RELATIONSHIP BETWEEN FEED MANAGEMENT TOWARD STRESS RESPONSES AND WELFARE OF HORSES IN MALAYSIA

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Eric Lim Teik Chung, PhD
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Stabling practices significantly alter horses' dietary and natural feeding behaviour, leading to the development of abnormal oral behaviours (AOB) such as oral stereotypy (OS) and redirected behaviour (RB). The present study has four studies. Study 1 was a cross-sectional study that were conducted in seven established equine facilities in Klang Valley, Malaysia, using 207 working horses consisted of equestrians, leisure riding, endurance, polo sport and patrolling. Data on the management and feeding practices were collected in each facility, and the horses behaviours were observed. In Study 2, a proximate analysis was conducted on feedstuffs to determine the nutritional composition and the horses' total daily dietary intake from each working group (n = 207), and their interaction with the two types of AOB (OS and RB). In Study 3, blood samples were collected from 48 horses representing low and high AOB levels from each of the horse working groups for the determination of haematology and biochemical parameters, plasma cortisol, ghrelin, and leptin concentrations, as well as the telomere length expression. Lastly, Study 4 determined the effect of a high forage, high fiber (HFHF) and high concentrate, low fiber (HCLF) diets on patrolling horses with high and low AOB levels. Findings found the amount of hay fed greatly influenced the expression of abnormal behaviors. Whereas, horses with longer working hours were more vulnerable to OS. The blood biochemistry profile was different between high- and low AOB horses. Plasma ghrelin and leptin were exclusively influenced by AOB. Telomere length was not associated with AOB. The data were further supported by the feeding experiment with HFHF and HCLF diets, which indicated that the type of diet affects the performance of AOB through their influence on ghrelin and leptin hormones except on telemere length. Hence, the present study provides novel findings that ghrelin and leptin hormones could be used as potential stress biomarkers in horses suffering from AOB.

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

HUBUNGAN ANTARA PENGURUSAN MAKANAN DAN TINDAK BALAS TERHADAP STRES DAN KEBAJIKAN KUDA DI MALAYSIA

Oleh

FARAH HANIS BINTI JUHARI

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Amalan mengandangkan kuda dapat mengubah tingkah laku pemakanan dan pemakanan semula jadi kuda dengan ketara, yang menjurus pada perkembangan tingkah laku abnormal oral (AOB) seperti stereotaip oral (OS) dan tingkah laku terarah semula (RB). Eksperimen ini dijalankan dalam empat kajian. Kajian 1 melibatkan kajian keratan rentas yang dijalankan di tujuh fasiliti ekuin yang terdapat di Lembah Klang, Malaysia yang terdiri daripada equestrian, aktiviti berkuda santai, daya tahan, sukan polo dan meronda. Data berhubung dengan amalan pengurusan dan pemakanan di kandang telah dikumpulkan. Dalam kajian 2, melibatkan analisis hampiran (proksimat) dijalankan pada bahan makanan untuk menentukan komposisi pemakanan dan jumlah pengambilan diet harian kuda bagi setiap kumpulan kerja (n = 207) dan interaksi kuda dengan dua jenis AOB (OS dan RB).. Dalam Kajian 3, sampel darah dikumpulkan daripada 48 kuda yang mewakili tahap AOB rendah dan tinggi daripada setiap kumpulan kerja kuda untuk menentukan parameter hematologi dan biokimia, kortisol plasma, ghrelin, dan kepekatan leptin, serta ekspresi panjang ukuran telomer.. Sementara itu, Kajian 4 melibatkan ujian pemakanan diet tinggi serat dan tinggi foraj (HFHF) dan diet tinggi pati dan rendah fiber (HCLF) untuk menerangkan kesan pemakanan terhadap AOB, profil darah, hormon plasma, dan panjang ukuran telomer relatif pada kuda peronda dengan tahap AOB yang tinggi dan rendah. Penemuan mendapati jumlah rumput kering yang diberi sangat mempengaruhi ekspresi tingkah laku yang tidak normal. Manakala, kuda dengan waktu kerja yang lebih panjang lebih terdedah kepada tingkah laku stereotaip oral. Profil biokimia darah adalah berbeza antara kuda AOB tinggi dan rendah. Plasma ghrelin dan leptin secara eksklusif dipengaruhi oleh AOB. Manakala tiada perkaitan dikesan antara ukuran panjang telomer dengan AOB. Data ini disokong oleh eksperimen pemakanan menggunakan diet HFHF, yang menunjukkan bahawa jenis diet mempengaruhi prestasi AOB melalui pengaruhnya terhadap hormon ghrelin dan leptin. Oleh itu, kajian ini merumuskan bahawa hormon ghrelin dan leptin berpotensi untuk digunakan sebagai biopenanda stres bagi kuda yang mengalami AOB.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVATIONS

AOB	Abnormal oral behaviour
OS	Oral stereotypy
RB	Redirected behaviour
LS	Locomotory stereotypy
SD	Standing alert
SA	Stand dozing
RP	Recumbency position
DM	Dry matter
CP	Crude protein
CF	Crude fibre
EE	Ether extract
NFE	Nitrogen free extract
Ca	Calcium
P	Phosphorus
DE	Digestible energy
HFHF	High forage and high fibre
HCLF	High concentrate low fibre
RBC	Red blood cells
Hb	Hemoglobin
PCV	Packed cell volume
MCV	Mean corpuscular volume
MCHC	Mean corpuscular hemoglobin concentration
WBC	White blood cells
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase

AST	Aspartate aminotransferase
GGT	Gamma-glutamyltransferase
CK	Creatine kinase
A:G	Albumin:Globulin ratio
ELISA	Enzyme-linked immunosorbent assay
eHBB	Equine haemoglobin subunit beta

CHAPTER 1

INTRODUCTION

1.1 Introduction

The equine industry in Malaysia is still small and volatile compared with that in other developed countries such as the United Kingdom, the United States, or Australia as well as other Southeast Asian countries, for instance, the Philippines and Thailand (Horse Production in the Philippines from 2005 to 2017, 2017; Developing Asian Equestrian Nations, 2018). Nevertheless, interest in the equestrian activity in Malaysia has been growing moderately since the late 20th century (Bashir, 1993). Currently, there are approximately 4.12 thousand horses in Malaysia, with the majority of horse-riding activities centralized in the Klang Valley region. Malaysia has five active turf clubs that are specific for horse racing (Developing Asian Equestrian Nations, 2018). In addition, other equine activities such as endurance, show jumping, dressage, polo, and leisure horseback riding are also popular in the country. The Malaysia equestrian team is actively participating in the Southeast Asian Games where the equestrian coaching and training in Malaysia are adapted from Equestrian Australia (James, 2017). This is because Australia is the major horse exporter to Malaysia (Developing Asian Equestrian Nations, 2018). Other exporter countries include New Zealand, the United States, and Great Britain (Selangor Turf Club, 2019). Hence, the knowledge on the management practices, horse care, and nutrition is commonly adapted either from Australia or the United Kingdom. To date, there is no precise and consistent information on horse management in Malaysia and this includes scientific information on the horses' behaviour and welfare.

Stereotypy is a repetitive behaviour pattern with no significant goal or function. It is considered to be abnormal as it only seen in the captive population and completely absent in its wild counterparts (Mason, 1991; Garner et al., 2003; Mason and Rushen, 2006). This behaviour is believed to be manifested by the state of the animal being frustrated, bored, or stressed because of the suboptimal condition of the housing condition and also could be a clinical symptom of the central nervous system dysfunction (McBride and Hemmings, 2009; Roberts et al., 2017). Once developed, animals persist in performing stereotypic behaviours are willing to work hard to perform the behaviour, even if it may cost a large amount of energy (Novak et al., 2015). Additionally, stereotypic behaviour is observed in the clinical psychological disorders: autism, schizophrenia, and attention-deficit hyperactivity disorder (Turner, 1997; Garner et al., 2003; Morrens et al., 2006; Cunningham and Schreibman, 2008).

The channelling hypothesis by Lawrence and Terlouw (1993) on stereotypy proposes that free-ranging animals' foraging behaviours are richly stimulated by a variety of environmental stimuli compared to poor housing conditions. Hence, the highly motivated foraging behaviour is modified into simpler sequences that are allowed by the restricted environment. Supporting this idea, Fernandez (2021) introduced the foraging loop hypothesis, which suggested that the lack of stimuli (i.e., visual of food) in the foraging system makes the animal unable to complete the behavioural sequences, thus, locked in a repeated loop of stereotypy such as pacing. Therefore, animals are believed to demonstrate stereotypic behaviours in an attempt to cope with stressful conditions (McBride and Cuddeford, 2001; Pomerantz et al., 2012).

In horses, stereotypic behaviour is classified into two primary forms, which are the oral (OS) and locomotory stereotypic (LS) behaviours (Figure 1.1) (Sarrafchi and Blokhuis, 2013; Roberts et al., 2017). Common OS reported in horses are crib-biting/wind sucking, and licking, while weaving is the most common LS (Sarrafchi and Blokhuis 2013). Another type of abnormal behaviour that is also reported in stabled horses is redirected behaviour (RB). Coprophagy, wood chewing, and bed eating are some examples of RB in horses that are fed with a low fibre and high concentrate diet (Hothersall and Casey, 2011). Nutritional imbalance and other external factors such as limited access to turnout and lack of exercise may exacerbate the expression of these behaviours (Krzak et al., 1991; Waters et al., 2002b). The OS and RB are among the most prevalent abnormal oral behaviour (AOB) in stabled horses (Seabra et al., 2021). Both OS and RB are generally used as an indicator of poor welfare associated with stabling. Many studies suggest that keeping horses in the stable for 21 to 24 hours a day was associated with significantly increased risk of behavioural problems especially AOB (Hockenhull et al., 2014; Leme et al., 2014; Chung et al, 2018). The time spent in the stable may be influenced by the horse's type of work. For instance, horses that were used for show jumping and hire demonstrated the lowest occurrence of abnormal behaviours as they spend less than 12 hours a day in the stable box (Leme et al., 2014). Among the equestrian disciplines, endurance horses were less likely to develop AOB because they spent significantly longer time outside for training and competing as well as were kept in pasture compared with dressage horses which were more susceptible at 11.7% and 27.5% respectively (McGreevy et al., 1995b). However, a survey-based study in the United States reported that horses also engaged in crib-biting despite being allowed to socialise with other horses during turnout (Albright et al., 2009). A questionnaire survey on UK leisure horse owners by Hockenhull et al. (2014), reported that 48% of the respondent horses had OS and 52% with RB such as eat bedding or self-inflicted biting. Whereas, a survey by Leme et al. (2014), reported that 56% of horses from 10 equestrian centres in Atibaia, São Paulo State, Brazil, exhibited abnormal behaviours of which 30% were OS such as coprophagia, licking, wood chewing, crib-biting and wind sucking. The current trends in equine behaviour and welfare research focus mostly on the OS especially crib-biting and/or wind sucking compared to RB with the exception of wood chewing due to its association with crib-biting (Hothersall et al., 2009; Sarrafchi et al., 2013). Despite this, RB is still considered undesirable among horse owners due to the adverse effects on horse health.

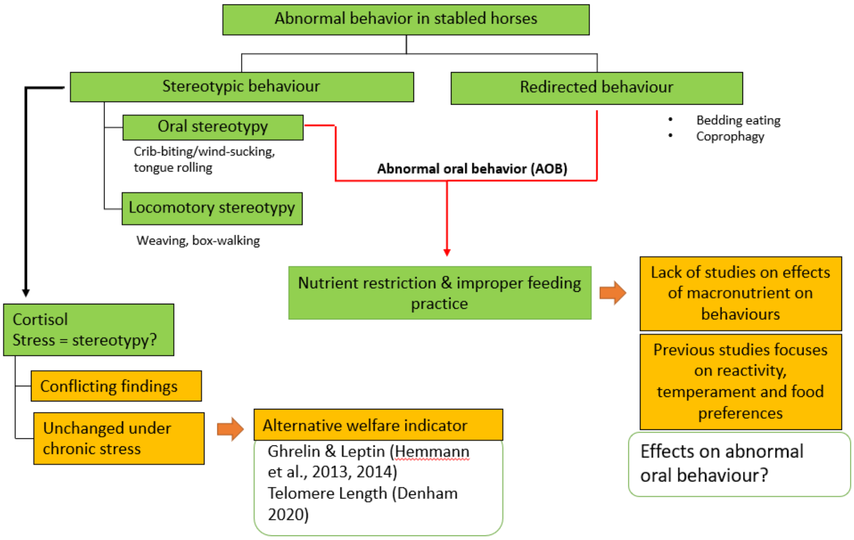

Figure 1.1 : Abnormal behaviour in horses. The present study focused on the oral stereotypic and redirected behaviours that were categorised as abnormal oral behaviour (AOB). In general, AOB was postulated to be caused by nutrient restriction and improper feeding practices, with macronutrients as the underlying factors

Management stressors such as nutrient restriction and inappropriate feeding strategies that limit foraging and food manipulation were major contributors to the development of OS and RB (Whisher et al., 2011; Sarrafchi and Blokhuis, 2013; Gussek et al., 2018). Pica and crib-biting horses had haematology and biochemistry parameters that differed from normal horses, indicating a nutritional imbalance or deficiency in the diet (Aytekin & Onmaz, 2010; Omidi et al., 2018; Li et al., 2020). Unbalanced macronutrient provision, such as fibre, protein, and lipids, underpins the development of AOB (McBride et al., 2001; Nicol et al., 2005; Hothersall et al., 2012). However, there is data paucity on the influence of nutrients on AOB in horses. Earlier studies on the impact of nutrients on horse behaviours focused on the determination of reactivity, temperament, and food preferences. According to Carranza et al. (2009), the reactivity of horses can be improved by increasing the dietary fats content. Alternatively, Nicol et al. (2005) reported that foals supplemented with a fat-and-fibre diet were less stressed compared to those fed with a starch-and-sugar diet. This lower reactivity in horses could be attributed to the elevation of blood glucose after meals following high-starch diets (Bulmer et al., 2015). On the other hand, the effects of macronutrients on the food preferences of horses were first published by Redgate et al. (2014) who indicated that horses preferred protein and hydrolysed carbohydrates over fats. van den Berg (2016) further confirmed that energy content did not influence horses' feed preferences. This is because, horses would compensate for low nutrients or calories in their diet by increasing feed intake (Edouard et al., 2010). In the same vein, feral horses were found to have complex feeding behaviours, where they consume a wide variety of plant species that are low in calories and high in fibre (Harris, 2007; Hampson et al., 2010). In addition, van den Berg et al. (2016a, 2016b) posited that organoleptic cues such as smell and taste would influence the choice of diets. For instance, horses were reported to prefer silage to haylage and hay (Müller and Udén, 2007). A recent study found that lateralization, odour, and excitability trait influenced feed preferences in ponies (Vinassa et al., 2020). At present, no study has been conducted to compare the influence of nutrients on AOB among different horse working groups. In working horses, information on the daily requirements and the recommended nutrient intake in working horses is crucial for their health and wellbeing in different conditions. The condition is often worse in tropical regions owing to the high environmental temperature and humidity leading to negative effects on horses.

Numerous physiological measurements have been used to study the relationship between stress and stereotypic behaviour in animals. The stress hormone cortisol has been studied extensively to determine the relationship between stress and stereotypic behaviour. Studies on the relationship between cortisol and stereotypic behaviour have faced many challenges due to the inconsistency of the findings. Furthermore, established stereotypic behaviour and chronic stress such as living in unfavourable housing conditions do not usually elicit changes in the cortisol level (Broom, 2017). Therefore, it is suggested that it would be beneficial to include some other welfare indicators in stereotypic behaviour studies. For example, ghrelin and leptin hormones were found to increase and decrease respectively under acute and chronic stress in humans and rats, which make these hormones putative candidates as a welfare indicator (Muhammad et al., 2018; Stone et al., 2020).

Mainly produced by the ghrelinergic cells located in the gastrointestinal tract, the ghrelin hormone is responsible for regulating food intake, energy expenditure, taste sensation, and controls hunger peripherally (Sakata et al., 2002; Müller et al., 2015). Several studies demonstrated that ghrelin modulates the food reward and motivation systems via activation of the mesolimbic dopamine pathway (Jerlhag et al., 2006; Jerlhag, 2008; Skibicka et al., 2011; Vestlund et al., 2019). Due to their unique asynergic relationship, changes in leptin concentration are often measured to complement the ghrelin study. Leptin is an adipokine hormone, which is mainly synthesized by white adipose tissue; as a result, this hormone has a positive correlation with the body fat percentage (Al Maskari and Alnaqdy, 2006; Fujita et al., 2019). In contrast to ghrelin, leptin mediates energy homeostasis by reducing food intake through its action on the central nervous system to regulate the sensation of satiety, and motivation behaviour at the hypothalamic sites (Blundell et al., 2001; Figlewicz, 2003; Morton et al., 2005).

The production of ghrelin and leptin hormone are regulated by food intake (Menzies et al., 2013). The ghrelin hormone peaked before meals, whereas leptin increased postprandially after meals (Müller et al., 2015). For instance, rats fed on fixed scheduled showed peak of plasma ghrelin before meal compared to ad libitum fed rats (Drazen et al., 2006). On the contrary, serum leptin demonstrated a postprandial reduction after meals in control and refeeding cattle (Delavaud et al., 2002). The relationship between ghrelin-leptin and eating disorders has been studied for two decades in humans. The elevation of ghrelin has been linked with eating disorders such as anorexia and bulimia nervosa, and psychiatric illnesses such as post-traumatic stress disorder (PTSD) and depression in humans (Wittekind and Kluge, 2015; Stone et al., 2020). In contrast, binge eating had elevated leptin level but was reduced in anorexia nervous compared to healthy person (Monteleone et al., 2004; Cassioli et al., 2020). Hence, the incorporation of ghrelin and leptin could shed some light on the complex developmental processes of the equine oral abnormal behaviours chiefly the stereotypies.

Apart from the endocrine system, stress can also modify the biochemical environment at a cellular level, as seen in the progressive loss of telomere sequences also known as telomere erosion in somatic tissues (Boukamp, 2001; Shalev, Entringer, et al., 2013). Telomere plays an important role in protecting the chromosome from end-to-end fusion, recombination, and degradation (Wai, 2004). Human telomere length decreases with age at a rate of 24.8-27.7 base pairs per year whereas 7000 base pairs per year in mice (Whittemore et al., 2019; Shammas, 2011). However, humans studies have suggested that early exposure and chronic stress are associated with accelaration of telomere erosion (Puterman et al., 2016; Li et al., 2017; Mayer et al., 2019; Lu et al., 2019). For that reason, the telomere length serves as an indicator of the cellular aging process to predict the onset of diseases and earlier mortality (Shalev, Entringer, et al., 2013). The regulation of telomere is controlled by genetic as well as environmental factors (Srinivas et al., 2020; Fernandes et al., 2021). Several other factors such as oxidative damage, exposure to stress, psychiatric illness, age-related diseases, and unhealthy lifestyle could also affect the telomere length (Shalev et al., 2020). Telomere length has been applied in numerous stress-related studies for human and animal welfare. Recent development in the study of horses has shown that telomere length could be used as a potential indication of health and welfare (Denham, 2020).

1.2 Problem statement

The high prevalence of AOB in horses has been linked to dietary concerns. However, it is unclear how dietary intake influences the expression of AOB in horses, and the pathophysiology of AOB in horses remains a conundrum. Recent research on the relationship between ghrelin and leptin, and OS offers new insights into some of the complex mechanisms underlying the AOB in horses. Therefore, this study aims to develop an understanding of how dietary intake is associated with the expression of AOB in working horses.

1.3 Hypothesis

1. OS would have the highest prevalence of abnormal behaviours in the studied population.
2. The total dietary intakes of horse working groups would varies depending on the workload, and are inversely associated with the likelihood of OS and RB.
3. There would be significant differences in haematology and biochemistry parameters between low and high levels of AOB among horse working groups.

4. AOB and working groups would have a significant influence on plasma ghrelin and leptin concentrations as well as telomere length.
5. The feeding treatment of high forage high fiber (HFHF) would reduce plasma cortisol and ghrelin and simultaneously increase the plasma leptin concentration and the rate of relative telomere length.

1.4 Objective

The general objective of this study was to investigate the prevalence of AOB among the horse population in Malaysia and to explore the physiological characteristics of AOB as well as its association with dietary intake. The specific objectives were:

1. To investigate the prevalence of abnormal behaviours and AOB population in different working horses.
2. To evaluate the feeding management and dietary intake of AOB among horse working groups.
3. To measure haematology and biochemistry parameters in low and high AOB among horse working groups.
4. To analyse the ghrelin and leptin hormone concentration and telomere length in low and high AOB among horse working groups.
5. To elucidate the effect of the HFHF diet using the 15 g DM/kg BW formula on the expression of AOB, concentrations of ghrelin, leptin, and cortisol, and relative telomere length.

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