Seasonal changes in serum calcium, phosphorus, and vitamin D concentrations in llamas and alpacas

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**Objective**—To evaluate the interaction of season and age on serum calcium, phosphorus, and vitamin D₃ concentrations in llamas and alpacas.

**Animals**—23 clinically normal llamas and 7 alpacas.

**Procedures**—Animals were assigned to 1 of the 3 following groups on the basis of age at the start of the study: adult (age, > 24 months; n = 8), yearling (> 12 but < 20 months; 5), and neonate (< 6 months; 17). Twelve serum samples were obtained at monthly intervals. Calcium, phosphorus, and vitamin D₃ concentrations were measured, and the calcium-to-phosphorus concentration (Ca:P) ratio calculated. Effect of season and age on each of these variables was determined.

**Results**—Vitamin D₃ concentrations varied significantly as a function of season; the highest and lowest concentrations were detected September through October and February through March, respectively. The seasonal decrease in vitamin D₃ concentration was significantly greater in neonates and yearlings, compared with adults. Serum phosphorus concentration decreased as a function of age, with the most significant seasonal change detected in the neonate group. The Ca:P ratio in neonates varied between 1.1 and 1.3 except during winter months when it increased to ≥ 2.0.

**Conclusions and Clinical Relevance**—Mean vitamin D₃ concentration varied by > 6 fold in neonatal and yearling llamas and alpacas and > 3 fold in adult animals as a function of season. These results support the hypothesis that seasonal alterations in vitamin D₃ concentrations are a key factor in the development of hypophosphatemic rickets in llamas and alpacas. (Am J Vet Res 2001;62:1187–1193)

Vitamin D-responsive hypophosphatemic rickets, characterized by swollen joints, a shifting leg lameness, reluctance to walk, kyphosis, and angular limb deformities, has been described in young rapidly growing llamas and alpacas. Vitamin D₃ concentration in serum is a key factor in the development of hypophosphatemic rickets. Affected animals typically have a low serum phosphorus concentration (< 2 mg/dl), which is believed to be a result of a low serum vitamin D₃ concentration. There appears to be a seasonal prevalence to this condition, with most cases in Australia diagnosed during the winter months. Clinical observations also suggest that crias born in fall or winter are more likely to develop this condition, compared with crias born in spring or summer. On the basis of these observations, we hypothesized that llamas and alpacas are unable to synthesize sufficient endogenous vitamin D₃ during the winter months to support normal skeletal growth. Low vitamin D₃ concentrations result in development of vitamin D-responsive hypophosphatemic rickets.

Vitamin D is a unique nutrient in that it can be supplied in the diet in 1 of 2 forms: vitamin D₂, which is of plant origin, or vitamin D₃, which is of animal origin. In addition, in animals with adequate exposure to ultraviolet light, vitamin D₃ is endogenously synthesized in the skin. Clinical syndromes have been reported associated with seasonal decreases in serum concentration of vitamin D₃ in sheep. Serum vitamin D₃ concentrations in sheep have also been associated with seasonal variations in solar radiation. The objective of the study reported here was to determine seasonal variations in, and affects of age, sex, and month of birth on, serum calcium, phosphorus, and vitamin D₃ concentrations in llamas and alpacas.

**Materials and Methods**

Animals and groups—Twenty-three llamas and 7 alpacas from the Oregon State University research herd were used for this study. Animals were assigned to 1 of the 3 following groups on the basis of age at the start of the study: adult (age, > 24 months; n = 8), yearling (> 12 but < 20 months; 5), and neonate (< 6 months; 17). Mean ages at the start of the study for the adult, yearling, and neonate groups were 80 months (range, 45 to 132 months), 13.4 months (12 to 20 months), and 1.8 months (1 to 5 months), respectively. The adult group consisted of 4 llamas (3 females, 1 male) and 4 alpacas (3 females and 1 male), the yearling group comprised 4 llamas (1 female and 3 males) and 1 alpaca (female), and the neonate group comprised 15 llamas (7 females, 8 males) and 2 alpacas (1 female, 1 male). Month of birth was defined for all animals in the neonate group.

Most of the llamas and alpacas were housed at 1 of 2 outdoor locations on the basis of age, sex, and physiologic state (eg, pregnant, lactating). Three other locations were used intermittently to house a limited number of animals for brief times for purposes of breeding and separation of males and females. All locations were within the Corvallis, Oregon area at 44.3°N latitude and 123.2°W longitude. Animals were cared for under protocols approved by the Oregon State University Animal Care and Use Committee. A common grass hay was used as the primary forage fed at all locations (Table 1). To address energy and mineral needs, 1 of 3 commercial grain supplements was also fed to all animals according to physiologic needs.
logic state, and animals were provided a mineral salt supplement ad libitum (Table 2). In addition, growing neonates and lactating females received between 100 and 250 g of a corn-oats-barley grain mix for additional energy. Estimated intake of supplemental vitamin D3 from these sources for animals in a maintenance, lactating, or neonatal state was 10 g/d.0.22 kg of food/45 kg of body weight. §Average consumption was 0.12 kg/d. †Average daily consumption was 0.22 kg/d.

Sample collection and analyses—For each animal, blood was collected by jugular venipuncture into evacuated tubes on a monthly basis for 12 consecutive months. Serum was harvested and stored at –70°C until analyzed. The first aliquot of serum was removed for Ca, P, vitamin D3, and vitamin E on a monthly basis for 1 year, because neonates were added to the study as they were born, and sample collection continued for 12 months for each of these animals.

Serum concentrations of calcium and phosphorus were measured in each sample as described. The calcium-to-phosphorus concentration (Ca:P) ratio was calculated. Serum concentration of vitamin D3 was measured at a reference endocrinology laboratory as described. Intra- and interassay coefficients of variation were 7.6 and 18.7%, respectively, for 5 sets of analyses conducted on samples of pooled llama serum. Mean vitamin D3 concentration in pooled serum was 65.3 nmol/L (26.1 ng/ml). Daily solar radiation data from the National Weather Service Hyslop Field weather station in Corvallis, Ore were collected for analysis.

Statistical analyses—Seasonal effects on daily solar radiation, serum calcium, phosphorus, and vitamin D3 concentrations, and the Ca:P ratio were analyzed by use of ANOVA for repeated measures. Main effects in the solar radiation model included year, month, and their interaction term. Main effects for the dependent variable models included age group, month, and age group by month interaction; subplot factors included month of birth, chronologic and initial age, sex, and species where applicable. For each dependent variable, llama nested within age group was subjected to 5 covariance structures. The best covariance structure of the data was determined by comparing derived parameter estimates for each evaluation. When model effects were determined significant, monthly mean separations were analyzed by use of least significant differences. Significance was set at P ≤ 0.05. Data were reported as mean ± SEM.

Results Effect of age group—None of the llamas and alpacas in this study developed clinical signs of rickets. Season had a significant effect on serum calcium, phosphorus, and vitamin D3 concentrations (Fig 1). Season also significantly affected the Ca:P ratio. We did not detect differences in these variables between years of the study. Accordingly, data collected for both years were classified by month of collection irrespective of year of collection. Age group significantly influenced serum concentrations of calcium and phosphorus and the Ca:P ratio. Age group did not, however, affect vitamin D3 concentration. Neonatal llamas and alpacas had higher mean serum calcium (10.0 mg/dl) and phosphorus (8.1 mg/dl) concentrations and a lower Ca:P ratio (1.34), compared with the yearling (calcium, 9.5 mg/dl; phosphorus, 6.5 mg/dl; Ca:P ratio, 1.57) and adult (8.8 mg/dl; 5.3 mg/dl; 1.79) groups. Mean differences in calcium and phosphorus concentrations between the yearling and adult groups were also significant. An age group by month of year interaction was observed for serum calcium concentration and the Ca:P ratio.

Effect of season—Total daily solar radiation significantly varied by month during a given year (Fig 2). Mean solar radiation was not significantly different between the 2 years of the study (1994, 324 langleys; 1995, 309 langleys), nor was there an interaction between month and year of study. Accordingly, solar radiation for the 2 years was averaged, and a mean monthly value was used for statistical analyses. July had the highest mean solar radiation value, and November, December, and January had the lowest. Amount of solar radiation in May, June, and August was not significantly different; solar radiation in these months ranked second to July. Amount of solar radiation in April and September was not different, and solar radiation in these 2 months ranked third. Amount of solar radiation differed significantly in March, October, and February; amount in these months ranked fourth, fifth, and sixth, respectively.

The association between serum vitamin D3 concentration and season was further examined by computing the correlation coefficient between these variables for each age group. Serum vitamin D3 concentration and season were significantly associated in all age groups (neonate, r = 0.24; yearling, r = 0.52; adult, r = 0.53). When vitamin D3 concentrations for each animal were normalized and expressed as a percentage

Table 1—Nutrient content of grass hays fed to llamas and alpacas during each year of the study

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>1994</th>
<th>1995</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (%)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Table 2—Nutrient content of commercial grain and mineral supplements fed to llamas and alpacas

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Maintenance grain</th>
<th>Lactating/ neonate grain</th>
<th>Mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>8.0</td>
<td>16.0</td>
<td>NA</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>1.0</td>
<td>2.0</td>
<td>NA</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>25</td>
<td>145</td>
<td>8.0</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.5</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>15</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>Manganese (ppm)</td>
<td>105</td>
<td>NA</td>
<td>85</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>140</td>
<td>NA</td>
<td>125</td>
</tr>
<tr>
<td>Selenium (ppm)</td>
<td>4.5</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Vitamin A (U/kg)</td>
<td>17,600</td>
<td>17,600</td>
<td>19,800</td>
</tr>
<tr>
<td>Vitamin D3 (U/kg)</td>
<td>3,300</td>
<td>2,200</td>
<td>3,080</td>
</tr>
<tr>
<td>Vitamin E (U/kg)</td>
<td>770</td>
<td>550</td>
<td>7,040</td>
</tr>
</tbody>
</table>

Values reported on a percentage dry matter basis.

Values reported on an as fed basis.

*Average consumption was 0.12 kg/d. †Average daily consumption was 0.22 kg/d. ‡Estimated average consumption for all animals except neonates and lactating females was 10 g/d. NA = Not applicable.
of mean yearly vitamin D₃ concentration, vitamin D₃ concentration and season remained significantly associated in all age groups (neonate, $r = 0.50$; yearling, $r = 0.71$; adult, $r = 0.82$). In the adult group, serum vitamin D₃ concentration was significantly associated with both calcium ($r = 0.41$) and phosphorus ($r = 2.5$).
–0.31) concentrations. In the yearling group, vitamin D₃ concentration was also significantly associated with calcium ($r = 0.51$) and phosphorus ($r = 0.39$) concentrations. However, in the neonate group, vitamin D₃ concentration was significantly associated ($r = 0.28$) with phosphorus concentration only; both serum calcium ($r = 0.27$) and phosphorus ($r = 0.38$) concentrations were associated with month of the year.

**Effect of species**—Because of the limited number of yearling and neonatal alpacas, effect of species on measured variables could only be assessed in the adult group. Relative to adult llamas, adult alpacas had lower serum calcium (7.81 mg/dl vs 8.74 mg/dl) and vitamin D₃ (60.6 nmol/L [24.2 ng/ml] vs 160.1 nmol/L [64.0 ng/ml]) concentrations. When data for individual animals were assessed, however, mean serum vitamin D₃ concentrations and seasonal variations in vitamin D₃ concentration in 2 adult alpacas were similar to values in adult llamas, whereas in the other 2 adult alpacas, vitamin D₃ concentrations were low and seasonal variation minimal (Fig 2). Both of the alpacas with low vitamin D₃ concentrations were black; the other alpacas were silver-gray or brown.

**Values for neonatal llamas**—Serum calcium and phosphorus concentrations in neonatal llamas were significantly influenced by month and age. Vitamin D₃ concentration did not, however, significantly affect phosphorus concentration. Serum vitamin D₃ concentration was significantly influenced by month, age, month of birth, and sex. Male crias had higher serum vitamin D₃ concentrations than females, but this was confounded by the earlier months of birth for males.

Because of the limited number of neonatal and yearling alpacas, data from these groups were not statistically analyzed. Qualitative changes in calcium, phosphorus, and vitamin D₃ concentrations and the Ca:P ratio as a function of month of year and age group were similar between llamas and alpacas.

To evaluate interactions between month of birth and serum calcium, phosphorus, and vitamin D₃ concentrations and changes in the Ca:P ratio, crias were assigned to 1 of 2 risk groups on the basis of their month of birth. Crias born between September and February ($n = 9$) were classified as high risk, whereas those born between March and August ($n = 6$) were classified as low risk. Vitamin D₃ concentrations in high- and low-risk groups differed significantly ($P < 0.05$) between 1 and 7 months of age.

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not significant. Serum vitamin D₃ concentration was significantly influenced by risk group, chronologic age, and risk group by age interaction (Fig 3). Serum vitamin D₃ concentrations were significantly lower in high-risk crias at 1 through 6 months of age, compared with low-risk crias.

In low-risk crias, serum calcium (r = -0.48), phosphorus (r = -0.53), and vitamin D₃ (r = -0.35) concentrations were negatively correlated with chronologic age. In contrast, there was no association between chronologic age and serum phosphorus concentration in high-risk crias. A slight negative association (r = -0.21) between chronologic age and serum calcium concentration and a positive association (r = 0.51) between age and vitamin D₃ concentration were detected for high-risk crias. Serum calcium, phosphorus, and vitamin D₃ concentrations were also significantly associated (r = 0.34, 0.40, 0.36, respectively) with month of year in high-risk crias. In low-risk crias, however, only vitamin D₃ concentration was significantly associated (r = 0.40) with month of year.

Discussion
Seasonal changes in serum vitamin D₃ concentration have been observed in such phylogenetically diverse species as sheep, elephant seals, horses, cows, dromedary camels, and humans. Because vitamin D₃ can either be formed de novo from ultraviolet irradiation of 7-dehydrocholesterol in the skin or obtained from animal-based dietary sources, seasonal changes in serum vitamin D₃ concentration are a reflection of alterations in vitamin D availability from both sources. This interplay between dietary sources and de novo synthesis is reflected in the seasonal changes in serum vitamin D₃ concentrations in sheep maintained in an environment with relatively constant (eg, Sinai Desert, latitude 31° N) and highly variable (eg, northern Scotland, latitude 56° N) ultraviolet light exposure. Sheep maintained in the Sinai Desert have mean (± SD) vitamin D₃ concentrations of 92.75 ± 22 nmol/L (371 ± 8.8 ng/ml) in the winter and 101.75 ± 22.75 nmol/L (407 ± 9.1 ng/ml) in the summer. In comparison, corresponding winter and summer vitamin D₃ concentrations for sheep maintained in Northern Scotland are 30 and 88 nmol/L (12 and 35 ng/ml), respectively. Differences in peak vitamin D₃ concentrations are probably a reflection of differences in breed, pigmentation, ultraviolet light intensity, and assay methodology. An evaluation of dietary and total serum vitamin D₃ and 25-hydroxyergocalciferol concentrations suggests that biosynthesis is more important than diet as a source of vitamin D₃ in sheep and, most likely, other grazing animals.

Although we detected a clear correlation between amount of solar radiation and serum vitamin D₃ concentration, there was a temporal lag between peak radiation and maximum vitamin D₃ concentrations in adult llamas. The peak solar radiation period was in June and July, whereas peak vitamin D₃ concentrations were detected 2 to 4 months later. One possible explanation for this time lag is that the vitamin D₃ synthesized during periods of peak solar radiation was initially sequestered in the liver to replenish hepatic stores. Only as the storage capacity of the liver was exceeded did serum vitamin D₃ concentration increase. A similar time lag between peak solar radiation periods and serum vitamin D₃ concentrations has been observed in sheep and alpacas.

Results of studies with indoor- and pasture-housed sheep fed different diets indicate wide variations between peak (summer) and nadir (winter) serum vitamin D₃ concentrations. The magnitude of seasonal changes in vitamin D₃ concentration may be influenced by a number of factors, including degree of dietary supplementation, physiologic state, exposure to light, and coat color. In sheep that did not receive supplemental dietary vitamin D₃, serum peak vitamin D₃ concentration varied approximately 3-fold from the nadir concentration, whereas in pasture-housed white-faced pregnant ewes, peak and nadir serum vitamin D₃ concentrations varied 13-fold. In the present study, we detected a > 6-fold seasonal change in vitamin D₃ concentrations in neonatal llamas; the nadir concentration was < 25 nmol/L (< 10 ng/ml) in February, and the peak was > 175 nmol/L (> 70 ng/ml) in September. We detected a smaller variation between peak and nadir concentrations in adult llamas; minimum vitamin D₃ concentrations were consistently > 50 nmol/L (20 mg/ml) from February to April. The underlying cause for the difference in minimum and maximum vitamin D₃ concentrations between neonatal and adult llamas is unresolved. A reasonable hypothesis, however, is that metabolic requirements were decreased and the period for vitamin D synthesis prolonged in adult llamas, compared with neonates.

Comparison of vitamin D₃ concentrations between adult llamas and alpacas indicated that vitamin D₃ concentration in alpacas was significantly less than in llamas. Although this difference was significant, the biological importance of this observation is less clear. Specifically, 2 of the lighter colored alpacas had seasonal changes in vitamin D₃ concentrations similar to those observed in llamas. In contrast, the 2 black alpacas had extremely low vitamin D₃ concentrations throughout the year. These results suggest that the difference in serum concentration of vitamin D₃ between adult llamas and alpaca had less to do with differences in coat color.

Phosphorus concentration decreased as a function of increasing age in growing llamas and alpacas. This age-related change has been reported in llamas. During periods of moderate to high light availability, serum phosphorus concentrations in neonates were typically in the range of 8.0 to 9.5 mg/dl. The important clinical observation in the present study was the pronounced seasonal change in serum phosphorus concentrations in neonatal llamas; mean serum phosphorus concentration was < 6 mg/dl in February. Although phosphorus concentrations of 5.0 to 6.5 mg/dl are within the reference range for yearling and adult llamas, these concentrations are low for neonates. Accordingly, it is important that reference ranges established for neonates be used when evaluating mineral status in this group.

Mean calcium concentration decreased slightly, but not significantly, as a function of increasing age and...
did not change significantly with season. Although the shift toward lower calcium concentrations with increasing age in llamas has been reported, its clinical relevance, if any, is not known. Of greater diagnostic value is the seasonally related change in the Ca:P ratio. In rapidly growing neonates during periods of moderate to high light availability, the Ca:P ratio varied from 1.1 to approximately 1.3. In contrast, this ratio increased to 2.0 during the winter months. As neonatal llamas aged, the Ca:P ratio increased to >2.0, which is a typical ratio in many mature animals. Accordingly, if serum vitamin D₃ concentration cannot be easily measured, measurement of serum calcium and phosphorus concentrations and calculation of the Ca:P ratio can provide useful information for the evaluation of llamas and alpacas with clinical signs of hypophosphatemic rickets. On the basis of our results, serum phosphorus concentrations <7 mg/dl and a Ca:P ratio of >1.5 in rapidly growing neonatal llamas and alpacas are associated with vitamin D₃ concentrations <50 nmol/L (20 ng/ml). Physical examination, radiographic assessment of the physis, and measurement of serum vitamin D₃ concentration would be appropriate in such animals to confirm a diagnosis of hypophosphatemic rickets secondary to inadequate vitamin D₃ concentrations.

A limitation in using calcium and phosphorus concentrations and the Ca:P ratio as a diagnostic tool for the indirect assessment of vitamin D₃ status and diagnosis of hypophosphatemic rickets, however, is that the minimum serum vitamin D₃ concentration required to prevent the development of rickets is not known. Previous results suggest that vitamin D₃ concentrations <10 nmol/L (4 ng/ml) are commonly encountered in llamas and alpacas with clinical signs of rickets. A second limitation is the variability of calcium and phosphorus concentrations and the Ca:P ratio as predictors of vitamin D₃ concentrations.

Our observation that crias born in the fall and winter months have lower vitamin D₃ and phosphorus concentrations during the rapid growth phase (ie, 0 to 6 months of age), compared with crias born in the spring and summer months, is consistent with results of a previous study. Low serum vitamin D₃ and phosphorus concentrations during the period of rapid skeletal development in llamas is likely an important contributory factor to slow growth, development of skeletal abnormalities, or both. The functions of vitamin D in bone metabolism and homeostatic regulation of calcium and phosphorus concentrations in growing and adult animals is well established. Of interest in the present study was the observed dichotomy between defined low- and high-risk crias relative to the relationships between measured variables, season, and age.

Serum calcium, phosphorus, and vitamin D₃ concentrations in low-risk crias decreased with chronologic age, which was consistent with observations from another study characterizing biochemical variables in clinically normal llamas of different ages. The higher vitamin D₃ concentration at 1 month of age in low-risk crias (146.4 nmol/L [58.6 ng/ml]), compared with high-risk crias (34.3 nmol/L [13.7 ng/ml]), may be attributable to a greater degree of transfer of maternal vitamin D₃ via the colostrum or placenta. That vitamin D₃ concentrations remained high in low-risk crias as they aged was most likely a function of exposure to solar radiation. Increased exposure to solar radiation would potentially increase vitamin D₃ reserves, thus allowing serum vitamin D₃ concentrations to be maintained within physiologic needs even when biosynthesis of vitamin D₃ was minimal. Although we detected significant seasonal variation in vitamin D₃ concentrations, the nadir concentration in low-risk crias was sufficient to prevent metabolic derangements, as evidenced by serum calcium and phosphorus concentrations within reference ranges. Moreover, we did not detect a seasonal variation in calcium and phosphorus concentrations in low-risk crias.

In contrast, low vitamin D₃ concentrations detected during the first 6 months of life in high-risk crias may be attributable to low colostral or placental transfer of vitamin D₃ and low exposure to solar radiation as a function of season. We hypothesize that low vitamin D₃ concentrations in the adult group during the winter months may account for a decrease in degree of transfer of maternal vitamin D₃. As a direct result, high-risk crias would have minimal vitamin D₃ reserves to buffer the impending seasonal decrease in such stores. Serum vitamin D₃ concentration in high-risk crias would then be totally dependent on biosynthesis, as evidenced by the greater correlation between vitamin D₃ concentration and month of year in this group, compared with the low-risk group. Significant associations between serum calcium and phosphorus concentrations and month of year in high-risk crias suggest that vitamin D₃ concentrations may be nearing a critically low limit such that metabolic functions become perturbed. Evidence for this in the present study includes detection of lower serum phosphorus concentrations in high-risk crias between 3 and 6 months of age, compared with low-risk crias of a similar age. Results from this study suggest that crias born in the fall and winter have a greater potential for development of hypophosphatemic rickets than crias born in the spring and summer. On the basis of our data, dietary vitamin D supplementation of crias, particularly those born in the fall, and pregnant llamas and alpacas, particularly those expecting to give birth in the fall, may be warranted.

References