UNIVERSITI PUTRA MALAYSIA

THE INFLUENCE OF THOROUGHBRED RACING ON SPECIFIC SERUM BIOCHEMISTRY PARAMETERS IN RACING HORSES IN SELANGOR

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THE INFLUENCE OF THOROUGHBRED RACING ON SPECIFIC SERUM BIOCHEMISTRY PARAMETERS IN RACING HORSES IN SELANGOR

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A project paper submitted to the

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "The Influence of Thoroughbred Racing on Specific Serum Biochemistry Parameters in Racing Horses in Selangor", by Mohamad Hafizi bin Saidon 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 – Final Year Project.

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DEDICATION

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to our heart.

My humble effort I dedicate to my loving

FATHER & MOTHER,

Whose affection, love, encouragement and prays of day and night make me able to finish my task,

Along with all hard working and respected

SUPERVISORS

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Words could not express my gratitude but I hope Allah will repay all the efforts to everyone who helped me.

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PENGARUH PERLUMBAAN THOROUGHBRED KEPADA PARAMETER SERUM BIOKIMIA YANG KHUSUS DALAM KUDA LUMBA DI SELANGOR

oleh

Mohamad Hafizi bin Saidon

Penyelia: Dr Nurul Hayah binti Khairuddin

Penyelia bersama: Dr. Sumita Sugnaseelan, Dr. Shri Kanth Kanaesalingam, Dr. Noraniza Mohd. Adzahan

Jangka pendek perlumbaan kuda berintensiti tinggi baka Thoroughbred menyebabkan ketinggian parameter serum biokimia seperti laktid, glukosa, creatine kinase (CK), transaminase aspartik (AST) dan alanine transaminase (ALT) dalam serum. Laktid dan glukosa adalah bahan metabolik bagi otot manakala CK, AST dan ALT adalah parameter bagi enzim otot. Kajian ini dijalankan untuk mengukur kecergasan terhadap parameter dan membandingkan kenaikan setiap kategori. Sampel darah kuda diambil daripada kuda yang memenangi tempat pertama dan ketiga melalui kaedah venipuncture leher sejurus selepas perlumbaan. 36 kuda telah disampel daripada 18 perlumbaan trek dalam lingkungan jarak 1100m, 1200m, 1300m, 1400m dan 1600m.

Berdasarkan kajian, nilai purata bagi laktid mempunyai peningkatan sebanyak 30 kali ganda. Kenaikan purata nilai untuk AST, ALT, dan tahap glukosa ialah dua kali ganda. Walaubagaimanapun, nilai purata bagi CK berada dalam julat normal. Peningkatan besar laktid boleh diterangkan dengan penglibatan otot dalam glikolisis anaerobik bagi mengimbangi permintaan tenaga yang tinggi semasa perlumbaan. CK kekal dalam julat normal dengan AST dan ALT meningkat sedikit menunjukkan enzim yang diperolehi adalah berasalkan daripada hati di mana ia adalah normal bagi kuda selepas melakukan aktiviti. Hiperglisemia adalah disebabkan oleh tindakan antagonistik insulin kepada catecholamines, glukocorticoids, hormon pertumbuhan dan glukagon. Analisis statistikal mencadangkan bahawa perubahan antara kuda menduduki tempat pertama dan ketiga itu tidak membawa perbezaan yang jelas antara kumpulan untuk semua parameter juga terhadap perbezaan jarak, jantina, dan tahap umur . Paras laktid menunjukkan kapasiti oksidatif otot kuda di mana ketinggian paras laktid boleh menunjukkan penglibatan awal glikolisis anaerobik.

Cadangan bagi kajian yang sama untuk masa hadapan ialah dengan mendapatkan pembolehubah yang lebih membezakan antara kuda. Contohnya seperti sampel antara kuda di tempat pertama dan di tempat ke-sepuluh. Tahap biokimia laktid mencerminkan glikolisis anaerobik perlumbaan kuda baka Thoroughbred mencadangkan tindak balas langsung kepada keletihan otot.

Kata kunci: serum biokimia, perlumbaan Thoroghbred, laktid, prestasi, glikolisis anaerobic

ABSTRACT

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

THE INFLUENCE OF THOROUGHBRED RACING ON SPECIFIC SERUM BIOCHEMISTRY PARAMETERS IN RACING HORSES IN SELANGOR

by

Mohamad Hafizi bin Saidon

2017

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Co-supervisor: Dr. Sumita Sugnaseelan, Dr. Shri Kanth Kanaesalingam, Dr. Noraniza Mohd Adzahan

Short duration high intensity thoroughbred racing causes the elevation of serum biochemistry parameters such as lactate, glucose, creatine kinase (CK), aspartate transaminase (AST) and alanine transaminase (ALT) in the serum. The lactate and glucose are metabolic fuel of the muscles. CK, AST and ALT parameters are muscle-derived enzymes. This study was conducted to measure the influence of exercise to these parameters and to compare the increments of each categories. Blood was sampled from horses that won first and third placing. Method of blood collection was via jugular venipuncture shortly after the race. 36 horses were

sampled from 18 thoroughbred track races, which distances range from 1100m, 1200m, 1300m, 1400m, and 1600m.

Based on the study here, it was observed that the mean value for lactate had a substantial increase by approximately 30 folds. The mean value for AST, ALT, and glucose levels elevated two-folds. However, the mean value for CK is within normal range. The substantial increase of lactate could be explained by muscles engaging in anaerobic glycolysis to compensate the high energy demand for high intensity racing in a short duration. CK remained within normal range with AST and ALT showing slight elevations indicating the enzymes were liver-derived which is normal for clinically fit horses post exercise. Physiologic hyperglycemia is caused by insulin-antagonistic actions of catecholamines, glucocorticoids, growth hormone, and glucagon. Statistical analysis suggested that changes between the first placing horse (Winning horse) and the third placing horse (Show horse) did not pose a significant difference between groups for all parameters so as the differences in distance, gender, and age. Lactate levels indicate the oxidative capacity of muscles in horses, so a higher level of lactate could indicate earlier engagement of anaerobic glycolysis.

Future recommendations for similar study, suggest obtaining variables that are more differentiated such as between the winning horse and horses placed tenth instead. Determination of biochemistry level of lactate would reflect the anaerobic glycolysis of racing thoroughbred horses hence suggest indirect response to muscle fatigue.

Keyword: Serum biochemistry, thoroughbred racing, lactate, performance, anaerobic glycolysis

1.0 INTRODUCTION

Thoroughbred racehorses run at high speeds of around 18 m/s or 64 km/h over distances of 800 to 5000 metres. A large number of physiological and anatomical features act in concert to endow the horse with extraordinary athletic capacity. Maximal athletic performance is dependent upon integrated functioning of these physiological and anatomical features (Hinchcliff and Geor, 2004; Evans, 2007). However, there are limits to maximal performance of horses, and there is evidence that these limits have been reached or will soon be so, particularly for thoroughbred racehorses (Denny, 2008; Pieramati et al., 2011). Fatigue is a complex chain of events, with central as well as peripheral contributions. Short-duration, high-intensity exercise such as is performed in thoroughbred racing is not limited by availability of substrates but, more likely, by failure of energy production associated with an increase in protons and a decrease in adenosine triphosphate (ATP). Current studies have focused on which parameters could be used to determine the future outcome of performing horses to which this information can be used to further optimise the sports industry of equine racing.

Lactate and glucose are metabolic fuels of the horse muscles which are used during any type of exercise. During short duration high intensity exercises, anaerobic glycolysis seems to be the most dominant pathway for energy production and glucose regeneration (Hodgson, 1985). Lactate is the product of anaerobic glycolysis and this is used for the further production of glucose which is required by the muscles. The self-limiting nature of anaerobic power output means the horse can only maintain maximal speed for about 600 to 800 m. After this distance, energy supply falls back to slower aerobic pathways, necessitating a reduction in speed of exercise (Hodgson et al., 1985; McMiken, 1983).

Creatine kinase is a muscle specific enzyme which increases usually due to skeletal muscle injury. The increase in this parameter after a track race would indicate that the horse is not fit for racing at given

intensity and would lead to injury. This enzyme however has a short half-life and thus must be interpreted with another muscle enzyme with longer half-life which for example is aspartate transaminase. Aspartate transaminase has a lower specificity to muscle tissue thus when interpreted in conjunction with creatine kinase could provide great information on the function and health of skeletal muscles. Alanine transaminase is categorised under a muscle-derived enzyme as well as a liver-derived enzyme. However in equine studies, the liver has only little influence on alanine transaminase levels when compared to muscles.

This study was conducted to observe the changes of all of the stated parameters and to compare the differences in elevations between the first and third placings. This information could then be used to evaluate these parameters more specifically and associate this information to determine the performance of the horse and potential victory. Differences in other factors such as gender, distance, and age are also compared to see if these independent variables could affect the horse's performance.

2.0 LITERATURE REVIEW

2.1 Energy metabolism

The most important pathways for vertebrate locomotion are those concerned with energy production and muscular movement requires transformation of chemical energy stored in metabolic substrates to kinetic energy of muscular contraction (Gerard *et al*., 2014). All pathways integral to energy supply are concerned with the ultimate production of adenosine triphosphate (ATP) (Gerard *et al*., 2014). Under normal conditions, there is a finite source of ATP within muscle, sufficient to maintain muscular activity for only a short duration (Astrand and Rodahl, 1986; Lindholm, 1979). Therefore, it is necessary to resynthesise ATP for continuous muscular exertion and this is done by pathways of aerobic and anaerobic phosphorylation.

Fatigue is a complex and intricate physiologic response to exercise, leading to the inability to sustain further activity at the current intensity (Gerard *et al*., 2014). Acute fatigue is directly related to energy production in muscles and occurs in events requiring maximal work effort in short durations and is termed anaerobic fatigue (McMiken, 1983). Fatigue in response to high-intensity exercise is likely caused by a combination of factors, including depletion of ATP, decreased intracellular pH, and accumulation of lactate in muscles (Essen-Gustavsson, 1999; Hodgson *et al*., 1985; McMiken, 1983).

2.2 Lactate

Measurement of blood lactate can provide information relating to both the horse's aerobic and anaerobic capacity as lactate is produced as a result of anaerobic muscle metabolism (Franklin *et al*., 2014). The onset of blood lactate accumulation, or also known as lactate threshold, is the point where there is a

rapid increase in lactate and several studies have confirmed that high lactate threshold is associated with superior performance (LeuLeu *et al*., 2005; Lindner, 2010; Courouce *et al*., 1997).

Measurement of post-exercise lactate concentration indicates anaerobic capacity (McGowan *et al*., 2008) and in human athletes higher lactates are an indicator of superior sprinting ability (Green and Dawson, 1993; Weinstein *et al*., 1998). Some equine studies have also found that blood lactate rises more rapidly and reaches higher concentrations in faster horses (Harkins *et al*., 1993; Saibene *et al*., 1985; Bayly *et al*., 1987) and higher post-exercise blood lactate has been correlated with superior performance in Standardbred trotters (Rasanen *et al*., 1995).

It has been suggested that transport of lactate from plasma into red blood cells (RBCs) result in reduced plasma lactate levels, thereby facilitating outflow from the muscles (Posso *et al*., 1995). Individual variability in monocarboxylate (eg: Lactate) transporters not only occurs in RBC membranes but also in muscle cells, greatly influencing lactate accumulation (Koho *et al*., 2006) which explains why some horses experience fatigue sooner than others.

2.3 Glucose

Glucose is produced in the liver, to a lesser extent in the kidneys, or is absorbed from dietary sources and is a principal component in energy metabolism (Walton, 2014). Production of glucose is accomplished primarily in the liver through gluconeogenesis (generation of glucose from non-carbohydrate carbon substrates such as lactate) and glycogenolysis (biochemical breakdown of glycogen to glucose). Transient hyperglycemia by physiologic response can be due to insulin-antagonistic actions of catecholamines, glucocorticoids, growth hormones, and glucagon (Walton, 2014).

Both aerobic and anaerobic pathways are generally active during exercise, the relative contribution within each muscle depends on the nature, intensity level and duration of the activity, the muscle's fiber-type composition, the availability of oxygen and substrates, and the relative concentrations of intermediary metabolites that may potentially activate or inhibit selected enzymes (Valberg, 1996). Hence, at the

beginning of exercise when oxygen is abundant, energy production depends largely on metabolism of glycogen via aerobic pathways (Valberg, 1996) but within a few minutes, glucose and free fatty acid (FFA) concentrations rise in the blood and following 20–30% glycogen depletion, there is a shift towards β-oxidation of free fatty acids (Davie *et al*., 1999). With higher energy demands, the muscle ATP/ADP ratio decreases, providing a stimulus for energy production via anaerobic mechanisms (Rivero and Piercy,2014).

2.4 Muscle derived enzymes

2.4.1 Creatine kinase and Aspartate transaminase

Health and function of the muscular system of athletic horses is routinely assessed via measurement of serum activity of creatine kinase (CK) and aspartate transaminase (AST) (McKenzie, 2014). Serum CK activity provide specific indication of skeletal muscle injury in the horse although having a brief half-life. AST can remain persistently for days but must be interpreted in conjunction with CK and other liver enzymes such as alanine transaminase (ALT) to account for its lesser tissue specificity (Stockham and Scott, 2008). Snow and Harris (1988) have concluded that horses with elevated muscle enzymes are unlikely to perform optimally, thus screening for elevations following exercise will be valuable in the detection of poor performance. Serum muscle enzyme activities have been shown to increase following races (Poso *et al*., 1983; Snow *et al*., 1983b). Despite increases, values remained within normal limits (Snow and Harris, 1988).

2.4.2 Alanine transaminase

Alanine transaminase (ALT) is found in the liver, muscle (cardiac and skeletal), kidneys, and erythrocytes. Many consider ALT to be a muscle specific enzyme as the liver has minimal ALT activity and contributes little to serum ALT (Cornelius *et al*., 1959). Other studies have investigated that a marked increase in ALT was considered to reflect an improved ability to metabolise pyruvate to alanine, reducing formation of lactate (Guy and Snow, 1977b).

3.0 MATERIALS AND METHODS

3.1 Animals

Blood were collected from 36 clinically healthy Thoroughbred horses that won either first or third placing from a participation of a two-day race in Selangor Turf Club. Each day had a number of 9 races.

32 geldings, 4 mares, aged between 4 to 10 years old has competed in thoroughbred track racing which 10 horses participated in 1100m, 4 horses participated in 1200m, 8 horses participated in 1300m, 8 horses participated in 1400m, and 6 horses participated in 1600m. Blood of horses were immediately sampled by jugular venipuncture after the race approximately 10 to 15 minutes after the completion of the track race. Track races started at 1:00 pm for both days (29th January 2017 and 30th January 2017) and ended around 6:00 pm. Weather was partly cloudy and sunny on both days.

3.2 Blood sampling

Blood samples were taken from the jugular vein, when horses were in the post-race area. Ambient temperature and resting environment were noted during the time of sampling. Jugular vein were occluded and swabbed with alcohol to sterilise the venipuncture region. Samples were collected in a single 4ml plain tube.

Sampled blood was allowed to clot for 20 minutes and was centrifuged at 3400 × g for 10 minutes. Serum was separated and transferred into 1ml Eppendorf™ tubes. The serum was then stored at -20℃ before serum is analysed.

3.3 Serum analysis

The serum samples were sent to the clinical pathology laboratory in Faculty of Veterinary Medicine, Universiti Putra Malaysia for serum analysis. The analysis of lactate, glucose, creatine kinase, aspartate transaminase, and alanine transaminase were done using Siemens Dimension Xpand Plus® integrated serum chemistry analyser.

3.4 Statistical analysis

Mean concentrations of lactate, glucose, creatine kinase (CK), aspartate transaminase (AST), and alanine transaminase (ALT) against race placings, age, gender and distance ran were tabulated into Microsoft® Excel 2010. Statistical analysis, means and standard errors of mean were computed using SPSS® 23 for Windows® Microsoft. The results are expressed as the mean standard error of the mean (SEM). The comparison of means in lactate, glucose, CK, AST, and ALT concentrations were evaluated between race placings, gender, age, and distance ran.

4.0 RESULT

Of the 36 horses sampled in this study, half (50%) of the horses were placed first and the other half (50%) were placed third. Out of all the horses involved, there were 10 (27.78%) horses that competed in 1100m, 4 (11.11%) horses competed in 1200m, 8 (22.22%) horses competed in 1300m, 8 (22.22%) horses competed in 1400m, and 6 (16.67%) horses competed in 1600m.

There were 8 (22.22%) horses that were 4 years old, 9 (25%) horses were 5 years old, 6 (16.67%) horses were 6 years old, 7 (19.44%) horses were 7 years old, 3 (8.33%) horses were 8 years old, 1 (2.78%) horses was 9 years old, and 2 (5.56%) horses were 10 years old. 32 (88.89%) geldings were involved in this study as well as 4 (11.11%) mares.

The winning times of each horses placed first were recorded and the average overall speed was calculated among these 18 horses placed first.

4.1 Lactate and glucose concentration against placings

Horses that were placed first had a mean lactate concentration of 33.82 ± 0.82 mmol/L and a mean glucose concentration of 11.35 ± 0.85 mmol/L while the horses that were placed third had a mean lactate concentration of 34.49 ± 1.26 mmol/L and a mean glucose concentration of 10.76 ± 0.46 mmol/L. There is no significant association between the mean lactate concentrations of horses placed first and horses placed third (see Figure 4.1). There is also no significant association between the mean glucose concentrations of horses placed first and horses placed third.

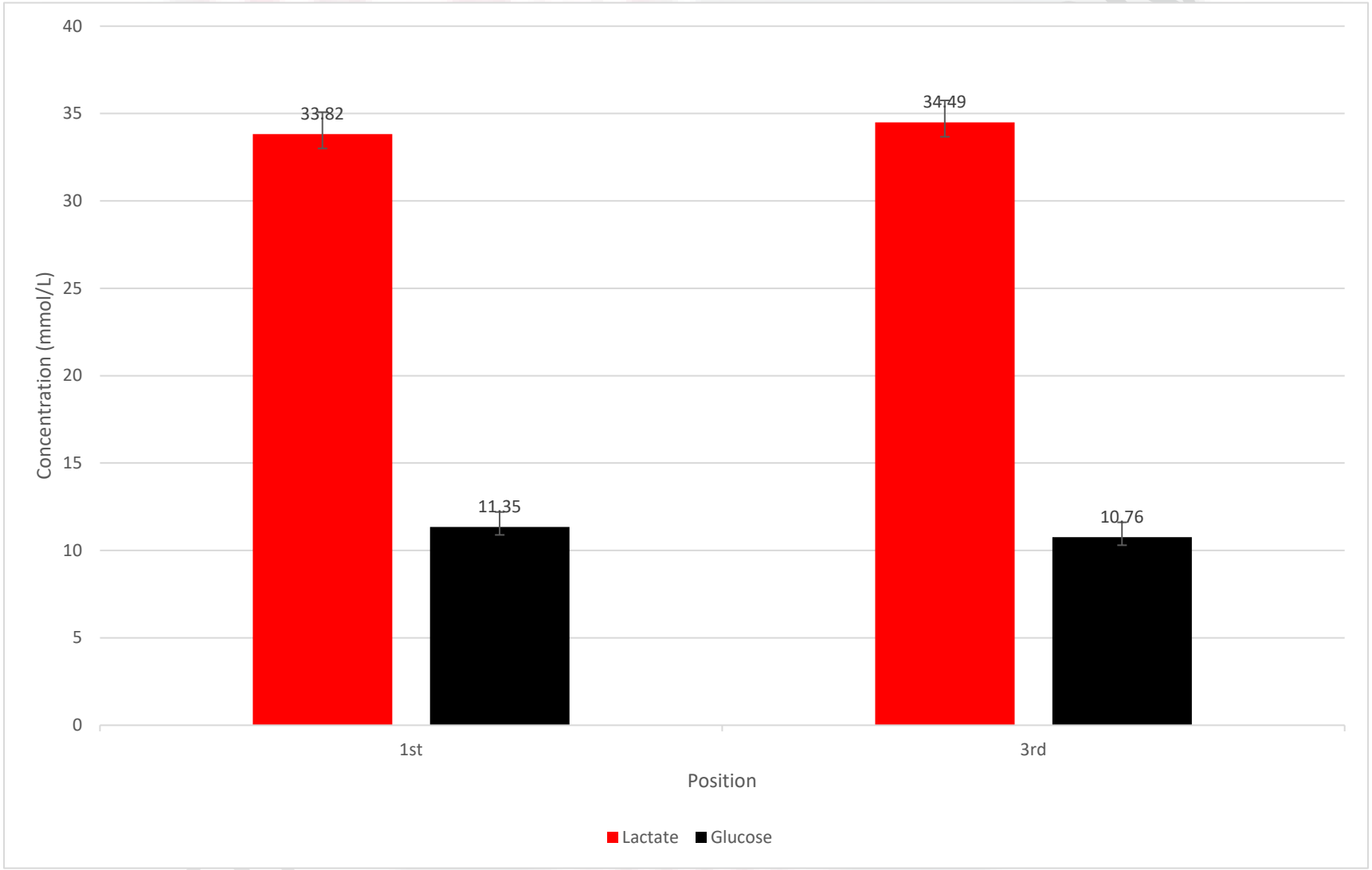

Figure 4.1 Comparative mean lactate and mean glucose concentrations (mmol/L) of horses placed first and horses placed third

4.2 Creatine kinase, aspartate transaminase, and alanine transaminase concentration against placings

Horses that were placed first had a mean creatine kinase concentration of 357.35 ± 17.77 U/L, mean aspartate transaminase concentration of 405.83 ± 20.22 U/L, and a mean alanine transaminase concentration of 18.33 ± 0.87 U/L. Horses that were placed third had a mean creatine kinase concentration of 276.50 ± 12.72 U/L, mean aspartate transaminase concentration of 357.28 ± 20.41, and a mean alanine transaminase concentration of 16.00 ± 1.86 U/L (see Figure 4.2). There is no significant association of the mean concentrations of creatine kinase, aspartate transaminase, and alanine transaminase between horses placed first and horses placed third.

4.3 Lactate and glucose concentrations against gender

When mean lactate and mean glucose concentrations were placed against gender, mean lactate concentrations were 34.18 ± 0.83 mmol/L for geldings and 33.93 ± 1.24 mmol/L for mares. Mean glucose concentrations were 10.96 ± 0.53 mmol/L for geldings and 11.85 ± 0.89 mmol/L for mares (see Figure 4.3). There is no significant association between the means of lactate and glucose concentrations between geldings and mares.

4.4 Creatine kinase, aspartate transaminase, and alanine transaminase concentration against gender

Mean concentrations of creatine kinase against gender are 307.94 ± 12.86 U/L for geldings and 376.50 ± 42.88 U/L for mares. Mean concentrations of aspartate transaminase for geldings are 377.16 ± 15.20 U/L and 416.75 ± 57.04 U/L for mares. Mean concentrations of alanine transaminase are 17.03 ± 1.14 U/L for geldings and 18.25 ± 1.89 U/L for mares (see Figure 4.4). There is no significant association between all three parameters against both genders.

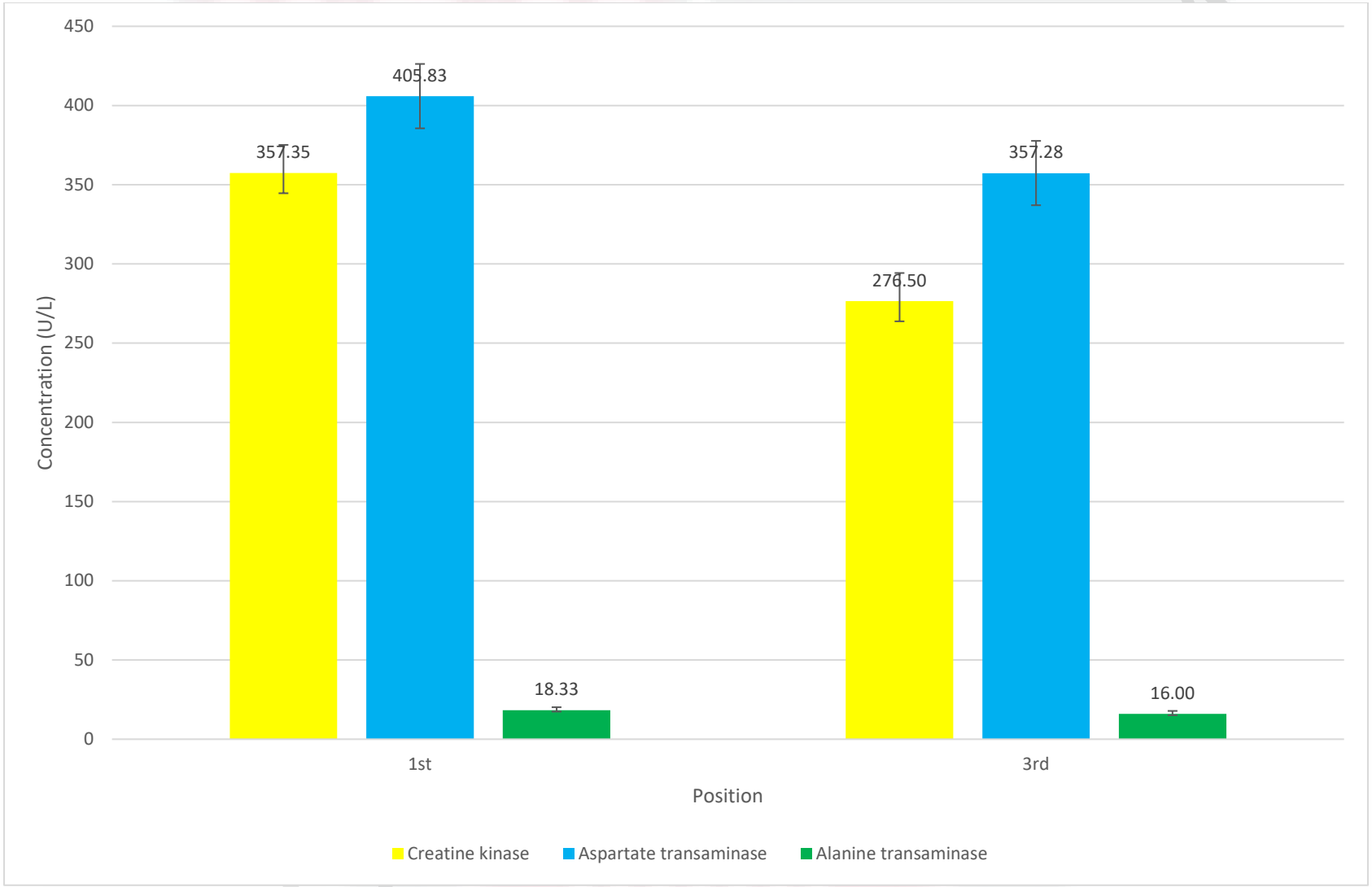

Figure 4.2 Comparative mean creatine kinase, aspartate transaminase, and alanine transaminase concentrations (U/L) of horses placed first and third

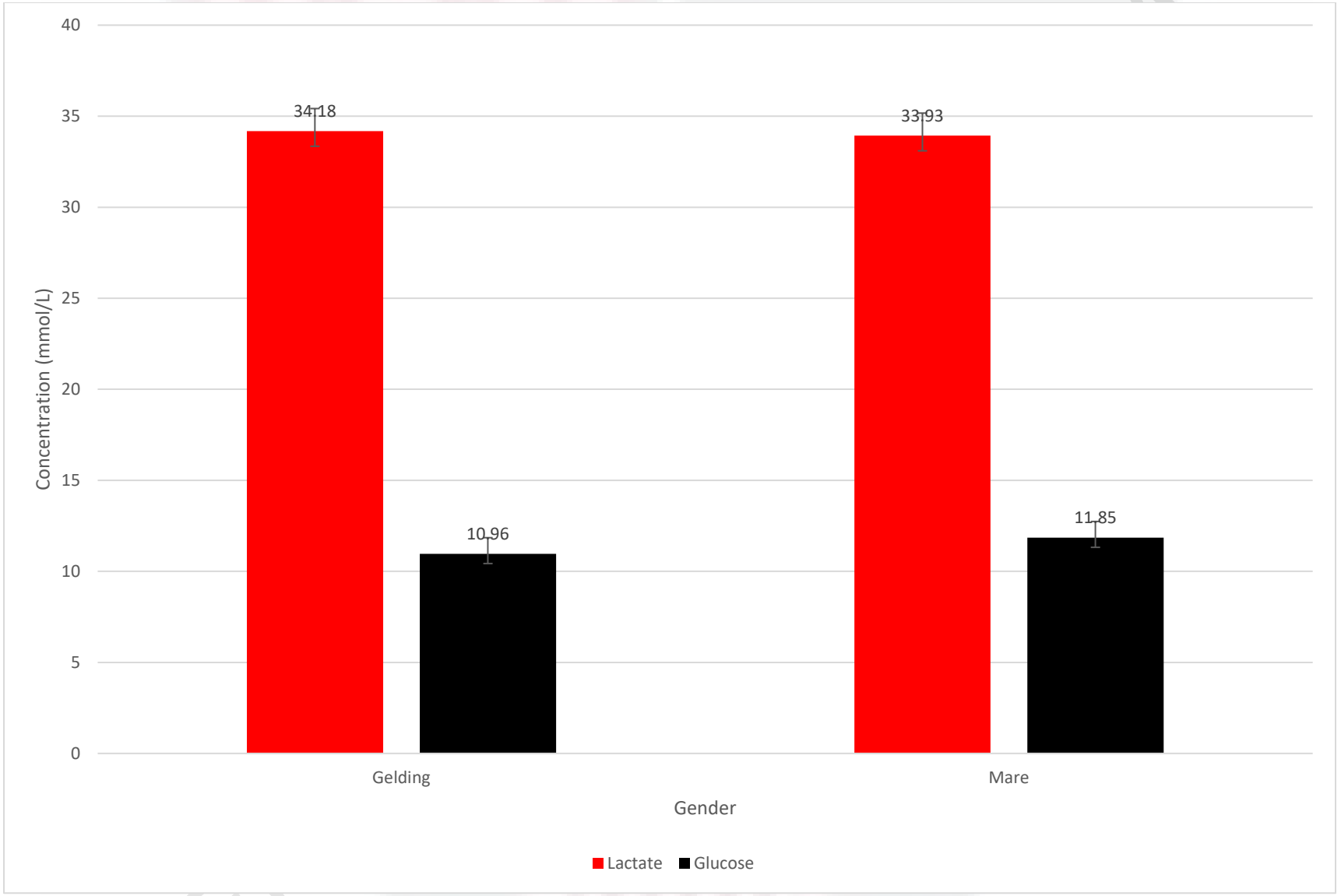

Figure 4.3 Comparative mean lactate and mean glucose concentrations (mmol/L) between geldings and mares

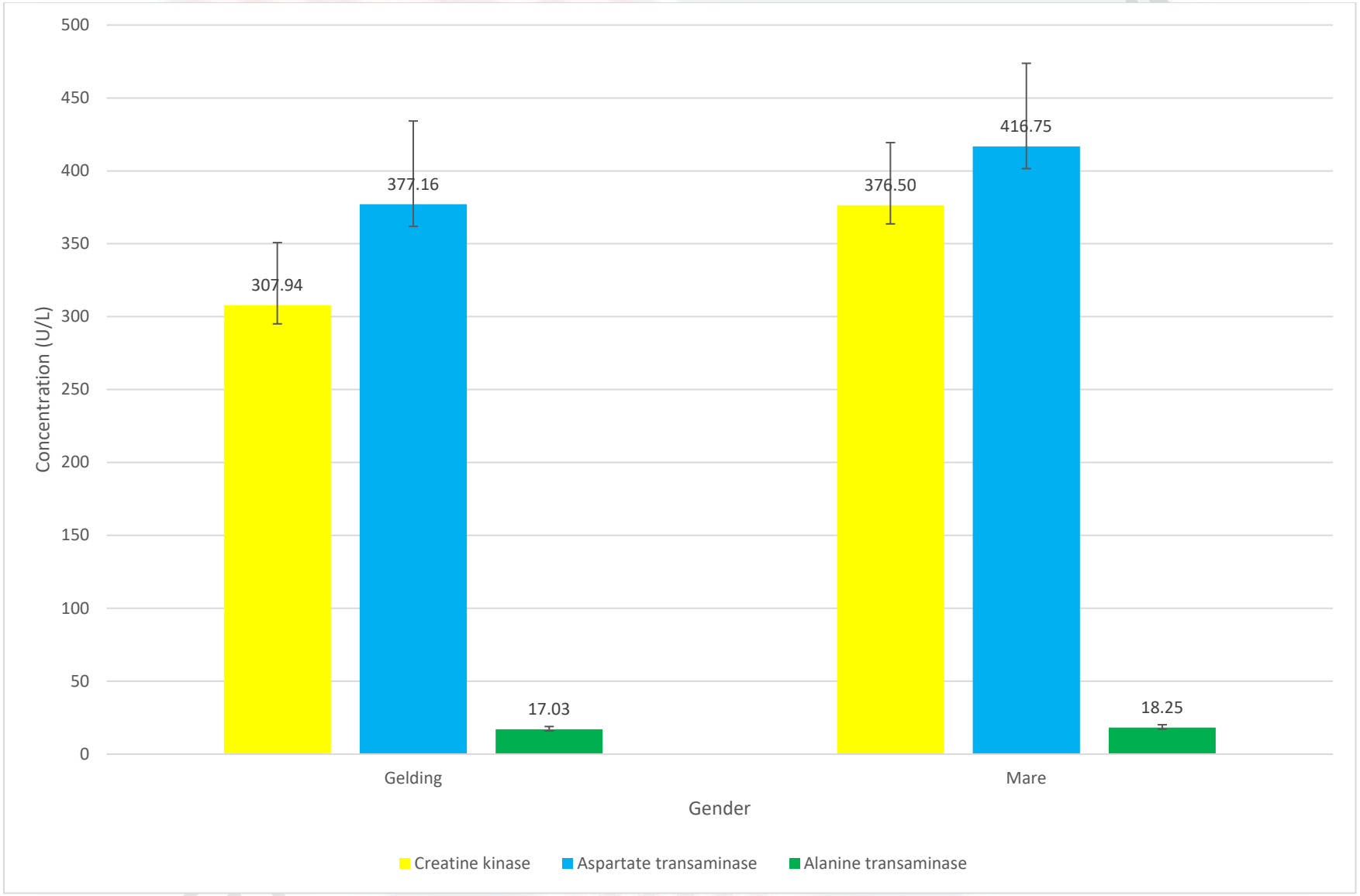

Figure 4.4 Comparative mean creatine kinase, aspartate transaminase, and alanine transaminase concentrations (U/L) between geldings and mare

4.5 Lactate and glucose concentrations against distance

Distances varied between each races and ranged from 1100m, 1200m, 1300m, 1400m, and 1600m. The values of mean lactate and mean glucose concentrations against each distance are put in table 4.5 and displayed in figure 4.5.

Distance (m)	Mean lactate concentrations (mmol/L)	Mean glucose concentrations (mmol/L)
1100	31.90 ± 1.34	10.82 ± 0.75
1200	33.70 ± 1.18	14.5 ± 3.29
1300	35.14 ± 2.32	10.23 ± 0.74
1400	34.16 ± 1.17	10.20 ± 0.44
1600	36.90 ± 1.10	11.40 ± 0.40

Table 4.5: Mean lactate and glucose concentrations with respective distances.

There is no significant association of mean lactate between and within all distances ran. There is also no significant association of mean glucose concentrations between and within all distances ran.

4.6 Creatine kinase, aspartate transaminase, and alanine transaminase concentration against distance

Mean creatine kinase, aspartate transaminase, and alanine transaminase concentrations were also placed against distance and the data is shown in table 4.6 and figure 4.6.

Distance (m)	Mean CK concentrations (U/L)	Mean AST concentrations (U/L)	Mean ALT concentrations (U/L)
1100	352.10 ± 33.72	364.2 ± 35.17	21.2 ± 2.66
1200	310.67 ± 55.48	372.50 ± 38.98	12.50 ± 4.17
1300	329.63 ± 17.45	398.38 ± 34.46	17.38 ± 1.29
1400	283.75 ± 19.48	377.25 ± 29.17	15.13 ± 1.19
1600	282.00 ± 10.98	399.83 ± 27.29	16.00 ± 0.93

Table 4.6: Mean CK, AST, and ALT concentrations with respective distances.

There is no significant association of mean creatine kinase, aspartate transaminase, and alanine transaminase concentrations between and within all distances ran.

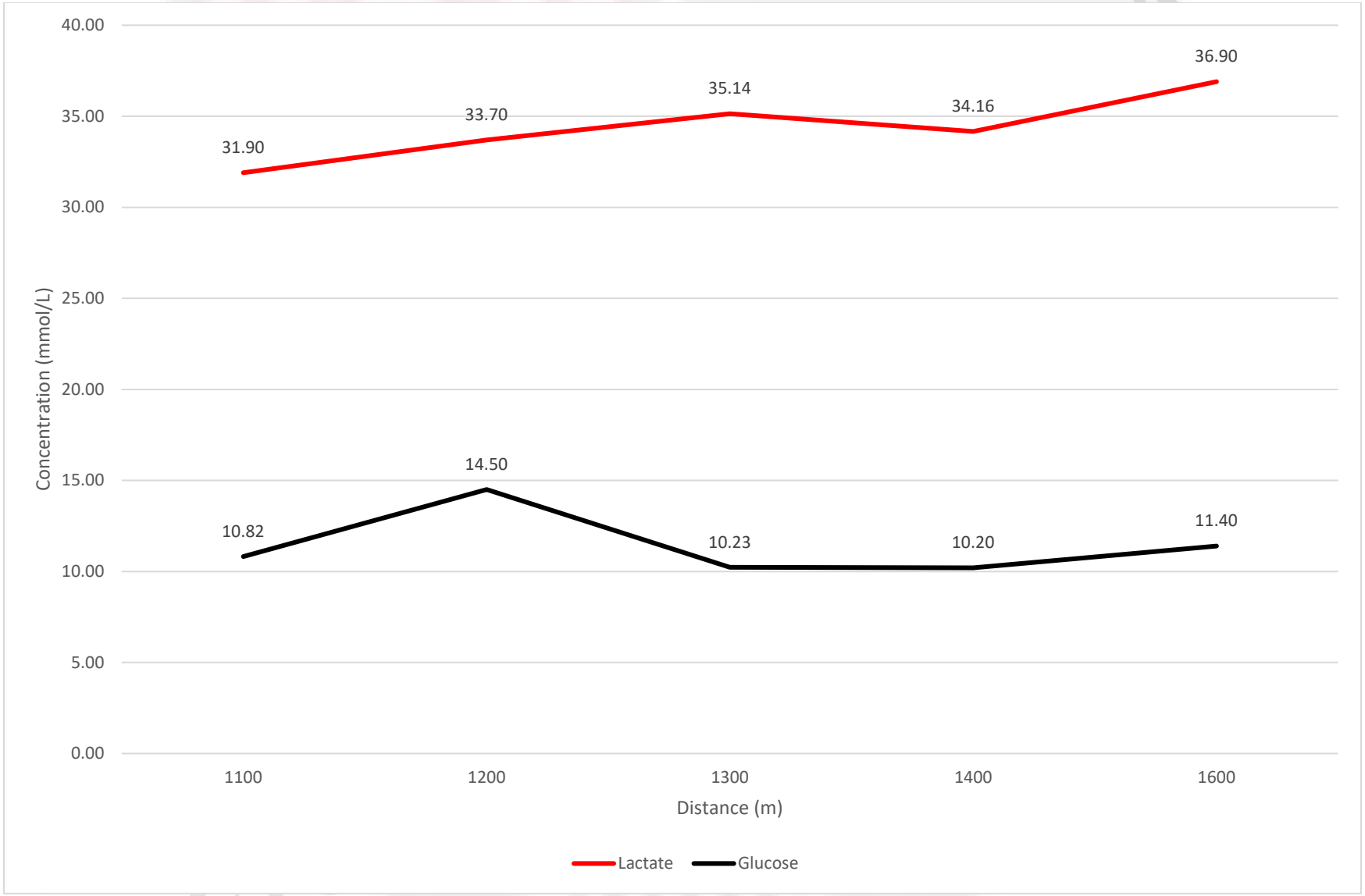

Figure 4.5 Comparative mean lactate and mean glucose concentrations (mmol/L) between all distances

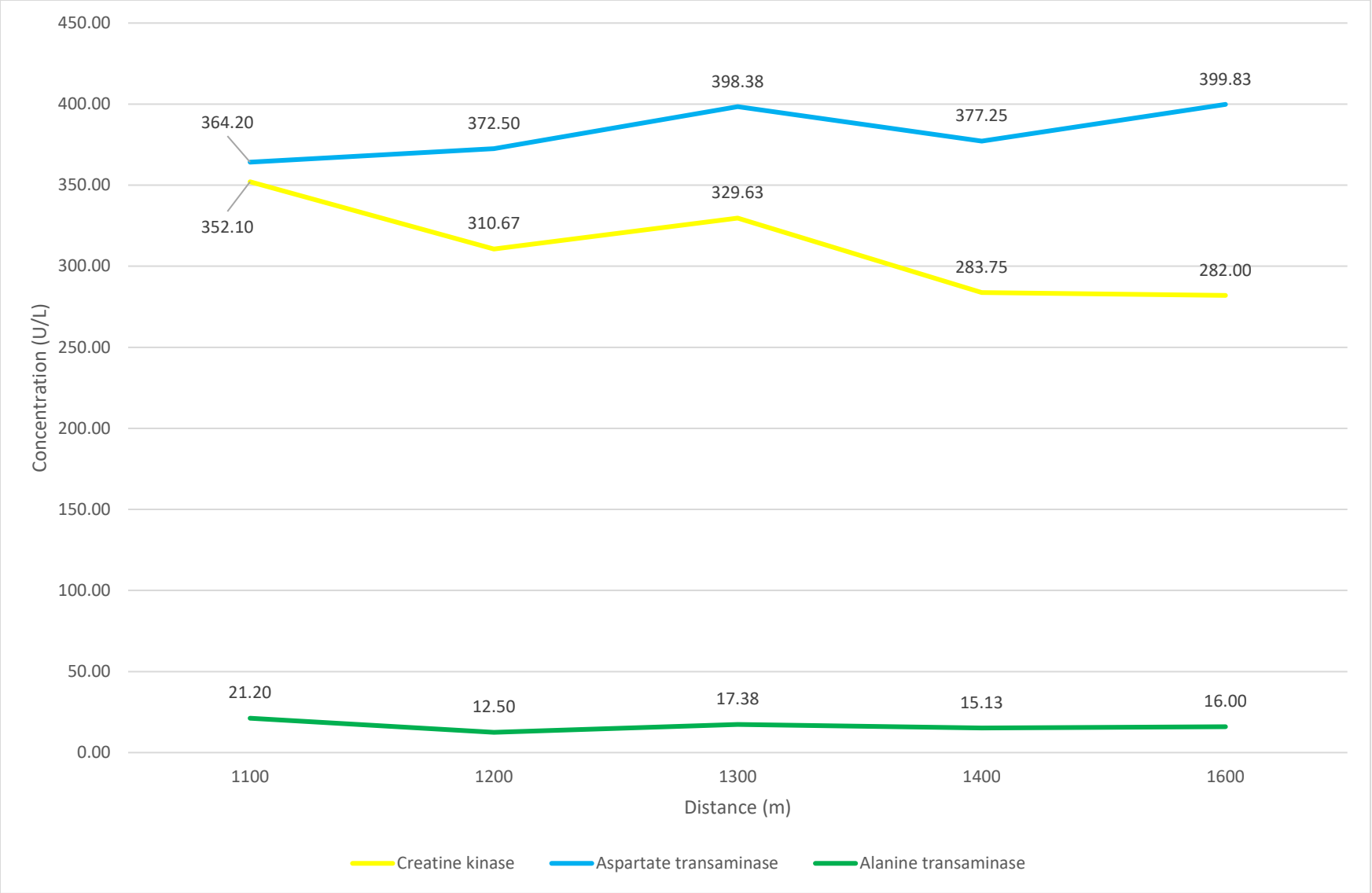

Figure 4.6 Comparative mean creatine kinase, aspartate transaminase, and alanine transaminase concentrations (U/L) between all distances

4.7 Lactate and glucose concentrations against age

Among all horses that were involved in this study, there were horses aged 4 years old to horses aged 10 years old. The mean lactate concentrations and mean glucose concentrations with their respective age is shown in table 4.7. The comparison of the mean lactate and glucose concentrations are plotted in figure 4.7.

Age (years)	Mean lactate concentrations (mmol/L)	Mean glucose concentrations (mmol/L)
4	34.83 ± 1.32	11.3 ± 0.78
5	34.96 ± 2.09	9.76 ± 0.41
6	33.80 ± 1.06	10.70 ± 0.74
7	32.2 ± 1.55	12.61 ± 2.02
8	36.8 ± 2.00	11.33 ± 1.22
9	26.2 ± 0.00	13.7 ± 0.00
10	35.8 ± 1.10	9.80 ± 0.10

Table 4.7: Mean lactate and glucose concentrations with respective ages.

There is no significant association of mean lactate between and within all ages. There is also no significant association of mean glucose concentrations between and within all ages.

4.8 Creatine kinase, aspartate transaminase, and alanine transaminase concentration against age

The mean concentrations of creatine kinase, aspartate transaminase, and alanine transaminase were also put against age and the results are tabulated in table 4.8. The mean for each parameters were also compared between each age and are displayed in figure 4.8.

Age (years)	Mean CK concentrations (U/L)	Mean AST concentrations (U/L)	Mean ALT concentrations (U/L)
4	337.25 ± 24.50	365.38 ± 15.84	17.25 ± 1.39
5	323.38 ± 26.16	380.67 ± 31.65	17.78 ± 1.54
6	272.50 ± 23.76	409.83 ± 40.07	13.33 ± 2.95
7	336.57 ± 37.24	415.14 ± 38.91	15.86 ± 1.06
8	290.33 ± 63.59	330.00 ± 32.62	22.00 ± 8.50
9	314.00 ± 0.00	380.00 ± 0.00	29.00 ± 0.00
10	295.50 ± 1.50	421.00 ± 59.00	17.00 ± 0.00

Table 4.8: Mean CK, AST, and ALT concentrations with respective ages.

There is no significant association of mean creatine kinase, aspartate transaminase, and alanine transaminase concentrations between and within all ages.

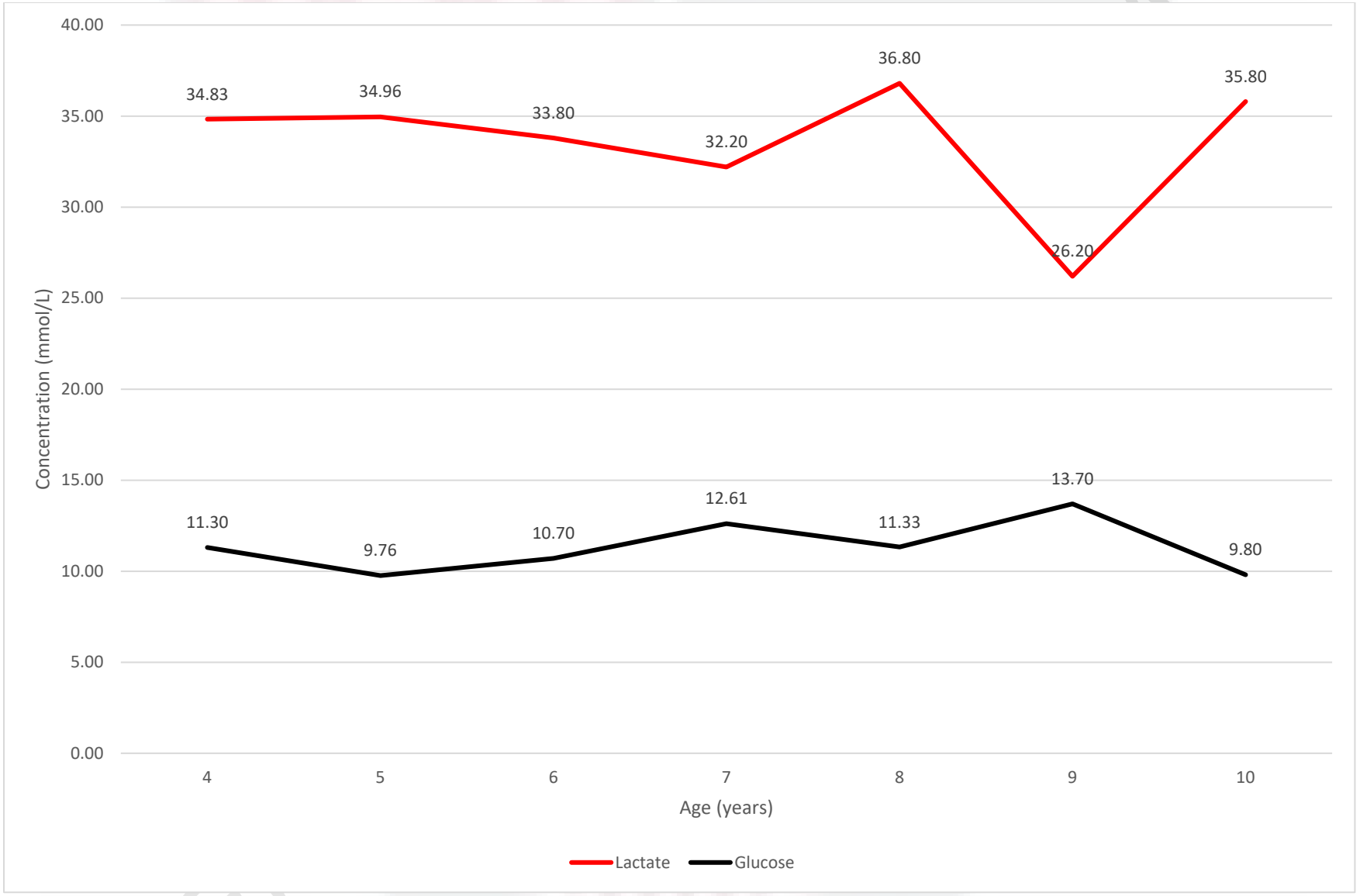

Figure 4.7 Comparative mean lactate and mean glucose concentrations (mmol/L) between all ages

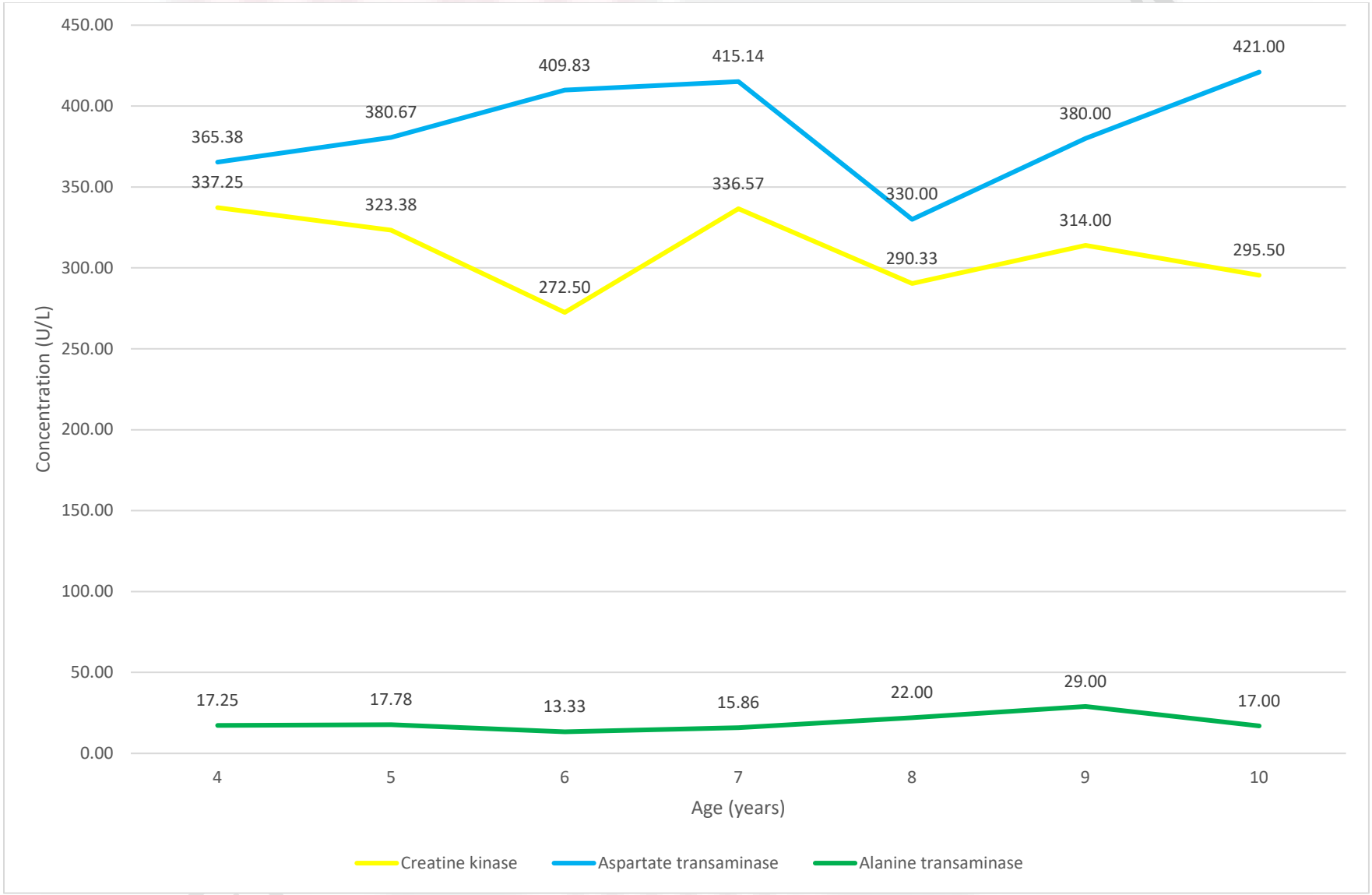

Figure 4.8 Comparative mean creatine kinase, aspartate transaminase, and alanine transaminase concentrations (U/L) between all ages

4.9 Lactate concentrations against average overall speed

The winning times of each champion horse were taken and the average overall speed is calculated according to their respective distances ran. The mean lactate concentration is then placed against the average overall speed and is shown in figure 4.9. The data is also recorded in the table below (Table 4.9).

Average overall speed (ms^{-1})	Mean lactate concentration (mmol/L)
16.30	31.50 ± 0.00
16.37	33.30 ± 0.00
16.47	35.90 ± 0.8
16.48	34.70 ± 0.00
16.50	33.80 ± 0.00
16.51	40.10 ± 0.00
16.52	30.70 ± 0.00
16.67	30.08 ± 1.60
16.69	36.40 ± 0.00
16.72	37.60 ± 0.00
16.73	32.50 ± 0.00
16.88	39.40 ± 0.00
16.90	33.20 ± 0.00
17.39	33.50 ± 0.00

Table 4.9: Mean lactate concentrations according the horse average overall speed

There is no significant association between all of the average overall speed from the slowest (16.30 ms^{-1}) to the fastest (17.39 ms^{-1}). There was also no consistent increase or decrease of lactate concentration.

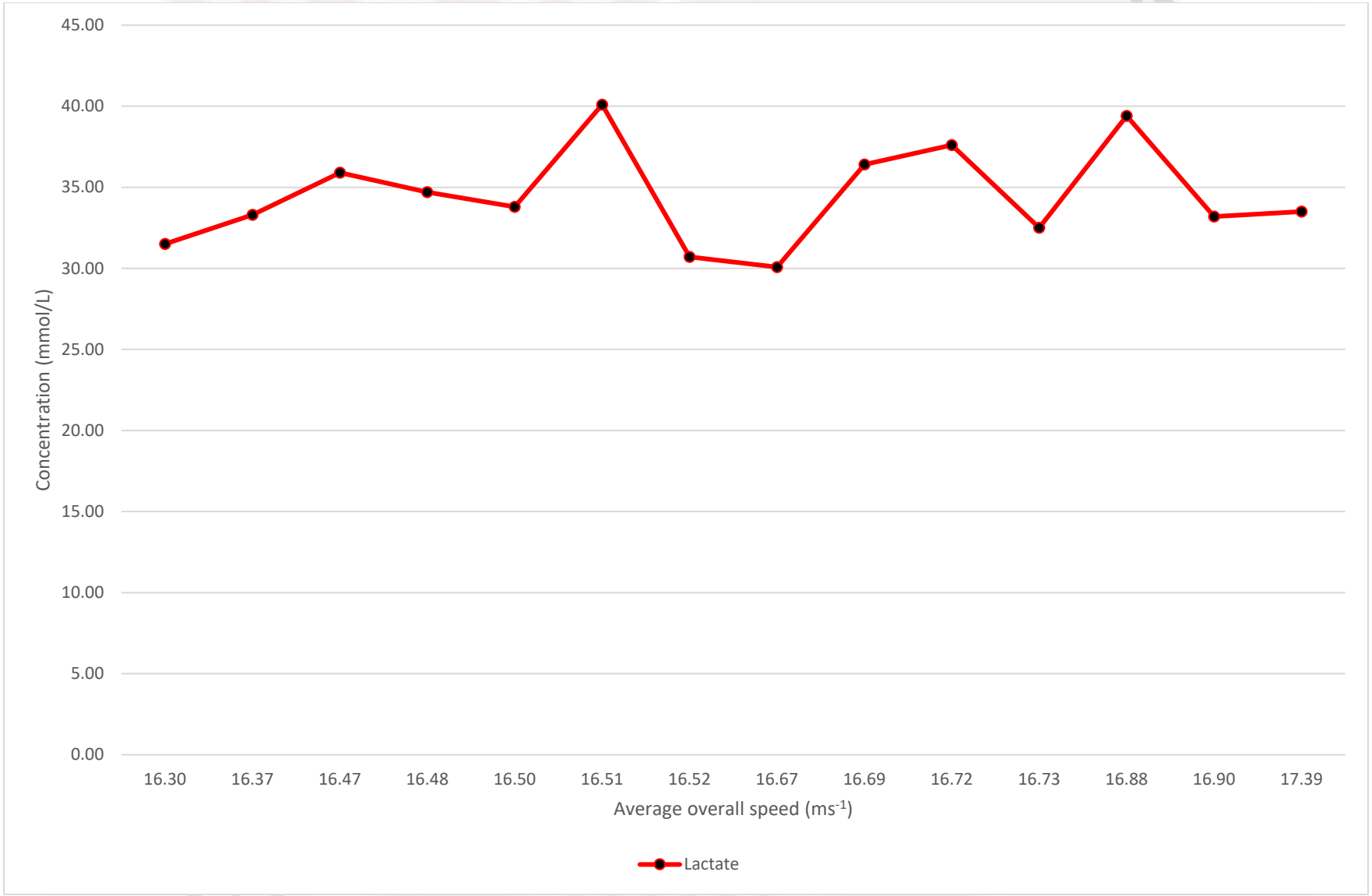

Figure 4.9 Comparative mean lactate concentration between all the average overall speeds between champion horses

5.0 DISCUSSION

The present study was undertaken to establish the level of lactate, glucose, creatine kinase, aspartate transaminase, and alanine transaminase in the serum immediately after a track race in thoroughbred horses. Several factors were taken into consideration, namely (i) the comparison of serum parameters between horses placed first and third, (ii) the comparison of serum parameters between geldings and mares, (iii) the comparison of serum parameters between all the various distances ran from 1100m to 1600m, (iv) the comparison of serum parameters between horses of different ages from 4 years old to 10 years old, and (v) the comparison of serum parameters between champion horses and their average overall speed ran throughout the race.

The lactate levels of each horse has elevated substantially by approximately 20 folds (Total mean lactate concentration = 34.16 ± 0.74 mmol/L) from the reference range which is 1.11 – 1.78 mmol/L. During short duration high intensity exercises such as thoroughbred track racing, the main supply of energy as soon as oxygen depletes within the muscles is through anaerobic glycolysis. This process occurs within the muscle cells to produce lactate which is then oxidized by the liver to regenerate glucose as a source for adenosine triphosphate (ATP) utilisation of the muscles. At the start of a race, muscles utilize energy from glycogen stores in the muscle in the presence of oxygen. However, when muscles require high amounts of energy in very short durations, oxygen and glycogen becomes depleted very quickly and anaerobic glycolysis becomes dominant for primary energy production. Anaerobic glycolysis produces a free hydrogen ion byproduct which consequently decreases the intracellular pH of muscle cells and could ultimately lead to fatigue of the muscles reducing it's capability to further contract thus significantly affecting performance. After lactate is produced, they are actively transported across the sarcolemma into the bloodstream with the aid of monocarboxylate transporters (MCT). This would go on until a point where

this process becomes saturated thus having a limit to the lactate efflux. Studies have found that training prior to racing could increase the number of MCT proteins on the sarcolemma thus helping the efflux of lactate from the muscle, helping the body to utilise the lactate more rapidly (Donovan and Brooks, 1983; Phillips, 1995). Another study supporting this evidence is by Yamano *et al*., in 2002 and Hodgson *et al*., in 1986 which proved that well-trained horses have significantly lower blood lactate concentrations during an exercise and are able to run faster and longer before reaching their limits (Valberg, 1999).

Anaerobic capacity is termed as the point at which the dominant energy production of the muscles switch from aerobic to anaerobic. Conclusively from this, we can state that a horse with a higher anaerobic capacity would indirectly have a better performance due to a delayed response to fatigue. Lactate levels peak around 12 minutes post exercise rapidly and levels decline about 1 or 2 days post exercise gradually. Delayed reduction of lactate levels could mean that the horse is not fit or any other underlying clinical problems and this would cause muscle soreness until the lactate is fully oxidised. A similar study by Snow et al., in 1983 reported having lactate levels ranging from 25 to 30 mmol/L in trained horses post exercise which could mean that lactate levels in Malaysia are almost as similar to horses abroad.

The values of glucose obtained in this study was elevated approximately by two folds (11.06 ± 0.48 mmol/L) from the reference range value which is 3.3 – 5.5 mmol/L and this elevation was consistent with each horse. There are many conditions and factors that could lead to hyperglycemia in horses and it can be present either transiently or persistently. A laboratory assessment of glucose metabolism in equine clinical pathology by Raquel in 2014 have reported that a physiologic factor is included to be a reason for transient hyperglycemia. This is caused due to insulin-antagonistic actions of catecholamines, glucocorticoids, growth hormone, and glucagon post physiologic stress. However, this elevation does not induce any clinical abnormalities and would reside within 2 to 4 hours.

Creatine kinase levels obtained in this study (Total mean creatine kinase concentration = 315.77 ± 12.72 U/L) were in the normal reference range values which are 100 – 500 U/L while the aspartate transaminase (AST) levels (Total mean AST concentration = 381.56 ± 14.742 U/L) and alanine

transaminase (ALT) levels (Total mean ALT concentration = 17.17 ± 1.03 U/L) has elevated by approximately two folds from the reference range which is 120 – 160 U/L and <10 U/L respectively. Creatine kinase is a muscle specific enzyme while the other two enzymes AST and ALT are also liver-derived thus this could explain the elevations obtained in this study is not muscle-derived but only liver-derived. Creatine kinase would increase most probably only during a skeletal muscle injury and with the values remaining within normal values despite the substantial increase in lactate and the highly energy demanding exercise, no damage had occurred on the integrity of the muscle tissues which could also mean that the horses that participated in the race are fit for the activity and are clinically normal. The slight elevations of liver enzymes are considered normal and does not produce any negative effects and would return to baseline within a few hours. A study by Rose et al., in 1983 also reported minimal hepatic enzymes post-exercise.

6.0 CONCLUSION

Based on the study conducted, it can be assumed that all the horses that participated in the race and obtained a placing of either first or third are clinically and physically fit for the activity. Significant elevations in lactate levels are considered a good indicator of well-trained horses after a thoroughbred track race. Serum levels of creatine kinase (CK), aspartate transaminase (AST), and alanine transaminase (ALT) can still be used to detect underlying diseases such as muscle damage after an exercise but could less be related to performance.

With this information we could also conclude that gender and age (4 to 10 years old) does not play a significant role in performance thus when selecting horses for racing purposes we could neglect these factors. From this study, we could assume that training efficiency and frequency would probably be the main factors that would contribute to performance.

7.0 RECOMMENDATIONS AND LIMITATIONS

Despite the increases in the serum parameters of lactate, glucose, aspartate transaminase (AST), and alanine transaminase (ALT) post-exercise, there was no significant association when compared against placings, distances ran, gender, age, and average overall speed for champion horses.

Future studies should sample horses of more differentiated variables for example sampling horses placed first and horses placed tenth instead or sampling horses aged 4 years old and horses aged 18 years old. Other than that, instead of a single-time sampling, should consider a double-sampling, or even triple sampling for example sampling the same horse before the race, immediately after the race, and one day after the race. With this we could observe the increase from the baseline of specific individuals and we could also observe the clearance pattern of these parameters.

However, limitations exist such as only horses placed first, second, and third are required to proceed to the post-race area for blood collection while the other horses go back to their respective stables thus making it difficult to obtain the samples. In addition, to obtain samples a day before or after the race would require us to track the horse's origin and sample the horses at their respective states as horses that race in Selangor Turf Club come from various states across the country.

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