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Original Research

Biological markers and metabolic energy indexes of show jumping horses during a field exercise test in Portugal

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ABSTRACT

Background: Show jumping is a popular and complex equestrian modality practiced by a athletes of different levels. However, most studies focus on elite athletes.
Aims/objectives: The aim of this work was to assess the effect of a show jumping test (SJT), designed for novice athletes, on the physiological biomarkers and energy indexes of a group of horses, bred and trained in Portugal.
Methods: In this prospective cross-sectional study, thirteen horses and their riders performed a SJT with obstacles set at 80 cm height using a heart rate monitor (M430 with H10 sensor). Physical examination and blood samples, for complete blood count and sera biochemical analysis (total protein, albumin and muscle enzymes), were performed at four timepoints. Metabolic energy indexes – energy expenditure (EE), cost of transport (CoT) and metabolic power (Pmet) – were estimated based on heart rate and speed registered during the SJT.
Results: Exercise affected the animals' heart rate, respiratory rate, rectal temperature, glycaemia, lactatemia, albuminemia, and the number of white blood cells, neutrophils, lymphocytes and erythrocytes, and also haemoglobin and haematocrit values ($P \leq 0.05$). Estimated energy expenditure was 431.0 ± 222.5 J/kg/min, CoT was 0.10 ± 0.05 beats·kg·m·103 and Pmet was 0.02 ± 0.01beats/min/kg.
Conclusion: The SJT significantly influenced some of the studied biomarkers and the animals performed a submaximal effort and maintained an aerobic metabolism during exercise. This work offers some references for future assessment of novice showjumping horses, which can be used for future comparative analysis and for optimizing training programs.

1. Introduction

Show jumping is one of the three Olympic equestrian sports, requiring speed, dexterity and agility to overcome an obstacle [1]. This athletic ability is the combination of all the horses' physio-anatomical characteristics, some of which can be honed with training and experience, of both the horse and rider [2]. When designing an optimal training programme, it is necessary to consider the energy expenditure and the aerobic and anaerobic contributions to the metabolism during exercise [3]. For this reason, field exercise tests, which recreate the duration, difficulty and type of exercises the athlete is expected to perform, are routinely used in equine sport medicine to assess fitness level and detect potential health issues [4,5]. However, the direct measurement of oxygen consumption (VO_2), an indicator of metabolic energy indexes, is a challenging procedure during a field exercise test. A simpler alternative is the use of a cardiac monitor integrated with a global positioning system (GPS), which provides data on both heart rate (HR) and speed, parameters that exhibit a linear relationship with VO_2.

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This method enables the estimation of energy expenditure (EE), cost of transport (CoT) – which indicates the amount of energy required to move the body – and metabolic power (Pmet) [6,7]. Establishing the energy indexes associated with exercise may facilitate comparative analyses and assist in optimising training, monitoring progress, and preventing injuries.

During submaximal exercise, aerobic metabolism is the primary source of energy. However, as the intensity of exercise increases, and if oxygen consumption (VO_2) does not rise proportionally or reaches its maximal limit (VO_2 max), the energy demands are increasingly met through anaerobic metabolism, resulting in lactate accumulation and the onset of fatigue [8]. Thus, blood lactate is used to estimate the anaerobic component of energy production and is considered a marker of fitness [9–11].

Other biomarkers that can help assess the equine athlete's fitness condition, are red blood cell count [12,13], serum proteins [14], and muscle activity related enzymes – aspartate amino transferase (AST) and creatinine kinase (CK) [15–17]. These biomarkers reflect adaptative changes to exercise and may assist in the design of appropriate training programs and in monitoring the athlete's progress, thereby preventing the onset of overtraining.

The aim of this study was to assess the levels of fitness biomarkers in a group of Portuguese show jumping horses and quantify metabolic energy indexes, such as EE, CoT, and Pmet during show jumping simulation test for novice riders and horses. We hypothesised that these animals would perform at a submaximal level of exercise and that the aerobic component would be the main contributor to the energy metabolism.

2. Materials and methods

2.1. Ethical approval

The study protocol was approved by the Ethics and Animal Welfare Committee of Lusófona University (Approval number, 108/2021).

2.2. Animals

A convenient sample of thirteen clinically healthy show-jumping horses, with ages between 6 and 17 years old (11.54 ± 4.18 years) and weighing 563.1 ± 40.3 kg, were enrolled in the present study. The group of horses included one Lusitano, 11 cross-bred Lusitanos and 2 Arabian horses that were selected based on their physical condition and fitness level, with each being capable of jumping at a similar height (80–100 cm). The horses were ridden by their respective riders, who, although inexperienced in the show jumping discipline, were undergoing preparation for a mandatory show jumping examination involving fences of 80 cm in height.

All horses belonged to the same riding centre, located in Mafra, Portugal (N 38° 56′ 12.8862″, W 9° 19′ 39.5292″) and were housed in individual stalls (3.0 × 3.0 m) and their diet consisted of three daily meals (8 a.m., 12 p.m., 5 p.m.) of a commercial feed, with 12.70 % crude protein, and two daily portions of ryegrass hay. Feed and hay quantities were adjusted to individual needs, according to the horses' body weight, body condition and level of physical effort. Water was provided *ad libitum*.

At the time of this study, all horses were physically active, having trained regularly for show jumping for the last six months. The horses were exercised five to six days a week and their daily training routine consisted of 50 min of flat groundwork in different gaits (walk, trot and canter), and show jumping exercises with varying degrees of complexity.

2.3. Show jumping test

To assess the horses' physical condition, a show-jumping test (SJT) was conducted in a 130 × 60 m outdoor grass arena. The course was designed in accordance with the specifications provided by the trainer, which reflected the experience of the horse and rider, as well as the level of forthcoming competitions or events. Prior to testing, the animals underwent a 15 min warm-up that included flatwork and jumping over three obstacles—two verticals and one oxer of 80 cm height.

As previously stated, the horses were ridden by their regular riders (mean weight: 66.8 ± 14.8 kg), and all horse–rider pairs completed the same show jumping course with the following specifications: eight obstacles at a height of 80 cm (three verticals and five oxers); a total track length of approximately 700 m; and completion of the course in the shortest possible time. A schematic representation of the course is provided in Fig. 1.

The SJT was performed in January (winter in the Northern hemisphere), during the morning (7 a.m. - 11 a.m.), with an average temperature of 6°C [5-8°C] and average relative humidity of 73 % [72-75 %]. The test protocol followed the animal welfare principals described by Coelho et al. [18].

After the exercise test the horses cooled down for 5 min and returned to the stall approximately 30 min after having completed the SJT, where they had the remaining of the hay given in the morning to eat and water *ad libitum*.

2.4. Heart rate monitoring

During the SJT, the horses wore a heart monitor (M430 with H10 sensor, Polar Electro; Lake Success, NY, USA) with a Global Positioning System (GPS). The collected data was transferred to a computer and analysed using the Polar Flow software to determine average and peak speed, average and peak heart rate (HR), as well as covered distance.

2.5. Data collection

Data from each animal were collected at four different timepoints: at rest in the box before exercise without a saddle or any apparatus (T0); immediately after the JST (T1); after 30 minutes of recovery (T2); and after 240 minutes of recovery (T3). Data collection began at approximately 9 a.m. and finished at 4:30 p.m.

At each timepoint a full physical examination was performed, and blood samples were collected. Heart rate and respiratory rate (RR) were measured simultaneously by the same two individuals using a stethoscope, while rectal temperature (RT) was measured using a digital thermometer.

Blood samples were collected aseptically from the jugular vein, using 25 × 0.8 mm disposable needles and the collected blood was stored in tubes with EDTA (BD Vacutainer) and in tubes with a clot activator (BD Vacutainer). Samples were refrigerated and shipped to the laboratory, where they were processed within four hours of collection.

Plasma glucose and blood lactate were measured using the hand-held stable side portable devices Glucomen Areo 2 K (Menarini) and Lactate pro 2 (Arkray), respectively.

The EDTA sample was processed immediately upon arrival at the laboratory using the IDEXX ProCyte Dx haematological analyser. Haematocrit, erythrocyte count, haemoglobin concentration and other haematometric indices, such as Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC), as well as white blood cell (WBC) count were measured.

The samples obtained from the tubes without anticoagulant were centrifuged (Hettich Universal 320 R) for 10 min at 2000 g, and the

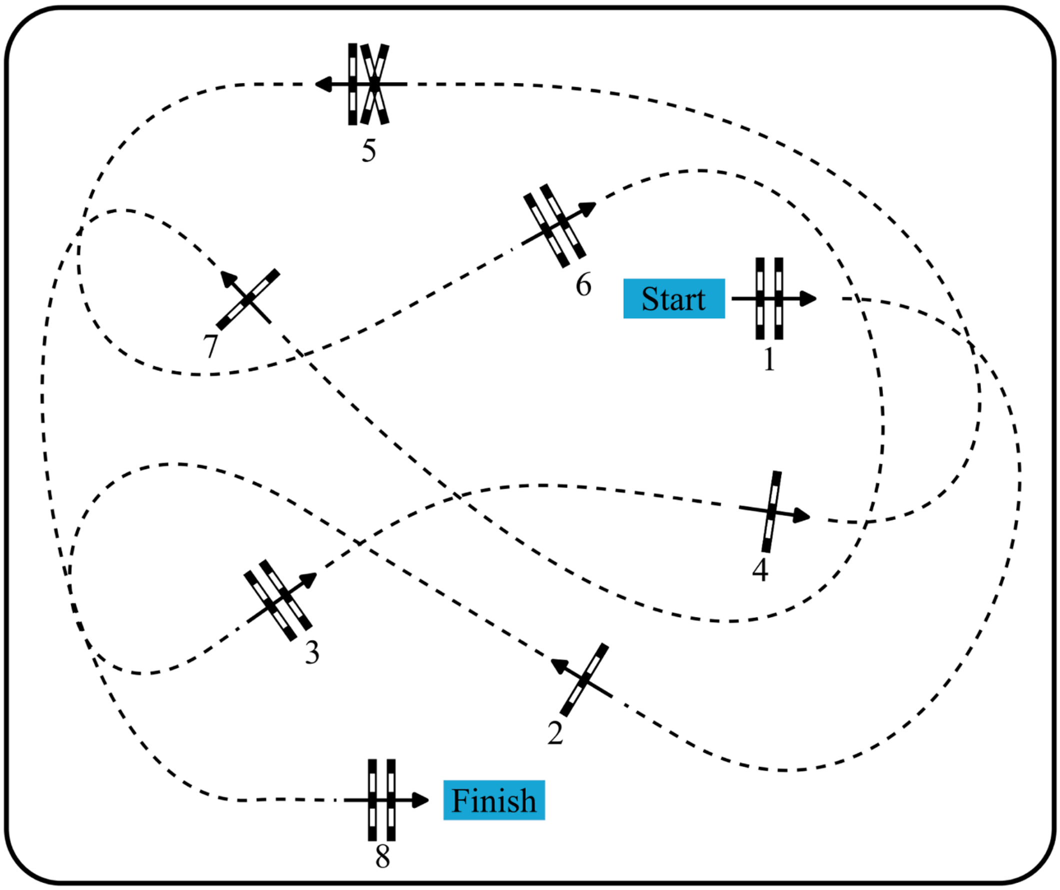

Fig. 1. Course identification of the show jumping simulation test containing three verticals and five oxers of 80 cm height.

obtained serum was frozen at −80 °C, until processed. Serum biochemistry concentrations of total protein (TP), albumin (ALB), creatine kinase (CK) and aspartate aminotransferase (AST) were determined using commercial kits on a Spotchem EZ SP-4430 automatic biochemistry analyser.

2.6. Energy indexes

Energy expenditure (EE), cost of transport (COT) and metabolic power (Pmet) were determined using the formulas described below (Figs. 2–4). Calculations were performed considering the resting heart rate (HR) of each animal, the total weight (kg) comprising of the sum of the weight of the horse, rider and accessories, the covered distance during exercise (m) and the duration of exercise (min).

2.7. Statistical analysis

The statistical analysis was performed with the PROC MIXED software, SAS 9.1 (SAS Institute Inc., Cary, North Caroline, USA). Normality was assessed using the Kolmogorov-Smirnov test and the influence of physical exercise on the measured biomarkers was assessed with analysis of variance for repeated measures, followed by comparison of the means using the Tukey test. The results were expressed as means ± standard deviation and were considered significant when $P < 0.05$.

$$\text{EE (J/min)} = 0.0566 \times \text{HR}^{1.9955}$$

Fig. 2. Formula used to estimate Energy Expenditure [19].

$$\text{COT (beats/m} \times 10^{3}) = (\text{HR} - 35)/\text{kg/m} \times 10^{3}$$

Fig. 3. Formula used to assess Cost of Transport [7].

$$\text{Pmet (beats/min/kg)} = (\text{HR}-35)/\text{min/kg}$$

Fig. 4. Formula used to determine Metabolic Power [20].

3. Results

All animals cleared the SJT and no refusals to jump were observed. The course was completed in 4.9 ± 2.3 min on average and distance travelled during the exercise test was 840 ± 190 m.

The recorded values for HR, RR, RT, glucose, and blood lactate at the four time points are presented in Table 1. The highest values for HR, RR, RT, and lactate were observed at T1, followed by a progressive return to baseline levels by T3. Glucose concentrations were highest at T0, decreased at T1, and subsequently increased progressively up to T3.

The WBC and lymphocyte count peaked at T1, whilst neutrophil count was the highest at T3. The highest values of erythrocyte count, haemoglobin and haematocrit were measured at T1. The SJT did not influence mean corpuscular volume and mean corpuscular haemoglobin concentration (Table 2).

The only serum biochemical parameter elevated in response to exercise was albumin, which peaked at T1 and returned to baseline levels

Table 1
Physiological parameters, glycaemia, and lactatemia (mean ± standard deviation) of horses performing a show-jumping field test.

Parameters	Time				*P*
	T0	T1	T2	T3	
HR (beats/min)	38.85 ± 6.61[b]	98.31 ± 15.79[a]	40.08 ± 5.42[b]	38.62 ± 6.08[b]	<0.0001
RR (breaths/min)	12.08 ± 4.01[b]	74 ± 18.07[a]	16.77 ± 5.69[b]	11.62 ± 5.82[b]	<0.0001
RT (°C)	37.27 ± 0.33[b]	38.06 ± 0.55[a]	37.89 ± 0.33[a]	36.82 ± 0.5[b]	<0.0001
Glucose (mg/dL)	92.62 ± 13.45[a]	73.08 ± 7.34[b]	75.62 ± 7.86[b]	91.08 ± 11.61[a]	<0.0001
Lactate (mmol/L)	1.13 ± 0.26[b]	2.38 ± 0.94[a]	1.41 ± 0.35[b]	1.15 ± 0.29[b]	<0.0001

Note: Different letters in the same line indicate statistically significant differences between timepoints according to the Tukey test ($P \leq 0.05$); T0: at rest; T1: immediately after exercise; T2: at 30 minutes of recovery; T3, at 240 minutes of recovery. HR, heart rate; RR, respiratory rate; RT, rectal temperature. P: significance value of the analysis variance for repeated measures.

Table 2
Blood count parameters (mean ± standard deviation) of the horses that performed a show-jumping field test.

Parameters	Time				*P*
	T0	T1	T2	T3	
WBC (x10^3/μL)	6.2 ± 1.58[b]	7.73 ± 0.66[a]	6.46 ± 0.87[b]	7 ± 1.19[ab]	0.0071
Ne (x10^3/μL)	3.19 ± 0.91[b]	3.86 ± 0.49[ab]	3.38 ± 0.28[ab]	3.96 ± 0.82[a]	0.0113
Lympho (x10^3/μL)	2.66 ± 0.77[b]	3.47 ± 0.61[a]	2.73 ± 0.61[b]	2.65 ± 0.62[b]	0.0072
Ery (x10^6/μL)	7.85 ± 0.92[b]	10.28 ± 0.59[a]	7.77 ± 0.65[b]	7.97 ± 0.75[b]	<0.0001
Hb (g/dL)	12.52 ± 1.40[b]	16.58 ± 0.78[a]	12.59 ± 1.01[b]	12.78 ± 1.07[b]	<0.0001
Hct (%)	35.01 ± 4.61[b]	47.85 ± 2.91[a]	35.53 ± 3.04[b]	35.66 ± 3.35[b]	<0.0001
MCV (fL)	44.65 ± 3.44	46.64 ± 3.18	45.78 ± 2.94	44.83 ± 3.07	0.3665
MCHC (g/dL)	34.47 ± 5.43	34.68 ± 0.86	35.44 ± 0.89	35.87 ± 0.97	0.5949

Note: Different letters on the same line indicate statistically significant differences between timepoints according to the Tukey test ($P \leq 0.05$); T0: at rest; T1: immediately after exercise; T2: at 30 minutes of recovery; T3, at 240 minutes of recovery. WBC, white blood cell count; Ne, total neutrophil count; Lympho, total lymphocyte count; Ery, total erythrocyte count; Hb, haemoglobin concentration; Hct, haematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration. P: significance value of the analysis variance for repeated measures.

Table 3
Serum biochemistry parameters (mean ± standard deviation) of horses performing a show-jumping simulation test.

Parameters	Time				*P*
	T0	T1	T2	T3	
AST (IU/L)	269.69 ± 74.38	297.23 ± 77.31	277.77 ± 72.78	284.46 ± 82.19	0.8256
ALB (mg/dL)	2.9 ± 0.2[b]	3.18 ± 0.18[a]	2.98 ± 0.2[ab]	2.97 ± 0.2[b]	0.0052
TP (mg/dL)	5.61 ± 0.47	5.79 ± 0.4	5.63 ± 0.35	5.76 ± 0.39	0.5667
CK (IU/L)	144.92 ± 57.45	194.69 ± 66.01	170.46 ± 49.85	173.92 ± 40.87	0.1535

Note: Different letters on the same line indicate statistically significant differences between timepoints according to the Tukey test ($P \leq 0.05$); T0: at rest; T1: immediately after exercise; T2: at 30 minutes of recovery; T3, at 240 minutes of recovery. AST, aspartate aminotransferase concentration; ALB, albumin concentration; TP, total proteins concentration; CK, creatine kinase concentration. P: significance value of the analysis variance for repeated measures.

by T3 (Table 3).

Table 4 presents the mean values and standard deviations of the parameters recorded by the heart rate monitor (H10 sensor/M430 Polar) during the show jumping simulation test, along with the estimated energetic parameters calculated from heart rate data collected during the field test.

Table 4
Heart rate and velocity, and energy expenditure parameters of the horses during the show-jumping simulation test collected using the heart monitor H10 Polar.

Parameters	Values
HRpeak (bpm)	109.5 ± 34.4
HRm (bpm)	85.9 ± 20.9
Vpeak (km/h)	26.9 ± 2.8
Vm (km/h)	11.6 ± 1.3
EE (J/kg/min)	431.0 ± 222.5
CoT (beats/kg/m.10^3)	0.10 ± 0.05
Pmet (beats/min/kg)	0.02 ± 0.01

Note: Data are expressed as mean and standard deviation. HRpeak, peak heart rate; HRm, average heart rate; Vpeak, peak velocity; Vm, average velocity; EE, energy expenditure; CoT, cost of transport; Pmet, metabolic power.

4. Discussion

This study aimed to assess the physical conditioning of a group of Portuguese show jumping horses and their response to a field simulation test by measuring physiological, haematological and metabolic parameters. We confirmed our hypothesis that all animals would perform at a submaximal level of exercise and that they would be able to successfully complete the obstacle course while relying mainly on the aerobic energy pathway.

In equine exercise physiology HR is well correlated to VO_2 and, since its monitoring can be easily achieved using non-invasive methods, it is a good indicator of oxygen demand [21,22]. In the present study, higher HR values were observed immediately after exercise, at T1, (98.31 ± 15.79 beats/min) when compared to other timepoints. This increase is a well-documented response to exercise [23–25]. Furthermore, the use of heart monitors with a GPS system allows the correlation of HR with speed [21], and during submaximal exercise in treadmill tests there is a linear relationship between heart rate and speed [26]. On the other hand, higher HRs do not always correlate with higher speeds [27]. In show jumping, this correlation is not always evident, possibly due to an increase in HR during the jump, regardless of its speed [28]. The HR registered during the SJT were fairly low, which is consistent with the speed at which the horses completed the course. This could be explained by the low height of the obstacles and may reflect the horse's adaptation to the imposed level of exercise.

The increase in HR and RT observed at T1 are consistent with a higher metabolic demand during exercise. The rise in respiratory rate (RR), concurrent with the increase in HR observed at T1, further supports the increased muscular demand for oxygen. Around 70-80 % of the energy produced by the muscles is released in the form of heat that is dissipated through sweating and vasodilation so that the athlete is able to perform the exercise [29]. Additionally, the observed tachypnoea immediately after exercise would also help dissipate heat [30–32,17].

The exercise performed by this group of athletes here presented can be characterized as aerobic, since the lactate values at T1 (2.38 ± 0.94 mmol/L) did not reach the anaerobic threshold of 4 mmol/L [33]. In fact, the highest lactate value recorded in the present study was lower than those reported in similar works where lactate values of 3.9 ± 0.70 mmol/L and of 3.54 ± 1.98 mmol/L were described with obstacles between 80 - 120 cm and between 80 and 100 cm, respectively [34,35]. Lactate concentrations exceeding the anaerobic threshold, reaching 5.00 ± 0.46 mmol/L and 9.04 ± 0.9 mmol/L, have also been reported in show jumping events involving obstacles between 120 cm and 150 cm in height [1,23]. In these studies, there was a predominance of anaerobic metabolism resulting in the accumulation of large amounts of lactate [36]. Thus, exercise intensity can impact blood lactate concentration and, although, show jumping events are not typically associated with long exercise periods or increased speed, anaerobic metabolism may be required in more complex events or when the animals fitness level is not compatible with the required exercise [11,23,27].

In both humans and horses, glycaemia levels are affected by the intensity and the duration of exercise [23,37]. In this study, the observed equine athletes presented higher glucose values at T0 and T3. At T1, a significant decrease in glycaemia was observed, possibly due to increase uptake by muscle cells and eventual intramuscular glycogen depletion due to activation of glycolytic pathways. However, given the intensity of the exercise the former is the more probable explanation. The increase observed again at T3 could be the result of hepatic glycogenolysis stimulation, of fat metabolism activation or a blood volume decrease [12], as similar findings were reported in other studies [1,17]. However, in our study, it is more likely that values returned to baseline due to a decrease in blood glucose uptake by the muscles.

The SJT led to a significant increase in Hct, Ery and Hb which can be explained by sympathetic activation and catecholamine release with subsequent splenic contraction [12,31,35,38,13]. This mechanism ensures a correct oxygen and energy supply to the muscles and the removal of catabolites [13,35]. Additionally, as body temperature rises, fluid loss occurs, either through sweating or via the respiratory system, leading to a decrease in blood volume and consequently an increase in the haematocrit [16].

At T1 the animals presented increased white blood cell, neutrophil and lymphocyte counts when compared to rest, possibly due to cortisol release (stress leukogram) and due to catecholamine release with secondary splenic contraction (physiological leukogram) [39,12]. At T3, although the Ne count remained higher than at T1, lymphocyte count had returned to baseline values, which could be associated to cortisol release, a hormone responsible for maintaining homeostasis and in charge of creating an adaptative response to exercise [40,41].

No large-scale hydrolytic changes occurred during the exercise simulation test, as no changes were observed in total proteins. However, the significant increase in ALB at T1 could be associated with hypervolemia and the transport and metabolism of fatty acids [42,43].

Increases in muscle enzymes can occur during exercise with prolonged anaerobic metabolism. In this work serum concentrations of AST and CK were not significantly influenced by the SJT, suggesting that no muscle lesion occurred in the studied group of horses, which indicates they're adaptation to the level of exercise performed [44].

As previously mentioned, HR was used to calculate EE, CoT and Pmet, since VO_2 measurement during the field exercise test was impractical. In general, the values found for EE in the jumping horses in this study were similar to those described in Warmblood horses at walk (~438.4 J/kg/min, at 15 % Vmax) [3]. However, they were much lower compared to when the same group of Warmbloods were worked at trot (~982.8 J/kg/min, at 30 % Vmax), canter (~1712.8 J/kg/min, at 50 % Vmax) and gallop (~2447.3 J/kg/min, at 70 % Vmax) [3]. Physical effort is intimately related to energy expenditure. The higher the intensity, the higher the HR and consequently the greater the EE. Thus, athletes who are well adapted to the imposed effort level will present lower HR and, consequently, lower EE.

In this group of horses the CoT was lower than that described for steeplechase horses - 0.319 and 0.408 beats/kg/m x 10^3 [45], jumping horses ~0.25 beats/kg/m x 10^3 [46], and horses on a treadmill without inclination both while walking (~0.8 beats/kg/m x 10^3) and trotting (~0.6 beats/kg/m x 10^3) [7]. It would be expected that show jumping horses would have a higher CoT, as this exercise requires a vertical displacement of the horse's centre of mass against gravity, compared to other modalities that require only trot or canter [46]. Thus, the obtained results may be due to the low height of the obstacles.

The metabolic power (Pmet) is associated with acceleration during exercise [45,46]. In show jumping, the acceleration and deceleration phases occur particularly during the approach to an obstacle and after transposing it. Perhaps due to the height of the obstacles and the speed of the exercise, the Pmet values observed in this group of horses were lower than those reported for steeplechase horses, in which the athletes are required to jump comparatively higher fences at greater speeds over a prolonged period of time [46].

This study presents some limitations. The findings described in this study must be interpreted based on the type of athletes included in the sample. Firstly, the riders and horses were inexperienced which influenced the height of the obstacles and has probably affected the approach chosen during the jumps, and can affect speed. Another limitation was the inclusion of breeds not considered to have an innate aptitude for show jumping and therefore are not usually included in studies of this sport modality. Thus, the reported results should not be extrapolated to experienced horses competing at higher levels. Nonetheless it is important to characterise the metabolic energy indexes associated with low intensity exercise since this data can serve as base for other comparative studies and can assist in the tailoring of training programs for non-elite athletes.

Lastly, although difficult to perform during a field exercise test, the direct measurement of VO_2 during exercise is considered the reference standard for estimating energy indexes. The correlation between HR and VO_2 is well described and serves as an alternative to assess energy metabolism, but this method is not as accurate.

The return to baseline of the physiological and haematological parameters observed in our data at T2 and T3 is consistent with findings reported in the literature for horses that are well conditioned to the performed level of exercise [1,31,35]. In addition, there were no clinical signs of muscle damage associated with SJT and the athletes were able to successfully complete the obstacle course. Although not commonly used in non-elite athletes, simulation tests can serve as valuable tools for assessing fitness and refining training programmes.

5. Conclusion

The present study focused on a group of show jumping horses competing at a novice level. Despite the relatively low height of the obstacle course, variations in several of the analysed biomarkers were observed in response to exercise, demonstrating their potential utility in the assessment of fitness in animals performing at a comparable level. The horses completed the SJT without difficulty, maintaining an aerobic metabolism profile and performing at a submaximal intensity level. Understanding and characterising the physiological responses of athletes within specific equestrian disciplines is crucial for the optimisation of fitness programmes and safeguarding the health of the horses competing at various levels.

Conflict of interest statement (From Feb 2025 this will be required as a separate submission file on the online editorial manager system)

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Ethics in publishing statement

This research presents an accurate account of the work performed, all data presented are accurate and methodologies detailed enough to permit others to replicate the work.

This manuscript represents entirely original works and or if work and/or words of others have been used, that this has been appropriately cited or quoted and permission has been obtained where necessary.

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All authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

CRediT authorship contribution statement

J. Simões: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation. **A.M. Santos:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **C. Santos:** Methodology. **A.S. Silva:** Methodology. **C. Vintém:** Methodology. **J. Fonseca:** Methodology. **C. Coelho:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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