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Serum Calcium-Phosphorus Product as a Diagnostic Method for Vitamin D Deficiency and Toxicity in Camelids

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ABSTRACT

Camelids have been known to be especially susceptible to vitamin D-responsive rickets due to their evolutionary adaptation to downregulate *in vivo* vitamin D production. The objective of this study was to provide parameters for the use of calcium-phosphorus product as an alternative to the more expensive traditional vitamin D testing for diagnosis of vitamin D deficiency and toxicity in camelids. This retrospective study used published and unpublished data to produce Pearson's correlations, general linear models, and contingency tables to determine relationships between serum calcium, serum phosphorus, calcium-phosphorus product, and vitamin D as well as other factors. It was found that vitamin D controls phosphorus concentration more so than calcium, that there is a difference between age groups in vitamin D physiology, and that calcium-phosphorus product is a valid measure of vitamin D. It was determined that the calcium-phosphorus product threshold for diagnosis of vitamin D deficiency in juvenile camelids is 50 mg^2/dL^2 while a previous study found a calcium-phosphorus product threshold of 60 mg^2/dL^2 for vitamin D toxicity in adult camelids. This calcium-phosphorus product method of vitamin D testing has great implications on maintaining healthy production camelids.

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Chapter 1

Literature Review

Rickets is a disease affecting young animals, including children, that is the result of failure to mineralize growing bones, often leading to curvature of the long bones in the body and other bone abnormalities. The disease is caused by deficiencies, imbalances, or abnormal metabolism of vitamin D, calcium, or phosphorus (Dittmer & Thompson, 2010). Camelids (used only to refer to llamas and alpacas for purposes of this text) are especially susceptible to vitamin D-responsive rickets due to their reduced ability to synthesize vitamin D and their seasonal variations in vitamin D levels (Van Saun, 2006; Van Saun, 2009b). It is important to understand the role of vitamin D in the body, vitamin D-responsive rickets, and the implications of vitamin D-responsive rickets on production camelids in order to ensure the health and efficiency of these animals. The objective of the present study is to determine diagnostic ranges of the calcium-phosphorus product (CPP) that indicate vitamin D deficiency and toxicity in different age groups of camelids.

Role of Vitamin D in the Body

Vitamin D, also known as calciferol or colloquially as the "sunshine vitamin," is a fat-soluble, steroid hormone that comes in two forms: vitamin D_2 and vitamin D_3 (Bowen, 2019; Dittmer & Thompson, 2010; National Institutes of Health, 2020). Most animals, except for dogs and cats, can synthesize vitamin D in their skin (Zafalon et al., 2019). Animals can also obtain

vitamin D in their diet. Most can metabolize either form of vitamin D, except chickens which can only metabolize vitamin D_3 (Soares, 1995). Understanding the role of vitamin D in the body comes from an understanding of its biological pathway and functions.

Biological Pathway

Vitamin D metabolism in most animals begins in the skin or with the ingestion of vitamin D. When the skin of an animal is exposed to ultraviolet radiation (290-315 nm in wavelength), 7-dehydrocholesterol is converted to previtamin D_3 (Holick, 2007). Upon exposure to heat in the body, previtamin D_3 is isomerized to vitamin D_3 (cholecalciferol) (Holick, 2007; Lips, 2006). Vitamin D_2 (ergocalciferol) is formed in plants and can then be ingested as can vitamin D_3 from animal sources (Lips, 2006). Once formed, vitamin D_3 binds vitamin D-binding protein and is transported to the liver for further conversion or stored in fat or the blood. In the liver, vitamin D_3 is converted to 25-hydroxyvitamin D_3 ($25(OH)D_3$) which is its main circulating and storage form (Dittmer & Thompson, 2010). Next, the $25(OH)D_3$ is transported to the kidneys where it is converted by the enzyme 25-hydroxyvitamin D-1αhydroxylase to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$ or 1,25-dihydroxycholecalciferol), which is vitamin D's active form, but is not commonly circulating. $25(OH)D_3$, and $1,25(OH)_2D_3$ act as negative feedback molecules of vitamin D activation while parathyroid hormone acts as a positive feedback molecule due to its role in increasing serum calcium concentrations (Holick, 2007). $1,25(OH)_2D_3$ binds to vitamin D receptors which are type II nuclear receptors. These vitamin D receptors must heterodimerize with retinol X receptors in order to function. Once $1,25(OH)_2D_3$ is bound

to the vitamin D receptor, the vitamin-receptor complex binds to vitamin D-responsive elements in the DNA, giving vitamin D a mechanism to control gene expression (Dittmer & Thompson, 2010).

Functions

Vitamin D biologic activity targets calcium and phosphorus metabolism in the intestines, bones, and kidneys, and is associated with the function of the parathyroid gland.

Intestine

Vitamin D action in the intestine is to promote cellular uptake of calcium via transient receptor potential vanilloid 6 (TRPV6) (Dittmer & Thompson, 2010). Once in the cell, calcium is transported across the cell by calbindin D. Vitamin D upregulates expression of TRPV6 and calbindin D, which leads to an increase in calcium absorption. Vitamin D also upregulates the expression of Na-P_i receptors to increase phosphorus uptake.

Bones

In bones, vitamin D promotes calcium release to maintain blood calcium concentrations through interactions that lead to increased osteoclast concentration and activity. Vitamin D upregulates expression of the receptor activator for NF-

κB ligand (RANKL) on osteoblasts. When RANK receptors on preosteoclasts bind to these RANKL receptors, they are converted to osteoclasts which produce hydrochloric acid to release calcium from the bone (Laird, 2010). Vitamin D also causes chondrocytes to differentiate into osteoblasts. This differentiation in conjunction with raising calcium and phosphorus levels in local bone caused by vitamin D leads to mineralization of bone growth plates leading to bone growth (Gil et al., 2018). Vitamin D may facilitate the invasion of blood vessels carrying osteoblasts and osteoclasts into growing bone which further promotes bone growth (Masuyama et al., 2006).

Kidneys

In the kidneys, vitamin D acts as a negative feedback signal of its own production and acts in the transport of calcium and phosphorus into the distal convoluted tubules via mechanisms similar to those in the intestine (Dittmer & Thompson, 2010; Johnson & Kumar, 1994).

Parathyroid Gland

The parathyroid gland, which secretes parathyroid hormone (PTH), and vitamin D have a tight biological relationship. PTH promotes the conversion of vitamin D to its active form in the kidney and, much like vitamin D, enhances expression of RANKL. PTH and vitamin D both increase calcium absorption in

the kidney and calcium secretion from bone. However, when considering phosphorus, vitamin D increases absorption in the kidney while PTH promotes secretion (Lofrese et al., 2020). Vitamin D functions to decrease PTH secretion in a negative feedback loop by decreasing growth factors of parathyroid cells, increasing inhibitors of parathyroid cells, and increasing calcium-sensing receptors on the parathyroid gland. Due to this relationship, hypocalcemia induced by vitamin D deficiency leads to hyperparathyroidism (Dittmer & Thompson, 2010).

Vitamin D-Responsive Rickets

As previously described, rickets is a disease characterized by failure of mineralization of bones, especially of the growth plates, and can be caused by deficiency, imbalances, or abnormal metabolism of either vitamin D, calcium, or phosphorus (Dittmer & Thompson, 2010). Abnormalities in metabolism that cause rickets can be genetically based with some genetic disorders affecting vitamin D biosynthesis or renal tubular phosphate reabsorption (Acar et al., 2017). Imbalances include deviation from the recommended 2:1 ratio of calcium to phosphorus in the animal's diet. Nutrient deficiencies, either absolute or induced, are the main cause of rickets (Loughrill et al., 2017). Causes of deficiencies include, but are not limited to, underfeeding of calcium, lack of exposure to sunlight (leading to lack of production of vitamin D), or strontium inhibition of $1,25(OH)_2D_3$ synthesis in the body (Dittmer & Thompson, 2010; Omdahl & DeLuca, 1971). Rickets is seen only in growing bones and therefore only affects young, growing animals, including children. Signs of rickets include long bone curvature,

lethargy, depressed appetite, lameness, hunched back, decreased growth rate, swollen joints, loss of condition, increased recumbency, and poor weight gain (Dittmer & Thompson, 2010; Whitehead, 2013; Whitehead & Cebra, 2014). Vitamin D deficiency is one of the more common causes of rickets (Dittmer & Thompson, 2010). Important information in understanding vitamin D-responsive rickets include its etiology and pathogenesis, diagnostic methods, and therapeutic approaches and prevention methods.

Etiology and Pathogenesis

In camelids and most other species, vitamin D-responsive rickets is not caused directly by vitamin D, but rather inadequate absorption of calcium followed by malabsorption of phosphates due to low levels of vitamin D. In other words, vitamin D-responsive rickets is a subset of hypophosphatemic rickets, as low levels of phosphorus are the central cause of the disease (Van Saun, 2014; Van Saun & Cebra, 2014). The pathogenesis of vitamin D-responsive rickets begins with a deficiency in vitamin D, which leads to poor calcium absorption. Poor absorption of calcium leads to low levels of serum-ionized calcium in the blood which is recognized by calcium receptors on the parathyroid gland, causing the gland to increase levels of parathyroid hormone (Holick, 2006). PTH functions to increase blood calcium levels through reabsorption in the distal convoluted tubule and collecting ducts in the kidney. PTH also decreases the reabsorption of phosphates by the kidney leading to phosphaturia (Holick, 2006; Khan et al., 2020). Enhanced expression of RANKL on osteoblasts caused by PTH increases calcium mobilization from the skeleton to keep blood calcium levels normal. The osteoblasts

continue their normal function of producing osteoid, used to form bone, but due to inadequate levels of phosphorus, bone cannot be mineralized, resulting in rickets. This lack of mineralization leads to weakening of fast-growing long bones which often curve under the weight of the body (Holick, 2006). Several risk factors make certain species or individuals more susceptible to developing rickets through this mechanism.

Risk Factors

Host and environmental factors put certain animals at risk of developing vitamin D-responsive rickets. Compared to animals with lighter pigmentation and a thinner coat, animals with darker pigmentation, thicker coats, or both are less able to synthesize vitamin D in their skin due to the pigment's and coat's ability to absorb ultraviolet radiation, making it unavailable for vitamin D synthesis (Dittmer & Thompson, 2010; Norman, 1998). Altitude, latitude, and season also put animals at risk. Lower altitudes, higher latitudes, and less sunlight during winter months each lead to less ultraviolet radiation being available for vitamin D synthesis (Dittmer & Thompson, 2010).

When considering camelids specifically, alongside pigmentation and fleece thickness effects, their unique physiology puts these species at increased risk of vitamin D-responsive rickets. Camelids originate from the high altitude, low latitude regions of South America and therefore were exposed to high levels of ultraviolet radiation. It is hypothesized that they evolved to downregulate vitamin D synthesis in their bodies to adapt to these high-radiation conditions.

When these animals are taken out of their native habitat into regions with lower levels of ultraviolet radiation (low altitude, high latitude), they may be unable to synthesize vitamin D in adequate amounts (Van Saun, 2014). Camelids, as well as other animals, also show seasonal variation in vitamin D synthesis with lower vitamin D production in the fall and winter months. This variation, in conjunction with lowered ability to produce vitamin D in both crias and dams, puts crias born in the colder months at increased risk of vitamin D deficiency. Crias born in the winter months have been shown to have lower vitamin D levels than crias born in warmer months. This difference may be attributed to low placental transfer of vitamin D and receiving colostrum and milk with inadequate vitamin D levels (Smith & Van Saun, 2001).

Diagnostic Methods

Diagnosis of vitamin D-responsive rickets can be obtained using a combination of signalment, physical examination, radiologic and post-mortem examination, blood testing, and histological examination.

Physical Examination

Physical examination, after obtaining a signalment and suspecting rickets, often shows physical signs including the presence of angular bone deformities such as curvature of the long bones, thoracic lordosis, and joint swelling, all

caused by the weakening of bones characteristic of rickets, as well as a "rosary bead" feel to the rib cage caused by enlarged costochondral junctions (Dittmer & Thompson, 2010; Whitehead and Cebra, 2014). However, physical examination is limited in its diagnostic capability, offering a positive predictive value of only 60.9% in human children (Ozkan, 2010).

Radiography and Post-mortem Examination

Radiographic imaging and post-mortem examination will show similar bone deformities and are the most definitive methods of diagnosing rickets (Van Saun & Cebra, 2014). These deformities include widening of the physeal growth plate of fast-growing bones, flaring and cupping of the metaphysis, thinning of the cortical bone layer, decreased radiographic density due to poor bone mineralization, enlargement of costochondral junctions, and pathologic fractures (Ewband et al., 2013; Stieger-Vanegas et al., 2013). Findings that may only be found upon post-mortem examination include thickening of physeal cartilage, erosion of articular cartilage, and spontaneous fractures (Dittmer & Thompson, 2010).

Blood Testing

Blood tests indicating low levels of vitamin D (specifically $25(OH)D_3$; the circulating form) and hypophosphatemia (in camelids: <50 nmol/L and <0.65

mmol/L, respectively) may indicate rickets, but this method by itself has limitations as rickets is not the only disease with these results (Stieger-Vanegas et al., 2013). For example, hypophosphatemia can be seen with advanced renal disease and hypovitaminosis D has been associated with heart and autoimmune diseases (Brunelli & Goldfarb, 2007; Caccamo et al., 2018). Hypocalcaemia may or may not be present due to tight regulation of serum calcium (Stieger-Vanegas et al., 2013).

Histological Examination

Changes of the distal radial growth plate seen on histological examination may also be used to diagnose rickets. Fibrosis of the metaphysis, clustering of chondrocytes, unmineralized osteoid, and elongated vessels in the epiphyseal plate may indicate rickets (Long et al., 1984; Anonymous, 2014).

Therapeutic Approaches and Prevention Methods

Both therapeutic approaches and prevention methods for vitamin D-responsive rickets focus on supplementation to improve nutritional status as well as other methods. Ensuring adequate levels of vitamin D, either through oral or intramuscular injection supplementation or through feed, can keep vitamin D at adequate levels to prevent vitamin D-responsive rickets or can return animals to adequate levels to stop and possibly reverse the disease process (Van Saun, 2009a). Bringing vitamin D levels to an adequate

state can allow for remineralization of the bone (Huh & Gordon, 2008). Phosphate supplementation along with adequate vitamin D may also be beneficial to ensure adequate CPP for bone mineralization (Stieger-Vanegas et al., 2013). Because vitamin D synthesis is dependent on exposure to ultraviolet radiation, ensuring adequate sun exposure may also help in treatment and prevention (Dittmer & Thompson, 2010). Corrective surgery of limb deformities caused by rickets may be implemented in humans and other animals such as crias (Gizard et al., 2017; Whitehead & Cebra, 2014).

For camelids, specific supplementation schemes and surgical methods have been found for the prevention and treatment of vitamin D-responsive rickets. Intramuscular supplementation of 1500-2000 IU/kg body weight every three months, oral gel supplementation of 33,000 IU every two weeks or 100,000 IU monthly, or dietary intake of 30-40 IU/kg body weight daily supplies adequate vitamin D to camelids (Van Saun, 2009a). Supplementation of late-pregnant dams within a month of birthing to improve vitamin D levels in colostrum or supplementation of the cria shortly after birth can be used to improve vitamin D status and prevent vitamin D-responsive rickets (Van Saun, 2013).

Combined with vitamin D supplementation, surgical correction in crias experiencing limb deformities from rickets, specifically carpal valgus, may be used for treatment (Whitehead & Cebra, 2014). The type of surgery varies by age and severity of deformities. Surgical procedures include periosteal stripping possibly combined with partial ulnar osteotomy, transphyseal bridging of the radial growth plates in older crias or crias with carpus valgus angled at >10 degrees, or corrective osteotomy in older crias

whose radial growth plates have closed (older than 9 months of age). Despite these treatments, older crias experiencing stunted growth due to rickets may remain stunted.

Chapter 2

Introduction

Vitamin D imbalance (either deficiencies or toxicities) in camelids is becoming more of interest due to camelids' increasing popularity for use in production. Camelids' evolutionary adaption to downregulate vitamin D production, their increasing popularity in low-UV regions, and the risk of rickets development and associated production losses in deficient animals have brought vitamin D deficiencies to the forefront of camelid nutritional care (Van Saun, 2014). Although less likely to occur than vitamin D deficiency, vitamin D intoxication can occur with excessive supplementation and can lead to production losses. Therefore, it must be taken into consideration when assessing camelid health (Van Saun & Cebra, 2014).

Diagnoses of vitamin D deficiencies and toxicities are relatively straightforward when using a combination of blood testing, visual examination, histology, and/or radiographic and ultrasound imaging. Vitamin D deficiencies are diagnosed as serum $25(OH)D_3$ concentrations <50 nmol/L, possibly in combination with serum phosphorus levels <0.65 mmol/L. Visual and radiographic observation may show angular limb deformities, thinning of cortical tissue in long bones, and pathological fractures, but these are not diagnostic on their own because they may be the result of other diseases. Rickets may also be seen in growing animals and is diagnostic of deficiencies in either vitamin D, calcium, or phosphorus (Van Saun & Cebra, 2014). Vitamin D toxicity presents as serum $25(OH)D_3$ concentrations >600 nmol/L and hepatic and kidney levels >85 nmol/L, possibly in combination with calcium levels >2.6 mmol/L (hypercalcemia), phosphorus >2.8 (hyperphosphatemia), blood urea nitrogen >12.8 mmol/L (azotemia), and/or creatinine levels >248 μmol/L (Van Saun & Cebra, 2014; Gerspach et al., 2010; Chamorro et al.,

2013). Histology, ultrasound imaging, and radiography may show soft tissue mineralization due to the elevated circulated calcium associated with vitamin D toxicity (Van Saun & Cebra, 2014).

Although serum vitamin D testing makes diagnosis of vitamin D deficiencies and toxicities rather simple, cost, which is approximately $65 per animal, makes widespread testing impractical. Testing of serum calcium and phosphorus, molecules closely associated with vitamin D, costs only $19 each and therefore are much more cost-effective testing methods (Anonymous, 2019). Testing for serum calcium and phosphorus and calculating the CPP may offer an alternative way to diagnose vitamin D toxicity and deficiency. In human pediatric patients with renal disease, CPP and vitamin D therapy were both associated with soft tissue calcification, a sign also associated with vitamin D toxicity (Milliner et al., 1990). Data in camelids also correlated increased CPP levels with vitamin D levels for use as a diagnostic tool in vitamin D toxicity cases (Van Saun, unpublished). These data offer evidence of the association between vitamin D and CPP and corroborates CPP's potential use in diagnosing vitamin D deficiency and toxicity.

The intent of the present study is to determine diagnostic ranges of serum CPP that indicate vitamin D toxicity and deficiency in different age groups of camelids. This study will focus on the use of CPP to diagnose vitamin D deficiency, but toxicity will also briefly be addressed. An analysis of variants was used in this retrospective study on data presented in both published and unpublished works investigating vitamin D levels and metabolism in camelids. By defining CPP levels indicative of vitamin D toxicity and deficiency, diagnosis of vitamin D imbalance using CPP will be more cost effective which may lead to more frequent diagnosis and treatment and better prevention of vitamin D-associated diseases, leading to less economic loss for camelid producers.

Chapter 3

Materials and Methods

In this retrospective study, data from various published and unpublished studies and a herd toxicity case were used for analysis. Statistical analysis was done using SAS®□ software.

Description of Studies

Several studies and a herd toxicity case were used to collect data for this study and a brief description of each is provided below.

Seasonal Changes in Serum Calcium, Phosphorus, and Vitamin D Concentrations in Llamas and Alpacas (Study 1)

In this study, Smith & Van Saun (2001) were researching how seasonal variation affected calcium, phosphorus, and vitamin D in different age groups (neonate, yearling, and adult; as described in the **Sample Collection** section) of camelids. Another focus of the study was the susceptibility of crias born during different seasons (Fall/Winter versus Spring/Summer) to hypophosphatemic rickets and effect on coat color on concentrations described. This study found that, for each age group, vitamin D and phosphorus concentration were lowest during winter months and highest during warmer months. All animals showed variation in these concentrations according to month, but neonates showed the most fluctuation. Darker fleeced animals (black and dark brown) had lower

vitamin D concentrations compared to lighter fleeced animals (white). In terms of risk, Fall/Winter crias showed lower concentrations of vitamin D than Spring/Summer crias until each group reached approximately 9 months of life. Age and month of birth accounted for 56% of the variation in vitamin D in these animals. This means that Fall/Winter crias are more susceptible to hypophosphatemic rickets as they do not have the opportunity to store vitamin D during the winter while they are experiencing their rapid growth phase.

Oral Toxicity in Alpaca Herd (Studies 2 & 5)

In this case, an alpaca herd was experiencing lethargy, weight loss, decreased growth, and a high death rate. It was found that the herd's mixed grain supplement, which was formulated for vitamin D levels of 13,000 IU/lb feed, actually contained vitamin D levels of 191,000 IU/lb, meaning each animal received 47,750-95,000 IU vitamin D/day for up to 10 months. Pathology findings showed serum vitamin D levels >400-800 nmol/L (reference range 50-200 nmol/L), mild to moderate elevation in serum phosphorus, no hypercalcemia, normal renal and liver function tests, soft tissue mineralization in 48.5% of euthanized or dead animals, significant renal mineralization in C-T scans of crias, and growth rates for crias in the 5th percentile (Van Saun, unpublished; Gerspach et al., 2010). Soft tissue mineralization was significantly associated (P =0.03) with exposure time and amount of pellet consumed. In the present study, two sets of data were obtained from this case: serum concentrations collected for the feed

company involved (study 2) and serum concentrations collected by the herd veterinarian (study 5).

Toxicity Study (Study 3)

This study sought to determine the risk of vitamin D toxicity in camelids (Van Saun & Smith, unpublished). Vitamin D was administered in a single dose of 8,000 IU/kg body weight, 16,000 IU/kg, 32,000 IU/kg, and 64,000 IU/kg. Control animals were not administered any vitamin D. Serum vitamin D, calcium, and phosphorus were measured. It was found that serum vitamin D concentrations increased with increasing dosage and phosphorus levels spiked shortly after administration before reaching similar concentrations for each dosage. Changes were seen in calcium concentration as well. These increases in calcium and phosphorus could potentially have adverse effects. Clinical toxicity was not seen in most animals (except for the 64,000 IU/kg animal which showed soft tissue mineralization), indicating that clinical toxicity is unlikely from a single dosage in amounts usually used by producers. However, this does not rule out the possibility of clinical toxicity with extremely high dosages or multiple supplementations over a short period of time.

Feeding Trial Study (Study 4)

To determine the appropriate daily supplementation rate of vitamin D, camelids were fed supplemented camelid pellets with varying vitamin D concentrations (Van Saun & Smith, unpublished). Feed supplementation of vitamin D was administered at 0 IU/kg body weight (control), 3.3 IU/kg, 6.6 IU/kg (NRC recommendation for other species), and 13.2 IU/kg. At these dosages, serum vitamin D remained fairly similar for all groups, so supplementation doses were increased by a factor of 10 at week 7 of the trial. Calcium levels remained fairly consistent and similar between groups while phosphorus levels increased throughout the trial. It was concluded that 30-40 IU/kg body weight is the daily recommended dosage.

Injection Study (Study 6)

This study was conducted to determine the appropriate dosage of injectable vitamin D for supplementation (Van Saun & Smith, unpublished). Each animal was given an injection of 1,000 IU/kg body weight, 2,000 IU/kg, or 4,000 IU/kg while one group was given an oral dose of 4000 IU/kg and another group (control) received no vitamin D. Injection led to a spike in phosphorus and little change in calcium compared to control, suggesting vitamin D controls phosphorus more tightly than calcium. It was found that up to 90 days post-injection, vitamin D levels were significantly elevated and it was suggested that proper injection dosage is 1,500-2,000 IU/kg body weight. Oral supplementation at 4,000 IU/kg

showed a lower increase in vitamin D levels when compared to injection at the same dosage, indicating poor absorption and utilization of oral vitamin D.

Data Collection Method

The procedures for sample collection and serum analysis are described in Smith & Van Saun (2001) and Van Saun et al. (1996) and are summarized below.

Sample Collection

Llama and alpaca subjects were split into groups based on age at the time the study began (Smith & Van Saun, 2001). These groups were adults (age ≥ 24 months), yearlings (also called teens; age between 12 and 20 months), and neonates (also called juveniles; age <6 months). Neonates were further divided based on month of birth into a low risk group (March through August) and a high risk group (September through February), with risk defining the crias' risk of developing rickets. For 12 consecutive months, blood was drawn from the jugular vein of each animal and the serum was collected and stored at -70°C.

Serum Analysis

Serum was sent to commercial laboratories with the particular laboratory used varying by study location (Van Saun et al., 1996). To determine serum calcium concentration, one portion of each sample was reacted with o-

cresolphthalein in alkaline solution. An analyzer set to 550 nm used absorbance to determined calcium concentration of the sample. For serum inorganic phosphorus analysis, ammonium molybdate in acidic solution was reacted with a portion of serum. The same analyzer used to determine calcium concentration was set to 360 nm and used absorbance to determined serum phosphorus concentration. Serum $25(OH)D_3$ levels were used to determine vitamin D concentration via a validated commercially available radioimmunoassay. The sample was exposed to vitamin D binding protein and competed with a defined amount of hydrogen labeled vitamin D for binding sites, and a means comparison to a standard curve was used to determine sample vitamin D concentration.

Statistical Analysis

Data on individual test subjects and serum mineral and vitamin D levels were added to a spreadsheet. Data for each individual consisted of individual ID number, the study the individual participated in (study 1, 2, 3, 4, 5, or 6 as previously described), species (llama or alpaca), age in months, month of birth, age group (adult, yearling, or juvenile as previously described), study site, sex, year of data collection, month of data collection, serum calcium concentration, serum phosphorus concentration, and serum vitamin D concentration. Serum CPP was calculated for each individual and added to the data set. Data analysis was conducted using SAS®□ software using GLM, FREQ, and MEANS procedures. Means for continuous variables were calculated and a Pearson's correlation was obtained. An ANOVA using repeated measures was conducted to

determine how variables influenced either vitamin D concentrations or CPP. Contingency tables were produced for several thresholds of CPP and selectivity, specificity, positive predictive value, and negative predictive value were calculated.

Chapter 4

Results

Results for the present study include demographic data, factors influencing vitamin D and CPP, and contingency table calculations.

Demographic Data

A total of 1,188 observations were collected for this study; 1,109 of these observations were used for data analysis. Observations that were not used were those that did not have complete data. Of the 1,188 observations, 363 were contributed by juvenile animals, 263 by yearling animals, and 562 by adult animals. Minimum, maximum, mean, median, middle quartile, standard deviation, and standard error values for serum calcium concentration, serum phosphorus concentration, serum vitamin D concentration, and CPP for each age group can be found in Table 1.

Study 1 contributed 353 observations with 201 (56.94%) observations from juveniles, 60 (17.00%) from yearlings, and 92 (26.06%) from adults. In study 2, there were 145 observations with 30 (20.69%) being obtained from juveniles, 41 (28.28%) obtained from yearlings, and 74 (51.03%) obtained from adults. Study 3 contained 159 adult observations and study 4 contained 31 adult observations. Yearlings contributed 4 (21.05%) observations and adults contributed 15 (78.95%) observations of the 19 observations in study 5. In study 6, 481 observations were obtained. 132 (27.44%) of these observations were from juveniles, 158 (32.82%) from yearlings, and 191 (39.71%) from adults. Demographic data by study are summarized in Table 2.

Table 1. Demographic Data for Each Age Group: Pctl= percentile, Group 0= age group 0 (juveniles), Group 1= age group 1 (yearlings), Group 2= age group 2 (adults), Ca= Serum calcium concentration in units of mg/dL, P= Serum phosphorus concentration in units of mg/dL, vitD= Serum vitamin D concentration in units of nmol/L, CaP= Calcium-phosphorus product in units of mg^2/dL^2

Table 1. Demographic Data for Each Age Group

group	N Obs	Variable	N	Minimum	Maximum	Mean	Median	25th Pctl	75th Pctl	Std Dev	Std Error
0	363										
		Ca	360	7.70	12.00	9.80	9.90	9.30	10.30	0.78	0.04
		P	360	1.70	14.40	7.69	7.75	6.25	9.10	2.11	0.11
		vitD	344	1.00	684.00	121.04	64.50	23.00	159.50	141.06	7.61
		CaP	360	14.79	148.32	75.73	77.22	59.45	91.84	22.51	1.19
1	263										
		Ca	260	7.40	11.10	9.51	9.50	9.15	10.00	0.70	0.04
		P	260	1.40	11.80	6.53	6.70	5.50	7.80	1.79	0.11
		vitD	255	2.00	1340.00	167.92	124.00	43.00	222.00	175.28	10.98
		CaP	260	12.60	105.84	62.20	63.48	51.30	74.47	17.95	1.11
2	562										
		Ca	546	4.80	10.70	8.67	8.70	8.30	9.10	0.72	0.03
		P	546	0.70	16.10	6.05	5.90	4.90	7.10	1.94	0.08
		vitD	510	4.00	3848.00	403.47	188.50	97.00	439.00	585.86	25.94
		CaP	546	6.86	160.59	52.44	51.33	41.76	60.35	17.90	0.77

Table 2. Demographic Data for Each Study: Frequency= number of observations in age group, Percent= percent of total observations, Row Pct= percent of observations in a study, Col Pct= percent of observations in age group, Group 0= age group 0 (juveniles), Group 1= age group 1 (yearlings), Group 2= age group 2 (adults)

Table 2. Demographic Data for Each Study				
study	**group**			
	0	**1**	**2**	**Total**
1				
Frequency	201	60	92	353
Percent	16.92	5.05	7.74	29.71
Row Pct	56.94	17.00	26.06	
Col Pct	55.37	22.81	16.37	
2				
	30	41	74	145
	2.53	3.45	6.23	12.21
	20.69	28.28	51.03	
	8.26	15.59	13.17	
3				
	0	0	159	159
	0.00	0.00	13.38	13.38
	0.00	0.00	100.00	
	0.00	0.00	28.29	
4				
	0	0	31	31
	0.00	0.00	2.61	2.61
	0.00	0.00	100.00	
	0.00	0.00	5.52	
5				
	0	4	15	19
	0.00	0.34	1.26	1.60
	0.00	21.05	78.95	
	0.00	1.52	2.67	
6				
	132	158	191	481
	11.11	13.30	16.08	40.49
	27.44	32.85	39.71	
	36.36	60.08	33.99	
Total				
	363	263	562	1188
	30.56	22.14	47.31	100.00

Factors Influencing Vitamin D and CPP

Pearson's correlation data for the juvenile age group showed that serum vitamin D concentration was correlated with serum phosphorus concentration (r^2 = .236; *P* <.0001) and CPP (r^2 = .235; *P* <.0001), but not serum calcium concentration (r^2 = .088; *P* = .102). In these animals, CPP was correlated with serum calcium concentration (r^2 = .456; *P* <.0001) and serum phosphorus (r^2 = .966; *P* <.0001). In yearling animals, serum phosphorus concentration (r^2 = .243; *P* <.0001), serum calcium concentration (r^2 = .142; *P* = .023), and CPP (r^2 = .267; *P* <.0001) were correlated with serum vitamin D concentration. CPP was correlated with serum calcium (r^2 = .327; *P* <.0001) and serum phosphorus (r^2 = .956; *P* <.0001) in yearlings. Serum vitamin D concentration was correlated with serum phosphorus concentration (r^2 = .302; *P* <.0001), serum calcium concentration (r^2 = .137; *P* =.002), and CPP (r^2 = .334; *P* <.0001) in adult animals. Calcium concentration (r^2 = .237; *P* <.0001) and phosphorus concentration (r^2 = .966; *P* <.0001) were correlated with CPP in adults.

In modeling factors that influence serum vitamin D from all age groups in studies 1, 4, and 6 (those in which animals were not exposed to toxic levels of vitamin D) it was found that vitamin D was influenced by CPP (*P* <.0001), age group (*P* = .003), a CPP and age group interaction (*P* = .004), sex (*P* <.0001), an age group and sex interaction (*P* <.0001), and study (*P* <.0001). This model was highly significant (*P* <.0001) and explained 26.9% of data variation. Least squares means evaluation of serum vitamin D concentrations showed that juveniles (302.6 ± 22.4 nmol/L) had a lower vitamin D concentration that yearlings (388.1 ± 24.3 nmol/L; *P* <.0001) and adults (348.0 ± 25.4

nmol/L; P = .025). Yearlings' serum vitamin D concentrations tended to be higher than adult vitamin D concentrations (P =.073).

When considering influences on CPP from all age groups in studies 1, 4, and 6, CPP was influenced by age group (P <.0001) and a vitamin D and age group interaction (P = .028), but not vitamin D (P = .203). The model used explained 38.1% of data variability and was significant (P <.0001). CPP in juveniles (79.1 ± 15.0 mg^2/dL^2) was higher than yearlings (63.9 ± 15.0 mg^2/dL^2; P <.0001) and adults (47.3 ± 15.0 mg^2/dL^2) based on least squares mean evaluation. In yearlings, CPP was higher than in adults (P <.0001).

Models for the influence of factors on vitamin D using data from studies 1, 2, and 6 (studies that contained juvenile data) and only considering the juvenile age group were significant (P <.0001) and explained 18.8% of data variation. In these data, vitamin D was only influenced by CPP (P = .001). Influences on CPP using the same study and age group criteria were determined by a significant model (P <.0001) explained 20.6% of data variation. CPP was influenced by vitamin D (P = .0007), study (P <.0001), and a vitamin D and study interaction (P = .060).

In modeling factors that influence serum vitamin D from juveniles in studies 1, 2, and 6 it was found that vitamin D was influenced by CPP below 50 mg^2/dL^2 (P = .039), but not exposure to high levels of vitamin D (P = .312). This model was significant (P = .005) and accounted for 3.8% of the variation in the data. In juveniles not exposed to high vitamin D and that had CPP below 50 mg^2/dL^2, the least squares mean value of serum vitamin D concentration (56.9 ± 26.8 nmol/L) was lower than that of juveniles not exposed to high vitamin D and that did not have a CPP below 50 mg^2/dL^2 (132.6 ± 8.2

nmol/L; P = .007). Juveniles not exposed to high vitamin D and did not have a CPP below 50 mg^2/dL^2 had higher levels of serum vitamin D than that of animals exposed to high vitamin D and had a CPP below 50mg^2/dL^2 (43.1 ± 35.9 nmol/L; P = .016).

In a model considering yearling animals in studies 1, 2, 5, and 6, a significant (P = .002) model was generated that described 5.9% of data variation. In these animals, serum vitamin D was influenced by CPP below 50 mg^2/dL^2 (P = .013). When considering least squares mean values, yearlings not exposed to high vitamin D with CPP below 50 mg^2/dL^2 had vitamin D levels (78.3 ± 25.1 nmol/L) lower than animals not exposed to high vitamin D without CPP below 50 mg^2/dL^2 (184.1 ± 13.2 nmol/L; P = .0002) and animals that were exposed to high vitamin D and did not have CPP below 50 mg^2/dL^2 (199.9 ± 28.8 nmol/L; P = .002).

For adults in all studies, serum vitamin D was influenced by exposure to high vitamin D (P <.0001) and tended to be influenced by CPP below 50 mg^2/dL^2 (P = .079). This model was significant (P <.0001) and explained 15.5% of data variation. Least squares mean values of serum vitamin D for adults not exposed to high vitamin D and that did have CPP below 50 mg^2/dL^2 (175. 2 ± 43.3 nmol/L) was lower than that of animals exposed to high vitamin D with CPP below 50 mg^2/dL^2 (555.3 ± 60.2 nmol/L; P <.0001) and animals exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (692.5 ± 43.4 nmol/L; P <.0001). For adults not exposed to high vitamin D and that did not have CPP below 50 mg^2/dL^2, serum levels of vitamin D (213.8 ± 51.2 nmol/L) were lower than that of animals exposed to high vitamin D with CPP below 50 mg^2/dL^2 (P <.0001) and animals exposed to high vitamin D without CPP below 50 mg^2/dL^2 (P <.0001). Adults exposed to high vitamin D with CPP below 50 mg^2/dL^2 tended to have serum

vitamin D lower than that of animals exposed to high vitamin D and CPP not below 50 mg^2/dL^2 (P = .065).

Models were generated for each age group in a manner similar to that previously described, but instead modeled the influences of variables on CPP. All models were significant (P <.0001) and explained 44.5% of variation in juvenile data, 61.3% of variation in yearling data, and 50.2% of variation in adult data.

For juveniles, CPP was influence by CPP below 50 mg^2/dL^2 (P <.0001), exposure to high levels of vitamin D (P = .013), and an exposure and CPP below 50 mg^2/dL^2 interaction (P = .002). Juveniles not exposed to high vitamin D levels with CPP below 50 mg^2/dL^2 had a least squares mean CPP level (39.0 ± 2.8 mg^2/dL^2) that was lower than animals not exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (82.5 ± 1.0 mg^2/dL^2; P <.0001) and animals exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (63.4 ± 4.3 mg^2/dL^2; P <.0001). Juveniles not exposed to high vitamin D with CPP not below 50 mg^2/dL^2 had higher CPP levels than animals exposed to high vitamin D with CPP below 50 mg^2/dL^2 (41.1 ± 4.3 mg^2/dL^2; P <.0001) and animals exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (P <.0001). High vitamin D exposed juveniles with CPP below 50 mg^2/dL^2 had CPP lower than juveniles exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (P = .0003).

CPP was influenced by CPP below 50 mg^2/dL^2 (P <.0001) and an exposure to high vitamin D and CPP interaction (P = .033) for yearling animals. Least squares mean CPP levels were lower for yearlings not exposed to high vitamin D with CPP below 50 mg^2/dL^2 (37.1 ± 1.6 mg^2/dL^2) than yearlings not exposed to high vitamin D with CPP not

below 50 mg^2/dL^2 (71.2 ± 0.8 mg^2/dL^2; *P* <.0001) and animals exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (63.3 ± 1.9 mg^2/dL^2; *P* <.0001). Yearlings not exposed to high vitamin D with CPP not below 50 mg^2/dL^2 had CPP levels higher than those of animals exposed to high vitamin D with CPP below 50 mg^2/dL^2 (38.4 ± 3.4 mg^2/dL^2; *P* <.0001) and animals exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (*P* = .0002). Animals exposed to high vitamin D with CPP below 50 mg^2/dL^2 had CPP levels lower than that of yearlings exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (*P* <.0001).

In adults, CPP levels were influenced by CPP below 50 mg^2/dL^2 (*P* <.0001) and exposure to high vitamin D (*P* = .0003). Adults not exposed to high vitamin D with CPP below 50 mg^2/dL^2 had least squares mean CPP levels (37.9 ± 1.0 mg^2/dL^2) lower than that of animals not exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (61.6 ± 1.1 mg^2/dL^2; *P* <.0001), animals exposed to high vitamin D with CPP below 50 mg^2/dL^2 (41.6 ± 1.4 mg^2/dL^2; *P* = .030), and animals exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (66.2 ± 1.0 mg^2/dL^2; *P* <.0001). Adults not exposed to high vitamin D with CPP not below 50 mg^2/dL^2 had CPP levels higher than animals exposed to high vitamin D with CPP below 50 mg^2/dL^2 (*P* <.0001) and lower than that of adults exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (*P* = .002). For animals exposed to high vitamin D with CPP below 50 mg^2/dL^2, CPP levels were lower than that of animals exposed to high vitamin D with CPP not below 50 mg^2/dL^2 (*P* <.0001).

Using data from all studies and age groups and considering exposure to high vitamin D, models for vitamin D concentration were significant and explained 21.6% of data variation. Serum vitamin D was influenced by exposure to high vitamin D (*P*

<.0001), age group (*P* <.0001), and an exposure and age group interaction (*P* <.0001). Juveniles not exposed to high vitamin D had a least squares mean serum vitamin D concentration (126.4 ± 21.7 nmol/L) lower than that of not exposed adults (192.7 ± 23.4 nmol/L; *P* = .038) and exposed adults (642.5 ± 24.9 nmol/L; *P* <.0001). Exposed juveniles had vitamin D concentrations (65.4 ± 70.3 nmol/L) lower than exposed adults (*P* <.0001) and tended to have lower concentrations than adults that were not exposed to high vitamin D (*P* = .086). Vitamin D concentrations for exposed adults were higher than those for not exposed yearlings (164.0 ± 26.6 nmol/L; *P* <.0001), exposed yearlings (186.1 ± 57.4 nmol/L; *P* <.0001), and not exposed adults (*P* <.0001).

A model similar to that previously described, but with CPP as the dependent variable, yielded a significant model (*P* <.0001) explaining 27.0% of data variation. CPP was influenced by exposure to high vitamin D (*P* <.0001), age group (*P* <.0001), and an exposure and group interaction (*P* <.0001). Least squares mean of CPP concentration was higher for not exposed juveniles (77.86 ± 1.0 mg^2/dL^2) compared to exposed juveniles (52.2 ± 3.4 mg^2/dL^2; *P* <.0001), not exposed yearlings (63.3 ± 1.3 mg^2/dL^2; *P* <.0001), exposed yearlings (57.2 ± 2.8 mg^2/dL^2; *P* <.0001), not exposed adults (48.0 ± 1.1 mg^2/dL^2; *P* <.0001), and exposed adults (57.9 ± 1.2 mg^2/dL^2; *P* <.0001). Exposed juveniles had CPP levels lower than not exposed yearlings (*P* = .003). For not exposed yearlings, CPP levels were higher than those of exposed yearlings (*P* = .049), not exposed adults (*P* <.0001), and exposed adults (*P* = .002). Exposed yearlings had CPP levels higher than not exposed adults (*P* = .002). Exposed adults had CPP levels higher than not exposed adults (*P* <.0001).

A mixed procedure model of serum vitamin D concentration for juveniles in low risk and high risk groups (as defined in the **Materials & Methods** section) yielded a significant model (P <.0001). Vitamin D was influenced by risk group (P = .041) and CPP (P <.0001). Although a similar model for CPP showed that CPP was not influenced by risk group, but was influenced by vitamin D (P = .0002), the model only tended to be significant (P = .079) so these are findings that cannot be claimed with certainty.

Other mixed procedure models for juveniles in low and high risk groups also considered CPP risk threshold (defined as values equal to or below 50, 60, or 65 mg^2/dL^2) influences on vitamin D concentration. All models were significant (P <.0001).

For CPP threshold of 50 mg^2/dL^2, vitamin D was only influenced by risk group (P = .024). Vitamin D was influenced by CPP risk (P = .0007) and tended to be influenced by risk group (P = .090) for CPP threshold of 60 mg^2/dL^2. When defining CPP risk threshold as 65 mg^2/dL^2, vitamin D was influenced by CPP risk (P = .002) and tended to be influenced by risk group (P = .090).

Mixed procedure models were generated using similar parameters to those described above, but considered influences on CPP. Only CPP risk threshold of 65 mg^2/dL^2 yielded a significant model (P = .047). CPP was only influenced by CPP risk (P <.0001).

Contingency Table Calculations

Contingency tables were generated for juveniles using CPP thresholds of 50, 55, 60, 62.5, and 65 mg^2/dL^2 (values evaluated in a previous, unpublished toxicity diagnosis

study) and a vitamin D threshold of 40 nmol/L (a value slightly higher than rachitic). Contingency tables for each CPP threshold can be found in Table 3. These values were used to calculate sensitivity, specificity, positive predictive value, and negative predictive value. These values are summarized in Table 4. For CPP threshold of 50 mg^2/dL^2, sensitivity was 18.9%, specificity was 98.2%, positive predictive value was 89.5%, and negative predictive value was 59.9%. Sensitivity was 26.7%, specificity was 95.5%, positive predictive value was 82.8%, and negative predictive value was 61.6% for CPP threshold of 55 mg^2/dL^2. CPP threshold of 60 mg^2/dL^2 yielded a sensitivity of 31.1%, a specificity of 92.8%, a positive predictive value of 77.8%, and a negative predictive value of 62.4%. For CPP threshold of 62.5 mg^2/dL^2, sensitivity was 34.4%, specificity was 89.2%, positive predictive value was 72.1%, and negative predictive value was 62.7%. At a CPP threshold of 65 mg^2/dL^2, sensitivity, specificity, positive predictive value, and negative predictive value were 36.7%, 86.5%, 68.8%, and 62.7% respectively.

Table 3. Contingency Tables for CPP Thresholds: CPP Risk 0= CPP equal to or below threshold, CPP Risk 1= CPP above threshold, Vitamin D Category 0= serum vitamin D equal to or below 40 nmol/L, Vitamin D Category 1= serum vitamin D above 40 nmol/L

Table 3. Contingency Tables for CPP Thresholds			
CPP Threshold 50 mg^2/dL^2			
CPP Risk	**Vitamin D Category**		**Total**
	0	1	
0	17	2	19
1	73	109	182
Total	90	111	201
CPP Threshold 55 mg^2/dL^2			
CPP Risk	**Vitamin D Category**		**Total**
	0	1	
0	24	5	29
1	66	106	172
Total	90	111	201
CPP Threshold 60 mg^2/dL^2			
CPP Risk	**Vitamin D Category**		**Total**
	0	1	
0	28	8	36
1	62	103	165
Total	90	111	201
CPP Threshold 62.5 mg^2/dL^2			
CPP Risk	**Vitamin D Category**		**Total**
	0	1	
0	31	12	43
1	59	99	158
Total	90	111	201
CPP Threshold 65 mg^2/dL^2			
CPP Risk	**Vitamin D Category**		**Total**
	0	1	
0	33	15	48
1	57	96	153
Total	90	111	201

Table 4. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value for CPP Thresholds: PPV= Positive predictive value, NPV= Negative predictive value

Table 4. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value for CPP Thresholds				
CPP Threshold (mg^2/dL^2)	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
50	18.9	98.2	89.5	59.9
55	26.7	95.5	82.8	61.6
60	31.1	92.8	77.8	62.4
62.5	34.4	89.2	78.1	62.7
65	36.7	86.5	68.8	62.7

Chapter 5

Discussion

Correlation results offer evidence that vitamin D controls phosphorus concentrations more tightly than calcium in all age groups of camelids. This points to a unique aspect of camelid physiology compared to most animals, such as cattle, small ruminants, and poultry, in which the function of vitamin D is more so to control calcium homeostasis (Hurst et al., 2020). Phosphorus was also a higher contributor to CPP compared to calcium, another indication of the unique importance of phosphorus in camelids. The rarity of hypocalcemia in camelids in addition to these findings indicating that there is tight control and importance of phosphorus in camelids may point to an evolutionary adaptation to grazing on low-phosphorus, high-calcium grasses, but this is only conjecture (Van Saun & Cebra, 2014).

The correlation between vitamin D and phosphorus, as well as the correlation between vitamin D and CPP, became more positive with age. Least squares mean evaluations also indicated differences in serum vitamin D concentration and CPP in clinically normal animals and animals exposed to high vitamin D based on age group. Age group also often had an influence on serum vitamin D and CPP in general linear models. All these findings point to an age effect on vitamin D. This indicates that each age group must be examined separately when it comes to vitamin D and associated parameters rather than evaluating all animals identically. Further, juvenile risk group also influenced serum vitamin D, indicating that juveniles should also be examined differently based on month of birth when considering vitamin D status.

Assessment of exposure to high vitamin D showed that serum vitamin D concentration was not influenced by exposure in juvenile and yearling animals, but was influenced by exposure

in adults. This may indicate that there is a mechanism for the degradation of vitamin D, a phenomenon documented in other species such as the dairy cow, in the early rumen environment (Hymøller & Jensen, 2010). The degradation of vitamin D maybe one of the factors that puts these animals at increased risk of developing vitamin D-responsive rickets. It may also indicate that younger animals may be able to tolerate higher supplementation of vitamin D as a rickets preventative measure before concern of vitamin D toxicity arises. However, further research would be needed to potentiate these claims.

In most analyses, serum vitamin D was influenced by CPP. However, CPP was not always influenced by serum vitamin D. This indicates that CPP is a reliable measure of vitamin D concentration, but there is more variability in using vitamin D as a measure for CPP. It indicates that abnormal CPP should not affect the vitamin D status of an animal, meaning treatment for abnormal CPP can focus on improving calcium and phosphorus status without concern for vitamin D toxicity or deficiency. CPP's influence on vitamin D is a positive finding for the purposes of this study because it validates the use of CPP as a diagnostic tool for vitamin D deficiency and toxicity.

A previous study found that, for adult camelids, the CPP threshold for vitamin D toxicity is 60 mg^2/dL^2, but it did not determine a threshold for vitamin D deficiency, leading to the need for the present study (Van Saun, unpublished). When considering sensitivity, specificity, positive predictive value, and negative predictive value, no single CPP threshold yielded the highest value for all four parameters. CPP threshold of 50 mg^2/dL^2 had the highest specificity and positive predictive value, 65 mg^2/dL^2 yielded the highest sensitivity, and 62.5 mg^2/dL^2 and 65 mg^2/dL^2 yielded identical and greatest negative predictive value, but negative predictive value for all thresholds were fairly similar. For general use of CPP as a diagnostic tool for vitamin D

deficiency, CPP threshold of 50 mg^2/dL^2 would be the proper limit to use considering it yielded the greatest accuracy of all values with a specificity of 98.2%. This means that if a juvenile has a CPP above 50 mg^2/dL^2, it can be claimed with 98.2% certainty that the animal is not at risk of rickets. Using this metric will more often than not identify animals that do not require further diagnostic testing, eliminating them as a concern for rickets. This allows for focus and resources to be allotted to animals who are at risk of vitamin D deficiency. Sensitivity would not be a proper metric to use because it could only be claimed with a maximum of 36.7% certainty that a juvenile with a CPP below or equal to 65 mg^2/dL^2 is vitamin D deficient. Using a metric with such a high level of uncertainty may lead to use of other diagnostic methods when not necessary and possibly unnecessary treatment with vitamin D supplementation, putting the animal at risk of developing vitamin D toxicity.

Comparison of Results with Published Data

Two case studies and one study assessing vitamin D in rachitic camelids provide a means of evaluating the results of the present study in comparison to a real-world context. Stieger-Vanegas et al. (2013) reported serum phosphorus levels of 1.7 mg/dL in a 5-month-old juvenile with rickets. Serum calcium concentration was reported to be within reference range (8.1 mg/dL to 9.9 mg/dL) (Steiger-Vanegas et al., 2013; Anonymous, 2016). A 7-month-old rachitic juvenile was reported as having a serum phosphorus of 1.1 mg/dL (McClanahan et al., 2006). Calcium concentration was not reported, but it is assumed that serum calcium concentration was within reference range. Van Saun et al. (1996) reported mean values of 9.7 ± 0.26 mg/dL serum calcium and 3.6

± 0.40 mg/dL serum phosphorus in rachitic camelids. It was also reported that serum calcium between rachitic and non-clinical animals was not significantly different while serum phosphorus was significantly different between rachitic and non-rachitic animals.

In all cases, serum phosphorus, but not calcium, was severely affected when these animals experienced vitamin D-responsive rickets. This indicates that in these cases, vitamin D correlated more closely with phosphorus concentration, leading to the assumption that vitamin D controls phosphorus concentration more than calcium concentration. The results of the present study also indicate this close relationship between vitamin D and serum phosphorus and a comparatively loose relationship between vitamin D and serum calcium. These published studies substantiate the claim made by the present study that there is a unique association between vitamin D and serum phosphorus in camelids.

Although these published studies did not report CPP, calculations using the reference ranges indicate that the camelids in the case studies had a CPP of 13.77 to 16.83 mg^2/dL^2 and 8.91 to 10.89 mg^2/dL^2 (Steiger-Vanegas et al., 2013; McClanahan et al., 2006). The mean value of CPP for the camelids in Van Saun et al. (1996) was approximately 34.92 mg^2/dL^2. The present study reports a threshold of 50 mg^2/dL^2 in order to report with good certainty that a juvenile is not vitamin D deficient. These animals were well below the threshold identified in this study, meaning they would have been suspected as vitamin D deficient based on threshold criteria and sent for further testing. These findings validate the use of 50 mg^2/dL^2 as a threshold for CPP in suspected vitamin D deficiency.

Chapter 6

Conclusion

The objective of this study was to find thresholds for use of CPP as a diagnostic method for vitamin D deficiency and toxicity. In this retrospective study, it was found that in camelids vitamin D selectively controls phosphorus more so than calcium and that differences in vitamin D physiology can be found in different age groups of camelids. The most important finding of this study is that CPP is a valid indicator of vitamin D status and that a CPP threshold of 50 mg^2/dL^2 should be used to determine if a juvenile is at risk of developing rickets while a CPP threshold of 60 mg^2/dL^2 should be used to determine vitamin D toxicity in adult camelids (Van Saun, unpublished). The use of CPP in diagnosing vitamin D imbalance is critical as it provides a less expensive alternative to traditional vitamin D testing. This CPP method of diagnosis gives producers greater opportunity for early diagnosis and treatment to combat economic losses due to vitamin D-responsive rickets and vitamin D toxicity diseases to maintain herd health in production camelids.

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ACADEMIC VITA

MARTA E. BAKAJ

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Education

Pennsylvania State University

Bachelor of Veterinary and Biomedical Sciences with a Minor in Psychology *May 2021*

- Honors: Schreyer Honors College; Pennsylvania State University's Dean's List (Fall 2017-Fall 2020); Phi Eta Sigma National Honor Society; Gamma Sigma Delta Agricultural Science Honor Society

Work Experience

Dr. Mimnagh Veterinary Associates, Hershey, PA *May 2020-July 2020*

Veterinary Assistant

- Assisted veterinarians during appointments and surgeries on a daily basis by obtaining patient history, restraining and preparing animals, monitoring anesthesia, and completing tasks requested by the veterinarian
- Cared for animals at the clinic through administering medications, drawing blood, and husbandry tasks
- Aided clients by reviewing appointment findings, preparing medication, and answering questions

Nottingham Animal Hospital, Trenton, NJ *Summers of 2017-2018*

Veterinary Intern and Assistant

- Learned restraint methods, vaccine administration, biological sample analysis, and animal care
- Observed and assisted with castration and oral surgeries, laser treatments, and daily routine checkups

Penn's Cave & Wildlife Park, *Centre Hall, PA* *March 2019-November 2019*

Wildlife Tour Guide

- Interacted with various wildlife species such as elk, timber wolves, and bobcats on a daily basis
- Attained knowledge about species specific care, biologically, natural history, and endangerment status
- Educated approximately 90 visitors a day about wildlife species, animal care, and conservation

Volunteering

Shaver's Creek Environmental Center, *Petersburg, PA* *October 2019-present*

Animal Care Volunteer

- Learned and demonstrated raptor and herp handling, care, and behavior training through operant conditioning and positive reinforcement on a weekly basis
- Educated members of the community about raptor and herp conservation and natural history
- Maintained a clean environment and performed husbandry tasks in enclosures

Research Experience

Dr. Robert Van Saun Ruminant and Camelid Nutritional Research, *State College, PA* *February 2020-present*

Honors Thesis Researcher

- Used statistical analysis to establish ranges of the calcium-phosphorous product indicating vitamin D deficiency in different age groups of camelids
- Attained extensive knowledge on biological pathways of vitamin D and vitamin D-associated diseases

Dr. Victoria Braithwaite Cognitive Ecology and Behavior Lab, *State College, PA* *May 2019-October 2019*

Research Assistant

- Cared for over 100 fish several times a week through feedings, water testing, and tank cleaning tasks
- Assisted in experimentation and data recording involving zebra fish maze tasks

Leadership

Runkle and Ritner Residence Halls, *State College, PA* *May 2019-present*

Resident Assistant for Earth House Special Living Option and New Student Orientation

- Organized events individually or with up to eleven other resident assistants that align with university extracurricular education goals
- Mediated discussion, negotiated various solutions to resolve conflicts, enforced residence hall policies, created community, and served as a mentor for up to 65 residents