



**UNIVERSITI PUTRA MALAYSIA**

***METABOLIC CRISIS INDEX AS A PREDICTOR FOR  
METABOLIC CRISIS IN ENDURANCE HORSES***

**LAWAN ADAMU**

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**METABOLIC CRISIS INDEX AS A PREDICTOR FOR  
METABOLIC CRISIS IN ENDURANCE HORSES**

By

**LAWAN ADAMU**

Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of doctor  
of philosophy  
January, 2014

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## DEDICATIONS

*This thesis is dedicated to the memory of my late mother and father Amina (Kingga) and Adam Musa Duniya, and also to my late brothers Abba, Ma'aji and to my late uncle Ramadan Musa Duniya. To my late guardian Idirisa Kafaran and to Abubakar Musa (Dodo). To Mohammed Abdullahi Helma and Hajara Usman.*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of doctor of Philosophy

## METABOLIC CRISIS INDEX AS A PREDICTOR FOR METABOLIC CRISIS IN ENDURANCE HORSES

By

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January, 2014

**Chair: Associate Professor Datuk Bashir Ahmad B. Fateh Mohamed, PhD.**

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In Malaysia, equine endurance race is an event conducted on natural tracks over distances ranging from 40 to 120 km over a period of 24 hours. This event, under challenging physical conditions involves both aerobic and anaerobic metabolisms of the horse. The intense physical activity over an extended period often causes susceptible horses to develop metabolic crises resulting in elimination from races. Metabolic crisis is the extreme changes associated with the combination of the physical parameters that result in the complex and detrimental physiological and biochemical alterations leading to elimination of endurance horses from the race. Metabolic abnormalities in horses under extreme conditions are reflected by abnormalities in physical and blood parameters during and after physical activity. The development of metabolic crises in horses during endurance races is unpredictable. This is due to the fact that there is no established method to determine, before the race that a horse is not fit to participate in an endurance race. It was postulated that the physical and blood parameters of horses that develop metabolic crisis and eliminated during endurance races differed significantly from those of horses completing the race successfully. Thus the objectives of this study were to determine the risk factors for horses to develop metabolic crises during endurance races and to develop a method to predict before the race, that a horse may potentially develop the crises. The subjects for the study were horses participating in 19 endurance races held between March 2010 and December 2012 in several states of Malaysia. Three hundred and seventy five Arabian ( $n = 152$ ) and Arabian cross ( $n = 223$ ) endurance horses aged from 6 to 15 years and weighing between 350 and 450 kg involved in these races were selected for the study. The height of these

horses ranged from 14 hands 2 inches and 16 hands 1 inch. There was no sex preference for horse selection and only apparently healthy horses were included in the study. Of these horses 253 developed metabolic crises and 122 completed the race successfully. Among the 253 eliminated endurance horses, 122 were selected randomly from establishments A, B, and C to represent this category. Sixty one horses in each independent category was represented by pre and post-ride completed, pre and post-ride metabolic. All horses were physically examined and physical data such as skin recoil, mucous membrane colour, capillary refill time and intestinal motility were obtained before the race and after 20 to 30 minutes of recovery period. Blood samples were collected at pre- and post-race periods to determine haematological, plasma electrolyte and plasma biochemical parameters including glutathione reductase (GR), serum amyloid-A (SAA) and interleukin-6 (IL-6). The mean environmental humidity and ambient temperature during the endurance races were  $71.73 \pm 4.05$  % and  $29.06 \pm 1.1^{\circ}\text{C}$  respectively. The study showed that Arabian cross horses were more prone to develop metabolic crises than pure Arabian horses. Among the parameters analysed in this study, the significant ( $p < 0.05$ ) findings in horses with metabolic crisis were increased packed cell volume ( $0.66 \pm 0.19 \text{ LL}^{-1}$ ), creatine kinase ( $1988 \pm 1447 \text{ UL}^{-1}$ ) and IL-6 ( $3.25 \pm 3.95 \text{ ng/ml}$ ), decreased GR ( $10.91 \pm 3.95 \text{ ng/ml}$ ) and chloride ( $89.6 \pm 4.4 \text{ mmol/L}$ ). These changes are characteristic of metabolic crises in horses are associated with muscle damage due to physical activity, increased sweating without water replenishment, and the inherent inability of horses to neutralise the increased reactive oxygen species production during endurance races. Using the plasma biochemical parameters which are strongly associated with metabolic crises with a correlation coefficient of ( $r = 0.8790$ ;  $P < 0.001$ ), a new method called Metabolic Crisis Index (MCI) was developed as a predictor for horses with potential to develop metabolic crisis in endurance races. This index, tested in an endurance competition and by using retrospective data from previous and on going competitions indicated a higher value greater than 5.5 for those eliminated and lower value below 5.5 for those that completed the race successfully, this proved to be accurate in the prediction of metabolic crisis in endurance horses. The MCI is an innovative and simple method use as a prediction method that will assist the equine endurance society to reduce the rate of elimination and to safeguard against serious medical problems during endurance races in the tropics.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

## INDEKS KRISIS METABOLISME SEBAGAI PERAMAL KRISIS METABOLISME PADA KUDA TAHAN LASAK

Oleh

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Di negara Malaysia, perlumbaan kuda lasak adalah satu acara yang dijalankan pada laluan semula jadi yang panjangnya antara 40 hingga 120 km dalam tempoh 24 jam. Acara yang dijalankan dalam keadaan fizikal mencabar melibatkan metabolisme aerobik dan anerobik kuda. Aktiviti fizikal tinggi dalam suatu tempoh yang lama, kerap menjadikan kuda rentan untuk mendapat krisis metabolisme yang mengakibatkan pengguguran daripada perlumbaan. Krisis metabolisme adalah suatu perubahan melampau berkaitan dengan gabungan parameter fizikal yang membawa kepada perubahan fisiologi dan biokimia kompleks dan memudaratkan. Keabnormalan metabolisme pada kuda dalam keadaan keterlaluan ini mencerminkan keabnormalan fizikal dan darah pada masa dan selepas aktiviti fizikal. Perkembangan gangguan metabolisme kuda ketika dalam perlumbaan tahan lasak tidak dapat diramalkan. Ini disebabkan ketiadaan kaedah yang boleh diguna untuk menentukan sebelum perlumbaan, kesihatan kuda untuk bertanding dalam perlumbaan tahan lasak. Adalah dipostulat yang parameter fizikal dan darah kuda mengalami krisis metabolisme dan digugurkan daripada perlumbaan tahan lasak berbeza daripada yang terdapat pada kuda berjaya menamatkan perlumbaan. Justeru itu, objekif kajian ini ialah untuk menentukan faktor risiko pada kuda untuk mengembangkan krisis metabolisme semasa perlumbaan tahan lasak dan untuk membangunkan suatu kaedah untuk meramalkan kuda berpotensi mengembangkan krisis tersebut. Subjek untuk kajian ini ialah kuda yang menyertai 19 perlumbaan tahan lasak yang dijalankan di antara bulan Mac 2012 dan Disember 2013 di beberapa negeri di Malaysia. Tiga ratu tujuh-puluh-lima ekor kuda Arab ( $n = 152$ ) and kacukan Arab ( $n = 223$ ) tahan lasak berumur 6 hingga 15 tahun dan berat badan antara 350 dan 450 kg telah dipilih untuk kajian ini. Tinggi kuda

antara 14 tangan 2 inci dan 16 tangan 1 inci. Pemilihan kuda tidak mengambil kira jantinya dan kuda yang nampak sihat dipilih untuk kajian. Daripada kesemua kuda ini, 253 ekor mengalami krisis metabolisme dan digugur daripada pertandingan sambil 122 ekor berjaya menamatkan perlumbaan. Satu ratus dua-puluh-dua ekor kuda daripada tiga pertubuhan yang digugurkan dipilih secara rawak. Kuda yang dipilih ini diperiksa fizikalnya dan data seperti, pantulan kulit, warna membran mukus, masa pengisian semula kapilari, kemotilan usus telah diperolehi sebelum perlumbaan dan pada tempoh 20 hingga 30 minit pemulihan. Sampel darah dikumpul daripada kuda pada sebelum dan selepas perlumbaan untuk menentukan parameter hematologi, elektrolit dan biokimia serum termasuk glutathion reduktase (GR), amiloid-A serum (SAA) dan interleukin-6 (IL-6). Min kelembapan dan suhu ambien persekitaran pada masa perlumbaan masing-masing adalah  $71.73 \pm 4.05 \%$  dan  $29.06 \pm 1.1 \text{ } ^\circ\text{C}$ . Kajian ini menunjukkan kuda kacukan Arab adalah lebih mudah untuk mendapat krisis metabolisme daripada kuda Arab tulen. Di kalangan parameter yang dianalisis dalam kajian ini, penemuan paling tererti ( $p < 0.05$ ) pada kuda mengalami krisis metabolisme adalah peningkatan isipadu sel padat ( $0.66 \pm 0.19 \text{ L L}^{-1}$ ), kreatin kinase ( $1988 \pm 1447 \text{ UL}^{-1}$ ) dan IL-6 ( $3.25 \pm 3.95 \text{ ng mL}^{-1}$ ) dan penurunan GR ( $10.91 \pm 3.95 \text{ ng mL}^{-1}$ ) dan klorida ( $89.6 \pm 4.4 \text{ mmol L}^{-1}$ ). Perubahan ini adalah cirian untuk krisis metabolisme dan ia berkaitan dengan kerosakan otot disebabkan aktiviti fizikal, peningkatan perpeluhan tanpa minum air, ketakupayaan semula jadi kuda untuk meneutralkan spesies oksigen reaktif yang meningkat semasa perlumbaan. Dengan menggunakan parameter biokimia serum yang terkait kuat dengan krisis metabolisme pada pekali korelasi  $r = 0.8790$ ; ( $P < 0.001$ ), suatu kaedah baharu yang beri nama Indeks Krisis Metabolisme (MCI) telah dihasilkan sebagai peramal kepada kuda berpotensi untuk mengembangkan krisis metabolisme dalam perlumbaan tahan lasak. Indeks Krisis Metabolisme apabila diuji dalam satu pertandingan kuda tahan lasak dan dengan menggunakan data retrospektif daripada pertandingan telah lepas menunjukkan nilai MCI lebih tinggi daripada 5.5 dalam kuda yang digugurkan dan kurang daripada 5.5 dalam kuda yang berjaya menamatkan perlumbaan. Indeks Krisis Metabolisme ini terbukti tepat dalam meramalkan kejadian krisis metabolisme pada kuda yang bertanding dalam perlumbaan tahan lasak. Indeks Krisis Metabolisme ini adalah suatu kaedah peramalan krisis metabolisme yang inovatif dan mudah dilakukan yang akan dapat membantu untuk mengurangkan pengguguran daripada pertandingan dan masalah perubatan teruk di kalangan kuda yang bertanding dalam perlumbaan tahan lasak dalam suasana tropika.



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I certify that a Thesis Examination Committee has met on 20<sup>th</sup> January, 2014 to conduct the final examination of Lawan Adamu on his thesis entitled "Metabolic crisis index as a predictor for metabolic crisis in endurance horses" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATIONS</b>	i
<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	iv
<b>ACKNOWLEDGEMENTS</b>	vi
<b>APPROVAL</b>	viii
<b>DECLARATION</b>	x
<b>LIST OF TABLES</b>	xvi
<b>LIST OF FIGURES</b>	xviii
<b>LIST OF ABBREVIATIONS</b>	xix
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
1.1 Endurance Sport	1
1.1.1 Endurance Sport in Malaysia	3
1.1.2 Constraints	3
1.2 Hypothesis, Research Questions and Objectives of Study	4
<b>2 LITERATURE REVIEW</b>	<b>6</b>
2.1 Endurance Horses	6
2.2 Metabolic Crises	7
2.3 Performance Indicators	8
2.4 Factors Determining Performance in Endurance Races	8
2.4.1 Physical Parameter	8
2.4.2 Heart Rate	9
2.4.3 Cardiac Recovery Index (CRI)	11
2.4.4 Respiration	12
2.4.5 Body Temperature	12
2.4.6 Dehydration Indicators	12
2.4.7 Capillary Refill and Mucous Membranes	13
2.4.8 Jugular Refill	13
2.4.9 Intestinal Motility	13
2.5 Mechanism of Sweat and Electrolytes Loss	13
2.6 Mechanism of Lactate Alterations in Blood and Muscles	15
2.7 Mechanism of Glucose Alterations in Blood and Muscles	17
2.8 Creatine Kinase and Aspartate Transaminase	17
2.9 $\gamma$ -glutamyltransferase	18
2.10 Uric Acid	18
2.11 Antioxidants	20

2.12	Free Radicals	20
2.13	Workload and Adaptive Condition	20
2.14	Tissue Trauma and Inflammatory Response in Exercise	21
2.15	Acute Phase Response During Exercise	21
2.16	Control of Inflammatory Response	22
2.17	Anti-Inflammatory Cytokines	23
2.18	Markers of Acute and Physical Activity	23
2.19	Musculoskeletal Properties	24
2.20	Haematological and Biochemical Properties of Muscle	25
2.21	Breed Predisposition of Muscle	26
2.22	Energy Source	27
2.23	Aerobic Metabolism	28
2.24	Anaerobic Alactic Metabolism	29
2.25	Anaerobic Lactic Metabolism	29
2.26	Interaction Between Energy Metabolic Pathways	29
2.27	Fatigue	30
2.28	Thermoregulation, Heat Loss	30
2.29	Climatic Factors	31
2.30	Summary	32
<b>3</b>	<b>GENERAL MATERIALS AND METHODS</b>	<b>33</b>
3.1	Horses	33
3.2	Criteria for Good and Poor Performance	33
3.3	Climatic Factors	35
3.4	Blood Sample	35
3.5	Statistical Analysis	36
<b>4</b>	<b>RISK FACTORS FOR METABOLIC CRISIS IN ENDURANCE HORSES</b>	<b>37</b>
4.1	Introduction	37
4.2	Materials and Methods	38
4.3	Statistical Analysis	39
4.4	Results	39
4.4.1	Elimination of Horses	39
4.4.2	Risk Factors in the Elimination of Horses During Endurance Races	40
4.4.3	Horse Riders	41
4.4.4	Endurance Race Tracks	41
4.5	Discussion	41
4.5.1	Physical Parameters	41
4.5.2	Endurance Races	49
4.5.3	Risk Factors	52



<b>5</b>	<b>HAEMATOLOGICAL PARAMETERS OF ENDURANCE HORSES WITH METABOLIC CRISIS</b>	<b>54</b>
5.1	Introduction	54
5.2	Materials and Methods	55
5.2.1	Blood Sample	55
5.3	Statistical Analysis	55
5.4	Results	55
5.4.1	Erythrocyte Parameters	55
5.5	Leucocyte and Thrombocytes Counts	60
5.5.1	Completed versus Eliminated	60
5.6	Discussion	60
<b>6</b>	<b>BIOCHEMICAL PARAMETERS OF ENDURANCE HORSES WITH METABOLIC CRISIS</b>	<b>65</b>
6.1	Introduction	65
6.2	Materials and Methods	66
6.2.1	Blood Sample	66
6.3	Statistical Analysis	66
6.4	Results	67
6.4.1	Biochemical Parameters	67
6.4.2	Horse Riders	67
6.4.3	Endurance Race Tracks	67
6.4.4	Electrolytes	68
6.5	Discussion	68
<b>7</b>	<b>ACUTE PHASE PROTEIN, ANTIOXIDANT AND CYTOKINE IN ENDURANCE HORSES WITH METABOLIC CRISES</b>	<b>77</b>
7.1	Introduction	77
7.2	Materials and Methods	78
7.2.1	Blood Sample	78
7.3	Statistical Analysis	78
7.4	Results	78
7.4.1	Horse Riders	79
7.4.2	Endurance Race Tracks	79
7.5	Discussion	83
<b>8</b>	<b>PREDICTION AND VALIDATION OF METABOLIC CRISES IN ENDURANCE HORSES</b>	<b>86</b>
8.1	Introduction	86
8.2	Materials and Methods	87
8.2.1	Blood Sample	88
8.3	Statistical Analysis	88
8.4	Results	89
8.4.1	Horse Riders	90
8.4.2	Endurance Race Tracks	90
8.5	Validation Test	95

8.6	Prospective and Retrospective Study to Validate the Metabolic Crisis Index	100
8.7	Discussion	100
8.8	Conclusion	101
<b>9</b>	<b>GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION</b>	<b>102</b>
9.1	Haematological Parameters	102
9.2	Biochemical Parameters	103
9.3	Electrolytes	104
9.4	Acute Phase Proteins, Interleukin-6 and Glutathione Reductase	104
9.4.1	Acute Phase Protein	104
9.4.2	Interleukin-6	105
9.4.3	Glutathione Reductase	105
9.4.4	Metabolic Crisis Index	105
9.4.5	Conclusion	106
9.4.6	Recommendations for Future Research	106
	<b>REFERENCES</b>	<b>108</b>
	<b>BIODATA OF STUDENT</b>	<b>123</b>
	<b>LIST OF PUBLICATIONS</b>	<b>124</b>

## LIST OF TABLES

Table	Page
3.1 Schedule of endurance competitions in Malaysia (2010 - 2012)	34
4.1 Physical parameters of endurance horses competing in 40 km race	42
4.2 Physical parameters of endurance horses competing in 80 km race	43
4.3 Physical parameters of endurance horses competing in 120 km race	44
4.4 Correlations between physical parameters of horses competing in 40, 80 and 120 km endurance race	45
4.5 Mean values of speed in the various loops and heart rate between the successfully completed and eliminated Arabian and Arabian cross endurance horse	46
4.6 Effect of physical parameters between the successfully completed and eliminated Arabian and Arabian cross endurance horse	47
4.7 Effects of the Risk factors between the successfully completed and eliminated Arabian and Arabian cross endurance horses	48
5.1 Erythrocyte parameters of endurance horses competing in 40 km race	57
5.2 Erythrocyte parameters of endurance horses competing in 80 km race	58
5.3 Erythrocyte parameters of endurance horses competing in 120 km race	59
5.4 Leucocyte and thrombocyte counts of endurance horses competing in 40 km race	61
5.5 Leucocyte and thrombocyte counts of endurance horses competing in 80 km race	62
5.6 Leucocyte and thrombocyte counts of endurance horses competing in 120 km race	63
6.1 Blood biochemical parameters of endurance horses competing in 40 km race	69
6.2 Blood biochemical parameters of endurance horses competing in 80 km race	70
6.3 Blood biochemical parameters of endurance horses competing in 120 km race	71
6.4 Blood electrolytes of endurance horses competing in 40 km race	72
6.5 Blood electrolytes of endurance horses competing in 80 km race	73
6.6 Blood electrolytes of endurance horses competing in 120 km race	74
7.1 Blood glutathione reductase, serum amyloid-A and interleukin-6 of endurance horses competing in 40 km race	80
7.2 Blood glutathione reductase, serum amyloid-A and interleukin-6 of endurance horses competing in 80 km race	81
7.3 Blood glutathione reductase, serum amyloid-A and interleukin-6 of endurance horses competing in 120 km race	82

8.1	Likelihood ratios to estimate the prediction ability conferred by each variable	96
8.2	Ranges of predicted metabolic crises index for elimination of endurance horses from race	97
8.3	Validation of MCI using values from ongoing competition	98
8.4	Validation of MCI using values from previous competitions	99



## LIST OF FIGURES

Figure	Page
8.1 PCV changes in endurance horses at pre and post race periods; 1 = pre-ride successfully completed; 2 = post-ride successfully completed; 3 = pre-ride metabolic and 4 post-ride metabolic; Error bar = 1 standard deviation; * = Significant Predictor.	90
8.2 CK changes in endurance horses at pre and post race periods; 1 = pre-ride successfully completed; 2 = post-ride successfully completed; 3 = pre-ride metabolic and 4 post-ride metabolic; Error bar = 1 standard deviation; * = Significant Predictor.	91
8.3 CHLORIDE changes in endurance horses at pre and post race periods; 1 = pre-ride successfully completed; 2 = post-ride successfully completed; 3 = pre-ride metabolic and 4 post-ride metabolic; Error bar = 1 standard deviation; * = Significant Predictor.	92
8.4 GR changes in endurance horses at pre and post race periods; 1 = pre-ride successfully completed; 2 = post-ride successfully completed; 3 = pre-ride metabolic and 4 post-ride metabolic; Error bar = 1 standard deviation; * = Significant Predictor.	93
8.5 IL-6 changes in endurance horses at pre and post race periods; 1 = pre-ride successfully completed; 2 = post-ride successfully completed; 3 = pre-ride metabolic and 4 post-ride metabolic; Error bar = 1 standard deviation; * = Significant Predictor.	94
8.6 Receiver operating characteristic curves for the various groups of the pre and post race goodness of fit of the predictors; 1 = pre-ride successfully completed; 2 = post-ride successfully completed; 3 = pre-ride metabolic and 4 post-ride metabolic	95

## LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AERC	American endurance ride conference
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate dependent kinase
APP	Acute phase proteins
APR	Acute phase reaction
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BW	Body weight
Ca <sup>2+</sup>	Calcium
CAT	Catalase
CK	Creatine kinase
Cl <sup>-</sup>	Chloride
CoC	Certificate of capabilities
CRP	C-reactive protein
CRT	C-reactive protein
CRT	Cardiac recovery index/Capillary refill time
Cu <sup>+</sup>	Copper
DNA	Deoxyribonucleic acid
EDTA	Ethyl diaminetetraacetic acid
ELISA	Enzyme-linked immune sorbent assay

Fe <sup>2+</sup>	Ferrous
Fe <sup>3+</sup>	Ferric
FEI	<i>Fédération Équestre Internationale (FEI)</i>
FFAs	Free fatty acids
FOR	Functional overreaching
g	grams
GGT	Gamma glutamyl transaminase
GR	Glutathione reductase
GSH-Px	Glutathione peroxidase
h	Hour
H <sup>2</sup> O <sup>2</sup>	Hydrogen peroxide
Hb	Hemoglobin
HSPs	Heat shock proteins
IL-6	Interleukin-6
I-R	Ischemia and reperfusion
IU	International unit
K <sup>+</sup>	Potassium
Kg	kilogram
Km	Kilometer
L	liter
m	meter
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
μg	microgram
μl	microliter
mg	milligram
min	minute
ml	milliter

mmol	milimole
mRNA	Messenger ribonucleic acid
NAD <sup>+</sup>	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NFOR	Non functional overreaching
ng	nanogram
O <sub>2</sub> <sup>-</sup>	Oxygen free radical
O <sub>2</sub>	Oxygen
OBLA	Onset of blood lactate accumulation
°C	Degree Celsius
OS	Oxidative stress
OT	Over training
PCV	Packed cell volume
%	Percentage
RBC	Red blood cells
ROS	Reactive oxygen species
SAA	Serum amyloid A
SDF	Synchronous diaphragmatic flutter
Std Dev	Standard deviation
UA	Uric acid
V <sub>200,140,170</sub>	Speed or velocity at heart rate of 200, etc beats per min
V <sub>LA4</sub>	Velocity at a blood lactate concentration of 4mmol L <sup>-1</sup>
VO <sub>2max</sub>	Maximal Oxygen uptake
WEC	World endurance championship
XO	Xanthine oxidase



# CHAPTER 1

## INTRODUCTION

### 1.1 Endurance Sport

Endurance race is an equestrian sport involving controlled long-distance races ranging between 40 and 160 km over a 24-h period. It is one of the intercontinental competitions acknowledged by the *Fédération Équestre Internationale (FEI)*. In endurance races, the horses are required to stop periodically at veterinary check point for assessment of fitness before they are allowed to continue the race. The winning horse is the first one to cross the finish line after passing all fitness tests. The long distance ride of endurance races is arduous and this may compromise the health status and performance of the horses. As a result the horses may develop metabolic crises and/or musculoskeletal abnormalities, which result in the elimination of these horses from the race. Metabolic crisis is the extreme changes associated with the combination of the physical parameters that result in the complex and detrimental physiological and biochemical alterations leading to elimination of endurance horses from the race.

Endurance is the ability to delay physical and psychogenic exhaustion and fatigue (Piccione et al., 2010c). Endurance race or sporting activity is the measure of the ability of a horse to withstand the rigors of speed under maximal aerobic and anaerobic conditions (McMiken, 1983; Piccione et al., 2010b). In endurance sports, the distance of race varies between 40 and 160 km. The races are divided into different stages; each stage comprises a distance of 20 to 40 km depending on the class of the race. The competition regulations require that horses should have a recovery period of 20 to 30 minutes and compulsory resting period of 30 to 50 minutes after each stage. During this period the horse is cooled with cold water until a heart rate of equal to or less than 64 beats per minute (bpm) is attained, with normal cardiac, respiratory, musculoskeletal and hydration status before presentation for evaluation by the line veterinarian at the veterinary gate. Only horses that comply with the criteria of the evaluation are considered fit and allowed to proceed with the next stage of the race. If otherwise, these horses are eliminated from the race and sent to the clinic for observation and treatment.

Generally, endurance horses are eliminated from the race due to either lameness or metabolic crises. In Malaysian endurance competitions, approximately 54 and 28 % of the horses were eliminated because of metabolic crises and lameness respectively (Lawan et al., 2012). Lameness in endurance horses prevails as a result of tendonitis, laminitis and musculoskeletal disorders. Metabolic crises ensues because of inability of endurance horses to maintain a heart rate of equal to or less than 64 beats per minute (bpm) and in combinations with severe changes in the mucus membrane, skin tent, capillary refill time and intestinal motility.

Severely congested mucous membrane is apparently reddened, severely dehydrated skin fold requires more than 3 seconds to return to its normal position, no motility or sound is indicated when the sounds are reduced or absent and severe capillary refill time is when the blood takes a prolonged time period of more than 3 seconds for the pinkness to reappear in the mucous membranes of the mouth, after the removal of the thumb pressure from the gum.

During races, horses show homeostatic changes, such as energy diminution and alterations in fluids, electrolytes and acid-base balance, which result in negative consequences on both performance and health of the horse. These changes manifest as metabolic problems and exhaustion characterised as elevated heart rate, dehydration and gastrointestinal signs (Carlson, 1983; Geor et al., 1996; Flaminio and Rush, 1998; Castejn et al., 2006; Robert et al., 2010; Schott II, 2010). The elimination of endurance horses from the races is also associated with changes in haematological and biochemical parameters, antioxidant activity, concentration of acute phase proteins and cytokine, which are biomarkers of metabolic crises in endurance horses. These parameters are essential to the tissues and organs, playing a role in maintenance of health of the organism (Castejn et al., 2006). For example the improvement of oxygen carrying capacity by increasing the blood oxygen content is important for the endurance of the horses (Castejn et al., 2006). The blood components also function to maintain the osmolality of the blood, and changes of some of the parameters are indicators of the hydration status of the animal (Funkquist et al., 2000; Kearns et al., 2002). The blood components also function in the maintenance of blood pH through its buffer capacity (Rose and Hodgson, 1994; Piccione et al., 2008).

Endogenous stresses and metabolic crises were due to biochemical alterations that may subsequently lead to acid-base disorders like metabolic alkalosis, excessive sweat, electrolyte loss and hyperthermia (Art and Lekeux, 2005; Castejn et al., 2006; Fielding et al., 2009; Piccione et al., 2010a). This can result in fatigue and muscle weakness, dehydration and poor performance (Sampieri et al., 2006). In fact metabolic changes experienced during grueling endurance events have been attributed to depletions of some biochemical components in working muscles, which may cause skeletal muscle damage (rhabdomyolysis) and increase in muscle enzyme concentrations in the blood (Hodgson et al., 1994; Fallon et al., 2001; Piccione et al., 2008).

Circulating acute phase proteins, antioxidants and proinflammatory cytokines are intimately linked to exercise performance and recovery. Acute phase proteins (APP) are found in abundance during the acute phase reaction in the liver, and are the innate, non-specific major response to many disorders (Gruys et al., 2005; Petersen and Pedersen, 2006). Acute phase reaction (APR) is stimulated by pro-inflammatory cytokines. Exercise-induced increase in blood APR is typified by changes in pro-inflammatory cytokines (Fallon et al., 2001; Peeling et al., 2008). In endurance horses after strenuous rides the APR increased significantly (Hee-

gaard et al., 2000; Cywinska et al., 2012). Antioxidants on the other hand are enzymes, vitamins and minerals that have to be synthesized abundantly and endogenously in the body to counteract the effects of reactive oxygen species (ROS) during arduous endurance races (Robson-Ansley et al., 2009; Williams and Burk, 2012; Adamu et al., 2013a). The ROS has tremendous biological activities and is largely produced by the contracting excitable muscle tissues (Pederson et al., 2005; Robson-Ansley et al., 2009). It also activates and promotes metabolic alterations leading to local and systemic effects (Gruys et al., 2005; Petersen and Pedersen, 2006; Tizard, 2009; Cywinska et al., 2012).

### **1.1.1 Endurance Sport in Malaysia**

Equine sporting events have become competitive in Malaysia, and are gaining international recognition especially when Malaysia was invited to participate in the world endurance championship (WEC) in Dubai in 1998. The equine endurance activities in Malaysia are recognised by the world body, the *Fédération Équestre Internationale (FEI)*, an international governing council for equestrian sports. As a result Malaysia was invited to organize the world endurance competition in 2008, held in Terengganu, which attracted 142 foreign participants from 44 countries. This was the first endurance competition of international standard to be held in a hot humid climate, attributing to Malaysia's ability to organise an event of such stature. Fortunately the country is blessed with the climate and excellent infrastructure for endurance sport. This was the impetus for the country to organise regular endurance events, which are now being conducted almost monthly.

### **1.1.2 Constraints**

The number of endurance horses in Malaysia is on the increase, particularly from the interest and participation of government organizations, private clubs and individuals from major cities of Malaysia. From this gaining interest, individuals and organizations are now acquiring high performance horses from many countries across the world in order to increase the competitiveness of these endurance events. In spite of the purchase of high-quality horses, the level of performance of horses in endurance competitions is still low. This was quite obvious because many horses failed the qualification requirement by not obtaining certificate of capabilities (CoC) from FEI, which requires the horses, among others, to successfully complete a classified number of rides at selected distances within a time period over a specified speed. Due to the strict requirements the number of horse abled to obtain CoC and proceed to compete is small. This is quite evident during the 2008 WEC in Terengganu where only a small number from Malaysia were allowed to participate because of lack of CoC.

During local endurance competitions, the number of horses eliminated from the race was very high compared to international competitions, suggesting that the horses were poorly conditioned and not fit to compete, inspite of being trained in the environment of the competitions. Those horses that participated were prone to develop metabolic crises and musculoskeletal injuries and eliminated (Lawan et al., 2012).

Metabolic crisis is a complex of abnormal changes which include abnormalities in the heart rate, changes in the color of the mucus membrane, capillary refill time, gastrointestinal symptoms and hydration status during strenuous long distance races. To determine the development of these crises, the evaluation of the performance markers in the blood samples and physical parameters of horses that were eliminated and successfully completed is necessary. The data and information obtained from the evaluations can be assessed and analysed to determine the cause of the disorders and eventually used to improve performance of these endurance horses.

## **1.2 Hypothesis, Research Questions and Objectives of Study**

The hypothesis of the study is:

The values of performance markers differ significantly between endurance horses of different performance levels.

To validate the hypothesis, the following research questions must be addressed:

- I. What is the current performance status of Malaysian endurance horses?
- II. What are the markers that can be used in the validation of endurance horses of different performance levels that can be used for the screening and selection of horses prior to an endurance competition?
- III. Is there any relationship between the performance markers with the performance levels?
- IV. Which performance parameters can consistently be used to classify and distinguish endurance horses of different performance levels?

Therefore, the objectives of this study were to:

1. Classify local endurance horses in Malaysia into different performance levels.
2. Determine the baseline performance markers using standard laboratory test

for the identification of endurance horses that complete the race successfully and those that developed metabolic crises.

3. Determine physical, haematological and biochemical parameters that can be used as indicators of fitness for horses to participate in endurance races.

4. Determine the risk factors for horses to develop metabolic crises during endurance races.

5. Determine the potential of acute phase proteins, antioxidants and cytokines as performance fitness markers.

6. Develop a method for determination of horses that may potentially develop metabolic disorders during endurance races.



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