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OBSERVATION ON LACTIC ACID MODIFICATIONS, DEPENDING ON TRAINING PERIODS AND SPEEDS, IN SPORT HORSES, USED AT EFFORT TEST

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Abstract

In the actual stage, on international level and in our country, is growing the importance of horses for equestrian sports. The researches were carried out on sports horses from Romanian Sport Horse and English Thoroughbred breeds. In this study, in order to evaluate aerobe energetic capacity of the horses, by determining the value of V_{La4}, there was used a standard effort test. There was completed an instrumental method to determine the lactic acid, based on enzymatic reactions and Screen Master Plus spectrophotometer was used for analyses. In can be observed that only for heating stage of training, the average value of lactic acid increases from first test to the third test. At all other effort stages the situation is opposed. Testing the differences significations for the first two testing periods, it was find that between all compared values the differences are very significant. For the third testing period, for all compared lactic acid values the differences are significant, excepting the differences testing between the lactic acid values at 400 m/minute stage and 450 m/minute stage, where the differences are very significant.

Key words: *effort test, lactic acid, sport horse.*

INTRODUCTION

At the present stage at worldwide level and in our country, the importance of the horse for equestrian sports is increasing. As a result, horse breeding technology must also adapt to these requirements and an important place must be given to sport horse dressage and specific training. Horse training mean the physical and psychological preparation of the horse for trials (tests), so that the animal can show at some point all the power it is capable of, without harming its health. Training activities try to reduce the fat layer, to improve the functionality of internal organs, to get muscles to be used to strong and continuous tensions.

When it comes to assessing the physiological adaptations that occur within the muscles of a horse due to training, several approaches are possible: 1. follow-up of the evolution of performance parameters, such as speed, heart rate and blood lactic acid, throughout consecutive standardized exercise tests on a, for example, two-weekly basis, 2. follow-up of muscle morphometric by means of ultrasound (Van de Winkel et al., 2016) 3. longitudinal follow-up of specific parameters, such as muscle fiber typing, total glycogen content, enzymatic activity, in serially harvested muscle biopsies (Vermeulen et al., 2017).

Trainers are familiar with the appearance of lactic acid following muscle effort, even if there are different opinions about the effect it has. Lactic acid is produced in muscles as a consequence of the generating of anaerobic energy and can be metabolized in muscles where it accumulates during critical periods with maximum effort (Dunnett, 2016.)

Lactic acid is an indicator of the effort made by horses during training and it has nothing to do with fatigue or muscle damage and is not harmful. Lactate is a source of energy and helps reduce the acidity of muscle cells subjected to intense training by eliminating hydrogen ions. High levels of lactic acid in the blood can be correlated with high athletic performance, not fatigue.

After training, horses become accustomed to the type of effort required and have normal metabolic levels of acidosis and lactatemia, depending on the effort required they have undergone. However, one hour after effort, only acidemia was eliminated, while hyperlactatemia was still present (Gomes et al., 2020)

There are several training methods, to prepare the horse for speed trials, endurance or strength tests. Training is like dressage an art, because it addresses to an animal that has a phenotypic appearance and an energetic possible temperament that varies from one individual to another, so that the training methods must be adapted to each individual in a special way.

The sport horse is submit to great efforts and as a result there are made progressive morpho-functional changes of the body, based on a rational, planned and systematic training program. In the process of formation and development of the energetic capacity, based on some schemes and judicious progressions is ensured the maximum improvement of the physiological functions.

At the basis of sport horse optimal preparation must be the following specific principles (Nicolescu, 1950).

a) The principle of sports training. It involves the repeated effort demand of the muscular system, which determines the improvement of the superior nervous activity, the elaboration and establishment of the necessary conditional connections, of the motor qualities and skills the morpho-functional improvement of all the organs and systems of sport horses.

b) The principle of systematic character. In horse training there must be a cadence. The interruption leads to the decrease of working capacity, as a result of the abolition of the previously elaborated and unmaintained connections. There may also be a regression of motor skills, due to the involution of all morpho-functional progressive changes in the body: muscles, cardiovascular and respiratory system, which reduce their activity after decreasing of formed reflexes. If arrhythmias occur in the preparation of the horse, it is first recommended to restore the coordination capacity (easy exercises in manege) and then to do more intense exercises to increase the functional level of the body.

c) The principle of assuring the optimal effort interval and the alternation of effort moments with relaxation ones. During the training period (daily, weekly, monthly, seasonal, annually) the effort periods must alternate with relaxation periods, in order to ensure good training and normal maintenance and health.

The animal's body goes through 3 phases in the training program: fatigue (reduced effort capacity), restoring the work capacity to the initial level and increasing the effort capacity. Each level has different durations, depending on the intensity and size of the effort and the grade of horse training. The alternation of work with breaks depends on the purpose of the training program and the horse preparation level.

d) The principle of progressive tasks increasing. This principle aims the widening of the work capacity limits, which is achieved by continuously increasing of functional body state, allowing the practice of more difficult movements and increasing training tasks. The progressive increase effort must be foreseen in the training program in all its stages and especially in the horses that are preparing for very heavy trials.

e) The principle of maximum effort capacity. Normally, the horse can perform maximum tasks at the end of the training period, when a series of morpho-functional changes occur that allow to increase the intensity of the effort. (Nicolescu T. 1950). But there are certain situations, in the case of horses prepared for heavy trials (complete riding trial), when during the training period maximum effort tests are introduced (rapid ascent of the slopes), which determines a demand of the body up to maximum limits.

f) The principle of multilateralism, which has as fundamental objective the complete and complex development of the horse. Based on this principle, the systems and devices required during training are developed and the superior nervous activity is perfected. For this purpose, the motor qualities of the animal are developed: speed, strength and breathing, based on various types of training.

MATERIALS AND METHODS

Using horses for sport requires preparation and optimization of physical and mental qualities, both contributing to achieving the desired performance.

Energy metabolism in sport horses is strongly influenced by the intensity and duration of exercise. Thus, in short duration and high intensity efforts, most of the chemical energy needed for muscle contraction is supplied by lactic anaerobic metabolism. This metabolic pathway lead to lactic acid byproduct, whose accumulation, in muscle and blood, will influence the level of serum electrolytes (Şovărel et al., 2014)

The research was carried out on a number of 15 sport horses from the Romanian Sport Horse and Pure English Blood breeds.

To evaluate the aerobic energy capacity by establishing the value of V_{La4} (the horse speed at which the blood lactate concentration reaches 4 mmol and appear the anaerobic metabolic threshold "artificially determined"), a standard test exercise was used, adapted to the specific conditions of the training ground, for three different workouts periods.

The V_{La4} parameter is considered as a reference parameter in horses because it is related to aerobic capacity (Harkins et al., 1993; Persson & Lydin, 1983).

The importance of establishing V_{La4} lies in the fact that it is a method of accurate assessment of the degree of physical training in the sport horse, offering the possibility to constantly check the improvement of aerobic exercise capacity.

The test was repeated in 3 different periods (1st, 2nd and 3rd), during which the horses were gradually trained. This test-exercise is detailed in the Table 1, and can be performed both on the treadmill and on the field.

Table 1. Standardized test-exercise for the determination of V_{La4} (adapted from Lindner, 1997)

No.	Parameter	Specification
1	Heating	5 minutes to trot
2	Effort level	4
3	Duration of each level	5 minutes
4	Speed in the first level	350 m/minute
5	Increase speed from level to level	With 50 m/minute
6	Rest between levels	1 minute
7	The moment for collecting blood samples	After heating and as soon as possible after each level
8	Recovery phase	10 minutes (step, trot)
9	Sample processing	On the spot and within 30 minutes of sampling

In order to maintain a constant speed during the effort levels, the distance of the course was measured and marked with flags, and each rider, by successive tentative, was framed in the time necessary to cover it. These tentative took place 3 days in advance of the date of the test-exercise. In order to minimize any errors related to the horse-rider relationship, each horse was ridden by the person who prepared it until the time of the test, and the field of exercise was the one used daily for training. The blood sample were harvested from jugular vein. To minimize the stress associated with taking blood samples, the jugular vein was catheterized, using a catheter 18 G/L, 32 mm, which was attached to the skin using adhesive tape.

The blood was collected in Li-heparin vacutainers. The plasma was immediately separated by centrifugation and placing it in separate tubes, immersed in water and crushed ice in an isothermal bag at 4°C. Plasma lactate dosing was performed on site within a maximum of 30 minutes of sampling.

The protocol recommended by Lindner (1997) for plasma lactate dosing provides this immediate separation of erythrocytes, as glycolysis continues in blood samples. If the samples cannot be processed on the spot, it is recommended to take the blood on sodium fluoride and store it at 4°C until dosing. Even under these conditions, the lactate should be dosed within a maximum of 48 hours. In order to determine the concentration of lactic acid, there was selected a specific, instrumental method, based on enzymatic reactions. The principle of the method consists in the transformation of lactic acid into pyruvic acid under the action of lactat-oxidase (LOD), a process in which rises an equivalent amount of hydrogen peroxide. The hydrogen peroxide formed is coupled with 4-aminoantipyrine (in the presence of peroxidases), forming a coloured compound. The intensity of the colour is proportional to the concentration of lactic acid.

Blood samples were collected in vacutainer tubes, on lithium-heparin with the addition of fluoride. The most difficult part of this analysis is stabilizing the level of lactic acid to the corresponding concentration level to that in vivo. The in vitro increase of lactate concentration is prevented by reducing glycolytic activity, which is why the blood is collected on sodium fluoride (or it is deproteinized and the precipitate is separated immediately by centrifugation). After sampling, lactic acid was immediately measured from the separated plasma. For the analyses it was used the Screen Master Plus spectrophotometer.

RESULTS AND DISCUSSIONS

Regardless of the test period in which the investigations were made, the lactic acid content was the lowest in the heating phase and increased in relation with the speed of movement.

The lactic acid values determined in different phases of the exercise test (from heating to increasing speeds from 350 m/minute to 500 m/minute), determined in 3 different periods, are presented in Table 2.

Table 2. Average lactic acid values on the exercise test

Testing period	n	Lactic acid values ($\bar{x} \pm s\bar{x}$ mmol/l)				
		Heating	350 m/min	400 m/min	450 m/min	500 m/min
1	15	0.46 ± 0.019	1.86 ± 0.032	3.84 ± 0.07	5.08 ± 0.12	10.61 ± 0.39
2		0.60 ± 0.04	1.83 ± 0.06	2.61 ± 0.05	4.05 ± 0.08	7.74 ± 0.17
3		0.65 ± 0.03	1.63 ± 0.46	2.44 ± 0.05	3.62 ± 0.10	4.12 ± 0.03

It is observed that only at the heating phase the average value of lactic acid increases from the first test to the third test. At all other levels of effort the situation is reversed, the value of lactic acid decreasing from the first test to the third.

It is observed that in the first testing period, at a speed of 400 m / min., the value of the lactic acid concentration it is close to V_{La4}, that is 3.84 mmol/l, which means that the training intensity approaches the optimal level.

In the same test period, at higher speeds (450 m/min. and 500 m/min. the value of V_{La4} was exceed, obtaining 5.08 mmol/l and 10.61 mmol/l, respectively, which may show that the training intensity is not appropriate (figure 1).

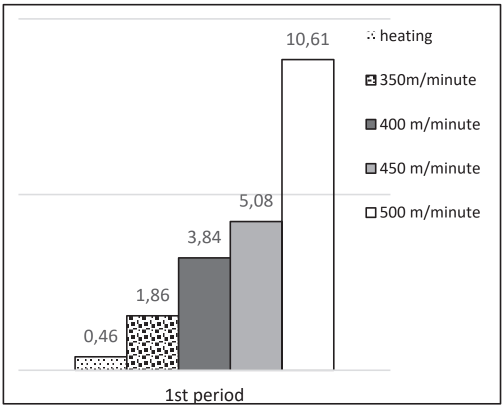

Figure 1. Average lactic acid values at first period of the exercise test

It is observed that in the second test period, the optimal value of the lactic acid concentration (V_{La4}) was not reached at heating and speeds of 350 m/minute and 400 m/minute, but at a speed of 450 m/minute it is optimal (4.05 mmol/l). (figure 2).

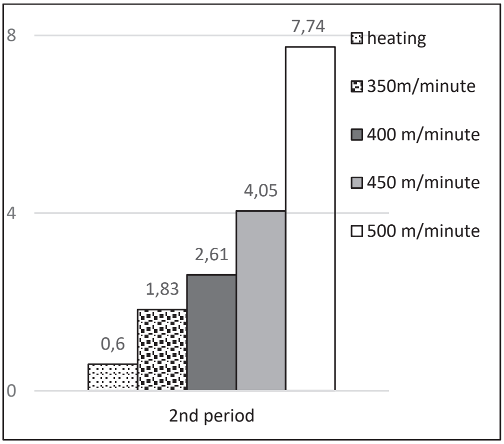

Figure 2. Average lactic acid values at second period of the exercise test

The optimal value of V_{La4} was exceeded, at a speed of 500 m/minute, approaching a double of it (7.74 mmol/l). This may indicate an incorrect intensity of effort and an inadequate training structure

In the third test period it is observed that the value of the lactic concentration was not reached up to the speed of 450 m/minute. At a speed of 450 m/min, the value obtained is close to V_{La4}, and at a speed of 500 m/minute it can be said that it reached the optimum value (figure 3).

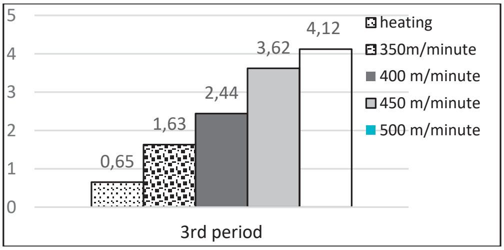

Figure 3. Average lactic acid values in the third period of the exercise test

Testing the significance of the differences for the first and the second test periods, it was find that between the value from heating and the value achieved at a speed of 350 m/minute the differences are very significant (Tables 3 and 4). It can also be seen that between the values compared at speeds of 350 and 400 m/minute, 400 and 450 m/minute and 450 and 500 m/minute, the differences remain very significant (Tables 3 and 4).

Testing the significance of the differences for the third test period, we find that between all the compared values, the differences are significant, except for testing the differences between the values from the 400 m/minute and the 450 m/minute level, where the differences are very significant (table 5).

Table 3. Significance of differences for the first test period

Specification	I					
	$\overline{X}_1$	$\overline{X}_2$	d	t calculated	table t (t α) P<0.001	Signification
Heating-350 m/min	0.46	1.86	-1.4	**-12.2**	**3.67**	Very significant differences ***
350 -400 m/min	1.86	3.84	-1.98	**-12.2**		Very significant differences ***
400 -450 m/min	3.84	5.08	-1.24	**-5.59**		Very significant differences ***
450 -500 m/min	5.08	10.61	-5.53	**-15.23**		Very significant differences ***

Table 4. Significance of differences for the second test period

Specification	II					
	$\overline{X}_1$	$\overline{X}_2$	d	t calculated	table t (t α) P<0.001	Signification
Heating-350 m/min	0.60	1.83	-1.23	**-7.65**	**3.67**	Very significant differences ***
350 -400 m/min	1.83	2.61	-0.78	**-4.63**		Very significant differences ***
400 -450 m/min	2.61	4.05	-1.44	**-7.86**		Very significant differences ***
450 -500 m/min	4.05	7.74	-3.69	**-15.48**		Very significant differences ***

Table 5. Significance of differences for the third test period

Specification	III						
	$\overline{X}_1$	$\overline{X}_2$	d	t calculated	table t (t α) P<0.01	table t (t α) P<0.001	Signification
Heating-350 m/min	0.65	1.63	-.098	**-2.755**	**2.756**	-	Significant differences **
350 -400 m/min	1.63	2.44	-0.81	**-2.23**			Significant differences **
400 -450 m/min	2.44	3.62	-1.18	**-5.99**	-	**3.67**	Very significant differences ***
450 -500 m/min	3.62	4.12	-0.5	**-2.73**	**2.756**	-	Significant differences **

CONCLUSIONS

Adaptation to effort for sport horse with performances, involves physiological adjustments in the striated muscles, respiratory system, cardiovascular system, endocrine system and all organs ad body systems. In order to cope and reach the optimal and maximum potential, the animal's body subjected to high efforts, is necessary to carry out a training with appropriate intensity, which will lead to certain biochemical changes in the blood.

As a result of the training performed, the blood lactic acid values changed, being around the critical value at a speed of 400 m/minute (V_{La4} = 3.84 ± 0.07 mmol/l), in the first test period, reaching the optimum value at a speed of 450 m/minute (4.05 ± 0.08 mmol/l), in the second test period and slightly exceeding this value at a speed of 500 m/minute (4.12 ± 0.013 mmol/l), in the third test period.

The maintaining of the training program results in the obvious improvement of the aerobic energy capacities.

In order to maintain the health of the animals and to improve the sports performances, it is necessary to carry out periodic analyses of the biochemical components in the horses and of the main physiological indices, both before and after the effort, in order to notice the situations that would alter the state of health of the animals subjected to too much effort, thus giving the possibility to establish a certain training regime. It is also necessary to establish an optimal training regime (daily and monthly), with the progressive increase of the effort to which the animals are subjected (increasing the speed of movement in the test-exercise).

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