



## Effects of supplementation with *Saccharomyces cerevisiae* and aerobic training on physical performance of Mangalarga Marchador mares

Tiago Resende Garcia<sup>1</sup>, Adalgiza Souza Carneiro de Rezende<sup>1</sup>, Pablo Trigo<sup>2</sup>, Juliano Martins Santiago<sup>3</sup>, Fernando Queiroz de Almeida<sup>4</sup>, Rosângela Antunes Terra<sup>1</sup>, Mayara Gonçalves Fonseca<sup>1</sup>, Francisco Castejón<sup>2</sup>

<sup>1</sup> Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Zootecnia, Belo Horizonte, MG, Brasil.

<sup>2</sup> Universidad de Córdoba, Córdoba, España.

<sup>3</sup> Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, Serra Talhada, PE, Brasil.

<sup>4</sup> Universidade Federal Rural do Rio de Janeiro, Instituto de Veterinária, Departamento de Medicina e Cirurgia Veterinária, Seropédica, RJ, Brasil.

**ABSTRACT** - The objective of this study was to assess the effects of supplementation with *Saccharomyces cerevisiae* on the performance of Mangalarga Marchador equines and to verify the effectiveness of the aerobic training protocol adopted. The study used 14 Mangalarga Marchador mares in a completely randomized split-plot design. The plots were made up of two treatments (groups Probiotic and Control) and the split plots comprised two maximal-effort physical tests applied both before and after the six weeks of training. The animals' spirometric parameters, heart rate, and plasma lactate levels were assessed. No difference was found between the Probiotic and Control groups in any of the parameters assessed. A difference was found in the physical tests for time-to-fatigue, aerobic and anaerobic thresholds, speed at which plasma lactate levels reached 2 and 4 mmol/L, speed at which the heart rate reached 200 bpm, and the lactate level at 30 min of the recovery period. Supplementation with *Saccharomyces cerevisiae* has no effect on physical conditioning of Mangalarga Marchador mares and the aerobic training protocol adopted increases the animals' aerobic and anaerobic capacity.

Key Words: equine, nutrition, probiotic, spirometry

### Introduction

Horse breeding and use have a prominent position in both developed and developing countries (CEPEA, 2006) and Brazil holds the fourth largest equine herd in the world with 5.5 million animals (FAO, 2010). In this context, the Mangalarga Marchador breed is responsible for the largest and most representative equine herd in Brazil (CEPEA, 2006) and the Brazilian Mangalarga Marchador Horsebreeders' Association (ABCCMM) is the largest equine breed association in Latin America.

Besides marcha competitions, the main competition of this breed, cavalcades, endurance rides, and functional and handling contests are part of ABCCMM's competitions. All these trials require great energy expenditure and the good performance of animals in such competitions is highly dependent on adequate nutrition associated with proper training.

The use of probiotics aiming to improve animal health has become routine, while research has shown their benefits

in the better absorption of equine diets (Moura et al., 2009; Moura et al., 2011; and Rezende et al., 2012). These authors justify the benefits of adding probiotics to equine diets to their effect on the fermenting activity and the stabilization of the cecum-colon microbiota, which potentiates nutrient absorption.

In equine sports the physical conditioning of animals plays an important role in getting good results; it is closely related to the physical ability of the animal and the training protocol adopted. The physical conditioning can promote or maintain maximum performance, develop technical skills, minimize the occurrence of injuries, and maintain willingness and enthusiasm for exercise (Boffi, 2007).

The present study aimed to assess the effects of *Saccharomyces cerevisiae* supplementation and six weeks of aerobic training on the physical performance of Mangalarga Marchador mares.

### Material and Methods

The experiment was carried out in the city of Rio de Janeiro/RJ, Brazil. The experimental procedures were assessed and certified by the Ethics Committee on Animal Experimentation of Universidade Federal de Minas Gerais (CETEA - UFMG), under protocol 206/2008.

Received July 30, 2014 and accepted November 24, 2014.

Corresponding author: adalgizavetufmg@gmail.com

<http://dx.doi.org/10.1590/S1806-92902015000100004>

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Fourteen clinically healthy non-pregnant Mangalarga Marchador mares between four and nine years-old weighing between 320 and 413 kg were used. The mares had never been trained. The study followed a completely randomized split-plot design. The plots were made up of two treatments (Probiotic and Control groups) with seven repetitions per treatment, while the split plots comprised two maximal-effort physical tests applied before and after the six weeks of training.

The animals were housed in individual masonry stalls with feeding troughs for hay and concentrate, and drinking troughs for free access to water. The horses were fed a diet made up of coastcross (*Cynodon dactylon*) hay and commercial concentrate (Itambé® Expander Atleta feed). The mares were weighed at the beginning of the experimental period and every week afterwards. At each weighing, the daily diet was calculated as 2.5% of the live weight of each animal, which represented the dry matter intake until the next weighing. The amount of concentrate provided represented 50% of that value. These values were determined for horses in moderate work, according to the NRC (2007). The concentrate feed was split into three daily portions supplied at 06.00 h, 12.00 h, and 18.00 h, while hay was split into two portions supplied at 08.00 h and 19.00 h. Mineral salt was freely available.

The experimental trial was divided into two phases. In the first phase, which lasted 15 days, the mares adapted to the facilities, handling, and to the treadmill exercises (Galloper Sahinco®) wearing a spirometry mask (MetaVet 1.0 - Cortex Biophysik), and to the automated walker (Sahinco®). The first treadmill maximal ergospirometry test was applied at the end of the first phase. In the second phase, which lasted 42 days, the mares underwent aerobic training and the Probiotic group, besides being fed the standard diet, started to receive 20 g *Saccharomyces cerevisiae* (Biosaf Sc 47® - Lesaffre Feed Additives) daily (minimum of  $1.9 \times 10^9$  cfu/g) and also the concentrate for the first time.

The mares trained for six weeks, Mondays through Saturdays, alternating the exercise on the treadmill and the automated walker. The training protocol on the treadmill consisted of an initial 10-min warm-up period, comprising five minutes of walking (1.6 m/s) and five minutes of marching (3.5 m/s). Next, the treadmill was sloped at 3° and the animals marched for 30 min at a specific speed for each animal that corresponded to 80% of their aerobic threshold determined in the first ergospirometry test, ranging from 3.6 to 4.4 m/s. Cool-down was carried out for 10 min, consisting of five minutes of marching (3.5 m/s) and five minutes of walking (1.6 m/s), with no treadmill slope. The exercise in the walker consisted of 60 min walking at 1.6 m/s, comprising 30 min clockwise and 30 min counter-clockwise.

In the maximal-effort tests, performed before and after the six weeks of training, the mares warmed up for 10 min, consisting of five minutes of walking at 1.6 m/s and five minutes of marching at 3.5 m/s, with the treadmill flat. After the warm-up period, the speed was raised to 4 m/s and the treadmill was set to a 3° slope. From this point on, the speed was progressively raised by 1 m/s every other minute until the animals could not keep up with the treadmill speed, indicating they had reached fatigue. Next, the mares underwent a 10-min cool-down period, consisting of five minutes of marching (3.5 m/s) and five minutes of walking (1.6 m/s), with the treadmill remaining at a 3° slope.

During the tests, the animals wore a heart rate monitor (Equine - Polar®) to assess this parameter before and after the tests. Before each test, the horses' left jugular vein was cannulated so blood could be collected in the last 15 s at each speed and at 10, 30, and 60 min into the recovery period.

On test days, the first (baseline) blood sample was collected at 16.00 h using vacuum blood collection needles (Vacutainer BD®) and vacuum tubes with sodium fluoride (Equine - Polar®) so that the plasma lactate levels were determined. The blood samples were centrifuged at 3,000 rpm for 10 min to separate the plasma. Next, 1 mL plasma aliquots were stored in polypropylene Eppendorf tubes and stored at -18 °C for further analyses. Plasma lactate level analyses were carried out using a reagent kit (Katal® for lactate and Biosystem® for uric acid) and a spectrophotometer (BTS 315 - Biosystem®).

During the tests, the animals wore a spirometry mask to measure oxygen intake ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), respiratory rate, and tidal volume every 10 s. The aerobic and anaerobic thresholds were determined from these data (Aunola and Rusko, 1988).

The gas analysis system was calibrated before the tests using a gas mixture with  $\text{O}_2$  and  $\text{CO}_2$  levels that set the measuring range. Oxygen intake and  $\text{VCO}_2$  were corrected for normal temperature, pressure, and humidity conditions.

The speed indices at which the heart rate reached 150 beats per minute (bpm) ( $S_{150}$ ) or 200 bpm ( $S_{200}$ ) were estimated through linear regression of the heart rate values as a function of the mares' speed during the tests from the speed of 6 m/s. The estimates of the speed at which plasma lactate levels reached 2 mmol/L ( $\text{SLa}_2$ ) and 4 mmol/L ( $\text{SLa}_4$ ), as well as the plasma lactate levels at which the heart rate reached 150 bpm ( $\text{La}_{150}$ ) and 200 bpm ( $\text{La}_{200}$ ), were obtained through exponential regression of the horses' lactate levels during the progressive steps in each test. The results were subjected to analysis of variance and the means were compared by Fisher's test at 5% probability using the SISVAR (version 5.0) and SAEG (version 9.1) software.

## Results and Discussion

No difference ( $P>0.05$ ) was found between the Probiotic and Control groups in any of the assessed parameters. However, differences ( $P<0.05$ ) were found between the maximal-effort physical tests for time-to-fatigue, aerobic and anaerobic thresholds,  $SLa_2$ ,  $SLa_4$ , and  $S_{200}$  (Table 1).

Rezende et al. (2012) did not observe effects of daily supplementation with 20 g probiotics on physical performance of Andalusian horses subjected to six weeks of training.

In contrast with the results found in present work, Glade and Campbell (1990) observed that a culture of live yeast added to horse diet led to lactate and heart rate reduction in trained animals and suggested that the increased aerobic capacity of supplemented animals was a result of the improvement in nitrogen balance, inducing an increase in muscle mass and metabolic efficiency by increasing muscle vascularity and, consequently, cardiovascular capacity.

The results of the present study also differed from those of Art et al. (1994), who observed an increase in aerobic energy generation in horses supplemented with probiotics. According to Poole and Erickson (2008), better aerobic energy usages were accompanied by improvement in heart response, with increases in  $O_2$  supply to the muscles, metabolism and product removal, and were directly related to exercise intensity.

According to Rezende et al. (2012), different responses observed in studies using probiotics in horses may be due to differences in the amount of live yeast supplement given to the animals, as there are no studies suggesting the ideal dose to be used.

A positive influence of the probiotic nutritional effect on horse performance in this study may not have been

evidenced because the animals from both groups were trained properly.

Another factor to be considered was that animal diets from both groups were balanced and provided the necessary nutrients for horses undertaking moderate activity (NRC, 2007). If animals were receiving low-fiber and low-nutrient diets, performance would have been affected. It should also be noted that the horses in this study had relatively constant body weight, indicating that dietary energy requirement during the training program was adequately met by adjustments in the diet, following the weekly assessment of animal body conditions. This suspicion agreed with Furtado et al. (2010), who concluded that use of yeast may be beneficial in the diet for horses that are fed a low-quality diet.

The aerobic and anaerobic thresholds, determined both through spirometry and through the lactate levels ( $SLa_2$  and  $SLa_4$ ), had very similar values. The two methods to determine the aerobic and anaerobic limits have been shown to be appropriate in assessing equine physical performance (Amann et al., 2004). Both in humans and in equines, the anaerobic threshold, determined from lactate, occurs at maximal  $VO_2$  percentages similar to the anaerobic threshold determined through spirometry (McDonough et al., 2002).

The training performed at 80% of the aerobic threshold resulted in the increase of the aerobic and anaerobic thresholds of mares and, consequently, of  $SLa_2$  and  $SLa_4$ . This result showed that the mares gained physical conditioning, which is in line with Castejón et al. (2006), who reported that, during aerobic training, the speed at which the horse starts accumulating lactate in the blood gradually increases, i.e., as conditioning increases, so does  $SLa_2$  and  $SLa_4$ .

Table 1 - Mean spirometric parameters, heart rate, and plasma lactate levels of Mangalarga Marchador mares before and after aerobic training for the Probiotic (Pr) and Control (C) groups and their respective coefficients of variation (CV)

Parameter	Before training		After training		CV (%)
	Pr	C	Pr	C	
Time-to-fatigue (s)	600b	644b	726a	716a	5.0
Aerobic threshold (m/s)	4.90b	5.00b	5.80a	5.50a	7.8
$SLa_2$ (m/s)	4.90b	4.80b	5.80a	5.60a	9.1
Aerobic threshold HR (bpm)	169	180	175	179	8.6
$S_{150}$ (m/s)	3.90	4.30	4.60	4.40	15.5
Lactate <sub>150</sub> (m/s)	1.63	1.87	1.47	1.50	19.6
Anaerobic threshold (m/s)	5.80b	6.00b	6.50a	6.30a	8.2
$SLa_4$ (m/s)	5.90b	6.00b	6.50a	6.40a	7.5
Anaerobic threshold HR (bpm)	186	188	192	193	8.1
$S_{200}$ (m/s)	6.20b	6.60b	7.60a	7.20a	14.3
Lactate <sub>200</sub> (m/s)	7.20	6.81	8.43	6.50	7.5

Means followed by different letters in the rows differ between the physical tests according to Fisher's test ( $P<0.05$ ).

$SLa_2$  - speed at which the lactate plasma level reached 2 mmol/L; HR - heart rate;  $S_{150}$  - speed at which HR reached 150 bpm; Lactate<sub>150</sub> - lactate level at the speed at which HR reached 150 bpm;  $SLa_4$  - speed at which the plasma lactate level reached 4 mmol/L;  $S_{200}$  - speed at which HR reached 200 bpm; Lactate<sub>200</sub> - lactate level at the speed at which HR reached 200 bpm.

In the after-training test, the mares reached the highest speed when the heart rate was 200 bpm ( $S_{200}$ ), agreeing with Rogers et al. (2007), who found that  $S_{200}$  is sensitive to the improvement in training and represents one of the most widely used variables to estimate the anaerobic threshold. However, the authors point out that these values have been higher than the anaerobic threshold determined through spirometry or based on lactate ( $SLa_4$ ), which was also found in the present study.

On the other hand, no difference ( $P>0.05$ ) was found regarding the speeds at which the mares reached 150 bpm heart rate ( $S_{150}$ ) (Table 1). The values of speed at 150 bpm represented 84% of the aerobic threshold ( $SLa_2$ ) in the pre-training test and 79% in the after-training test, which shows that at 150 bpm the mares were at a speed that characterized eminently aerobic exercise. Heart rate is strongly correlated with speed in maximal exercises, but weakly correlated with low- and medium-intensity exercise (Babusci and López, 2007). Evans (2004) showed that the heart rate during trot and canter may greatly vary due to the interference of psychogenic factors and its response to the exercise intensity is not linear.

No difference was found ( $P>0.05$ ) in the plasma lactate levels when the mares' heart rate was 150 and 200 bpm both before and after training (Table 1). Nevertheless, the speed at which the mares reached 200 bpm heart rate was higher in the pre-training test while maintaining the same lactate levels. This result shows that plasma lactate deposition after training was lower in more intense exercises.

The plasma lactate levels at the moment when the mares reached 150 bpm heart rate were low, close to baseline values (Table 1). This shows that the production and elimination of lactate were balanced at that moment and, therefore, no lactate accumulated in the muscle, which indicates that the exercise was eminently aerobic, matching the result of the  $S_{150}$  variable.

No difference ( $P>0.05$ ) was found in the lactate levels at maximal speed both before and after training and also at 10 and 60 min into the recovery period (Table 2). In the after-training test, the plasma lactate level was higher than

in the test before training only at 30 min into the recovery period.

Although lactate at maximal speed did not differ ( $P>0.05$ ), in the after-training test the mares reached higher speeds, which resulted in a higher time-to-fatigue (Table 1). This may indicate that lactate accumulated more slowly in the blood after training, matching the increases in the aerobic and anaerobic thresholds and allowing the mares to work longer and faster.

Regarding the similarity in lactate levels at 10 min of the recovery period, because the tests were performed at maximal effort, the mares reached maximal lactate levels. This fact, associated with the longer time-to-fatigue in the after-training test, might be responsible for the similarity in maximum lactate levels in the tests before and after training at 10 min of recovery.

McGowan et al. (2002) correlated horse blood lactate levels after exercise with the time running on the treadmill ( $r = 0.71$ ). In the present study, even though the mares exercised for longer and faster in the after training test, the plasma lactate levels at 10 and 60 min of recovery were the same.

Since the mares performed greater effort during the after-training test, the lactate metabolism rate may have been slower, which justifies the difference in lactate levels at 30 min into the recovery period.

No difference ( $P>0.05$ ) was found in the maximal heart rate and at 10, 20, 30, 60, and 120 min after training (Table 3).

Similarly to lactate, the longer time-to-fatigue in the after-training test (Table 1) may be responsible for the similar values in maximum heart rate and during the recovery period, since, according to Evans (2004), training promotes an increase in the speed at which  $HR_{max}$  is reached. Thus, the cardiovascular system adapted to the greater physical activity by the skeletal muscle and to the metabolic demand during the after-training test by readjusting the blood flow so as to increase the availability of oxygen and energetic substrates for the synthesis of adenosine triphosphate (ATP), which is in line with Babusci and López (2007).

Table 2 - Mean maximum lactate levels ( $Lactate_{max}$ ) and lactate levels at 10, 30, and 60 min after exercise by Mangalarga Marchador mares before and after aerobic training in the Probiotic (Pr) and Control (C) groups, with their respective coefficients of variation (CV)

Parameter	Before training		After training		CV (%)
	Pr	C	Pr	C	
Lactate <sub>max</sub> (mmol/L)	16.5	13.7	17.1	15.4	3.9
Lactate 10' (mmol/L)	14.8	12.6	16.6	16.2	20.7
Lactate 30' (mmol/L)	9.2b	7.4b	11.3a	10.1a	4.8
Lactate 60' (mmol/L)	3.7	3.6	5.7	4.7	38.8

Means followed by different letters in the rows differ between the physical tests according to Fisher's test ( $P<0.05$ ).

Table 3 - Mean maximum heart rate (HR<sub>max</sub>) and heart rate (HR) at 10, 30, 60, and 120 min after exercise by Mangalarga Marchador mares before and after aerobic training in the Probiotic (Pr) and Control (C) groups, with their respective coefficients of variation (CV)

Parameter	Before training		After training		CV (%)
	Pr	C	Pr	C	
HR <sub>max</sub> (bpm)	226	223	230	226	4.3
HR 10' (bpm)	127	120	120	120	18.3
HR 20' (bpm)	101	99	101	105	24.0
HR 30' (bpm)	88	84	87	80	32.8
HR 60' (bpm)	56	56	62	65	25.6
HR 120' (bpm)	43	42	44	43	18.0

## Conclusions

Supplementation with *Saccharomyces cerevisiae* has no effect on the physical conditioning of Mangalarga Marchador mares. The aerobic training protocol adopted raises the aerobic and anaerobic capacity of Mangalarga Marchador mares.

## Acknowledgments

The authors thank Universidade Federal de Minas Gerais, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROCAD), Conselho Nacional de Desenvolvimento Científico e Tecnológico, Escola de Equitação do Exército, Universidade Federal Rural do Rio de Janeiro, Associação Brasileira de Criadores de Cavalo Mangalarga Marchador, Rações Itambé, BET Laboratories, Lesaffre do Brasil, Hertape Calier, and Haras Santorini.

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