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Daily access to pasture turnout prevents loss of mineral in the third metacarpus of Arabian weanlings[1]

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ABSTRACT: Seventeen Arabian weanlings were used to determine the influence of housing on third metacarpal bone mass. Animals were separated into three treatment groups: Pasture (n = 6), Stall (n = 5), and Partial-Pasture (n = 6). Radiographs of the left third metacarpus were taken every 28 d to determine radiographic bone aluminum equivalence (RBAE). Serum was collected every 14 d and analyzed for osteocalcin, carboxyterminal telopeptide of type I collagen (ICTP), and keratan sulfate. Hip and wither height, BW, and cannon circumference were measured every 28 d. Lateral RBAE in the pastured group increased linearly from d 0 to d 56 ($P = 0.001$). In the Pasture group, total RBAE increased from d 0 to 56 ($P = 0.05$) and medial RBAE tended to increase from d 0 to d 28 ($P = 0.06$). The Partial Pasture group increased from d 0 to 56 in medial ($P = 0.02$) and tended to increase in total RBAE ($P = 0.08$). Although the Stall group demonstrated an increase in total RBAE from d 0 to 56 ($P = 0.04$), the Partial Pasture group tended to have greater total RBAE than the Stall group at d 28 ($P = 0.08$), and the Pasture group had greater lateral RBAE at d 28 ($P = 0.005$) and 56 ($P = 0.007$) than did the Stall group. At d 28, medial RBAE was greater in the Pasture ($P = 0.003$) and Partial Pasture ($P = 0.05$) groups than in the Stall group. Pasture and Stall groups tended to decrease in osteocalcin ($P = 0.06$), whereas Partial Pasture weanlings decreased ($P = 0.01$) from d 0 to 56. All treatment groups decreased from d 0 to 56 in ICTP ($P < 0.01$). Pastured weanlings decreased from d 0 to 42 in serum keratin sulfate ($P < 0.05$), whereas the Stall group decreased from d 0 to 56 ($P = 0.05$). All treatment groups increased in wither height ($P \leq 0.01$), hip height ($P \leq 0.001$), and BW ($P \leq 0.01$). Both the Pasture and Partial Pasture weanlings demonstrated greater cannon circumference than Stall weanlings on d 28 ($P \leq 0.05$) and 56 ($P \leq 0.005$). These data demonstrate that pasture rearing or 12-h daily turnout is beneficial to maintaining and increasing bone mineral content in weanling Arabian horses.

Key Words: Bone, Horses, Housing, Osteocalcin

Introduction

According to Wolff's Law, bone adapts to the forces placed on it by altering its architecture and mass (Woo et al., 1981). Thus, as habitual loading increases, such as with exercise, so does bone mass. Likewise, as loading decreases, bone mass also decreases. Numerous studies have demonstrated that depriving animals of exercise is detrimental to bone strength. Laying hens housed in battery cages had 54% weaker bones than those housed in percheries (Knowles and Broom, 1990). Woo et al. (1981) observed that exercised swine had a 35% increase in maximum-load strength of the femur over those swine not undergoing exercise. Hoekstra et al. (1999) found that yearling Arabian horses demonstrated a decrease in the rate of bone formation, an increase in bone resorption, and a decrease in bone mineral content when kept in stalls compared with control horses maintained on pasture. McCarthy and Jeffcott (1992) found that yearling horses exercised on a treadmill demonstrated an increase in bone density compared to those not exercised.

In the horse industry, horses are often stalled for 3 to 4 mo before a sale. This practice could leave horses with weaker bones that are more prone to injury at the onset of training. Nielsen et al. (1997) found that as mineral content of the third metacarpus decreased, the incidence of bone-related injuries increased. The hypothesis of this study was that stalling weanlings results in a negative effect on bone mass. The first objec-

Table 1. Calculated daily nutrients (DM basis) in Arabian weanlings maintained on Pasture, in 3.1- × 3.1-m stalls, or on pasture for 12 h and in stalls for 12 h for 56 d after weaning (d 0)

Nutrient	Stall		Pasture		Partial Pasture	
	Nutrient content	Calculated total daily intake	Nutrient content	Calculated total daily intake	Nutrient content	Calculated total daily intake
DE	3.06 Mcal/kg	14.7 Mcal	3.0 Mcal/kg	14.3 Mcal	3.02 Mcal/kg	14.5 Mcal
CP	14.0%	670.0 g	11.9%	570.0 g	12.9%	620.0 g
Ca	0.27%	12.8 g	0.16%	7.8 g	0.21%	10.3 g
P	0.33%	15.9 g	0.33%	16.0 g	0.33%	15.9 g

tive was to determine whether housing weanling horses in stalls impedes bone and cartilage development compared with that in weanling horses maintained on pasture. The second objective was to determine whether 12-h daily turnout of stalled weanlings could prevent the loss of bone mass.

Materials and Methods

Animal Management

Seventeen Arabian weanlings from the Michigan State University Horse Teaching and Research Center were stratified by age and randomly assigned to three treatment groups. Horses remained on the study for 56 d. Before the start of the study, weanlings were kept outside with their dams. Horses were weaned at three time periods, and two horses from each treatment group were weaned at each period. The first period began on August 25, the second on September 21, and the third on October 19. The Pasture group (n = 6, mean age = 136 d) was maintained on a 433- × 54-m pasture allowing free access to exercise. The Stall group (n = 5, mean age = 134 d) was housed in 3.1- × 3.1-m stalls with no free access to exercise. The Partial-Pasture group (n = 6, mean age = 135 d) was maintained with the pasture group for 12 h and housed in 3.1- × 3.1-m stalls for 12 h each day. Each group was individually fed 0.9 kg corn, 0.85 kg oats, and 0.05 kg 40% protein pellets (Kent Feeds, Muscatine, IA) twice daily (0700 and 1900) to meet NRC requirements. Horses on pasture had ad libitum access to a mixed grass pasture, and the stalled horses had ad libitum access to alfalfa and grass mixed hay (Table 1). Water and trace mineral salt blocks were available at all times to all horses.

Bone Mineral Content

On d 0, 28, and 56, radiographs of the left third metacarpal were taken to determine radiographic bone aluminum equivalence (**RBAE**) (Meakim et al., 1981). Dorsal-palmar radiographs were taken at 17 mA, 0.08 s, 70 kV at 71 cm focal film distance, using mobile x-ray equipment and medical x-ray film. The radiographic cassette was positioned against the palmar surface of the leg, allowing the beam to be parallel to the ground and centered at the midpoint of the metacarpal region.

An aluminum stepwedge penetrometer was used as a reference standard for each radiograph. Radiographs were scanned at the level of the nutrient foramen, to determine RBAE, using the Bio-Rad GS-700 Imaging Densitometer (Bio-Rad Laboratories, Hercules, CA). Logarithmic regression was used to determine RBAE of the lateral and medial cortices (mm Al) using the thickness of the stepwedge and the maximum optical density readings of these cortices (Meakim et al., 1981). Total RBAE (mm^2 Al) was used to determine density and volumetric changes using the total area of bone divided by the total area of the aluminum stepwedge (Nielsen and Potter, 1997). The total area under the stepwedge corresponding to the steps with thickness of 14, 17, 20, 23, and 26 mm Al and the total area of the third metacarpal bone curve were used. The measured area of the stepwedge was 1,270 mm^2. The total RBAE of the third metacarpal bone was determined by multiplying the area of the third metacarpus (mm × optical density) by 1,270 mm^2 and dividing by the scanned area of the stepwedge (mm × optical density).

Serum Analyses

Every 2 wk (d 0, 14, 28, 42, 56) blood was collected via jugular venipuncture. Blood was allowed to clot for 1 to 2 h and was subsequently centrifuged at 1,340 × *g* for 15 min at room temperature. Serum was collected and stored in a –20°C freezer until it was analyzed. Serum was analyzed for osteocalcin, carboxyterminal telopeptide of type I collagen (**ICTP**), and keratan sulfate. Intraassay CV were determined using unknown samples (7.4% for serum osteocalcin, 5.1% for keratan sulfate, 9.5% for ICTP).

Osteocalcin. Osteocalcin was quantified using Novocalcin ELISA kits (Metra Biosystems, Mountain, View, CA) according to the manufacturer's instructions. Serum was diluted between 1:10 and 1:15, depending on the sample. Each sample was run in duplicate. Standards and controls were reconstituted using 0.5 mL of 1× wash buffer. Twenty-five microliters of standard, sample, or control was added to each well with 125 μL of anti-osteocalcin antibody. This mix was incubated at room temperature for 2 h and then each well was washed three times with 300 μL of 1× wash buffer. Enzyme conjugate (150 μL) was added to each well and incubated for 1 h at room temperature. Each well was

then washed with 300 μL of 1× wash buffer. The final step was the 40-min incubation of 150 μL of working substrate solution. Each plate was then read at 405 nm optical density on a Spectra Max 340 plate reader (Molecular Devices, Sunnyvale, CA).

ICTP. Carboxyterminal telopeptide of type I collagen was quantified using ICTP ^{125}I RIA Kit (Incstar Corp., Stillwater, MN) according to the manufacturer's instructions. Each sample, standard, control, and nonspecific binding tube was run in duplicate. The tubes were set up as follows: nonspecific binding tubes, 100 μL of sample and 200 μL of distilled water; standards (0 and A–F), 100 μL of standard and 200 μL of ICTP antiserum; controls and samples, 100 μL of serum and 200 μL of ICTP antiserum. Each tube was incubated for 2 h at 37°C with 200 μL of ^{125}ICTP. Separation reagent (500 μL) was added to each tube and incubated for 30 min at 25°C. Tubes were then centrifuged for 30 min at $2,000 \times g$ at 10°C. The supernate was decanted and placed in the 1290 GammaTrac Gamma Counting System (TM Analytic, Elk Grove Village, IL).

Keratan Sulfate. Keratan sulfate was quantified by an ELISA with an inhibition step previously described (Williams et al., 1988). The keratan sulfate standard (generously provided by R. J. Todhunter, Cornell University, Ithaca, NY) was diluted at 1:24 and serially diluted onto an uncoated plate. Samples were diluted to 1:4 (70 μL of sample + 210 μL of PBS-Tween 20:1% BSA buffer) and serially diluted to 1:32 in the uncoated plate. Both the standard and the samples were diluted with PBS-Tween 20:1% BSA, pH 5.3. For the inhibition step, a 1:12,000 dilution of the anti-keratan sulfate monoclonal antibody (ICN Pharmaceuticals, Costa Mesa, CA) was made and 140 μL was added to each well. Each plate was covered and incubated, with shaking, for 1 h at room temperature. The plates were then stored at 4°C overnight. Coating buffer was removed from the coated plates with three washes of PBS-Tween 20, pH 5.3, for 5 min. Two hundred microliters from each well from the inhibition step was transferred to the washed, coated plates. The coated plate was covered and incubated, with shaking, for 1 h at room temperature. Ten minutes prior to use, the horseradish peroxidase-conjugated anti-mouse IG antibody (second antibody) (ICN Pharmaceuticals) was diluted in PBS-Tween 20:1% BSA, pH 5.3, at a 1:1,000 dilution. After 1 h of incubation, the inhibition mixture in the coated plates was removed and 5-min washes were performed three times. The second antibody (200 μL) was added to each well and incubated, with shaking, for 1 h at room temperature. After 1 h of incubation, the inhibition mixture in the coated plates was removed and 5-min washes were performed three times. *O*-Phenylenediamine was added (200 μL/well) and incubated at room temperature for 15 min (Sigma Chemical, St. Louis, MO). To stop the substrate-enzyme color development, 50 μL/well of 2 *M* H$_2$SO$_4$ was added. The plates were then read with the Spectra Max 340 plate reader (Molecular Devices Corp.).

Growth Measurements

Wither height, hip height, BW, and cannon circumference were measured every 28 d. Wither and hip heights were measured using an altitude stick from the ground to the point of the hip or the withers. Body weight was estimated using a weight tape placed around the girth. Cannon circumference was measured with a measuring tape wrapped around the midsection of the third metacarpus.

Statistics

Differences among treatments, day of study, and day × treatment interactions were determined using a two-factor ANOVA (PROC MIXED, SAS Inst. Inc., Cary, NC). The blocking effect of weaning in three periods was included in the model. This was added to account for any differences that may have existed due to weaning time. An LSMEANS statement was included in the analysis to obtain treatment means, difference between means, and standard errors. Serum osteocalcin was normalized (d-0 values subtracted from other days to examine changes from d 0) to account for treatment differences that existed at d 0. A *P*-value of less than 0.05 was considered significant, and a trend was investigated at a *P*-value of less than 0.10. Orthogonal polynomial contrasts were used to investigate linear and quadratic trends in growth and cannon bone measurements over time within each treatment group.

Results

Bone Mineral Content

Medial RBAE. Medial RBAE tended to increase in the Pasture group from d 0 to 28 (*P* = 0.06, Figure 1). The Partial Pasture group had an increase from d 0 to 56 (*P* = 0.02). The Stall group was lower than the Pasture (*P* = 0.003) and Partial Pasture groups (*P* = 0.05) on d 28 and remained lower than the Partial Pasture group (*P* = 0.01) on d 56.

Lateral RBAE. The pastured weanlings had increased lateral RBAE on d 28 compared to d 0 (*P* = 0.05) and continued to increase to d 56 (*P* = 0.001, Figure 2). This resulted in a significant linear increase in lateral RBAE (*P* = 0.005). Partial-Pasture weanlings had lower lateral RBAE than Pasture weanlings on d 56 (*P* = 0.02). The Stall weanlings tended to have lower lateral RBAE than the Partial-Pastaure weanlings on d 28 (*P* = 0.08). The Stall group had lower lateral RBAE than Pasture weanlings on d 28 (*P* = 0.005) and 56 (*P* = 0.007).

Total RBAE. Pasture weanlings had greater total RBAE on d 56 than on d 0 (*P* = 0.05) and tended to have greater total RBAE than Stall weanlings on d 28 (*P* = 0.08, Figure 3). The Partial-Pasture group tended to have greater total RBAE on d 56 than on d 0 (*P* = 0.08) and had greater total RBAE than the Stall weanlings on d 28 (*P* = 0.05). The total RBAE of Stall weanlings was greater at d 56 than at d 0 (*P* = 0.04).
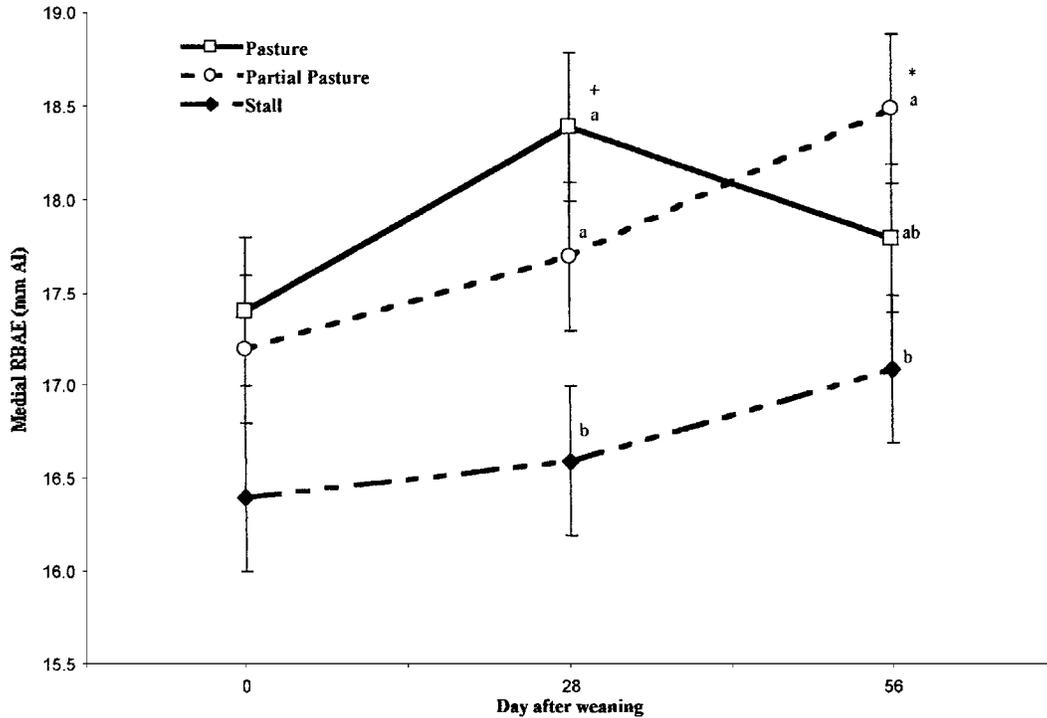
Figure 1. Medial radiographic bone aluminum equivalence (RBAE) (mm Al) vs day of project in weanling Arabians maintained on pasture, in 3.1- × 3.1-m stalls, or on pasture for 12 h and in stalls for 12 h for 56 d after weaning (d 0). *Different from d 0 ($P < 0.05$). †Different from d 0 ($P < 0.10$). [a,b]Treatments with different superscripts at a given day differ ($P < 0.05$).
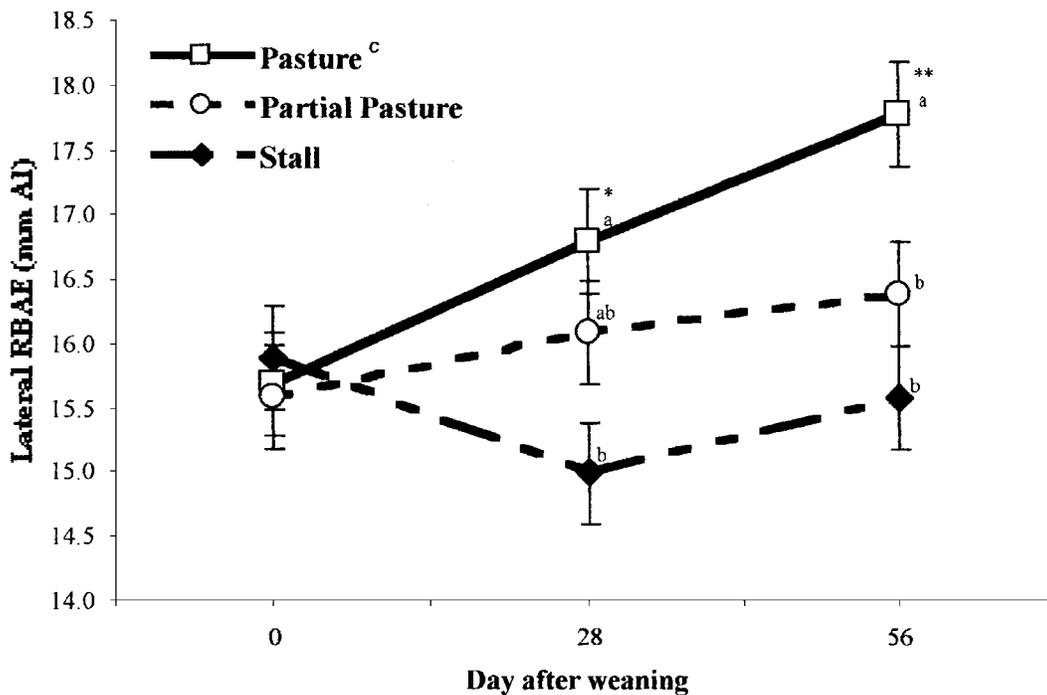

Figure 2. Lateral radiographic bone aluminum equivalence (RBAE) (mm Al) vs day of project in weanling Arabians maintained on pasture, in 3.1- × 3.1-m stalls, or on pasture for 12 h and in stalls for 12 h for 56 d after weaning (d 0). *Different from d 0 ($P < 0.05$). **Different from d 0 ($P < 0.01$). [a,b]Treatments with different superscripts at a given day differ ($P < 0.05$). [c]Linear increase significant for Pasture at $P = 0.005$.
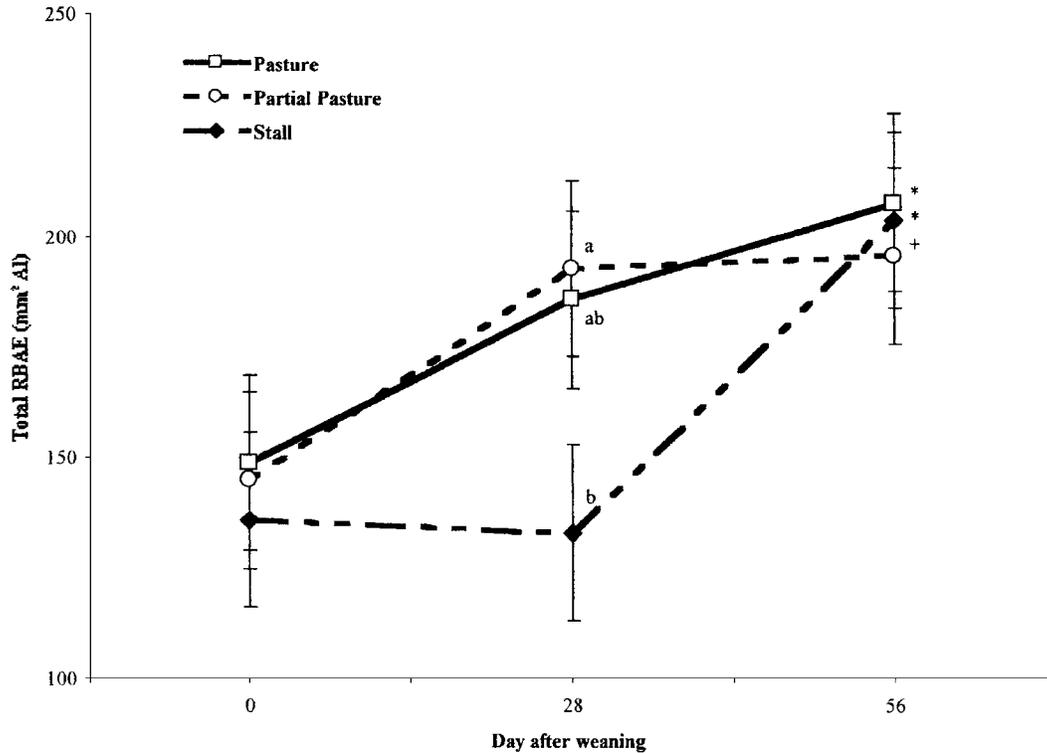
Figure 3. Total radiographic bone aluminum equivalence (mm^2 Al) vs day of project in weanling Arabians maintained on pasture, in 3.1- × 3.1-m stalls, or on pasture for 12 h and in stalls for 12 h for 56 d after weaning (d 0). *Different from d 0 ($P < 0.05$). †Different from d 0 ($P < 0.10$). [a,b]Treatments with different superscripts at a given day differ ($P < 0.05$).

Serum Markers

Osteocalcin. Serum osteocalcin of Pasture weanlings was lower at d 42 than at d 0 ($P = 0.006$) and remained low at d 56 ($P = 0.06$, Table 2). Partial-Pasture weanlings had lower osteocalcin concentrations at d 56 that at d 0 ($P = 0.01$). The Stall group tended to have lower serum osteocalcin concentrations at d 56 than at d 0 ($P = 0.06$). On d 42, Stall weanlings had greater serum osteocalcin concentration than Partial-Pastured weanlings ($P = 0.05$).

ICTP. All treatment groups had lower serum ICTP concentrations at d 56 than at d 0 ($P < 0.002$), with no treatment differences observed (Table 2).

Keratan Sulfate. No treatment differences were observed in serum keratan sulfate concentration (Table 2). The Pasture group had decreased serum keratan sulfate concentrations at d 28 ($P = 0.04$), which remained low to d 42 ($P = 0.05$) compared with d 0. Serum keratan sulfate concentrations in Stall weanlings decreased from d 14 to 56 ($P = 0.05$). There was no change in the serum concentration of the Partial-Pasture weanlings.

Growth Measurements

All treatment groups increased in wither height, hip height, and BW from d 0 to 56 (Table 3). In cannon circumference, both Pasture and Partial-Pasture groups had linear increases from d 0 to 56 ($P = 0.0001$), whereas the circumference in Stall weanlings did not increase. Both Pasture and Partial-Pasture weanlings had greater cannon circumferences than Stall weanlings on d 28 ($P < 0.05$) and 56 ($P = 0.004$, Table 3).

Discussion

Bone Mineral Content

Bone growth is rapid in foals and weanlings and any alteration during development may have a large impact on bone strength later in life (Nunamaker et al., 1990). Additionally, exercise may be advantageous in growing bone due to its more responsive nature in its adaptation to stress than mature bone (Raub et al., 1989). In the present study, total bone mineral content (RBAE) and that in the lateral cortex increased in the Pasture group, and there was a trend for an increase in mineral content of the medial cortex. The Stall group remained lower than the Pasture group in the medial and lateral views and tended to be lower in total bone mineral content. These data are similar to those from a study investigating the influence of housing on yearling horses (Hoekstra et al., 1999). In that study, pastured horses had greater bone mineral content than stalled horses on d 28 and 56 in the lateral and medial cortices.

Table 2. Serum osteocalcin, carboxyterminal telopeptide of type I collagen (ICTP), and keratan sulfate (KS) in Arabian weanlings maintained on pasture, in 3.1×3.1 m stalls, or on pasture for 12 h and in stalls for 12 h for 56 d after weaning (d 0)

Treatment	Day					SEM
	0	14	28	42	56	
	Osteocalcin, ng/mL[g]					
Pasture	121[ae]	115[ab]	109[ab]	74[ce]	89[abc]	12
Partial Pasture	155[af]	114[b]	106[b]	89[be]	113[b]	12
Stall	145[ef]	124	133	126[f]	109	13
	ICTP, ng/mL					
Pasture	20[a]	17[b]	18[b]	14[c]	16[bc]	0.9
Partial Pasture	19[a]	16[b]	16[b]	16[b]	15[b]	0.8
Stall	19[a]	17[bc]	18[ab]	14[d]	15[cd]	0.8
	KS, ng/mL					
Pasture	537[a]	587[a]	465[b]	460[b]	491[ab]	12
Partial Pasture	496	508	493	502	475	12
Stall	486[ab]	497[a]	490[ab]	460[ab]	434[b]	13

[a,b,c,d]Days with different superscripts within rows differ ($P < 0.05$).
[e,f]Treatments with different superscripts within columns differ ($P < 0.05$).
[g]Serum osteocalcin concentrations from d 0 were used as a covariate due to significant pretreatment differences.

A Partial-Pasture group was used to determine how much time on pasture was necessary to prevent the bone loss seen by Hoekstra et al. (1999). The present study indicates that the Partial-Pasture horses had greater medial and total RBAE and tended to have greater lateral RBAE than the Stall group. These data demonstrate the benefits of 12-h daily turnout on bone strength, in that the results in the Partial-Pasture group are similar to those of the Pasture group.

The increases of bone mineral content in the Pasture and Partial-Pasture groups, and the differences found between both groups and the Stall group, are supported by previous studies looking at effects of exercise or immobilization on the equine third metacarpus. McCarthy and Jeffcott (1992) observed an increase in bone mineral content of exercised Thoroughbred yearlings. By wk 12, exercised horses had greater bone mineral content than unexercised horses. This increase in the exer-

Table 3. Wither height, hip height, weight, and third metacarpal circumference in Arabian weanlings maintained on pasture, in $3.1\text{-} \times 3.1\text{-m}$ stalls, or on pasture for 12 h and in stalls for 12 h for 56 d after weaning (d 0)

Treatment	Day of study			SEM	Contrasts[f]	
	0	28	56		Linear	Quadratic
	Wither height, cm					
Pasture	119.9[a]	122.1[a]	125.1[b]	1.2	0.01	NS
Partial Pasture	119.7[a]	124.4[b]	124.9[b]	1.2	0.006	NS
Stall	119.2[a]	122.9[b]	125.1[b]	1.4	0.007	NS
	Hip height, cm					
Pasture	127.0[a]	129.7[b]	131.8[c]	1.1	0.03	NS
Partial Pasture	124.7[a]	127.5[b]	132.0[c]	1.1	0.0002	NS
Stall	123.8[a]	127.1[b]	129.5[c]	1.2	0.008	NS
	Weight, kg					
Pasture	186[a]	205[b]	235[c]	7	0.0001	NS
Partial Pasture	195[a]	209[a]	228[b]	7	0.002	NS
Stall	188[a]	208[b]	238[c]	8	0.0001	NS
	Cannon circumference, cm					
Pasture	15.2[a]	15.9[bd]	16.6[cd]	0.2	0.0001	NS
Partial Pasture	15.5[a]	15.9[bd]	16.5[cd]	0.2	0.005	NS
Stall	15.3[ab]	15.2[ae]	15.6[be]	0.2	NS	NS

[a,b,c]Days with different superscripts within rows differ ($P < 0.05$).
[d,e]Treatments with different superscripts within columns differ ($P < 0.05$).
[f]Contrast: Linear = linear effect within treatments; Quadratic = quadratic effect within treatments. Contrasts were not considered significant (NS) if $P > 0.10$.

cised horses was determined to be due to the horse exercising to its maximum potential (galloped for up to 1.2 km or until exhaustion). In the present study, when the Partial-Pasture horses were brought to the pasture every morning they regularly ran off to join the other weanlings, and after all the horses were together they often ran and chased one another. This was probably enough loading on the bone to initiate new bone formation. This parallels Jeffcott's (1991) suggestion that growing horses should be pastured for 12 h/d to develop normal bones and joints. Pratt (1982) stated that the most efficient way to increase bone strength is to run short, vigorous sprints. Therefore, it is likely that even less than 12 h of turnout per day may be adequate.

The Stall group became very active in their stalls after d 28 of the study. Their activity included jumping, bucking, and rearing, as well as running around in small circles. This increase in activity may explain the increase in total RBAE of the Stall group from d 0 to 56. This activity may have provided enough strain on the third metacarpus to increase the mineral content. The increase in bone mineral content may not have been great enough to see in the lateral and medial views separately, but in the combined views it was great enough to detect in the total RBAE. In the medial and lateral cortices the Stall group demonstrated no changes with time, although they remained lower than the Pasture group.

The only concern as to whether 12 h of turn out is as beneficial as continuous turnout was that Partial-Pasture weanlings demonstrated lower lateral RBAE than Pasture weanlings on d 56. The Partial-Pasture weanlings demonstrated no increase in lateral RBAE, which may account for the difference between them and Pasture weanlings in lateral RBAE on d 56. However, the reason for a lack of an increase in the lateral RBAE of the Partial-Pasture weanlings is unclear.

Serum Analyses

All treatment groups had, or tended to have, decreased serum osteocalcin concentrations. On d 42, Stall weanlings demonstrated greater serum osteocalcin concentration than the Pasture and Partial-Pasture weanlings. This may be explained by the increase in activity of the Stall weanlings after d 28. The decrease in osteocalcin concentration with age observed in this study corresponds with other studies in which a decreased serum osteocalcin concentration with an increase in age in equine was observed (Lepage et al., 1990, 1991; Hope et al., 1993). This decrease in bone formation corresponded with the decrease in modeling and remodeling with increasing age (Maenpaa et al., 1988; Fraher, 1993). The osteocalcin concentrations in this study fell within the range reported by Lepage et al. (1991).

All treatment groups had decreased serum ICTP concentration. This corresponds with numerous studies indicating that ICTP concentration decreases with age in equines (Price et al., 1995a,b), canines (Allen et al., 1998), and humans (Crofton et al., 1997; Zanze et al., 1997). Price et al. (1995a) reported serum ICTP concentrations of 15 female Thoroughbreds less than 1 yr of age to be 13.7 to 26.7 µg/L. In the present study, serum ICTP concentrations of 14.4 ng/mL to 19.9 ng/mL fell within the range Price et al. (1995a) reported.

Stalled and pastured weanlings had decreased serum keratan sulfate concentrations over 56 d. This decrease parallels other studies demonstrating decreases in keratan sulfate concentration with increasing age (Roughley and Lee, 1994; Okumura et al., 1997; Todhunter et al., 1997). Okumura et al. (1997) reported keratan sulfate concentrations in 15 foals to be approximately 4,000 ng/mL at 3 mo of age, decreasing to 500 ng/mL at 6 mo of age. In the present study, serum keratan sulfate concentrations fell within the range observed by Okumura et al. (1997).

The bone and cartilage serum markers we measured reflect systemic skeletal metabolism, which may have masked any specific changes that were occurring in bones of the lower limb and associated joints. Any treatment differences that were observed may have been due to changes occurring in other parts of the body and not necessarily changes in the third metacarpus as a response to housing. Also, during the growth phase of young horses, modeling and remodeling occur at a very high rate, allowing for appositional growth and strengthening of long bones, the rate of formation exceeding the rate of resorption (Nunamaker et al., 1990; Fraher, 1993). The high concentrations of these serum markers during rapid growth could prevent detection of adverse environmental changes, such as stalling, on bone metabolism. Cartilage turnover also occurs at a faster rate in young animals than in older animals (Leipold et al., 1989). In 8- to 10-mo-old dogs predisposed to osteoarthritis, no change in keratan sulfate was observed (Leipold et al., 1989). Thus, keratan sulfate was a poor marker for indicating a predisposition to osteoarthritis, but the effects may have been due to the age of the dogs masking any changes that were occurring. The serum markers in the current study were found to be inconclusive but mirror the results of previous studies indicating that bone and cartilage turnover decreases with age (Price et al., 1995a,b; Okumura et al., 1997).

Growth Measurements

The Pasture and Partial-Pasture weanlings had greater cannon bone circumference at d 56 than on d 0, but Stall horses exhibited no change. Hence, the Pasture and Partial-Pasture groups demonstrated larger cannon circumferences than the Stall horses on d 28 and 56. Sherman et al. (1995) measured the breaking strength and cortical cross-sectional area of the third metacarpal bone in 24 Thoroughbreds (2 to 4 yr of age) with various training backgrounds. Horses with a more extensive training background had greater peripheral thickening and greater area moment of inertia. By look-

ing at the correlation between area moment of inertia and failure load, Sherman et al. (1995) found that this increase in size corresponds with an increase in strength. Woo et al. (1981) agreed with Sherman et al. (1995) in that the increase in femoral cross-sectional area of exercised swine compared to an unexercised group is directly related to bone strength. The increase in cannon circumference of Pasture and Partial-Pasture weanlings in the present study may correspond with the increase in bone mineral content observed in the radiographs and hence is likely associated with an increase in bone strength (El Shorafa et al., 1979; Fleming et al., 1994).

Nutrition

There were nutritional differences in the present study. Stall weanlings had access to hay, whereas the Pasture weanlings had access to the pasture grasses and the Partial-Pasture weanlings had access to both hay and pasture grasses. The nutrition table (Table 1) indicates an inverse Ca:P ratio, but no detrimental effects of this inverted ratio were noted. Nutrition was not considered to have had a part in the results observed. Hoekstra et al. (1999) performed a study similar to ours, but the study took place during the winter months. During winter, no grass was available in the pasture, and consequently all horses had free access to alfalfa-grass hay and were fed the same amount of concentrate (Hoekstra et al., 1999). Results observed in the Hoekstra et al. (1999) study were very similar to those reported here. Regardless of this, we recognize that, in addition to differences in exercise, there are confounding factors between treatment groups including nutrition and exposure to sunlight. However, these same confounding factors would be present in any operation where a decision has to be made as to whether to stall young horses or provide access to pasture.

Implications

We conclude that stalling weanlings without exercise prevents maximal mineral deposition in the third metacarpus. This may prove detrimental to some horses when they enter training later in life. Stalling horses while "sale prepping" is a common occurrence in the horse industry. Horses are often stalled for 3 to 4 mo before a sale to improve hair coat and prevent injuries. This "prepping" likely yields horses with weaker bones that are more prone to injury after training commences. Exercise is particularly important during skeletal maturation so that after this process is complete the bone can withstand increases in strain associated with training and competition. Results of the present study indicate that if stalling is necessary, 12-h daily turnout is enough to prevent decreases in bone mineral content. However, even a shorter time outside may provide the same results.

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