

EVALUATION OF A COMMERCIAL VITAMIN AND MINERAL SUPPLEMENT IN MILK REPLACERS ON SERUM NUTRIENTS IN PRE-RUMINANTS

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Abstract

Since opening in May 1972, the San Diego Zoo Safari Park has hand-raised hundreds of ruminant neonates. In order to improve success rates and generate appropriate protocols with any hand-reared neonate species, it is important to document each animal's daily status and changes implemented to husbandry practices with each experience. Milk formula composition and consumption, solid-food intake, body weights, and fecal output and consistency are all closely monitored by animal care staff. This paper evaluated the effectiveness of a commercially available vitamin and mineral supplement in milk replacers on serum and whole blood nutrient concentrations in pre-ruminant animals hand-raised between 01-Sept-13 and 01-Dec-14. The inclusion of the vitamin and mineral supplement in eight milk-replacer formulas increased the average dietary concentrations of copper, iron, vitamin E, and selenium by 77.5, 798.0, 391.0, and 81.0%, respectively. However, 50% of the formulas were still below the recommended concentration for both dietary copper and iron as suggested in NRC (2001). At least 91.6, 88.5, and 97.5% of the neonate hoofstock hand-raised on the formulas were within or above the adequate range for serum copper, iron, and whole blood selenium concentrations. Ninety-seven percent of the serum vitamin E concentrations were within the ranges reported for plasma vitamin E concentrations in five related families of artiodactyla.

Introduction

Following the decision to hand-raise an animal, usually due to dam neglect or medical issues with the calf, each hoofstock neonate was relocated from the exhibit to the Animal Care Center (ACC) at the San Diego Zoo Safari Park. The neonate was first examined by the veterinary staff and received treatment for any medical problems. Detailed records were kept on each animal, noting body weight, formula composition and intake, feeding frequency, urine output, and stool frequency and quality. The calves remained at the ACC until weaned at 3 to 4 mo of age.

Historically, milk-replacer formulas have been formulated to mimic values reported for the species dam's milk from available data or analysis, or the animals' are offered a suitable milk replacer from an appropriate model species. All of the neonatal ruminant milk formulas are deficient in iron and have been supplemented. The majority of the formulas are whole goat's milk-based with varying ratios of commercial milk replacers added. Two iron supplements have been used at the San Diego Zoo Safari Park. Vi-Sorbin (vitamin-iron preparation with sorbitol; Pfizer Inc., Pfizer Animal Health, New York, NY, 10017), and Lixotinic (vitamin and mineral supplement for horses, dogs, and cats; Zoetis Inc., Kalamazoo, MI, 49007). Visorbin was discontinued and Lixotinic was the suggested replacement. Lixotinic was used until concerns arose with feeding any products containing ruminant tissue to a ruminant animal. Lixotinic contained ruminant-derived liver paste. The concern was over the potential of exposing our animals to products from cattle infected with Bovine Spongiform Encephalopathy (BSE).

In 2005, a new liquid iron supplement from Kirkman Labs was identified as appropriate to use for ruminants. Each vitamin and mineral supplement was added daily to the first bottle for each neonate until it was weaned. With the Vi-sorbin product, each animal received 0.66 mg Fe/kg BW (0.33 ml/kg BW), and with Lixotinc each animal received at 1.1 mg Fe/kg BW (0.44 ml/kg BW). The dose established for the Kirkman's liquid Iron was 1.05 mg Fe/kg BW (0.3 ml/kg BW).

In May 2014, several species of neonatal ruminants that were being raised at the ACC, were reported to have low serum copper levels (< 0.7 mg/L and < 0.8 mg/L for sheep, and bovine and caprine, respectively, Table 1). The neonatal ruminants (n = 9) that were copper deficient received a treatment of copper sulfate solution in their bottles, daily for one month. The nine animals represented 39% of hand-raised animals that had blood mineral and trace mineral panels between April-September 2013. These same animals had follow-up lab results performed, and all but one animal's serum copper level was adequate (> 0.76 mg/L).

Maternal deficiencies of phosphorus, manganese, cobalt, copper, zinc, and selenium can result in deficiencies in the fetus and newborn calf (NRC, 2001). The fetus has the ability to concentrate some of these minerals, particularly copper and selenium providing some protection against marginal deficiencies in the dam (NRC, 2001). A number of ruminant species housed in large field exhibits (25-65 acres or 10-26 hectares) at the San Diego Zoo's Safari Park have been documented with chronic low-copper levels. This issue is currently being addressed with feeding a custom formulation of a High-Copper Herbivore pellet (40-50 mg/L) as compared to the custom standard High-fiber Herbivore pellet (20-30 mg/L). Affected animals can also be given a copper bolus opportunistically during a medical procedure requiring an immobilization. It is not evident if serum mineral or vitamin deficiencies seen with some of the neonatal ruminant species at the Safari Park are due specifically to a dam's mineral status, or formulas with dietary deficiencies, or if both issues will have to be addressed simultaneously to mitigate the problem.

Nutrition was asked to review the hand-rearing formulas (Table 2) for deficiencies and to consider dietary modifications to address the low serum copper. Vibra-Life Fortifier (Hampel Animal Care, Germantown, WI, 53022), a commercially available vitamin and mineral powder supplement was identified. A sample of this supplement was sent to an independent laboratory for analysis (Table 3).

Methods

The nutrient recommendations for dairy calves fed a milk replacer were deemed appropriate for the species of ruminants most commonly hand-raised at the ACC (Table 4). The supplement was added to the existing formulas used at the ACC at the recommended rate of 0.6 g per L of milk formula (including formulas containing a commercial milk replacer). This vitamin and mineral supplement was added daily to the first bottle for each neonate until the animal was weaned. Table 2 shows the nutrient concentrations of the supplemented formulas compared to the NRC (2001) recommended nutrient concentrations for dairy calves. Since the concentration of iron in the formulas was also increased with the new supplement, the Kirkland liquid Iron was discontinued to mitigate any excessive dietary iron. The normal protocol for hand-raising hoofstock was followed during the 15 month period when the use of the Vibra-Life supplement was implemented.

Blood samples were routinely taken after an animal had nursed formula for 24 h to determine if its immunoglobulin G (IgG) levels were appropriate for determining passive immunity. Also, blood was drawn, by the veterinary staff, when animals were ill, and at four or more weeks of age when the animals were scheduled to receive a vaccine to protect against clostridium bacterium infection. Blood was collected into acid-washed tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ, 07417, USA) for serum mineral, and vitamin E analysis and K2 EDTA tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ, 07417, USA) for whole blood selenium analysis. The blood samples are analyzed for blood chemistry and serum-macro and trace-element screens, including calcium, phosphorus, iron, copper, magnesium, potassium, sodium, and zinc (California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, UC Davis, Davis CA, 95616). Analysis for vitamin E (serum) and selenium (whole blood) was often requested to help with assessing the health status of the neonates. Blood samples were collected when the animals were at least 4 wks old with most samples collected between 4 to 6 wks.

This post-natal period allowed for nutrient normalization in the tissue of the neonates receiving the supplement. Serum copper, iron, vitamin E and whole blood selenium were the nutrients of particular interest because chronic deficiencies have been observed in Safari Park animals, and due to their importance for growth. The ranges of each of these four nutrients plus standard deviations were determined for 20 different species of ruminant hoofstock for the combined total of 39 individual animals (Table 4).

No statistical analysis to determine significant findings were performed. The objective was to evaluate if the supplement improved serum copper to adequate levels, and produced adequate serum iron and vitamin E, and blood selenium levels in pre-ruminant hoofstock.

Results and Discussion

Table 2 lists the nutrient concentrations of eight supplemented milk-replacer formulas used at the ACC, compared to the NRC (2001) nutrient recommendations for dairy calves. Although, all milk formulas are prepared to meet all the nutritional needs of the neonates, only minerals, trace minerals, selenium, and vitamin E are highlighted for the purpose of this project.

Table 1 lists the adequate serum or whole blood concentrations of copper, iron, vitamin E, and selenium in ruminants. Reference ranges from the California Animal Health and Food Safety Laboratory (Davis, CA) and Puls (1994) which are the reference values used by SDZG Veterinary and Nutritional Services Departments to evaluate the health status of animals.

Across all species (n = 20), the average for serum copper was 1.10 mg/L (range = 0.41-2.20 mg/L, S.D. = 0.34 mg/L), serum iron was 2.22 mg/L (range = 0.54-4.20 mg/L, S.D. = 1.04 mg/L), serum vitamin E was 2.97 mg/L (range = 0.36-6.80 mg/L, S.D. = 1.56 mg/L), and whole blood selenium was 0.38 mg/L (range = 0.15-0.67 mg/L, S.D. = 0.15 mg/L; Table 4).

Copper

The recommended concentration for copper in milk-replacer formulas for dairy calves is 10 mg/L (NRC, 2001). The copper concentrations in the eight formulas ranged between 3.97-12.83

mg/L, and were lower than the recommended concentration by 60%, to above the recommended concentration by 28% (Table 2). The formulas containing less than 10 mg/L were the Whole Cow's Milk:Powdered Goat's Milk 11:1, Whole Goat's Milk:Powdered Goat's Milk 18:1, Whole Goat's Milk:Doe's Match:Whey:Water 20:1:1:3, and Whole Cow's Milk (Table 2). When the Vibra-Life supplement was first used in September 2013, the first three formulas were not currently being fed, and subsequently were not evaluated for the copper concentration with an assumption the level would be sufficient. Adding the vitamin and mineral supplement to Whole Goat's Milk at the advised rate did not raise the copper concentration to the NRC (2001) recommendation; however, there were concerns increasing the rate of supplementation would increase the concentration of dietary vitamin A above the reported safe limit. The presumed safe limit for vitamin A is 66,000 IU/kg of dietary DM for lactating and non-lactating cattle (NRC, 2001), but safe limits specifically for young calves have not been established. Supplementation levels of several times the requirement are common in commercial milk replacers (NRC, 2001).

The animal with the lowest serum copper concentration (0.41 mg/L) was on the formula with a copper concentration of 8.80 mg/L. The highest serum copper concentration of 2.20 mg/L occurred in one animal that received the formula with dietary copper concentration of 12.83 mg/L. Puls (1988) list serum concentrations of 2.5 mg/L, 1.8 mg/L, and 5.0 mg/L as high, and 10.0 mg/L, 7.5 mg/L, and 20.0 mg/L as toxic for bovine, caprine, and ovine, respectively. Collectively, 91.6% or 100% of the 39 animals had adequate or greater serum copper concentrations when using the CAHFS lab (0.70 – 1.50 mg/L) or Puls (1988; 0.32 – 2.00 mg/L) reference ranges, respectively (Table 4).

Iron

The recommended concentration for iron in milk-replacer formulas for dairy calves is 100 ppm (NRC, 2001). The iron concentrations in the eight formulas ranged between 60-114 ppm, and are below the recommended concentration by 60%, to above the recommended concentration by 14.4% (Table 2). A dietary iron concentration of 95 ppm is recommended for growth in goats (NRC, 2007). A study by Lindt and Bloom (1994) suggested that a diet with 50 mg iron/kg DM was sufficient to support growth although the carcass remains pale (NRC, 2001). The formulas containing less than 100 mg/L were the Whole Cow's Milk:Powdered Goat's Milk 11:1, Whole Goat's Milk:Powdered Goat's Milk 18:1, Whole Goat's Milk:Doe's Match:Whey:Water 20:1:1:3, and Whole Cow's Milk (Table 2).

The animal with the lowest serum iron concentration (0.54 mg/L) was on the formula with a iron concentration of 107 mg/L, although other animals of the same species had adequate serum iron concentrations (1.3 mg/L or above, Table 4). The highest serum iron concentration of 4.2 mg/L occurred in one animal that received the formula with the highest dietary iron concentration of 114 ppm. Puls (1988) lists serum concentrations of 400-600 mg/L, and 1800-2500 mg/L as high and toxic, respectively, for bovine (Table 1). Collectively, 88.5% of the 39 animals were within or above adequate serum iron concentrations when referencing either CAHFS (1.30-2.50 mg/L) or Puls (1988; 1.30-2.50 mg/L) reference ranges, respectively (Table 1).

Vitamin E

The recommended concentration for vitamin E in milk-replacer formulas for dairy calves is 50 ppm (NRC, 2001). Vitamin E concentrations in the eight formulas ranged between 74.0-222.0

ppm, exceeding the recommended level by 150-400% (Table 2). Vitamin E is one of the least toxic vitamins due in part to its relatively low absorption (NRC, 2001). Toxicity studies have not been conducted with ruminants, but data from rats suggest an upper limit of approximately 75 IU/kg of body weight per day (NRC, 2001).

The average serum vitamin E concentration (2.97 mg/L) in the neonatal ruminants was similar to means seen in Dierenfeld (1989) for plasma vitamin E concentrations of 1.90, 1.92, 2.03, 2.09, and 3.01 mg/L, for camelidae, bovidae, giraffidae, cervidae, and tragulidae, respectively. The range in serum vitamin E concentrations seen in the neonatal ruminants (0.36-6.80 mg/L, Table 4) was also similar to the wide ranges seen in the Dierenfeld (1989) study, where plasma vitamin E ranges of 1.0-5.4, 0.5-8.5, 0.9-7.0, 0.4-6.7, 0.7-5.0 mg/L were reported for camelidae, bovidae, giraffidae, cervidae, and tragulidae, respectively.

The hand-raised animal with the lowest serum vitamin E concentration (0.36 mg/L) was on the formula containing the highest concentration of 222.0 mg/L; but there was another animal of the same species that had a serum vitamin E level of 2.70 mg/L. One animal's blood results came back with vitamin E as undetectable, and received the formula with a vitamin E concentration of 198 ppm; however, other animals of the same species had serum vitamin E concentrations between 1.6-5.4 mg/L.

Selenium

The recommended concentration for selenium in milk-replacer formulas for dairy calves is 0.3 ppm (NRC, 2001). The selenium concentrations in the eight formulas ranged between 0.67 – 1.10 ppm (Table 2), and are all above the recommended concentration by 223-367%. NRC (2001) states that chronic toxicity can occur when cattle are fed diets with 5 to 40 mg of selenium/kg (DM) for several weeks or months. Puls (1988) list serum concentrations of 0.8-2.5 mg/L and 3.5-4.1 mg/L as high and toxic for bovine, respectively, and 3.0 mg/L and above as toxic to caprine. Collectively, 97.4% of 30 animal samples had more than adequate selenium concentrations when referenced with Puls (1988; 0.03 – 0.15 mg/L) for bovine, plus 80.0% and 20% had adequate, and more than adequate selenium concentrations for ovine (Table 4).

Summary

There will be many opportunities in the future allowing animal care staff to collect more information (i.e., milk formula composition, supplementation, and consumption, solid food intake, body weights, fecal output and consistency) and further improve hand-rearing successes with neonatal ruminants. A complicating factor affecting mineral, and vitamin status of a neonate animal is its health status. If an animal has a medical issue, its nutrient regulatory biochemical pathways may be compromised.

Although the majority of the animals had adequate serum copper and iron concentrations, based on the findings in this case study, dietary copper and iron need to be improved in four formulas, and an alternate commercial or custom vitamin and mineral supplement should be considered.

All eight formulas contained vitamin E and selenium concentrations in excess of the recommended concentrations for dairy calves (NRC, 2001). Serum vitamin E and whole blood

selenium concentrations were adequate or above adequate for the majority of the hand-raised ruminant neonates.

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Table 1. Reference ranges of adequate serum copper, iron, and selenium concentrations for bovine, caprine, and ovine.

Species	Serum Copper		Serum Iron		Serum Selenium
	CAHFS ¹	Puls ²	CAHFS	Puls	Puls
Bovine	0.80 – 1.50	0.32 – 1.20	1.30 – 2.50	1.30 – 2.50	0.025 – 0.150
Caprine	0.80 – 1.20	0.80 – 1.20	1.50 – 2.50	nr ³	nr
Ovine	0.70 – 1.50	0.70 – 2.00	1.70 – 2.20	1.66 – 2.22	0.08 – 0.50

¹California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, UC Davis, Davis, Ca.; values reported as mg/L.

²Puls, 1988; values reported as mg/L.

³nr = Not reported.

Table 2. Nutrient composition (DMB) of selected milk-replacer formulas with VibraLife¹ vitamin and mineral supplement used for hand-rearing pre-ruminants at the San Diego Zoo Safari Park as compared to nutrient recommendations for dairy calves.

Milk Replacer Formulas²									
Nutrient	WGM	WGM:DM 11:1	WGM:DM 14:1	WGM:DM:H2O 15:2:1	WGM:DM 18:1	WGM:PGM 18:1	WCM:PGM 11:1	WGM:DM:WHEY:H2O 20:1:1:3	Nutrient Requirements of Dairy Calf⁵
Calcium, %	1.04	1.01	1.01	1.00	1.02	1.02	0.98	0.91	1.0
Phosphorus, %	0.86	0.84	0.84	0.84	0.84	0.85	0.80	0.72	0.7
Ca:P ratio	1.2:1	1.2:1	1.2:1	1.2:1	1.2:1	1.2:1	1.2:1	1.25:1	1.4:1
Magnesium, %	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.07
Potassium, %	1.60	1.77	1.74	1.81	1.72	1.57	1.38	1.48	0.65
Sodium, %	0.38	0.61	0.57	0.66	0.55	0.36	0.36	0.46	0.4
Iron, ppm	86.80	106.97	103.88	114.38	101.43	65.34	59.91	91.24	100
Copper, ppm	8.00	11.81	11.22	12.83	10.75	6.27	3.97	8.80	10
Zinc, ppm	92.00	78.76	80.75	78.28	82.33	75.32	80.94	74.71	40
Selenium, ppm	1.10	0.72	0.78	0.67	0.83	0.83 ³	0.85 ⁴	0.80	0.3
Vitamin E, IU/kg	108.0	198.0	185.0	222.0	174.0	81.0 ⁴	74.0 ⁴	162.0	50

¹VibraLife (Hampel Animal Care, Germantown, WI, 53022)

²100% of the formula ingredients have a value for nutrient listed and ingredient ratios are measured on a ‘by weight’ basis; Formula ingredients: **WGM** = Whole Goat’s Milk (Meyenberg, Turlock, Ca, 95381); **DM** = Doe’s Match (Land O’ Lakes Animal Milk Products Co., Shoreview, MN, 55126); **PGM** = Powdered Goat’s Milk (Meyenberg, Turlock, Ca, 95381); **WCM** = Whole Cow’s Milk (Wholesome Farms, Sysco San Diego, Poway, Ca, 92064); **WHEY** = Whey Isolate Powder (The Milky Whey Inc., Missoula, MT, 59801); **H2O** = Water (tap).

³33% of the formula ingredients have a value for nutrient listed.

⁴67% of the formula ingredients have a value for nutrient listed.

⁵NRC (2001) nutrient concentrations for a 45 kg dairy calves fed milk replacer containing 4.75 kcal ME at rate of 0.53 kg DM (1.0% BW) per day.

Table 3. Nutrient concentrations (DMB) of Vibra-Life¹ vitamin and mineral supplement.

Nutrient	Guaranteed analysis²	Commercial laboratory Analysis³
Calcium, %	3.5 -4.5	3.32
Phosphorus, %	0.5 – 0.85	0.83
Ca:P ratio	nr ⁴	1.70
Magnesium, %	nr	1.81
Potassium, %	nr	6.39
Sodium, %	nr	0.21
Iron, mg/L	nr	19100.00
Copper, mg/L	nr	952.00
Zinc, mg/L	nr	17700.00
Selenium, mg/L	240.0	na ⁵
Vitamin E, IU/kg	22,000	na

¹Hampel Animal Care, Hamel Corp., Germantown, WI, 53022.

²Manufacturer's label.

³Dairy One, Ithaca, New York, 14850.

⁴nr = not reported.

⁵na = not analyzed.

Table 4. Serum copper, iron, vitamin E, and selenium concentrations for 20 pre-ruminant species fed milk-replacers containing Vibra-Life vitamin and mineral supplement.

Species	Sample Size	Serum Copper, mg/L		Serum Iron, mg/L		Serum Vitamin E, mg/L		Blood Selenium, mg/L	
		Range ¹	S.D. ²	Range	S.D.	Range	S.D.	Range	S.D.
Addra Gazelle (<i>Nanger dama ruficollis</i>)	1	1.10	--	3.40	--	2.50	--	0.50	-
Armenian Mouflon (<i>Ovis aries orientalis</i>)	1	1.30	--	1.90	--	0.96	--	0.36	-
Blackbuck (<i>Antilope c. cervicapra</i>)	8	0.74 – 1.50	--	0.54 - 4.00	1.20	0.89 – 4.60 ⁴	1.60	0.28 – 0.59 ⁴	0.13
Domestic Cattle (<i>Bos t. taurus</i>)	1	0.70	--	1.70	--	na ³	--	na	--
East African Sitatunga (<i>Tragelaphus s. spekii</i>)	1	0.87	--	2.40	--	2.10	--	0.17	--
Eastern Bongo (<i>Tragelaphus eurycerus</i>)	2	1.20 – 1.30	0.07	2.70	--	5.20	--	0.41	-
Eastern Giant Eland (<i>Taurotragus derbianus gigas</i>)	1	1.80	--	0.92	--	3.10	--	0.15	--
Eastern White Bearded Gnu (<i>Connochaetes taurinus albojubatus</i>)	1	0.76	--	2.00	--	1.90	--	0.43	--
Fringe-eared Oryx (<i>Oryx beisa callotis</i>)	1	1.30	--	na	--	6.80	--	0.57	--
Grant's Gazelle (<i>Nanger g. granti</i>)	2	1.00 – 1.10	0.07	1.50 – 2.80	0.92	2.50 – 2.80	0.21	0.63 – 0.69	0.04
Kenya Impala (<i>Aepyceros melampus rendilis</i>)	2	0.99 – 1.30	0.22	1.50 – 3.60	1.48	3.30	--	0.67	--

Table 4 (cont.). Serum copper, iron, vitamin E, and selenium concentrations for 20 pre-ruminant species fed milk-replacers containing Vibra-Life vitamin and mineral supplement.

Species	Sample Size	Serum Copper, mg/L		Serum Iron, mg/L		Serum Vitamin E, mg/L		Blood Selenium, mg/L	
		Range ¹	S.D. ²	Range	S.D.	Range	S.D.	Range	S.D.
Nubian Ibex (<i>Capra nubiana</i>)	1	1.40	--	1.40	--	4.50	--	0.35	--
Scimitar-horned Oryx (<i>Oryx dammah</i>)	1	1.10	--	0.64	--	2.10	--	0.38	--
South African Greater Kudu (<i>Tragelaphus s. strepsiceros</i>)	2	0.96 – 1.30	0.24	1.40 – 2.50	0.78	2.40 – 2.60	0.14	0.26 – 0.30	0.03
South Africa Sable Antelope (<i>Hippotragus n. niger</i>)	1	1.10	--	1.50	--	na	--	na	--
South African Springbok (<i>Antidorcas m. marsupialis</i>)	3	0.88 – 2.20	0.58	1.40 – 3.90 ⁵	1.39	3.20 – 3.80 ⁵	0.42	0.39 – 0.42 ⁵	0.02
Southern Gerenuk (<i>Litocranius w. walleri</i>)	1	0.41 – 0.72	0.22	2.30 – 2.50	0.14	5.80	--	0.52	--
Thomson's Gazelle (<i>Eudorcas t. thomsonii</i>)	6	0.57 – 1.50	0.32	0.87 – 3.80	1.08	1.60 – 5.60 ⁶	1.45	0.23 – 0.30 ⁶	0.03
Transcaspian Urial (<i>Ovis aries arkal</i>)	2	0.71 – 0.80	0.06	3.90 – 4.20	0.21	0.36 – 2.70	1.65	0.38 – 0.40	0.01
Turkomen Markhor (<i>Capra falconeri heptneri</i>)	1	0.74	--	1.40	--	3.80	--	0.43	--
Across all species	39	0.41 – 2.20	0.34	0.54 – 4.20	1.04	0.36 – 6.80⁷	1.56	0.15 – 0.69⁷	0.15

¹Single value represents result for 1 animal.

²S.D. = Standard deviation; cells with hyphens were not calculated.

³na = not analyzed.

⁴Values listed represent results for 5 of 8 animals.

⁵Values listed represent results for 2 of 3 animals.

⁶Values listed represent results for 5 of 6 animals; 1 animal had no detectable Vitamin E concentration.

⁷Range value represents 30 of 39 animals.