Clinical Evaluation of Vitamin A, ß-Carotene, Vitamin E and Cortisol Levels in Health and Selected Diseases in Camels (*Camelus Dromedarius*) in Egypt

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Abstract: Vitamin A, β-carotene, vitamin E and cortisol levels were investigated in 133 of dromedary camels. Healthy camels (n= 52); according to seasons were divided into spring (n=13), summer (n=13), autumn (n=13) and winter (n=13); according to sex divided into males (n=27), females (n=25) and according to age into young (under 3years old n=17) and adult (n=35). The general mean values of serum vitamin A, β-carotene, vitamin E and cortisol were 2.090±0.063 µmol/L, 0.096±0.014 µmol/L, 4.042±0.222 µmol/L, and 90.631±6.363 nmol/L, respectively. Selected diseased camels (n= 81), including simple indigestion (n= 9), rumen acidosis (n= 15), rumen alkalosis (n= 11) and parasitic diarrhea (n=11) were examined. Young camels and adult female, especially in summer season had low levels of vitamin A, while they had low levels of vitamin E in winter. Adult males had low β-carotene level in winter and spring. In all camels cortisol level was high during summer and autumn. It is recommended to give adequate supply of vitamin A, β-carotene and vitamin E to camels in risk of simple indigestion occurrence during transport or changing of rations. In cases of rumen acidosis, rumen alkalosis and parasitic diarrhea; beside the correction of rumen& blood acid-base imbalance and dehydration; the administration of vitamin A, β-carotene and vitamin E in combination with anti-stress should be put in consideration in treatment of such cases.
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1. Introduction

Vitamin A (retinol) does not occur per se in plants, but its precursors the carotenes occur in several forms. Carotenes are orange-yellow pigments that occur in green leaves and to a lesser extent in corn grain. Four of these compounds, alpha-carotene, beta-carotene, gamma-carotene and cryptoxanthin (the main carotenoid of corn), are of particular importance because of their pro-vitamin A activity. Vitamin A activity of beta-carotene is substantially greater than that of other carotenoids **(Bauernfeind, 1981 and Weiss *et al.*, 1994)**.

One mg of ß-carotene, the precursor of vitamin A; is equivalent to 400 IU of vitamin A. Liver is the storage site of vitamin A, while adipose tissue is the main storage of ß-carotene **(Akar and Gazioglu, 2006)**.

Principle functions of retinol are to sustain normal epithelium, form retinal photo-chemicals, enhance immune functions and protect against infections and probably some cancers, it is important as a dietary supplement for animals including ruminants, since it is the only vitamin which may be deficient to ruminant under farm conditions **(Bondi, 1987)**.

The function of vitamin E is to scavenge the free radicals generated by normal metabolic processes and to prevent attacking of poly unsaturated fats in cell membrane which result in cellular injury **(Bennett and Brown, 2003)**. Factors affecting vitamin pool size are type of diet, rate of absorption, utilization and excretion **(Mc Dowel, 1989)**.

Cortisol is used as an indicator of stress in case of transport for long distance **(Saleem, 2006)**, stress and pain **(Thuer *et al.*, 2007)**, in surgical stress **(Mudron *et al.*, 1994)** and in cases of dehydration and hot weather **(Kataria *et al.*, 2000)**.

Only few references are dealing with vitamin A, β-carotene, vitamin E and cortisol levels in healthy camels, while scanty numbers of literature about their levels in diseased camels were available.

This work was applied to investigate vitamin A, β-carotene, vitamin E and cortisol levels in healthy and selected diseased camels to be used as a guide in clinical examination, diagnosis and treatment of diseased camels.

2. Material and Methods:

This study was performed on 133 camels to evaluate the serum level of vitamin A, ß-carotene and vitamin E, and cortisol. Blood samples of 52 apparently healthy camels were collected; in spring (n=13), summer (n=13), autumn (n=13) and winter (n=13). According to sex were divided into males (n=27), females (n=25) and according to age into young (under 3years old n=17) and adult (n=35). The same constituents were estimated in 81 samples from diseased camels which included simple indigestion (n=9), rumen acidosis (n=15), rumen alkalosis (n=11) and parasitic diarrhea (n=11).

The diagnosis of diseased cases depended on case history, clinical and laboratory examination of specific constituents in rumen fluid and fecal matter according to methods described by **Baraka *et al*., 2000; Baraka and Illek, 2003; Baraka, 2006 a, b& c** and methods described by **Kaufmann, 1996.**

Blood, rumen fluid and fecal matter samples were collected from camels in early morning before feeding. Blood was centrifuged at 3000 rpm for 20 min and sera were placed into sterile tubes and then frozen at -20^{0}C.

Serum levels of vitamin A, vitamin E and ß-carotene were performed by a method described by **Bouda *et al.* (1980)** using automated analyzer (Fluorescence spectrophotometer, Perkin – Elmer 204), cortisol levels were measured using ELISA technique by the specific kits produced by Lachima Company, CZ.

The obtained data were calculated and tabulated in both conventional units and SI units to facilitate the comparison with previous available papers.

Statistical analysis:

Statistical analysiswas applied using SPSS Statistical Computer Software, Copyright (c) **SPSS Inc., 2007** version 16.0., based on one-way ANOVA, with *post hoc* Duncan multiple comparison test. Differences at $p<0.05$ were considered significant.

3. Results:

Levels of serum vitamin A, ß-carotene, vitamin E and cortisol under effect of seasons (Table 1) showed that the highest level of vitamin A was recorded in winter, while the lowest level was in summer. Serum ß-carotene level was high in autumn and low in winter. Vitamin E was in lowest levels in winter and summer, while highest level was in spring. Stress of heat and humidity in summer and autumn caused the increase in level of cortisol, while its levels reduced in winter and spring.

Effect of sex and age (Table 2) revealed that Vitamin A and E levels in males were higher than that in females and ß-carotene and cortisol levels were higher in females (she-camels). With reference to the age Vitamin A and E levels in adult camels were higher than that in young camels, while ß-carotene and cortisol levels were higher in young than adult camels.

The general mean values of vitamin A, ß-carotene, vitamin E and cortisol all over the year (Table 3) which considered as control values were 2.09±0.06, 0.10±0.014, 4.13±0.27µmol/L and 94.17±7.13 nmol/L; which equal to 59.89±1.81, 5.16±0.75, 0.18±0.01 and 3.41±0.26 µg/dl , respectively.

Concerning to mean values all over the study, vitamin A correlated negatively with ß-carotene and vitamin E (r = - 0.04 and r = - 0.18, respectively), while positively correlated with cortisol (r = 0.16). Beta carotene positively correlated with vitamin E and cortisol (r = 0.22 and r = 0.49 respectively). Vitamin E negatively correlated with cortisol (r = - 0.23).

In diseased camels (Table 3) in comparison with general mean of healthy camels' values; a unique maintenance of vitamin A levels near to the normal was recorded. Levels of ß-carotene dramatically reduced in cases of simple indigestion. Levels of vitamin E increased in cases of parasitic diarrhea; with reduction in cases of simple indigestion and rumen acidosis. The highest levels of cortisol were recorded in cases of diarrhea and rumen acidosis.

Table 1: Serum vitamin A, β-carotene, vitamin E and cortisol levels in healthy camels under effect of seasons

Parameters (No.)	Units	Spring (13)	Summer (13)	Autumn (13)	Winter (13)
Vitamin A	(µmol/L)	2.22±0.15 ^{a*}	1.70±0.08 ^{a}	2.30±0.150 ^{b}	2.36±0.23 ^{b}
	(µg/dl)	63.26±4.16	48.71±2.35	65.79±4.30	67.54±6.71
ß-carotene	(µmol/L)	0.09±0.03 ^{b}	0.10±0.01 ^{b}	0.18±0.048 ^{ab}	0.02±0.01 ^{a}
	(µg/dl)	4.57±1.40	5.59±0.75	9.57±2.58	1.18±0.48
Vitamin E	(µmol/L)	5.65±0.41 ^{b}	3.46±0.54 ^{ab}	4.62±0.48 ^{ab}	2.87±0.45 ^{a}
	(mg/dl)	0.24±0.02	0.15±0.02	0.20±0.02	0.12±0.02
Cortisol	(nmol/L)	77.64±9.25 ^{a}	106.51±14.63 ^{b}	101.48±14.78 ^{b}	78.50±13.12 ^{a}
	(µg/dl)	2.82±0.34	3.86±0.53	3.68±0.54	2.85±0.48

*Means in rows with different superscript letter are statistically different at p<0.05.

Table 2: Serum vitamin A, β-carotene, vitamin E and cortisol levels in healthy camels under effect of sex and age

Parameters (No.)	Units	Sex		Age	
		Male (27)	Female (25)	Young (17)	Adult (35)
Vitamin A	(µmol/L)	2.44±0.13 ^{ab*}	1.94±0.14 ^{a}	1.74±0.10 ^{a}	2.34±0.14 ^{b}
	(µg/dl)	69.91±3.72	55.64±4.10	49.86±2.87	67.05±4.04
ß-carotene	µmol/L)	0.07±0.02 ^{ab}	0.11±0.02 ^{a}	0.19±0.06 ^{b}	0.06±0.01 ^{ab}
	(µg/dl)	3.87±1.18	5.75±1.02	10.11±3.17	3.39±0.65
Vitamin E	(µmol/L)	4.17±0.41 ^{b}	3.43±0.39 ^{ab}	2.65±0.33 ^{a}	3.51±0.43 ^{ab}
	(mg/dl)	0.18±0.02	0.15±0.02	0.11±0.01	0.15±0.02
Cortisol	(nmol/L)	84.53±10.14	88.23±15.82	82.73±11.59	77.30±16.64
	(µg/dl)	3.06±0.37	3.20±0.57	2.98±0.42	2.80±0.60

*Means in rows with different superscript letter are statistically different at p<0.05.

Table 3 : Serum vitamin A, β-carotene, vitamin E and cortisol levels in camels suffering from selected digestive disorders

Parameters (No.)	Units	Simple indigestion (9)	Rumen acidosis (15)	Rumen alkalosis (11)	Diarrhea (11)	General mean (52)
Vitamin A	(µmol/L)	2.05±0.14	2.08±0.24	1.80±0.13	1.92±0.34	2.09±0.06
	(µg/dl)	58.74±4.01	59.60±6.88	51.58±3.74	55.01±9.71	59.89±1.81
ß-carotene	(µmol/L)	0.01±0.01	0.13±0.10	0.03±0.02	0.15±0.09	0.10±0.014
	(µg/dl)	0.43±0.27	7.04±5.48	1.83±1.13	8.28±4.84	5.16±0.75
Vitamin E	(µmol/L)	3.41±0.62 ^{a*}	3.43±0.38 ^{a}	4.41±0.43 ^{ab}	6.10±1.22 ^{b}	4.13±0.27
	(mg/dl)	0.14±0.03	0.15±0.02	0.19±0.02	0.26±0.05	0.18±0.01
Cortisol	(nmol/L)	74.55±16.89 ^{a}	101.53±18.37 ^{ab}	68.89±10.83 ^{a}	148.70±41.75 ^{b}	94.17±7.13
	(µg/dl)	2.70±0.61	3.68±0.67	2.50±0.39	5.39±1.51	3.41±0.26

*Means in rows with different superscript letter are statistically different at $p<0.05$.

4. Discussion:

Concerning vitamin A level, similar values were recorded by **Abbas and Ali, 2001**, while higher values were recorded by **Agab *et al.* 1992, Al-Senaidy, 1996, Bogin, 2000,** but Lower values were recorded by **Romdane *et al.*, 2002**.

Stressful conditions, such as hot weather, increase body requirement for vitamin A reduce ß-carotene conversion to vitamin A (**Mc Dowell, 1989**); that explains the reduced levels of vitamin A in summer and reduced ß-carotene levels in winter.

The carotene content of most feedstuffs is quite variable and subject to large losses under less than ideal harvest and storage conditions. Vitamin A and the precursor carotenoids are rapidly destroyed by oxygen, heat, light and acids. Presence of moisture and trace minerals accelerates destruction of vitamin A activity in feeds (**Olson, 1990**).

Although, **Abbas and Ali (2001)** found that sex had no significant effect on the vitamin A level; males had higher levels than that in females with an inverse relation with ß-carotene. Sex showed insignificant effect on vitamin levels as mentioned by **Mohamed, 2006**.

Level of vitamin A increased with age, in agreement with that recorded by **Romdane *et al.* (2002)** and ß-carotene levels decreased with age. Ruminant animals are born with very low liver reserves of vitamin A. Therefore, it is critical that calves receive adequate amounts of colostrum, which contains high levels of vitamin A activity, within a few hours after birth. Colostrum deprivation during the first 24 hours of life impairs overall absorption of vitamins A, D and E through seven days of age (**Blum *et al.*, 1997**).

Serum ß-carotene levels were high in young camels and she-camels and especially in autumn and summer. Higher values were recorded by **Al-Senaidy (1996, a)**. Although, negligible plasma ß-carotene in the camel is in accord with findings by **Ghosal and Dwaraknath (1976)** who mentioned that plasma carotenes were absent in camels and confirmed by **Snow *et al.* (1992)** who mentioned that ß-carotene concentration in the plasma of camels were generally below the level of detection.

In regard to vitamin E level in camels it was found to be lower than in other ruminants, which can be attributed to nutritional factors. Similar values for vitamin E in camels kept under different conditions were recorded by **Higgins and Kock, 1986**, higher values were recorded by **Al-Senaidy, 1996b,** but, **Romdane *et al.*, 2002** recorded lower levels in camels.

Adult camels had higher levels of vitamin E than young as mentioned by **Mohamed, 2006**. But levels in dry seasons were higher than that in rainy seasons. High level of vitamin E in sera of suckling camel calves may be due to high level in milk of dams (**Osama, 1990**). **Romdane *et al.* (2002)** found that vitamin E level reduces by increase in age, cachectic and little lipid reserve camels.

In comparison between obtained values of vitamins A and E; inverse relationship was recorded, it can be referred to that retinol enhances the oxidation of α-tocopherol in the gut and influence the permeability of the intestinal mucosa, therefore, the relatively low level of α-tocopherol in the camel may promote an increase in retinol level, or vise versa (**Chow, 1979**). Deterioration of vitamin E in dry pasture by ultra-violet rays is of special importance in the etiology of vitamin deficiency and high levels of polyunsaturated fatty acids in diet (**Rice *et al.*, 1983**). Regarding a negative effect of vitamin A on vitamin E utilization and the suitability of currently used standard activity values for vitamin E supplements for ruminants have been raised. High dietary levels of vitamin A have depressed vitamin E utilization in most animals (**Schelling *et al.*, 1995**).

Cortisol level in camels was higher in summer than winter, which can be explained by that stress of dehydration (**Kataria *et al.*, 2000**); lowest levels were in winter and spring when climate stress is reduced. The level in females is higher than males and in young more than adult camels.

Volatile fatty acids are formed as a result of fermentation processes in the rumen and vitamin synthesis parallels other fermentation processes; type of roughage fed had a direct effect on the amount of vitamin synthesis in the rumen. Several papers indicate that appreciable amounts of carotene or vitamin A may be degraded in the rumen. Various studies with

different diets have indicated preintestinal vitamin A disappearance values ranging from 40% to 70% (**Ullrey, 1972**).

Vitamin A levels was reduced in cases of simple indigestion and rumen acidosis according to the degree of acidosis and its consequent changes in metabolism and dehydration resulting from the loss of intravenous cellular fluid into the rumen and from osmotic diarrhea (**Baraka *et al.*, 2005, Baraka, 2006 a**).

In rumen acidosis cellulolytic bacteria are sensitive to low rumen pH, with their activities being impaired when pH is 6.2 and complete inhibition at pH 6 (**Shi and Weimer, 1992; Weimer, 1993**). The toxicity to cellulolytic bacteria of low rumen pH is due to the metabolic inhibition by VFA anions (**Russell and Wilson, 1996**), impairment of glucose transport (**Chow and Russell, 1992**) and reduced numbers of cellulolytic bacteria adhered to feed particles (**Hoover, 1986**) and reduce lipolysis (**Demeyer and Van Nevel, 1995**).

In cases of rumen alkalosis a significant increase in the rumen pH (7.86±0.36) as a result of abnormal rates of fermentation and decomposition ended by putrefaction (**Mousa *et al.*, 1994, Baraka, 2006 b**); and rumen pH between 7 and 7.5 was found with prolonged anorexia (**Smith, 1990**) and continuous ingesting a large amount of saliva which containing bicarbonate and urea to be recycled in the rumen. Macrocytic hypochromic anemia can be referred to the reduced food and water intake due to anorexia (**Smith 1990, Kaneko 1997, Baraka, 2006 b**).

Parasitic diarrhea causes dehydration and in diarrheic camels serum ß-carotene concentration increase as that intestinal inflammation prevents the conversion of ß-carotene into vitamin A causing a reduced vitamin A level (**Agab *et al.*, 1992**).

Presence of parasitic infestation prevents the proper absorption and utilization of ß-carotene as, primary site of vitamin A and carotene absorption is the proximal jejunum (**Olson, 1991**). Normal pancreatic, liver function and adequate fat intake are required for absorption of vitamin A and its precursors.

Vitamin E levels decreased in cases of simple indigestion and rumen acidosis; and not markedly changed in rumen alkalosis. Beside the changes which may cause reduction in vitamin E; the deficiencies in vitamin E have been demonstrated to occur even when ruminants consume diets with supra-nutritional amounts of the vitamin (**Enser *et al.*, 1999, Kasapidou *et al.*, 2001**). On the other hand, it is uncertain if the deficiencies arise as a result of degradation of vitamin E in the rumen, impaired intestinal uptake of the vitamin or increased metabolism post absorption.

Some studies indicate that pre-intestinal vitamin E losses may be as high 42% in sheep (**Alderson *et al.*, 1971**) and up to 52% in cattle (**Shin and Owens, 1990**). In contrast, other studies indicate that vitamin E is not catabolised to any significant extent (**Astrup *et al.*, 1974 and Leedle *et al.*, 1993**). In healthy ruminants losses of vitamin E in the rumen were 21 and 9% of intake for low- and high-vitamin E diets respectively (**Chikunya *et al.*, 2004**).

Cortisol levels increased sharply in parasitic diarrhea because of dehydration stress and continuous debilitation caused by parasites like *trichostrongylus spp*., *nematodirus spp*., *hemonchus spp*., *trichuris spp. and monezia spp.* (**Nafie *et al.*, 1992, Haroun *et al.*, 1996 and Baraka 2006c**); followed by rumen acidosis in which hemconcentration and metabolic acidosis are common sequels. While Finberg et al. (1979) mentioned that rates of excretion of cortisol and corticosterone were much higher in camels than in other mammals. Dehydration did not cause any significant changes in blood levels of cortisol; **Kataria *et al.* (2000)** found that the stress of dehydration highly increased cortisol level. In cases of rumen alkalosis and simple indigestion the levels decreased, which need further study to be explained.

Conclusions

Young camels and adult female, especially in summer season had low levels of serum vitamin A, while they had low levels of vitamin E in winter. Adult males had low β-carotene level in winter and spring. In all camels cortisol level was high during summer and autumn. It is recommended to give adequate supply of vitamin A, β-carotene and vitamin E to camels in risk of simple indigestion which can occur during transport or change of rations. In cases of rumen acidosis, rumen alkalosis and parasitic diarrhea; beside the correction of rumen& blood acid-base imbalance and dehydration; the administration of vitamin A, β-carotene and vitamin E in combination with anti-stress should be put in consideration in treatment of such cases.

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