
Volume 30 • Number 8 • August 2010
ELSEVIER
Journal of
Equine
Veterinary
Science
www.j-evs.com

ORIGINAL RESEARCH

A Marine Mineral Supplement Alters Markers of Bone Metabolism in Yearling Arabians

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ABSTRACT

This study tested whether the supplement (Aquacid), high in calcium and other minerals, can alter markers of bone metabolism and mineralization of the equine third metacarpus bone. Radiographs were taken of the left third metacarpus of 14 yearlings. Radiographic bone aluminum equivalence (RBAE) of each cortex was calculated to estimate mineral content. Blood samples were also taken at this time. Horses were ranked according to RBAE and gender, were pair-matched, and randomly assigned to two treatment groups. Each group was provided one of two mineral supplements in addition to their normal diet. The treated group (Aq) received 75 g Aquacid/horse/d, which provided an additional 15 g of calcium. The control group (Co) received 39.5 g of limestone to provide similar amounts of calcium. The study lasted for 112 days, with blood being taken every 28 days. At day 56 and 112, additional radiographs was taken to track changes in RBAE. Blood was analyzed for osteocalcin (a bone formation marker) and serum C-telopeptide crosslaps of type I collagen (a bone resorption marker) to detect alterations in bone metabolism. Using day 0 values as a covariate for bone markers, there was a trend ($P = .07$) for osteocalcin concentrations to be greater in Aq horses than in Co. Likewise, C-telopeptide crosslaps of type I collagen concentrations were greater ($P < .0001$) in Aq horses than in Co. There were minimal differences in RBAE values. These findings suggest Aquacid, while not altering bone mass, increases bone turnover and may aid in repairing damaged bone and preventing injuries.

Keywords: Equine; Bone; Calcium; Osteocalcin; CTX1

INTRODUCTION

A major issue faced by trainers of athletic horses is the prevention of injuries. Although injuries can be in many forms, the majority of them are skeletal injuries.[1] Manipulation of diet and exercise are two management factors used to influence skeletal integrity.[2] Although exercise seemingly can have a greater effect on skeletal strength,[3] nutrition certainly does play a role in bone health. Numerous dietary supplements have been suggested to improve various health parameters, including skeletal health, and are available commercially; however, many of these have yet not been proven in a controlled scientific study to improve bone health.[4] Without a controlled study, it is not possible to determine whether supplementation has an effect.

Aquacid (Marigot Ireland Ltd, Currabinny, Carrigaline, Co., Cork, Ireland) is a supplement produced from a marine plant that is known to have approximately 20% to 26% of calcium. Besides calcium, it is notably high in silicon and boron, but low in phosphorus, although it does contain multiple other minerals. Aquacid has been provided to horses as a gastric buffer as well as for improving skeletal health. It has been speculated that supplemental calcium can reduce the severity of ulcers[5] and can also improve skeletal development, particularly when the dietary calcium supply is marginally adequate.[6] Thus, in cases where calcium is limited, providing additional calcium is beneficial. However, when the supply of dietary calcium meets recommended dietary amounts,[7] there is little evidence to date to suggest that adding additional calcium would be beneficial.

The objective of this study was to test whether supplementation of a calcified seaweed supplement (Aquacid) can have a positive effect on the mineralization of the equine third metacarpus (MCIII) bone and markers of bone metabolism as compared with supplementation of limestone that provides an equivalent amount of calcium when fed with a diet containing the National Research Council (NRC)-recommended[7] amount of calcium.

MATERIALS AND METHODS

Before treatment assignment, 14 yearlings (four geldings and 10 fillies) had dorsal-palmar and lateral-medial radiographs taken of their MCIII metacarpus. A dorsal-palmar view was taken with the cassette positioned against the palmar aspect of the leg, and the beam was centered on the midpoint of MCIII and directed parallel to the ground in the mid-sagittal plane. A lateral-medial view was taken with the cassette placed medially, and the beam was

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0737-0806/$ - see front matter

doi:10.1016/j.jevs.2010.07.003

centered on the midpoint of MCIII and directed parallel to the ground 90° from the mid-sagittal plane. For all views, the x-ray machine was set at 70 kV, with an exposure time of 0.16 seconds and a focal length of 90 cm. An aluminum stepwedge penetrometer was attached to each radiographic cassette to allow for standardization of the radiographs. Radiographs were scanned with a Bio-Rad GS-800 densitometer (Bio-Rad Laboratories, Hercules, CA, USA), and a logarithmic regression was formed using known thickness of steps on the aluminum penetrometer attached to each radiograph to determine bone optical density, which was expressed as radiographic bone aluminum equivalence (RBAE).[8] Maximum optical density values for all cortices were recorded in millimeters of aluminum, 1 cm distal to the nutrient foramen. Total RBAE was then calculated on the dorsal-palmar radiograph using the area under the curve concept[9] and was also reported in millimeters of aluminum. Samples of blood were also taken at this time. The horses were stratified according to RBAE and gender and then pair-matched and randomly assigned to two treatment groups, which were housed in separate pastures with vegetation consisting primarily of orchard grass. Each horse was fed an average of 1.6 kg of oats, which was divided into two equal feedings of 0.8 kg. Oats and pasture were sampled on day 0, 56, and 112 for analysis of calcium and phosphorus concentrations by Equi-Analytical (Ithaca, NY, USA). A sample of Aquacid was sent to Western Analysis, Inc. (Salt Lake City, UT, USA) to determine the complete elemental composition. In addition to their daily ration, each group was supplemented with a mineral supplement as well. The treated group received the calcified seaweed supplement at the rate of 75 g Aquacid/d/horse mixed in with their daily concentrate allotment of oats. The 75 g Aquacid provided an additional 15 g of calcium and a negligible amount of phosphorus (0.07 g) per horse each day. The control group received 39.5 g of limestone mixed in with their oats to provide a similar dose of calcium (15 g) and a negligible amount of phosphorus (0.008 g). The additional 15 g of calcium provided by Aquacid and limestone was deemed an amount that was sufficient to reveal treatment differences but that would not greatly alter the calcium to phosphorus ratios of the diets. Horses remained a part of the study for 112 days, with blood samples being taken every 28 days. At days 56 and 112, additional sets of radiographs were taken for determination of changes in the mineral content of MCIII. The blood samples were analyzed for concentrations of osteocalcin (OC; a marker of bone formation) and serum C-telopeptide crosslaps of type I collagen (CTX-1), which is an enzyme immunoassay for quantitative assessment of bone resorption, to help detect any alterations in bone metabolism. Serum OC concentration was determined using a commercial competitive immunoassay (MicroVue Osteocalcin; Quidel Corporation, San Diego, CA, USA). On all days, serum samples were diluted with double-deionized water in a ratio of 1:5. The concentrations of serum C-telopeptide crosslaps of type I collagen were determined using a commercial immunoassay (Serum Crosslaps; Immunodiagnostics Systems, Scottsdale, AZ, USA). Data were normally distributed and analyzed with PROC MIXED analysis in SAS 9.1 (SAS Institute, Cary, NC). To account for variation in initial samples, values for day 0 were used as a covariate in the analyses. Main effects in the statistical model were horse, treatment, and day; and the interaction of treatment*day was also analyzed. Significance was considered at $P \leq .05$, and trends toward significance were considered as $P < .1$. The protocol was approved by the Michigan State University Institutional Animal Care and Use Committee (AUF# 07/07-114-00).

RESULTS

From the analysis of the pastures, it was found that horses obtained an average of 39 g of calcium and 19 g of phosphorus from the forage and grain provided to them. This meets or exceeds the 2007 NRC[7] guidelines of 32 g calcium and 19 g phosphorus for horses of this age and size. Both the Aquacid and the limestone were mixed in with the grain portion of the diet and were readily consumed with no signs of refusals. Hence, combined with their respective mineral supplement, total daily calcium intake for both groups was 54 g per horse, whereas phosphorus intake remained unchanged. The horses were receiving 122% of their daily calcium requirement with their basal diet alone. With supplementation, they were receiving 169% of their daily calcium requirement. In both the cases, animals were meeting, but not exceeding, their daily phosphorus requirements.

No overall treatment effects were seen in any of the views of the MCIII (Table 1). Likewise, there were minimal differences on different days, with only a trend ($P = .06$) for a difference in the total RBAE. Similarly, day x treatment interactions were not seen except for in the total RBAE ($P = .03$). These changes in total RBAE were reflective of a 14% decrease in total RBAE in the limestone control horses from day 56 to 112 ($P = .01$), which also produced a trend for the Aquacid-treated horses to have an 11% greater total RBAE than the limestone control horses on day 112 ($P = .08$).

An overall trend ($P = .07$) for serum OC concentration to be 6.2 ng/mL more in horses treated with Aquacid than in control horses treated with limestone (Table 2) was seen. The day effect was highly significant ($P < .0001$) but there was no day x treatment effect. Overall concentrations of CTX-1 were 0.10 ng/mL more in the Aquacid treated horses than in the limestone control horses ($P = .001$); the day effect was also highly significant ($P < .0001$), with concentrations generally decreasing over

Table 1. Radiographic bone aluminum equivalence (RBAE) in millimeters of aluminum (dorsal, palmar, lateral, medial) or millimeters squared of aluminum (total) of the third metacarpal of horses supplemented with either the Aquacid treatment or limestone control during the course of a 112-day study with day 0 values used as a covariate and the significance of treatment (Trt), day, and day × Trt interactions

View	Variable	Limestone (SEM)	Aquacid (SEM)	*P* values
Dorsal RBAE	56	16.3 (0.3)	16.4 (0.3)	
	112	16.4 (0.3)	16.5 (0.3)	
	Trt			.71
	Day			.80
	Day × Trt			.89
Palmar RBAE	56	15.2 (0.3)	15.2 (0.3)	
	112	15.0 (0.3)	14.6 (0.3)	
	Trt			.60
	Day			.15
	Day × Trt			.43
Lateral RBAE	56	19.4 (0.4)	19.4 (0.4)	
	112	19.3 (0.4)	19.4 (0.4)	
	Trt			.95
	Day			.75
	Day × Trt			.97
Medial RBAE	56	20.8 (0.4)	20.9 (0.4)	
	112	20.2 (0.4)	20.8 (0.4)	
	Trt			.36
	Day			.47
	Day × Trt			.55
Total RBAE	56	604 (23)	574 (23)	
	112	522 (23)	584 (23)	
	Trt			.57
	Day			.06
	Day × Trt			.03

the course of the study but, once again, there were no day x treatment interactions ($P = .20$).

DISCUSSION

Providing calcium supplementation when calcium requirements are not met or are only marginally met has been shown to positively affect equine bone.[6] However, when nutritional requirements are met in a balanced ratio, making minor alterations in diet do not typically result in considerable changes in indices of equine bone health.[3] In human beings, calcium supplementation may prevent loss of bone mineral density in postmenopausal women and may reduce vertebral fractures, and there is evidence to suggest dose-dependent benefits of vitamin D both with and without calcium for retaining bone mineral density.[10] However, even in human beings, there are minimal data suggesting that supplementing calcium beyond requirements, regardless of the source, can produce demonstrable effects on bone or markers of bone metabolism. As a result, the findings in this study were somewhat surprising. The basal diet of the horses in this study exceeded the daily requirements for calcium of these animals and it also met their requirements for phosphorus. Although exceeding minimal requirements, the amount of calcium provided with supplementation would be considered within a normal range of dietary intake. Even with supplementation, the horses were provided equivalent amounts of calcium and phosphorus, and so the main difference between treatments seems to be not only the source of the calcium, but also the other minerals present only in Aquacid – many of which have previously been shown to be beneficial for bone health.

The mineral source in this study had minimal effects on RBAE. This measurement evaluates bone optical density, which is an estimate of bone mineral content, by using radiographic photodensitometry.[8] Although this measurement is not as precise as some other techniques used to measure bone mineral content or density, it has been used in numerous studies evaluating nutrition[2,6,11,12] and

Table 2. Serum osteocalcin (ng/mL) and serum C-telopeptide crosslaps of type I collagen (CTX-1; ng/mL) in horses supplemented with either the Aquacid treatment or limestone control during the course of a 112-day study with day 0 values used as a covariate and the significance of treatment (Trt), day, and day × Trt interactions

Measurement	Variable	Limestone (SEM)	Aquacid (SEM)	*P* values
Osteocalcin	28	51.1 (2.6)	59.7 (2.7)	
	56	56.0 (2.5)	60.7 (2.7)	
	84	39.7 (2.7)	44.3 (2.7)	
	112	41.5 (2.5)	48.4 (2.6)	
	Trt			.07
	Day			<.0001
	Day × Trt			.62
CTX-1	28	0.30 (0.02)	0.40 (0.02)	
	56	0.39 (0.02)	0.48 (0.02)	
	84	0.22 (0.03)	0.36 (0.02)	
	112	0.26 (0.02)	0.30 (0.02)	
	Trt			.0009
	Day			<.0001
	Day × Trt			.20

exercise,[2,12-14] with differences in RBAE typically being noted more often with alterations in exercise. In this study no differences were observed in the RBAE of any of the individual cortices, there was, however, a day x treatment interaction ($P = .03$) with the total RBAE. This difference was influenced by a decrease in total RBAE from day 56 to day 112 in the limestone control group ($P = .01$). The cause of such a decrease is unknown and probably should not be over-interpreted as it may simply be an artifact. Although reflecting a positive advantage for the Aquacid treated group, given that there was no change in total RBAE during this period, this lack of a response suggests limited influence of that treatment on this variable.

In contrast, the changes in markers of bone turnover were fairly interesting. The day differences are expected in growing horses because markers of bone turnover generally decrease as animals reach maturity.[15] However, more interestingly, the trend ($P = .07$) for the Aquacid treated horses to have greater OC concentrations as compared with the limestone controls, combined with the highly significant increase ($P = .0009$) in CTX-1 seen with the treated group, suggests that the bone turnover was enhanced with Aquacid supplementation. Similar enhanced bone turnover was reported when bull calves were supplemented with sodium zeolite A.[16] Additionally, broodmares supplemented with sodium zeolite A showed a trend for increased OC as compared with control broodmares.[17] Considering that sodium zeolite A supplementation was reported to decrease injuries to horses in race training[18] and that no differences were seen in RBAE between supplemented and unsupplemented horses,[19] a possible explanation for the decreased injury rate in race horses is enhanced bone turnover that resulted in the repair of subclinical damage associated with training and racing before it becomes clinical. This similarity in response (minimal alterations in bone mineral content but enhanced bone turnover) of the Aquacid treated horses in this group to the sodium zeolite A treated calves raises the question as to whether it is the source of calcium that caused the treatment differences seen in the current study or whether it was some other mineral. Although Aquacid contains approximately 20% to 26% of calcium, it also contains numerous other minerals. Minerals that are known to have an influence on bone and present in notable concentrations in Aquacid include silicon, magnesium, and boron.

Sodium zeolite A is a bioavailable source of silicon[20] and it is the silicon in sodium zeolite A that is believed to have caused the beneficial effects in the race horse study.[18] The silicon content of Aquacid, as determined by Western Analysis, Inc., was 1,544 ppm and potentially this silicon played a role in the positive response seen in the treated horses. This is similar to the concentration of silicon in sodium zeolite A, which has been reported to be 1,632 ppm.[16]

Boron was present in a smaller amount (43 ppm) than calcium and silicon, but this amount might still play a role in bone metabolism. In human beings, many individuals do not consume more than 1 mg of boron per day although it has been suggested that the acceptable safe range for adult human beings could be between 1 and 13 mg/d.[21] At 43 ppm, the 75 g of Aquacid would provide an additional 3.2 mg/d/horse. Numerous laboratories have shown that low boron intake results in impaired bone health.[22] Recent research has shown that boron stimulates bone formation.[23] Additionally, it was seen that rats fed a boron-deficient diet (0.1 ppm) had decreased maximal femur fracture force compared with rats supplemented with boron at 3 ppm, confirming that boron is beneficial to cortical bone strength and also trabecular bone microarchitecture.[24]

Obviously there are several minerals besides calcium that may have been responsible for the altered bone metabolism in the Aquacid treated horses. However, trying to determine which individual mineral or minerals are responsible for the response would be difficult, if not impossible. This is often the case when evaluating the effect of specific minerals on various parameters of equine health or performance. Creating purified diets for an animal as large as a horse is not practical and given that there are many interactions between minerals that can alter mineral absorption, thoroughly determining the specific effect of a single mineral can be challenging – especially when the amounts are fed at a practical level.

It should be noted that there have been concerns related to health when iodine is fed in excess. Considering that Aquacid is derived from a marine algae source, one concern is whether it is high in iodine as some marine mineral sources contain greatly elevated amounts.[7] Aquacid contains less than 11 ppm of iodine, hence iodine toxicities would not be an issue.

CONCLUSION

In conclusion, supplementing Aquacid to young horses in an amount designed to provide calcium above NRC[7] requirements produced altered bone metabolism compared with that in horses supplemented with limestone designed to provide an equal amount of calcium. Whether this altered bone metabolism was because of the source of calcium or because of additional dietary silicon or boron being provided by the Aquacid cannot be determined from this study. Although differences in the amount of bone mineral present in the MCIII as determined through radiographic photodensitometry were negligible, enhanced bone turnover may provide a means to remove and repair old or damaged bone before a clinical injury manifests itself. However, determining whether supplementing Aquacid could result in injury reduction to horses in training was beyond the scope of this study.

ACKNOWLEDGMENTS

The authors acknowledge Michael Ryan and Denise O'Gorman of Marigot Ireland Ltd, Currabinny, Carrigaline, Cork, Ireland, for support during the development and implementation of this project.

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