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Body development of weaned foals of Mangalarga Marchador breed fed probiotics or phytase supplemented diets

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ABSTRACT. The aim of the study was to assess the average daily gain (ADG), the growth rate of linear measurements and cortical index changes of the third metacarpus (ICOR) of foals from 173 ± 8 to 363 ± 12 day of life. Sixteen Mangalarga Marchador weaned colts were divided by age and allotted randomly to one of four dietary treatments (n = 4): A – basal diet (*Cynodon* pasture + 1.2 kg · 100 kg^{-1} body weight (BW) of concentrate + water and mineral mix *ad libitum*); B – basal diet + 5 g · day^{-1} of yeast culture (10^9 cfu · g^{-1} *Saccharomyces cerevisiae* Sc47); C – basal diet + 2 g · day^{-1} of bacteria (10^8 cfu · g^{-1} *Bifidobacterium bifidum, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus plantarum*) and yeast culture (10^5 cfu · g^{-1} *S. cerevisiae*); and D – basal diet + 544 FTU of phytase per kg of concentrate per day. Data was analysed as repeated measures and fixed effects of age, treatment and their interactions accordingly to SAS® procedures. The additives did not affect the ADG or growth rate of linear measurements ($P > 0.05$); however the effect of age was observed. Furthermore, the ICOR was greater in 273 day-old foals fed diet B, which may suggest an increase in mineral uptake and bone development. So it can be concluded that a 100-day diet supplementation with *S. cerevisiae* Sc47 may increase bone development of weaned foals, however more studies are necessary to assure the beneficial effect of yeast addition on equine growth rate.

Introduction

Mangalarga Marchador is the most prevalent horse breed raised in Brazil. Like other light breeds such foals grow quickly during their first year of life (Rezende et al., 2000a,b; Cabral et al., 2004). In tropical climates, in which the rainy season occurs in the summer, many stud farms allow for the adoption of extensive raising systems, which differ from temperate climate studs in which stalls are necessary. In Brazil, a lot of animals are kept on pastures for the most of the year so deterioration of grasses nutritional values has an influence on them.

Foal body growth is generally evaluated on the basis of body weight average gain as well as on changes in whither and hip height, body length, chest girth and cannon circumference (Rezende et al., 2000a; Cabral et al., 2004; Pinto et al., 2005; Santos et al., 2005). Maximum height of adult equine is mostly determined genetically, but body development (size and growth rate) of young animals may be influenced by sex, date of birth (month), climate, nutrition and management (Saastamoinen, 1990).

When probiotics are administered in adequate amounts, they exert health benefits for the host (FAO/WHO, 2002). In the equine species, this additive is used to improve diet quality, body growth, physical condition but also for prevention and/or recovery from gastrointestinal diseases. Studies conducted on horses have shown that the use of probiotics improves nutrient digestibility, especially fibre (Kim et al., 1991; Medina et al., 2002; De Fombelle et al., 2003; Jouany et al., 2009; Moura et al., 2009, 2011; Furtado et al., 2010). However, more studies are needed to assess the indirect effects of these nutritional benefits on foals body growth (Glade, 1991a,b; Perrone et al., 2013), especially in animals raised under particular climatic conditions like in Brazil.

Phytase is an enzyme used throughout the world as a nutritional additive for monogastric animals, especially for poultry and pigs, due to its deleterious effects on mineral utilization (P, Ca, Mg, Zn, Fe, Mn). Few studies have been conducted to examine the effects of this enzyme type supplementation on horses (Patterson et al., 2002; Hainze et al., 2004; Van Doorn et al., 2004; Dunnet et al., 2009). Among them were also Mangalarga Marchador foals, which have higher mineral requirements (Moura et al., 2009, 2011).

The objective of this study was to assess the body development (weight, linear measurements and bone development) of weaned Mangalarga Marchador foals fed probiotics or phytase supplemented diets. It was hypothesized that the growth of weaned foals fed diet supplemented with probiotics or phytase would be greater than the growth of foals fed unsupplemented diet.

Material and methods

Animals, treatments and experimental design

All applied experimental procedures were approved by the Ethics Committee for Animal Experimentation of The Federal University of Minas Gerais (UFMG), Brazil. The study was carried out at a private stud farm (Montes Claros, Minas Gerais, Brazil) from 13 April to 16 December. Sixteen colts were used, all from the same stallion and breed lineage ('*Catuni*'), which is considered to be the one of the first established lineages of the Mangalarga Marchador breed that started to be selected in the early nineteenth century (Costa et al., 2005).

Foals were weaned after 173 ± 8 days weighing 178 ± 20 kg. Prior to weaning, foals were kept on a single pasture with their dams and received only a mineral mix *ad libitum*. Weaning was conducted in groups of four foals from April to May. Before this trial, all animals were treated for internal and external parasites. Treatment against endoparasites was repeated every two months and against ectoparasites – each month. At weaning, all foals were divided by age, and four foals were allocated randomly to one of four dietary treatment groups: A – basal diet; B – basal diet + 5 g · day^{-1} of yeast culture (10^9 cfu of *Saccharomyces cerevisiae* NCYC Sc47; Biosaf©; Lesaffre Feed Additives, Marcq-en-Baroeul, Nord-Pas-de-Calais, France); C – basal diet + 2 g · day^{-1} of bacteria and yeast culture (10^8 cfu of four bacteria strain (*Bifidobacterium bifidum, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus plantarum*) and 10^5 cfu of *Saccharomyces cerevisiae*; Florafort©; Laboratório Vitafort Ind. e com. de Produtos Veterinários Ltd., Ribeirão Preto, São Paulo, Brazil); and D – basal diet + 544 phytase units (FTU) (5000 FTU per g; Natuphos©; BASF, Ludwigshafen, Germany) per kg of pelleted concentrate per day.

Foals were kept on 8 ha Bermuda grass (*Cynodon* spp.) pasture, in a continuous grazing system, with free access to water and mineral mix (Hiposal 80©; Total Alimentos, Três Corações, Minas Gerais, Brazil). A pelleted concentrate (Equisul Lactação 16 Floc©; Total Alimentos, Três Corações, Minas Gerais, Brazil) was offered every day in a proportion of 1.2 kg per 100 kg body weight and divided into two meals (7:30 and 15:30).

The additives, phytase or probiotics (yeast or yeast with bacteria mixture), were administered directly into the horse mouth at every morning feeding. The probiotic composed of a yeast culture was in the form of powder, dehydrated by lyophilization, from the same batch, stored at room temperature and protected from sunlight and humidity. The probiotic composed of a bacteria and yeast culture was in the form of paste, stored at 4 ºC and used up to four months from its preparation. The phytase supplement was in the form of lyophilized powder from a single batch and stored at 4 ºC.

The adaptation to the facilities, managament and diets lasted the first 14 days of the experiment. Foal body development was monitored in sum for 200 days (from weaning at 173 ± 8 to 363 ± 12 day of life).

Body growth evaluation and diet analyses

At weaning and every 30 days until the end of the experiment, the foals were weighed after the morning feeding and measured with a Lydthin measuring stick (wither height – WH, hip height – HH, body length – BL and barrel height – BH) and with a tape (chest girth – CG and cannon circumference – CC), as illustrated on Figure 1. Data published by Gonçalves et al. (2012) was used to estimate the percentage of the values observed for all body measurements, except for barrel height, in relation to the expected values for Mangalarga Marchador adult males. Body condition was scored through the entire study, using the method of Carrol and Huntington (1988): 0 – very thin to 5 – obese.

Foals at 235, 273, 308, 339 and 368 days of life were radiographed in the dorso-palmar position of the right metacarpus (Mtc III). The cortical index (ICOR) was calculated in accordance to Rezende et al. (2000b):

$$\text{ICOR} = [(\text{total bone width} - \text{medular cavity witdh}) / \text{total bone width}] \times 100.$$

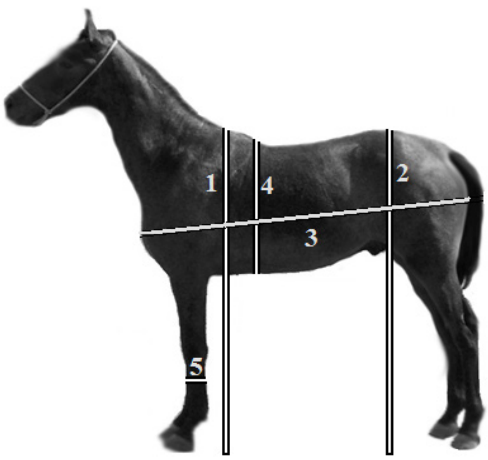

Figure 1. Linear measurements of foal body: wither height (1), hip height (2), body length (3), barrel height and chest girth (4), cannon circumference (5)

For the age effect comparisons, individual ICORs measured at the end of each sampling period were converted to percent change in relation to previous or initial value of each period. The individual mean percentage changes were then averaged across each age period and the means (absolute % values and % changes) between ages compared by orthogonal contrasts.

For the diet effect comparisons, individual ICORs measured at each sampling period (age) were converted to percentage change in relation to the control one. For comparisons the same procedures for age were used.

Feed samples were collected to determine their chemical composition in the Animal Nutrition Laboratory of The Federal University of Minas Gerais (UFMG), Department of Animal Science (Table 1). Diets were analysed for the following parameters: dry matter (DM), crude protein (CP), ether extract (EE), calcium (Ca), phosphorus (P) and oxalate (AOAC International, 1995), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (Van Soest et al., 1991). The digestible energy (DE) of the concentrate and forage harvested in August was determined by Moura et al. (2011). Forage DE from each month of the study (April, June, October and December) was estimated by the NRC (2007) formula:

$$\text{DE (Mcal)} = 2.118 + 0.01218 \times \text{CP} - 0.00937 \times \times \text{ADF} - 0.00383 \times (\text{NDF} - \text{ADF}) + 0.04718 \times \text{EE} + + 0.02035 \times \text{NFC} - 0.0262 \times \text{ash}$$

where: CP, NDF, ADF, EE, NFC (nonfibre carbohydrate) and ash are expressed as %.

Statistical analysis

All analyses were performed by the SAS® package version 6 (1998), SAS Institute Inc., Cary, NC, USA. Data relative to the average daily gains

Table 1. Chemical composition of feedstuffs

Feed	Month	DM[1], %	DE[2], Mcal · kg^{-1}	CP[3]	Lysine	EE[5]	Ca	P	Starch	NFC[6]	NDF[7]	ADF[8]	Lignin	Oxalate
				%										
Forage	April	30.35	1.81[a]	8.47		3.00	0.47	0.39	16.25	8.14	70.79	35.08	6.89	0.39
	June	43.96	1.80[a]	5.33		3.11	0.55	0.34	26.33	9.23	73.13	36.08	7.47	0.20
	August	60.27	1.15[b]	3.67	0.36[4]	1.65	0.63	0.34	22.94	4.26	82.25	45.62	9.99	0.30
	October	32.49	1.96[a]	11.15		3.76	0.52	0.34	27.70	9.09	68.01	33.16	6.93	0.39
	December	28.06	1.98[a]	9.87		2.44	0.38	0.35	10.56	12.92	66.11	28.64	5.82	0.19
Concentrate		89.97	3.65[b]	18.89	0.87	4.36	1.42	1.17	27.90	35.69	31.90	11.27	–	–

[1]DM – dry matter; [2]DE – digestible energy ([a]estimated by the formula (NRC, 2007)): DE = 2.118 + 0.01218 × CP – 0.00937 × ADF – 0.00383 × (NDF – ADF) + 0.04718 × EE + 0.02035 × NFC – 0.0262 × ash; [b]estimeted by Moura et al. (2011)); [3]CP – crude protein; [4]according to NRC (2007); [5]EE – ether extract; [6]NFC – nonfibre carbohydrates, NFC = 100 – (CP) + (NFD – NDICP) + EE + ash, where NDICP – neutral detergent insoluble crude protein (NFC include sugars, starches, fructans, galactans, pectins, β-glucans and organic acids (Van Soest et al., 1991)); [7]NDF – neutral detergent fibre; [8]ADF – acid detergent fibre

of body weight and linear measurements, and cortical index was analysed as repeated measures and the fixed effects of age, diet and their interaction tested considering the initial measurement of ech of these variables as covariates. The covariance structure with the smallest (compound symmetry) Akaike's value was used. The error term was animal within diet. Means were compared by orthogonal contrasts. The level of significance was set at 5%. Means were calculated accordingly to the ANOVA procedure. Body measurements were regressed onto age by the PROCREG procedure.

Results

There was no effect of dietary treatment and its interaction with age ($P > 0.05$) on body weight (BW) absolute values and body linear measurements (Table 2), neither on average daily/monthly gains (Table 3) of foals from 173 (weaning) to 363 day of life. Average body condition score across the experimental period was 3.0, and no differences were observed between treatment, age or between age interaction treatment throughout the study ($P > 0.05$).

The results for absolute values of BW and linear measurements of all foals in relation to time (Table 2) significantly fit the following regression models, where: X – age (days):

BW (kg) $= 99.0136 + 0.484437 \times X$; $R^2 = 98.53\%$;
WH (cm) $= 109.375 + 0.07227 \times X$; $R^2 = 99.15\%$;
HH (cm) $= 114.305 + 0.06753 \times X$; $R^2 = 98.68\%$;
BL (cm) $= 97.7318 + 0.106213 \times X$; $R^2 = 98.16\%$;
BH (cm) $= 41.8868 + 0.03491 \times X$; $R^2 = 97.81\%$;
CG (cm) $= 117.006 + 0.0918974 \times X$; $R^2 = 93.36\%$;
CC (cm) $= 15.4305 + 0.00984063 \times X$; $R^2 = 98.47\%$.

Average daily gains (ADG) and monthly gains of linear measurements were only influenced by foal age (Table 3). With the exception of WH and CC, the average monthly gains of linear measurements for all foals significantly fit the regression models.

The ADG regression model for all foals over time was:

$$Y = 3.0308 - 0.01647 \times X + 0.00002586 \times X^2;$$
$$R^2 = 82.97\%$$

where: Y – ADG (kg · day^{-1}) and X – age (days).

The hip height (HH) gain regression model for all foals over time was:

Table 2. Absolute values of body weight and linear measurements for weaned Mangalarga Marchador foals fed unsupplemented or probiotics/phytase supplemented diets

Age, days	Group[1] A	B	C	D	General means
Body weight, kg (% relative to adult[2])					
173 ± 8	177.8 ± 19.6 (39.5)	165.5 ± 16.9 (36.8)	177.8 ± 19.6 (39.5)	173.0 ± 16.1 (38.6)	173.5 ± 17.0 (38.6)
205 ± 8	201.5 ± 22.8 (44.8)	188.5 ± 17.7 (41.9)	210.3 ± 15.9 (44.8)	196.3 ± 14.6 (43.6)	196.6 ± 18.6 (43.8)
232 ± 12	219.5 ± 27.7 (48.8)	208.3 ± 20.3 (46.3)	230.3 ± 18.5 (51.2)	210.3 ± 20.8 (46.7)	217.1 ± 21.7 (48.2)
267 ± 11	231.0 ± 27.4 (51.3)	224.5 ± 21.6 (49.9)	246.0 ± 17.6 (54.7)	227.8 ± 17.0 (50.6)	232.3 ± 20.9 (51.6)
302 ± 12	244.8 ± 30.4 (54.4)	235.3 ± 21.6 (52.3)	259.0 ± 18.8 (57.5)	240.8 ± 15.0 (53.5)	244.9 ± 21.8 (54.4)
332 ± 12	259.0 ± 29.4 (57.5)	248.8 ± 18.1 (55.3)	271.8 ± 17.9 (60.4)	253.8 ± 14.7 (56.4)	258.3 ± 20.6 (57.4)
363 ± 12	275.0 ± 25.6 (61.1)	263.0 ± 15.1 (58.4)	286.3 ± 16.3 (63.6)	265.3 ± 13.6 (58.9)	272.4 ± 18.9 (60.5)
Wither height, cm (% relative to adult[3])					
173 ± 8	121.4 ± 3.6 (80.9)	118.6 ± 3.3 (79.1)	123.9 ± 3.1 (82.6)	119.0 ± 3.5 (79.3)	121.5 ± 3.7 (81.0)
205 ± 8	123.5 ± 2.9 (82.3)	121.9 ± 3.2 (81.3)	126.0 ± 2.8 (84.0)	122.9 ± 2.5 (81.9)	123.9 ± 3.3 (82.6)
232 ± 12	125.5 ± 3.2 (83.6)	124.6 ± 3.7 (83.1)	128.4 ± 2.3 (85.6)	124.9 ± 2.6 (83.3)	126.3 ± 3.6 (84.2)
267 ± 11	128.1 ± 2.8 (85.4)	127.6 ± 3.7 (85.1)	131.3 ± 2.2 (87.5)	128.3 ± 2.5 (85.5)	129.2 ± 3.5 (86.1)
302 ± 12	131.3 ± 3.8 (87.5)	129.9 ± 3.4 (86.6)	133.9 ± 2.7 (89.3)	130.5 ± 2.9 (87.0)	131.8 ± 3.6 (87.9)
332 ± 12	132.4 ± 3.5 (88.3)	131.3 ± 3.1 (87.5)	135.4 ± 2.7 (90.3)	132.0 ± 2.7 (88.0)	133.2 ± 3.5 (88.8)
363 ± 12	134.3 ± 2.6 (89.5)	133.6 ± 3.5 (89.1)	136.6 ± 2.4 (91.1)	134.4 ± 3.6 (89.6)	135.0 ± 3.2 (90.0)
Hip height, cm (% relative to adult[3])					
173 ± 8	125.3 ± 3.0 (83.9)	123.4 ± 3.0 (82.6)	127.1 ± 2.9 (85.1)	126.0 ± 3.8 (84.3)	125.4 ± 3.2 (83.9)
205 ± 8	127.5 ± 3.6 (85.3)	126.3 ± 3.0 (84.5)	129.3 ± 2.9 (86.5)	128.5 ± 3.3 (86.0)	127.9 ± 3.1 (85.6)
232 ± 12	130.3 ± 3.6 (87.2)	128.5 ± 3.4 (86.0)	131.9 ± 1.9 (88.3)	131.0 ± 4.2 (87.7)	130.4 ± 3.3 (87.3)
267 ± 11	132.3 ± 3.0 (88.5)	131.3 ± 3.1 (87.9)	134.6 ± 1.8 (90.1)	134.0 ± 3.9 (89.7)	133.0 ± 3.0 (89.0)
302 ± 12	134.5 ± 4.5 (90.0)	133.6 ± 2.9 (89.4)	136.8 ± 2.5 (91.6)	135.5 ± 3.7 (90.7)	135.1 ± 3.3 (90.4)
332 ± 12	136.4 ± 3.1 (91.3)	134.9 ± 3.1 (90.3)	138.3 ± 1.9 (92.6)	137.3 ± 3.7 (91.9)	136.7 ± 3.0 (91.5)
363 ± 12	137.4 ± 2.6 (92.0)	136.5 ± 3.0 (91.4)	139.6 ± 2.5 (93.4)	139.0 ± 3.6 (93.0)	138.1 ± 2.9 (92.4)

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Table 2. continued

Age, days	Group[1]				General means
	A	B	C	D	
Body length, cm (% relative to adult[3])					
173 ± 8	114.6 ± 6.0 (75.4)	113.4 ± 2.6 (74.6)	117.4 ± 5.6 (77.2)	114.5 ± 4.7 (75.3)	115.0 ± 4.7 (75.6)
205 ± 8	118.0 ± 4.0 (77.6)	116.5 ± 2.0 (76.6)	121.9 ± 4.4 (80.2)	119.8 ± 4.0 (78.8)	119.0 ± 3.9 (78.3)
232 ± 12	123.8 ± 5.7 (81.4)	122.0 ± 3.8 (80.3)	125.5 ± 5.1 (82.6)	123.3 ± 2.8 (81.1)	123.6 ± 4.2 (81.3)
267 ± 11	128.0 ± 4.7 (84.2)	126.0 ± 3.7 (82.9)	129.9 ± 4.3 (85.5)	126.0 ± 3.9 (82.9)	127.5 ± 4.1 (83.9)
302 ± 12	130.3 ± 6.0 (85.7)	127.9 ± 3.6 (84.1)	131.9 ± 3.7 (86.8)	129.1 ± 4.1 (84.9)	129.8 ± 4.2 (85.4)
332 ± 12	132.8 ± 4.4 (87.4)	131.3 ± 3.7 (86.4)	135.1 ± 4.5 (88.9)	132.4 ± 2.0 (87.1)	132.9 ± 3.7 (87.4)
363 ± 12	135.3 ± 5.8 (89.0)	133.4 ± 2.7 (87.8)	137.5 ± 4.1 (90.5)	135.4 ± 1.3 (89.1)	135.4 ± 3.8 (89.1)
267 ± 11	141.8 ± 6.5 (81.3)	141.2 ± 6.8 (80.9)	146.8 ± 2.4 (84.1)	142.1 ± 3.8 (81.4)	143.0 ± 5.2 (81.9)
302 ± 12	144.7 ± 7.8 (82.9)	143.0 ± 5.4 (81.9)	147.6 ± 1.9 (84.6)	143.8 ± 2.9 (82.4)	144.7 ± 4.9 (82.9)
332 ± 12	147.7 ± 5.3 (84.6)	145.2 ± 4.2 (83.2)	149.5 ± 2.1 (85.7)	145.6 ± 4.0 (83.4)	147.0 ± 4.0 (84.2)
Barrel height, cm					
173 ± 8	47.8 ± 3.1	46.6 ± 1.8	48.1 ± 1.3	47.3 ± 2.3	47.5 ± 2.1
205 ± 8	49.1 ± 2.6	48.1 ± 1.8	50.8 ± 0.9	48.1 ± 1.4	49.0 ± 1.9
232 ± 12	50.3 ± 3.3	49.1 ± 2.4	51.9 ± 0.9	49.8 ± 1.3	50.3 ± 2.2
267 ± 11	51.4 ± 2.2	50.4 ± 1.3	53.3 ± 0.9	52.0 ± 1.2	51.8 ± 1.7
302 ± 12	51.9 ± 3.0	51.6 ± 1.8	54.3 ± 0.6	52.6 ± 0.9	52.6 ± 1.9
332 ± 12	53.3 ± 2.1	52.1 ± 1.8	55.1 ± 0.5	52.8 ± 1.0	53.3 ± 1.8
363 ± 12	54.3 ± 1.9	53.4 ± 1.7	55.6 ± 0.6	53.6 ± 1.5	54.2 ± 1.6
Chest girth, cm (% relative to adult[3])					
173 ± 8	130.5 ± 3.8 (74.8)	127.1 ± 3.6 (72.8)	133.4 ± 5.1 (76.4)	129.3 ± 6.1 (74.1)	130.1 ± 4.9 (74.5)
205 ± 8	136.5 ± 4.8 (78.2)	133.9 ± 6.0 (76.7)	140.4 ± 3.9 (80.4)	135.9 ± 4.9 (77.9)	136.7 ± 5.1 (78.3)
232 ± 12	140.7 ± 6.2 (80.6)	136.8 ± 5.5 (78.4)	145.7 ± 2.9 (83.5)	138.8 ± 5.1 (79.5)	140.5 ± 5.7 (80.5)
267 ± 11	141.8 ± 6.5 (81.3)	141.2 ± 6.8 (80.9)	146.8 ± 2.4 (84.1)	142.1 ± 3.8 (81.4)	143.0 ± 5.2 (81.9)
302 ± 12	144.7 ± 7.8 (82.9)	143.0 ± 5.4 (81.9)	147.6 ± 1.9 (84.6)	143.8 ± 2.9 (82.4)	144.7 ± 4.9 (82.9)
332 ± 12	147.7 ± 5.3 (84.6)	145.2 ± 4.2 (83.2)	149.5 ± 2.1 (85.7)	145.6 ± 4.0 (83.4)	147.0 ± 4.0 (84.2)
363 ± 12	150.6 ± 4.0 (86.3)	147.3 ± 4.9 (84.4)	151.4 ± 2.3 (86.8)	147.9 ± 2.2 (84.7)	149.3 ± 3.7 (85.6)
Cannon circumference, cm (% relative to adult[3])					
173 ± 8	17.4 ± 0.5 (95.6)	16.7 ± 0.6 (91.7)	17.4 ± 0.3 (95.6)	17.0 ± 0.5 (93.4)	17.1 ± 0.5 (93.9)
205 ± 8	17.6 ± 0.6 (96.7)	17.0 ± 0.6 (93.4)	17.8 ± 0.5 (97.8)	17.2 ± 0.7 (94.5)	17.4 ± 0.6 (95.6)
232 ± 12	17.9 ± 0.6 (98.3)	17.3 ± 0.5 (95.0)	18.1 ± 0.4 (99.4)	17.7 ± 0.3 (97.2)	17.7 ± 0.5 (97.2)
267 ± 11	18.2 ± 0.4 (100.0)	18.0 ± 0.6 (98.9)	18.5 ± 0.1 (101.6)	18.1 ± 0.3 (99.4)	18.2 ± 0.4 (100.0)
302 ± 12	18.4 ± 0.4 (101.1)	18.3 ± 0.8 (100.5)	18.8 ± 0.3 (103.3)	18.5 ± 0.4 (101.6)	18.5 ± 0.5 (101.6)
332 ± 12	18.5 ± 0.4 (101.6)	18.5 ± 0.8 (101.6)	19.1 ± 0.2 (104.9)	18.7 ± 0.3 (102.7)	18.7 ± 0.5 (102.7)
363 ± 12	18.9 ± 0.4 (103.8)	18.7 ± 0.9 (102.7)	19.2 ± 0.3 (105.5)	18.9 ± 0.4 (103.8)	18.9 ± 0.5 (103.8)

[1]A – control diet (*Cynodon* pasture + 1.2 kg · 100 kg^{-1} BW of concentrate + water and mineral mix *ad libitum*); B – diet + 5 g · day^{-1} of yeast culture (10^9 cfu · g^{-1} *S. cerevisiae* Sc47); C – diet + 2 g · day^{-1} of bacteria (10^8 cfu · g^{-1} *B. bifidum*, *E. faecium*, *L. acidophilus*, *L. plantarum*) and yeast culture (10^5 cfu · g^{-1} *S. cerevisiae*); and D – diet + 544 FTU of phytase per kg concentrate per day; [2]expected body weight of Mangalarga Marchadors adult males: 450 kg; [3]linear measurements of Mangalarga Marchador adult males raised in the region of Montes Claros, Minas Gerais, Brazil (Gonçalves et al., 2012): 150.0 ± 2.9 cm (wither height), 149.4 ± 2.9 cm (hip height), 152.0 ± 4.0 cm (body length), 174.5 ± 6.0 cm (chest girth), 18.2 ± 0.7 cm (cannon circumference)

$$Y = -0.7291 + 0.02892 \times X - 0.000064238 \times X^2; \quad R^2 = 0.9133$$

where: Y – HH gain (cm · $month^{-1}$) and X – age (day).

The barrel height (BH) gain regression model for all foals over time was:

$$Y = 2.5241 - 0.004926 \times X; \quad R^2 = 0.6767$$

where: Y – BH gain (cm · $month^{-1}$) and X – age (day).

The chest girth (CG) gain regression model for all foals over time was:

$$Y = 39.6238 - 0.241526 \times X + 0.00038426 \times X^2; \quad R^2 = 0.9648$$

where: Y – chest girth gain (cm · $month^{-1}$) and X – age (day).

The estimated highest gain in hip height (HH) was 2.71 cm · $month^{-1}$ at 204 day of life, and the estimated lowest rate of CG gain was 1.70 cm · $month^{-1}$ at 299 day of life (Table 3). The body length (BL) and barrel heigh (BH) gains of the Mangalarga Marchador foals had a reduction in growth rate of –0.012605 cm

Table 3. Average daily gain of body weight and average montly gains of linear measurements for weaned Mangalarga Marchador foals fed unsupplemented or probiotics/phytase supplemented diets

Parameter[1]	Diet[2]				Age, days						*P*-value	
	A	B	C	D	204 ± 7	229 ± 11	264 ± 10	299 ± 12	329 ± 12	360 ± 12	diet	age
ADG, kg · day^{-1}	0.52 ± 0.02	0.52 ± 0.02	0.53 ± 0.02	0.50 ± 0.02	0.66 ± 0.03^{D}	0.70 ± 0.03^{E}	0.42 ± 0.03^{B}	0.36 ± 0.03^{A}	0.46 ± 0.03^{B}	0.50 ± 0.03^{C}	0.9184	<0.0001
cm · month^{-1}												
WHG	2.15 ± 0.10	2.35 ± 0.10	2.16 ± 0.10	2.21 ± 0.10	2.67 ± 0.21^{D}	2.10 ± 0.21^{C}	2.89 ± 0.21^{E}	2.39 ± 0.21^{D}	1.42 ± 0.21^{A}	1.83 ± 0.21^{B}	0.5273	<0.0001
HHG	2.01 ± 0.08	2.17 ± 0.08	2.06 ± 0.08	2.21 ± 0.08	2.71 ± 0.21^{F}	2.33 ± 0.21^{D}	2.54 ± 0.21^{E}	2.04 ± 0.21^{C}	1.64 ± 0.21^{B}	1.39 ± 0.21^{A}	0.3398	0.0002
BLG	3.26 ± 0.19	3.26 ± 0.19	3.28 ± 0.19	3.39 ± 0.19	4.04 ± 0.36^{E}	4.21 ± 0.36^{F}	3.89 ± 0.36^{D}	2.39 ± 0.36^{A}	2.73 ± 0.36^{C}	2.52 ± 0.36^{B}	0.9563	0.0004
BHG	1.09 ± 0.06	1.15 ± 0.06	1.20 ± 0.06	1.12 ± 0.06	1.62 ± 0.17^{F}	1.12 ± 0.17^{D}	1.39 ± 0.17^{E}	0.83 ± 0.17^{A}	0.89 ± 0.17^{B}	0.98 ± 0.17^{C}	0.6364	0.0247
CGG	3.24 ± 0.20	3.20 ± 0.20	3.21 ± 0.20	3.18 ± 0.20	6.18 ± 0.43^{E}	3.73 ± 0.43^{D}	2.40 ± 0.43^{B}	1.70 ± 0.43^{A}	2.42 ± 0.43^{B}	2.81 ± 0.43^{C}	0.9969	<0.0001
CCG	0.26 ± 0.03	0.31 ± 0.03	0.30 ± 0.03	0.29 ± 0.03	0.31 ± 0.05^{C}	0.36 ± 0.05^{D}	0.40 ± 0.05^{E}	0.32 ± 0.05^{C}	0.14 ± 0.05^{A}	0.20 ± 0.05^{B}	0.7566	0.0126

[1]ADG – average daily gain, WHG – wither height gain, HHG – hip height gain, BLG – body length gain, BHG – barrel height gain, CGG – chest girth gain, CCG – cannon circumference gain; [2]see Table 2; $^{A–F}$ – values with different superscripts for each main effect (diet or age) are significantly differerent at $P \le 0.05$

Table 4. Absolute values and changes on cortical index of third metacarpus of weaned Mangalarga Marchador foals fed unsupplemented or probiotics/phytase supplemented diets

Parameter	Diet[1]				Age, days					*P*-value		
	A	B	C	D	235	273	308	339	368	diet	age	diet x age
Cortical index, %	57.849 ± 0.864	58.794 ± 0.884	58.388 ± 0.846	56.578 ± 0.840	55.354 ± 0.569^{A}	57.036 ± 0.569^{B}	57.666 ± 0.569^{B}	58.844 ± 0.569^{C}	60.612 ± 0.569^{D}	0.3201	<0.0001	–
Cortical index change, %	–	1.633 ± 0.364	0.932 ± 0.376	–2.198 ± 0.361	–	1.041 ± 0.433	1.104 ± 0.433	2.042 ± 0.433	3.004 ± 0.433	0.9260	0.2787	0.0144

[1] see Table 2; cortical index change calculated for diet as main effect, % – values were calculated as percentage change in relation to control diet value at each sampling period (diet means (overall from all sampling periods) were compared by orthogonal contrasts); cortical index change calculated for age as main effect, % – values were calculated by subtracting each value at the end of each period (age) by initial value within that period. Values are expressed as percentage change; $^{A–D}$ – values with different superscripts for each main effect (diet or age) are significantly differerent at $P \le 0.05$

and –0.0049 cm · month^{-1}, respectively, according to the regression analysis.

Cortical index was not influenced by dietary treatment but was higher in 368-day old foals (60.61 ± 0.57%) in comparison to 273-day old ones (55.35 ± 0.57), indicating again the age effect (Table 4). The cortical index for all foals over time significantly fit the following regression model:

$$Y = 46.7146 + 0.0367598 \times X; R^2 = 96.84\%$$

where: Y – cortical index (%) and X – age (day).

The monthly cortical index changes were not dependent on either dietary treatment or age; however an interaction ($P < 0.05$) between cortical index and age was observed (Table 4). This effect was only observed on certain combinations between age and dietary treatment. Only at 273 day of life, the monthly change on cortical index was higher in foals reciving additional yeast treatment (4.1175 ± 0.8754) when compared to the control group (1.1604 ± 0.8718), to the bacteria + yeast treated group (1.6463 ± 0.8658) and to the phytase supplemented group (–0.1891 ± 0.8673).

Discussion

The growth of all foals in presented study was similar to that observed in other studies conducted on Mangalarga Marchador foals (Rezende et al., 2000a; Cabral et al., 2004) and general obtained means were as follows: 0.48 kg · day^{-1} for average daily gain (ADG); 0.07 cm · day^{-1} for wither height (WH) and hip height (HH); 0.11 cm· day^{-1} for body length (BL); 0.03 cm · day^{-1} for barrel height (BH); 0.09 cm · day^{-1} for chest girth (CG) and 0.01 cm· day^{-1} for cannon circumference (CC).

Although probiotic or phytase may be beneficial for horse nutrition and health, such effects related to ADG and body linear growth were not observed in this study after 200 days of supplementation. These findings agreed with those stated by Bennett et al. (1991), who found no difference in ADG and WH of yearlings fed diets supplemented with yeast culture. On the other hand, the current findings are in disagreement with Mason (1993) and de Léon and Samudio (2002), who observed higher body weight, ADG and WH in weaned foals fed diet supplemented with *Saccharomyces cerevisiae* for 120 days compared to animals fed basal diet.

These contradictory results can be explained by the difference in growth rate of examined breeds, age of foals, composition of commercial products and the quality of the feedstuffs used in related studies. Glade (1991a,b) observed possitive changes in growth estimated by WH measurements in foals from mares supplemented with *S. cerevisiae*, which showed superior values from 42 to 56 days in comparison to foals from control mares. The use of additive probiotic composed of species-specific microorganisms (Yuyama et al., 2004) and in minimal viable concentration superior to 7 log10 CFU · g^{-1} for each microorganism present in the product (Vanbelle et al., 1990) may be relevant in the attempt to obtain results with probiotic supplementation.

The lowest rate of ADG was 0.36 kg · day^{-1} which was observed for 299-day old foals, coinciding with the harshest period of the regional dry season. The climate of the northern region of Minas Gerais, where Montes Claros is located, is mild semiarid with total absence of rains from May to August. Despite the positive effects of probiotics or phytase supplementation on nutrient digestibility, especially fibre, previously observed in foals from the same species (Moura et al., 2009, 2011), growth rates remained low due to the overall lower quality of dry season pastures (3.67% crude protein and 9.99% lignin; Table 1). The negative effects of nutrient deficiency on foal growth were most pronounced in weight gain, followed by CG, CC and, finally, WH (Rezende et al., 2000a). So, the positive response to probiotic supplementation, when forages with poor quality were offered in the diet, was questionable.

According to the NRC (2007), the use of supplements does not replace basic animal nutrition recommendations. In this sense, the negative influence of diet energy and protein deficiency on foal growth cannot be overcomed by supplementation. If in this study, the addition of other forage types with higher nutritional value, such as lucerne or other tropical legumes (Silva et al., 2012) had been used, normal foal development should have been observed during the dry season.

A temporary improvement in cortical index was confirmed in foals supplemented with *S. cerevisae* Sc47 after 273 days of life, expressed by the interactive effect between age and diet (Table 3). A permanent effect on bone development had been expected with the two probiotics studied, because, in a previous study conducted on Mangalarga Marchador foals, the probiotic-supplemented foals showed an improvement in the uptake of minerals, but no effect of phytase supplementation was observed (Moura et al., 2011). Additionally, Sindhu and Khetarpaul (2001) observed a sharp reduction in the contents of phytic acid, polyphenols and trypsin inhibitor activity with *Lactobacillus casei* or *Lactobacillus plantarum*, alone or associated with *Saccharomyces boulardii in vitro* inoculations (containing 10^6 cells · ml^{-1} broth), which should have also lead to some improvement in mineral uptake.

Moreover, the concentration of the microorganisms in supplements must be considered as a factor that interferes with the possible positive effects of probiotics on body growth. The *S. cerevisiae* concentration in the tested probiotics varied from 10^9 cfu · g^{-1} in the probiotic composed only of the yeast culture (Sc47 strain) to 10^5 cfu · g^{-1} in the probiotic composed of the bacteria and yeast culture. These values should have accounted for benefits in bone development, but only a transitory effect was observed in this experiment.

The absence of a phytase effect could be related to the fact that the dietary calcium and the calcium:phosphorus ratio were balanced to meet the nutritional requirements for foals. Therefore, the phytase catalytic action could have been limited by the deleterious effects of calcium and inorganic phosphorus (Singh, 2008). Further studies in which horse diets will be balanced according to the expected quantity of phosphorus released by enzyme supplements (Dunnett et al., 2009) are needed.

Conclusions

The probiotics and phytase tested under the presented experimental conditions did not influence the body weight and linear measurement growth rates in Mangalarga Marchador foals. Bone development was increased only temporarily by the *Saccharomyces cerevisiae* culture (Sc47 strain) supplementation indicating a possible beneficial effect of this probiotic on mineral balance.

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References

AOAC International, 1995. Official methods of analysis of AOAC International vol. 1. 16th Edition. Arlington, VA

Bennett K., Loch J.C., Lattimer E.M., Green E.M., 1991. Effect of yeast culture supplementation on weight gains, skeletal growth and bone density of third metacarpal in yearling Quarter horses. J. Anim. Sci. 69, Suppl 1, 324 (Abstr.)

Cabral G.C., Almeida F.Q., Quirino C.R., Pinto L.F.B., Santos E.M., Corassa A., 2004. Morphometric evaluation of Mangalarga Marchador horses: linear measures. R. Bras. Zootec. 33, 989–1000

Carroll C.L., Huntington P.J., 1988. Body condition scoring and weight estimation of horses. Equine Vet. J. 20, 41–45

Costa M.D., Bergmann J.A.G., Resende A.S.C., Fonseca C.G., Faria F.J.C., 2005. Study on genetic subdivision of the Mangalarga Marchador horse breed. Arq. Bras. Med. Vet. Zootec. 57, 272–280

de Fombelle A., Varloud M., Goachet A.-G., Jacotot E., Philippeau C., Drogoul C., Julliand V., 2003. Characterization of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets. Anim. Sci. 77, 293–304

de Léon L., Samudio A.D., 2002. Effect of a dietary live-yeast-based supplementation of *Saccharomyces cerevisiae* on the development of Thoroughbred foals during the growth phase. In: International Seminar Microbiology Applied to Animal Nutrition. Guadalajara (México). Available from: lfa-america.com/infortec/info_tec27.html

Dunnett C., Dunnett M., McLachlan F.K., 2009. The effect of a feed ingredient with endogenous phytase activity on phosphorus availability in equine diets. In: A. Lindner (Editor). Applied Equine Nutrition and Training. Equine Nutrition and Training Conference (ENUTRACO) 2011. Wageningen Academic Publishers, Wageningen (The Netherlands), pp. 177–189

FAO/WHO, 2002. Guidelines for the Evaluation of Probiotics in Food. London, Ontario (Canada), pp. 11

Furtado C.E., Barboza E.D., Brandi R.A., Ribeiro L.B., Oliveira A.A.M.A., 2010. Use of yeast in equine fed diets with hay with different nutritional qualities. R. Bras Zootec. 39, 2194–2199

Glade M.J., 1991a. Dietary yeast culture supplementation of mares during late gestation and early lactation: Effects on milk production, milk composition, weight gain and linear growth of nursing foals. J. Equine Vet. Sci. 11, 89–95

Glade M.J., 1991b. Effects of dietary yeast culture supplementation of lactating mares on the digestibility and retention of the nutrients delivered to nursing foals via milk. J. Equine Vet. Sci. 11, 323–329

Gonçalves R.W., Costa M.D., Rezende A.S.C., Rocha Júnior V.R., Leite J.R.A., 2012. Effect of inbreeding on morfhometric traits in Mangalarga Marchador horses. Arq. Bras. Med. Vet. Zootec. 64, 419–426

Hainze M.T.M., Muntifering R.B., Wood C.W., McCall C.A., Wood B.H., 2004. Faecal phosphorus excretion from horses fed typical diets with and without added phytase. Anim. Feed Sci. Tech. 117, 265–279

Jouany J.-P., Medina B., Bertin G., Julliand V., 2009. Effect of live yeast culture supplementation on hindgut microbial communities and their polysaccharidase and glycoside hydrolase activities in horses fed a high-fiber or high-starch diet. J. Anim. Sci. 87, 2844–2852

Kim S.M., Kim C.M., Lee H.K., Park W.P., Lim Y.J., Kim B.J., Chung T.Y., 1991. Evaluation of nutrient values of some feedstuffs, and the effects of yeast culture supplementation on digestibilities of nutrients and blood parameter in horse. Korean J. Anim. Nutr. Feedstuffs 15, 272–280

Mason T.R., 1993. Effect of tall oil and yeast culture on growth rate of wild horses. Res. Bull., McNesse State University, Lake Charles, LA (USA)

Medina B., Girard I.D., Jacotot E., Julliand V., 2002. Effect of a preparation of *Saccharomyces cerevisiae* on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. J. Anim. Sci. 80, 2600–2609

Moura R.S., Saliba E.O.S., Almeida F.Q., Lana A.M.Q., Silva V.P., Rezende A.S.C., 2009. Feed efficiency in Mangalarga Marchador foals fed diet supplemented with probiotics or phytase. R. Bras. Zootec. 38, 1045–1050

Moura R.S., Saliba E.O.S., Almeida F.Q., Lana A.M.Q., Moreira D.C.A., Silva V.P., Moss P.C.B., Perreira R.V.G., Costa M.L.L., Rezende A.S.C., 2011. Apparent digestibility of diets with probiotics or phytase in Mangalarga Marchador foals. Arch. Zootec. 60, 193–203

NRC, 2007. Nutrients Requeriments of Horses. 6th Edition. The National Academies Press. Washington, DC

Patterson D.P., Cooper S.R., Freeman D.W., Teeter R.G., 2002. Effects of varying levels of phytase supplementation on dry matter and phosphorus digestibility in horses fed a common textured ration. J. Equine Vet. Sci. 22, 456–459

Perrone G.M., Pérez A., Caviglia J., Barbará A.C., 2013. Effects of live yeast (*Saccharomyces cerevisiae* Strain 1026) supplementation on the closure of articular growth plates in Quarter Horse foals. J. Equine Vet. Sci. 33 261–265

Pinto L.F.B., Almeida F.Q., Quirino C.R., Azevedo P.C.N., Cabral G.C., Corassa A., 2005. Multivariate analysis of the body measures in Mangalarga Marchador foals: principal components analysis. R. Bres. Zootec. 34, 589–599

Rezende A.S.C., Sampaio I.B.M., Legorreta G.L., Moreira D.C.A., 2000a. Effect of two different nutritional programs on development in Mangalarga Marchador foals. R. Bras. Zootec. 29, 495–501

Rezende A.S.C., Sampaio I.B.M., Legorreta G.L., Moreira D.C.A., 2000b. Effect of two different nutritional programs on orthopedic alterations in Mangalarga Marchador foals. J. Equine Vet. Sci. 20, 651–656

Saastamoinen M., 1990. Factors affecting growth and development of foals and young horses. Acta Agr. Scand. 40, 387–396

Santos E.M., Almeida F.Q., Vieira A.A., Pinto L.F.B., Corassa A., Pimentel R.R.M., Silva V.P., Galzerano L., 2005. Lactation in Mangalarga Marchador mares: yield production and composition of milk, and weight gain of suckling foals. R. Bras. Zootec. 34, 627–634

Silva V.P., Rezende A.S.C., Saliba E.O.S., Costa M.L.L., Pereira R.V.G., Lima J.T.M., 2012. Intake and digestibility of concentrate and legume hay diets fed to foals. R. Bras. Zootec. 41, 655–661

Sindhu S.C., Khetarpaul N., 2001. Probiotic fermentation of indigenous food mixture: effect on antinutrients and digestibility of starch and protein. J. Food. Compost. Anal. 14, 601–609

Singh P.K., 2008. Significance of phytic acid and supplemental phytase in chicken nutrition: a review. World Poultry Sci. J. 64, 553–580

van Doorn D.A., Everts H., Wouterse H., Beynen A.C., 2004. The apparent digestibility of phytate phosphorus and the influence of supplemental phytase in horses. J. Anim. Sci. 82, 1756–1763

Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597

Vanbelle M., Teller E., Focant M., 1990. Probiotics in animal nutrition: a review. Arch. Tierernähr. 40, 543–567

Yuyama T., Yusa S., Takai S., Tsubaki S., Kado Y., Morotomi M., 2004. Evaluation of a host-specific Lactobacillus probiotic in neonatal foals. Int. J. Appl. Res. Vet. Med. 2, 26–33