healthy and dexamethasone-treated cattle. Am. J. Vet. Res. 46, 2434-2436.
45. Roy K, De, A. U. and Sengupta, C. 2000. Evaluation of glutathione and ascorbic acid as suppressors of drug-induced lipid peroxidation. Indian J. Exp Biol. 38(6):580-6
46. Sakamoto, M., Kobayashi, S., Katoo, K. and Shimazono, N. J. 1981. The effect of vitamin C deficiency on complement system and complement components. J. Nutr. Sci. Vitaminol, 27, 367-378.
47. Sidiqu, H. H., Aroba, R. B. and Mahlta, N. K. 1972. Effect of hypoxia on blood glucose, liver glycogen and adrenal ascorbic acid in mice of varying environmental temperature. Indian J. Med. Res. 60, 153.
48. Sidorov, V. T. 1985. Increasing natural immunity and preventing stress in young male cattle and quinea pigs by means of pharmacological substances. Sornik Trudov Belorusskii Nauchno Isseldovatel Skii Institut Zhivotnovostva.
49. Siegel, B. V and Morton, J. T. C. 1977. Vitamin C and the immmune response. Experimentia. 15, 393.
50. Stocker, R. and Frei, B. 1991. Oxidants and antioxidants. In: Oxidative stress (Sies, H. ed.), pp. 213-243, Academic Press, U.K.
51. Teistina, A. Y. 1965. Effect of vitamin P on ascorbic acid metabolism in rats during long-term exposure to high environmental temperature. Vop. Pitan. 24, 35.
52. Umar, I. A., Wuro-Chekke, A. U., Gidado, A. and Igbokwe, I. O. 1999. Effects of combined parenteral vitamins C and E administration on the severity of aneamia, hepatic and renal damage in Trypanosomiasis bruei infected rabbits. Vet. Paras. 85, 43-47.
53. Verde, M. T. and Piquer, J. G. 1986. Effect of stress on the corticosterone and ascorbic acid (vitamin C) content of the blood plasma of rabbits. J. Applied Rabbirt Res. 9, 181.
54. Volkova, O. I. and Preobazhenskii, S. N. 1983. Effect of vitamins on young male cattle during transportation. Sbornik Nauchnkh Moskovkoi Veterianroi Akademii. 64-65. White A, Estrada M, Walker K, Wisnia P, Filgueira G, Valdes F, Araneda O, Behn C, Martinez R. 2001. Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. Comp Biochem Physiol A Mol Integr Physiol. 128(1):99-104
55. White, A., Estrada, M., Walker, K., Wisnia, P., Filgueria, G., Valdes, F., Araneda, O., Behn, C., Martinez, R. 2001. Role of exercise and ascorbate on plasma antioxidant capacity in thorougbred racehorses. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 128(1), 99-104.

Chapter 2

Vitamin C concentrations in blood plasma, tissues and urine of camels (*Camelus dromedarius*) in Sudanese herds

H.E. Mohamed [1] and A.C. Beynen[2]

[1]*Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum, Khartoum North, Sudan;*

[2]*Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Summary

There is suggestive evidence that a low status of ascorbic acid in ruminants is related with decreased disease resistance. In a first attempt to identify conditions in camels that could affect their health, an inventory was made of ascorbic acid concentrations in plasma and tissues as related to breed, gender, sexual activity and season. A total of 3429 camels was studied and subsamples were used for selected comparisons. The highest concentrations of ascorbic acid were found in adrenals (152 mg/100 g wet tissue) and the lowest in heart (8 mg/100 g), the levels being unrelated with season. Arabi camels had higher plasma concentrations of ascorbic acid (6.42 µg/ml) than did Anafi and Bishari camels, the latter breed showing the lowest concentrations (3.24 µg/ml). Female camels of the Anafi breed had higher concentrations urinary ascorbic acid than did their male counterparts. It is suggested that in camels the main elimination route of vitamin C is with urine. Female and male Arabi camels that were sexually active had 52 and 23% lower plasma ascorbic acid concentrations than did their sexually inactive counterparts. It is suggested that especially Bishari camels during the breeding season might be sensitive to disease.

Introduction

Ascorbic acid (vitamin C) is an antioxidant involved in the defence against free radicals (ROSE and BRODE 1993) and modulates the immune response as it influences phagocytic cell mobility and chemotaxis (BENDLICH 1993; VOJDANI et al. 2000). L-ascorbic acid is synthesized in the liver of many species of mammals, including ruminants (HORNIG 1975). The vitamin C status of ruminants may be important in relation to their health and disease. Cows with endometritis (KOLB et al. 1994) and calves with pneumonia (JAGOS et al. 1977) have low ascorbic acid concentrations in blood plasma. Intravenous administration of ascorbic acid has been shown to be beneficial for calves infected with either bovine rhinotracheitis or herpesvirus-1 (DUBESKI et al. 1996). Thus, in ruminants a low vitamin C status not only may be the result of infectious disease but also may be protective against infection. Supplementation with vitamin C of ruminants has to be done by injection as orally administered vitamin C will be degraded by rumen bacteria. In the light of the observations in cows, it could be suggested that vitamin C status also plays a role in disease resistance in camels. This study aimed at identifying the association, if any, between season, breed or sex and vitamin C status of camels (*Camelus dromedarius*) kept under natural grazing conditions in Sudan. Thus, the objective of this study is of a descriptive nature while it focusses on vitamin C. It was anticipated that the present study would provide clues as to situations in which the vitamin C status in camels could easily become compromised.

Materials and methods

Samples were analyzed from a total of 3429 camels (2009 males and 1420 females) that were derived from thirteen herds kept in the Butana area in eastern Sudan. The herds belonged to the Lahawiyin tribe. The camels sampled were aged 6-10 years. During a total of 31 sampling days, 5-10 persons were involved in the collection of the various samples from a total of 1163 camels. The animals had been driven to 5 different

slaughterhouses for slaughtering according to Islamic principles. The camels did not have access to feed and water for a period of 18 – 24 hours prior to slaughter. Fasting for up to 24 hours may not influence vitamin C status (HORNIG 1975). Blood samples were taken from the jugular vein into heparinized vacutainer tubes. The samples were immediately put on ice. Immediately after the camels were killed, samples from liver, adrenals, kidney, lung, spleen and heart were collected. Samples were immediately put in liquid nitrogen (-70 ^{0}C). Blood and tissue samples were transported over distances up to 180 km to the laboratory for further processing. Slaughtering was done under veterinary inspection and samples used for analysis were derived from animals approved for human consumption. A total of 53 carcasses was found to be unsuitable for consumption and the corresponding samples were discarded. From a total of 2319 camels blood samples were taken only, and while they were in the herds. The camels were sampled as described above, but were in the fed state. A total of 828, ear-tagged camels was sampled twice, both during the breeding and non-breeding season.

All animals sampled were apparently healthy and had been fed on natural range vegetation. The samples were categorized according to gender and breed, i.e. camels bred for either riding (Bishari or Anafi breed) or for packing and/or meat production (Arabi breed). Categorization was based on advice given by the owners and/or workers in the slaughterhouses. Arabi camels have a heavy posture, a large hump and grey color. Bishari camels are relatively small with light posture and pinkish appearance. Anafi camels are similar to Bishari's, but have lighter color and longer neck. Samples from Arabi camels were taken both in the dry (January-May) and rainy season (July-September). In the period November-July, the males typically show sexual arousal by "flehmen" and females by high frequency of urination. The age of the camels was estimated to range between 6 and 10 years as based on their teeth formation. Younger animals generally are not slaughtered in Sudan, but are exported to Egypt or the Gulf region. Body weight of the camels was estimated by measuring chest size and the regression formula of WILSON (1984). The mean body weight ± SD for the Anafi (n=1007), Bishari (n=497) and Arabi camels (n=1925) was 390 ± 24, 402 ± 20, and 516 ± 36 kg respectively.

The blood samples were centrifuged in a refrigerated centrifuge. Immediately after centrifugation, a 2.55 % (v/v) solution of metaphosphoric acid was added to the plasma samples (plasma: acid solution = 2:1) and the mixtures were frozen until thawed prior to analysis. The storage period was less than 1 week. Ascorbic acid was measured using high-pressure liquid chromatography and electrochemical detection according to the method of BEHRENS and MADERE (1987). The limit of detection for ascorbic acid was 0.4 µg/ml of the final assay sample and the coefficient of variation was on average 5.9 % for both plasma and tissues. In animal tissues, ascorbic acid may be oxidized to dehydroascorbic acid (HORNIG 1975), which was also determined in this study.

The tissue samples were minced and 5 g was homogenized in 10 ml of 10 % (v/v) trichloro acetic acid. The mixture was diluted with the acid solution up to a volume 20 ml and then centrifuged at 3000 rpm for 20 min. The supernatant was analyzed for ascorbic acid as described above.

Urine was collected from Anafi camels only; the animals were not withheld from feed and water prior to sampling. A foley catheter was used for urine collection from the females. Urine from males was collected during spontaneous voiding or after stimulation. A 100-ml sample was mixed with an identical volume of 0.6 M trichloro acetic acid solution

to de-proteinize the urine. The supernatant was filtered through a 0.45-µm membrane filter and ascorbic acid analysis was performed as described above.

In this study, sample size was large so that it was assumed that possible confounding factors would be equally distributed when the data were categorized according to season, breed, sexual activity or sex. For various comparisons rounded numbers of samples were used, the selection of samples within the present set being at random. The numbers were generally based on desired statistical power. Statistical analysis of the data was performed using Student's t test or Duncan's multiple range test. Statistical significance was pre-set at $P < 0.05$.

Results

To study the influence of season, Arabi camels were sampled during both the dry and rainy season. Six hundred, non sexually active males were selected for each season. Table 1 shows that season did not affect tissue ascorbic acid contents. On a wet weight basis, the highest levels of ascorbic acid were found in adrenals and liver, whereas the lowest concentrations were seen in heart.

Table 1. Ascorbic acid concentrations in tissues and plasma of male Arabi camels slaughtered either during the dry (January-May) and rainy season (July-September)

	Dry season (n = 300)	Rainy season (n = 300)
Tissue (mg ascorbic acid/100 g wet tissue)		
Adrenal	151 ± 12.1	153 ± 10.2
Liver	60 ± 6.3	60 ± 5.8
Spleen	44 ± 4.6	46 ± 4.4
Kidney	17 ± 2.8	16 ± 2.1
Lung	13 ± 2.6	12 ± 3.7
Heart	8 ± 1.3	7 ± 1.1
Plasma (µg/ml)	5.44 ± 0.91	5.00 ± 0.98

Means ± SD.

Table 2. Ascorbic and dehydroascorbic acid contents in plasma from different breeds of camels sampled during the rainy season (July-September)

Breed	Males (n = 250/breed)		Females (n = 247/breed)	
	Ascorbic acid	Dehydroascorbic acid	Ascorbic acid	Dehydroascorbic acid
		(µg/ml)		
Arabi	6.45 ± 0.12	0.75 ± 0.22	6.38 ± 0.14	0.91 ± 0.32
Bishari	3.67 ± 0.46	0.42 ± 0.11	2.81 ± 0.64	0.29 ± 0.03
Anafi	4.83 ± 0.42	0.66 ± 0.21	4.12 ± 0.30	0.54 ± 0.19

Means ± SD. Ascorbic acid or dehydroascorbic acid values in the same column or in the same row are significantly different (P<0.05).

Plasma concentrations of ascorbic and dehydroascorbic acid in different breeds are given in Table 2. The apparently healthy animals were randomly selected, but were not sexually active. Arabi camels had significantly higher levels of ascorbic acid than did the Bishari and Anafi breeds. A sex difference was seen only in Bishari camels, the males having significantly higher values than the females. Dehydroascorbic concentrations were lowest in the Bishari camels. Among Anafi camels, females had significantly higher concentrations of urinary ascorbic acid than males (Table 3).

Table 3. Ascorbic acid concentrations in plasma, liver and urine in Anafi camels sampled during the rainy season (July-September)

Sample	Males (n = 250)	Females (n = 260)
Liver (mg/100 g)	56 ± 6.1	53 ± 5.4
Plasma (µg/ml)	4.87 ± 1.00	4.78 ± 1.22
Urine (mg/l)	3.22 ± 0.97	5.33 ± 0.25*

Means ± SD. All camels came from the same herd and were aged 6-8 years.
**Significantly different (P<0.05) from the values for males (P<0.05).*

Table 4 shows that the plasma level of ascorbic acid in Arabi camels of both sexes was significantly decreased during the breeding season as compared to the non-breeding season. The opposite was seen for the plasma level of dehydroascorbic acid.

Table 4. Plasma ascorbic and dehydroascorbic acid contents in Arabi camels that were sampled both during the breeding (October-February) and non-breeding season (April-June)

	Males (n = 409)		Females (n = 419)	
	Ascorbic acid	Dehydroascorbic acid	Ascorbic acid	Dehydroascorbic acid
	(µg/ml)			
Breeding	3.94 ± 1.30	0.91 ± 0.21	2.04 ± 0.77	0.76 ± 0.11
Non breeding	5.11 ± 1.71	0.61 ± 0.11	4.22 ± 0.97	0.43 ± 0.09

Means ± SD. Ascorbic acid or dehydroascorbic acid values for males versus females or for breeding versus non-breeding are significantly different ($P<0.05$).

Discussion

This paper presents vitamin C concentrations in various tissues of camels browsing on natural vegetation in the desert. The study is a descriptive one and thus ascorbic acid levels in many tissues were determined even though the rationale from a metabolic point of view was not always clear. Plasma concentrations of ascorbic acid in camels have been reported earlier (SOLIMAN et al. 1975; SNOW et al. 1992) and the values correspond well with those obtained in this study. The rainy and dry season had no differential effect on tissue and plasma ascorbic acid contents. This observation could imply that ascorbic acid synthesis and catabolism do not depend on season. Ascorbic acid is synthesized from glucose, but this process is not important in quantitative terms (HORNIG 1975) so that it is unlikely that gluconeogenesis in ruminants would limit ascorbic acid synthesis. However, in sheep fed stall rations plasma ascorbic acid levels were lower than during grass feeding (CAKALA et al. 1974). In contrast, KOLB et al. (1991) showed that values of liver ascorbic acid in bulls and oxes were highest in December when they were kept inside. Thus, there may be an effect of diet on vitamin C metabolism in ruminants.

BARAKAT and ABDALLAH (1965) analyzed ascorbic acid in livers obtained from Egyptian camels without known feeding history, and found an average value of 58 mg/100 g wet tissue, which is almost identical to the present data. The tissue contents of ascorbic acid in the camels became lower in the order of adrenals, liver, spleen, kidney, lung and heart. In contrast to our findings, KOLB et al. (1989) showed in cows that the ascorbic acid level in spleen was higher than that in liver.

There was a clear relation between breed and plasma ascorbic acid concentration. The Arabi camels displayed higher concentrations than did their Bishari and Arabi counterparts. The Bishari camels had the lowest concentrations of ascorbic acid in plasma and also had the lowest values of plasma dehydroascorbic acid. These observations point at genetic differences in vitamin C metabolism among the three camel breeds. VERMA et al. (1993) and HAAG and HOFMANN (1987) reported for cattle that serum ascorbic acid depends on genotype.

Female Anafi camels had higher concentrations of ascorbic acid in urine than did the males, and it is likely that the females excrete more ascorbic acid. This finding is consistent with data published by NESENI (1938), showing that that urinary ascorbic acid contents were higher in female than male cattle. However, PALKINAS (1941) reported that

there was no sex difference as to ascorbic acid in urine of calves. It is likely that the main excretory route for vitamin C in camels is via the urine as has been shown for sheep (KOLB et al. 1994).

There was an influence of sex not only on urinary excretion of ascorbic acid, but also on plasma levels. Male camels had higher plasma concentrations of both ascorbic and dehydroascorbic acid than did the females. Similar results have been found by SNOW et al. (1992) in camels from the United Arab Emirates and by SOLIMAN et al. (1975) in Egyptian breeds of camels. The lower levels in sexually active animals may be caused by enhanced utilization of vitamin C.

In conclusion, this study shows that the lowest concentrations of ascorbic acid in blood plasma were seen in the Bishari camels, and that the values may become even lower during the breeding season. Given the evidence suggesting that low plasma vitamin C levels are associated with disease in ruminants (KOLB 1992), it would follow that camels, especially the Bishari breed, have a low disease resistance during the breeding season.

Acknowledgement

H.E. Mohamed was supported by The Netherlands Foundation for Nutrition and Health Research.

References

BARAKAT, M.Z.; ABDALLAH, A., 1965: J. Food Sci. 39, 185-187.
BEHRENS, W.; MADERE, R., 1987: Anal. Biochem. 165, 102-107.
BENDLICH, 1993: J. Dairy Sci. 76, 2789.
BENDLICH, A., 1993: J. Dairy Sci. 76, 2789-2794.
CAKALA, S.; BORKOWSKI, T.; ALBRYCHT, A., 1974: Pol. Arch. Weter. 17, 117.
DUBESKI, P.L.; OWENS, F.N.; SONG, W.O.; COBURN, S.P.; MAHU, J.D., 1996: J. Anim. Sci. 74, 1358.
HAAG, W.; HOFMANN, W., 1987: Tierartzliche Umschau. 42, 956-963.
HORNIG, D., 1975: Ann. N.Y. acad. Sci. 258, 103.
JAGOS, P.; BOUDA, I.; DVORAK, R., 1997: Vet. Med. (Praha) 22, 133.
KOLB, E., 1992: Tierärztl. Umschau 47, 163.
KOLB, E.; KOUIDER, S.; KUBA, M.; LEO, M.; WAHREN, M.; VÖLKER, L., 1994: Schweiz. Arch. Tierheilk. 136, 95.
KOLB, E.; DITTRICH, H.; DOBELEIT, G.; SCHMALFUSS, R.; SIEBERT, P.; STAUBER, E.; WAHREN, M., 1991: Berl. Münch. Tierärztl. Wochenschr. 104, 387.
KOLB, E.; WAHREN, M.; DOBLEIT, G.; GRUNDER, G., 1989. Arch. Exper. Vet. Med. 439, 327-334.
NESENI, R., 1938: Prag. Tierärztli. Arch. 18, 137.
PALKINAS, P., 1941. Kolrebol Osszehansolo. 29, 267.
ROSE, R.C.; BRODE, A.M., 1993: FASEB J. 7, 1135.
SNOW, D.H.; BILLAH, A.M.; RIDHA, A.; FRIGG, M., 1992: Prod. Ist. Int. Camel Confr. 263-270.
SOLIMAN, M.K.; YOUSSEF, G.W.; MANSOUR, S.A., 1975: Egypt. J. Vet. Sci 12, 107
VERMA, R.P., BHAG, H.K., MISHRA, R.R. 1993: Indian J. Dairy Sci. 46, 162-165

VOJDANI, A.; BAZARGAN, M.; VOJDANI, E.; WRIGHT, J., 2000: Cancer Detect. Prev. 24, 508.
WILSON, R.T., 1984: The Camel. Longman, New York.

Chapter 3

Vitamin C in Plasma and Leukocytes of Camels with Different Reproductive Status

H.E. Mohamed[1], H.M. Mousa[1] and A.C. Beynen[2]

[1]*Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum, Khartoum North, Sudan*

[2]*Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Abstract

The relationships between reproductive cycle and vitamin C contents in plasma and leukocytes were studied in Sudanese camels browsing on local vegetation. Vitamin C status was found to be lowest during pregnancy and highest during lactation. Estrus versus non-estrus was associated with high vitamin C status. Brucellosis-positive camels showed a reduction in the levels of ascorbic acid in plasma and leukocytes.

Introduction

The reproductive cycle has phases that can be considered stressful for animals. Different types of stress may lower the vitamin C status of animals [1], which in turn diminishes their disease resistance [2]. We have described vitamin C status in Sudanese camels in different situations [3,4], but we are not aware of reports on the relation between reproductive status and ascorbic acid concentrations in plasma and leukocytes. Thus, this investigation was carried out to describe vitamin C status throughout the reproductive cycle of camels. In addition, we studied the influence of brucellosis, an infectious disease causing abortion and infertility.

Materials and methods

The female camels (*Camelus dromedarius*) studied were of the Arabi breed and kept in 30 different herds of the Lahawiyin tribe. They grazed at the low woodland savanna in Central Sudan. The camels were selected for sampling on the basis of their reproductive status. Another, large group of female camels was subjected to a brucellosis test. Only apparently healthy animals were used; their estimated age was 6-10 years.

In collaboration with the owners, 280 non-pregnant, non-lactating females were selected and sampled once either during estrus or non-estrus. Camels in estrus were identified by signs of restlessness, vagina swelling and mucous discharge, and their attraction to males. Pregnant camels (n=430) were identified by cocked tail and confirmation was obtained by measurement of serum progesterone; concentrations higher than 1 ng/ml were taken as evidence of pregnancy. The ear-tagged pregnant camels were followed up to the stage of 4-5 months in lactation when they were sampled again. Another group of non-pregnant, non-lactating camels (n=280) was also sampled. Out of a group of 1200 female camels, with different reproductive status, 42 animals were found to be brucellosis positive.

Blood was collected from the jugular vein. Samples were taken during the rainy season (July-September) while the animals were in the fed state. Ascorbic acid in plasma and leukocytes was analyzed as described [5]. Serum progesterone was determined with a commercial test combination (Boehringer Mannheim GmbH, Germany). Plasma glucose was determined for animals that were followed throughout pregnancy and lactation; the glucose oxidase method was used (test combination, Boehringer Mannheim GmbH). Serum was tested

immediately for brucellosis at the field laboratory using the Rose Bengal Test and a positive outcome was confirmed by the standard agglutination test according to Alton et al. [6].

It was assumed that by following up the same animals and by using large sample sizes, there was equal distribution of possible confounding factors among the comparisons. Duncan's multiple comparison test or Student's t-test was used to identify significant differences between groups. The level of statistical significance was pre-set at $P<0.05$.

Results

Female camels during estrus instead of non-estrus showed increased ascorbic acid concentrations in plasma and leukocytes (Table 1).

Table 1. Vitamin C contents of plasma and leukocytes in female Arabi camels as related to reproductive status

Status	Ascorbic acid	
	Plasma (mg/l)	Leukocytes (mg/l blood)
Non-oestrus (n=280)	3.13 ± 0.66^{a}	30.7 ± 1.34
Oestrus (n=280)	5.89 ± 1.88^{b}	40.3 ± 2.11
Pregnant (n = 430)	3.77 ± 0.81^{c}	32.7 ± 0.91^{c}
Lactating, non-pregnant (n = 290)	5.01 ± 0.68^{d}	52.2 ± 1.31^{d}
Non-pregnant, non-lactating (n=280)	4.34 ± 0.70^{e}	45.6 ± 1.01^{e}

Means ± SD. Means in the same column having different superscripts are significantly different ($P < 0.05$)

The reproductive cycle was related with the content of ascorbic acid in plasma and leukocytes. The highest levels were seen in lactating camels and the lowest during pregnancy while intermediate levels were found in non-pregnant, non-lactating animals that were not typified according to the stage of the estrus cycle (Table 1). Brucellosis was found to be associated with a reduction of the ascorbic acid contents of leukocytes and plasma (Table 2).

Table 2 Effect of brucellosis on vitamin C in leukocytes and plasma from female Arabi camels

	Ascorbic acid	
	Plasma (mg/l)	Leukocytes (mg/l blood)
Negative (n=1158)	4.22 ± 0.79	44.39 ± 2.11
Positive (n=42)	3.02 ± 0.99[a]	30.22 ± 1.98[a]

Means ± SD. [a] Significantly different from Brucellosis-negative animals (P<0.05).

Discussion

There was a significant increase in plasma and leukocyte ascorbic acid during lactation, whereas during pregnancy the levels were relatively low. Vitamin C is produced from glucose and it could be suggested that glucose availability was limiting vitamin C synthesis during pregnancy. However, there was no clear association between vitamin C status and the glucose level. Plasma glucose concentrations (means ± SD) were 5.38 ± 0.64, 4.34 ± 0.64 and 5.50 ± 0.91 for the pregnant, lactating and non-pregnant camels, respectively. Thus, in the lactating camels, high plasma ascorbic acid was associated with low glucose. During lactation there is extra need for glucose to serve as precursor for lactose synthesis, which is reflected by low plasma glucose. In contrast to our findings, Naziroglu and Gur [7] did not find a difference between pregnant and non-pregnant cows with respect to plasma vitamin C levels. Camels in estrus were found to have increased vitamin C levels in plasma and leukocytes. Miszkiel et al. [8] found in cattle, in different stages of estrus and pregnancy, that ascorbic acid concentrations were directly correlated with those of progesterone.

In the present study, 42 out of 1200 animals (3.5 %) were found to be brucellosis positive. Previous reports on brucellosis in camels in Sudan have estimated the prevalence ranging from 1.9 – 7.5 % [9], but El-Ansary et al. [10] found no positive cases in the 64 camels studied. Brucellosis was associated with reduced levels of ascorbic acid in plasma and leukocytes. It is likely that the bacterial infection had caused a decrease in vitamin C status [4] even though at the moment of blood sampling the camels were apparently healthy.

It is clear that vitamin C status of camels is related with the reproductive cycle. The low vitamin C status of pregnant camels might impair their disease resistance.

References

1. Nordberg, J. and Arner, E.S. (2001) Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic. Boil. Med. 31, 1287-1312.
2. Field, C.J., Johnson, I.R. and Schley, P.D. (2002) Nutrients and their role in host resistance to infection. J. Leukoc. Biol. 71, 16-32.
3. Mohamed, H.E. and Beynen, A.C. (2002) Vitamin C concentrations in blood plasma, tissues and urine of camels (*Camelus dromedarius*) in Sudanese herds. J. Anim. Physiol. a. Anim. Nutr. (in press).
4. Mohamed, H.E. and Beynen, A.C. (2002) Ascorbic acid contents in blood plasma, erythrocytes, leukocytes and liver in camels (*Camelus dromedarius*) without or with parasite infections. Int. J. Vitam. Nutr. Res. (in press).
5. Behrens, W. and Madere, R. (1987) A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic acid and dehydroascorbic acid in tissues, biological fluids and foods. Anal. Biochem. 165, 102-107.
6. Alton, G. G., Jones, M., Angus, R. D. and Verger, J. M. (1988) Techniques for the Brucellosis Laboratory. Institut De La Recherche d'Agronomie, Paris.
7. Naziroglu, M. and Gur, S. (2000) Antioxidants and lipid peroxidation levels of blood and cervical mucus in cows in relation to pregnancy. Dtsch. Tierärztl. Wochenschr. 107; 374-376.
8. Miszkiel, G., Skarzynski, D., Bogacki, M. and Kotwica, J. (1999) Concentrations of catecholamines, ascorbic acid, progesterone and oxytocin in the corpora lutea of cyclic and pregnant cattle. Reprod. Nutr. Dev. 39, 509-516.
9. Agab, H., Abbas, B., Ahmed, H. and Maoun, I.E. (1994) First report on the isolation of Brucella abortus biovar 3 from camels (*Camelus dromedarius*) in the Sudan. Rev. Elev. Med. Vet. Pays Trop. 47, 361-363.
10. El-Ansary, E.H., Mohamed, B.A., Hamad, A.R. and Karom, A.G. (2001) Brucellosis among animals and human contacts in eastern Sudan. Saudi Med. J. 22, 577-579.

Chapter 4

Ascorbic Acid Concentrations in Milk from Sudanese Camels

H.E. Mohamed[1], M.H. Mousa[1] and A.C. Beynen[2]

[1]*Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum, Khartoum North, Sudan*

[2]*Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Abstract

The present study in Sudanese camels was done to describe the associations between vitamin C concentrations in milk, and either breed, stage of lactation, parity or the presence of mastitis. Arabi camels had higher ascorbic acid levels in milk than did either Anafi or Bishari camels. Milk ascorbic acid levels were higher for camels more than 180 days in lactation than for those earlier in lactation. Multiparous versus primiparous camels had higher ascorbic acid concentrations in their milk. The ascorbic acid content of colostrum was higher than that of milk. Mastitis was associated with a decrease in the ascorbic acid content of both milk and blood plasma.

Introduction

Vitamin C status of animals may be a determinant of their disease resistance (FIELD et al. 2002). We have described vitamin C concentrations in plasma and leukocytes of Sudanese camels in relation to characteristics such as breed, sex, sexual activity and season (MOHAMED and BEYNEN 2002a) and parasite infections (MOHAMED and BEYNEN 2002b). Vitamin C concentrations in plasma and leukocytes were found to be higher in lactating camels than in their dry counterparts (MOHAMED et al. 2002). There are reports on the vitamin C content of camel milk (FARAH et al. 1992; KNOESS 1977; MEHAIA and AL-KAHNAL 1989), but there was no information on the ascorbic acid content of camel milk in relation to the stage of lactation. Thus, in this study vitamin C levels were measured in colostrum and in milk from camels up to 360 days in lactation. Parasite infections (MOHAMED and BEYNEN 2002b) and brucellosis (MOHAMED et al. 2002) were associated with low vitamin C status of Sudanese camels. In this study on ascorbic acid in camel milk we also addressed the question whether mastitis affects vitamin C status.

Materials and Methods

In this study, a total of 2586 camels (*Camelus dromedarius*) from 15 herds of the Lahawiyin tribe were used. The first author was familiar with the camel owners and belongs to the same tribe. Animals were allowed to graze in the centre of Butana, Sudan during the rainy season and they migrated to the Elshowak region during the dry season. The camels were mainly fed on natural vegetation, but during the dry season dietary supplements consisted of sorghum stalk and sesame cakes.

To assess the relation between breed and ascorbic acid in colostrum, samples were taken from Arabi, Anafi and Bishari camels at 4 or 5 days after parturition. The animals were of different parities. Milk samples were taken from the same, ear-tagged animals when they were 4-5 months in lactation. Blood samples were taken at the same time as the milk samples. Blood samples were taken from primiparous Arabi camels in the dry state and another group was followed throughout lactation while milk and blood samples were taken at different stages. The animals were ear-tagged for identification. Another group of camels was used to determine the influence of parity. On the basis of information of the owners, the animals were classified as primi- or multiparous.

One thousand primi- or multiparous, lactating Arabi camels that were 6-9 months in lactation were checked for the presence of mastitis using the California Mastitis Test.

Positive camels were verified to have clinical signs such as redness and increased temperature of the udder in acute cases and fibrosis in chronic cases. Blood and milk samples were taken for ascorbic acid analysis.

All samples were taken during the rainy season (July-September). Blood was taken from the jugular vein while the animals were in the fed state. Blood samples were kept on ice and centrifuged at 4 ^{0}C. The camels were milked by hand. Milk samples were put on ice and transported to the laboratory. Samples of plasma and milk were prepared and analyzed for ascorbic acid as described previously (BEHRENS and MADERE 1987).

It was assumed that possible confounding factors would not affect interpretation of the results because either the same animals were followed and/or sample size was large. To identify group differences, Duncan's multiple comparison test or Student's t-test was used. The level of statistical significance was pre-set at $P<0.05$.

Results

As shown in Table 1, significant breed differences were observed for colostrum and milk L-ascorbic acid contents.

Table 1. Colostrum, milk and plasma L-ascorbic acid levels in three breeds of camels

	Breed		
	Arabi (n=219)	Anafi (n=204)	Bishari (n=197)
Plasma (mg/l)	5.53 ± 1.41^a	4.42 ± 1.22^b	3.21 ± 1.00^c
Colostrum (mg/l)	54.8 ± 6.7^a	44.5 ± 5.9^b	42.3 ± 6.1^c
Milk (mg/l)	47.8 ± 5.3^a	40.9 ± 4.0^b	39.1 ± 3.7^c

Data presented as means ± SD. Means in the same row having a different superscript are significantly different ($P<0.05$).

Arabi camels had the highest concentrations. Plasma ascorbic acid also was highest in the Arabi camels. Table 2 shows a significant effect of stage of lactation on milk vitamin C levels.

Table 2. Milk and plasma ascorbic acid levels as affected by the stage of lactation in primiparous Arabi camels

	Stage of lactation, days				
	Dry (n=230)	6-89 (n=310)	90-179 (n=221)	180-269 (n=200)	270-360 (n=233)
Plasma (mg/l)	$3.98^{a} \pm 0.81$	$3.91^{a} \pm 0.87$	$4.10^{a} \pm 0.99$	$4.40^{b} \pm 1.09$	$4.94^{b} \pm 1.18$
Milk (mg/l)	-	$44.2^{a} \pm 4.2$	$44.2^{a} \pm 3.9$	$46.7^{b} \pm 4.0$	$48.4^{b} \pm 3.8$

Data presented as means ± SD. Means in the same row having a different superscript are significantly different (P < 0.05).

The levels were higher after 180 days in lactation than before. Plasma ascorbic acid concentrations showed the same trend. Table 3 shows that multiparous versus primiparous female camels had similar plasma ascorbic acid concentrations, but had significantly higher concentrations in milk.

Table 3. Milk and plasma ascorbic acid levels as affected by parity in Arabi camels

	Primiparous (n=171)	Multiparous (n=255)
Plasma (mg/l)	4.44 ± 0.91	4.35 ± 0.89
Milk (mg/l)	44.9 ± 5.8	46.3 ± 4.7^{a}

Means ± SD. [a] Significantly different from primiparous animals (P<0.05).

Udder condition markedly affected milk and plasma vitamin C concentrations. Table 4 shows that mastitis was significantly associated with reduced ascorbic acid levels.

Table 4. Plasma and milk ascorbic acid levels as affected by mastitis in Arabi camels

	Healthy (n=510)	Mastitis (n=490)
Plasma (mg/l)	4.43 ± 1.01	2.71 ± 0.91^{a}
Milk (mg/l)	47.4 ± 5.2	26.8 ± 4.4^{a}

Means ± SD. [a] Significantly different from healthy animals (P<0.05).

Discussion

Milk production of camels may vary between 1800 and 12700 kg for a lactation period between 9 and 18 months. As to milk production by camels, BACHMANN and SCHULTHESS (1987) have studied the effect of breed, KARUE (1994) has investigated the effect of stage of lactation on daily production and FIELD (1979) has reported the effect of parity. The present study shows that milk concentrations of ascorbic acid are on average 44.2 mg/l before 180 days in lactation and 47.6 mg/l thereafter. The present values for milk vitamin C are higher than those reported by FARAH et al. (1992), who obtained a mean value of 37 mg/l in indigenous Kenyan camels. For Saudi breeds of camels, SAWAYA et al. (1984), using only 11 camels, found a mean value of 24 mg/l. KNOESS (1977), MEHAIA and AL-KAHNAL (1989) and KON (1959) have reported similar low levels.

Ascorbic acid concentrations were found to be on average 10 % higher in colostrum than in milk obtained at 4-5 months after parturition. HIDIROGLOU et al. (1995) obtained a level of 16 mg/l in colostrum and 8 mg/l in milk of Canadian Holstein cows. It would appear that newborn camel and dairy calves depend on colostrum as a source of vitamin C. There is evidence that vitamin C stimulates the immune response in calves (CUMMINS and BRUNNER 1989). WITNAH and RIDDELL (1977) also reported low ascorbic acid concentrations in cow milk. From a metabolic point of view, it is interesting to study the species difference.

The incidence of mastitis in the 1000 Arabi camels was found to be 51%. This figure is similar to that reported by ABDURAHMAN et al. (1995) showing in a field survey using 170 camels that the incidence of mastitis was 43.5%. It is clear that udder condition markedly affected the vitamin C content of both milk and blood plasma from camels. This finding is in contrast to recent data obtained in cows (SANTOS et al. 2001). In any event, in camels mastitis may lower vitamin C status as has been shown for other types of infection (MOHAMED and BEYNEN 2002b; MOHAMED et al. 2002).

Acknowledgement

H.E. Mohamed was supported by The Netherlands Foundation for Nutrition and Health Research.

References

ABDURAHMAN, O.A.; AGAB, H.; ABBAS, B.; ASTROM, G.;1995: Acta. Vet. Scand. 36, 423-431.

BACHMANN, M.R.; SCHULTHESS, W.; 1987: Milchwissenschaft. 42, 766-768.

BEHRENS, W.; MADERE, R.; 1987: Anal. Biochem. 165, 102-107.

CUMMINS, K.A.; BRUNNER, C.J.; 1989: J. Dairy Sci. 72, 129-134.

FARAH, Z.; RETTENMAIER, R.; ATKINS, D.; 1992: Int. J. Vitam. Nutr. Res. 62, 30-33.

FIELD, C.R.; 1979: Camel growth and milk production in Marsabit District, Northern Kenya. In: The Camelid: An All-purpose Animal. Scandinavian Institute for African Studies. Uppsala, Sweden.

FIELD, C.J.; JOHNSON, I.R.; SCHLEY, P.D.; 2002: J. Leukoc. Biol. 71, 16-32

HIDIROGLOU, M.; IVAN, M.; BATRA, T.R.; 1995: J. Dairy Sci. 44, 399-402.

KARUE, C.N.; 1994: The dairy characteristics of the Kenyan camel. In: Hameauxe Dromedaries, Naulchott, Mauritanie.
KNOESS, K.H.; 1977: World Anim. Rev. 22, 39-44.
KON, S.K.; 1959: Milk and milk Products for Human Nutrition. FAO Nutrition Services, Rome.
LARANJA DA FONESCA, L.F.; 2001: J. Dairy Sci. 84, 134-139.
MEHAIA, M.A.; AL-KAHNAL, M.A.; 1989: Nutr. Rep. Intl. 39, 351-357.
MOHAMED, H.E.; BEYNEN, A.C.; 2002a: J. Anim. Physiol. a. Anim. Nutr. (in press)
MOHAMED, H.E.; BEYNEN, A.C.; 2002b: Int. J. Vitam. Nutr. Res. (in press)
MOHAMED, H.E.; MOUSA, H.M.; BEYNEN, A.C.; 2002: Int. j. Vitam. Nutr. Res. (submitted)
SANTOS, M.V.; LIMA, R.F.; RODRIGUES, P.H.; BARROS, S.B.; LARANJA FONESCA, L.F.; 2001: J. Dairy Sci. 84, 134-139.
SAWAYA, W.N.; KHALIL, J.K.; AL-KAHNAL, A.; AL-MOHAMED, H.; 1984: J. Food Sci. 49, 744-747.
TOUTAIN, P.L.; BECHU, D.L.; HIDIROGLOU, M.; 1997: Am. J. Physiol. Regul. Inter. Com. Physiol. 273, 1585-1597.
WITNAH, C.H.; RIDDELL, W.H.; 1977: J. Dairy Sci. 20, 9-84.

Chapter 5

Vitamin C Status of Dromedary Camels and their Calves during the First Month after Parturition

H.E. Mohamed[1], H.M. Mousa[1] and A.C. Beynen[2]

[1]*Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum, Khartoum North, Sudan*

[2]*Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Abstract

We measured plasma vitamin C levels during the first four weeks postpartum in camel calves and their dams. In the calves plasma ascorbic acid declined as from birth and had stabilized after four weeks. In the dams, plasma ascorbic acid rose from parturition and reached a steady value after four weeks.

Introduction

The foetus of mammals that are able to synthesize ascorbic acid can also synthesize the vitamin [1], but colostrum and milk are nevertheless still considered important as an exogenous source of ascorbic acid [2]. Ascorbic acid intake by dairy calves may stimulate their immune response [3]. As far as we know, no research has been conducted on vitamin C status of camel calves. Therefore, this investigation was designed to measure vitamin C levels in newborn camel calves during the first month of life.

Material and methods

Camels (*Camelus dromedarius*) of the Arabi breed, and approaching parturition, were studied at the Camel Research Centre, Elshowak, Sudan. After delivery, 10 calves weighing 29.7 ± 2.7 kg (mean ± SD) were fed with colostrum for two days and then with milk at an intake level of 10% of their body weight. They received half of their allowance in the morning (09.00 h) and the other half in the afternoon (17.00 h), and had free access to a starter diet composed of alfalfa, sorghum and sesame cake. Calves were weighed weekly. The mothers were fed ad libitum on *Acacia mellifera* and sorghum stalk. Dams and calves were kept in individual pens.

Each week, after the morning and afternoon meal of the calves, blood samples were taken from the dams and the calves by puncture of the jugular vein. The dams were milked by hand and samples were taken. Ascorbic acid levels in plasma and in milk were measured as described [4].

The data were statistically analysed to identify significant changes with time using Duncan's multiple comparison test. The values for different time points were considered to be independent. Ascorbic acid concentrations in plasma from dams and calves were compared with Student's t-test. The level of statistical significance was pre-set at $P<0.05$.

Results

During the four-week postpartum period, growth of the calves on average was 0.50 ± 0.10 kg/day. Table 1 shows the plasma levels in the calves during the first four weeks of life and the corresponding plasma and milk levels in the dams.

Table 1. Plasma and milk ascorbic acid levels after parturition

	Parturition	Week 1	Week 2	Week 3	Week 4
Plasma (mg/l)					
Dams	2.30 ± 0.64^{a}	2.71 ± 0.51^{a}	3.42 ± 0.71^{b}	3.61 ± 0.79^{b}	3.52 ± 0.83^{b}
Calves	9.65 ± 1.72^{a}	4.74 ± 0.81^{b}	3.53 ± 0.75^{c}	2.35 ± 0.30^{d}	2.21 ± 0.21^{d}
Milk (mg/l)	49.1 ± 3.4^{a}	34.0 ± 5.9^{b}	24.2 ± 3.9^{c}	22.2 ± 3.0^{d}	21.3 ± 2.9^{d}

Data presented as means ± SD for 10 animals. For each animal, the plasma values of the morning and afternoon samples were averaged. Means in the same row with different superscript letter are significantly different ($P < 0.05$).

Plasma ascorbic acid in the calves decreased with age, but in the dams there was an increase during the postpartum period. The ascorbic acid level in milk fell with time after parturition. Plasma ascorbic acid at parturition was four-fold higher in the calves than in the dams, but four weeks later the dams had higher levels.

Discussion

The time courses of ascorbic acid concentrations in plasma from the calves and the dams, and also the time course of milk ascorbic acid, most likely have physiological relevance. During the first weeks of life, prenatal storage, liver biosynthesis, colostrum and milk have to meet the camel calves' ascorbic acid requirement. The high plasma level in newborn calves could be necessary to stimulate the immune system [5] and/or protect against free radical damage [6]. A high plasma level is supported by the high ascorbic acid concentration in colostrum when compared with that in milk. In an earlier study, we have also shown higher ascorbic acid concentrations in colostrum than in milk [7]. The low plasma level in the dams at parturition could imply that ascorbic acid synthesis may not keep pace with the requirement of the foetus. Pregnant camels have been found to show lower plasma ascorbic acid levels than their lactating counterparts [8] which could point at less disease resistance in the former. The postpartum drop of plasma ascorbic acid in the calves and the concurrent rise in the dams cannot be easily explained. The ascorbic acid concentrations in plasma of the dams and in milk, as found in the present study with camels fed a stall ration, were generally lower than those seen in camels ranging on natural vegetation [7].

The present observations in camels may be compared with data from cows. The ascorbic acid plasma levels in Canadian Holstein calves at birth and in the first, second, third and fourth week of life were 7.65 ± 1.73, 3.47 ± 0.75, 2.23 ± 0.72 and 1.35 ± 0.20 mg/l (means ± SD, n=6), respectively [9]. Plasma ascorbic acid in the cows was low and

fluctuated between 1.7 and 2.6 mg/l. The ascorbic acid concentration in colostrum at calving was 16 ± 1.02 mg/l and dropped to 8.0 ± 1.31 mg/l on day 2 and then stabilized at 9.1 ± 1.8 mg/l until 28 days post calving [9]. In another study [10], it was also found that plasma ascorbic acid concentration was lower in periparturient cows than in their calves, but they were similar at 28 days postpartum [10]. Clearly, the levels of ascorbic acid in camel calves and their dams are higher than those in their dairy cow counterparts.

Acknowledgement

H.E. Mohamed was supported by The Netherlands Foundation for Nutrition and Health Research.

References

1. Ching, S., Mahan, D.C., Ottobre, J.S. and Dabrowski, K. (2001). Ascorbic acid synthesis in fetal and neonatal pigs and in pregnant and postpartum sows. J. Nutr. 131, 1997-2001.
2. Dobinsky, O., Itze, L., Pospisil, J. and Pospisil, M. (1979). Vitamin C levels in the early postnatal period in calves and their mothers. Vet. Med. (Praha) 24, 385-390.
3. Cummins, K.A. and Brunner, C.J. (1989). Dietary ascorbic acid and immune response in dairy calves. J. Dairy Sci. 72, 129-134.
4. Behrens, W. and Madere, R. (1987). A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic acid and dehydroascorbic acid in tissues, biological fluids and foods. Anal. Biochem. 165, 102-107.
5. Nordberg, J. and Arner, E.S. (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic. Biol. Med. 31, 1287-1312.
6. Field, C.J., Johnson, I.R. and Schley, P.D. (2002). Nutrients and their role in host resistance to infection. J. Leukoc. Biol. 71, 16-32.
7. Mohamed, H.E., Mousa, H.M. and Beynen, A.C. (2002). Ascorbic acid concentrations in milk from Sudanese camels. J. Anim. Physiol. a. Anim. Nutr. (submitted).
8. Mohamed H.E., Mousa, H.M. and Beynen, A.C. (2002). Vitamin C in plasma and leukocytes of camels with different reproductive status. Int. J. Vitam. Nutr. Res. (submitted).
9. Toutain, P. L., Bechu, D. and Hidiroglou, M. (1997). Ascorbic acid desposition in plasma and tissues of calves. Am. J. Physiol. Regul. Integr. Comp. Physiol. 273, 1585-1597.
10. Hidiroglou, M., Ivan, M. and Batra, T. R. (1995). Concentrations of vitamin C in plasma and milk of cattle. Ann. Zootech. 44, 399-402.

Chapter 6

Ascorbic acid contents in blood plasma, erythrocytes, leukocytes and liver in camels (*Camelus dromedarius*) without or with parasite infections

H.E. Mohamed[1] and A.C. Beynen[2]

[1]*Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum, Khartoum North, Sudan;*

[2]*Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Abstract

Healthy camels (*Camelius dromedarius)* and those naturally infected with trypanosomiasis, sarcoptic mange, and helminthiasis were compared as to ascorbic acid contents of red blood cells, white blood cells, whole blood, plasma and liver. The camels were kept under natural grazing conditions in Sudan. A low status of vitamin C was found in camels with parasite infections, especially in animals with Trypanosomiasis. It is suggested that the low vitamin C status in infected camels is caused by increased utilization and /or decreased synthesis of vitamin C.

Introduction

Sudan has a dromedary population of about 2.5 million, representing 14% of the total world population. Trypanosomiasis, sarcoptic mange and helminthiasis are endemic and have a negative impact on camel welfare and productivity. Stress of disease imposed on animals may lower plasma ascorbic acid levels [1], whereas in non-ruminants dietary supplements of ascorbic acid may provide disease resistance [2]. In an attempt to identify conditions that are associated with enhanced sensitivity to disease in Sudanese camels, we have reported concentrations of ascorbic acid in plasma and tissues as related to season, camel breed, gender and sexual activity [3]. The present study was done to investigate the relationship between specified parasite infections and ascorbic acid status in camels. We measured the contents of ascorbic acid in blood plasma, red blood cells, white blood cells, whole blood and liver from apparently healthy and infected camels kept under practical conditions in Sudan.

Materials and methods

Animals

The study was performed during the period of August-December 2000 using about 2000 Arabi camels *(Camelus dromedarius)* of both sexes from 30 herds. Herd owners belonged to the tribes of Lahawiyin and Shukriya. Camels were kept browsing on natural vegetation in the Tambul area, 145 km southeast of Khartoum. The camels, aged 5-10 years, had been moved to a slaughterhouse in Tambul. All samples were collected over a 13-week period. Ante-mortem inspection of camels was performed to identify infected individuals. Direct microscopic examination for the presence of *Trypanosomis evasi* was done as described [4]. Animals were considered helminthiasis positive when ova were detected in grab samples of faeces [4]. Skin scrapes and hair samples were collected to check for the presence of mites (*Sarcoptes scabiei var. cameli*) as described [4]. Blood samples were collected prior to slaughtering and liver samples immediately after killing the animals. Out of the 2157 camels examined, there was a total of 1017 healthy camels sampled and there were 902 infected camels of which 388 had helminthiasis, 306 had mange and 208 had trypanosomiasis. Animals with combinations of parasites (n = 238) were not included.

Ascorbic acid analyses

Blood samples were collected from the jugular vein into heparinized tubes. Upon centrifugation, plasma was collected and erythrocytes were washed three times by centrifugation in ten volumes of phosphate-buffered saline, containing 154 mM NaCl and 12.5 mM Na_2HPO_4 in deionized water with pH adjusted to 7.4. The buffy coat of white cells was carefully removed after each wash. A solution containing 2.55% (v/v) metaphosphoric acid was added to the plasma samples (plasma: acid solution = 2:1, v/v) and the mixture was frozen until thawed just prior to analysis. The period of frozen storage was less than 1 week. Ascorbic acid in the various samples was detected using high-pressure liquid chromatography and electrochemical detection according to the method of Behrens and Madere [5]. The detection limit was 0.4 ug ascorbic acid/ml of assay sample and the coefficient of variation was 5.9 % for plasma and liver samples.

Statistical analysis

It was assumed that the large sample size had caused equal distributions of possible confounding factors, such as genetic and environmental factors, within the four categories of infection status. The data were subjected to two-way ANOVA and no statistically significant effect of gender and no interaction between gender and infection status were found. Thus, the data from males and females were pooled and assigned to one of four categories of infection status. Duncan's multiple comparison test was used to identify significant differences among the four categories of camels. The level of statistical significance was pre-set at $P<0.05$.

Results

The vitamin C levels in blood fractions and liver for all camels are presented in Table 1. When expressed on a volume basis, the leukocytes had higher ascorbic acid contents than the other blood constituents. The blood values are higher than expected on the basis of ascorbic acid concentrations in plasma and erythrocytes, as has been reported by others [6]. Due to repeated washing, the erythrocytes may have lost ascorbic acid. There was no effect of sex in the four categories of Arabi camels. Ascorbic acid contents in all blood fractions and also in liver were significantly reduced when the camels were infected with parasites. The lowest values were seen in the camels with trypanosomiasis.

Table 1: Ascorbic acid contents in erythrocytes, leukocytes, plasma, whole blood and liver of camels without or with parasite infections

Measure	Healthy animals (n=1017)	Helminthiasis (n=388)	Sarcoptic mange (n=306)	Trypanosomiasis (n=208)
RBC, mg/l	4.4 ± 1.1^{a}	2.9 ± 0.9^{b}	2.7 ± 1.0^{b}	1.9 ± 0.5^{c}
WBC, mg/l	43.4 ± 2.5^{a}	34.2 ± 2.5^{b}	30.3 ± 3.8^{c}	23.3 ± 1.8^{d}
Blood, mg/l	21.4 ± 1.3^{a}	20.4 ± 0.9^{b}	17.9 ± 1.2^{c}	14.9 ± 1.3^{d}
Plasma, mg/l	5.8 ± 1.2^{a}	3.6 ± 0.9^{b}	2.9 ± 0.9^{c}	1.8 ± 0.4^{d}
Liver, mg/100g	62.9 ± 2.0^{a}	53.4 ± 1.9^{b}	50.2 ± 2.2^{c}	33.2 ± 3.3^{d}

RBC = red blood cells; WBC = white blood cells; ascorbic acid content expressed per l of blood
Means ± SD. Values within the same row with different superscript letter are significantly different ($P<0.05$).

Discussion

As far as we know, this is the first report on ascorbic acid levels in blood and liver in camels with natural infections. It is clear that the camels with trypanosomiasis, sarcoptic mange and helminthiasis had lower plasma concentrations of ascorbic acid than did the healthy controls. In keeping with our data, Kouider and Kolb [7] reported that lambs with various types of parasites had low plasma concentrations of ascorbic acid. From the present case-control study, cause-and-effect relationships cannot be identified. However, the observed low ascorbic acid concentration in plasma from camels with parasite infections probably is a consequence of the disease. Gameel [8] reported that experimentally-induced fascioliasis resulted in a reduction of the plasma ascorbic acid contents in sheep.

The decrease in vitamin C status upon infection could relate to impaired synthesis and/or enhanced utilization of ascorbic acid. The synthesis of ascorbic acid may be diminished upon infection, but there is also evidence that infection is associated with higher rates of utilization of ascorbic acid [9]. High levels of ascorbic acid in leukocytes are required for their motility and protection against oxidative damage [9].

In summary, this study shows that camels with natural parasite infections have a low status of vitamin C, especially in the case of trypanosomiasis. Parasite eradication in camel herds would be expected to raise vitamin C status and thereby enhance disease resistance, and thus have an impact beyond the negative, direct effects of the parasites.

Acknowledgement

H.E. Mohamed was supported by The Netherlands Foundation for Nutrition and Health Research.

References

1. Hidiroglu, M., Ivan, M., Lessard, J.R.C. (1977). Effect of ration and inside versus outside housing on plasma levels of ascorbic acid, lactic acid, glucose and cholesterol in Hereford steers wintered under practical conditions. Can. J. Anim. Sci. 57, 519-529.
2. Gadient, M., Wegger, I. (1985). Ascorbic acid in intensive animal husbandry. Nutr. Abstr. Rev. 5531.
3. Mohamed, H.E. and Beynen, A.C. (2002) Vitamin C concentrations in blood plasma, tissues and urine of camels (*Camelus dromedarius*) in Sudanese herds. J. Anim. Physiol. a. Anim. Nutr. (in press)
4. Manual of Veterinary and Advisory Service (1977). Technical Bulletin. No. 18, 2nd edition, 58-61.
5. Behrens, W.A. and Madere, R. (1987) A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic acid and dehydroascorbic acid in tissues, biological fluids and foods. Anal. Biochem. 165, 102-107.
6. Soliman, M.K., Toussef, G.W., Mansour, S.A. (1975). Ascorbic acid content of erythrocytes, plasma, leukocytes and whole blood of healthy camels. Egypt. J. Vet. Sci. 12, 107-110.
7. Kouider, S.A., Kolb, E. (1994). Contents of ascorbic acid, glucose, protein, ALT and AST in blood plasma of healthy lambs and those of abdomasal, intestinal and lung parasites before and after i.v. injection of ascorbic acid solution. Tierärtzliche Umschau 49, 299-302.
8. Gameel, A.A. (1982). Plasma ascorbic acid levels in sheep experimentally infected with *Faciola hepatica*. Z. Parazienkd. 669, 321-326.
9. Thaxton, J.P., Pardue, S.L. (1984). Ascorbic acid and physiological stress. In: Ascorbic acid in Domestic Animals. Edited by I. Wegger, F.J. Tagwerk and J. Moustgaard, Copenhagen, Royal Danish Agr. Soc. 153-161.

Chapter 7

The Effect of Habitual Diet on L-ascorbic Acid Concentrations in Plasma and Leukocytes of Sudanese Camels

H.E. Mohamed[1] and A.C. Beynen[2]

[1]*Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum, Khartoum North, Sudan;*

[2]*Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.*

Abstract

There is suggestive evidence that a low status of ascorbic acid in camels enhances their risk for infectious diseases. This study was carried out to find clues as to the role of diet in affecting ascorbic acid status. In a cross-over trial with feeding periods of three weeks, six camels (*Camelus dromedarius*) were fed either a composite of their habitual diet or alfalfa. The simulated habitual diet contained grass (hummra), *Acacia mellifera* and *Blepharis persica*. The habitual diet significantly lowered ascorbic acid concentrations in plasma and leukocytes. It is concluded that camels kept on natural desert vegetation may not have optimal disease resistance due to a diet-induced low ascorbic acid status.

Introduction

We have shown in camels, kept under natural grazing conditions in Sudan, that parasite infections are associated with low ascorbic acid status [1]. It is likely that the parasite infections had lowered ascorbic acid status. In sheep, infection with *Fasciola hepatica* produced a decrease in plasma ascorbic acid concentrations [2]. In non-ruminants it has been shown that dietary supplements of ascorbic acid provide disease resistance [3]. Thus, a low status of ascorbic acid may enhance the risk for contracting infectious disease.

In ruminants, even though they are able to synthesize vitamin C from glucose, the composition of the ration could influence the concentration of plasma ascorbic acid. Kolb et al. [4] showed that values of liver ascorbic acid in bulls and oxes were highest in December when they were kept inside and fed a stall ration. Thus, there might be an effect of diet on vitamin C metabolism in ruminants. It is possible that camels consuming their habitual diet and kept under practical conditions have a diet-dependent ascorbic acid status. To test this possibility the present experiment was carried out.

Materials and methods

Six non-pregnant, non-lactating female Arabi camels (age, 10.3 ± 1.7 years; mean ± SD) with a mean body weight of 450 kg (SD = 24.5) were used. Prior to the commencement of the experiment, the camels were injected intramuscularly with oxy-tetracycline hydrochloride (10 mg/kg body weight) and subcutaneously with antiparasite medication (Ivomec, 200 mg/kg body weight). The experiment was carried out during the dry season at the Camel Research Centre at Elshowak, southeast of Khartoum. The maximum and minimum temperatures during the study were 31 and 18 ^{0}C and relative humidity was between 17 and 61 %. The camels were individually housed in steel pens with sand as bedding.

The trial had a cross-over design with three camels per treatment sequence. Each dietary treatment lasted three weeks. All camels went through a 14-day pre-experimental period during which they were fed green alfalfa (*Medicago sativa L.*) as sole source of nutrition. The alfalfa was obtained from a local market and fed in fresh form. Each camel received 5 kg dry matter of alfalfa twice a day at 7 a.m. and 2 p.m. The habitual diet was simulated by a mixture consisting of fresh *Acacia mellifera*, *Aristidia funiculata* (hummra) and *Blepharis persica* in a 2:1:1 ratio on as fed basis. The composition of the habitual diet was based on field observations. Each camel received 5 kg dry matter of the mixture two times a day. Water was freely available. During the experiment, the camels were either fed

alfalfa or the habitual diet. Initial body weight of each camel was measured immediately after its arrival with the use of a balance. At the end of the feeding periods, body weights were also determined.

The macronutrient composition of the feedstuffs was analysed according to the Weende methods. Blood samples were collected and processed as described [1]. Plasma and leukocyte vitamin C levels were determined according to Behrens and Madere [5].

Student's paired t-test was used to identify a diet effect. The level of significance was pre-set at $P < 0.05$.

Results

Table 1 shows the analysed composition of the alfalfa and the calculated composition of the habitual diet.

Table 1. The macronutrient composition of the two rations

	Alfalfa ration	Habitual diet[1]
Dry matter, g/kg diet	956	872
Crude protein, g/kg dm[2]	173	114
Crude fiber, g/kg dm	287	328
Crude fat, g/kg dm	29	29
Ash, g/kg dm	123	102
Nitrogen-free extract, g/kg dm	388	427

[1] *Mean for 4 samples of each diet component*
[2] *dm = dry matter*

When the camels were consuming the simulated habitual diet, they ingested somewhat more crude fiber and less crude protein than when they ate the alfalfa diet.

The two diets were consumed completely throughout the experiment. There was no influence of dietary treatment on body weight. At the end of the experiment, body weight was 450 ± 27.5 kg (n=6).

At the end of the pre-experimental period, plasma and leukocyte ascorbic acid concentrations were 4.23 ± 1.04 µg/ml and 43.2 ± 3.97 µg/ml, respectively (means ± SD, n=6). Table 2 shows that the habitual diet produced a significant decrease in plasma and leukocyte ascorbic acid levels.

Table 2. Ascorbic acid concentrations in plasma and leukocytes of camels fed the two rations

Ascorbic acid	Alfalfa ration	Habitual diet
Plasma (µg/ml)	5.39 ± 1.11	3.90 ± 0.97*
Leukocytes (µg/ml)	46.17 ± 3.85	39.85 ± 3.97*

Means ± SD, n=6.
[*] *Habitual versus alfalfa diet: $P < 0.05$.*

Discussion

The current study shows that the type of diet influences blood plasma and leukocyte vitamin C levels in camels. Our results are consistent with those of Rasmussen et al. [6], showing that a ration rich in alfalfa hay raised the plasma ascorbic acid values in lambs. In an other study with lambs there was no effect of ration on blood vitamin C [7]. In a study using Rahmani rams, Abdelhamid et al. [8] revealed that a ration containing clover hay, rice straw and concentrate resulted in an increase in plasma vitamin C.

It is clear that the composition of the diet might affect vitamin C status in ruminants, including camels. The design of the studies does not give information as to specific feedstuffs and/or nutrients affecting vitamin C metabolism. A diet change may influence ascorbic acid synthesis and/or excretion, but the mechanisms remain obscure. This study shows that Sudanese camels kept under natural grazing condition may have a diet-induced, low vitamin C status. As a consequence, these camels may not have optimal disease resistance [3].

References

1. Mohamed, H.E. and Beynen, A.C. (2002) Ascorbic acid contents in blood plasma, erythrocytes, leukocytes and liver in camels (*Camelus dromedarius*) without or with parasite infections. Int. J. Vitam. Nutr. Res. (in press).
2. Gameel, A.A. (1982). Plasma ascorbic acid levels in sheep experimentally infected with *Fasciola hepatica*. Z. Parazienkd. 669, 321 - 326.
3. Field, C.J., Johnson, I.R. and Schley, P.D. 2002. Nutrients and their role in host resistance to infection. J. Leukoc. Biol. 71, 16-32.
4. Kolb, E., Dittrich, H., Dobeleit, G., Schmalfuss, R., Siebert, P., Stauber, E. and Wahren, M. 1991. Content of beta-carotene, vitamin E and ascorbic acid in blood plasma of female calves, cattle, bulls, castrates and ox throughout the course of the year. Berl. Münch. Tierärztl. Wochenschr. 104: 387-391.
5. Behrens, W.A. and Madere, R. (1987) A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic acid and dehydroascorbic acid in tissues, biological fluids and foods. Anal. Biochem. 165, 102 – 107.
6. Rasmussen, R.A., Cole, C.L., Miller, M. and Thorp, F. 1944. Plasma ascorbic acid values of sheep of various ages fed different rations. J. Anim. Sci. 3, 340 – 345.
7. Weir, W.C., Pope, A.L., Phillips, P.H. and Bohstedt, G. 1949. The effect of low carotene winter ration on the blood, milk and liver concentrations of vitamin A and C of ewes and their lambs. J. Anim. Sci. 8, 381 – 391.
8. Abdelhamid, A.M., El-Shinnawy, M.M. and Farrag, F.H.H., 1990. Effect of feeding sheep on spoiled rice straw, clover hay and concentrate mixtures. Arch. Anim. Nutr. 7, 637 – 646.

Chapter 8

Bioavailability of Ascorbic Acid in Camels (*Camelus dromedarius*) following Administration by Different Routes

H.A. Elsheikh[1], H.E. Mohamed[1] and H.M. Mousa[2]

[1]*Faculty of Veterinary Medicine, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan*

[2]*Faculty of Veterinary Science, University of Khartoum, P.O. Box 32, Khartoum North, Sudan*

Proceedings of the Third Annual Meeting for Animal Production Under Arid Conditions, Vol. 1: 180-186

Bioavailability of Ascorbic Acid in Camels (*Camelus dromedarius*) Following Administration by Different Routes.

H.A. Elsheikh[1], H.E. Mohamed[2], and H.M. Mousa[2]

[1]Faculty of Veterinary Medicine, Jordan University of Science and Technology, P.O. Box 3030 Irbid 22110, Jordan. [2]Faculty of Veterinary Science, University of Khartoum, P.O. Box 32, Khartoum, North Sudan

ABSTRACT

This study reports the bioavailability of ascorbic acid in four adult female camels following administration at a dose of 50 mg/kg intravenously (i.v.), subcutaneously (s.c.) or orally (p.o.). The half-life of elimination following i.v. Injection (178.3 min.) was longer than after s.c. (145.2 min) or p.o. administration (110.0 min) ($P < 0.05$). Following s.c. injection, the maximum concentration (C_{max}) obtained (28.4 mcg/ml) was higher than that for p.o. (14.29 mcg/ml) ($P < 0.01$). However, the time to reach C_{max} (T_{max}) was shorter after p.o. (75.5 min) compared to the s.c. route (181.5 min) ($P < 0.01$). The bioavailability following s.c. injection was found to be much greater than after giving the drug orally. It can be concluded that, if ascorbic acid administration is justified in camels, the s.c. route is more suitable than the i.v. or the oral route.

Key words: Ascorbic acid, Bioavailability, Dromedary Camel.

INTRODUCTION

Ascorbic acid plays an important role as an antioxidant and important in the formation of connective tissue (Kanter, 1998). It has also been suggested to be of value in alleviating both physiological and pathological stress (Jaeschke, 1984; Hemingway, 1991). Camels are mostly raised under pastoral husbandry conditions in hot dry environments and are therefore exposed to various degrees of stress.

Accordingly, administration of ascorbic acid may be of value in camels to alleviate stress. Knowledge of ascorbic acid

pharmacokinetics in camels is necessary in the determination of the dose, the interval of dosing and the appropriate route of administration. As part of our investigation of ascorbic acid in camels it was thought to be of interest to investigate the bioavailability of this vitamin following its administration intravenously (i.v.), subcutaneously (s.c.) or orally (p.o.).

MATERIALS AND METHODS

Animals

Four clinically healthy adult female camels (*Camelus dormedarius*), four to six years of age and weighing 380-425 kg were used. They were bought From Omdurman Animal Market (Sudan) and kept for three weeks for acclimatization before starting the experiment. The animals were fed sorghum stalks and grain, dry grass and drinking water *ad libitum***.**

Drug administration and sample collection

One week before the administration of ascorbic acid, samples of blood were collected from each animal to establish baseline concentration. The drug was given intravenously (i.v.), subcutaneously (s.c.) or orally (p.o.) at a dose rate of 50 mg/kg. A washout period of 14 days was allowed between drug administrations. Blood samples (5 ml) were collected in heparinized syringes before treatment and at 5, 10, 20, 40, 60 and 90 min and at 2, 3, 4, 6, 8, 10, 12, 18 and 24 h after i.v. administration and at 0, 15, 30, 45, 60 and 90 min and 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 h after p.o. or s.c. injection. Immediately after separation of plasma, metaphosphoric acid solution was added and the treated plasma samples were then stored at –20 °C prior to analysis within one week.

Drug analysis

Ascorbic acid concentration in the plasma was determined spectrophotometrically according to the method described by McGown et al., 1982. The limit of quantification of the method was

1.2 mcg/ml. The intra-assay coefficient of variation was less than 6%.

Pharmacokinetic and statistical analysis

For the purpose of Pharmacokinetic analysis, the baseline concentration of ascorbic acid (4.52 ± 0.96 mcg/ml) was subtracted from all values obtained. Pharmacokinetic analysis of the plasma concentration versus time data was performed using non-linear regression analysis. The i.v. data were best fitted to biexponential equations. Following p.o. or s.c. administration, plasma concentrations of ascorbic acid versus time were analyzed by adopting a one compartment open model. Pharmacokinetic parameters were calculated according to the equations described by Baggot (1977).

All values of parameters are presented as mean ± SD. Harmonic means were calculated for half-lives of elimination. Wilcoxon's rank sum test was used to test for significant differences in the half-lives. The other parameters were analyzed using one way analysis of variance or student's *t*-test.

RESULTS AND DISCUSSION

The data of mean plasma concentration versus time in camels given ascorbic acid i.v., s.c. or p.o. at a dose of 50 mg/kg is shown in Fig. 1. Following i.v. injection ascorbic acid plasma level declined in a biexponential manner. Thus, a two compartment open model fit the individual plasma levels data. The absorption of the drug was rapid following p.o. or s.c. administration. Higher maximum concentration was reached after s.c. injection, but the decline in concentration was slower than after the p.o. route which is indicative of the higher bioavailability after s.c. administration.

Table 1, represents some selected pharmacokinetic parameters. The half-life of elimination after i.v. injection (178.4 min) was longer than after s.c. (145.2 min) or p.o. administration (110.0 min) ($P < 0.05$). The elimination half-life in this work in camels, following i.v. Injection was longer than in sheep (100.8 min) by 76.9% (Black and Hidiroglou, 1996), and considerably shorter than that reported for horses after injection by the same route (297 – 774 min) (Loscher *et*

al., 1984; Snow and Frigg, 1990), which indicates species variations in the elimination of the drug.

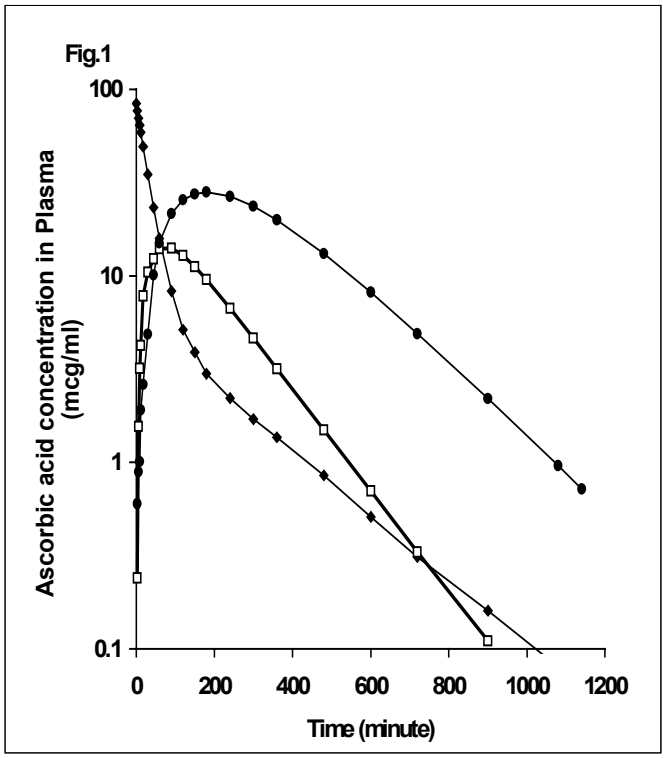

Fig. 1: Means plasma concentration versus time after administration of ascorbic acid in four female camels at a dose rate of 50 mg/kg intravenously (◆), subcutaneously (•) or orally (◊). Each point represents the mean of four observations. Standard deviation was less than 10%.

Following s.c. injection, the maximum concentration (C_{max}) obtained (28.4 mcg/ml) was higher than that obtained after p.o. administration (14.29 mcg/ml) ($P < 0.01$). However, the time to reach C_{max} (T_{max}) was shorter for p.o. (75.5 min) compared to the s.c. route (181.6 min) ($P < 0.01$). The bioavailability following s.c. injection (340.8%) was found to be much higher than after giving the drug orally (85.5%).

Table 1: Pharmacokinetic parameters of ascorbic acid (mean ± SD) following intravenous (i.v.), subcutaneous (s.c.) and oral (p.o.) administration in four female camels (dose 50 mg/kg).

Pharmacokinetic Parameter	I.V.	S.C.	P.O.
$t_{1/2\beta}$ (min)	178.4±15.4	145.20±8.44*	110.04±11.39*
C_{max} (mcg/ml)	-	28.38±2.55	14.29±4.78**
T_{max} (min)	-	181.60±10.44	75.48±6.36**
AUC (mcg.h/ml)[1]	63.20±12.2	215.46±23.35	54.04±9.02
F(%)[2]	-	340.8	85.5

*Significantly different $P < 0.05$ compared to the value obtained after i.v. injection. **Significantly different $P < 0.01$ compared to the value obtained after s.c. injection. [1]AUC = area under the concentration versus time curve. [2]F = bioavailability obtained using the relationship $AUC_{s.c.}$ or $AUC_{p.o.}/AUC_{i.v.}$ x 100.

This may be due to the much slower absorption following s.c. injection ($t_{1/2a}$ was 89.2 min compared to 27.8 min for oral route), to a conservation mechanism, or to an impact of endogenous ascorbic acid synthesis since the drug takes more time to be eliminated after s.c. injection. Black and Hidiroglou (1996) reported bioavailability values ranging between 295.8 and 608.6%. after intramuscular administration of ascorbic acid in sheep.

The present study showed considerably higher bioavailability following p.o. administration, than in horses in which very poor absorption of ascorbic acid was reported (Loscher *et al.*, 1984). However, only repeated oral administration was effective in increasing ascorbic acid plasma levels in this animal species (Snow *et al.*, 1987). Loscher and his colleagues (1984) suggested that, the reason for the low systemic availability of ascorbic acid after oral administration in the horse may be related to: 1) Chemical or enzymatic destruction in the gastrointestinal fluid and/or mucosa; 2)

Absorption into the hepatic portal blood but subsequent metabolism in the liver before reaching the general circulation (a first pass effect); 3) Slow entry into the general circulation but relatively rapid excretion through the kidney as soon as it exceeds the endogenous concentration range.

No local reactions were observed following s.c. injection of ascorbic acid in camels of this study, whereas in horses serious swellings at the site of injection i.m. and s.c. were reported (Loscher *et al.*, 1984). Further studies are needed to determine the appropriate ascorbic acid concentrations needed in camels to alleviate stress. So that suitable doses of ascorbic acid in camels can be determined, and to investigate daily oral supplementation in feed to raise systemic ascorbic acid concentrations.

CONCLUSION

In conclusion the present study showed considerably high bioavailability of ascorbic acid in camels following subcutaneous administration compared to the intravenous and oral routes. Moreover, local irritation was not observed. Therefore, it can be suggested that, if ascorbic acid administration is justified in camels, the s.c. route is more suitable than the i.v. or the oral route.

REFERENCES

Baggot, J.D. 1977. Principles of drug disposition in domestic animals. W.B. Saunders Company, Philadelphia.

Black, W.D. and M. Hidiroglou. 1996. Pharmacokinetic study of ascorbic acid in sheep. Can. J. Vet. Res. 60: 216-221.

Hemingway, D.C. 1991. Vitamin C in the prevention of neonatal calf diarrhea. Can. Vet. J. 32: 184.

Jaeschke, G. 1984. Influence of ascorbic acid on physical development and performance of race horses. In: Proceedings of the workshop on ascorbic acid in domestic animals. Eds., Wegger, I., F.G., Tagwwerker and J. Moustgaard, p. 153-161.

Scandinavian Association for Agricultural Sciences and Royal Danish Agricultural Society, Copenhagen.

Kanter, M. 1998. Free radicals, exercise and antioxidant supplementation. Proc. Nutr. Soc. 57: 9-13.

Loscher, W., G. Jaeschke and H. Keller. 1984. Pharmacokinetics of ascorbic acid in horses. Equ. Vet. J. 16: 59-65.

McGown, E.L., M.G. Rusnak, C.M. Lewis and J.A. Tilloston. 1982. Tissue ascorbic acid analysis using ferrozine compared with the dinitrophenylhydrazine method. Anal. Biochem. 119: 55-61.

Snow, D.H. and M. Frigg. 1990. Bioavailability of ascorbic acid in horses. J. Vet. Pharmacol. Therap. 13: 393-403.

Snow, D.H., S.P. Gash and J. Cornelius. 1987. Oral administration of ascorbic acid to horses. Equ. Vet. J. 19: 520-523.

Chapter 9

Vitamin C Status of Sudanese Cattle and Sheep

H.E. Mohamed[1], H.M. Mousa[1] and A.C. Beynen[2]

[1]*Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum, Khartoum North, Sudan*

[2]*Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Abstract

Sudanese cattle and sheep were compared as to plasma, liver and urinary ascorbic acid concentrations. Cattle had lower hepatic ascorbic acid concentrations than sheep. Male cattle had lower plasma ascorbic acid levels and female cattle had lower urinary levels than their sheep counterparts. Concentrations of liver ascorbic acid were lower in females and urinary ascorbic acid was higher in females, irrespective of the species. It is concluded that cattle and sheep may differ as to ascorbic acid metabolism and status, but differences in environmental factors could have had an impact also.

Introduction

We have described vitamin C status in Sudanese camels (*Camelus dromedarius*) in relation to characteristics such as breed, gender, sexual activity and season [1] and also in relation to parasite infections [2]. From a comparative point of view, we wished to describe also vitamin C status of Sudanese cattle and sheep. Thus, we determined ascorbic acid concentrations of plasma, liver and urine from both female and male animals.

Materials and Methods

We analysed samples from 120 Butana cattle (*Bos indicus*) and 120 desert sheep (*Ovis aries*) that were slaughtered during the rainy season (July-September) at the Tambul abattoir which is located at 145 km southeast of Khartoum. Sampling was done within a 3-week period. The animals had been kept under natural grazing conditions. The age of the sheep and cows was estimated to be 1.5 – 2 years and 2-5 years, respectively. Castrated males were excluded. Upon arrival at the slaughterhouse, urine was collected in bags that had been attached to the animals. If necessary, urine release was stimulated by massage of the bladder. The cattle and sheep had no access to food or water for at least 18 hours before slaughtering. Blood samples were collected by puncture of the jugular vein before slaughter and liver samples were taken on the slaughter track. The samples were processed as described [1,2] and ascorbic acid was analysed by the method of Behrens and Madere [3].

Student's t-test was used to evaluate selected comparisons. The level of statistical significance was pre-set at $P < 0.05$.

Results

Gender had no significant effect on plasma ascorbic acid concentrations in both cattle and sheep, but liver ascorbic acid was significantly lower in females than in males (Table 1).

Table 1. Ascorbic acid contents in plasma , liver and urine from Sudanese cattle and sheep

Measure	Species	Males	Females
Plasma (mg/l)	Sheep	5.42 ± 1.11	4.99 ± 1.54
	Cattle	4.87 ± 1.00[b]	4.78 ± 1.22
Liver (mg/100 g wet weight)	Sheep	82.1 ± 11.6	77.8 ± 12.4[a]
	Cattle	60.0 ± 12.1[b]	53.1 ± 13.4[a,b]
Urine (mg/l)	Sheep	3.1 ± 1.4	4.3 ± 1.0[a]
	Cattle	2.9 ± 0.8	3.9 ± 1.0[a,b]

Means ± SD for 90 male and 30 female sheep and 32 male and 88 female cows. [a]Significant difference for females versus males within the same species and same measure (P<0.05). [b]Significant difference for cattle versus sheep within the same gender and same measure (P<0.05).

Urinary ascorbic acid concentration was higher for females than for males, irrespective of the species. Cattle had lower ascorbic acid concentrations in plasma, liver and urine than did the sheep, but the difference for plasma ascorbic acid in the females and that for urinary ascorbic acid in the males did not reach statistical significance.

Discussion

When the outcome of this study is compared with our earlier studies [1,2], it follows that vitamin C status in Sudanese camels is similar to that of Sudanese cattle, but differs from that in Sudanese sheep. The plasma ascorbic acid concentrations in the three species were similar, but the sheep had about 30% higher ascorbic acid levels in liver than did camels and cattle. Urinary ascorbic acid concentrations were of the same order of magnitude in the three species.

Liver ascorbic acid contents in Sudanese cattle are similar to those reported by Barakat and Abdalla [4]for Egyptian cattle, but for European cattle lower values have been published [5]. Genetic and environmental differences may play a role here. The vitamin C level in liver was significantly higher in males than in females, both in sheep and cattle. In camels we found similar values for hepatic ascorbic acid concentrations in males and females [1].

The present data indicate that female sheep and cattle may excrete more ascorbic acid than the males. This finding is consistent with data from Neseni [6] in cattle. In camels we also found that females had higher ascorbic acid concentrations in urine than did males [1]. The higher level of urinary ascorbic acid excretion in females could relate to females synthesizing more ascorbic acid than do males. The levels of urinary ascorbic acid of cattle and sheep in the present study differed somewhat from those obtained by Ugolini [7], who showed urinary ascorbic acid contents for cattle and sheep of 5.3 and 1.8 mg/l for cattle and sheep, respectively.

The present data indicate differences in ascorbic acid metabolism between cattle and sheep and also between females and males. As to the species difference, environmental in addition to genetic differences may be involved. For instance, the composition of the ration may influence vitamin C status in ruminants [8].

Acknowledgement

H.E. Mohamed was supported by The Netherlands Foundation for Nutrition and Health Research.

References

1. Mohamed, H.E. and Beynen, A.C. (2002) Vitamin C concentrations in blood plasma, tissues and urine of camels (*Camelus dromedarius*) in Sudanese herds. J. Anim. Physiol. a. Anim. Nutr. (in press).
2. Mohamed, H.E. and Beynen, A.C. (2002) Ascorbic acid contents in blood plasma, erythrocytes, leukocytes and liver in camels (*Camelus dromedarius*) without or with parasite infections. Int. J. Vitam. Nutr. Res. (in press).
3. Behrens, W. and Madere, R. (1987) A highly sensitive high performance liquid chromatography method for the estimation of ascorbic acid and dehydroascorbic acid in tissues, biological fluids and foods. Anal. Biochem. 165, 102-107.
4. Barakat, M.Z., Abdalla, A. (1965) The ascorbic acid content of edible liver. J. Food Sci. 39, 185-187.
5. Watts, P.S. (1950) Studies on vitamin A and C in bovines. Vitamin C in the liver, kidney, and plasma of cows, calves, and fetuses. J. Comp. Pathol. Therap. 60, 283-285.
6. Neseni, R. (1938) Vitamin C content of urine of domestic animals. Prag. Tierärztliche. Arch. 18, 137-152.
7. Ugolini, M. (1942) The vitamin C content of the urine of the most common, clinically healthy, domestic animals. Biochem. Terap. Sper. 29, 187-192.
8. Mohamed, H.E. and Beynen, A.C. (2002) The effect of habitual diet on L-ascorbic acid concentrations in plasma and leukocytes of Sudanese camels. Int. J. Vitam. Nutr. Res. (submitted).

General Discussion

The most important protozoal disease of camels is trypanosomiasis. *Trypanosomis evansi* is widespread throughout tropical and subtropical regions. The disease may be acute, causing early death if untreated, or become chronic resulting in loss of milk and meat, abortion, premature birth or infertility. Mange, caused by *Sarcoptes scabie var. cameli*, is often regarded

secondly of economic importance in dromedary camels. Mange is a chronic condition with high morbidity and low mortality. From the current study, it is clear that natural parasitic infections are associated with a marked reduction in vitamin C blood status. Trypanosomiasis had the greatest impact. It is suggested that parasite infections cause an increase in the utilization of ascorbic acid and/or reduction of the rate of hepatic synthesis. The lowering of plasma ascorbic acid may further decrease disease resistance. Different reports have been published on vitamin C in blood status as affected by infection in lambs (Kouider and Kolb, 1994), sheep (Gameel, 1982), cattle (Ziola, 1960; Kadziolka, 1962), and calves (Jagos et al. 1977, Dubeski et al. 1985). Viral infections are also known to cause a reduction in serum ascorbic acid concentrations (Beisel, 1982).

Ascorbic acid is present in high concentrations in leukocytes and is utilized at a higher rate during infection and pathogenesis (Thomas and Holt, 1978, Moser, 1987). It is required for neutrophil function (Anderson and Lukey, 1987). The protective effect of vitamin C may partly be mediated through its ability to reduce circulating glucocorticoids (Degkwitz, 1987). The suppressive effect of corticoids on neutrophil function in cattle is alleviated by vitamin C supplementation (Roth and Kaeberle, 1985). Ascorbate is required for glucocortocoid synthesis (Goodman, 1966; Dvorak, 1984). Supplementation of ascorbate may help to overcome cortisol-induced immunosuppresion in both humans and experimental animals. Plasma and liver concentration of ascorbate is critical for the immune response, but may be depleted during stress and disease. Supplementation of feed for non-ruminants with ascorbic acid may help to restore normal levels and thus may provide non-specific resistance against invading pathogens (Pardue and Thaxton, 1986, Wu et al., 2000). Vitamin C has recently received a great deal of attention because of its action on immune response and disease in animals, and consequently on their production. Low plasma vitamin C levels result in a compromised immune system and leads to reduction of animal production and increased susceptibility to diseases.

Camels are normally raised under pastoral husbandry conditions in a hot, dry environment and therefore are exposed to stress. Under normal conditions, the requirement for vitamin C is met by endogenous synthesis and there is no need for exogenous supplementation. Plasma ascorbic acid is low in calves stressed by weaning (Dubeski, 1992). High environmental temperature decreased plasma ascorbic acid in rabbits (Verde and Piquer, 1986). Breeding season may be regarded as a physiological stressor as the plasma level of vitamin C was found to be reduced in both female and male camels. It is suggested that especially Bishari camels during the breeding season might be sensitive to disease. Similar results have been shown in breeding, female camels in the United Arab Emirates (Snow et al., 1992). The present data indicate significant inter-breed variations in vitamin C blood status. Arabi camels had higher values than Anafi's and Bishari's. Breed variation has been reported previously for Indian crossbred cattle (Verma et al., 1993) and German cattle (Haag and Hofmann, 1987).

Camels are seasonally polyoestrous, with peak sexual activity from November to February in the Northern Hemisphere. With a continuous supply of sufficient food, however, they can be regarded as truly polyoestrous. There is a specific male breeding season ('the rut'), but the stud will mate and fertilize females at any time of the year (Arthur, 1992). Martin and

Mecca (1961) have studied the physiological role of high concentrations of ascorbic acid in endocrine tissues. For skin and bone, higher ascorbic acid levels parallel with the requirement for the biosynthesis of collagen (Schwartz, 1984). The relatively high concentrations in adrenals, liver and spleen as found in the present study can be interpreted as being parts of ascorbic acid body reserves. With respect to sex, the present study showed higher concentrations in urine from females when compared with male camels. This finding concurs with data for cattle published by Neseni (1938), but Palkinas (1942) reported no detectable amounts of ascorbic acid in urine of horned cattle. Yashin (1987) showed that young cattle excrete vitamin C mainly as dehydroascorbic acid. As stated by Toutain et al. (1997), most of ascorbic acid is eliminated by first-pass effect through the kidney when the plasma level was above a critical value. Under normal conditions 15% of the daily synthesised ascorbic acid is excreted in urine by rats (Burns et al., 1954) whereas in guinea pigs 8 % of the label ascorbic acid was recovered in urine (Burns et al., 1956). Therefore, urine is not an important route of ascorbic acid elimination in guinea pigs.

The rate of hepatic synthesis of ascorbic acid has not been determined in camels. Previous reports indicated a rate of hepatic synthesis of 2.5 mg/kg body weight in Holstein calves (Toutain et al. 1997) and 40-275 mg/kg/day in other mammals (Chatterjee, 1973). The dromedary camel has a remarkable ability to exploit the scanty feed and water in its natural habitat. These abilities include proper utilization of endogenous protein and recycling of urea when low protein diet is fed, and selective browsing (Rutagwenda et al., 1990a). However, in the present investigation, it was observed that green alfalfa when fed to camels, instead of feed of desert, raised vitamin C status. The increase might be attributed to a stimulating factor assisting in the biosynthetic pathways of ascorbic acid. Thus, the current study shows that the type of diet fed to camels can influence blood plasma and leukocyte vitamin C levels. This observation is consistent with data from Rasmussen et al. (1944), McHenry (1935) and Cakala et al. (1974), showing that the composition of the ration affects plasma ascorbic acid values in lambs. On the other hand, in other studies with lambs there was no effect of ration on blood vitamin C (Knight et al., 1941, Abdelhamid et al., 1990). There are various factors that contribute to the regulation of ascorbic acid metabolism such as intestinal absorption, biosynthesis, urinary excretion, and catabolism. It could be suggested that in ruminants the availability of glucose might limit ascorbic acid synthesis. However, the amount of glucose converted into ascorbic acid is only small. In addition, in pregnant and lactating camels there was no association between the plasma levels of ascorbic acid and those of glucose.

In contrast to horses, dromedary camels showed no local irritation when injected with vitamin C subcutaneously showed (Loscher et al., 1984). Even upon oral supplementation, the present study showed a higher bioavailability in camels than in horses (Loscher et al., 1984). Only repeated oral administration was effective in increasing ascorbic acid plasma levels in the horse (Snow et al., 1987). A higher bioavailability following subcutaneous injection was found than after oral administration. This may be attributed to the much slower absorption following injection, to a conservation mechanism, or to an impact of endogenous ascorbic acid synthesis since the vitamin takes more time to be eliminated after subcutaneous injection. However,

coated vitamin C, when supplemented orally to cattle, showed an increase in plasma levels (Hidiroglou, 1999, Mohamed, unpublished results).

Milk constitutes an important source of nutrition for desert inhabitants. Compared with other livestock in arid regions, the camel has advantages in milk production (Stiles, 1995). Previous reports have described the effect of stage of lactation (Field, 1979; Karue, 1994), breed of camel (Bachman and Schulthess, 1987) and season (Lakosa and Shokin, 1964) on milk yield in camels. When compared to other domestic animals, camels produce milk for a longer period. Camel milk has both nutritional and therapeutic potentials in treating different ailments (Chandan et a., 1968; Mal et al., 2001; Sarwar and Enbergs, 2001) and has long shelf live (Gnan, 2001). The ascorbic acid content of milk and colostrum in Sudanese camels, particularly Arabi, were found to be higher than those reported for Saudi breeds (Sawaya et al., 1984, Mehaia, 1994), Pakistani camels (Knoess, 1977) and Kenya camels (Farah et al., 1992). This may be attributed to genetic differences between breeds. The camel calves, born under harsh conditions, may need a source of disease resistance, which could be supported by vitamin C. Consistently with previous reports, colostrum versus milk showed higher ascorbic acid in all breeds of Sudanese camels (Hidiroglou et al., 1995, Toutain et al., 1997). The present study indicates that L-ascorbic acid was increased in plasma of lactating camels, despite of a lower plasma glucose level in these animals. As mentioned above, it is possible that the glucose needed for the synthesis of ascorbic acid represents only a small portion of the glucose turnover in ruminants (Abel 1992). Mastitis, which causes a reduction of milk yield and quality, in camels, has so far received little attention. Udder condition markedly affected milk ascorbic acid concentrations, as camels showing mastitis had lower levels as compared with healthy controls. Parity and stage of lactation only had a minor effect on the vitamin C milk level, which agrees with data for cows (Santos et al. 2001).

Signs of oestrous in a female camel include restlessness, bleating, vulval swelling and discharge of vaginal mucous. The female wags its tail when approached by a male, or when it hears the gurgling voice of the rutting male. Fertility of female camels may be as high as Bedouin herd owners claim that 80-90% of those mated in one season do produce calves. Poor nutrition and trypanosomiasis are probably responsible for infertility (Karimi and Kimenye, 1990). Brucellosis in camels has first been reported by Ahmed (1939). It contributes to loss of productivity, milk reduction and infertility. Brucellosis-positive camels were found to have lower vitamin C plasma contents than their Brucellosis-negative counterparts.

The mean gestation period in the camel is 375 days (Arthur, 1992). Pregnancy and lactation increases the demands for food and water, and exert an influence on fluid balance. Camels thrive and reproduce in arid and semi arid areas, although scarcity of food and water frequently occurs. The present study showed higher ascorbic acid levels in plasma during the oestrous than the non-oestrous period. This may be attributed to the role of vitamin C in the synthesis of progesterone. This finding is inconsistent with a report of McIntosh (1941). Bortree et al. (1942) stated that the plasma ascorbic acid values did not differ between pregnant and non-pregnant animals. However, Gosse (1934) recorded higher values for pregnant animals. Concerning the relationship between plasma ascorbic acid level and the reproductive

cycle contradictory results have been obtained (Phillips et al., 1941; Chattapodhyay et al., 1972 and Cheremisinov et a., 1981). It is interesting to note the higher ascorbic acid plasma levels in lactating versus non-pregnant and pregnant camels. This phenomenon has been found earlier by Pope et al. (1949) using 18 grades Hampshire Sphropshire ewes and could help to maintain high levels of this vitamin in milk and colostrum.

As mentioned above, ascorbic acid plays a role as anti-stress factor (Takahashi et al., 1991). Different stressors have been studied, such as transportation (Von Tunglen, 1986) and weaning (Sidrov, 1985, Soloveva, 1969; Cook, 1995). Ascorbic acid synthesis in farm animals may be significantly reduced at high temperature, overcrowding or physical activity (Hidiroglou et al., 1977; Verde and Piquer, 1986). The detrimental effects of stress associated with aberrant housing have been thoroughly investigated (Cummins and Brunner, 1991; Booker, 1960; Desmarais, 1960). The immuno-potentiating effect of vitamin C is evident (Bendlich et al., 1986; Anderson and Luky 1987; Degkwitz, 1987). Vitamin C is thought to be essential in increasing the phagocytic abilities of neutrophils, maintaining the structural integrity of the cells and consequently increasing the antibacterial and immunological functions. It is likely that the wealth of knowledge on vitamin C in relation to stress and immunity can be applied to husbandry of the camels. This thesis has identified conditions under which camels may be most susceptible to disease as caused by low vitamin C status.

Directions for future research

The investigations carried out in this thesis have identified factors associated with vitamin C status in camels. However, many questions remained unanswered. A few statements may be made.

- The basis for the high vitamin C contents in milk of the camel, when compared with dairy cows, is not clear.
- It is of interest to study the effect of racing or packing on camel's ascorbic acid status.
- It is important to investigate in camels the possible immuno-potentiating effect of vitamin C, especially under stressful conditions.
- Dietary supplements should be identified in order to raise vitamin C status in camels, especially in those animals that have low status.

References

Abdelhamid, A.M., El-Shinawy, M.M. and Farrag, F.H.H., 1990. Effect of feeding sheep on naturally spoiled rice straw, clover hay concentrate feed mixtures. Arch. Anim. Nutr. Berlin. 7, 637 – 646.

Abel, H. 1992. Review of ascorbic acid in cattle nutrition. In: Ascorbic acid in domestic animals. Ed. C. Wenk, R. Fenster and L. Volker.

Ahmed, M. R. 1939. The incidence of brucellosis in different domesticated animals in Egypt. Tech. Bull. 23, 210-231.

Anderson, R. and Lukey, P. T. 1987. A biological role of ascorbate in the selective neutralization of extracellular phagocyte-derived oxidants. Ann. N. Y. Acad. Sci. 498, 229-233.

Arthur, G. H,. 1992. An overview of reproduction in the camelids. Proc. Ist. Int. Camel Confr., Dubai, UAE, 109-113.

Bachmann, M. R. and Schulthess, W., 1987. Lactation of camels and composition of camel milk in Kenyaa. Milchwissenschaft. 42, 766-768.

Beisel, W. R. 1982. Single nutrient and immunity. Am. J. Clin. Nutr. Suppl. 35, 417-468.

Bendlich, A. 1993. Physiological role of antioxidants in the immune system. J. Dairy Sc. 76, 2789-2794

Booker, W. M. 1960. Relation of ascorbic acid to adrenocortical function during cold stress. Fed. Proc. 19, 94-96.

Bortree, A. L., Huffman, C. F. and Dunca, C. W. 1942. Normal varaitions in the amount of ascorbic acid in the blood of dairy cattle. J. Dairy Sci. 25, 983.

Burns, J. J., Dayton, P. G. and Schulenberg, S. 1956. Further observations on the metabolism of L-ascorbic acid 1-^{14}C in guinea pigs. J. Biol. Chem. 218, 15-21.

Burns, J. J., Mosbach, E. H. and Schulenberg, S. 1954. Ascorbic acid synthesis in normal and drug-treated rats, studied with L-ascorbic 1- C 14 acid. J. Biol. Chem. 207, 679-687.

Cakala, S., Borkowski, T. and Albrycht, A., 1974. Rumen acidosis in sheep induced with different doses of sucrose. Pol. Arch. Weter. 17, 117 – 130.

Chandan, R. C., Parry, R. M. and Shahani, K. M. 1968. J. Dairy Sci. 51, 606-607.

Chatterjee, I. B. 1973. Evolution and biosynthesis of ascorbic acid. Sc. 82, 1271-1272.
Chattopadhyay, R., Choudhury, G. and Sinha, R. 1972. Studies on the ascorbic acid content of blood plasma during different stages of oestrous cycle and early pregnancy in crossbred cows (Jersey X Hariana cross). Proc. Session Indian Sci. Cnogr. 59, 26-27.

Cheremisinov, G. A., Nezhdanova, A. G. and Vlasova, A., 1981. Biochemical values of blood in relation to reproduction function in cows. Veterinariya. 4, 53-55.

Cook, C. J. 1995. Oxytocin and prolactin suppress cortisol response to acute stress in both lactating and non-lactating sheep. J. Dairy Sci. 64, 327-339.

Cummins, K. A. and Brunner, C. J. 1991. Effect of calf housing on plasma ascorbate and endocrine and immune function. J. Dairy Sci. 74, 1582.

Degkwitz, E. 1987. Some effects of vitamin C may be indirect, since it affects the blood levels of cortisol and thyroid hormones. Ann. NY. Acad. Sci.

Desmarais, A. 1960. Ascorbic acid in cold acclimitization. Fed. Proc. 19, 88-93

Dubeski, P. L. 1992. B-vitamins for cattle: Availability plasma levels, and immunity. Ph.D. Dissertation, Oklahoma State University.

Dubeski, P. L. and Owens, F. N. 1995. Effects of weaning, fasting and B-vitamin injections on plasma B-vitamins concentrations in beef calves. Anim. Sci. Res. Report, Agricultural Experimental Station, Oklohama State University, p-933, 242-248.

Dvorak, M. 1984. Ascorbic acid, stress resistance and reproduction in swine. In: Workshop on Ascorbic Acid in Domestic Animals. Proc. Royal Danish Society, Copenhagen, pp. 80.

Farah, Z., Rettenmaier, R. and Atkins, D., 1992. Vitamin content of camel milk. Int. J. Vitam. Nutr. Res. 62, 30-33.

Field, C. R., 1979. Camel growth and milk production in Marsabit District, Northern Kenya. In: The Camelid. An All-purpose animal. Proc. Khartoum Workshop. Camel, De. 1979. Scand. Inst. African Stud. Uppsala, Sweden.

Gnan, S. O. 2001. Shellife of camel milk. 2001. The International Conference on Reproduction and Production of Camelids. 6th Annual Confr. Anim. Prod. Under Arid Cond. College of Food Systems, University of Arab Emirates, Al-Ain, 11-13 November, UAE. (abstract)

Gameel, A. A. 1982. Plasma ascorbic acid levels in sheep experimentally infected with *Faciola hepatica.* Z. Parazienkd. 669, 321-326.

Goodman, A. D. 1960. Studies on the effect of omega-methyl-pantothenic acid on corticosterone secretion in the rat. Endocrinology. 66, 420.

Gosse, K. H., 1936. Inaug. Diss. Hannover.

Guerrant, N. B. and Bechdel, S. I. 1941. Utilization and excretion of ascorbic acid by dairy cow. J. Dairy Sci. 24, 567-577.

Haag, W. and Hofmann, W. 1987. Investigation about content of ascorbic acid in the plasma and leukocytes of cattle. Tierärztl. Umschau. 42, 956-963.

Hidiroglou, M., Ivan, M. and Batra, T. R., 1995. Concentrations of vitamin C in plasma and milk of dairy cattle. Annal. Zootech. 44, 399-402.

Hidiroglou, M., Ivan, M. and Lessard, R. 1977. Effects of ration and inside versus outside housing on plasma levels of ascorbic acid, lactic acid, glucose and cholesterol in Hereford steers wintered under practical conditions. Can. J. Anim. Sci. 57, 5190-529

Hidirogou, M. 1999. Technical Note: Forms and route of vitamin C supplementation for cows. J. Dairy Sci. 82, 1831-1833.

Jagos, P., Bouda, J. and Dvorrak, R. 1977. Ascorbic levels in the bronchopneumonia of calves. Vet. Med. (Praha). 22, 133-136.

Kadziolka, A. 1962. Distribution of glycogen and vitamin C in the liver of cattle affected with parasitic cirrhosis (fasciolasis). Med. Wet. (Warszawa), 18, 93-99.

Karimi, S.K. and Kimenye, D.M. 1990. Some observations on reproductive performance of camels kept in Northern Kenya. Is it possible to improve reproductive performance in the camel? Proc. UCDBC Workshop, Paris.

Karue, C.N., 1994. The dairy characteristics of the Kenyan camel. In: Proc. Chameauxe Dromedaries, Animaux Laitiers. Conf. Naulchott, Mauritanie.

Knoess, K. H., 1977. The camel a meat and milk animal. World Anim. Rev. 22, 39-44.

Kolb, E. 1990. Einige neue Erkentnisse zum Stoffwechsel und zur Function von Ascorbinsäure. Z. gesammte Inn. Med. 45, 205-210.

Kouider, S. A. and Kolb, E. 1984. Contents of ascorbic acid, glucose, protein, ALT and AST in blood plasma of healthy lambs and those of abomasal, intestinal and lung parasites and after i.v. injection of ascorbic acid solution. Tierärztl. Umschau. 49, 299-302.

Lakosa, I..I. and Shokin, V.A. 1964. Milk production. In: Camels. Science. Technical Agricultural Publ. Kolos. Moscow. 113-120.

Losher, W., Jaeschke, G. and Keller, H. 1984. Pharmacokinetics of ascorbic acid in horses. Equine Vet. J. 16, 59-65.

Mal, G., Suchitra, D., Jain, S. V. K. and Sahani, M. S. 2001. Theraputic utility of camel milk as nutritional supplement against multiple drug resistance (m.d.r.) patients. The International Conference on Reproduction and Production of Camelids. 6th Annual Confr. Anim. Prod. under Arid Cond. College of Food Systems, University of Arab Emirates, 11-17 November, Al-Ain, UAE. (abstract).

Ogilio, M. 1942. The vitamin C content of the urine of the most common, clinically healthy domestic animals. Biochem. Terap. Sper. 29, 187-192.

Martin, G.R. and Mecca, C.E. 1961. Studies on the distribution of L-ascorbic acid in the rat. Arch. Biochem. Biophys. 93, 110-114.

McHenry, E.W. and Graham, M. 1935. Biochem. J. 29, 2013.

McIntosh, R. A., 1941. Ascorbic acid (vitamin C) for the treatment of impotency in bulls and sterility in cows. J. Comp. Med. 5, 267-268.

Moser, V. 1987. Uptake of ascorbic acid by leukocytes. Ann. N. Y. Acad. Sci. 498, 200-205.

Neseni, R. 1938. Vitamin C content of urine of domestic animals. Prag Tierärztl. Arch. 18, 137-152.

Palkinas, P. 1941. The amount of ascorbic acid in the blood of some herbivores domestic animals and in the urine of horned cattle. Kolrebol Osszehansolito. 29, 267.

Pardue, S. L. and Thaxton, J. P. 1986. Ascorbic acid in poultry: a review. World's Poult Sci. 42, 107-123.

Phillips, P. H., Lardy, H. A., Boyer, P. D. and Werner, G. H. 1941. The relationship of ascorbic acid to reproduction in the cow. J. Dairy Sci. 24, 153-158.

Pope, A., Philips, J., Bohsteadt, P. H., 1949. Vitamin A and C concentrations in the blood plasma of their lambs. J. Anim. Sci. 8, 57-66.

Rasmussen, R.A., Cole, C.L., Miller, M. and Thorp, F. 1944. Plasma ascorbic acid values of sheep of various ages fed different rations. J. Anim. Sci. 3, 340 – 345.

Roth, J. A. and Kaeberele, M. L. 1985. In vivo effect of ascorbic acid on neutrophil function in healthy and dexamethasone-treated cattle. Am. J. Vet. Res. 46, 2434-2436.

Rutagwenda, T., Lechner-Doll, M., Schwartz, H. J., Schultka, W. and Engelhard, W. V. 1990a. Dietary preference and degradability of forage on semi-arid Thornbrush by indigenous ruminants, camels and donkeys. Anim. Feed Sci. Technol. 31, 179-192.

Santos, M.V., Lima, F.R., Rodrigues, P.H., Barros, S.B. and Laranja Fonesca, L.F. 2001. Plasma ascorbic concentrations are not correlated with milk somatic cell count and metabolic profile in lactating and dry cows. J. Dairy Sci. 84, 134-139.

Sarwar, A. and Enbergs, H. 2001. Lysozyme activity in ilk of suckling dromedaries during early lactation period. The International Conference on Reproduction and Production of Camelids. 6th Annual Confr. Anim. Prod. Under Arid Cond. 11-13 November, College of Food Systems, University of Arab Emirates, Al-Ain, UAE. (abstract).

Sawaya, W. N., Khalil, J. K., Al-Kahnal, A. and Al-Mohammad, H. 1984. Chemical composition and nutritional quality of camel milk. J. Food Sci. 49, 744-747.

Schwartz, E. R. 1984. Effect of ascorbic acid on collagen structure and metabolism in normal and osteoarthritic tissue. In: Ascorbic Acid in Domestic Animals. (Eds.) I. Wegger, F.J. Tagwerk and J. Mousegaard. Royal Danish Agricultural Society, Copenhagen, Denmark.

Servetnik-Chalaia GK, Mal'tseva LM. 1981. Nature of the vitamin composition of mare's milk and koumiss depending on the time of year. Vop. Pitan. 4, 46-48.

Sidorov, V. T. 1985. Increasing natural immunity and preventing stress in young male cattle and guinea pigs by means of pharmacological substances. Sornik Belorusskii Nauchno Issledovotel Skii Institut Zhivotnodstova. 26, 143-149.

Snow, D. H., Billah, A. M., Ridha, A. and Frigg, M. 1992. Plasma concentrations of some vitamins in camels. Proc. Ist. Intl. Camel Confr. Dubai, UAE, pp. 335-338.

Snow, D. H., Gash, S. P. and Cornelius, J. 1987. Oral administration of ascorbic acid to horses. Equine Vet. J. 16, 59-65.

Soloveva, G. A. 1969. Disturbance of vitamin C metabolism in monkeys during acclimatization. Vop. Pitan. 28, 58-62.

Stiles, D. 1995. The advantage of camels over other livestock in drylands. In: Camel keeping in Kenya, Range Manage. Handbook Kenya. III. Min. Agric. Livest. Dev. Marketing Range Man. Div. Nairobi, Kenya.

Takahashi, K., Akiba, Y. and Horiguchi, M. 1991. Effects of supplemental ascorbic acid on performance organ weight and plasma cholesterol concentration in broilers treated with propythiouracil. Br. Poult. Sci. 32, 545-554.

Thomas, W. R. and Holt, P. G. 1978. Vitamin C and immunity: an assessment of the evidence. Clin. Exp. Immunol. 32, 370-379.

Toutain, P. L., Bechu, D. and Hidiroglou, M., 1997. Ascorbic acid disposition in plasma and tissues of calves. Amer. J. Physiol. Regul. Inter. Com. Physiol. 273, 1585-1597.

Verde, M. T. and Piquer, J. G. 1986. Effect of stress on the corticosterone and ascorbic acid (vitamin C) content on the blood plasma of rabbits. J. Appl. Rabbit Res. 9, 181.

Verma, R. P., Bhagi, H. K, Grag, R. C. and Mishra, R. R. 1984. Biochemical studies on reproductive status of Hariana cows. Livestock Advisor. 29, 29-34.

Von Tunglen, D. L. 1986. The effects of stress on the immunology of the stocker calf. Bovine Pract. 18, 109.

Wu, C. C., Doraijan, T. and Lin, T. 2000. Effect of ascorbic acid supplementation on the immune response of chickens vaccinated and challenged with infectious bursal disease virus. Vet. Immunol. Immunopathol. 74, 145-152.

Yagil, R., 1982. Camel Milk, FAO Production and Health Paper. 26, 9.

Yashin, A. V. 1987. Concentration of ascorbic acid and dehydroascorbic acid in urine of young vitamin C deficient male cattle. Nutr. Abstr. 182, 6658.

Ziola, T. 1960. Distribution of cholesterol and vitamin C in the adrenal cortex of sheep with cirrhosis of the liver due to fasciolasis. Med. Vet.

Summary

Vitamin C (ascorbic acid), which is a potent antioxidant, recently has received a great deal of attention because of its positive action on the immune response and disease resistance of animals. Brief reviews are presented on ascorbic acid metabolism in animals and its relation with stressful conditions. The original research in this thesis is concerned mainly with the identification of various factors that are related with vitamin C status in Sudanese camels (*Camelus dromedarius*) kept under natural grazing conditions. The study was conducted in the Butana area, Central Sudan. As indicators of vitamin C status, the concentrations of ascorbic acid in blood, leukocytes, organs and milk were used. Irrespective of the season, the highest tissue ascorbic acid levels were observed in adrenals and liver, and the lowest levels were in heart. There were breed variations in vitamin C status, Arabi camels having the highest plasma ascorbic acid concentrations and Bishari's the lowest, the Anafi's showing intermediate values. In the Sudanese camels, gender did not affect plasma ascorbic acid concentrations. However, female camels excreted more ascorbic acid with urine than did males. The breeding season was associated with a reduction of vitamin C status in both males and females. Estrus versus non-estrus was associated with high plasma and leukocyte ascorbic acid concentrations. Lactating Arabi camels had higher plasma and leukocyte ascorbic acid levels than did their pregnant counterparts. The stage of lactation was associated with plasma vitamin C levels, the levels being higher when the animals were more than 180 days in lacatation. The ascorbic acid levels in milk parallelled those in plasma. Multiparous camels had higher ascorbic acid concentrations in their milk than did primiparous animals. New-born calves had higher plasma ascorbic acid concentrations than their dams. In the calves, plasma ascorbic acid declined as from birth and had stabilized after four weeks. In the dams, plasma ascorbic acid rose from parturition and reached a steady value after four weeks. Colostrum contained more ascorbic acid than milk. The simulated habitual diet of Sudanese camels, when compared with alfalfa as sole source of nutrition, lowered plasma and leukocyte levels of ascorbic acid. Infections due to sarcoptic mange, helminthiasis and trypanosomiasis were associated with a lowering of vitamin C status, the latter infection having the greatest impact. Brucellosis was associated with a reduction of ascorbic acid status and was so mastitis. It is assumed that ascorbic acid status is a reflection of disease resistance. The data may also indicate that infectious diseases may further lower disease resistance and that female camels in non-estrus versus those in estrus, pregnant versus lactating, non-pregnant camels, breeding versus non-breeding animals and Bishari versus Arabi and Anafi camels might be more sensitive to disease. Dietary supplements should be identified that enhance vitamin C status in Sudanese camels as their habitual, free-range diet tends to lower the status.

Summary (in Dutch)

Vitamine C (ascorbinezuur), hetgeen een krachtige anti-oxidant is, heeft recentelijk veel aandacht gekregen vanwege haar positieve effect op de immuunfunktie en weerstand tegen ziekten bij dieren. Korte overzichten worden gegeven inzake de ascorbinezuurstofwisseling bij dieren en de relatie met stressvolle situaties. Het originele onderzoek in dit proefschrift is hoofdzakelijk gericht op de identificatie van diverse factoren, die verband houden met de vitamine C status van Sudanese kamelen (*Camelus dromedarius*), die onder gebruikelijke vrij grazende condities worden gehouden. Het onderzoek werd uitgevoerd in Butan, in centraal Sudan. De ascorbinezuurconcentraties in bloedplasma, leukocyten, organen en melk zijn als indicatoren van de vitamine C status gebruikt. Onafhankelijk van het seizoen werden de hoogste ascorbinezuurconcentraties gevonden in de bijnieren en lever, terwijl de concentraties in het hart het laagst waren. Er waren verschillen in vitamine C status tussen de rassen. Arabi-kamelen hadden de hoogste ascorbinezuurconcentraties in plasma en de Bishari's hadden de laagste; de Anafi-kamelen hadden tussenliggende concentraties. Het geslacht van de Sudanese kamelen had geen invloed op het plasma-ascorbinezuurgehalte. Vrouwelijke dieren scheidden echter meer ascorbinezuur uit met de urine dan mannelijke dieren. Het voortplantingsseizoen werd geassocieerd met een lage vitamine C status bij zowel mannelijke als vrouwelijke kamelen. Oestrus, vergeleken met anoestrus, was parallel aan hoge ascorbinezuurconcentraties in plasma en leukocyten. Lacterende Arabi-kamelen hadden hogere gehalten aan ascorbinezuur in plasma en leukocyten dan drachtige dieren. Het lactatiestadium werd geassocieerd met de plasmaspiegel van ascorbinezuur; de spiegels waren hoger wanneer de dieren langer dan 180 dagen in lactatie waren. Het verloop van het ascorbinezuurgehalte van de melk ging samen met dat van het plasma. Multipare kamelen hadden hogere ascorbinezuurconcentraties in de melk dan primipare dieren. Pasgeboren kalveren hadden hogere plasmaspiegels dan hun moeders. Bij de kalveren nam de ascorbinezuurconcentratie in het plasma af vanaf het moment van geboorte en bereikte een nieuwe evenwichtsconcentratie na 4 weken. Bij de moeders steeg de plasmaspiegel vanaf de geboorte en bereikte ook een stabiel niveau na 4 weken. Biest bevatte meer ascorbinezuur dan melk. De gesimuleerde, habituele voeding van Sudanese kamelen, vergeleken met luzerne als enige bron van voeding, verlaagde de ascorbinezuurconcentratie in het plasma en in de leukocyten. Infecties veroorzaakt door *Sarcoptes spp*., *Helminthus spp*, en *Trypanosomas spp*. waren geassocieerd met een verlaging van de vitamine C status. De verlaging was het grootst bij dieren met een infectie op basis van trypanosomen. Brucellosis en mastitis gingen samen met een daling van de vitamine C status. Het uitgangspunt is dat de vitamine C status de weerstand weerspiegelt. De huidige gegevens suggereren aldus, dat infectieziekten de weerstand verder verlagen. De gegevens geven ook aan dat dieren in anoestrus, vergeleken met die in oestrus, drachtig versus lacterende, seksueel-actieve versus niet-actieve dieren en Bishari-kamelen vergeleken met Arabi- en Anafi-kamelen, gevoeliger zijn voor het ontstaan van ziekten. Voedingssupplementen dienen geidentificeerd te worden teneinde de vitamine C status van Sudanese kamelen te verhogen daar hun habituele voeding de vitamine C status neigt te verlagen.

Curriculum Vitae

Name: Hassab Elrasoul Hussein Mohamed Elkhidir
Place of Birth: Elgunied, Butana Province, Sudan.
Date of Birth: January 1, 1968
Marital Status: Single
E-Mail Address: hhgunied@yahoo.com

Academic Qualifications:

1. Bachelor of Veterinary Science, University of Khartoum, February 1994.
2. Master of Veterinary Science (M.V.Sc.), University of Khartoum, October 1996. Title of thesis: Distribution of Vitamin C in different tissues and plasma of camels, local and exotic breeds of ruminants.

Practical experiences:

1. Veterinarian, Ministry of Agriculture, Khartoum State, 1994-1997
2. Veterinarian, Al-Rajhi Agricultural Company, Saudi Arabia, 1997-1998.
3. Lecturer, Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum, 1998-2001.

Conferences and Workshops attended:

1. 8th Arabian Veterinary Conference, 24-27 March 1998, Khartoum, Sudan.
2. Fourth Annual Conference on Camel Production under Arid Conditions , Al-Ain, United Arab Emirates, 1998
3. Animal Wealth in Sudan, 4th Scientific Conference, 6-8 April 1999, Khartoum, Sudan
4. International Society for Analytical Cytology (ISAC) XX International Congress, 19-24 May, 2000, Montpellier, France
5. International Conference on Reproduction and Production of Camelids. University of United Arab Emirates, Faculty of Food Systems, United Arab Emirates, Al-Ain, 11-13 November 2001
6. Arab European Veterinary Medicine Conference, 6-19 November 2001, Khartoum, Sudan

References:

1. Prof. Hassan Mirghani Mousa, Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, King Saud University, Buraydah, Al Gaseem, Saudi Arabia
2. Prof. Anton C. Beynen, Department of Animal Nutrition, Faculty of Veterinary Science, University of Utrecht, Netherlands E-mail: a.c.beynen@vet.uu.nl
3. Dr. Ahmed Gubara Abdelrahim, Department of Biochemistry, Faculty of Veterinary Science, University of Khartoum. Tel: 00249-13-318255
4. Bernard Faye, Laboratoire d'Ecopathologie, Institut National de la Recherche Agronomique, Saint-Genes-Champanelle, France. E-mail: faye@cirad.fr