Thiamine (vitamin B1) status in the blood of young, pregnant, lactating and racing dromedary camels (Camelus Dromedarius) in UAE

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SUMMARY

Thiamine was determined in the blood of camel calves at days 1-5, weeks 1-6, months 3-4 and 7-9 and at one year, in the blood of breeding camels at late pregnancy, early, mid and late lactations and in the blood of racing camels at the ages of 2, 3, 4, 5, and >6 years. A total number of 1315 camels were used in this study. Thiamine was analyzed in the blood of these camels using HPLC with fluorescent detector. Normal camel calves were born with high levels of thiamine (97.8µg/l) in the blood. This level decreased by advancement of age to attain the lowest values by the time of weaning (Group A). Stress of pregnancy and lactation decreased the thiamine level in the blood of breeding camels (Group B); however, polioencephalomalacia (PEM) was not reported in this group. Very low thiamine level was seen in the blood of breeding camels (Group B); however, polioencephalomalacia (PEM) was seen among these young racing camels. PEM-affected camels responded very well to intravenous injections of 2 to 4 years (Group C) and PEM was seen among these young racing camels. PEM-affected camels responded very well to intravenous injections of thiamine given at the early course of disease at the rate of 8mg/kg BW/day for three days.

Keywords: Thiamine, blood, HPLC, young, pregnant, lactating, racing, camels, polioencephalomalacia, deficiency.

Introduction

Thiamine (Vitamin B1), a thiazole and pyrimidine rings connected by methylene bridge, is the first in the vitamin B group to be discovered, characterized and purified. The biologically active form, thiamine pyrophosphate (TPP), is a co-factor for a number of key enzymes in tricarboxylic acid cycle and in pentose phosphate pathway. Also it is involved in lipid and protein metabolism, blood formation and biosynthesis of acetylcholine for nerve transmission [7, 15]. Under normal conditions, ruminants meet thiamine requirement mainly from microbial synthesis, while their young obtains it from milk, which contains normally high amount of the vitamin [1, 4].

Polioencephalomalacia (PEM) is a well-established disease of ruminants with distinct clinical signs, pathological lesions and biochemical changes in blood and tissues; the disease responds to thiamine treatment [1, 6, 10]. There are number of factors that may favor this disease, including thiamine deficiency, increased thiaminases in the rumen, excess consumption of sulfate in water or feed, lead poisoning, amprolium and thiabendazole medication [2, 3, 8, 9, 10, 18]. On the other hand, low level in diet and stress factors may predispose animals to subclinical thiamine deficiency manifested by reduced growth rate, decreased appetite, low performance and low fertility rate [20, 21].

Camel racing is a very popular sport in the Gulf countries. The camels are offered high energy diets for power boosting...
during the racing season. PEM has been noticed in sporadic cases of racing camels [22] and the disease has been experimentally induced by oral administration of amprolium, a coccidiostat and a well known vitamin B1 antagonist [23]. Like other ruminants, camels showing PEM are responsive to thiamine if administered early in the course of the disease [1, 14]. Moreover, thiamine, administered to camels showing low blood vitamin level and low racing performance, show dramatical improvement in the tract [22, 24].

The effect of factors such as age, racing, pregnancy and lactation on blood thiamine level has been poorly studied in animals and never investigated in the camel. The objective of this work is to look into the effects of these factors on thiamine levels in apparently healthy young, lactating, pregnant, non-lactating non-pregnant and racing camels. Few clinical conditions and sub clinical thiamine cases were also reported in this study.

Materials and Methods

CLIMATIC CONDITIONS

The average temperatures during this experiment were 33.7, 29.5, 23.5, 18.5, 17.2, 21.1, 23.5°C; and the relative humidity were 37, 43, 53, 67, 67, 66, 58% in the months of September, October, November, December 2005, January, February and March 2006 respectively.

ANIMALS

A total of 1315 apparently healthy camels were used in this study. They were categorized into five groups: camels in groups A, B, and C looked apparently healthy.
- Group A: 228 camel calves of different sex and age: 1-5 days, 1-6 weeks, 3-4 months, 7-9 months and around 1 year.
- Group B: 262 female breeding camels, sub-grouped into: late pregnant (12 months), lactating (1-5 days, 1-6 weeks, 3-4 months, 7-9 months) and non-lactating-non-pregnant (controls).
- Group C: 807 racing camels at the age of 2, 3, 4, 5, >6 years and control camels at 5 and >6 years of age (non-racing). Blood was collected at about five days from the commencement of racing.
- Group D: 6 racing camels showing clinical PEM. Blood was collect at the same day when the animals manifested clinical signs of the disease.
- Group E: 12 camels: 6 mothers that gave birth to 6 camel calves and both showed low thiamine levels in the first 1-5 days (i.e. less than their respective normal groups of dams and calves).

SAMPLES

Blood samples were collected by venipunture from jugular vein into EDTA vacutainer tubes at around 8h a.m. The analysis is usually performed within ½ to 1h. If analysis is delayed for 1 or 2 days the samples are kept frozen at -20°C.

Rhodes grass and water samples were collected from the camel farms and sent to the food research centre for mineral analyses.

CHEMICAL METHODS

Thiamine pyrophosphate (TPP), the physiologically active form of vitamin B1, in whole blood was analyzed by HPLC (Alliance 2495; Waters Co., U.S.A) using commercial vitamin B1 reagent kit (Chromsystems, Munchen, Germany). The kit consist of mobile phase (Cat No: 35001), extraction buffer (Cat No: 37003), precipitation reagent (Cat No: 37004), derivatization reagent 1 (Cat No: 35005), derivatization reagent 2 (Cat No: 35006), neutralization reagent (Cat No: 35009), stabilization reagent (Cat No: 35007), calibration standard (Cat No: 37008) and two levels of controls (Cat No: 00033, 00035).

All reactions were carried out in dark brown 1.5 mL eppendorf test tubes. To 200 µL EDTA blood, 100 µL extraction buffer was added. The mixture was shaken by vortex for 2 seconds and 300 µL of precipitation reagent was added, mixed then centrifuged for 5 minutes at 9000 g. The supernatant was added into a new light protected vial containing 200 µL of derivatization reagent and mixed briefly. To this mixture 100 µL neutralization reagent and 100 µL of stabilization reagent were added and mixed. The mixture was let to stand for 20 minutes at room temperature and 50 µL of this mixture was injected in the HPLC system. The calibration standard and two levels of controls were treated the same and run with the samples. The theory behind the method is that thiamine is sensitive to the alkaline solutions used during the digestion and splits to thiazole ring and then oxidized to the fluorescent compound thiochrome which is detected by the fluorescent detector at EX 367 nm and EM 435 nm. The retention time at a flow rate of 1 mL/min is about 3 minutes.

METHOD VALIDATION

Defined amounts of thiamine pyrophosphate (17.3, 22.4, 54.9, 160.3 µg/L) were added (in quadruplicates) at a ratio of 1:1 to 16 blood samples pooled from freshly collected blood specimens. The percentage recovery was calculated for each set as 94%, 92%, 98% and 89% respectively. The method is linear within concentrations of 10.15 – 203.06 µg/L. The lowest validated detectable limit for thiamine pyrophosphate in matrix is 15.23 µg/L. The intra-assay coefficient of variation (n=10) is 3.9% and the inter-assay coefficient of variation (n = 10) is 4.9%.

ANALYSIS OF FEEDSTUFFS

Grinded feed samples (0.5 g) were digested with 7 mL 65% nitric acid and 1 mL of hydrogen peroxide in a microwave oven (Milestone, UK) controlled by a built-in computer program. The digested feed samples were transferred into 50 mL volumetric flasks and diluted to the mark with deionized water. The samples were analyzed by the Inductively Coupled Plasma Emission Spectrometer (ICP-OES; Vista-MPX, Varian, Australia). Feed and water were analyzed for S, Cu, Mo, Na, K and Pb content.
STATISTICAL ANALYSIS

Data was analyzed using Minitab Statistical Software 13.2. ANOVA was used to test the difference in blood thiamine within each group of camels, the t test was used for testing difference within sex (Group A, Group C) and correlation coefficient to test the relationship between blood thiamine level and age (Group A).

Results

MINERAL CONTENTS IN FEEDSTUFFS AND WATER

The mineral contents in feedstuffs and water are given in Table I. Both Lucerne and Rhodes grass were high in sulfur and low in copper. Mineral contents of water were within acceptable limits.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rhodes (DM)*</th>
<th>Lucerne (DM)*</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur</td>
<td>0.47%</td>
<td>0.48%</td>
<td>33.1 mg/L</td>
</tr>
<tr>
<td>Copper</td>
<td>2.8 ppm</td>
<td>4.6 ppm</td>
<td>0.002 mg/L</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.6 ppm</td>
<td>2.2 ppm</td>
<td>0.007 mg/L</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.2%</td>
<td>1.1%</td>
<td>113 mg/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.7%</td>
<td>1.65%</td>
<td>7.0 mg/L</td>
</tr>
<tr>
<td>Lead (BDL)**</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
</tbody>
</table>

* DM = on dry matter basis.
** BDL = Below Detection Limit.

Table I: Feed and water mineral analysis.

THIAMINE CONTENT IN THE BLOOD OF MALE AND FEMALE CAMELS

The effect of sex in blood thiamine was tested in young (Group A) and racing (Group C) camels. There was no significant effect of sex in both groups as shown in figure 1 (P > 0.05). Therefore, the results for these two groups were pooled for both sexes and presented in Table 2 (Group A) and Table 4 (Group C).

THIAMINE IN THE BLOOD OF CAMEL CALVES

Table 2 shows the level of thiamine in the blood of apparently normal male and female camel calves from birth to one year of age. Blood thiamine level was very high in the newly born camel calves and the level dropped gradually and significantly with age to reach the lowest values by weaning. There is a negative correlation between blood thiamine and increase of age in this group (r = -0.924; P < 0.05).

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of animals</th>
<th>Mean blood Thiamine level (µg/L)</th>
<th>STD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 5 days</td>
<td>22</td>
<td>97.8a</td>
<td>17.7</td>
</tr>
<tr>
<td>1 – 6 weeks</td>
<td>87</td>
<td>77.7b</td>
<td>17.4</td>
</tr>
<tr>
<td>3 – 4 months</td>
<td>20</td>
<td>60.3c</td>
<td>8.1</td>
</tr>
<tr>
<td>7 – 9 months</td>
<td>12</td>
<td>48.9 d</td>
<td>4.6</td>
</tr>
<tr>
<td>1 year</td>
<td>87</td>
<td>35.9e</td>
<td>6.2</td>
</tr>
</tbody>
</table>

a, b, c, d, e Values with different superscript in a same column are significantly different (P < 0.05).

Table II: Blood thiamine status in camel calves at different ages (group A).

THIAMINE IN THE BLOOD OF BREEDING CAMELS (GROUP B)

Table 3 shows thiamine status in the blood of breeding camels at different physiological status. The mean thiamine level in the blood of non-lactating non-pregnant breeding camels was significantly higher than all groups (P < 0.05), followed by the late pregnant females. There was a significant decrease of blood thiamine level with advancement of lactation (P < 0.05).

THIAMINE IN THE BLOOD OF RACING CAMELS (GROUP C)

Table 4 shows thiamine level in the blood of racing camels at different ages. The lowest blood thiamine was reported at the ages of 2, 3 and 4 years with no significant difference between these age groups (P > 0.05). Racing camels at the age of 5 and >6 years had similar but significantly higher blood thiamine than that of the younger camels (P < 0.05). The thiamine status in the blood of non-racing camels at both ages (5, >6 years) were significantly higher than that of all racing groups (P < 0.05).
BLOOD THIAMINE STATUS IN DROMEDARY CAMELS

**Camels showing PEM (group D)**

The animals showing PEM were all racing camels at the age of 2 to 4 years. They exhibited staggering gate and muscle tremors in the first day. 24 hours later, two untreated camels became apparently blind and sternally recumbent which are common sign of PEM in camels. Their mean blood thiamine was 21 ± 10.4 µg/L (5.4 – 37 µg/L). All the six animals recovered within 3-4 days after receiving 8 mg/kg/d thiamine hydrochloride intravenously (T500; 200 mg/mL, Jaapharm; Canada) in three consecutive days and their blood thiamine returned to normal by day three (78.6 ± 17.4 µg/L; 60.7 – 104.6 µg/L).

**Borderline deficient camels (group E)**

Figure 2 shows the thiamine status in the blood of normal breeding camel (1), their calves (2), borderline deficient camels (3) and their calves (4).
red to that of normal camels and their calves at day 1-5 of delivery (Group A). The border line deficient breeding camels exhibited a significantly lower blood thiamine than that of normal breeding camels (37.4 vs. 58.6 µg/L; P < 0.05). Calves from borderline deficient dams also had lower blood thiamine compared to those from normal camels (41.9 vs. 97.8 µg/L; P < 0.05). The thiamine status was significantly higher in normal camel calves than their mothers (P < 0.05), however, the difference between dams and calves in the border line deficient group was not significant (P > 0.05).

**Discussion**

This study points to many factors that can affect the blood thiamine level in the camel. Among others, age seemed to be determinant. Newly born calves (1-5 days) from healthy mothers attained the highest blood thiamine values. This may not be an effect of suckling colostrum, which is richer in vitamin B1 than milk [19], but rather indicates an active transport of thiamine from mother to fetus during gestation [5, 25]. In the first 6 weeks of life the blood thiamine level decreased slightly and with advancement of age, the level dropped progressively to reach its lowest value at weaning age (around one year). It is possible that the diet of the weaning camel calves, a dry fibrous grass which is low in protein and energy, can not sustain the rumen micro-flora for thiamine synthesis [6, 7] and at the same time these calves are deprived of milk which is a rich source of thiamine [1].

Recently born camel calves to mothers with low blood thiamine levels (37.4 ± 3 µg/L) also exhibited low blood thiamine compared to normal calves at their age (41.9 vs. 97.8 µg/L). This indicates that this group of pregnant camels could not actively mobilize enough thiamine to the fetus as the normal pregnant camels do.

The non-pregnant non-lactating breeding camels displayed the highest blood thiamine than the other breeding groups although they received the same diet and water. This finding supports the work of TINSON et al. [22] who reported high mean blood thiamine level (74 µg/L) in 16 experimental non-racing, non pregnant, non lactating camels. On the other hand, the stress of pregnancy and the preferential mobilization of thiamine to support the fetal growth seemed to have a direct effect on the blood thiamine status of pregnant camels. Indeed, the very high thiamine level in the blood of lactating mothers, the continuous need for milk synthesis look to be more demanding for thiamine than pregnancy. In recent calved group (1-5 days) the vitamin content in blood was less than those at late pregnancy and the levels dropped continuously thereafter to reach its lowest values in late lactating camels. The diet of breeding camels, Rhodes grass (Table 1), was high in sulfate (0.47%) and exceeded the maximum tolerant limit, (0.4%), defined by NCR [16] for beef cattle. Sulfide production increases under such high sulfate intake and has the potential to produce PEM lesions either by direct neurotoxic effect on the brain [11] or by splitting thiamine into pyrimidine and thiazole moieties, mimicking thiaminase activity [4]. Despite the stress of pregnancy, lactation and the high sulfate content of diet, PEM was not noticed in the breeding camels, but subclinical cases were reported among the recently calved mothers and their young. These subclinical thiamine-deficient mothers and their calves did not show any clinical signs of PEM thereafter, only it was observed that the calves did not grow as fast as those from normal dams did. Low thiamine status in blood is not necessary to produce the clinical condition of PEM [10, 12, 17].

Thiamine was high in the adult non-racing camels (group C) similar to that of the non-pregnant non-lactating breeding camels in group B (P>0.05), and significantly higher than that of racing camels. This finding is in general agreement with reports of TINSON et al. [22] and WERNERY et al. [24]. However, the blood thiamine in the young racing camels was lower than that reported by WERNERY et al. [24]. These young camels were new to the stress of racing and to the type of feed. Traditionally, the racing camels are given about one kg high energy diet composed of, concentrates, Lucerne, dates, honey and ghee. It is well known that high-energy diets lower rumen pH and alter the normal balance of rumen microorganisms thus favouring production of thiaminases that split thiamine [3, 6]. Lucerne was reported also to cause acute PEM in 10 camels used in a donor program [22]. In addition to that racing camels are purged with magnesium sulfate (500 g for young and 1kg for adults), or with a plant ‘Harmal’ (*Zygophyllum qatarense*) rich in sulfur [22] six days before the start of any race. This acidogenic high energy diet, along with the residual sulfur from magnesium sulfate or Harmal, the Lucerne and the diarrhea usually occurring as a result of stress just before the start of race might be the potential factors triggering PEM in the young racing camels. Adult camels (5 years or more) seemed to be more adaptive to the racing regime, tract stress and to the trainers’ practices and thus more refractory to PEM. Such adaptation is common in ruminants raised in sulfur rich pasture and water [4, 13].

Racing camels, which developed PEM, were responsive to treatment by thiamine hydrochloride (T500; 200 mg/mL, Jaapharm, Canada) when administered intravenously at early stages of the disease and at the rate of 8 mg /kg BW/d for three consecutive days. This is in line with previous observations [10, 14, 22, 24]. On the other hand oral doses of the vitamin were reported not be effective [22].

We concluded that the age of camel calves, stress of pregnancy, stage of lactation, age of racing camel, type of feed and stress of tract, ameliorate the thiamine level in the blood without posing major effect and rarely precipitates clinical or sub clinical PEM. These factors should be considered whenever thiamine level is assessed in the blood of camels.

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References