Recent Research in Llama Nutrition

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Vitamin D Related Problems in Llamas and Alpacas

Abnormal bone growth is a commonly diagnosed problem in young growing animals of all domestic species and is usually related to nutritional deficiencies. A rickets syndrome in juvenile llamas and alpacas characterized by a shifting leg lameness and enlargement of the joints, most noticeably the carpus, has been described. Affected crias have variably shown a slowed growth rate, reluctance to move, and kyphosis. Radiographic evidence of physeal ectasia and low serum phosphorus concentrations were consistent with a diagnosis of rickets. The Camelid Research Group at Oregon State University has been investigating the role of vitamin D in hypo-phosphatemic rickets since 1993. Our initial involvement in this activity was the result of an inquiry from a local practitioner who was not convinced that phosphorus supplementation was correcting the identified problem. Since our initial study we have completed 4 additional studies dealing with various aspects of etiology, treatment, and prevention of this syndrome. The following are summaries of the pertinent findings from these studies.

Study 1: Role of Vitamin D - In our first study we defined the role of vitamin D in the hypophosphatemic rickets syndrome. Twenty clinical and 10 age and sex matched non-clinical control llamas and alpacas from 9 farms were compared in Study 1 (1). While serum calcium (Ca) concentrations did not differ between groups, serum phosphorus (P) concentrations in the control group (9.0 ±0.9 mg/dl) were higher ($P<0.001$) than in the clinical group (3.4 ± 0.2 mg/dl). Serum 25-OH cholecalciferol concentrations in the control and clinical groups, 83.2 +/- 20.2 nmol/l and 7.4 +/- 1.9 nmol/l respectively, differed ($P<0.001$) supporting the hypothesis that insufficient vitamin D_3 (Vit D) production played an important role in the development of rickets in the llama and alpaca. Vitamin D concentrations and month of birth accounted for 80% of the variation in serum P concentrations.

Study 2: Seasonality Effects - As a result of the observed seasonal incidence of the problem (primarily November to March) it was hypothesized that insufficient sunlight during the winter months resulted in decreased *de novo* Vit D production. In study 2, serum Ca, P, and D_3 concentrations in 30 llamas and alpacas were measured at monthly intervals for 12 months (2). Analysis of samples collected from 13 of these llamas (5 juveniles, 4 teenagers, and 4 adults) for 12 months showed no seasonal change in serum Ca concentrations. In contrast, P and D_3 concentrations declined significantly during the winter. The magnitude of the decline was most pronounced in the youngest animals with mean P and D_3 concentrations decreasing from a peak of 9.6 mg/dl P and 183 nmol/l D_3 during September to a minimum of 4.9 mg/dl P and 15 nmol/l D_3 in February. The results support the hypothesis that vitamin D and serum phosphorus concentrations vary significantly as a function of season. These results have important implications for the management of Fall-born crias.

Study 3: Parenteral Supplementation- The objective of this project was to establish an appropriate level of injectable vitamin D supplementation to provide safe and therapeutically useful serum concentrations of vitamin D for the prevention of vitamin D deficiency disease in llamas. Within this objective, we also wanted to determine if there would be any age differences in response to vitamin D supplementation. Thirty llamas and alpacas were assigned to 1 of 5 treatment groups based on level of Vit D supplementation (0; 1,000; 2,000; 4,000 IM; and 4,000 Oral IU/kg). All animals were given a single injection or oral bolus. Each treatment group was further subdivided by age (juvenile, teenage, adult).

Intramuscular injection of a commercial vitamin D_3 preparation increases serum vitamin D concentration above placebo injected controls for a period of 90-120 days from treatment. Based on our assessment of these data, we would recommend that a dosage between 1,500 to 2,000 IU Vitamin D_3/kg body weight seems to maintain clinically appropriate serum concentrations to prevent deficiency disease. An interesting finding confirming other unpublished data shows a significant age effect on calcium and phosphorus concentrations in the llama. Vitamin D concentrations were not influenced by age. Relative changes in serum vitamin D concentrations following a single oral or intramuscular administration of vitamin D at a rate of 4,000 IU/kg found a lower than anticipated oral bioavailability of vitamin D following a single treatment.

Study 4: Vitamin D Toxicity - Given the usual adage that if a little is good, a lot is better; we initiated a study to assess the degree of toxicity vitamin D has in the llama and alpaca. Initially 12 llamas and alpacas were assigned to 1 of 4 treatment groups with varying levels of a single vitamin D intramuscular injection (0; 8,000; 16,000; 32,000 IU/kg). Serum Vit D concentrations showed a dose-dependent response; however, no clinical evidence of Vit D toxicity was appreciated. Following these results, a single animal was treated with 64,000 IU/kg and again no toxicity was observed on clinical or postmortem evaluation. A third trial using 9 llamas at 3 Vit D treatments (0; 2,000; 8,000 IU/kg) was initiated using an emulsified form of Vit D, as was used in the previous supplementation studies. Again dose-dependent Vit D responses were observed, but no clinical evidence of toxicity. Further data analyses are pending for this study, but preliminary impressions suggest that llamas and alpacas seem fairly resistant to Vit D toxicity.

Study 5: Oral Vitamin D Supplementation - The current study being completed is an attempt to determine a reasonable level of dietary supplementation of Vit D to maintain what would be considered appropriate serum concentrations of Vit D to prevent the rickets syndrome. A total of 32 llamas and alpacas were separated into 3 age groups (juvenile, teenager, adult) and assigned to 1 of 4 dietary treatment groups differing in amount of oral Vit D supplementation (0; 3.3; 6.6; 13.7 IU/kg body weight). After 6 weeks of the feeding trial it was apparent that these supplementation levels were not sufficient and the supplementation rates were increased 10x (0; 33; 66; 137 IU/kg) and fed for an additional 6 weeks. The original dosing scheme was set to 0.5x, 1x and 2x the NRC recommended dosages for sheep and calves. Increases in serum 25-OH cholecalciferol concentrations were noticed within 1 week of feeding the higher levels in all treatment groups. Preliminary data would suggest adequate serum Vit D concentrations may be maintained with daily supplementation at 33 IU/kg, but further data analyses are needed. Preliminary data from this study supports our earlier findings that bioavailability of oral Vit D seems to be low in llamas and alpacas.

1. Van Saun, RJ and Smith, BB. Evaluation of vitamin D status in llamas and alpacas with hypophosphatemic rickets. *JAVMA* 1996;209:1128-1133.
2. Smith, BB and RJ Van Saun. Hypophosphatemic rickets in the South American Camelids: Interaction of calcium, phosphorus, and vitamin D. pp. 79-94, *In:* Proceedings of 2nd European Symposium on South American Camelids, Gerken, M and C Renieri (eds), Camerino, Italy, August 30 - September 2, 1995.

Hepatic Lipidosis in Camelids

Hepatic lipidosis is a well-known syndrome in periparturient dairy cattle and sheep, anorexic cats, and a sporadic problem with fat horses and ponies. This pathologic process has also been recognized in most other species, including humans. There are limited reports of hepatic lipidosis in llamas and alpacas in the literature; however, producer groups and practicing veterinarians have an increased interest in the pathogenesis of this disease process. We have dealt with three different herd-based problems with hepatic lipidosis in which multiple animals have been lost in the past year. These cases have prompted projects attempting to further characterize and define the pathogenesis of this disease process.

A retrospective study of 31 histologically confirmed cases of hepatic lipidosis in llamas and alpacas submitted to Oregon State University Veterinary Diagnostic Laboratory revealed a predominately middle aged, pregnant or lactating female population to be affected. However 22.6% of the cases were male and age ranged from 5 months to 18 years. In these cases there was no significant association with any infectious, parasitic, or toxic causative agent. Biochemical measures associated with negative energy balance, liver dysfunction, and muscle damage were consistently elevated. Lipemia and ketonemia were not consistently associated with hepatic lipidosis in this retrospective study population compared to the two literature reports. These data suggest similarities in the pathogenesis of hepatic lipidosis in camelids to other species and not just ruminants. A common theme in most cases of hepatic lipidosis in camelids is a period of anorexia prior to clinical signs.

Although llamas and alpacas have a pregastric fermentation system similar to other ruminant animals, their metabolic processes are far less understood. Therefore direct extrapolation of information pertaining to hepatic lipidosis in dairy cattle may not be appropriate to camelids. As a consequence, we have initiated a study attempting to better characterize metabolic responses to feed restriction in llamas. It was our hope that we could generate a model of hepatic lipidosis. Preliminary findings suggest the llama is fully capable of metabolically adapting to feed restriction and we were not able to induce significant fatty infiltration of the liver. These data and our clinical impressions would suggest that hepatic lipidosis in llamas is primarily a secondary disease process in a debilitated or severely stressed animal.

Nutritional Diagnostics

Serum minerals assessment- Clinical chemistry measures to determine concentrations of various macro- and micromineral elements are used routinely in the diagnostic endeavors of all animals. Most of the reference values used are derived from adult animal populations. Based on

data generated in other studies, we have found age to be a significant factor in serum concentrations of calcium and phosphorus. A study was undertaken to determine serum minerals and vitamin E concentrations in clinically normal llamas grouped by age, sex, pregnancy status, and stage of gestation. A total of 270 samples were collected from 21 farms in Oregon with the animals ranging in age from < 1 month to > 15 years. Selected llamas did not have previous health problems and met specific health criteria on examination.

Mean values and reference ranges for most of the minerals and vitamins were similar to previously reported values. Male versus female differences were not identified for any measurements. Age was a significant variable for Ca, P, Fe, and Se concentrations, as well as Ca-to-P ratio and total iron binding capacity. Identified age-based effects were modeled by use of linear regression. Copper and Zn concentrations and % transferrin saturation did not differ as a function of age. Serum Vit E concentration was influenced by an age by sex interaction and stage of gestation. Clinical diagnosis of metabolic disease may be improved with use of age-based reference values, especially for neonates.

1. Smith, BB, RJ Van Saun, PJ Reed, AM Craig and A Youngberg. Normal blood mineral and vitamin E concentrations in the llama (*Lama glama*). *Am J Vet Res* 1998; August (in press).

Hepatic trace minerals - Trace minerals are indirectly or directly associated with a tremendous variety of metabolic processes. Deficiency diseases affect almost every physiologic function and include immune dysfunction (Cu, Zn, Se); developmental abnormalities (Cu, Mn, I); abortion (Cu, I, Se); retained placenta (Cu, Se, I); and metabolic disturbances (Co, Fe, Zn, I). A number of ultra-trace minerals (Cr, Si, As, Mo) have been identified as being required; however, good data as to specific requirements are lacking. Significant economic losses result from llama fetal and neonatal deaths or disease processes. Many problems are linked to decreased immune function that is influenced by trace mineral status. Diagnosis of abortion losses still remains below 45% and a significant portion of these "idiopathic" abortions have been hypothesized to be related to nutritional causes. Many trace mineral deficiencies can result in abortion, stillbirths or weak neonates.

During the early postnatal period, almost all essential nutrients are adequately provided for by milk consumption. However, a number of critical micronutrients, namely Cu, Fe, Zn and Se, are insufficiently provided by milk consumption alone, thus requiring additional sources to meet daily needs. These sources may include dry feed consumption or mobilization of tissue reserves, if available, or both. Recent research data has confirmed a fetal liver concentrating ability for these nutrients in finding fetal hepatic mineral concentrations to exceed maternal values. Fetal hepatic nutrient reserves could potentially play a critical role in maintaining adequate micromineral concentrations to support daily nutrient requirements in the milk-fed postnatal animal.

Scant data are available documenting fetal or neonatal liver mineral concentrations for camelids. One study alluded to a lack of fetal concentrating ability in alpacas based on a single sample, however, their data were presented on a wet weight basis (1). In this report the authors stated that fetal hepatic concentrating ability has not been documented for sheep or goats. In contrast, our

preliminary data suggests that sheep fetuses have a similar capability in concentrating minerals in the liver for postnatal use.

Incidence rate of the primary disease processes afflicting the postnatal cria, namely diarrhea and pneumonia, has been linked to functionality of the immune system. In the early postnatal animal this involves both passive and active immune function and integrity of epithelial barriers. A number of nutrients including vitamins A and E and the minerals Se, Cu, Zn, and Mn have been linked to proper immune system function. Immune system dysfunction has been implicated in facilitating the occurrence of postnatal disease. Much of the variability in severity and time of occurrence of postnatal disease may be explained through time frame for tissue mineral depletion and subsequent immune system dysfunction. There is a critical need for this database to improve our ability to better interpret current nutritional status and its association with disease incidence in the neonate.

At present few laboratories, if any, have any reference values for fetal or neonatal mineral concentrations for livestock species. Most labs assume that adult values are adequate for assessing mineral status of the fetus or neonate. Use of adult standards for tissue mineral concentration evaluations may be totally inappropriate for younger animals given differences in metabolic rates and tissue water content. Most of the mineral data reported from diagnostic laboratories are on a wet weight basis. Is this appropriate in evaluating fetal and neonatal data? Data from cattle has shown fetal liver dry matter content is significantly lower than mature cows (Van Saun, unpublished data 1994). Based on the data presented, comparison of fetal and adult liver mineral concentrations should be on a dry matter basis only. Given these data, the next question would relate to whether or not there is any difference in fetal liver dry matter across gestation. From these same data, fetal age ranged from 3.7 to 9.8 months based on measured crown-to-rump length. Liver dry matter content was linearly ($P < .0001$) related to fetal age with dry matter content increasing as gestation progressed. Fetal age accounted for 79% of the variation in fetal liver dry matter content. These data suggest that dry matter determinations need to be done on each individual sample. Use of a single mean fetal dry matter value, although better than using wet weight basis, would be inappropriate in determining hepatic mineral concentrations on a dry matter basis. A similar data set needs to be generated for llamas to help facilitate our diagnostic efforts and supplementation recommendations for trace minerals.

1. McMillan, E. and D. Paynter. Trace element studies in Australian alpacas, pp. 1-3 In: Camelids Proceedings 257, July 1995, Deakin University, Geelong, Victoria, Aust.