

Evaluation of cholesterol and vitamin E concentrations in adult alpacas and nursing crias

Andrea S. Lear, Stacey R. Byers, Jessica A.A. McArt, and Robert J. Callan

Abstract—The objective of this study was to determine if serum cholesterol and vitamin E concentrations change with production and physiologic state in alpacas. Blood was collected from 3 groups of alpacas. An adult female group was sampled in the periparturient period and once monthly until their offspring were weaned. Crias born to the females were sampled after birth, then once monthly until weaning. A group consisting of males was sampled once monthly throughout the study period. Serum vitamin E and cholesterol concentrations were measured and vitamin E to cholesterol ratios was calculated. Vitamin E concentrations were similar throughout the different physiologic states. Cria vitamin E concentrations closely correlated to that of their dam. Significant cholesterol concentration fluctuations in crias occurred after 4 weeks of life possibly due to milk fat content. After weaning, the cholesterol concentrations became similar to the adult animals within study. Vitamin E concentrations varied with age in crias as they transitioned from a milk to forage based diet. Cholesterol fluctuated with altered physiologic and metabolic demands, most noticeable in the crias. Further studies are needed to determine if vitamin E to cholesterol ratios would be more appropriate to fully assess the vitamin E status in nursing crias.

Keywords—alpaca, cholesterol, vitamin E

I. INTRODUCTION

Vitamin E is a lipid soluble vitamin responsible for a variety of functions throughout the body. Vitamin E deficiencies can manifest in different medical conditions such as nutritional myopathy and neuropathy [1,2]. Mammals do not synthesize vitamin E; therefore they must consume it in their diets. The most abundant dietary sources for herbivores are fresh green forages [3,4].

After absorption of vitamin E through the gut, it is transported via lipoproteins such as very low-density lipoproteins (VLDL), high-density lipoproteins (HDL), and cholesterol. Due to this lipid soluble nature of vitamin E, some reference laboratories evaluate the ratio of vitamin E to cholesterol, for example in cattle [5,6,7]. The effect of cholesterol concentration on vitamin E is unknown in camelids and it is unknown if cholesterol concentration varies with alterations in metabolic demands during pregnancy and lactation. Therefore comparison of animals under various metabolic demands is needed.

Research indicates vitamin E does not readily cross the placenta in ruminants and swine thus neonates of these species are considered deficient at birth [8,9,10]. It is likely

that camelid neonates are also deficient at birth but this has not been verified. Vitamin E is found in high concentrations in colostrum and milk of ruminants and swine [11,12]. However, some ruminant neonates require additional supplementation to prevent vitamin E related conditions such as myopathies due to insufficient concentrations in colostrum and milk [1,13,14]. It is unknown if low vitamin E concentrations in female camelids lead to a similar condition in crias, increasing their susceptibility to conditions such as nutritional myopathy or diaphragmatic paralysis [2].

Monitoring vitamin E through gestation and lactation in females and their crias would assist in identifying potential risk periods for vitamin E deficiency in pregnant and juvenile camelids. The objective of this study was to evaluate serum vitamin E and cholesterol concentrations under different metabolic conditions in adult and juvenile alpacas.

II. MATERIALS AND METHODS

An observational, cohort study was conducted using alpacas from a single ranch in north central Colorado. Three groups of 6 alpacas each, representative of the entire herd, were randomly selected. Group 1 consisted of 6 adult male alpacas housed in 2 adjacent dirt paddocks. These animals were maintained on a grass hay diet with no additional supplementation. The males were used as a comparison group due to expected stable metabolic demands. Group 2 included 6 pregnant females. These animals were housed together in a single dirt paddock. They were fed grass hay and a pelleted ration¹ manufactured specifically for the herd. Group 3 was comprised of the crias born to the females from group 2.

Blood was collected from group 1 once monthly from May to November 2012. Alpacas in group 2 were sampled at 30 and 14 days prior to expected parturition date, within 12 hours after parturition, monthly for 6 months, and 1 month after the cria was weaned (approximately 6 months after birth). Blood was collected from group 3 within 12 hours of birth, at 2 weeks of age, then monthly until 1 month after weaning. The jugular furrow in the right cranio-ventral neck was swabbed with alcohol, and 6 mL of blood was collected from the jugular vein and placed in non-additive (red top) blood collection tubes².

The blood tubes were placed in the upright position, allowed to clot, and stored at 4°C. Serum was collected from the tubes following centrifugation at 1,000 X g for 10 minutes

and then transferred to 2 mL freezer vials and stored at -20°C until vitamin E and cholesterol analyses were performed.

Vitamin E concentrations were measured using high-pressure liquid chromatography³ at the Colorado State University (CSU) Veterinary Diagnostic Laboratory. Due to a large variation in vitamin E concentrations using this method in a previous study, measurements were performed in triplicate and the mean concentration used in the statistical analyses [15]. The analyzer was calibrated according to the manufacturer's recommendations before each batch of samples. Cholesterol concentrations were measured at the CSU Clinical Pathology Laboratory using a Hitachi 917 chemistry analyzer⁴ according to the manufacturer's guidelines. Representative feed samples including hay, pelleted feed, trace mineral supplements were collected and analyzed for vitamin E concentration at a commercial laboratory⁵.

Descriptive statistical analysis was performed using Microsoft Excel⁶. Repeated measure ANOVA was performed using the PROC MIXED procedure in SAS⁷ to evaluate cholesterol and vitamin E concentrations for the different groups and time periods. Animal was used as the repeated measures subject over time. A P -value ≤ 0.05 was used to determine statistical differences.

1. Ranch-way Feeds, Fort Collins, CO
2. Becton Dickinson and Company, Inc. Sandy, UT
3. Waters 474 Scanning Fluorescence Detector, Waters Corporation, Milford, MA
4. Hitachi High Technologies America, Corporation. Wallingford, CT
5. Weld Laboratories, Inc., Greeley, CO
6. Microsoft, Redmond, WA
7. SAS Inst. Inc., Cary, NC

III. RESULTS

A total of 6 samples per adult male and 12 samples per female and cria were obtained over a period of 10 months due to the variation in parturition dates for the females. Throughout the 10-month sample period, no morbidity or mortality was observed in the animals included in the study. All crias were born without assistance and were observed to nurse within 2 hours of life. A mild amount of gross hemolysis of samples occurred at blood collection, which was not expected to affected outcome determination.

Mean vitamin E concentrations varied between the 3 groups of alpacas and over time (Figure 1). No differences were observed for the vitamin E concentrations amid the female group ($P = 0.27$), however differences were observed within cria ($P = 0.03$) and male ($P < 0.001$) groups over time. Vitamin E concentrations of crias were found to be similar to the dams throughout the study. The majority of vitamin E concentrations were less than the CSU Veterinary Diagnostic Laboratory's lower reference interval of 150 $\mu\text{g}/\text{dL}$ for camelids.

FIGURE 1. MEAN VITAMIN E CONCENTRATIONS ($\mu\text{G}/\text{DL}$) OVER TIME IN WEEKS FOR MALES ($N = 6$), FEMALES ($N = 6$), AND CRIAS ($N = 6$). TIME 0 AND 24 WEEKS DENOTES PARTURITION AND WEANING, RESPECTIVELY. THE 95% CONFIDENCE INTERVAL BARS ARE DISPLAYED FOR MEAN VITAMIN E CONCENTRATION. THE SOLID LINE AT 150 $\mu\text{G}/\text{DL}$ IS THE LOW-END REFERENCE INTERVAL FOR SERUM VITAMIN E CONCENTRATIONS REPORTED BY THE CSU DIAGNOSTIC LABORATORY

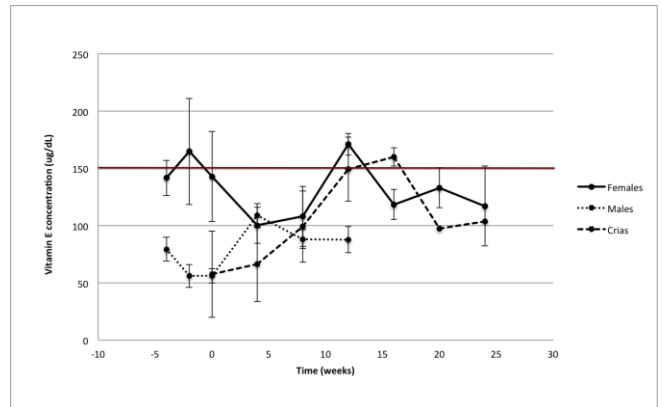
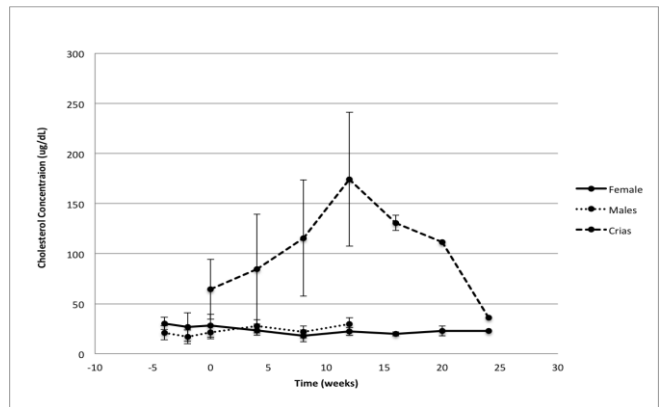


FIGURE 2. MEAN SERUM CHOLESTEROL CONCENTRATIONS ($\mu\text{G}/\text{DL}$) OVER TIME IN WEEKS FOR ADULT MALES ($N = 6$), FEMALES ($N = 6$), AND CRIAS ($N = 6$). TIME 0 AND 24 WEEKS DENOTES PARTURITION AND WEANING, RESPECTIVELY. THE 95% CONFIDENCE INTERVAL BARS ARE DISPLAYED FOR EACH MEAN CHOLESTEROL CONCENTRATION MEASUREMENT.



Mean cholesterol concentrations varied over time for the 3 alpaca groups (Figure 2). Mean cholesterol concentrations were different within the male and cria groups over time ($P < 0.001$) but not the female group. Cholesterol concentrations were found to be different in the female group only between the first (2 weeks prepartum) and fifth (approximately 4 months in lactation) samples ($P = 0.05$). Calculated vitamin E to cholesterol ratios did not vary in the males and female groups. Vitamin E to cholesterol ratios were found to be different amongst the cria group ($P < 0.001$). Table 1 displays the mean vitamin E concentrations of the feedstuffs fed to the entire alpaca herd used for this study.

TABLE I. VITAMIN E CONCENTRATIONS (MG/KG) ON A DRY WEIGHT BASIS OF FEEDSTUFFS FED TO THE ANIMALS IN THE STUDY.

Representative Hay Sample	Vitamin E (mg/kg)
Grass hay 1	135.33
Grass hay 2	175.33
Pellets	735.29

IV. DISCUSSION

The objective of this study was to evaluate serum vitamin E and cholesterol concentrations under different metabolic conditions in adult and juvenile alpacas. The serum vitamin E concentrations in the females did not vary throughout late gestation and lactation although there was a great deal of variation between individuals in the group. There were differences observed in the vitamin E concentrations for the males but not as much variation between animals. The physiological significance of the variations in the individual vitamin E concentrations within these two groups of adult animals is unknown at this time. The variation in vitamin E concentrations of the males from 5-10 weeks may be related to a new hay source for the animals. As observed in Table 1, the new hay samples (Hay-2) sample had a higher vitamin E concentration compared to Hay-1, which was fed at the time of initial sampling. This occurred at approximately 10 weeks into the sample period. Another source of variation in vitamin E concentrations due to feed may have occurred when the herd was evacuated for approximately 2 weeks due to a wildfire. This happened at week 2-4 for males and from 2 weeks prepartum to 2 weeks postpartum for females due to variation in parturition date. At that time an alternate feed source was being used, yet a representative sample was not collected and assayed. This time-feed source relationship cannot be evaluated in the females since their blood collection times were variable and were based on parturition time rather than a set schedule through the research project.

Crias were found to have large vitamin E concentration fluctuations over time. This fluctuation may be due to the expected low serum vitamin E concentrations at birth, which then rapidly increase with milk consumption [10,11]. A decrease near weaning was likely due to transitioning to a hay-based diet. This change in neonatal vitamin E status has been observed in calves and lambs [1,16]. The variation in vitamin E concentrations in the samples collected within 12 hours of life is expected to be due to partial absorption of vitamin E from the colostrum consumed before initial blood sample collection.

The consistently low serum vitamin E concentrations for all groups were surprising. As the hay vitamin E concentrations were low, it was not unexpected to see low serum concentrations in the males as they were fed a grass hay diet with no other form of supplementation. However, the female alpacas and crias were fed primarily a grass hay diet ad libitum with pellet supplementation daily that was formulated with additional vitamin E. Approximately 2 months prior to

the start of this research, the adult animals received a subcutaneous injection (5 mL/animal) of a commercially available vitamin A, D, and E supplement (containing 50 mg of vitamin E per mL) at shearing. No animals included in this study received additional supplementation of vitamin E during the sample period.

Vitamin E concentrations were measured in triplicate using high-pressure liquid chromatography due to the wide variability previously seen when evaluating serum vitamin E concentrations [15]. High-pressure liquid chromatography detects vitamin E in the DL-alpha tocopherol form, which can be easily denatured during preparation and analysis leading to inconsistency between concentrations of the same serum sample [15]. In addition, a small volume of serum was submitted for each sample in order to perform this test in triplicate, resulting in possible unevenness within the sample vitamin E concentrations. Other possible means of variability include the multistep process of preparing the sample for analysis as well as individual laboratory technician difference in sample preparation.

Throughout this study the majority of the animals were found to be vitamin E deficient in accordance with the CSU Diagnostic Laboratory reference interval (150-600 µg/dL). No clinical signs associated with low serum vitamin E, or morbidity or mortality was observed in the alpacas during the study period. As mentioned above, the low vitamin E concentrations were suspected to be due to the hay diet that was fed with no access to fresh forage. Once forage is cut, dried, and packaged as hay, the vitamin E concentration rapidly decreases within days below nutritional requirements, and animals fed a hay diet therefore require additional supplementation [3,4,17]. It is unclear if subclinical diseases due to the low serum vitamin E concentrations in this herd are occurring but not being detected. Alternatively, the normal reference intervals for vitamin E concentrations may need to be reevaluated in this species.

Serum cholesterol results were surprising when comparing the cria and the adult alpaca groups. It is not unexpected that cholesterol concentrations were clinically stable over time for the adult male alpacas. We expected to see a larger variation in serum cholesterol in the adult females as they transitioned into lactation. Based on lactating dairy cows, we expected to see an increase in metabolic demand leading to fat mobilization. A mild change in cholesterol occurred but due to the small sample size we are unable to determine if this is physiologically significant. This change in cholesterol concentration is observed in cattle and other camelid species [5,18]. Values then return to normal as lactation begins to decrease near weaning.

After week 4 of age, crias were observed to have an increase in mean serum cholesterol concentration that remained significantly elevated compared to the female and male serum cholesterol concentrations. Previous studies in llamas and alpacas have revealed an increase in milk fat composition occurring at 4 weeks of lactation and continuing through week 27 [18,19]. This was found to vary from that observed in lactating old-world camelids and ruminants where

milk fat composition did not vary with stage of lactation [20]. A recent study found that alpacas are most similar to llamas in their milk composition through lactation; this variability in the crias may be explained by milk fat composition [21]. The milk composition would then also explain the subsequent decline of the crias serum cholesterol concentration as weaning occurred.

In dairy cattle fluctuations in vitamin E concentrations are measured with cholesterol concentrations, since cholesterol fluctuates during the periparturient period [5,22]. The findings in this study conclude that vitamin E to cholesterol ratios are not needed to further evaluate vitamin E status in adult alpacas, as cholesterol was relatively consistent over time and through lactation. Due to the large increase in serum cholesterol concentrations observed in the crias the resulting serum vitamin E to cholesterol ratio is quite small, however the clinical significance of this is unknown. Due to the sample size of this study, we are unable to determine if a vitamin E to cholesterol ratio is more appropriate for use when evaluating vitamin E concentrations in nursing crias. Additionally there are no guidelines or reference intervals for this ratio in camelids to aid in further analyses. Follow up research should include a larger sample size and animals that are kept on fresh forage in addition to those on only a hay-based diet.

In conclusion, cholesterol was found to fluctuate considerably in crias over time. However, the physiologic significance of this finding is unknown. Additional studies are needed to determine if vitamin E to cholesterol ratio is more adequate to fully evaluate vitamin E status of nursing crias under 6 months of age. In addition, the majority of the animals enrolled in the study appeared to have vitamin E concentrations considered deficient based on our diagnostic laboratory reference intervals. We did not see clinical manifestations of the deficiency in this herd, yet there may be subclinical conditions that are not being recognized or the time period and sample size did not permit us to identify clinical conditions associated with vitamin E deficiency. Additional studies are needed to further identify if the reference intervals are correct or if alpacas fed a strictly hay-based diet need additional supplementation.

ACKNOWLEDGMENT

Research supported in part by the College Research Council through the United States Department of Agriculture.

REFERENCES

- [1] DC Van Metre and RJ Callan. Selenium and vitamin E. *Vet Clin North Am Food Anim Pract* 2001;17:373-402, vii-viii.
- [2] S Byers, G Barrington, D Nelson, G Haldorson, T Holt, and R Callan. Neurological causes of diaphragmatic paralysis in 11 alpacas (*Vicugna pacos*). *J Vet Intern Med* 2011;25:380-385.
- [3] TH Herdt. Blood serum concentrations of selenium in female llamas (*Lama glama*) in relationship to feeding practices, region of United States, reproductive stage, and health of offspring. *J Anim Sci* 1995;73:337-344.
- [4] TM Frye, SN Williams, and TW Graham. Vitamin deficiencies in cattle. *Vet Clin North Am Food Anim Pract* 1991;7:217-275.
- [5] TH Herdt, and TC Smith. Blood-lipid and lactation-stage factors affecting serum vitamin E concentrations and vitamin E cholesterol ratios in dairy cattle. *J Vet Diagn Invest* 1996;8:228-232.
- [6] MG Traber and I Jialal. Measurement of lipid-soluble vitamins-further adjustment needed? *The Lancet* 2000;355:2013-2014.
- [7] L Ford, J Farr, P Morris, and J Berg. The value of measuring serum cholesterol-adjusted vitamin E in routine practice. *Ann Clin Biochem* 2006;43:130-134.
- [8] M Hidirolou, E Farnworth, and G Butler. Effects of vitamin E and fat supplementation on concentration of vitamin E in plasma and milk of sows and in plasma of piglets. *Int J Vitam Nutr Res* 1993;63:180-187.
- [9] M Hidirolou. Mammary transfer of vitamin E in dairy cows. *J Dairy Sci* 1989;72:1067-1071.
- [10] JL Capper, RG Wilkinson, E Kasapidou, SE Pattinson, AM Mackenzie, and LA Sinclair. The effect of dietary vitamin E and fatty acid supplementation of pregnant and lactating ewes on placental and mammary transfer of vitamin E to the lamb. *Br J Nutr* 2005;93:549-557.
- [11] JD Quigley and JJ Drewry. Nutrient and immunity transfer from cow to calf pre- and postcalving. *J Dairy Sci* 1998;81:2779-2790.
- [12] A Pinelli-Saavedra, AM Calderon de la Barca, J Hernandez, R Valenzuela, and JR Scaife. Effect of supplementing sows' feed with alpha-tocopherol acetate and vitamin C on transfer of alpha-tocopherol to piglet tissues, colostrum, and milk: aspects of immune status of piglets. *Res Vet Sci* 2008;85:92-100.
- [13] J Maas, MS Bulgin, BC Anderson, and TM Frye. Nutritional myodegeneration associated with vitamin E deficiency and normal selenium status in lambs. *J Am Vet Med Assoc* 1984;184:201-204.
- [14] CG Rammell, KG Thompson, GR Bentley, and MW Gibbons. Selenium, vitamin E and polyunsaturated fatty acid concentrations in goat kids with and without nutritional myodegeneration. *N Z Vet J* 1989;37:4-6.
- [15] AS Lear, SR Byers, RJ Callan, and JA McArt. Evaluation of sample handling effects on serum vitamin e and cholesterol concentrations in alpacas. *Vet Med Int* 2014;2014:537213.
- [16] RJ Van Saun, TH Herdt, and HD Stowe. Maternal and fetal vitamin E concentrations and selenium-vitamin E interrelationships in dairy cattle. *J Nutr* 1989;119:1156-1164.
- [17] F Calderon, B Chauveau-Duriot, P Pradel, B Martin, B Graulet, M Doreau, and P Noziere. Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. *J Dairy Sci* 2007;90:5651-5664.
- [18] A Riek and M Gerken. Changes in Llama (*Lama glama*) milk composition during lactation. *J Dairy Sci* 2006;89:3484-3493.
- [19] DE Morin, LL Rowan, WL Hurley, and WE Braselton. Composition of milk from llamas in the United States. *J Dairy Sci* 1995;78:1713-1720.
- [20] H Zhang, J Yao, D Zhao, H Lui, J Li, and M Guo. Changes in chemical composition of Alxa bactrian camel milk during lactation. *J Dairy Sci* 2005;88:3402-3410.
- [21] EK Chad. Preliminary Investigation of the composition of alpaca (*Vicugna pacos*) milk in California. *Small Ruminant Research* 2014;In Press.
- [22] SJ LeBlanc, TH Herdt, WM Seymour, TF Duffield, and KE Leslie. Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. *J Dairy Sci* 2004;87:609-619.

AUTHORS' PROFILE

Andrea S. Lear Dr. Lear graduated with a Doctor of Veterinary Medicine degree from Auburn University, Auburn AL U.S.A, in 2011. She is currently performing a post-doctoral fellowship and candidacy for the American College of Veterinary Internal Medicine at Colorado State University, Fort Collins CO U.S.A. The author's major field of study includes topics in livestock medicine and nutrition.

Stacey R. Byers Dr. Stacey Byers received her DVM from Washington State University and completed her residency in large animal internal medicine at WSU in 2009 before joining the Livestock Medicine and Surgery service at Colorado State University. She works with a wide variety of livestock species, teaches veterinary students in both didactic and laboratory settings, and performs clinically oriented research.

Jessica A.A. McArt Dr. McArt is an Assistant Professor in the Department of Population Medicine and Diagnostic Sciences at Cornell University where she provides clinical service through the Ambulatory and Production Medicine Clinic. Her research focuses on the identification, epidemiology, and economics of periparturient diseases in dairy cattle.

Robert J. Callan Dr. Callan is a Professor at the Colorado State University College of Veterinary Medicine and Biological Sciences. He obtained his DVM from Oregon State University and has a PhD in virology from the University of Wisconsin, Madison. Dr. Callan provides clinical service and teaching in the Livestock Medicine and Surgery service at the James L. Voss Veterinary Teaching hospital. His research interests are in infectious and nutritional diseases and immunity in ruminants.