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Comparison of maximal lactate steady state with V_2, V_4, individual anaerobic threshold and lactate minimum speed in horses

[*Comparação da máxima fase estável do lactato com a V_2, V_4, o limiar anaeróbio individual e lactato mínimo em equinos*]

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ABSTRACT

The anaerobic threshold is a physiologic event studied in various species. There are various methods for its assessment, recognized in the human and equine exercise physiology literature, several of these involving the relationship between blood lactate concentration (LAC) and exercise load, measured in a standardized exercise test. The aim of this study was to compare four of these methods: V_2, V_4, individual anaerobic threshold (IAT) and lactate minimum speed (LMS) with the method recognized as the gold standard for the assessment of anaerobic threshold, maximal lactate steady-state (MLSS). The five tests were carried out in thirteen trained Arabian horses, in which velocities and associated LAC could be measured. The mean velocities and the LAC associated with the anaerobic threshold for the five methods were respectively: V_2 = 9.67±0.54; V_4 = 10.98±0.47; V_{IAT} = 9.81±0.72; V_{LMS} = 7.50±0.57 and V_{MLSS} = 6.14±0.45m.s^{-1} and LAC_{IAT} = 2.17±0.93; LAC_{LMS} = 1.17±0.62 and LAC_{MLSS} = 0.84±0.21mmol.L^{-1}. None of the velocities were statistically equivalent to V_{MLSS} ($P<0.05$). V_2, V_4 and V_{LMS} showed a good correlation with V_{MLSS}, respectively: r = 0.74; r = 0.78 and r = 0.83, and V_{IAT} did not significantly correlate with V_{MLSS}. Concordance between the protocols was relatively poor, i.e., 3.28±1.00, 4.84±0.30 and 1.43±0.32m.s^{-1} in terms of bias and 95% agreement limits for V_2, V_4 and LMS methods when compared to MLSS. Only LAC_{LMS} did not differ statistically from LAC_{MLSS}. Various authors have reported the possibility of the assessment of anaerobic threshold using rapid protocols such as V_4 and LMS for humans and horses. This study corroborates the use of these tests, but reveals that adjustments in the protocols are necessary to obtain a better concordance between the tests and the MLSS.

Keywords: equine exercise physiology, anaerobic threshold, maximal lactate steady state

RESUMO

O limiar anaeróbio é um evento fisiológico estudado em várias espécies. Sua mensuração possui vários métodos reconhecidos na literatura da fisiologia do exercício humano e equino, muitos deles envolvendo a relação entre a concentração sanguínea de lactato (LAC) e a carga de exercício. O objetivo do presente estudo foi comparar quatro desses métodos: V_2, V_4, limiar anaeróbio individual (LAI) e o teste do lactato mínino (LM) com o método reconhecido na literatura como o padrão ouro para a mensuração do limiar anaeróbio, a máxima fase estável do lactato (MFEL). Os cinco testes foram realizados em treze equinos árabes treinados, nos quais as velocidades e suas respectivas LAC puderam ser quantificadas. As velocidades médias e LAC associadas ao limiar anaeróbio aferido pelos cinco métodos foram respectivamente: V_2 = 9,67±0,54; V_4 = 10,98±0,47; V_{LAI} = 9.81±0.72; V_{LM} = 7,50±0,57 e V_{MFEL} = 6,14±0,45m.s^{-1}; e LAC_{LAI} = 2,17±0,93; LAC_{LM} = 1,17±0,62 e LAC_{MFEL} = 0,84±0,21mmol.L^{-1}. Nenhuma dessas velocidades foi estatisticamente igual à V_{MFEL} ($P<0,05$). A V_2, a V_4 e a V_{LM} mostraram uma boa correlação com a V_{MFEL}, respectivamente r = 0,74; r = 0,78 e r = 0,83, e a V_{LAI} não se correlacionou significativamente com a V_{MFEL}. A concordância entre os protocolos foi relativamente fraca, sendo 3,28±1,00; 4,84±0,30 e 1,43±0,32m.s^{-1} em termos de viés e limites de concordância a 95% para os métodos V_2, V_4 e LM comparados à MFEL. Muitos autores relataram a possibilidade da mensuração do limiar anaeróbio pelo uso de protocolos rápidos, como a V_4 e o LM, para humanos e equinos. O presente estudo corrobora a utilização desses testes, mas revela que ajustes nos protocolos são necessários para se obter uma melhor concordância entre os mesmos e a MFEL.

Palavras-chave: fisiologia do exercício equino, limiar anaeróbio, máxima fase estável do lactato

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INTRODUCTION

The anaerobic threshold (AT), a metabolic event associated with the increase in lactacidemia and changes in breathing, was theorized for humans and has been discussed at length for a number of decades in sport sciences (Aunola and Rusko, 1992; Myers and Ashley, 1997; Faude *et al*., 2009). The assessment of this threshold, by means of the quantification of some metabolic variables, particularly lactacidemia, has also been utilized for athletic horses (Bas *et al*., 2000; Rogers *et al*., 2007; Lindner *et al*., 2009; Lindner, 2010). There are various ways to measure this threshold, where the procedure known as the maximal lactate steady-state (MLSS) is considered the gold standard (Svedahl and MacIntosh, 2003). However, the thresholds determined by fixed lactacidemias, such as V_2 and particularly V_4, which is accepted by some authors as the anaerobic threshold (Bas *et al*., 2000), are often utilized due to their practicality and objectivity. Other methods for assessing the AT through lactacidemia, such as individual anaerobic threshold (IAT) and lactate minimum speed (LMS), were proposed for humans and there are few reports on LMS in horses (Gondim *et al*., 2007), and none or almost none regarding IAT. In humans, many studies were conducted to test the capacity of different methods predicting exercise intensity at threshold, which approximate those predicted by MLSS, and despite this, there is much controversy on the subject (Faude *et al*., 2009). The aim of this study was to compare the methods of assessing the anaerobic threshold based on lactacidemia, V_2, V_4, IAT and LMS with the MLSS method, which is considered the gold standard, in Arabian horses during a standardized exercise test performed on a high-speed treadmill.

MATERIALS AND METHODS

Animals: Thirteen adult, male and female Arabian horses were used, with a mean body weight of 381.85±22.88kg. The horses were maintained on a semi-extensive regimen in *Cynodon dactylon* pastures with water and mineral salts *ad libitum*. They were additionally fed *Cynodon dactylon* hay at a proportion of 60:40 (forage: concentrate) for a dry matter intake of 2% and concentrated formulation for the consumption of energy and nutrients proportional to the weight of each animal, according to the requirements for horses submitted to moderate exercise (National..., 2007).

Standardized exercise tests: Prior to the beginning of the experimental phase, the animals were submitted to 30 days of adaptation for handling and treadmill and were submitted to the 60 day training program, done with the objective of standardizing the physical capacities of the athletes. On the days of tests the animals were fed three hours prior to the tests. The tests were performed in the following order: progressive intensity, lactate minimum speed and maximal lactate steady-state, with six-day intervals between tests.

To obtain the blood samples to determine lactacidemia, the animals were submitted to central venous catheterization of the jugular. 25µL of blood was aspirated with an appropriate pipet and analyzed in a lactimeter (YSI 1500 Sport L-Lactate Analyzer. YSI Incorporated, USA).

Progressive intensity test: This test was used to measure V_2, V_4 and IAT. After 5min of warm-up at $4m.s^{-1}$ the treadmill was inclined 5% and the velocities were increased, every 2min, to 5, 6, 7, 8, 9, 10, 11, 12 and $13m.s^{-1}$, or until the exercise could not be sustained. Blood samples were collected 15 s before the end of each stage. Non-sustenance of the exercise was characterized by the inability of the animal to keep up with speed of the treadmill. Afterwards, there was an active recovery period at $3m.s^{-1}$ for 20min. Blood samples were also collected at 2, 5, 10 and 20min after exercise. V_4 was calculated by plotting the velocities and lactacidemias and approximating the exponential curve, where V_4 is the velocity at which lactacidemia is $4.0mmol.L^{-1}$. In the same manner, V_2 is the point where lactacidemia is 2.0 $mmol.L^{-1}$.

The IAT was calculated by plotting exercise times and lactacidemia, and then drawing a straight line parallel to the x-axis from the lactacidemia related to the fastest step of the progressive exercise. From the point where this new straight line intersects the recovery curve, a straight line is drawn tangent to the curve of progressive exercise. This tangent point is considered IAT (Tegtbur *et al*., 1993). Thus, the velocity and lactacidemia associated with IAT are determined (V_{IAT} and LAC_{IAT}).

Lactate minimum speed test: After 5min of warm-up (4m.s^{-1}, without inclination), hyperlactacidemia was induced when shifting the animal to galloping, with the treadmill inclined to 10%, at the highest speed possible, until the exercise could not be sustained. Afterwards, the animal was placed at a velocity of 4m.s^{-1}, and after two minutes of the first reduction in lactate concentration, the animal was submitted to an incremental exercise test with blood samples collected at each stage, similar to the progressive intensity test (initial stage at 5m.s^{-1} with increments of 1m.s^{-1} every 2min). The data from this incremental test were plotted and the point at which lactate concentration showed an inflection was considered LMS, calculated as the lowest point of the zero-derivative fitted curve. The velocity and lactacidemia associated with LMS (V_{LMS} and LAC_{LMS}) were thereby estimated.

Maximal lactate steady-state test: The horses were submitted to various exercise sessions with at least two days of rest in between sessions, with constant intensity and a duration of 30 min. Blood samples were drawn at the beginning and every 5min of the test. The first session had intensity equal to V_{LMS} and the others had intensities progressively lower by 5% of V_{LMS} until reaching the maximal intensity at which there was no change in lactacidemia above 1.0mmol.L^{-1} in the last 20min of the session. In this way, the velocity associated with MLSS (V_{MLSS}) and the mean lactacidemia of the last 20 min of the test (LAC_{MLSS}) could be defined.

Statistical analysis: All data are presented as means and standard deviations. The statistical comparisons between the velocities and lactacidemias were performed by analysis of variance for repeated measures with the Holm-Sidak test for comparisons with a control group (MLSS) as the post-hoc test. Pearson's correlation test was carried out for velocities associated with different assessment methods. To investigate the relation between velocities predicted by the different methods and V_{MLSS}, a simple linear model was fitted, and its equations and coefficients of determination were calculated. The concordance degree between the methods was determined calculating the bias and the 95% limit of agreement as described by Bland and Altman (1986). Differences were considered significant with $P<0.05$.

RESULTS

In general, all the methods determined velocities that were statistically higher than V_{MLSS}. When tested individually, no animal was capable of sustaining velocities equal to V_2, V_4 or V_{LMS} in the MLSS test, without producing an increase of 1mmol.L^{-1} in the last 20min of the test. The mean values of the velocities and lactacidemias for the different methods can be found in Tab. 1.

Table 1. Means and standard deviations of the lactacidemias and velocities associated with different protocols for measuring anaerobic threshold: velocity at which lactacidemia is 2.0 (V_2) and 4.0mmol.L^{-1} (V_4), individual anaerobic threshold (IAT), lactate minimum speed (LMS) and maximal lactate steady-state (MLSS)

Method	Lactacidemia (mmol.L^{-1})	Velocity (m.s^{-1})
V_2	-	9.67±0.54
V_4	-	10.98±0.47
IAT	2.17±0.93*	9.81±0.72
LMS	1.17±0.62	7.50±0.57
MLSS	0.84±0.21	6.14±0.45*

*Within a column, means have statistical difference ($P < 0.05$).

When comparing the methods, only the correlation between V_{IAT} and V_{MLSS} was not significant. The correlations (r) between V_{MLSS} and V_2, V_4 and V_{LMS} were 0.74; 0.78 and 0.83, respectively. The significant correlations can be seen in Figure 1.

In the fitted linear mode, the coefficients of determination (R^2) were 0.55, 0.61 and 0.69 for the predicted velocities by the V_2, V_4 and LMS methods. The equations and coefficients of the models are highlighted in Figure 1.

The biases and 95% limits of agreement between V_{MLSS} and the other methods were 3.53±0.70, 4.84±0.59 and 1.36±0.62, respectively, for V_2, V_4 and V_{LMS}. Bland-Altman diagrams of concordance between the different methods and MLSS are shown in Figure 2.

Regarding lactacidemias, the only method that predicted lactacidemias without statistical difference from LAC_{MLSS} was LMS.

V_{IAT} and LAC_{IAT} could not be calculated for four of the animals, because up to the last time of blood sampling (20min after exercise) lactacidemia had not reached levels below the last lactacidemia in the exercise, which made it impossible to determine the point where the straight line parallel to the x-axis intercepts the lactate curve and, consequently, to calculate the variables associated with IAT.

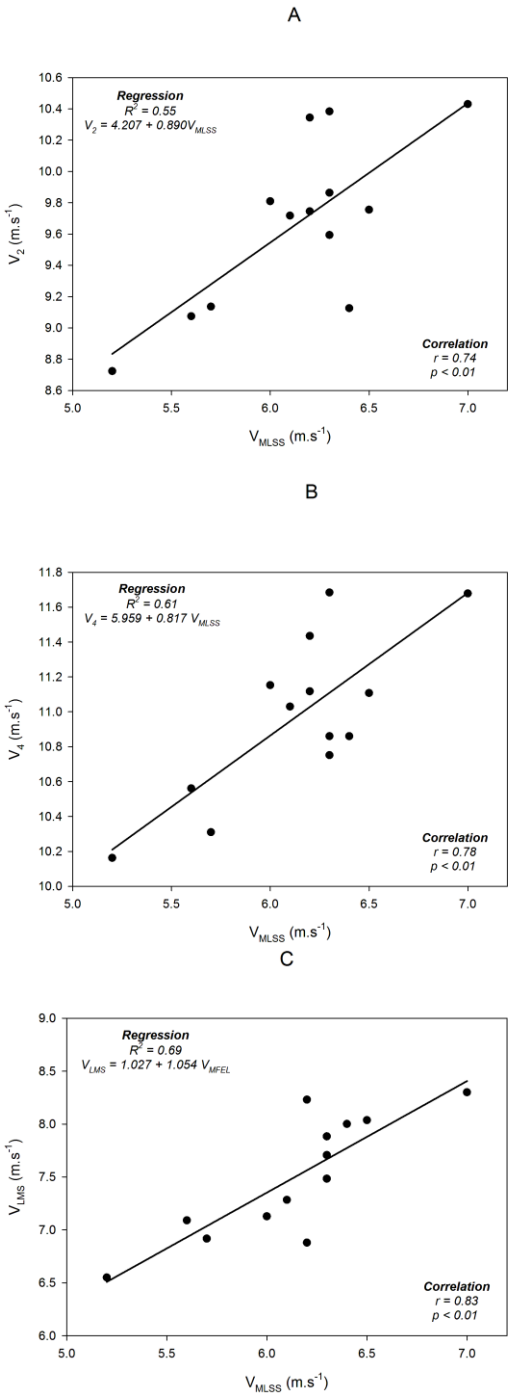

Figure 1. Scatter plots of predicted velocities by the different methods versus MLSS velocity. The coefficient of determination and straight line equation of the linear model fitted are shown. The Pearson correlation coefficients (*r*) and *p* values of the test are also shown. The solid lines represent the model fitted for variable. A: Plot of V_2 versus V_{MLSS}. B: Plot of V_4 versus V_{MLSS}. C: Plot of V_{LMS} versus V_{MLSS}.

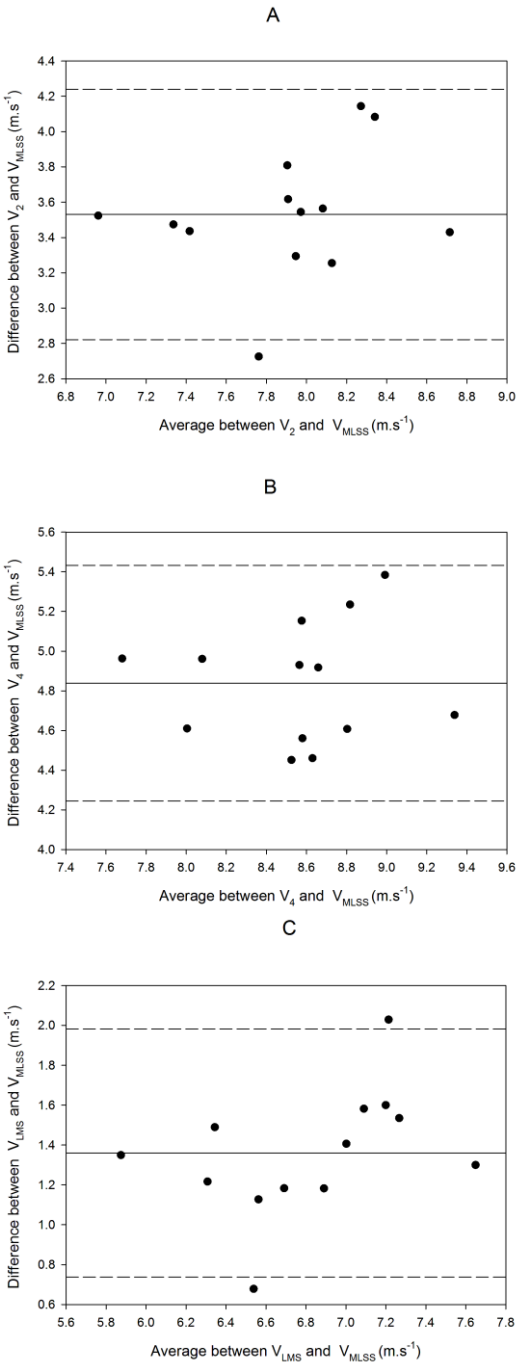

Figure 2. Results of the Bland-Altman analysis which demonstrated the concordance between predicted velocities through the different methods and the MLSS velocity. The bias, or mean difference, is represented by the solid line, and concordance limits at 95% by the horizontal broken line. A: Concordance between V_2 and V_{MLSS}. B: Concordance between V_4 and V_{MLSS}. C: Concordance between V_{LMS} and V_{MLSS}.

DISCUSSION

V_2 and V_4: There are various studies that have investigated the metabolic responses of athletes during constant exercise at threshold intensities in humans (Faude *et al*., 2009) and horses (Lindner, 2010). In our study, V_2 and V_4 values were always above the V_{MLSS} values, and in addition, the animals were not capable of sustaining those velocities in the MLSS test without an increment of 1 mmol.L^{-1}. However, a good correlation was found between the velocities predicted by V_2 and V_4 and V_{MLSS}, demonstrating that the two tests probably measure the same physical capacity. These findings corroborate the laboratory finding of the above mentioned study, in which horses thet exercised at intensities corresponding to V_2 on the treadmill were incapable of maintaining their lactacidemia without a variation of more than 1 mmol.L^{-1} between the 14th and 18th min of the constant intensity test, indicating that this intensity is greater than MLSS. However, the same study showed that in the field, the protocol utilized for measuring V_2 was capable of predicting velocities that could be sustained individually with constant exercise, without an increase of 1 mmol.L^{-1} between minutes 5 and 25 of the test, which did not occur in the present work.

There are some aspects that can explain this difference. In field tests, there are climate and rider influences that certainly alter the velocity results observed in the test. Studies are necessary to determine if these factors, inherent to the field test, influence incremental and constant load exercises differently and, consequently, their comparison. Additionally, this study used the method initially proposed for assessment of MLSS (Heck *et al*., 1985), which was later modified by investigators and which is currently more used and accepted by the scientific community in human sports (Beneke, 2003), using a protocol with 30 min of duration. This difference would probably alter the results of the comparison between the methods. Furthermore, the study mentioned did not show a correlation or concordance between the methods, which could have certainly made a better comparison.

Along the same comparison, there are some studies in humans that compare the thresholds found by different methods with those predicted by MLSS. Heck *et al*. (1985) found high correlations between V_4 and V_{MLSS}, using protocols of an incremental test with 5min stages; however, this study showed that if the protocols with shorter stages were used, the V_4 found is generally greater than V_{MLSS}. That could explain why in this study horses were unable to sustain V_4 for a long period without alteration of LAC. In addition, it must be noted that 5min stages would compromise the practicality of fixed threshold tests.

An ergometric rowing study demonstrated for V_4 what was found in this study for the thresholds of fixed velocity, meaning velocity values above those predicted by MLSS, and high correlation (r = 0.80) between them using 3 min stages (Beneke, 2003). For athletes in cycloergometry, a low correlation was reported (r = 0.57) between fixed velocities at 4 mmol.L^{-1} and those predicted by MLSS (Aunola and Rusko, 1992). As reported by Svedahl and MacIntosh (2003), there are arguments that support and that refute the use of fixed thresholds for the prediction of MLSS. These methods have advantages such as objectivity and practicality, but certainly ignore individual differences. For these fixed thresholds, some authors (Lindner and Boffi, 2007) reported that the duration of the stage influences the kinetics of lactacidemia during the incremental test in horses, and therefore, an increase in the duration of the stages could be recommended in our protocol, since there was a high correlation, but low concordance, between their predicted velocities and V_{MLSS}.

Individual anaerobic threshold (IAT): There are reports regarding humans showing that constant load exercises corresponding to V_{IAT} produce constant lactacidemias (McLellan and Jacobs, 1993; Urhausen *et al*., 1993). However, these studies do not show the correlation between the methods. Other studies exist that show a good correlation between V_{IAT} and V_{MLSS}, but critiques to the high heterogeneity of the physical condition of the athletes could be done (Faude *et al*., 2009). In a study with a homogeneous population of rowers, velocities corresponding to V_{IAT} were greater than V_{MLSS} (Beneke, 1995).

Some factors could explain the statistical difference and lack of correlation between the velocities predicted by IAT and by MLSS shown in our study. IAT is totally protocol-dependent,

where the initial point of the progressive intensity test, duration of the stages and final point of the test decisively influence the test (Svedahl and MacIntosh, 2003). It is likely that adjustments in these aspects of the protocol would lead to a better prediction of MLSS in horses. Another IAT criticism refers to the premise assumed by the test, which is that lactacidemia in IAT diminishes with the increase in aerobic capacity (Stegmann *et al*., 1981) and, therefore, predicts higher velocities. However, the physiological mechanisms and experimental tests for such fact are not consolidated (Denadai *et al*., 2004). One of the adaptations of the original protocol carried out by our research group that surely influences lactacidemia kinetics is the recovery method. While in the original study the authors used passive recovery, the present work used active recovery, which certainly influenced the plasma lactate concentration during its course. The IAT, besides showing no correlation, was not feasible in some animals, which is not reported in published studies that evaluated the method in human athletes. Because this method that has had very little study in equines, more studies are needed to resolve this issue.

Lactate minimum speed test: Our findings show a significant difference in the means of V_{LMS} and V_{MLSS}, but high correlation (r = 0.83) between the velocities, indicating that with adjustments this protocol can be used to predict the MLSS of horses on a treadmill. Additionally, the linear model with a reasonable coefficient of determination (0.69) can be adjusted, showing that under these conditions, the test has practical validity.

For horses, the study that reported the use of LMS in the field obtained results that could be tested later, and V_{LMS} was confirmed as being the effort intensity that did not cause a significant increase in lactacidemia in a constant load exercise of 10km (Gondim *et al*., 2007). In our work, this did not occur since the velocities predicted by LMS could not be sustained in constant exercise without an increase in LAC. This difference in findings can be explained by various factors. The tests of the authors mentioned were carried out in the field, and as discussed above, these tests are under the control of factors that can influence incremental and constant load exercises differently. Another factor to be considered is the method employed for confirmation of MLSS. Our study used the constant load protocol for a pre-defined time period, while the other authors used a fixed distance. This fact results in distinctions between the test load, which could influence the LAC kinetics, leading to different results in the confirmation of velocities in the MLSS test in the field or in the assessment of MLSS on a treadmill.

In humans, various studies consider LMS as being a valid method for estimating exact MLSS (Tegtbur *et al*., 1993; MacIntosh *et al*., 2002). Despite this, there are reports that this test is protocol-dependent and therefore difficult to standardize, where both the initial intensity and the duration of the stages of the incremental part of the test affect the intensity values predicted by LMS (Svedahl and MacIntosh, 2003; Faude *et al*., 2009). Other studies conducted in humans showed contradictory results. A moderate correlation between V_{LMS} and V_{MLSS} (r = 0.61) was reported (Jones and Doust, 1998), while other authors (Sotero *et al*., 2009) reported a high correlation (r = 0.87). In swimming rats, investigators found velocities associated with LMS that could be sustained in constant exercises without increase in lactacidemia (Araujo *et al*., 2007) and also stated that differences in the LMS protocol do not alter the results for final velocity. Differences between the species and in the muscle groups involved in swimming and running exercises could contribute to such differences.

Bland-Altman concordance analysis: the bias and 95% coefficient values can be considered high for all the methods, indicating low concordance, since 1.36-4.84m.s^{-1} certainly have a substantial influence in the comparison of competitive capacity or intensity of training prescription. These results demonstrate that despite there being a good correlation between the velocities determined by MLSS and V_2, V_4 and LMS, adjustments in the treadmill protocols are necessary to predict closer velocities. These adjustments are necessary to a greater degree for a fixed lactate threshold and to lesser degree for LMS.

Lactacidemias: Although researchers (Svedahl and MacIntosh, 2003) pointed out that the intensity of incremental exercise that produces a

particular lactacidemia is not necessarily equal to that which produces the same lactacidemia in constant-load exercise, many studies have compared LAC at the threshold of rapid predictive methods with LAC of MLSS. In our results, this could have been an additional indication that the LMS protocol utilized predicts exercise intensities at threshold closer to those predicted by MLSS, since there was no significant difference between a LAC_{LMS} and a LAC_{MLSS}.

CONCLUSION

Our results indicate that the protocols utilized here for V_2, V_4 and LMS can be utilized to predict MLSS due to the high correlations with the velocities predicted by the gold standard method, however, adjustments in the protocols are necessary, especially for V_2 and V_4 and to a lesser extent for LMS, in order to improve the concordance between the methods. Additionally, IAT appeared to be only partly feasible and its threshold velocities did not correlate with those of MLSS, indicating that more studies are needed in order to utilize this method in horses.

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