

Evaluation of Mangalarga Marchador foal development in the first year of life

Avaliação do desenvolvimento de potros da raça Mangalarga Marchador em seu primeiro ano de vida

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ABSTRACT

The purpose of this study was to examine the development pattern of Mangalarga-Marchador foals by determination of muscle development and deposition of adipose tissue associated with the evaluation of blood-based biomarkers. Thus, 72 Mangalarga Marchador foals (ages ranged from 1-12 mo) were randomly selected and divided into 12 age groups (n=6 per group), evaluated monthly. The thickness of the *Longissimus dorsi* (LD) and rump fat (RF) were determined through an ultrasound performed on each foal's left side. Such parameters were used to calculate the percentage of body fat (BF), fat mass (FM), and fat-free mass (FFM). Morphometric measurements were taken on the foal's right side using a tape measure and a hypsometer. Blood samples were collected for determinations of total proteins, albumin, triglycerides, cholesterol, urea, creatinine, and glucose. Morphometric measurements, the thickness of the LD and RF, weight, BF, FM, and FFM showed differences between age groups ($P < 0.001$). Muscle development and fat deposition in subcutaneous tissue were greater between six and 12 months and average weight gain and FFM were proportionally greater between one and six months. We concluded that morphometric measurements increase steadily and linearly during the first 12 months of life, indicating that it is feasible to use the linear regression formula. Moreover, significant differences between age and biochemical biomarkers may be attributed to nutritional and digestive physiology adaptations during these periods.

Keywords: Growth. *Longissimus dorsi*. Rump fat. Withers height. Fat mass.

RESUMO

O objetivo desse estudo foi examinar o padrão de desenvolvimento de potros Mangalarga Marchador através da determinação do desenvolvimento muscular e da deposição de tecido adiposo associado com avaliação de biomarcadores sanguíneos. Assim, 72 potros Mangalarga Marchador (idades entre 1 e 12 meses) foram selecionados ao acaso e divididos em 12 grupos etários (n=6/grupo), avaliados mensalmente. A espessura do *Longissimus dorsi* (LD) e da cobertura de gordura da garupa (RF) foi determinada através de ultrassonografia realizada do lado esquerdo de cada animal, tais parâmetros foram usados para calcular percentagem de gordura corporal (BF), massa de gordura (FM) e massa livre de gordura (FFM). Mensurações morfométricas foram obtidas do lado direito de cada potro, usando fita métrica e hipômetro. Amostras sanguíneas foram obtidas para determinações de proteínas totais, albumina, triglicérides, colesterol, uréia, creatinina e glicose. Medidas morfométricas, espessura de LD e RF, peso corporal, BF, FM e FFM mostraram diferenças entre os grupos etários ($P < 0,001$). O desenvolvimento muscular e a deposição de gordura no tecido subcutâneo foram maiores entre 6 e 12 meses e a média de ganho de peso e de FFM foi proporcionalmente maior entre 1 e 6 meses. Foi possível concluir que as medidas morfométricas aumentam de forma constante e linear nos primeiros 12 meses de vida, indicando que é factível usar fórmulas de regressão linear. Ainda, diferenças significativas entre idade e biomarcadores bioquímicos podem ser atribuídos às adaptações nutricionais e digestivas durante esses períodos.

Palavras-chave: Crescimento. *Longissimus dorsi*. Gordura da garupa. Altura de cernelha. Massa de gordura

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Introduction

One of the main sources of income for horse breeders is the sale of foals up to one-year-old. The longevity and future athletic performance of foals can be ensured by aiding their proper musculoskeletal development, albeit without accumulating excess fat reserves. Besides, knowledge of foal development patterns can enable the early selection of those with greater aptitude for sporting performance and functionality (Freeman, 2005; Pinto et al., 2005; Hunka et al., 2014).

Body condition score (BCS) is an indicator of the equine body status, based on fat deposition (Gobesso et al., 2014). In 1976, other authors (Westervelt et al., 1976) demonstrated that ultrasound measurements of rump fat (RF) are correlated with the fat percentage (FP) of horses; hence, ultrasound also began to be used in monitoring horse management, nutrition, and training (Gobesso et al., 2014).

The determination of FP in horse sheds reveals the metabolic adaptations in their different productive phases (Manso et al., 2009). The *Longissimus Dorsi* (LD) is one of the most frequently used muscles during exercises, which therefore allows for a better evaluation of the horse performance in races (D'Angelis et al., 2004). This muscle is considered easily accessible because of its superficial location between T14 and T18 in adult horses, enabling easy and good ultrasound (Scheven, 2010). In addition, knowledge of the levels of blood biomarkers reinforces understanding about foal development.

We aimed to study the development of Mangalarga Marchador foals, using morphometric and ultrasound measurements, determining muscle development and the deposition of adipose tissue, and analyzing blood biomarkers.

Materials and Methods

The study involved 72 Mangalarga Marchador foals aged between one and 12 months, from a farm located in the municipality of Feira de Santana, Bahia, Brazil (latitude 12.24 degrees S and longitude 39.03 degrees W). The foals were selected at random, according to the birth sequence at the property, and were divided into 12 age groups. The animals were weighed, morphometric measurements were taken, LD muscle and RF thickness were measured by ultrasound, and blood samples were collected.

All the animals were subjected to the same animal health management and feeding program. The deworming and vaccination protocols were started when the foals reached four months of age when they were vaccinated against rabies, encephalomyelitis, influenza, rhinopneumonitis, and equine tetanus, with reinforcement after 30 days. Deworming was carried out every three months, alternating the active ingredients of the products.

The foals were kept in paddocks with their mothers from birth to five months of age, after which they were weaned and housed in individual stalls during the daytime. At this age, their feed began to be supplemented with commercial feed¹ (minimum 18% of crude protein and 3,470 kcal/kg of digestible energy), 1% of live weight per day, divided into three times a day, and Tifton hay (*Cynodon nlemfuensis*) and mineral salt² at will. From 5PM to 7AM, the foals were kept in paddocks with free pasture access.

The LD and RF were measured using an ultrasound system³ and a linear transducer operating at a frequency of 5 Mhz. Because these are highly valuable animals intended for exhibitions, the region assessed by ultrasound was not shaved. The images were captured using commercial alcohol and coupling gel for B-mode ultrasound scanning, which was performed by a single operator, always on the left side of the foal.

The thickness of the LD muscle was measured at the height of the 17th and 18th ribs, with the transducer, positioned transverse to the thoracolumbar spine, following the methodology described by D'Angelis et al. (2004). The measurement was always taken from the image of the thickest portion of the cross-section of the muscle.

RF thickness was measured at the meeting point between two imaginary lines, the first from the ischium to the base of the tail and the second from the ilium to the lumbosacral spine (at the height of L6-S1), using a methodology adapted from Gentry et al. (2004). The FP was calculated based on the RF, using the formula $FP = 8.64 + (4.7 \times RF)$ (Westervelt et al., 1976). Fat mass (FM)

and fat-free mass (FFM) were calculated based on weight and FP (Abreu et al., 2009; Hunka et al., 2014).

The foals' morphometric measurements and weights were determined according to the previously described methodology (Cabral et al., 2004). The following were measured: withers height (WH), vertical distance from the highest point of the interscapular region (between the T5-6 spinous processes) to the ground; croup height (CH), vertical distance between the highest point of the croup (on the sacral tuberosity) to the ground; croup length (CL), distance between the cranial part of the iliac tuberosity and the caudal portion of the ischial tuberosity; chest girth (CG), the external circumference of the thoracic cavity, just below the withers, at the height of the ninth rib joint with the xiphoid process; the shin circumference (SC), measured on the middle region of the shin; head length (HL), distance from the nuchal crest to the central portion of the upper dental arch (tip of nose); elbow-to-ground distance (EGD), distance between the apex of the olecranon process and the ground; and lastly, rump width (RW), distance between the lateral ends of the right and left iliac tuberosities (region of the tips of the right and left hips). All the measurements were taken on the foal's right side, on even ground, using a tape measure and a horse height measuring stick⁴. Animal weight was estimated using a specific weigh-tape for horses, passing the tape over the chest right below the withers, at the point where the ninth rib joins the xiphoid process.

The serum biochemical profile was examined using blood samples collected aseptically under negative pressure by jugular venipuncture with disposable 25x0.8 mm needles. Blood samples were drawn into 9 ml tubes with a gel separator to determine serum total protein, albumin, triglycerides, cholesterol, urea, and creatinine; and into 4 mL sodium fluoride tubes to measure plasma glucose levels. All the samples were sent to the laboratory in a cooler and processed within an average time frame of 4 h between collection and laboratory analysis. The blood analyses were performed

using automated⁵ and semi-automated⁶ biochemistry analyzers for glucose, and commercial kits⁷.

Statistical analyses were performed to study the effects of age (in months) and sex (males vs. females, considering age as a blocking factor). The choice between parametric and non-parametric tests was based on the Kolmogorov-Smirnov normality test. The variables considered parametric (HL, WH, CH, EGD, CL, RW, weight, LD and RF thickness, FP, FFM, FM, creatinine, urea, and cholesterol) were subjected to an analysis of variance (One Way ANOVA), followed by Tukey's test for comparison between the age groups. The variables considered non-parametric (SC, CG, and triglycerides) were subjected to the Kruskal-Wallis non-parametric test, followed by Dunn's multiple comparison test for comparison between the age groups. A 5% level of significance was adopted, for a 95% confidence interval, with two-tailed analysis. The statistical analyses were performed using the SPSS 21.0 package for Windows. Based on the initial results, the variables HL, WH, CH, EGD, CL, RW, CG, and SC were subjected to linear regression as a function of age, using the GLM procedure of SAS-Statistical Analysis Software.

Results

Table 1 describes the morphometric measurements and weight, and all the analyzed variables show significant differences as a function of age. The weight and RF and LD thickness showed a growing and linear increase. Table 2 lists the average values of the LD and RF thicknesses, as well as the FP, FM, and FFM, which were calculated based on the RF and weight. Table 3 presents the averages of the biochemical variables according to age and blood biochemical analysis. Note that age development significantly influenced the foals' urea, cholesterol, and triglycerides levels.

Gender differences were noticed for HL ($P<0.0001$), WH ($P=0.0017$), CH ($P=0.0215$), and EGD ($P=0.0065$)

Table 1 – Morphometric measurements and body weight in Mangalarga Marchador foals in their first year of life

Parameters (m)	Age (months)												SEM	P
	1	2	3	4	5	6	7	8	9	10	11	12		
HL	0.47 ^a	0.512 ^{abc}	0.533 ^{bce}	0.553 ^{cdefg}	0.592 ^{defghijk}	0.575 ^{efghik}	0.592 ^{fg hijk}	0.599 ^{ghijk}	0.602 ^{hijk}	0.607 ^{jk}	0.623 ^{jk}	0.612 ^k	0.57	< 0.001
WH	1.032 ^a	1.11 ^{bc}	1.125 ^{cd}	1.178 ^{defg}	1.21 ^{efgh}	1.215 ^{efghij}	1.235 ^{ghij}	1.27 ^{hijkl}	1.28 ^{ijkl}	1.28 ^{kl}	1.315 ^{kl}	1.317 ^l	1.07	< 0.001
CH	1.063 ^a	1.143 ^{bc}	1.155 ^{cd}	1.215 ^{defg}	1.242 ^{efg}	1.242 ^{fg}	1.273 ^{ghij}	1.312 ^{hijkl}	1.313 ^{ijkl}	1.327 ^{kl}	1.36 ^{kl}	1.37 ^l	1.13	< 0.001
EGD	0.667 ^a	0.723 ^{bc}	0.743 ^{cd}	0.763 ^{defgh}	0.783 ^{efghijk}	0.785 ^{efghijk}	0.767 ^{ghijk}	0.792 ^{hijkl}	0.807 ^{ijkl}	0.812 ^{kl}	0.813 ^{kl}	0.828 ^l	0.56	< 0.001
CL	0.238 ^a	0.253 ^a	0.267 ^{ab}	0.253 ^a	0.278 ^{abce}	0.31 ^{bcddefg}	0.323 ^{cddefgh}	0.332 ^{defgh}	0.325 ^{efgh}	0.347 ^{fgh}	0.328 ^{gh}	0.368 ^h	0.55	< 0.001
RW	0.313 ^a	0.34 ^{ab}	0.35 ^{abc}	0.362 ^{abcd}	0.38 ^{bcddefgh}	0.362 ^{abcd}	0.38 ^{cddefg}	0.403 ^{defgh}	0.413 ^{efgh}	0.422 ^{fgh}	0.428 ^{gh}	0.433 ^h	0.52	< 0.001
SC	0.135 ^a	0.14 ^{ac}	0.136 ^a	0.1417 ^{abc}	0.148 ^{abcd}	0.15 ^{abcd}	0.152 ^{abcd}	0.158 ^{abcd}	0.167 ^b	0.162 ^a	0.165 ^c	0.167 ^d	0.16	< 0.001
CG	1.01 ^a	1.133 ^{ab}	1.173 ^{ab}	1.368 ^{abcde}	1.405 ^{abcde}	1.417 ^{abcde}	1.455 ^{abcde}	1.475 ^{bcde}	1.43 ^{abcde}	1.56 ^{cde}	1.592 ^{de}	1.555 ^e	2.19	< 0.001
BW (kg)	99.7 ^a	108.8 ^a	115.3 ^a	165.8 ^{bcd}	169.3 ^{cdefg}	171.7 ^{defg}	203.8 ^{efg}	205.2 ^{fg}	204.3 ^a	241.7 ^{hij}	246.5 ^{ij}	257.8 ^{ij}	6.45	< 0.001

NOTE: Lowercase letters denote significant differences between ages. HL (head length), WH (withers height), CH (croup height), EGD (elbow-to-ground distance), CL (croup length), RW (rump width), SC (shin circumference), CG (chest girth), BW (body weight), SEM (standard error of the mean).

Table 2 – Fat mass, fat-free mass, fat percentage values, and *Longissimus dorsi* and rump fat thicknesses in Mangalarga foals in their first year of life

Parameters (m)	Age (months)												SEM	P
	1	2	3	4	5	6	7	8	9	10	11	12		
LD*	1.75 ^a	1.78 ^a	1.96 ^{abcd}	2.04 ^{abcd}	2.01 ^{abcd}	2.10 ^{abcd}	2.26 ^{bcdfg}	2.28 ^{cdfg}	2.27 ^{dfg}	2.72 ^{efg}	2.43 ^{fg}	2.6 ^g	0.04	< 0.001
RF*	0.49 ^a	0.5 ^a	0.59 ^{abc}	0.59 ^{abc}	0.55 ^{ac}	0.58 ^{abc}	0.7 ^{abcd}	0.87 ^{bcde}	0.79 ^{abcde}	0.84 ^{cde}	0.94 ^{de}	1.05 ^e	0.27	< 0.001
FM (kg) ²	10.9 ^a	12.0 ^a	13.2 ^{ab}	18.9 ^{bc}	19.0 ^{bc}	19.5 ^{bc}	24.3 ^{cd}	26.1 ^{de}	25.2 ^{cd}	30.4 ^{def}	32.3 ^{ef}	35.3 ^{ef}	5.5	<0.001
FFM (Kg) ³ *	88.7 ^a	96.8 ^a	102.1 ^a	146.9 ^b	150.3 ^{bc}	152.1 ^{bc}	179.5 ^c	179.0 ^c	179.1 ^c	211.2 ^d	214.2 ^d	222.5 ^d	0.12	<0.001
FP (%) ¹ *	10.9 ^a	11.0 ^a	11.4 ^{ab}	11.4 ^{ab}	11.2 ^{ac}	11.4 ^{ab}	11.9 ^{abcd}	12.7 ^{bde}	12.3 ^{abcde}	12.6 ^{bcde}	13.0 ^{de}	13.6 ^e	0.026	<0.001

NOTE: Lowercase letters denote significant differences between ages. LD (*Longissimus dorsi*), RF (rump fat), FM (fat mass), FFM (fat-free mass), FP (fat percentage), SEM (standard error of the mean). *Values are presented in 10⁻³. 1.FP = 8.64 + (4.7 x RF) (Westervelt et al., 1976); 2. FM = body weight (kg) x FP/100; 3. FFM = body weight (kg) – FM.

Table 3 – Biochemical values in Mangalarga Marchador foals in their first year of life

Parameters	Age (months)												SEM	P-value
	1	2	3	4	5	6	7	8	9	10	11	12		
Urea (mg/dL)	22.7 ^{bc}	22.1 ^c	26.8 ^{abc}	37.4 ^{ab}	37.9 ^a	27.9 ^{abc}	34.6 ^{abc}	30.5 ^{abc}	24.8 ^{abc}	35.5 ^{abc}	37.4 ^{ab}	33.1 ^{abc}	1.28	0.041
Cholesterol (mg/dL)	121.3 ^{abcd}	138.2 ^{ab}	136.2 ^{abc}	151.3 ^a	106.5 ^{bcd}	101.2 ^{cd}	96.7 ^d	102.2 ^{bcd}	98.0 ^d	97.7 ^d	109.8 ^{bcd}	96.5 ^d	2.94	<0.001
Triglycerides (mg/dL)	30.1 ^{ab}	30.3 ^{ab}	46.1 ^{ab}	71.0 ^a	19.3 ^b	22.6 ^{ab}	18.9 ^b	22.5 ^{ab}	25.3 ^{ab}	23.4 ^{ab}	23.0 ^{ab}	23.2 ^{ab}	3.66	0.018
Creatinine (mg/dL)	1.49	1.47	1.48	1.46	1.48	1.37	1.36	1.30	1.41	1.25	1.42	1.44	0.28	0.815
Glucose (mg/dL)	163.0	149.1	130.9	143.1	149.0	150.3	152.7	149.9	140.5	137.2	150.8	145.6	3.66	0.958
Total protein (g/dL)	5.81	5.98	6.14	6.36	6.12	5.99	5.73	5.70	6.25	6.10	5.84	5.91	0.05	0.113
Albumin (g/dL)	2.81	2.92	2.89	2.99	2.94	2.66	2.70	2.74	2.75	2.89	2.93	2.73	0.03	0.303

NOTE: Lowercase letters denote significant differences between ages. SEM (standard error of the mean).

Discussion

The period of greatest development of the foal occurs in the first 12 months of life, starting at 10% of the weight of an adult at birth to 65% of the weight and 90% of the height of an adult when it reaches 12 months (Frape, 2004; Garcia et al., 2011). Height is directly related to bone development, which occurs earlier, followed by muscle development, and lastly, by fat deposition. Fat deposition in horses varies with advancing age and body weight, as well as nutrition (Pinheiro et al., 2007), and also according to different breeds and their abilities.

In this study, at one month of age, the foals presented 68% to 71% of the expected height of the adult, while at six months they had reached 80% to 83%, and at 12 months between 86% to 90% of the expected height of the adult. These findings are very similar to those reported in Mangalarga Marchador foals (Cabral et al., 2004), and Criollo foals (Moraes et al., 2017).

For official horse registration, the Brazilian Association of Mangalarga Marchador Horse Breeders (Associação Brasileira dos Criadores de Cavalos Mangalarga Marchador, 2017) recommends that the standard is for males of this breed to have a withers height greater than or equal to the croup height, while it is permissible for females to have a croup height up to two centimeters greater than the withers. In this study, all the foals had a croup height greater than the withers height in the first 12 months of

life, with a maximum difference of 5.5 cm in favor of the croup at 12 months. This difference was also reported previously, albeit to a lesser extent (Cabral et al., 2004). This discrepancy may be associated with a predominant lineage, even with the development pattern of the breed, or because foals are born with the croup higher than the withers, but the latter grows more, with higher values in adulthood (Cabral et al., 2004). Yet, for both variables (WH and CH) higher values were observed in males, also similar to Cabral et al. (2004).

The technique to measure LD thickness, which is measured at the height of the last two ribs, has been done (D'Angelis et al., 2004, 2007; Scheven, 2010). The total growth of the LD muscle in the foals in this study was 0.85 cm, indicating an increase of 32.7% in this time frame, with the period of greatest growth between six and 12 months, when the LD increased by 19.23%. These values are higher than those described by Ringmark et al. (2017), who found a 6.4% increase in the 24 months in Standardbred horses, indicating that there are differences in the growth rates in different periods.

The technique for measuring the thickness of the fat layer was adapted from Gentry et al. (2004). Due to the foals' different body sizes and conformations during the growth phase, it is infeasible to standardize the measurement points based on techniques that use distances in centimeters starting from anatomical points.

Thus, the references for the positioning of the ultrasound probe to measure the RF were determined from the meeting of two lines starting from anatomical points, so that regardless of the foal's size and conformation, the measurement can be taken at a single point. The RF increased by 15.52% between one and sixth months of age and by 44.76% between six and 12 months of age. Another study (Hunka et al., 2014) found RF thicknesses lower than those found in this study, reporting that the greatest RF thickness they found was 0.187 cm in five-month-old foals of the Quarter Horse breed. This difference in RF thicknesses may be attributed to differences among breeds, given the different abilities of Quarter Horse and Mangalarga Marchador breeds, the former being characterized by physical abilities for anaerobic exercises and the latter for aerobic exercises, with a greater fat reserve for energy metabolism.

Upon using the formula of several studies (Westervelt et al., 1976; Abreu et al., 2009; Manso et al., 2009; Hunka et al., 2014; Ringmark et al., 2017), an average body fat percentage (FP) was observed, based on the measurement of the RF thickness of 10.9% in the first month of life, 11.37% at six months and 13.58% at 12 months old. Another research (Manso et al., 2009) reported a fat percentage of about 10% in horses of different breeds younger than 18 months, and [15] found an FP of 9.52% in five-month-old Quarter Horse foals, unlike what was found in the present study, which again, can be attributed to differences among breeds and their abilities. Complementing, Manso et al. (2009) found mean RF values for horses of various breeds, up to 18 months old, of 0.37 cm and FP of 10%, while sports horses that participated in gait tests showed average RF of 1.305 cm and FP of 14.7%. A comparison of these data with those of the present study indicates that the Mangalarga Marchador foals presented average RF and FP already at 12 months of age, close to that of sports horses participating in gait tests.

Assuming that the average weight of an adult Mangalarga Marchador horse is approximately 450 kg, we can consider that the foal's weight reached 22.15% of the adult's weight in the first month of life, 38.15% at six months, and 57.28% at 12 months of age. According to Frappe (2004) and Garcia et al. (2011), horses are expected to reach 65% of the adult weight at 12 months of age, regardless of breed, which was not the case in this study. However, the proportion of 57.28% would be within the range recommended by Gallio et al. (2014), to prevent joint damage due to overweight in young animals.

The research showed that age developments significantly influenced the levels of urea, cholesterol, and triglycerides.

It was suggested that the type of management and feed influences the markers of protein metabolism, such as urea (Melo et al., 2013). Also, milk intake can strongly affect changes in serum levels of cholesterol, triglycerides, and glucose (Howard et al., 2007), and it was also stated that plasma concentrations of triglycerides and cholesterol can be altered by the absorption of dietary lipids (Fernandes et al., 2001; Durham, 2006). It should also be noted that the period of life in which these foals were studied coincides with the period when they undergo the greatest development of their gastrointestinal tract, favoring the processes of digestion and absorption of food, which are reflected in changes in their blood biomarkers.

Conclusions

We concluded that during the first 12 months of life the morphometric measurements of Mangalarga Marchador foals increase steadily and linearly, making it feasible to use the linear regression formula. Moreover, it was found that significant differences between age and biochemical biomarkers can be attributed to nutritional changes and the increase in digestive capacity resulting from the development of the gastrointestinal tract.

Manufacturers

¹Equitage Potro, Guabi Nutrição e Saúde Animal, Campinas, SP, Brazil.

²Coequi, Tortuga, Pecém, CE, Brazil.

³MyLab Five, Esaote, Monções, SP, Brazil.

⁴Walmur, Porto Alegre, RS, Brazil.

⁵PKL 125, Pontecagnano Faiano, SA, Italy.

⁶Bio 2000, Bioplus, Barueri, SP, Brazil.

⁷Bioclin, Belo Horizonte, MG, Brazil.

Conflict of Interest

The authors report no conflicts of interest. The authors are entirely responsible for the content and writing of this paper.

Ethics Statement

This study was approved by the Ethics Committee on Animal Use (CEUA-UFBA), under Protocol No. 76/2018.

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