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COMPARISON OF COMPLETED AND ELIMINATED HORSES ON HEART RATE, CREATINE KINASE, LACTATE AND ELECTROLYTES IN ENDURANCE RACE

ZULFITRI NAIM BIN ABDUL RAHIM

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It is hereby certified that we have read this project paper entitled "Comparison of Completed and Eliminated Horses on Heart Rate, Creatine Kinase, Lactate and Electrolytes in Endurance Race", by Zulfitri Naim bin Abdul Rahim and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 - Project

Assoc. Prof Dr. Noraniza Mohd Adzahan
DVM (UPM), MVM (UPM), PHD (UPM)
Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

Dr. Azalea Hani Othman
DVM (UPM), MPhil (QUEENSLAND), PHD (UPM)
Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

PERBANDINGAN KUDA BERJAYA DAN TERSINGKIR BERDASARKAN KADAR DENGUPAN JANTUNG, KREATIN KINASE, LAKTAT DAN ELEKTROLIT DALAM PERTANDINGAN

Oleh

ZULFITRI NAIM BIN ABDUL RAHIM

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Penyelia: Profesor Madya Dr Noraniza Mohd Adzahan

Penyelia Bersama: Dr Azalea Hani Othman

Kuda tahan lasak terdedah kepada tekanan berpanjangan ketika pertandingan walaupun selepas beberapa rejim latihan. Prestasi kuda akan ditentukan melalui kebolehan adaptasi terhadap latihan. Kajian ini bertujuan mengenal pasti perbezaan kadar dengupan jantung, kreatin kinase, laktat dan elektrolit di antara kuda yang berjaya dan tersingkir selepas pertandingan. Lima puluh satu kuda berumur 6 – 25 tahun telah bertanding dalam perlumbaan kategori 100 km. Kesemua kuda menjalani pemeriksaan fizikal dan diakhir perlumbaan, kuda tersingkir (n = 11) dan berjaya (n = 7) telah dipilih dan sampel darah telah diambil melalui suntikan vena jugular. Data kadar dengupan

jantung telah dikumpul berdasarkan rekod daripada buku log kuda. Sampel serum telah diekstrak dan dianalisa di makmal untuk kreatin kinase (CK), laktat darah, natrium (Na), kalium (K) dan klorida (Cl). Kesemua parameter dinilai menggunakan ujian-t sampel tidak bersandar and dihuraikan sebagai min dan sisihan piawai. Keputusan menunjukkan perbezaan ketara dalam kadar dengupan jantung dan laktat darah selepas perlumbaan ($p<0.05$). Kadar dengupan jantung dan laktat untuk kuda tersingkir, 67 ± 11 bpm dan 3.44 ± 1.39 mmol/L adalah lebih tinggi berbanding dengan nilai untuk kuda berjaya, 58 ± 2 bpm dan 1.61 ± 0.36 mmol/L. CK dan elektrolit selepas perlumbaan tidak terbukti mempunyai perbezaan ketara ($p>0.05$) di antara kuda berjaya dan kuda tersingkir. Kesimpulannya, kadar dengupan jantung dan laktat darah kuda boleh digunakan sebagai indikator penilaian semasa latihan untuk meramalkan potensi prestasi kuda dalam pertandingan.

Kata kunci: Kuda, sukan tahan lasak, kebolehan adaptasi, penilaian, prestasi

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of requirement of the course VPD 4999 – Project

COMPARISON OF COMPLETED AND ELIMINATED HORSES ON HEART RATE, CREATINE KINASE, LACTATE AND ELECTROLYTES IN ENDURANCE RACE

By

ZUFITRI NAIM BIN ABDUL RAHIM

2022

Supervisor: Associate Professor Dr Noraniza Mohd Adzahan

Co-supervisor: Dr Azalea Hani Othman

Endurance horses are subjected to prolonged stress during competition even after several conditioning regimes. Performance of these horses will be determined by the adaptability to the exercise. This study aims to determine the differences on heart rate, creatine kinase, lactate and electrolytes between successfully completed and eliminated horses after an endurance race. Fifty-one horses aged 6 – 25 years competed in 100 km

race category. All the horses were physically examined, and at the end of the race, eliminated (n = 11) and completed (n = 7) horses were selected and blood samples were collected via jugular venipuncture. Heart rate data were collected based on the record from horses' logbooks. Serum samples were extracted and analysed in laboratory for creatine kinase (CK), blood lactate, sodium (Na), potassium (K) and chloride (Cl). These parameters were tested statistically using independent-samples t- test and described as mean and standard deviation. The result showed significant differences in post-race heart rate and blood lactate ($p<0.05$). Heart rate and lactate of eliminated horses, 67 ± 11 bpm and 3.44 ± 1.39 mmol/L exhibited higher mean score compared to the completed horses, 58 ± 2 bpm and 1.61 ± 0.36 mmol/L respectively. Post-race CK and electrolytes are unable to be proven having significant difference ($p>0.05$) between the completed and eliminated endurance horses. In conclusion, heart rate and blood lactate of horses may be used as assessment indicators in endurance training to predict the horses' potential performance in endurance races.

Keywords: Horse, endurance, adaptability, assessment, performance

CHAPTER 1
INTRODUCTION

1.1 Overview

Endurance riding is a sport that tests the speed and tolerance of horses across a long distance that has been regulated by the *Fédération Equestre Internationale* (FEI) since 1982. The horses' performance is determined by their ability to complete the race in the shortest time, with the horses are still in good condition. Therefore, these horses are trained and conditioned to enable them to perform well in the competition. However, due to the prolonged stress and other contributing factors such weather, racing track, and terrain, endurance horses are subjected to elimination. Depending on the speed and course, the elimination rate in endurance events ranges from 10 to 60 % (Fielding *et al*., 2011). Hence, numerous previous study have addressed the issue of evaluating the racing capacity of these horses.

Among them is the use of heart rate, which is associated with the horses' major physiological adaptations that can directly affect exercise capacity and the efficiency of gas exchange, oxygen uptake and delivery to the active working muscles (Evans *et al*., 2006). In sport, the heart rate is the first important parameter as a horse can be deem unfit if the heart rate is persistently higher than required. This is because the horses with elevated heart rate are at high risk to develop metabolic disorders due to poor compensatory mechanism of the body. Besides that, this submaximal intensity activity will also cause muscle damage, which can be determined by the present of creatine kinase (CK) in the blood. This enzyme normally located in healthy cell and will leak into the bloodstream as the muscle injured. But, skeletal muscles are remarkable in adapting to certain stimuli, such as exercise (Fluck, 2006). Therefore, a proper training should able to reduce the CK appearance in the blood (Buzała *et al*., 2015).

Moreover, increase requirement of energy during the race, lead to production of lactate, end product of anaerobic glycolysis. Horse' body prevents accumulation of lactate by the Cori cycle, which uses blood lactate to produce glucose inside the liver as energy source for the muscles (Vermeulen *et al*., 2017). When the production of lactate exceeds the capacity of the Cori cycle, a rise of lactate in blood will be observed. Consequently, because the lactate induce acidification, it will cause fatigue in horses (Pösö, 2002). Hence, the lactate is also normally used to assess the horse fitness. In addition, horses also may loss about 10-15 litres per hour of body fluid in form of sweat as a result of evaporative heat loss during an endurance racing (McConaghy *et al*., 2010). As the horses secreting hypertonic sweat, it will also lead to loss of electrolytes, making electrolytes are useful marker to assess horses' hydration status.

Currently, the heart rate, creatine kinase, lactate and electrolytes value has been studied as keys performance of horses in multiple equestrian disciplines. However, there is limited data that specializes on these variables, particularly in comparing between the completed and eliminated horses in endurance competition.

1.2 Justification

There is limited data on post-race physical and biochemical parameters of endurance horses in relation to performance. Thus, this study was conducted to investigate the differences of completed and eliminated horses on heart rate, creatine kinase, lactate and electrolyte during an endurance race. Besides, according to literatures, these parameters are used to evaluate the horses' performance, hence this study will also determine the credibility of these parameter in assessing the performance of horses during endurance training and actual race.

1.3 Objective

The objectives of this study are:

1. to determine the differences in level of post-race heart rate between completed and eliminated endurance horses.
2. to determine the differences in level of post-race creatine kinase, lactate and electrolytes between completed and eliminated endurance horses.

1.4 Hypothesis

Null Hypothesis: There are no significant differences in heart rate, creatine kinase, lactate and electrolytes between completed and eliminated endurance horses.

Alternative hypothesis: There are significant differences in heart rate, creatine kinase, lactate and electrolytes between completed and eliminated endurance horses

CHAPTER 2
LITERATURE REVIEW

2.1 Endurance sport

Endurance riding is a sport under the equestrian discipline, where participants, which are riders and horses compete in races over a very long distance. Endurance tests the ability of rider to safely manage the stamina and the fitness of the horse over an endurance course in a competition against the track, distance, climate, terrain and the clock (Di Battista *et al.,* 2019). This sport conducted in various distances, usually between 80 km to 160 km, which divided into several phases of 20 to 40 km. Regardless the distance, the foundation of endurance sport lies on the good welfare management of the horses.

International-level endurance events are required to follow the FEI Regulations and FEI Endurance Rules. These events are divided into four categories, which are *Concours de Raid d'Endurance International* (CEI), *Concours de Raid d'Endurance International Officiel* (CEIO), *Championships and Games and Combined National/FEI events* (CEN/CEI). CEIs, CEIOs and Championships are conducted according the level of race distance and expertise or are known as "Star". There are 3 levels of star, which are 1, 2 and 3 Star.

All horses are required to undergo physical examination prior to the race and at the end of each loop by the veterinary officials. Horses must pass this compulsory inspection in order to proceed and complete the race. If horses presented with compromised metabolic and musculoskeletal conditions that indicate they are unfit to continue, the horses will be eliminated from the race and subjected to treatments immediately.

2.2 Veterinary control

All horses are required to undergo physical examination prior to the race and at the end of each loop by the veterinary officials. Hence veterinary control was established to ensure the welfare of competing horses are well-taken care of throughout the race. Physical examination is conducted to evaluate the metabolic parameters such as resting heart rate, mucous membrane, capillary and jugular refill, skin recoil, gut motility, muscle tone and soreness or injuries on the back, withers, girth area, body, or distal extremities (Adamu *et al.*, 2013c). The horses also will be trotted to assess the gait and presence of lameness.

If veterinarians determine that a horse's metabolic state or orthopaedic condition is unable to allow them to continue the ride, the horse will be removed from the ride. This elimination can occur before or during the ride, as well as at final veterinary inspection after completing the competition (Nagy *et al*., 2012). Horses will be eliminated due to metabolic reason if veterinarian considers that the horse's metabolic status is compromised, for example, longer capillary refill time, dehydration, absence of intestinal activity, and higher cardiac recovery index (CRI). Elimination for metabolic reasons can also happen solely as a result of a heart rate that is higher than what is specified by FEI in Rules for Endurance Event, which normally more 64 beats per minute (bpm).

The FEI also defines a horse will be eliminated due to lameness at any inspections during the course of competition, when presented with irregular gait, which consistently observable through evaluation by trotting the horse on a loose lead in hand straight out and back, without prior flexion or deep palpation; which must be perceived to cause pain, or threaten the immediate ability of the horse to safely perform

athletically. This horse will be removed from competition and will fail to qualify for the next phase.

2.3 Prevalence and risk factors of elimination in endurance horses

The popularity of endurance racing has continued to grow, accompanied by increasing number of participants competing at the international level. The number of FEI endurance events has grown from 16 in 1994 to 830 in 2021 (Nagy *et al.*, 2012 and FEI Annual Report, 2021). Endurance events in Malaysia also have gain recognition by the world, and the number of people participate in the competition has increase year by year. Unfortunately, the level of performance of horses in endurance races is still low and the rate of elimination is high. A study showed that up to 71.64 % of the horse population participated in one of the endurance races in Malaysia were eliminated from the endurance race mainly due to metabolic disorders (53.73 %) and lameness (17.91 %) (Adamu *et al.*, 2013b). Years later, Izzati (2018a) discovered that the rate of elimination of endurance horses in Malaysia from 2015 to 2016 had been decreased drastically with only 35.05 % of the horse were eliminated from the race, with lameness is the major cause of elimination followed by the metabolic disorders.

Nagy *et al.*, (2013) has published a report regarding the risk factors associated with the elimination in endurance race, mentioning the horse, rider, venue and environment are the contributors. Horse-related factors caused elimination are associated with the horse's sex, age, experienced and racing frequency. It was suggested an interval of at least 91 days between 2 rides was required to reduce the risk of elimination for lameness. Besides, if the horse's previous experience at a ride of longer distance than the current ride, it also reduce the risk of elimination. Rider's gender and experience may also influence the outcomes of the competition. Lastly, terrain such as

deep sand or soil, temperature and humidity also contribute to the elimination due to lameness and metabolic reasons.

2.4 Heart rate as first fitness parameter

In an endurance competition, the first crucial assessment of fitness is determined by the measurement of the heart rate (Bashir and Rasedee, 2009). The normal heart rate in a resting horse range between 28 – 40 bpm and will elevate during exercise (Evans *et al.*, 2006). According to the FEI, a horse must be presented to the vetting area with a heart rate of not more than 64 bpm in order to continue the race. If the heart rate is higher than the maximum heart rate, the veterinarian may demand that the horse undergo a second presentation, provided it is still within the permitted presentation time. If the heart rate is still exceeds the established limit at the second presentation, a second veterinarian will confirm it and the horse will not be allowed to proceed and is eliminated from the competition (FEI, 2022). Thus, a fast recovery of heart rate is very important for success in endurance events and horse with high heart rate indicates poor performance. The high heart rates appear to have an impact on tissue oxygenation and body thermoregulation, leading to anaerobic state and hyperthermia, subjecting a large percentage of these horses are eliminated from endurance competitions (Adamu *et al.*, 2012b). Heart rate also demonstrated to have a linear relationship between the speeds during submaximal activity (Mukai *et al*., 2003 and Younes *et al*., 2015). As the speed increased, the working muscle required faster oxygen supplies, thus the heart will pump blood faster and consequently will increase the heart rate.

2.5 Role of lactate in endurance horse

During high intensity exercise, lactate is formed when the body turns glucose into energy in the depletion of oxygen. This lactate is produced in the muscle cells and red blood cells. When the lactate develops depends largely on the individual's fitness. Therefore, blood lactate determination, as a measure of anaerobic threshold, has been found to be valuable for assessing the fitness in equine athletes (Williamson *et al*., 1996 and Gondim *et al.,* 2007). In fact, lactate production is a good indicator of muscle fatigue under various conditions (Pösö, 2002). The blood lactate concentration of 4 mmol/L and above is used to indicate unfit horse during endurance training program and able to predict the performance of the horse in an actual race (Kedzierski *et al.,* 2009 and Trilk *et al.,* 2010). Thus, a proper training program will capable to gradually reduce the blood lactate concentration and maintain the horse in aerobic state throughout the training or actual race.

In previous study, endurance horses during competition showed significant elevation of lactate concentration, where poor performance horses exhibit higher concentration compare to good performance horses (Adamu *et al*., 2012b). However in another study, contrarily good performance horses demonstrated to have higher post-race lactate concentration than poor performance horse (Adamu *et al*., 2010; 2013c). Besides, lactate concentration activity is also associated close to the glucose concentration, therefore increased in lactate production of the horses could be associated with poor glucose utilization by the metabolizing tissue (Ferraz *et al.,* 2008 and Adamu *et al.,* 2010).

2.6 Role of creatine kinase as muscle damage parameter

Creatine kinase is a type of enzyme found in the muscles, which helps the muscles to produce the phosphocreatine, which used as an energy source (Octura *et al*, 2014). Generally, the activities of these enzymes are low in plasma or serum because they are normally present in healthy cells. The CK activity during exercise depends largely on the duration and intensity of exercise (Buzała *et al.,* 2015). Therefore, prolonged strenuous activity like endurance race will eventually cause muscle damage and this condition can be determine by the increased appearance of CK in the blood that considered to be an indirect marker of muscle damage (Baird *et al*., 2012). Besides, the elevations of this enzyme activity in serum may also occur in association of increased cell permeability (Cardinet, 1997). Therefore, events related to strenuous exercise may lead to an increased skeletal muscle membrane leakage and it is noteworthy to report that elevated CK levels after exercise does not necessarily mean there is critical muscle injury (Cardinet, 1997). However, the enzyme activities may change with exercise due to increase in the metabolic process taking place in the muscle leading to transient changes in the muscle permeability without any significant muscle damage.

Previous studies reported that young horses have higher CK values than older as they were inexperienced (Adamu *et al.,* 2013c). However, this is contradicted with Klobučar (2019) that show significant higher CK in older horses. Apart from this, studies conducted at different distance categories, shown that by increasing the distance of the race, the CK value after race will become greater (Adamu *et al,* 2010 and Klobučar *et al*., 2019). Besides, horses exhibit a significant increase of CK prior to the competition also had a higher chance to be eliminated for lameness for every doubling of the CK value (Bollinger *et al.,* 2021). This could be contributed by the muscular

damage resulting from transportation stress, inadequate recovery breaks before the competition, overtraining-related muscle injury, or impaired renal elimination due to dehydration.

2.7 Changes of electrolytes in endurance race

Exercise will cause elevation of horses' body temperature and initiate the homeostasis process to stabilize it. It regulate the body temperature via production of sweat and the horses are known to produce hypertonic sweat, which mean they are not only excreting water and protein, but also excrete the sodium, chloride, potassium, calcium and magnesium through perspiration (Flaminio & Rush, 1998). These electrolytes aid in regulation of nerve and muscle function, and also importance for hydration maintenance.

Numerous factors have been linked to the elimination of endurance horses from the race, with dehydration, acid-base imbalances, and changes in electrolyte concentrations being the most commonly reported (Fielding *et al*., 2009; Muñoz *et al*., 2010; Nagy *et al*., 2013 & Izzati *et al.*, 2018a). However, it is also possible that certain horses have aberrant electrolyte concentrations or hydration status prior to the competition that can be influenced by many factors, for instance, transportation, illness and overnight stay prior to competition (Fielding & Magdesian, 2021). In addition, water and electrolytes intake, cooling technique, environmental conditions and difficulty of the track also have an impact on electrolyte changes (Larsson *et al.*, 2013). Hence, it is also important to note that maintaining electrolyte balance will enhance horses' performance.

CHAPTER 3
MATERIALS AND METHOD

3.1 Endurance venue and event

The study was conducted at Terengganu International Endurance Park (TIEP) during Kejohanan Kuda Lasak Piala Yang Dipertuan Muda Terengganu on 17 – 19 June 2022. This event was a combined national (CEN) and FEI (CEI*) events.

3.2 Horses

Fifty-one horses competed in an endurance competition of 100 km; out of this number, 27 were eliminated from the race and 24 completed the race successfully. For this study, 18 horses were conveniently selected to represent the 2 groups based on the status of the horses, which are eliminated (n = 11) or completed (n = 7). The race was conducted in accordance with FEI rules. The age and body weight of the horses ranged between 6 – 25 years old and 350 – 450 kg, respectively.

3.3 Veterinary inspections

Veterinary inspections were conducted before the race and within 15 minutes of recovery period at the end of each phase of the race on all competing horses and physical parameters were recorded (Figure 1). The race was divided into 4 phases and the distance of each phase in this study were 25, 30, 25 and 20 km, respectively. At the end of the race, the horses were classified as either successfully completed or eliminated. It was necessary for a horse to be able to retain normal gastrointestinal, respiratory, cardiac, or musculoskeletal state in order for it to be classified as completed. Horses subjected to elimination were those that failed to meet the above mentioned requirements and were thus disqualified from the endurance race (Figure 2).

Figure 1. Physical examination at pre-ride and at the end of each phase

Figure 2. Treated horse that was eliminated due to metabolic ailments

3.4 Horse riders

Rider must comply with the minimum weight requirements in order to compete. The weight measurement includes all riding equipments except for the bridle. The minimum weight for international level under FEI is 70 kg. Horses were ridden by professional riders based on the FEI rules and regulations.

3.5 Climatic factors and race track

The ambient temperature and humidity were recorded at an interval of one hour from the beginning of the race to the finish. The mean and standard deviation of temperature and humidity were 27.08 ± 1.91 °C and 84.45 ± 5.84 %, respectively during the period of the endurance race. The ambient temperature and humidity were measured using portable SNDWAY SW – 572 digital thermometer hygrometer. The geographical terrain was good and conducive water points were also provided at specific places along the track which was generally flat and lack debris of stones.

3.6 Data collection and blood sampling

Post-race heart rate were collected based on the record from the horses' log books. Post-race blood samples were obtained from all the horses via jugular venipucture into heparinized vacutainer tubes for biochemical analysis. The blood sample collection was performed immediately within 15 min of the recovery period. Blood samples collected were then centrifuged and plasma were extracted to be transported and analysed in the laboratory.

3.7 Blood biochemistry result

Blood biochemistry data includes the results of lactate, creatine kinase, and electrolyte, which are sodium, potassium and chloride. All parameters were analysed using BA 400 LED technology (BioSystems S. A., Barcelona, Spain).

3.8 Statistical analysis

The data were analysed using descriptive analysis (mean ± SD). Normality was tested using the Shapiro – Wilks and differences among the group were evaluated via independent-samples t-test using the statistical software package IBM SPSS statistics 27. Analyses were considered as significant at $P < 0.05$.

3.9 Ethical declaration

The procedures carried out on the animals in the study were approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM/IACUC/AUP-U047/2022).

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Overall rate of completion and elimination

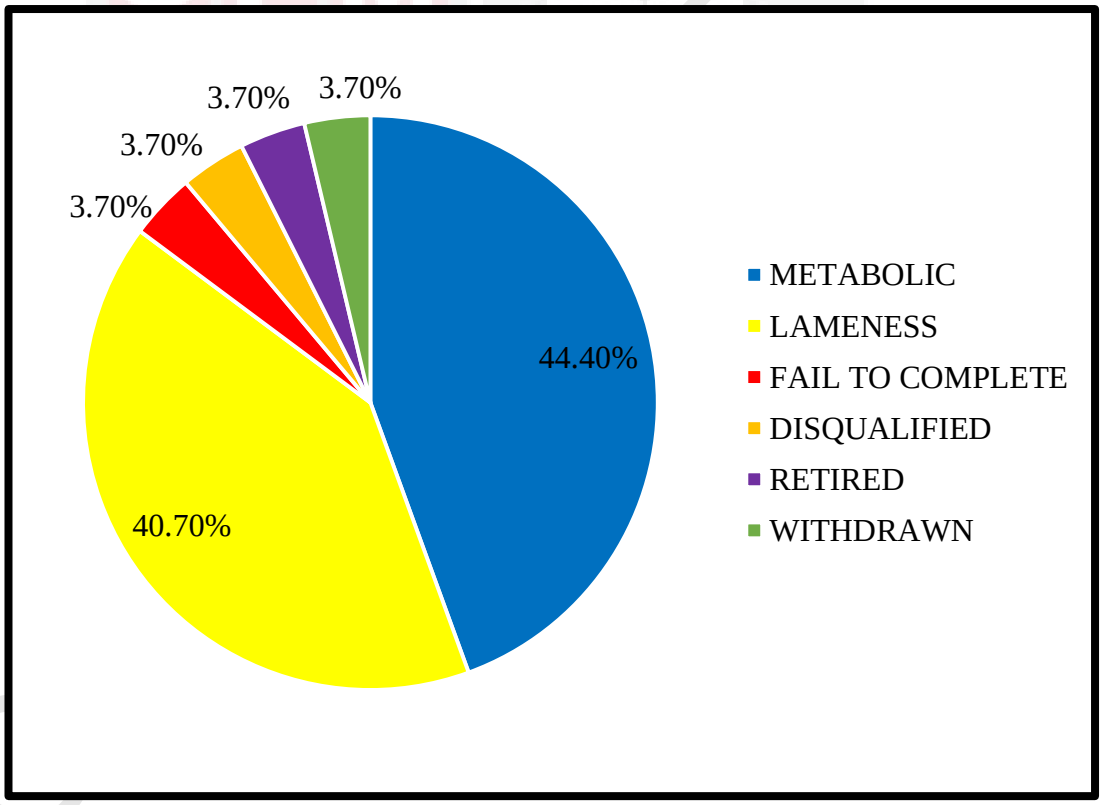
Fifty-one horses competed in an endurance race of 100 km, that categorized into two, which are CEI* (35 horses) and CEN (16 horses). However, 27 horses (52.94 %) had various physical and metabolic disorders and were eliminated from the race, whereas 24 horses (47.06 %) completed the race successfully. Among the successfully completed horses, 17 (70.8 %) of the horses competed in CEI* 100km, and 7 horse (29.2 %) take part in CEN 100 km; whereas for elimination, 18 (66.7 %) horses were from CEI* 100 km and 9 (33.3 %) were from CEN 100 km, respectively (Table 1).

The most common reasons for elimination in the race was metabolic (44.4 %), followed by the lameness (40.7 %) and a small percentage (3.7 %) of horses were eliminated from the race due to failure to compete, disqualified, withdrawal and retired, respectively (Figure 3). Elimination by metabolic disorder happened solely due to high heart rate at first and second presentation and when there were three and above of the metabolic parameters compromised. As the distance and speed increase, the horses were unable to compensate the metabolic demand of their body leading to deterioration of the parameters. Meanwhile, the lameness was contributed by continuous concussion, progressive muscle weakness and fatigue. Additionally, the high elimination in lameness might also associated to the racing frequency of the horses as the endurance events in Malaysia were organized every month and the majority of the horses involved was the same horse (Izzati *et al*., 2018a).

Table 1. Rate of completion and elimination of CEI* 100 km and CEN 100 km

		Status				Total	
		Completed		Eliminated			
		N	%	N	%	N	%
Race Categories	CEI	17	70.8	18	66.7	35	68.6
	CEN	7	29.2	9	33.3	16	31.4
Total		24	100.0	27	100.0	51	100.0

Figure 3. Distribution of cause of elimination in endurance horses



Moreover, rate of elimination was determined at each loop (Figure 4). There was higher elimination of the horses at the second loop (36 %), followed by the first loop (32 %) and third loop (28 %). At the fourth loop, there was only 1 horse (4 %) eliminated from the race. The cumulative effects of long distance races, specifically over rough and hard, concussive surfaces, aggravated by conformational weaknesses and poorly made shoes can escalate the incidence of bone, joint and tendon injuries and concussion that caused elimination due to lameness. Besides, excessive sweating and dehydration associated with elevated heart as the distance increased, might cause the metabolic imbalance that lead to elimination at each loop.

Horses' performance was also compared between age categories (Figure 5). The number of completed and eliminated horses was similar and highest at 6 to 10 years old. Likewise, the elimination is higher compared to completion in 11 to 15 years old and 16 to 20 years old category. This agrees with Adamu *et al.* (2013a; 2014) that indicated horses of age 6 to 10 years old performed well in the endurance competition of 120 km distance, while horses within the age range of 11 to 15 years old were mostly eliminated. The poor performance in young and older horses could be a result of genetic factors, environment, poor dietary management, or health issues that prevent the horses from performing. It might also be because horse owners and riders, are unable to choose the best training regimens for optimal performance (Adamu *et al.,* 2014). However, these findings are contradicted from Robert *et al.*, (2004) and Bollinger *et al.*, (2021) where they demonstrated that horses from the age of 10 and above were in their peak of performance.

Figure 4. Rate of elimination of endurance horses according to phases

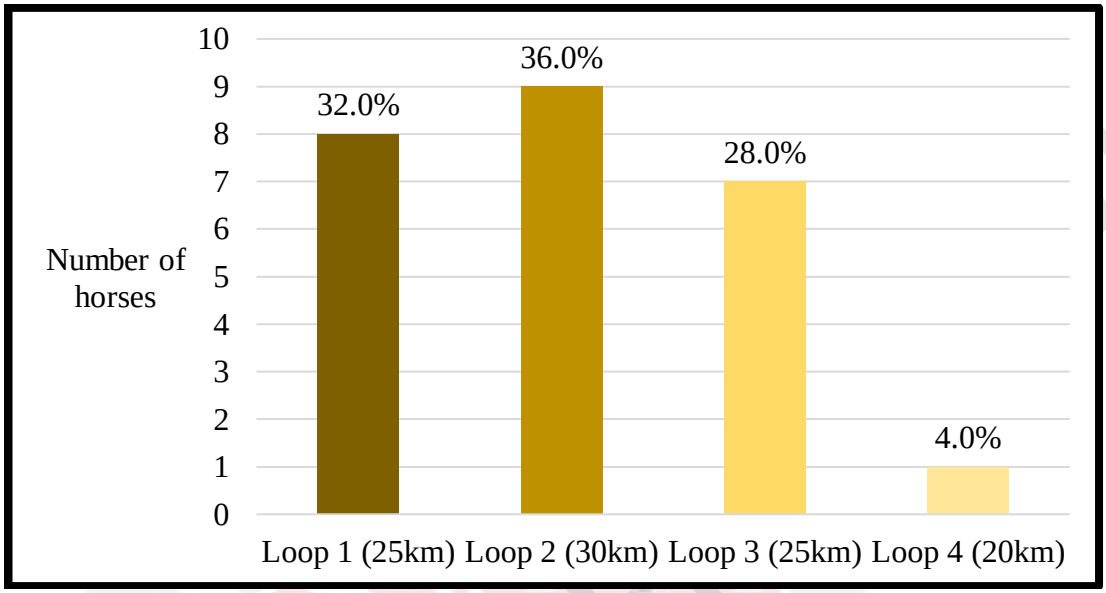
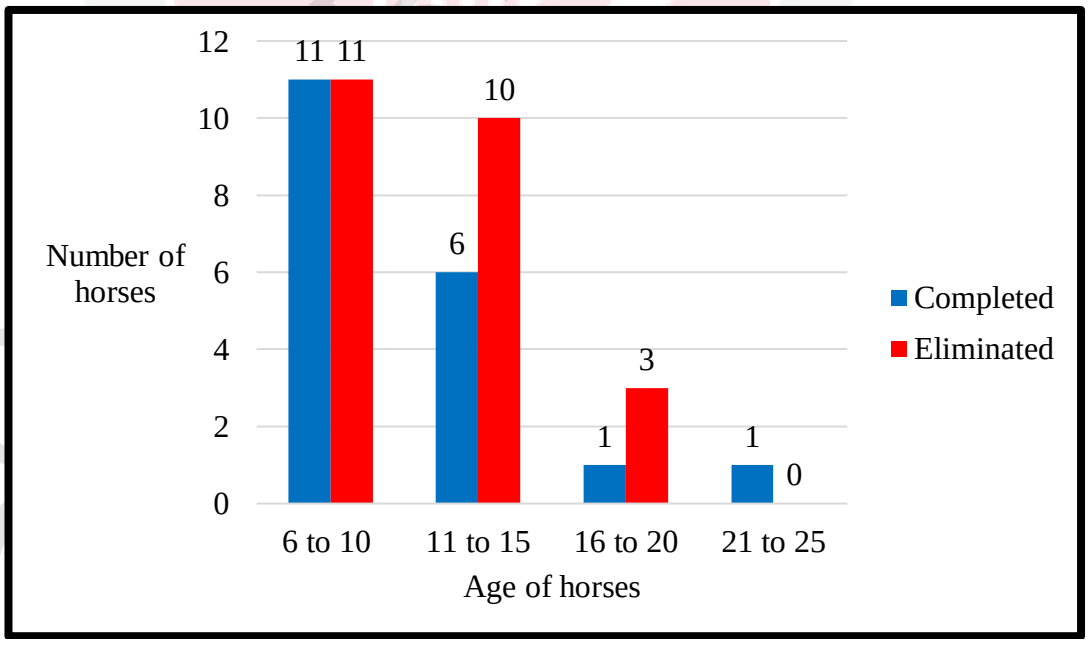
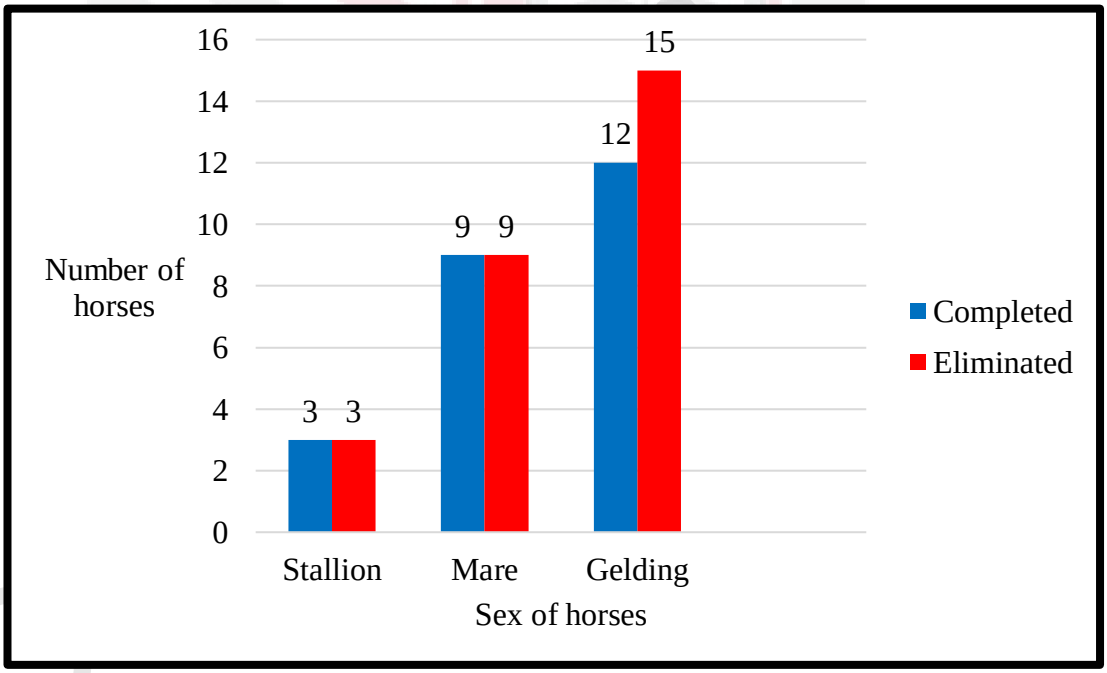


Figure 5. Age distribution of endurance horses based on performance status



Moreover, the completed and elimination rate according to sex, has shown that the stallion and mare have similar outcomes, which are 3 and 9 horses respectively (Figure 6). Conversely, in gelding category, the number of horses eliminated is higher than compare to the completed horses. Regardless the gender, the horses' performance depends largely on their ability to maintain high aerobic exercise capacity (Mukai *et al*., 2003 and Adamu *et al.*, 2013a).

Figure 6. Sex distribution of endurance horses based on performance status



4.2 Performance indicators of completed and eliminated horses

4.2.1 Heart rate

From the independent-samples t-test done, there is significant difference found between the completed and eliminated endurance horses. In Figure 7, the post-race heart rate of completed horses (58 ± 2 bpm) exhibits lower mean score compared to the eliminated horses (67 ± 11 bpm). The standard deviation of 2 of the completed horses also indicated that good performance horses had heart rate that did not vary too much between each other. Whereas, the eliminated horses had larger value of standard deviation which indicate some of the horses also had low heart rate as they were eliminated due to different reasons. Yet, this finding is in accordance to FEI Rule for Endurance Events that required horse to have heart rate not more than 64 bpm in order to complete the race.

During the race, there will be elevation of heart rate due to the demand by the active working muscle for oxygen and for thermoregulatory cooling. Poor performance horses were unable to adjust themselves, hence lead to the elevation of heart rate as the body attempt to compensate the needs. These were also putting them at a greater risk of developing hyperthermia and thumps brought on by changes in fluid and electrolyte status (Adamu *et al*., 2012a). In contrast, the completed horses are experienced and properly trained horses that have better adaptability toward this submaximal intensity activity due to the large heart size and high number of erythrocyte to carry oxygen (Janicki *et al*., 2013). These advantages help horses to compensate metabolic demand and lowering the heart rate. Indeed, low heart rate is reliable to determine a fit horse in response to strenuous endurance rides (Bashir and Rasedee 2009).

Figure 7. Mean score (mean ± SD) post-race heart rate of completed and eliminated endurance horses

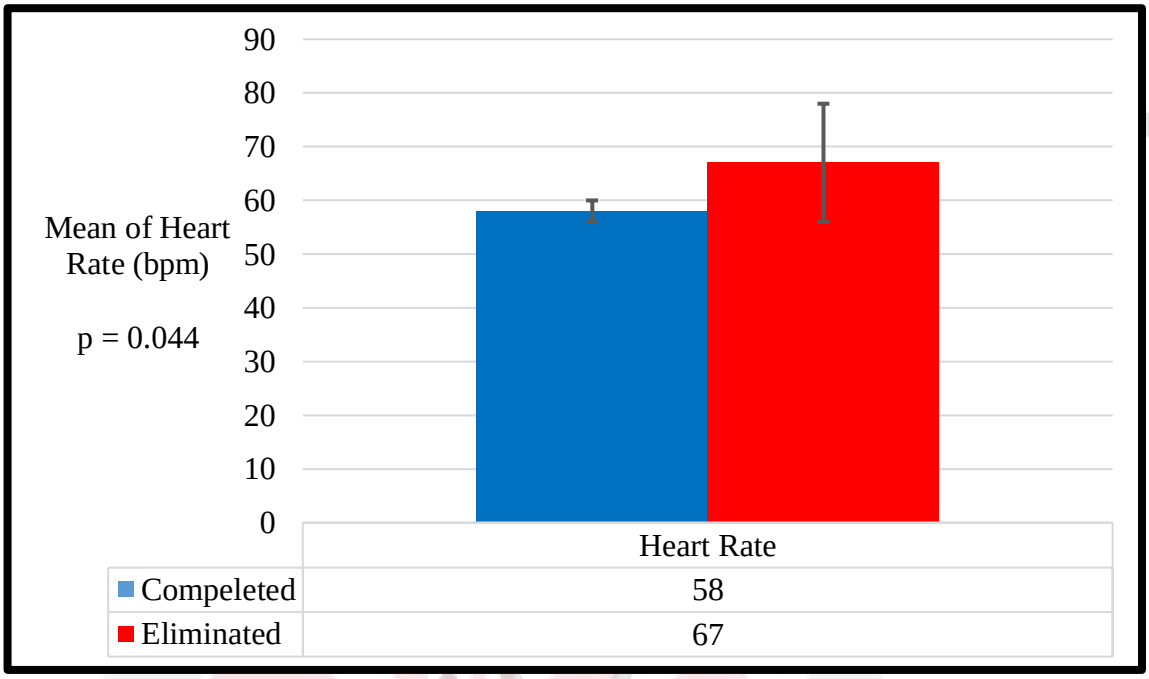
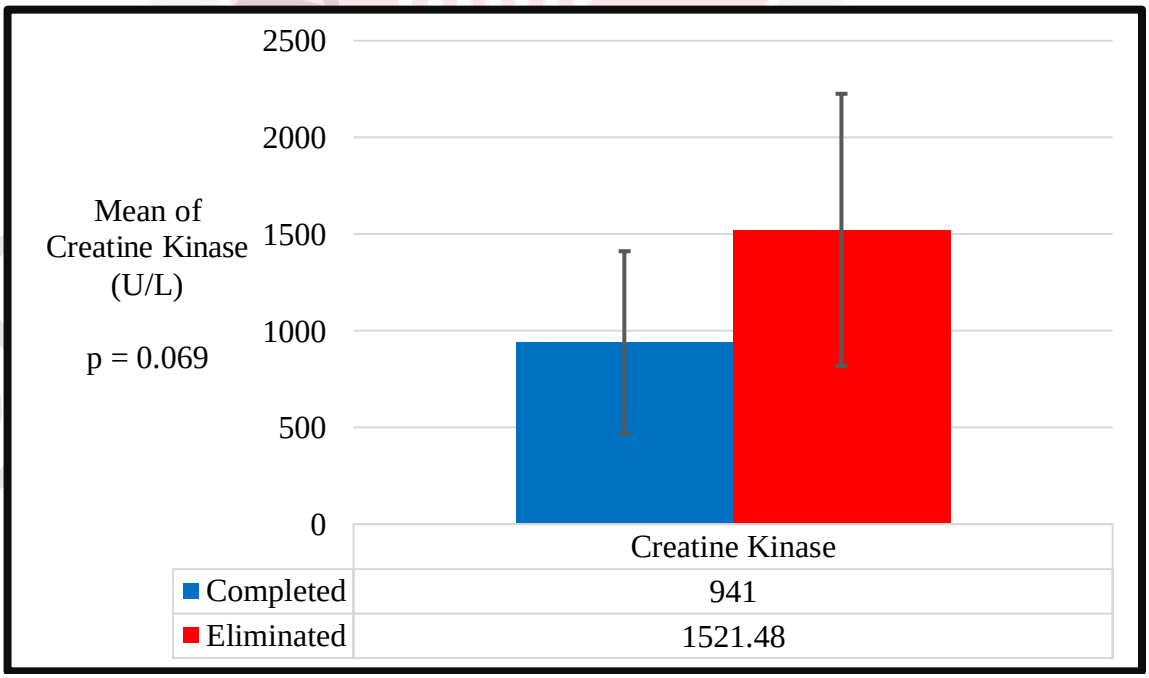


Figure 8. Mean score (mean ± SD) post-race creatine kinase of completed and eliminated endurance horses



4.2.2 Creatine kinase

The post-race CK tested statistically shows no significant differences between the completed and eliminated horses. In Figure 8, it demonstrate that the completed horses exhibit lower mean value of creatine kinase than the eliminated horses. The mean value of CK for completed horses was 941.00 ± 470.46 U/L, whereas it was 1521.48 ± 703.97 U/L for eliminated horses. The normal range for CK in horses is 300 – 500 U/L, which in this study, it indicates that both eliminated and completed horses had muscle damage and the elevation was beyond the range. These values in comparison to the range was double in the completed horses and it was triple in the eliminated horse.

As completed horses were rigorously trained, they have better ability to withstand prolonged stress during actual race and the CK activity slowly decrease with training because of adaptation of the muscle toward the exercise intensity. (Janicki *et al*., 2013; Noraniza *et al*., 2018). However, since the distance was too long, accompanied by the stress put onto the muscle due to surface of the track and terrain, some of this completed horses would gradually develop the muscle injury. Meanwhile, it is anticipated that the higher CK concentration in the eliminated horses might be consequence of poor training that might subjected the horses to exertional rhabdomyolysis and excessive damage (Izzati *et al*., 2018b). Additionally, the increase of CK in both groups of horse may also contributed by their body composition. Hence, the greater their muscle mass, the greater the CK value reflecting the muscle effort to finish the race.

4.2.3 Lactate

The normal value of the basal blood lactate concentration in horses is close to 1 mmol/L (Henderson, 2013). However, blood lactate concentration is expected to rise during exercise in horses. In this present study, post-race lactate concentration analysed was proven to have high significant difference at $p<0.05$ between the completed and eliminated endurance horses. From Figure 9, the mean value of completed horses (1.61 ± 0.36 mmol/L) was lower compared to the eliminated horses (3.44 ± 1.39 mmol/L). As proposed by literatures, the blood lactate of 4 mmol/L and above is used to indicate the unfit horses and start of anaerobic state (Lindner, 2010). Thus, the lactate concentration in the eliminated endurance horses in this study was within the aerobic to anaerobic threshold transitional range, which may signify the unfit threshold level in the horses during the competition (Fielding *et al*., 2009 and Adamu *et al.*, 2012b). These increased in lactate production is necessary to deliver energy at a faster rate and under condition where the oxygen delivery to the working muscle is limited. However, the accumulation of lactate will lead to fatigue and muscle weakness in horses and eventually cause the elimination.

Meanwhile, the mean value of lactate concentration for completed endurance horses could indicate the concentration required for aerobic performance in fit horses. These fit horses are normally develop from a proper training program that able to reduce the lactate concentration gradually and maintain a steady low state during the actual endurance race. Besides, increased fitness accompanied by good feeding regime also able to delay the glycogen depletion and decrease the rate of utilization, hence it will prevent excessive lactate formation in horses during the race (Bullimore *et al.*, 2000).

Figure 9. Mean score (mean ± SD) post-race lactate of completed and eliminated endurance horses

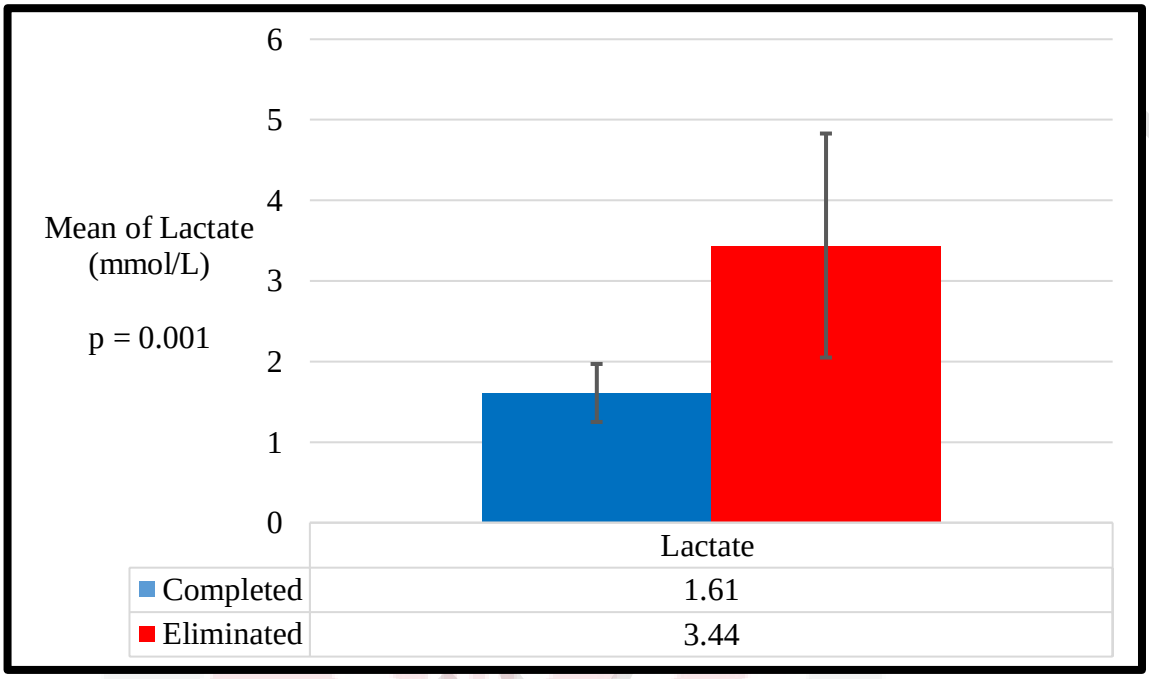
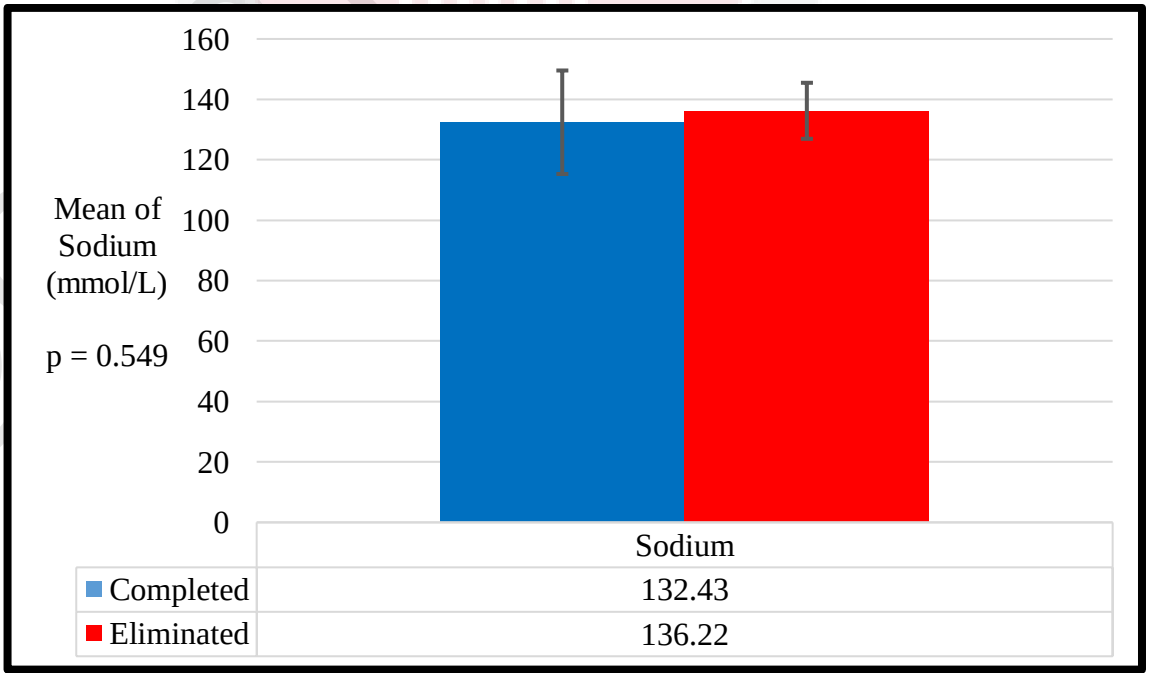


Figure 10. Mean score (mean ± SD) post-race sodium of completed and eliminated endurance horses



4.2.4 Electrolytes

Electrolyte parameters which are potassium, sodium and chloride were all failed to be demonstrated to have any significant differences between the completed and eliminated horses (Figure 10 – 12). The sodium level of completed horses (132.43 ± 17.13 mmol/L) and eliminated horses (136.22 ± 9.27 mmol/L), and chloride level of completed horses (98.03 ± 14.76 mmol/L) and eliminated horses (97.08 ± 7.50 mmol/L) were all in the normal range which are 132 – 144 mmol/L for sodium and 90 – 100 mmol/L for chloride, respectively. Unlike these two parameters, potassium level of both completed horses (2.27 ± 0.44 mmol/L) and eliminated horses (2.66 ± 0.41 mmol/L) were below the normal range, 3.0 – 4.5 mmol/L. However, the outcomes for these three electrolyte parameters are not significant. This is because horses' sweat is hypertonic, which means they are not only excreting the water during active activity, but also excreting the protein, sodium, chloride, potassium, calcium and magnesium. Therefore it is expected that horses will loss a large amount of electrolytes during a long distance race and their levels will be below the normal range, which were contrasted from the findings of this study.

This might has happened due to the electrolytes losses were masked by the dehydration. As the horse attempted to dissipate the heat from the body during the race, water and electrolytes were also be excreted, and the loss of electrolyte will suppress the thirst response and causing the horse to be dehydrated as they were reluctant to drink.

Figure 11. Mean score (mean ± SD) post-race chloride of completed and eliminated endurance horses

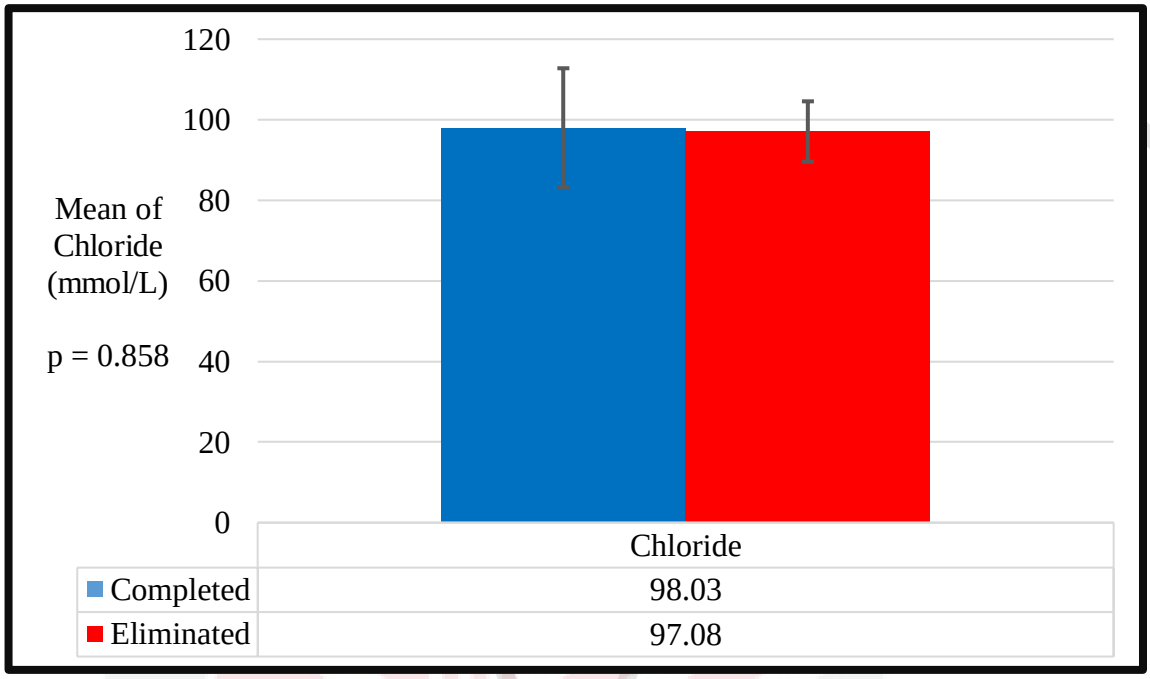
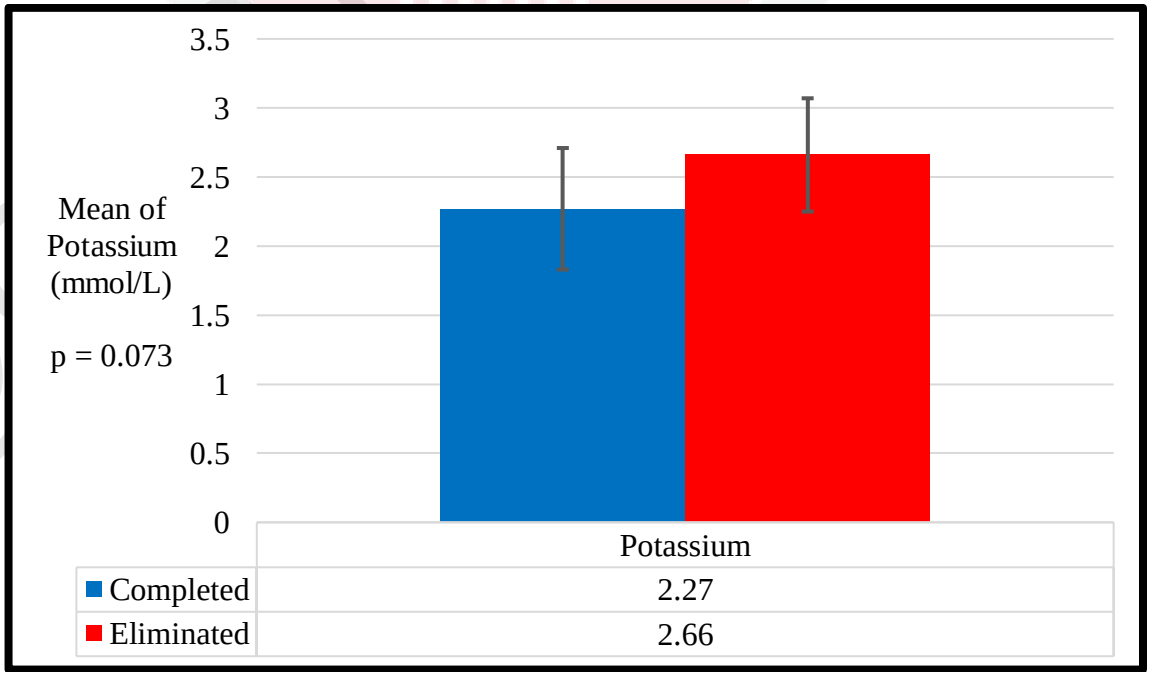


Figure 12. Mean score (mean ± SD) post-race potassium of completed and eliminated endurance horses



Therefore, these losses will lead to similar ratio of body fluid and electrolytes in blood. Apart from this, horses might also be supplemented with electrolytes paste orally prior to the race and during recovery period. Hence, this supplementation will increase the electrolytes levels in the body. These scenario may have account to the insignificant value of the electrolyte parameters.

CHAPTER 5
CONCLUSION, LIMITATION AND RECOMMENDATION

5.1 Conclusion

In conclusion, this present study have proven that there is significant difference between the completed and eliminated endurance horses on post- race heart rate. It also proved that there is significant difference between completed and eliminated endurance horses on post-race lactate, but not on creatine kinase and electrolytes. Therefore, these parameters will be useful for veterinarians or horse trainers to construct a training protocol or conditioning program while enabling the assessment of the horses' fitness during training and improve their performance during an actual endurance race.

5.2 Limitation

The main challenge of this study is the handling of the blood sample. This is because there was long interval period between blood sampling to analysis in the laboratory. As the samples were required to be transported more than 400 kilometres from TIEP, Terengganu to laboratory in Selangor, the long storing duration accompanied with chances of error in storing temperature might alter the result of blood parameters in this study.

5.3 Recommendation

This study suggest to use the heart rate in combination with lactate as assessment of fitness level in endurance horses in training and actual races. The training program should be established to gradually reduce the level of these two parameters by the end of the training and enhance the horse performance during the actual endurance

competition. If the horses able to maintain lower heart rate in aerobic state, it means that horses are fit.

Besides, given this study only analysed the post-race parameters, it is also recommended to measure the pre-race parameters in the future study. As the horses may have higher or lower value of physical or blood parameter initially before the race, comparing pre- and post-race levels may demonstrate the significant changes of the parameters.

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APPENDIX

Horse	Category	Status	Final loop	Heart Rate (bpm)	Creatine Kinase (U/L)	Lactate (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Potassium (mmol/L)
C01	CEI*	Completed	4	56	1771.61	2.0	133.9	102.3	2.5
C02	CEI*	Completed	4	60	1149.81	1.7	120.9	91.4	2.2
C03	CEI*	Completed	4	60	480.01	1.6	122.4	90.1	1.9
C04	CEI*	Completed	4	-	895.94	2.0	128.8	88.2	1.9
C05	CEN	Completed	4	-	1004.96	1.0	118.7	87.1	1.9
C06	CEI*	Completed	4	56	956.53	1.3	133.5	98.0	2.4
C07	CEN	Completed	4	60	328.12	1.7	168.8	129.1	3.1
E01	CEI*	Eliminated	1	48	979.83	1.6	122.3	90.6	3.0
E02	CEI*	Eliminated	1	60	2147.45	3.9	151.1	111.1	2.9
E03	CEN	Eliminated	2	68	881.48	1.5	148.9	108.4	2.6
E04	CEN	Eliminated	2	64	778.09	3.5	142.2	102.3	2.5
E05	CEI*	Eliminated	2	52	2184.51	2.1	135.5	98.3	3.0
E06	CEI*	Eliminated	3	80	2220.79	5.6	140.6	93.3	2.8
E07	CEN	Eliminated	2	72	2450.08	4.1	132	93.2	3.0
E08	CEI*	Eliminated	3	72	1156.43	5.5	140.5	99.0	2.5
E09	CEI*	Eliminated	3	80	894.62	3.0	131.1	92.6	1.6
E10	CEN	Eliminated	3	56	-	2.9	127	89.5	2.5
E11	CEI*	Eliminated	3	80	-	4.1	127.2	89.6	2.9

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.[a,b]	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Heart Rate	Equal variances assumed	9.110	.009	-1.544	14	.145	-8.145	5.277	-19.463	3.172
	Equal variances not assumed			-2.262	11.480	.044[c]	-8.145	3.601	-16.031	-.260
Creatine Kinase	Equal variances assumed	7.477	.016	-1.873	14	.082	-580.47841	309.85678	-1245.05511	84.09829
	Equal variances not assumed			-1.972	13.771	.069	-580.47841	294.42028	-1212.93330	51.97648
Lactate	Equal variances assumed	7.654	.014	-3.353	16	.004	-1.8221	.5434	-2.9741	-.6700
	Equal variances not assumed			-4.122	12.013	.001[c]	-1.8221	.4420	-2.7850	-.8591
Sodium	Equal variances assumed	.733	.405	-.612	16	.549	-3.7896	6.1887	-16.9091	9.3299
	Equal variances not assumed			-.537	8.274	.605	-3.7896	7.0538	-19.9625	12.3832
Chloride	Equal variances assumed	1.456	.245	.181	16	.858	.9468	5.2248	-10.1294	12.0229
	Equal variances not assumed			.157	8.003	.879	.9468	6.0180	-12.9298	14.8233
Potassium	Equal variances assumed	.123	.730	-1.919	16	.073	-.3922	.2044	-.8255	.0411
	Equal variances not assumed			-1.885	12.183	.083	-.3922	.2080	-.8447	.0603

a. Levene's Test is significant ($p<0.05$), suggesting a violation of the assumption of equal variances

b. Levene's Test is significant ($p>0.05$), suggesting the assumption of equal variances is satisfied

c. Independent-Samples T test, significant at ($p<0.05$)