



**NON-INVASIVE ASSESSMENT OF PROGESTERONE AND CORTISOL
LEVELS IN RELATION TO REPRODUCTIVE AND STRESS BEHAVIOURS
OF *Rusa unicolor* Kerr IN CAPTIVITY**

By

NOOR HAIDA BINTI ABDUL HAMID

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Master of
Science**

June 2022

FS 2022 58

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Sambar Deer, *Rusa unicolor* (KERR, 1792) are listed as 'vulnerable' in the IUCN Red List. In Malaysia, they are bred in semi-captive and captive environment under governmental management, but this has produced a variety of results. For example, breeding in zoos led to sambar deer becoming accustomed to human presence, whereas the current reintroduction programme by semi-captive centres was ineffective because sambar deer still face a high risk of extinction in the wild. This study aimed to assess sambar deer reproductive and stress behaviours and its progesterone and cortisol profiles in captivity while establish an ethogram on reproductive and stress behaviours. Also, to study the relationship between reproductive and stress behaviours with environmental parameters and correlate reproductive and stress behaviours of semi-captive sambar deer with progesterone and cortisol. In captive study, the frequency of each behaviour performed by 6 female sambar deers was calculated by session (morning, afternoon, evening) in the form of percentage. The reproductive and stress hormones in faecal samples were analysed using ELISA. Meanwhile, in semi-captive study, data on 8 female sambar deers were collected to assess their reproductive and stress behaviours. A Generalized Linear Model (GLM) was used to analyse each behaviour with the environmental parameters. Data for behavioural-hormonal correlation study were collected from 3 female sambar deers. Daily data on total frequency of each behaviours were correlate with concentration of progesterone and cortisol metabolites using Pearson Correlation Test. Study in captivity environment showed more reproductive behaviours in the morning and evening session. Sambar deer were suggested to be on anoestrus due to average reading of - 25.9 ng/g progesterone metabolites concentration. It could be suggested that, they were not in stress due to low cortisol metabolites concentration of - 0.3 ng/g. Study on semi-captivity environment showed more reproductive behaviours in the morning when there was no rain, the temperature was 28°C and without the presence of

workers. When there was no rain and an average temperature of 29°C, stress behaviours were more prevalent. Negative correlation between reproductive behaviours and progesterone levels was observed at the early, middle, and at the end of the month. The occurrence of oestrus among sambar deers in semi-captivity may last for 24 hours and had a shorter oestrus cycle (13 days). A behavioural-hormonal prediction model was constructed to help in predicting future reproductive event in captivity. The low level of positive correlation between stress behaviours and cortisol metabolite concentrations ($r = 0.32$) suggests that sambar deers living in a semi-captive environment are not under stress. Further study on other reproductive hormones to correlate with its behaviours needs to be conducted as it is very important to understand what these species are, and how they are currently managed in the wild and in captivity.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PENILAIAN BUKAN INVASIF PROGESTERON DAN KORTISOL
BERHUBUNG DENGAN TINGKAH LAKU REPRODUKTIF DAN STRES *Rusa*
unicolor Kerr DALAM KURUNGAN**

Oleh

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Rusa Sambar, *Rusa unicolor* (KERR, 1792) disenaraikan sebagai rentan kepada bahaya dalam Senarai Merah IUCN. Di Malaysia, mereka dibiakkan dalam separa kurungan dan kurungan di bawah pengurusan kerajaan, tetapi dengan hasil yang berbagai. Sebagai contoh, pembiakan di zoo menyebabkan rusa sambar menjadi terbiasa dengan kehadiran manusia, manakala program pelepasan semula oleh pusat separa kurungan tidak berkesan kerana rusa sambar masih menghadapi risiko kepupusan yang tinggi di hutan. Kajian ini bertujuan untuk menilai tingkah laku reproduktif dan stres rusa sambar serta profil progesteron dan kortisol dalam kurungan sambil mewujudkan etogram tentang tingkah laku reproduktif dan stres. Juga, untuk mengkaji hubungan antara tingkah laku reproduktif dan stress dengan faktor persekitaran dan mengaitkan tingkah laku reproduktif dan stres rusa sambar separa kurungan dengan progesteron dan kortisol. Dalam kajian kurungan, kekerapan setiap tingkah laku yang dilakukan oleh 6 ekor rusa sambar betina dikira mengikut sesi (pagi, tengah hari, petang) dalam bentuk peratusan. Hormon reproduktif dan stress dalam sampel najis dianalisis menggunakan ELISA. Sementara itu, dalam kajian separa kurungan, data ke atas 8 ekor rusa sambar betina telah dikumpul untuk menilai tingkah laku reproduktif dan stres mereka. Model Linear Umum digunakan untuk menganalisis setiap tingkah laku dengan faktor persekitaran. Data untuk kajian korelasi tingkah laku dan hormon dikumpul daripada 3 ekor rusa sambar betina. Data harian tentang jumlah kekerapan setiap tingkah laku dikaitkan dengan kepekatan metabolit progesteron dan kortisol menggunakan Ujian Korelasi Pearson. Kajian dalam persekitaran kurungan menunjukkan lebih banyak tingkah laku reproduktif pada sesi pagi dan petang. Rusa sambar dijangka mengalami anestrus kerana bacaan purata - 25.9 ng/g kepekatan metabolit progesteron. Turut dicadangkan bahawa, mereka tidak beradadalam keadaan tertekan kerana kepekatan metabolit kortisol yang rendah iaitu - 0.3 ng/g. Kajian terhadap persekitaran separa kurungan menunjukkan lebih banyak tingkah laku reproduktif pada waktu pagi apabila tiada hujan, suhu 28°C dan

tanpa kehadiran pekerja. Apabila tiada hujan dan suhu purata 29°C, tingkah laku stres lebih tinggi. Korelasi negatif antara tingkah laku reproduktif dan tahap progesteron dilihat pada awal, pertengahan, dan pada akhir bulan. Kejadian estrus di kalangan rusa sambar dalam separa kurungan mungkin berlangsung selama 24 jam dan mempunyai kitaran estrus yang pendek (13 hari). Model ramalan tingkah laku dan hormon telah dibina untuk membantu dalam meramalkan kejadian reproduktif masa depan dalam kurungan. Tahap toleransi positif yang rendah antara tingkah laku stress dan kepekatan metabolit kortisol ($r = 0.32$) menunjukkan bahawa rusa sambar yang hidup dalam persekitaran separa kurungan tidak mengalami tekanan. Kajian lanjut mengenai hormon reproduktif lain untuk dikaitkan dengan tingkah laku perlu dijalankan kerana ianya sangat penting untuk memahami spesies ini, dan bagaimana ia diuruskan secara liar dan dalam kurungan.



ACKNOWLEDGEMENTS

Alhamdulillah, I am very grateful to God for His wisdom, strength and continuous love towards me through good and difficult times in all the ways to finish this project.

I would like to express my gratitude towards my parents, my siblings, my husband, all my family members and beloved friends for the encouragement which help me in completing this research. Without their tremendous understanding and encouragement in the past few years, it would be impossible for me to complete my study. They serve as an inspiration for me to pursue this undertaking.

It is a genuine pleasure for me to express my deepest appreciation to my supervisor, Dr. Mohd Noor Hisham Mohd Nadzir for his willingness in taking me as his student and for the continuous support, patience, motivation, enthusiasm, and immense knowledge. All his dedication, patience, comments, knowledges and motivations throughout this project had been solely and mainly responsible for completing my work. Thanks for the opportunities given for me to explore a wide range of knowledge in animal physiology field. I am extremely thankful to all the supervisory committee including Dr. Mohd Noor Hisham Mohd Nadzir, Assoc. Prof. Dr. Geetha Annavi, Dr. Wan Nor Fitri Wan Jaafar and Assoc. Prof. Dr. Annas Salleh for their encouragement, insightful comments, and invaluable advice. Their constant encouragement throughout my journey really helps me to improve my performance. Their immense knowledge and plentiful experience have encouraged me in all time of my academic research and daily life.

In addition, I would like to thank the Zoo Negara Malaysia and Sungkai Wildlife Conservation Centre for the permissions to conduct the behavioural and faeces sample collection. Thanks to Mr. Junaidi Omar for helping and guiding me throughout my research in Zoo Negara. A big thanks as well to all staff of Animal Physiology Lab, Department of Biology, Faculty of Science and Histopathology Lab, Faculty of Veterinary Medicine. Lastly, I would like to thank all the conservation officers (DWNP) including Mr. Cosmas Ngau, Mrs. Hartini Ithnin and all staffs of Sungkai Wildlife Conservation Centre for their wide range of information and technical support on my study.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

WWF	World Wildlife Fund
IUCN	International Union for Conservation of Nature
PROG	Progesterone
ELISA	Enzyme-linked immunosorbent assay
Cor	Cortisol
HRP	Horseradish Peroxidase
OD	Optical Density
nm	Nanometre
μ l	Microlitre
$^{\circ}$ C	Degree Celsius
g	Gram
kg	Kilogram
ml	Millilitre
m	Meter
DWNP	Department of Wildlife and National Parks
%	Percent
GLM	Generalized Linear Model
AICc	Akaike's Information Criterion
Δ	delta
UPM	Universiti Putra Malaysia
ng	Nanogram/gram

CHAPTER 1

INTRODUCTION

1.1 Background of Study

It is reported that in the previous century, sambar deer lost more than 50 percent of its historical range and only a quarter of its present habitat is protected in the Peninsular Malaysia (Kawanishi et al., 2014). This species is listed as vulnerable by the International Union for Conservation of Nature Red List (IUCN 2015) due to drastic population decline in the wild throughout its geographical range, mainly driven by deforestation, hunting and overexploitation. This trend of declining occupancy and abundance of sambar deer is expected to continue in Peninsular Malaysia in the future unless a comprehensive study is conducted (Kawanishi et al., 2014) to boost sambar deer numbers in captivity and then reintroduce in the wild. Surveys done from 2009 to 2013 involving three national tiger conservation areas (Temenggor, Royal Belum State Park and Endau-Rompin) showed that sambar deer were detected in only four out of ten units surveyed (Ali et al., 2021). In addition, sambar deer were not caught by camera trap in chosen forests in Kelantan and Pahang. An effort to enhance the amount of sambar deer will also allow these regions to sustain a greater number of Malayan tigers, in line with our National Tiger Conservation Action Plan's target of increasing tiger numbers. Sambar deer were considered as the largest preferred tiger prey species. Better protection of sambar deer will not save the critically endangered endemic tigers in Malaysia automatically, but it would be one of the good steps in preparing a better conservation programme specially to increase their numbers in the wild. A government-initiated breeding program has been designed to restock sambar deer since the 1970s (Kawanishi et al., 2014). Nevertheless, there is still a lack of resources for conservation and improvement for the large-scale restoration and preservation of the original sambar habitat and for the effective reintroduction of captive sambar that has encountered little success with this attempt. There might be also a limited number of local expertise in breeding technology. Therefore, designing and implementing a suitable conservation and management plan requires data on reproductive biology, ecology, population, and interactions within species and other species, which is currently lacking is highly needed. Moreover, it is essential to maintain natural reproductive behaviour in captive animals to establish populations and maintain genetic diversity within the population (McPhee and Carlstead, 2010). Most deer species have developed their wild distribution in recent decades, leading to increased agricultural, environmental and social impacts (Roberts et al., 2015). Therefore, wild deer are increasingly seen as an invasive species that requires research and management (Davis et al., 2016). However, because of their solitary social structure, secretive nocturnal behaviour, and preference for deep tropical woods, sambar deer are difficult to study using conventional field methods. Sambar deer cannot be approached by anyone at any time. Humans are also considered as predators for wild animals. Therefore, as a prey animal, sambar deer are preconditioned towards a fight or flight response, and usually they will choose to run away and avoid the threat as they would have a much better chance of

survival. Providing adequate accommodation circumstances for a wild animal including sambar deer that will fulfil all its physical and psychological needs is very hard.

In the past few decades, there has been many acceptances on the role of animal behaviours in enhancing the success and effectiveness of conservation management programs (Greggor et al., 2019). Animal behaviour can be utilised as a tool in conservation efforts. It can act as an indicator of conservation issues as well as to understand and predict the effects of abiotic factors on wildlife populations. Furthermore, recent advancements in field endocrine techniques have made it possible to gather extensive data on an individual animal's growth, stress level, and reproductive status, which can be used to predict how populations will react in the present and the future to environmental changes (McCormick and Romero, 2017). Both behaviour and hormonal data were important to help the management in encouraging the life skills that improve survival once sambar deer has been reintroduced into the wild. Therefore, the ability to detect oestrus is important for mating at the right time. However hormonal profiles associated with breeding and stress condition such as progesterone and cortisol respectively are still not well documented for sambar deer. To overcome this, a better understanding on sambar deer reproductive and stress physiology is essential. The analysis of faecal hormone metabolites is one of the valuable tools for assessing sambar deer reproductive function and state of stress in captivity, which can then be used as an effective non-invasive method for detecting oestrus and oestrus cycle characterisation for effective captive breeding programs. Recent advances in non-invasive reproductive hormone measurements and pressure through faecal specimens reflect the state of endocrine activities that can provide an alternative way to study the relationship between reproductive and physiological stress reactions with behaviour (Sheriff et al., 2011; Cook, 2012). The stress response plays an important role in allowing animals to overcome the changes and challenges in dealing with both environmental certainty and uncertainty. The advantages of using non-invasive faecal samples to obtain hormonal profiles without animals being harmed or restrained are still not been tested in sambar deer. However, at the present time, little data on the reproductive features of these subspecies in Peninsular Malaysia has been recorded.

1.2 Problem Statement and Justification of Study

The major issue of drastic population declines in the wild is getting worse year by year and most people refuse to acknowledge the issues and the consequences. Sambar deer (*Rusa unicolor*) plays a big part in ecosystem as it is one of the more important prey for predators. Concerning to the decline of sambar deer population and the importance of this species to the ecosystem, especially to Malayan tigers, an effort to increase the number of sambar deer in the wild has been made to sustain a greater number of Malayan tigers. This is in line with our National Tiger Conservation Action Plan's target of increasing the tiger numbers in the wild. The conservational needs of sambar deer are an important strategy to support the dwindling number of Malayan tigers which prey

on sambar deer that was reintroduced into the wild. This is because sambar deer were considered as the largest preferred Malayan tiger prey species.

A government-initiated breeding program through *ex-situ* conservation to restock and to reintroduce sambar deer into the wilds was designed since the 1970s (Kawanishi et al., 2014). However, since 2015 Malayan tigers are still listed as critically endangered by the International Union for Conservation of Nature Red List (IUCN, 2015) with population remaining between 250-340 individuals. Based on these alarming statistical data, the questions that arises are, (i) whether the sambar deer that was released into the forest through the deer release program are able to survive in the wild before becoming the prey of the tiger or the deer is actually experiencing stress and eventually die before becoming the prey, (ii) the lack of resources for conservation and improvement for the large-scale restoration and preservation of the original habitat of sambar deer and (iii) the attempt of the reintroduction of captive sambar deer to the wild are still less successful. To date, the determinant for successful captive breeding of sambar deer is still unclear. There is only little information known about sambar deer conservation status, ecology, behaviour, and reproductive physiology which are required for better management and conservation. Moreover, non-invasive approach to study on reproductive and stress hormones were currently not available for sambar deer. The patterns of predictive behaviour regarding reproductive and stress hormonal profiles in sambar deer also have not yet been explored. Therefore, designing and implementing a suitable conservation and management plan requires data on reproductive biology, ecology, population, and interactions within species and other species, which is currently lacking is highly needed. A comprehensive behavioural-hormonal study will be a crucial step in preparing a better conservation programme specially to increase their numbers in the wild.

1.3 Study Objectives

This research was conducted to provide conservational needs and information through the behavioural and hormonal study of captive and semi-captive sambar deer. Thus, the objectives of this study were:

- i. to assess and update an ethogram on reproductive and stress behaviours of female sambar deer in captive environment.
- ii. to observe the reproductive and stress behaviours and assessing progesterone and cortisol secondary metabolites concentrations of female sambar deer in captive environment.
- iii. to assess the relationship of reproductive and stress behaviours with environmental parameters of female sambar deer in semi-captive environment.
- iv. to study the correlation between reproductive and stress behaviours with progesterone and cortisol secondary metabolites concentration of female sambar deer and developing the reproductive behavioural-hormonal prediction model in semi-captive environment.

It is hoped that this study will increase the understanding of sambar deer's basic needs in captivity such as the design and management of captive breeding facilities, which will improve the welfare and conservation effort of the sambar deer.

1.4 Hypothesis of Study

Sambar deer in captive environment were expected to show lack of movement and become less sexually active especially during the peak hour of frequent human contact. Despite of their less reproductive behaviour, the progesterone hormonal pattern could help in showing their oestrus status. It is expected that sambar deer in captive environment shows a high occurrence of stress behaviours due to the frequent human contact and thus increase the concentration of the cortisol hormone metabolites.

Meanwhile, sambar deer in semi-captive environment were expected to show high occurrence of reproductive behaviours and low occurrence of stress behaviour. Their movements were supposed to be not restricted with many disturbances. Whenever the reproductive behaviour increases, the progesterone metabolites concentration drops to the baseline indicating they are having their oestrus. Less occurrence of stress behaviour will show a low levels of cortisol metabolites concentration.

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PUBLICATION

Hamid, N.H.A., Nadzir. M. N. H. M., Omar, J., Annavi, G., Jaafar, W. N.F. W., & Salleh, A. (2022). Non-Invasive Measurement of Progesterone and Cortisol Metabolites in the Faeces of Captive Female *Rusa unicolor* at Zoo Negara, Malaysia and Its Reproductive and Stress Behaviour. *Pertanika Journal of Tropical Agricultural Science*, 30 (2). DOI: <https://doi.org/10.47836/pjst.30.2.40>





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