Determination of Vitamin E Status and Supplementation for Nyala (Tragelaphus angasi)

Wendy S. Graffam¹, Nancy A. Irlbeck¹, T. Grandin¹, C. Mallinckrodt¹, R.C. Cambre² and M. Phillips³

¹Department of Animal Science, Colorado State University, Fort Collins, Colorado
²National Zoological Park, Washington, DC
³Denver Zoological Gardens, Denver, Colorado

High incidence of white muscle disease in captive nyala has lead to speculation that they have high vitamin E requirements. Maintenance of nyala is often achieved through high vitamin E supplementation. Injectable vitamin E has been administered monthly (750 IU/injection) at Denver Zoological Gardens for years as an effective method of preventing vitamin E deficiency related deaths, yet, was evaluated to be effective in raising plasma vitamin E levels for only ten days post-injection. Four adult female, two juvenile females and one juvenile male nyala were trained to be bled in a handling crate designed to reduce stress. Animals were trained, using food rewards as positive reinforcement, to be bled biweekly. Baseline plasma vitamin E values were determined to be similar between individuals. Animals were supplemented with oral forms including: liquid water miscible vitamin E plus rice-hull powder (RHE) (1000 IU/d/group) in Protocol 1, liquid alone (500 IU/d/group) in Protocol 2, and caramelized vitamin E powder (CBE) (50 IU/d/animal) in Protocol 3. Preliminary results of liquid oral supplementation, show an increase in blood plasma vitamin E four fold higher than baseline. Protocol 1 had lower response than either protocol 2 or 3. Protocols 2 and 3 stimulated peak blood responses greater than injections and longer in duration due to daily administration. Orals are easier to administer than injections which require labor, personnel and stress to the animals. Oral supplementation was intended to reduce labor involved in vitamin E administration and was determined to be most effectively accomplished utilizing WM sprayed on the food items.

Key words: vitamin E, white muscle disease, captive nyala, requirements, supplementation

INTRODUCTION

Nyala (Tragelaphus angasi) have been maintained in zoological gardens for many years. Thomas, et al. (1986) reported that in 1962, only 26 nyala were maintained in four institutions in captivity worldwide; whereas, in 1982, 38 institutions maintained 288 animals, most of which were breeding. However, the dietary requirements of these animals are not well known. Nyala are secretive, forest dwelling animals, living in only the densest of cover of South Africa, are rarely seen and are known to have home ranges only near water sources (Cavendish, 1991; Grzimek, 1990; Thomas, et al., 1986; Whitfield, 1984; Grzimek, 1972).

Nyala have been documented to browse on leaves, fallen fruit and new shoots of grasses (Whitfield, 1984). Those items they selectively browse on are speculated to be naturally high in vitamin E. Young leaves, legumes and green plants (Lynch, 1991; McDowell, 1989) are known to be natural sources high in vitamin E. In captivity, many nyala have been lost to capture myopathy of which some speculate is due to a vitamin E deficiency. Capture myopathy is
characterized by acute onset muscular stiffness, weakness, tremors, paralysis, myoglobinuria and death often related to recent trapping, transportation or other stress (Spraker, et al., 1987) and has been documented in Africa since 1964 (Haigh et al., 1977).

A variety of species have been documented to die from capture myopathy including captive nyala (Liu, et al., 1982), elephant (Papas, et al., 1991) flamingos (Young, 1967), Swainson's hawks (Mainka, et al., 1994), blue herons (Nichols, et al., 1986), wild turkeys (Spraker, et al., 1987), mule deer (Dierenfeld and Jessup, 1990), dolphin (Colgrove, 1978), giraffe (Burton and Dierenfeld, 1990), brown pelican (Campbell and Montali, 1980), dama gazelle (Wallace and Bush, 1987), night herons (Carpenter, et al., 1979) elk (Lewis, et al., 1977), and pronghorn antelope (Chalmers and Barrett, 1977). Some researchers have reported no effect of vitamin E or selenium (Se) on capture myopathy in African wildlife (Wobeser, et al., 1976) while others have reported lesions associated with capture myopathy to be identical to vitamin E/Se induced myopathies (Liu, et al., 1982).

The desire to reduce the incidence of vitamin E related deaths has lead to the supplementation of this vitamin in many exotic animals. Supplementation is often in the form of injections or oral additives. Injections are not a feasible management protocol for most zoos due to the frequent handling of animals. Oral supplements are often given without any substantiating evidence of their efficacy in the desired species. Therefore, the objective of this study was to eliminate the need for monthly vitamin E injections to nyala at the Denver Zoological Gardens (DZG) by determination of the efficacy of various noninvasive forms of vitamin E supplementation. A secondary objective was to train animals to be handled, allowing collection of blood samples on a routine basis for vitamin E status determination.

MATERIALS AND METHODS

History

Documented cases of vitamin E deficiency related death in nyala are limited to those published by Liu et al. (1982) in which 32 animals died in a period of 9 years. Twenty one of these animals died of acute heart failure with evidence of myopathy. All animals had pale, white streaks in large muscle groups on necropsy. Myocardial degradation and necrosis were also observed in 9 animals. Alpha-tocopherol levels in affected animals were 0.03-0.08 mg/dl where clinically normal animals had levels at 0.09-0.24 mg/dl. Additionally, some animals died acutely when released into pastures at the onset of warm weather. Animals were seen running one day but were found dead the following day. Again, signs of vitamin E deficiency were seen in tissue and plasma values.

Several nyala at DZG have died acutely with similar signs as those listed above. Three calves, 9 months of age or younger died in this manner prior to 1982. Due to these three deaths, all nyala began to receive routine monthly injections of vitamin E (Schering-Plough Animal Health Vital E-300 -300 IU d-alpha-tocopherol/ml, Phoenix Vitamin E -200 mg d-alpha-tocopherol/ml) or vitamin E -Se (Schering-Plough BoSe - 68 IU d-alpha-tocopheryl acetate/ml). Injection dosages ranged from 200- 750 IU per injection.
Monthly injections required a large amount of stress to the animals. However, nyala eventually adjusted to being routinely injected with vitamin E via pole syringe every month by going immediately to the wall and waiting in a braced stance until injected, the amount of stress to the animals was still assumed to be significant. In an effort to reduce the stress to the animals and the labor involved in the catching the nyala, vitamin E was orally supplemented by top dressing the diet with vitamin E beadlets at a rate of 2.2 mg d, 1-alpha-tocopheryl acetate/kg body weight/day (Feed Products, Inc., Denver, CO) with the average female weighing 57-80 kg. With this supplementation regime in place (January 21, 1985), injections were stopped. In time, animals started to die again with similar lesions. Injections were reinstigated and the powdered supplement was reevaluated. Most of the powder was determined to be falling through the feed and remaining in the bottom of the feed dishes. Animals were receiving little or none of the vitamin E supplemented in this manner. It was suggested that a carrier would help to distribute the vitamin E throughout the diet. Vitamin E beadlets were mixed with rice hulls and sprinkled onto produce instead of dry pellets. This appeared to reduce the amount of supplement falling through to the bottom. In 1990, top dress composition was changed from 11,000 mg/kg top dress of d, 1-alpha-tocopheryl acetate to d-alpha-tocopheryl at 11,000 mg/kg. At the initiation of this study, animals were receiving vitamin E injections monthly and vitamin E top dressed onto produce daily.

Since 1982, 11 animals, 8 of which were under one year of age, have died of suspected vitamin E deficiency or related symptoms at DZG. Necropsies revealed pale streaked muscles and hearts along with congested lungs. Animals that did not die acutely were found to have little or no body fat stores and, often times, chronic lumpy jaw. Pathology reports indicated causes of death as: septicemia resulting from lumpy jaw, pneumonia, stress, and capture myopathy. Many of these deaths occurred acutely and without premonitory signs.

In other institutions, captive exotic animals diagnosed with a vitamin E deficiency before their deaths were treated with injectable vitamin E supplements. Administration of vitamin E served to improve the health of animals that had not progressed very far into the disease (Campbell and Montali, 1980). However, supplementation of animals in this manner is time consuming and may be stressful to the animals. Nyala at DZG had become accustomed to monthly injections but a less invasive method of supplementation was sought.

**Restraint and Behavior**

Seven nyala, four adult and two juvenile females and one juvenile male, ranging in age from 3 months to 10 years, were trained through habituation and conditioning to allow blood sampling and veterinary procedures in a specially designed crate. This procedure is described in detail in Grandin et al. (1995).

**Diet**

The basal nyala diet for the group (maximum 8 animals) consisted of 1.5 kg dehydrated alfalfa pellets (15% CP, 1.5% crude fat, 30% crude fiber) fed in the morning, 3.0 kg hoofstock pellet (14% CP, 3.5% crude fat, 16% CF), 3.0 kg alfalfa pellets fed in the afternoon with two chopped apples on which vitamin E powder in a rice hull carrier (RHE -Feed Products, Inc., Denver, Co. - 11,000 mg/kg) was sprinkled. Supplementation of RHE was fed at a rate of 2.2 mg/kg body
weight/day in a group setting and was initiated on January 21, 1985. Two feeding stations were available as were an automatic waterer, a trace mineral block and a salt block. Free choice grass hay (8.7% CP, 1.9 % crude fat, 29% CF) was available at all times. Straw bedding was present in one corner of each stall and was often seen being consumed. Monthly vitamin E injections were given at the dosage of 750 IU per adult animal (Schering-Plough Animal Health Vital E-300 -300 IU/ml).

**Blood Sampling and Analysis**

Individual animals adapted at different rates to being conditioned to be bled. Figure 1 demonstrates the timeline of events during this trial. Events such as first capture and first blood collection as well as the specific dates on which animals were bled or injected with vitamin E are shown in three concurrent timelines. Blood samples were obtained weekly until all animals were being sampled every week. Samples were taken weekly for 12 weeks (March 23, 1993 -June 17, 1993) and then biweekly for 11 weeks (June 17, 1993- January 1, 1994) until a plasma vitamin E baseline was established.

Animals were sampled every other week while on one of three vitamin E supplementation protocols after establishment of the plasma vitamin E baseline. Blood samples were taken via a 19 G butterfly catheter (Vacutainer Co., Franklin Lakes, NJ) into a syringe and manually transferred into a lithium-heparinized 10 ml tube (Vacutainer Co., Franklin Lakes, NJ). Blood samples were then immediately transported to Colorado State University for whole blood hemolysis testing (Preston, 1993). Remaining blood samples were separated, spun and stored in plastic cryogenic vials as whole blood, blood cells (after three washings with saline) and plasma, after being flushed with nitrogen, at -21° C until analyzed. No sample was stored for more than 6 months prior to analysis. All blood samples were analyzed for plasma copper, iron, zinc by standard flame atomic absorption spectroscopy, alpha- tocopherol and retinol (Miller and Chung, 1985), glutathione peroxidase (Agergaard and Jensen, 1982), and hematocrit values. Cholesterol and Creatine Phosphokinase (CPK) were determined by Colorado Veterinary Laboratory in Broomfield, CO within 14 days of blood collection.

**Supplementation Protocols**

Once a plasma vitamin E baseline was established (March 23, 1993 -January 1, 1994), animals were fed three dietary regimens of vitamin E supplementation (Figure 1). Protocol 1 (Jan 1, 1994 -April 1, 1994) consisted of the basal diet, powdered vitamin E top dress as RHE (2.2 mg/kg body weight/day -approximately 150 IU per animal daily), monthly injections (600 IU/injection), and one ml water miscible vitamin E (Stuart products, Inc. Emcelle tocochromol natural vitamin E supplement -500 IU d-alpha-tocopherol/ml) mixed with 9 ml water in a syringe and sprayed onto the hoofstock pellets in the afternoon daily. Animals consumed the hoofstock pellets as soon as offered. Therefore, hoofstock pellets were chosen as the carrier for this method of supplementation. This regime was presented to the animals as a group and provided approximately 221 IU/animal/day, not including monthly injections.

Protocol 2 consisted of the basal diet, monthly vitamin E injections, and supplemental liquid vitamin E sprayed on the hoofstock pellets as indicated in protocol 1 .This protocol ran from
April 1, 1994 through May 24, was also group fed, and provided approximately 71 IU per animal per day (500 IU to group) not including monthly injections.

Protocol 3 consisted of the basal nyala diet and individually fed yams containing 0.10 g of powdered, caramel and starch based vitamin E supplement (CBE -Roche Rovimix E, 50% SO). The caramel based vitamin E supplement contained 500,000 IU of d, 1-alpha-tocopheryl acetate per kg. This form of supplement was very sweet, in contrast to RHE which had been fed to the animals since 1985. No monthly injections were given during this time. Since it was very sweet and disguised in a yam, animals readily accepted these from the trainer or keeper's hand. This regime was continued from May 24 through June 31, 1994 and was given in addition to the liquid oral vitamin E being sprayed on the hoofstock pellets. Each animal received 50 IU per day from the powder.

**Statistical Analysis**

Data were analyzed by least squares, analysis of variance for repeated measures (Sokal, 1995) using the general linear models (GLM) procedure in SAS (SAS, 1988). Due to group feeding, measurement date and protocol were confounded. It was therefore assumed that no protocol by date interaction existed. Further analyses were performed using each individual animal as its own control by comparing each animal's baseline data with each type of vitamin E supplementation.

**RESULTS**

**Blood Sample Analysis**

Vitamin E plasma values of the nyala at the time baseline was being established is shown in Figure 2. In this figure, the injections effects can be easily seen as well as the rapid degradation of vitamin E plasma levels after injection. When blood samples were taken more than 10 days after an injection, no notable rise in plasma vitamin E was visible. Baseline appeared relatively stable at around 200 ug/dl. Published normals for cattle are > 400 ug/dl ( < 200 = deficient), sheep > 500 ug/dl ( < 100 = deficient) and goats 60-150 ug/dl (wild goat = 26 ug/dl) (Puls, 1994).

Figure 3 shows the plasma vitamin E averages for all animals on trial during the periods of protocols 1-3 (beginning January 1, 1994). Arrows indicated the dates of vitamin E injections that were given. Injections were given in the crate in March, April and May which are marked on the graph. The crate was utilized for injections in order to minimize any external stress on the animals and also to reduce the number of times the crate had to be moved out of the stall. When injections were given in the crate on bleed days, two weeks passed between that bleed and the next, thereby eliminating any possibility of seeing injection responses.

All values obtained for plasma vitamin E are represented as an average in Figure 4 in order to demonstrate the differences between protocol vitamin E levels and baseline. Effects from vitamin E injections are noticeable but have decreased effect on orally supplemented animals. Baseline values are visibly lower than the protocol supplementation regimes.
All animals were conditioned to be bled at varying rates. One animal was shipped out during the study and another animal died of unknown causes (LREbaby). Table 1 shows the number of blood samples collected from each nyala and the date which each animal went on study and off study along with any reason for premature removal.

Statistical analysis of the data indicated that the variation among animals were consistent over time. Therefore, the effect of individual protocols was similar on each animal. Weak to moderate correlations between variables (vitamin A & E, Cu, Fe, cholesterol, CPK, and glutathione peroxidase) ranging from .40 to -.06 were seen. However, due to sample size, all correlations can be accounted for by chance alone. Therefore, all measured variables were assumed to be independent of one another.

Least squares means for response variables (glutathione peroxidase, cholesterol, copper, vitamins A and E) for each protocol are presented in Table 2 for comparison of protocol effects. Table 3 shows the least squares means for plasma vitamin E by individual in order to demonstrate the amount of animal variability.

Vitamin E supplementation protocols increased plasma vitamin E levels significantly (P = .0001) compared to baseline. Protocols 2 and 3 were different from baseline and protocol 2 was more effective than protocol 3 in all animals except two animals (P < .05 for all except LGR, P = .1959, and LRE, P = .3876).

Some researchers have suggested that the use of alpha-tocopherol:cholesterol ratios would give a more standardized estimate of vitamin E status. This would adjust serum tocopherol values to the level of lipid fraction in the blood. The estimated minimum ratio for clinically normal zoo hoofstock is a value greater than 1 (Dierenfeld and Jessup, 1990). The average change in vitamin E per unit change (P = 0.1083) cholesterol was 2.920 with a standard error of 1.788. Cholesterol accounted for 94.1% of variability in vitamin E measurements. Therefore, in this study, the additional predictive power of tocopherol:cholesterol ratio is limited. Cholesterol may be of increased importance in situations where more unexplained variation exists, such as when no repeat measures are available.

Plasma alpha-tocopherol levels were significantly different for individual subjects (P = .0086). Plasma alpha-tocopherol varied significantly (P = .0001) between protocols (Table 4).

DISCUSSION

Vitamin E has many basic functions in mammals. Primarily, vitamin E functions as an antioxidant by quenching free radicals at the cellular membrane thereby maintaining the integrity of the cells and allowing them to function normally (Machlin, 1991; McDowell, 1989; Easter, 1986; Robbins, 1983). Glutathione peroxidase, a selenium containing enzyme, also acts as an antioxidant in the cell cytoplasm to enhance or supplement the function of vitamin E as an antioxidant. Vitamin E also stimulates the immune response, inhibits platelet aggregation and, therefore, slows blood coagulation (Veris, 1994; Machlin, 1991; McDowell, 1989; Sheffy and Schultz, 1979).
Wallach (1970) reported that 30-40% of deaths each year in captive zoo animals were a result of viruses, bacteria or parasites. He concluded that 60-70% of deaths resulted from poor management and husbandry; 25% from nutritional or nutritionally related problems and 35-45% die from poor understanding of the animals ecological or physiological needs.

Since diet selection of new leaves and shoots of grass may provide the animals with increased levels of vitamin E, this increased supply may have resulted in an increased requirement of vitamin E in nyala evolved over a long period of time. The elevation in vitamin E required by nyala may account for the necessity of large amounts provided by supplementation.

**Protocol Effects**

Nyala plasma vitamin E levels were dramatically increased following vitamin E injections but were seen to decrease to baseline within ten days post-injection (Figures 2-4). Ochoa, et al. (1992) suggests that the decline in plasma vitamin E may result from the saturation of blood and eventual deposition into the tissues. Njeru, et al. (1994) demonstrated a rapid increase in serum alpha-tocopherol following IM injection of d, 1-alpha-tocopherol with or without oral supplementation by 12 hr post-injection. In the same study, oral supplementation alone did not reach peak serum concentration until the seventh day. Jensen et al. (1988) also reported that a regular daily supply of vitamin E was preferable to intermittent, higher doses.

Plasma vitamin E was significantly increased with daily oral supplementation in nyala. Nearly all vitamin E values obtained during protocols 1-3 were above baseline. Animals readily consumed the all oral supplement protocols presented which was important due to the fact that the vitamin E in natural form will rapidly oxidize. The types of supplementation utilized in this study did not require an increase in the amount of time required for administration with the exception of the individually fed CBE in yams (protocol 3). Labor involved in giving injections was dramatically reduced with the availability of the crate.

Although plasma vitamin E values increased with all protocols, protocol 2 was the most effective in raising and maintaining the increase. Drops in plasma vitamin E were noted on one bleed in November (bleed 20), two bleeds in February (bleeds 25 and 26) and the last bleed associated with protocol 3 (bleed 36). It is not yet understood why these drops occurred but it has been speculated that the animals may have perceived an increase in stress due to their flighty nature. Although infrequent, the animals did act more skiddish on some days for unknown reasons. It is also possible that protocol 3 was less effective than initially observed and that enough time on this protocol was not allowed. More time to examine this protocol's effect was not available.

Peaks can be noted on Figures 2-4 which are related to an injection of vitamin E. During March, April and May, injections were given in the crate on the same day as blood sampling. The injections were given once the blood was taken. Animals were, in general, very cooperative to this procedure. Since the injection was given in conjunction with bleeding, there was a 14 day interval between injection and the next blood sample. Since the effect of vitamin E injections was only visible for ten days postinjection, any visualization of injection effects was not possible during this time.
Behavior

Nyala had been receiving injections of vitamin E for more than ten years at the initiation of this study. Animals had adapted to being injected by going immediately to the wall and standing in a braced position. Due to this prior acclimation to handling, animals were easily trained to enter the crate and, eventually, to be bled. Entrance into the crate and manipulation of the leg was not observed to create a large amount of stress in the animals. All animals willingly entered the crate and often were lined up to enter. Values for CPK were seen to remain low, (avg = 120 IU/l) as compared to normal values from CVL (sheep and cattle < 500 IU/l; horse = 95-503 IU/l), throughout the study with an occasional elevated value. Low values such as this are assumed to reflect little to no muscle damage or trauma before or during bleeding.

CONCLUSION

Nyala showed significant increases in plasma vitamin E levels taken under low stress conditions when supplemented with daily, oral d-alpha-tocopherol at a rate of 500 IU/group as a liquid sprayed on pellet or d-alpha-tocopheryl acetate at a rate of 50 IU/head/day fed individually as CBE. Individually feeding CBE to animals would not be possible in most groups of nyala due to their skiddish and shy nature. Therefore, spraying liquid vitamin E in its water miscible form is easily accomplished by any institution and serves as an effective means of supplying vitamin E to nyala without increased labor or handling of these animals.

ACKNOWLEDGEMENTS

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* All animals are female except RGR which was a juvenile male.
+ LREbaby was born during the study to LRE and died after a short duration on study.
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**Table 2.** Least squares means of response variables (vit A, vit E, Gx, Chol, Cu, Chol, Gx) from nyala blood samples during each protocol.
### TABLE 3. Least squares means of plasma vitamin E for individual animals while on each protocol

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* All animals are female except RGR which was a juvenile male. LREbaby was born during the study and died due to an unknown cause after a short period on study.
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<td>0.765</td>
<td>0.835</td>
</tr>
</tbody>
</table>

Vit A = vitamin A; Vit E = vitamin E; G Px = Glutathione Peroxidase; Chol = cholesterol; CPK = creatine phosphokinase; Cu = copper; Fe = iron
Figure 1. Time line of important events for duration of nyala study at Denver Zoological Gardens.
Figure 2. Average plasma vitamin E values for all nyala on study at Denver Zoological Gardens in 1993 (baseline establishment).
Figure 3. Average plasma vitamin E values for all nyala on study at Denver Zoological Gardens in 1994 (protocol tests).
Figure 4. Average plasma vitamin E values for all nyala on study at Denver Zoological Gardens for duration of study (1993-1994).