1999 Animal Science Research Report

EFFECT OF DIETARY CATION-ANION DIFFERENCE ON GROWTH AND SERUM OSTEOCALCIN LEVELS IN WEANLING HORSES

Pages 176-181

Authors:

S.R. Cooper, D.R. Topliff, D.W. Freeman, J.E. Breazile and R.D. Geisert

Story in Brief

Sixteen Quarter Horse weanlings were used in a split-plot design experiment to determine the effects of dietary cation-anion difference (DCAD) on skeletal growth parameters and serum osteocalcin concentrations. Experimental diets with a DCAD of +325 (High, H) and -52 (Low, L) were formed by supplementing Diet H with sodium bicarbonate and Diet L with calcium chloride. Weekly growth measurements were taken for body weight, heartgirth circumference, knee, shoulder, wither, hip and hock height between 150 and 330 d of age. (Venous blood samples were drawn the day before and the first day of each collection period at 8:00 a.m. using plain glass Vacutainers® for the subsequent analysis of osteocalcin.) Mean values for all parameters of growth did not differ significantly between males and females and were not altered by dietary treatment in this study. Sex did not influence serum osteocalcin levels as mean concentrations were similar between females and males. Furthermore, there was a significant correlation between osteocalcin and wither (r=.81), hip (r=.82), hock (r=.65) and shoulder (r=.72) height. Osteocalcin levels were similar among treatments H and L at 150 and 330 d of age, however, horses consuming Diet L had a lower osteocalcin concentration (28.97 ng/ml) than those on Diet H (33.18 ng/ml) at 240 d of age.

Key Words: DCAD, Horse, Osteocalcin, Growth

Introduction

Growing horses have a high degree of stress placed on the skeletal system, especially young racehorses which often begin training early in their 2-yr old year. Therefore, there is an enormous potential for skeletal injury and, in today's competitive horse industry, proper nutrition is imperative to optimize the full genetic potential of the animal. If manipulating the DCAD could be shown to improve calcium balance or retention, then skeletal demineralization may be minimized using this dietary approach. Therefore, the objectives of the present study were to evaluate the long-term effects of DCAD on parameters of growth and serum osteocalcin levels in an attempt to quantify changes in bone mineralization.

Materials and Methods

Sixteen Quarter Horse weanlings were used in a split-plot design experiment to determine the effects of dietary cation-anion difference (DCAD) on skeletal growth parameters and serum osteocalcin concentrations. Horses were blocked by age and sex and then randomly allotted to the two treatment groups (High or Low) with four fillies and four colts per treatment. The weanlings were pair weaned at 120 d of age and started on trial at 150 d of age. Horses were fed approximately 2.0% of their body weight per day in total ration. The diets consisted of a pelleted concentrate of corn, soybean meal and cottonseed hulls fed in a 70:30 ratio with native prairiegrass hay. Experimental diets, with a DCAD of +325 (High, H) and -52 (Low, L), were formed by supplementing Diet H with sodium bicarbonate and Diet L with calcium chloride (Table 1). The DCAD of the diets was calculated as meq (Na + K) - (Cl + S)/kg of diet DM. Rations were formulated to contain equivalent amounts of digestible energy (DE=2.9 Mcal/kg DM) on a calculated basis across treatments. Diets were analyzed and found to contain approximately equal amounts of CP, calcium, phosphorus, magnesium and sulfur. Weekly growth measurements were taken for body weight, heartgirth circumference, knee, shoulder, wither, hip and hock height. Venous blood samples were drawn the day before and the first day of each collection period at 8:00 a.m. using plain glass Vacutainers® . Samples were allowed to clot at room temperature and then centrifuged for 20 min at 2,500 rpm, after which the serum was removed and frozen for subsequent analysis of osteocalcin. Serum osteocalcin concentration was determined using a commercially available radioimmunoassay kit for measurement of human osteocalcin[1] and all samples were read on a Cobra II Auto-Gamma Counter[2]. Data for growth parameters and osteocalcin concentrations were analyzed using a general linear models procedure for repeated measures with time as the repeated variable (SAS, 1985). Least squares means over time were then calculated within a period and orthogonal contrasts were used to detect differences between treatment means. Polynomial regression analysis was performed on all data in order to determine best fit models over time for each parameter measured. Indicator variables (dummy variables) were used to determine differences in intercepts and slopes between treatments.

Results and Discussion

Growth Measurements. Mean values for all parameters of growth (body weight, heartgirth circumference, hip, shoulder, knee, hock and wither height) did not differ significantly between males and females in this study. This agrees with Cunningham and Fowler (1961) who found that both males and females tended to grow uniformly to 18 mo of age.

Body weight (BW) was similar ($P>.05$) between treatments (Table 2) and increased quadratically ($P<.01$) in response to increasing age. These results are similar to the response reported by Boren (1986), who indicated that weight gains in weanling Quarter Horses were best described by a second degree polynomial. This difference in the slope of the two lines would indicate that weanlings gained weight at a dissimilar rate. Mean body weight was higher in horses consuming Diet H vs Diet L at 150 and 240 d of age, however, no difference in body weight was detected at the end of the study. One reason for the observed difference in slope may be that the weanlings in the High group began the trial at a significantly higher mean weight and appear to be reaching their mature weight at an earlier age than those in the Low group.

Mean heartgirth circumference was not affected ($P>.05$) by dietary treatment (Table 2). Heartgirth increased quadratically ($P<.01$) with increasing age in both treatments. Initial heartgirth circumference differed significantly between treatments as indicated by the difference in intercepts. Further, the slopes for the regression of age on heartgirth were different ($P<.01$). The response over time exhibited by heartgirth paralleled changes in body weight. This finding may be explained by the fact that body weight and heartgirth were highly correlated ($r=.96$, $P<.001$) in these weanlings.

Mean values for height at the withers, which are similar to those reported by Boren (1986), did not differ ($P>.05$) between Diets H and L (Table 2). Wither height demonstrated a significant quadratic response with increasing age. Likewise, Boren (1986) found that height at the withers increased quadratically over time in growing horses. Initial wither height was significantly higher for weanlings consuming Diet H than for those consuming Diet L. However, the response of wither height to increasing age was similar between the two groups as no difference ($P>.05$) in slope was detected. These data indicate that weanling growth rate, as measured by height at the withers, is similar over time.

There were no significant differences in mean hip height or hock height between treatments (Table 2). Height at the hip and hock increased quadratically ($P<.01$) over time. In contrast, Boren (1986) reported that both hip and hock height were best described by a third degree (cubic) polynomial. The intercepts for the regression of age on hip and hock height were significantly higher ($P<.05$) for Diet H than Diet L but the slopes were similar ($P>.05$) in their response to increasing age.

Treatment did not significantly alter shoulder height as groups H and L had mean values of 93.75 and 92.80 cm, respectively (Table 2). These values are similar to that of Boren (1986), who found an average shoulder height of 93.95 cm in weanlings measured from 120 to 300 d of age. Regression analysis showed a significant quadratic effect of shoulder height over time. This differs from Boren (1986) who noted that height at the shoulder increased cubically with increasing age. Treatment means for initial shoulder height were the same ($P>.05$) between groups H and L. Further, the slopes for the regression of age on shoulder height were similar ($P>.05$) between treatments.

Mean height at the knee did not differ significantly between weanlings consuming Diets H and L (Table 2). Knee height remained unchanged in both treatments throughout the trial as initial and final values for all weanlings were 38.88 and 39.14 cm, respectively. These data would indicate that there is limited metacarpal growth after 5 mo of age. Similarly, Cunningham and Fowler (1961) discovered in Quarter Horses that 82% of the length from the knee to the ground was present at birth and that knee height reached maturity at 6 mo of age. No significant linear or quadratic effect was noted in either treatment. Boren (1986) reported that weekly measurements for height at the knee were best described by a fourth degree (quartic) polynomial and that knee height increased from 41.06 cm at 4 mo of age to 44.12 cm at 10 mo of age. It is interesting to note however, that the response curve in the previously mentioned study shows that knee height increases at a decreasing rate over time after approximately 170 d of age. Regression of age on knee height demonstrated that initial height at the knee was not significantly different between treatments H and L and that the weanlings' response over time was similar in that no difference ($P>.05$) in slope was found between the two groups.

Osteocalcin. Sex did not influence serum osteocalcin levels as mean concentrations were similar between females and males at 150 (13.02 vs 14.52 ng/ml), 240 (30.31 vs 31.83 ng/ml) and 330 (39.43 vs. 36.36 ng/ml) d of age, respectively. These results agree with Lepage et al. (1992) who found no significant effect of sex in Standardbred horses at ages of less than 6 mo and 6 to 18 mo.

Regression analysis showed that serum osteocalcin levels increased quadratically ($P<.05$) with increasing age in both treatments. This finding is similar to the response of the skeletal growth parameters (hip, hock, wither and shoulder height) in which a significant quadratic effect was observed over time. Furthermore, there was a significant correlation ($P<.01$) between osteocalcin and wither ($r=.81$), hip ($r=.82$), hock ($r=.65$) and shoulder ($r=.72$) height. Knee height and osteocalcin were not highly correlated ($r=.05$, $P>.10$) as cannon bone length did not change over time. These results suggest that as the weanlings approach puberty and skeletal growth occurs, there is an increase in total osteoblastic activity and bone formation indicated by the increase in osteocalcin concentration over time. In support of this observation, Sorva et al. (1997) demonstrated that peak osteocalcin levels were achieved at the time of maximal bone matrix formation and mineralization of bone in boys at puberty.

Mean osteocalcin concentrations for weanlings at 150, 240 and 330 d of age are given in (Table 3). Osteocalcin levels were similar ($P>.05$) among treatments H and L at 150 and 330 d of age. The response of osteocalcin to DCAD at 240 d of age differed from previous periods in that horses consuming Diet L had a lower ($P<.05$) osteocalcin concentration (28.97 ng/ml) than those on Diet H (33.18 ng/ml). Whether this decrease in osteocalcin is due to diet or a difference in growth during this period is unclear. Colle et al. (1988)

confirmed in humans that children with a short stature had lower levels of osteocalcin than an age-matched control group. These results would indicate that skeletal size has an effect on osteocalcin concentration. In the present study, weanlings in the high group began the trial at a higher ($P<.05$) hip and wither height and were taller at 240 d of age. This difference in skeletal maturity may have resulted in the lower osteocalcin levels observed on Diet L. Further research has also proposed that osteocalcin may function in the regulation of osteoclastic recruitment which would increase resorption of calcium and phosphorus from the bone (Carter et al., 1996). This mechanism would therefore suggest that osteocalcin is a predictor of overall bone turnover, which is comprised of both bone formation and resorption.

The results from the present study indicate that osteocalcin is positively correlated with indices of skeletal growth and that concentration increases with increasing age. These findings support the evidence that osteocalcin is a measure of osteoblastic activity which reflects the rate of bone formation. Furthermore, DCAD had no significant effect on growth performance as no difference was detected in any skeletal growth parameter. Further research is needed to quantify changes in bone metabolism in response to DCAD and correlate these data with serum osteocalcin concentrations.

Literature Cited

Boren, S.R. 1986. M.S. Thesis. Oklahoma State Univ., Stillwater.

Carter, S.D. et al. 1996. J. Anim. Sci. 74:2719.

Colle, M. et al. 1988. Acta Paediatr. Scand. 333:196.

Cunningham, K. and S.H. Fowler. 1961. Louisiana State Univ. Agr.Mech.

College Bull. 546.

Lepage, O.M. et al. 1992. Can. J. Vet. Res. 56:379.

SAS. 1985. SAS User□s Guide: Statistics (Version 5 Ed.). SAS Inst. Inc., Cary, NC.

Sorva, R. et al. 1997. Pediatr. Res. 42:528.

Table 1. Composition of treatment diets (as fed basis).

	Treatment	
Ingredient (%)	High	Low
Ground corn	40.00	40.20
Soybean meal	16.55	17.05
Cottonseed hulls	10.00	10.70
TM salt	.50	.50
Limestone	.80	---
Dicalcium Phosphate	.50	.30

Sodium Bicarbonate	1.50	---
Calcium Chloride	---	1.10
Chromic Oxide[a]	.15	.15
Prairie grass hay	30.00	30.00
DCAD	+325	-52
[a]Chromic Oxide was added as an indigestible marker.		

Table 2. Effect of DCAD on Growth Parameters[ab]

	Treatment		
Parameter	High	Low	S.E.M.
ADG, kg	.65	.67	.03
BW, kg	265.84	267.33	11.23
Wither height, cm	129.73	128.28	1.01
Hip height, cm	130.96	129.78	.93
Hock height, cm	55.93	55.28	.52
Shoulder height, cm	93.75	92.80	.80
Knee height, cm	39.12	38.90	.40
Heart girth, cm	144.50	142.32	2.13

[a]Values are least squares means.

[b]Means within a row do not differ (P>.05).

Table 3. Effect of DCAD on serum osteocalcin levels[a].

	Treatment		
Osteocalcin, ng/ml	High	Low	S.E.M.
150 days of age	14.39	13.15	.66
240 days of age	33.18[b]	28.97[c]	1.53
330 days of age	38.75	37.48	2.48

[a]Values are least squares means.

[b,c]Means within a row with different superscripts differ ($P<.05$).