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Effects of live yeast (Saccharomyces cerevisiae Strain 1026) : supplementation on the closure of articular growth plates in quarter horse foals

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Effects of Live Yeast (Saccharomyces cerevisiae Strain 1026)
Supplementation on the Closure of Articular Growth Plates in Quarter Horse Foals

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ON LINE ABSTRACT

The objective of this study was to determine whether dietary live yeast (*Saccharomyces cerevisiae strain 1026*) was able to shorten the time for growth plate closure. Twenty-four Quarter Horse foals, 8 months of age, were allotted to 2 treatment groups in a completely randomized experimental design. Horses were fed 0 or 20 g live yeast daily from 8 to 24 months of age. Growth plate closure was evaluated by dorso- palmar X – ray imaging at baseline, second study at 12 months of age and finally at 24 months of age. Blood and urine samples were taken at the same time to evaluate bone and mineral metabolism parameters, serum phosphorus, calcium, magnesium, alkaline phosphatase, serum hydroxyproline. Calcium, magnesium and phosphorus urinary fractional excretion were calculated. Closure scales of distal radius growth plate data were compared using the Wilcoxon´s test; bone markers were compared using Student´s *t*-test. Baseline radiographs showed that in 84% of foals the distal metacarpi were half closed or fully closed at 8 months; thus subsequent evaluations were limited to the distal radius. At 12 months of age, a positive but non-significant elevation in calcium urinary fractional excretion was observed in the supplemented group. At 24 months of age, radiographs showed a non-significant but positive effect in the live yeast group, with more than 50% of foals having closed distal radius growth plates. Although bone markers at 24 months of age did not differ ($p > 0.05$) between groups, some individual horses showed a positive effect with yeast supplementation.

ABSTRACT

To avoid osteoarticular injury and to minimize farm losses in animal use and sales, breaking in young horses is ideally done as soon as growth plates have closed. An experiment was performed to determine whether dietary live yeast could shorten the time for growth plate closure. Twenty-four female Quarter Horse foals, 8 months of age, were allotted to 2 treatment groups (12 animals each) in a completely randomized experimental design. Horses were fed 0 or 20 g live yeast daily from 8 to 24 months of age. Diets were regular for a stud farm in the area of the study in Argentina. Foals at 8 months of age had a very low baseline calcium urinary fractional excretion compatible with a primary calcium deficient diet for this age.

Growth plate closure was evaluated by dorsum-palmar radiographs at baseline. A second evaluation was performed when foals were 12 months of age and third evaluation when foals were 24 months of age. Blood and urine were sampled simultaneously at the same intervals to evaluate bone and mineral metabolism parameters (serum phosphorus, calcium, magnesium; alkaline phosphatase; and hydroxyproline). Calcium, magnesium and phosphorus urinary fractional excretions were also calculated. Closure scales of distal radius growth plate data were compared using Wilcoxon's test and bone markers were compared using Student´s *t*-test. Baseline radiological evaluation of growth plates showed that in 84% of foals the distal metacarpi were either half-closed or fully closed at 8 months; thus subsequent evaluations were limited to the distal radius. After 4 months of supplementation with live yeast at 12 months of age, a positive but non-significant elevation in calcium urinary fractional excretion was observed. At 24 months of age, radiographs showed a non-significant but positive effect in the live yeast group, with more than 50% of foals having closed distal

radius growth plates. Although bone markers at 24 months of age did not differ ($p > 0.05$) between groups, some individual horses showed a positive effect with yeast supplementation.

KEY WORDS

Quarter Horse; foal development; articular growth plates; yeast supplement, Saccharomyces.

1. INTRODUCTION

The breaking in of sport and working horses can begin at different times of foal development, depending on: genetic and individual factors, farm technical practice, feeding and nutritional management, the empirical observations of farm owners and horse trainers. But breaking in horses at early stages of development can potentially lead to osteoarticular injuries. Conversely, breaking horses later can potentially increase farm fixed costs through losses in animal use or sales.

The radiological evaluation of the closure of articular growth plates is the method of choice to decide the best time for putting a horse into training thus when growth plates are closed, breaking in can be started without risk of osteoarticular injury [1, 2].

In Thoroughbreds and Quarter Horses, the distal metacarpus (Mtc III) growth plate closes between 9 to 12 months of age and the distal radius growth plate closes between 24 to 30 months of age, although in Quarter Horses the total closure of Mtc III at 6 to 8 months of age has been reported [3, 4].

Supplementation with yeast cultures has been used in horses to stimulate microbial flora of the hindgut and to enhance digestibility of nutrients, such as fiber, crude protein, minerals, calcium and phosphorus, and dry matter. Enhanced digestibility and balance absorption of nutrients is of particular importance in developing foals [5, 6].

The particular mode of action of yeast supplementation can be explained by the stimulation of the microbiota in the different segments of horse gastrointestinal tract. The effect may begin in the fundic region of the stomach and the small intestine, thus explaining the increased amino acid and calcium absorption, and then continue in the ceco-colon complex where fibrolytic and microbial phytase activity promote phosphorus absorption [7, 8, 9, 10, 11, 12, 13].

Live yeast added to equine diets has been shown to improve the digestibility of nutrients, to increase absorption of minerals, and to increase growth rates. The effects of this dietary supplement have not been previously studied on horse growth plate closure [5, 6, 7].

The growth rate of horse can be evaluated by the age-related variations in the levels of bone metabolism markers. The evaluation of bone markers has been applied to the study of bone metabolism in both humans and animals. Bone markers and related biochemical parameters can be affected by age, breed and precocity or individual fast ageing [14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25].

We hypothesize that the growth plates of foals supplemented with live yeast will close earlier than those of unsuplemented ones. Our main goal was to evaluate the possible positive effects of live yeast feeding on growing foals on both the closure time of bone growth plates and on bone metabolism parameters.

2. MATERIALS AND METHODS

As the foals belonged to a private stud farm ("*Quebracho Herrado*") the owner granted the approval to the protocols and took care of animal welfare. No animals were harmed during the study.

Twenty-four female American Quarter Horse foals, with baseline sampling at 8 months age, were allotted to two treatment groups (n=12), in a completely randomized experimental design. One group was fed 20 g/d of live yeast (Y) (Yea-Sacc^{1026}, Alltech Inc., Nicholasville, KY) with a grain supplement beginning at 8 months of age and continued to 24 months of age, whereas the other group received no live yeast supplementation (NY). All foals were managed similarly, grazed the same mixed pasture (alfalfa *(Medicago sativa)*, orchard grass *(Dactilys glomerata)*, timothy canary grass *(Phalaris spp.)*, brome grass *(Bromus unioloides)* and perennial rye grass *(Lolium perenne)*) in spring and summer, pasture oats *(Avena sativa)* in winter, pasture soya *(Glycine max)* in summer,

alfalfa hay *ad libitum* and 1 kg of oats daily until 24 months of age. Diets were constant for a stud farm in the area of the study in Argentina.

Two foals in the NY group were sold and one additional foal entered the Y group before the second sampling. After the second sampling four foals of the Y group and three of the NY group were also sold. Thus, sixteen foals completed the study: nine in the Y group and seven in the NY group.

The closure of the growth plates was evaluated in each foal by dorsum- palmar radiological study according to the following schedule: both vertical and horizontal distances between diaphyses and epiphysis of the distal Mtc III were measured once at the beginning of the study; the same distance between diaphyses and epiphysis of the distal radius was measured at 12 months of age and again at 24 months of age. The following scale was used to evaluate growth plate closure (horizontal): complete closure (CX); ¾ closure (¾ X), ½ closure (½ X); ¼ closure (¼ X) and no closure (NX; completely open growth plate) [26]. The study ended when foals were 24 months of age. The time to closure was compared between treatment groups.

Blood and urine samples from each foal were collected as per the above schedule and analyzed. Urine was obtained by catheterization, at the same time with blood sampling. Phosphorus (Wiener Code. 1382001) [27], total proteins (Wiener Code. 1361003), serum albumin (Wiener Code. 1009300) [28], urea (Wiener Code. 1810058) [29] and alkaline phosphatase (AP, Wiener Code. 1361003) [30] were analyzed by colorimetric techniques. Serum calcium, magnesium, copper, and zinc were analyzed by atomic absorption spectrophotometer (Shimadzu AA 646). Ion concentrations were derived from standard calibration curves (50 and 100 µg/dl for Cu and Zn and 1.5 and 5 mg/dl for Mg, Merck Germany). The water used was double deionised with mili-pore system (Milli-q Plus), Serum hydroxyproline (Hypro) was determined by a modified method developed by Bergman and Loxley (1963) [31]. Calcium, phosphorus and magnesium urinary fractional excretions were calculated considering serum and urine creatinine.

2. 1. Statistical analysis

Biochemical parameters, minerals and bone markers from the two different treatment groups were compared using Student´s *t*-test for independent samples after ensuring data homosedasticity. Closure scales of distal radius growth plate data were compared using the Wilcoxon's rank-sum test for independent samples. Statistical significance was considered with $p < 0.05$.

3. RESULTS AND DISCUSSION

3. 1. Pre-experimental period

The baseline radiological evaluation of 19 foals (average 8 months age) showed that the foals distal Mtc III growth plates were between 1/2 X or CX, which precluded their radiological re-evaluation [31]. Thus, subsequent evaluations were limited to the distal radius.

In twelve fillies (average 8 months age), the blood and urine analyses before yeast supplementation showed that the resorption and formation bone turnover markers, serum Hypro and AP respectively, were elevated compared with reference values for this age. The serum mean value of phosphorus ($p < 0.05$) and calcium (non-significant) w ere lower in group 3 (½ X), respect to the other two groups (Table 1).

So at the baseline point foals, 8 months of age, had a very low CaFE compatible with a primary Ca-deficient diet for this age. Serum total protein, albumin, copper and zinc fell within our reference values (total proteins 5.94 ± 0.55 g/dl; albumin 3.4 ± 0.45 g/dl; Cu 150 ± 33 µg/dl; Zn 126 ± 36 µg/dl).

Measurement of bone markers has been applied for evaluation of metabolic bone diseases in human and animals [14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25]. Under normal bone development conditions the serum and urine profiles fall within homeostatic levels.

During the first month it is normal for foals to have high values of bone markers. Nevertheless, during the first year of life, it is common to have high values of bone markers in those foals growing at higher rate. In the latter case, both Hypro levels and AP activity are high, because bone modeling involves both resorption and formation processes. On the contrary, precocious animals show an earlier but simultaneous decrease in the formation and resorption bone markers [14, 15, 16, 17, 18, 19, 20, 24] compared to the reference values for their age. Under pathological conditions this fine equilibrium is lost, e.g. at early stages of foal skeletal development it is possible to find formation and resorption bone markers that are below the reference values, particularly AP, and this is commonly associated with low intake of trace elements that feature the foals raising conditions in this country, particularly copper and zinc [18, 25].

In this study, both formation and resorption bone markers were elevated at 8 months age compared with reference values, indicating a high growth rate and in line with the early closure of the distal metacarpal growth plates. Moreover, these values were lower than those expected for foals of 24 months of age, suggesting a breed-specific tendency for early development [15, 16, 17, 18, 19].

3. 2. Experimental period.

After 4 months of Y supplementation (12 months old), radiological evaluation showed that the distal radius growth plates were fully open in yearlings. Overall serum biochemical determinations showed that bone markers were higher than the reference values in both groups. Surprisingly, Hypro, a resorption bone marker, was higher in NY when compared to Y group, (p =0.005) and AP, a formation bone marker, was higher compared with reference values but no significantly higher between groups. Although CaFE values of both groups were lower than reference values, CaFE values tended to be higher in the Y (2.32 ± 0.48) than in NY (1.63 ± 0.69) group (p = 0.023), suggesting that foals in the first group had a more suitable net calcium absorption (Table 2).

In Argentina, reference values of calcium and phosphorus fractional excretion are lower than in other developing countries because of the extended soil calcium and phosphorus associated-deficiency [15, 16, 17, 18, 19]. High serum calcium levels, are not rare, however, these findings do not reflect an adequate input of this mineral but are associated with low serum phosphorus and PFE values.

Serum total proteins, albumin, copper and zinc were found to be within normal ranges (total proteins 5.94 ± 0.55 g/dl; albumin 3.4 ± 0.45 g/dl; Cu 150 ± 33 µg/dl; Zn 126 ± 36 µg/dl).

At 24 months of age, although distal radius closure rates did not differ (p = 0.6) between treatment groups, more than 50% of the fillies in the supplemented group had distal radius growth plates that were closed or near closure while more than 50% of the fillies in the NY group had distal radius growth plates that were half closed (Table 3). Early closure of the distal radius suggests that the bone growth plates in the Quarter Horse breed close earlier than those of other sport breeds, which typically close between 24 to 30 months of age [4].

At 24 months of age, bone and mineral metabolism parameters did not differ ($p > 0.05$) between treatment groups. However, AP ($p < 0.001$) and serum calcium were higher in Y group ($p < 0.01$) than reference values. Again Hypro, resorption bone marker, from NY group was higher ($p < 0.05$) than reference values. Biochemical data profile indicated an adequate absorption of nutrients in both experimental groups, NY and Y. Bone marker values from fillies aged 24 months were similar to those find in adults (Table 4).

4. CONCLUSIONS

Radiological findings and high bone marker in relation with reference values at 8 months of age suggest a breed tendency toward early development. After 4 months of supplementation with the live yeast, at 12 months of age, a positive elevation in urinary CaFE in the Y group (p = 0,023), and high bone markers in two groups were observed. At 24 months of age, radiological examinations showed a non-significant but positive effect in the Y group, with more than 50% of foals having

closed distal radius growth plates. But bone turnover markers means values obtained at 24 months of age did not differ ($p > 0.05$) between Y and NY groups.

Radiological and biochemical studies suggested that the *Quarter Horse* breed tended to develop early compared with other sport horse breed. Radiographs of *Quarter Horse* foals should be performed at earlier development stages to determine the safest and most cost-effective time to break in this breed.

Yeast supplementation at earlier stages of foal development, e.g., preweaning, might result in significant differences over the studied parameters.

6. ROLE OF THE FUNDING SOURCE

Alltech Inc. sponsored this paper, providing the live yeast ((Yea-Sacc^{1026}, Alltech Inc., Nicholasville, KY) and the funds for the collection and lab analysis of serum and urine samples; and X-ray films and developers.

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