Dissertation submitted in partial fulfilment for the Degree of Bachelor of Science Applied Animal Welfare Science

By Jack Anderson

March 2022

A double-blind, placebo-controlled study investigating the use of Pet Remedy in the reduction of stress and anxiety related indicators in horses prior to exercise

Abstract

Several potential situations could be deemed stressful to any horse; transport, competition, separation or herd changes. Stressful situations may contribute to performance and health concerns, highlighting the need for effective treatment. There are currently a broad range of stress treatments available for horses but no published evidence into their efficacy. I have therefore investigated the efficacy of one valerian-based product (pet remedy) in the treatment of stress in horses prior to exercise. To ensure accurate conclusions, a range of physiological and behavioural indicators were monitored. Baseline data was collected when horses were at rest to allow comparison to pre-exercise data. A double-blind placebo method was used with horses being treated topically with either water or pet remedy. Cortisol concentrations were monitored using salivary swabs and heart rates, (HR), were monitored by measuring cardiac beat to beat intervals. In addition, eye temperature was recorded using infrared technology (IRT) and behaviours were continuously monitored. 30 horses were used in this study, 10 mares and 20 castrated males. Stress and anxiety behaviours were rarely seen when the horses were at rest, 0.77% of the total time observed were stress behaviours seen by comparison, 7.9% was observed after application of pet remedy and 9.1% for the water placebo. In addition, rest and maintenance related behaviours were more commonly seen after the application of pet remedy. Chi square data analysis found a significant association between the bottle used and behaviours seen. There were statistical associations between eye temperature and age of horse as well as exercise levels. Eye temperature results did not correlate with other data sets questioning the efficacy of the methodology used. Mean heart rates were lowest at rest (30.46), pet remedy mean heart rates (32.3) were lower than that of the water placebo (35.66). In addition, there was a statistical variance found between the heart rate at rest and water placebo, evidencing the product keeps the heart rate close to at rest levels. Cortisol analysis found statistical association between cortisol concentration and sex of horses. Statistical tests found no variance between at rest data and either bottle of treatment.

This study has found pet remedy to be effective in the treatment of stress in horses prior to exercise. Limitations of the study include sample size; it is also recommended to focus data collection on a particular sex of horse as well as life stage. Other recommendations could also include adjustments to the methodology when using IRT to ensure accuracy of results.

Acknowledgements

Throughout the writing of this dissertation, I received a great deal of support and assistance from a variety of people that I would like to acknowledge.

I would firstly like to thank my supervisor, Grace Bell, whose academic and equestrian knowledge has been fundamental in the formulating of this research methodology and data analysis. I would like to acknowledge the staff at SOMA bioscience laboratory for their assistance in analysis of the horse salivary swabs. I would also like to thank the horse owners for trusting me with the use of their horses for this study I am very grateful to each owner for their participation in this study. Finally, I would like to thank my friends and family for supporting me in the completion of this dissertation.

Contents

Section 1: List of Figures and Tables

Section 2: Introduction and Aims

2.1: Introduction

The importance of understanding equine behaviour and health is paramount to improving and ensuring their welfare and meeting the requirements of animal welfare legislation. Equine welfare can be assessed by using a range of physiological and behavioural indicators (Gregic, 2020).

The domestic horse (*Equus ferus caballus*) is believed to have evolved from a fox-like species eolippus or dawn horse (*Hyracotheriuym*) approximately fifty million years ago (Houghton, 2003). The domestic horse is believed to have been in its current form for approximately two million years. Horses were originally a food source for humans, eventually being domesticated to carry goods with riding believed to have first started around 1500 BC and soldiers using horses in conflict from around 1000 BC (Houghton, 2003). The relationship between horses and humans has continued to develop, with equines being used in a variety of contexts from animal assisted therapies (AAT), to competition and leisure riding. Alongside this, horses continue to be used for traditional methods such as agricultural work worldwide. At present, leisure riding is the most common equestrian pursuit in the United Kingdom (UK) with approximately 847,000 horses kept nationally (British Horse Society, 2021), these are spread across 374,000 (British Equestrian Trade Association, 2022) homes in Britain. There is no available statistics on the number of horses being ridden in the UK.

Currently horses are exposed to a number of potential stressors including exercise (Henshall et al, 2022), training (Schmidt et al, 2010), competitions (Fazio et al, 2008) and veterinary treatment (Berghold et al, 2007). Typically, equestrian based research is focused on horses during or after exercise with physiological indicators generally used, as horses being ridden are unable to express natural behaviour. This research is timely as there is currently minimal research into anticipatory stress in horses prior to exercise, particularly leisure ridden horses. There has however been research into anticipatory stress in race horses. This research focused on the use of plasma cortisol concentrations and heart rate variability (HRV) and found cortisol concentrations did increase but this wasn't correlated with a change in HRV. The research concluded that the cause for this could have been initially higher cortisol concentrations than expected and summarised with the need for further research (Bohak et al, 2018). Evidencing the importance of this study.

Horse owners typically want to achieve high welfare for their horses as well as improve their overall performance. Physical exertion will affect the horse's homeostasis temporarily (Becker-Birck et al, 2013), it may also be altered in preparation for exercise which this study will explore further. Physiological methods for ascertaining stress levels typically include heart rate variability (HRV), this method monitors changes to rhythm as a stress response. Other methods include monitoring cortisol concentrations. Previously, plasma-based samples were used, however, more recently salivary samples have been developed avoiding the requirement for venepuncture which if done repeatedly could compromise horse welfare. Infrared thermography (IRT) has more recently been used as a non-invasive method for getting an animal's body surface temperature (Trindade et al, 2019). With studies also finding positive relationships between an increased eye temperature and salivary cortisol concentrations in horses (Cook et al, 2001), it is noted that this is the only study of its type evidencing the need for further research. There are alternative methods that have also been utilised including the horse grimace scale (HGS) (Costa, 2014) and spontaneous blink rates (Mott, 2020). Whilst some of these methodologies are new and emerging, all studies appear to use a range of methods to ensure accuracy of data, as well as allowing researchers to make correlations to accurately justify or disregard hypothesis.

There is a range of pheromone-based treatments available for the treatment of stress in horses, however, there is very little published literature into their efficacy. There have been published studies into the efficacy of pet remedy in the treatment of stress in dogs and rabbits. The only published dog study found that the product was not effective in reducing stress in dogs but only used behavioural indicators as an assessment method (Taylor & Madden, 2016). Research into the management of stress in rabbits found that pet remedy was effective in the reduction of stress and used both behavioural indicators and HR monitoring (Unwin et al, 2019). There is some research into the efficacy of valerian in the treatment of stress in dogs, but again this is minimal and more research is required (Buckley, 2019). Horses, rabbits and dogs fall within the same taxa Mammalia, whilst they have behavioural and physiological similarities these are minimal, evidencing the need for further research into the products efficacy across a range of species.

This research aims to investigate whether pet remedy is effective in reducing physiological and behavioural stress related indicators in horses prior to exercise. A double-blind placebo was utilised in order to account for any observer bias. Given the range of behaviours that could be displayed, a simplistic ethogram was compiled and observed behaviours were recorded. In addition, physiological indicators were measured, heart rate (HR), eye temperature (ET) and cortisol concentrations. It was predicted that stress indicators would increase prior to exercise when compared to 'at rest', baseline data. To make this comparison the same methodology was used for baseline data as it was for pre-exercise data, with the exclusion of the application of pet remedy or water placebo.

On review of current literature there are no studies to date that have tested the efficacy of pet remedy in the treatment of horses. I hypothesized that the product is effective in the treatment of stress in horses by reducing the frequency in which indicators are seen. These predictions are based upon previous research where either Pet Remedy (Unwin et al, 2019) or valerian (key active ingredient) has previously been found to reduce stress in other species (Murphy et al, 2010).

As part of this study a broad range of equestrian based research has been reviewed and analysed, due to the lack of available literature in some key areas additional non equestrian based research has also been considered. This is discussed within the literature review where emphasis on each methodology has been considered. This study includes the application of different behavioural and physiological techniques, these have been explained within the methodology section of this study. Including the order in which the tests were undertaken, timings and methods for the accurate recording of data. Results have been analysed per methodology and these have been discussed and interpreted within the discussion section of this study. The research concludes with the overall outcomes of the study, limitations and recommendations for the future.

2.2 Aims

Aim: To test the efficacy of a commercial valerian-based product for the treatment of stress in animals, by measuring the effects on behavioural and physiological stress related indicators and anxiety in horses prior to exercise once exercise equipment has been applied.

2.3 Objectives:

1. Horses have their heart variability monitored at rest and prior to exercise.
2. Horses have their cortisol concentrations monitored at rest and prior to exercise using salivary swab-based analysis.
3. Horses have their eye temperature recorded at rest and prior to exercise.
4. Horses have their behaviours monitored at rest and whilst being prepared for exercise
5. Horse owners will complete a horse personality-based questionnaire

Being able to accurately analyse and compare stress related indicators in horses at rest and prior to exercise will evidence whether there is a requirement for treatment and whether pet remedy is effective in the treatment of stress in horses.

H_0 – There will be no statistically significant difference in the behavioural and physiological indicators of stress when using a calming spray on horses prior to exercise.

H_1: There will be a statistically significant difference in the behavioural and physiological indicators of stress when using a calming spray on horses prior to exercise.

Section 3: Literature review

3.1 Stress related indicators

There are a variety of tested methods for measuring both physiological and behavioural stress related indicators in equines. Research in this area is predominantly focused on measuring stress by the use of cortisol analysis (CA) through saliva and plasma samples. Other methods include studying behavioural indicators, facial expression – horse grimace scale (HGS) (Costa, 2014), heart rate variability (HRV) (Lewinski, 2013), faecal immunoglobulin A (IGA) (Krueger et al, 2019) and spontaneous blink rates (SBR) (Mott, 2020). Research undertaken by Peeters (2013) found no correlation between stress levels in horses and performance, however they did find that riders with higher stress levels did get more penalties, evidencing that rider stress can impact their performance and therefore the performance of the horse (Peeters et al, 2012). This is supported by research undertaken by Kang (2016), who found correlation between horse and rider and how the stress levels were influenced by horse riding lesson programmes (HLP), indicating that if stress levels in the rider are lower this will have a positive impact on the horse's performance (Kang & Yun, 2016). By understanding and being able to identify stress related indicators in horses allows owners to make educated decisions to improve their horse's welfare, by doing so owners can also impact horse performance.

3.2 Anticipatory stress

Whilst there is a significant amount of research into stress related indicators in horses after exercise there is minimal research into these indicators in anticipation of exercise or other potentially stressful situations. Whilst research is minimal there is some published literature on this topic. Research undertaken by Bohak (2018) in anticipatory response before competition in race horses found an increase in cortisol concentrations and heart rate prior to competition indicating the horses were stressed in anticipation (Bohak et al, 2018). However, it should be noted that the sample size used in this study was small, only 8 horses. In addition, a noticeably high basal rate could suggest other factors influencing stress and therefore effecting validity of results. Research undertaken by Becker-Birck (2013) into cortisol release and HR variability found that HR increased in preparation for competition (Becker-Birck et al, 2013), unfortunately only a small sample of horses was used for this study, 13. Each horse had salivary samples taken 16 times over the course of the study ensuring the validity of the results.

Whilst there is minimal research into anticipatory stress in horses and other animals there is published research into anticipatory stress in humans. Research undertaken by Starcke (2008) tested stress levels in 20 students in anticipation of giving a public speech using a questionnaire and salivary cortisol analysis. The research found that all cortisol concentrations increased in anticipation of giving a public speech when comparing to basal concentrations (Starke & Oliver, 2008). This is supported by research undertaken by Edwards (2010) who found increased cortisol levels in female netball players prior to competition evidencing anticipatory stress (Edwards & Kurlander, 2010), however only a small sample was used in this study. Whilst these studies were on humans some direct comparisons can be made with horse related research as both are found within the same taxa and therefore do share some physiological similarities. Overall, however, there is a lack of published data on anticipatory stress in horses evidencing the requirement for further research.

3.3 Pheromone sprays

Whilst there is a plethora of research on the methods for ascertaining stress levels in horses there is little research on the effective management of it. There is currently a wide range of pharmaceutical interventions available for the treatment of stress in horses, these come in a range of forms from sprays, herbal supplements and syringe applied treatments.

A popular herbal supplement produced by Science Supplements is Pro Kalm, a syringe or daily fed supplement. Whilst there is no published evidence into its efficacy in the treatment of stress in horses it does contain ingredients that are known for stress related benefits. *Withania Somnifera* is referred to as ‘Indian Ginseng’ and has benefits for the treatment of stress in humans (Kulkarni & Ashish, 2008). It also contains *Passiflora incarnata*. Whilst widely used for the treatment of stress and anxiety in humans, research undertaken by Dhawan (2001) found little evidence to prove its efficacy (Kamaldeep et al, 2001), this was supported by Miroddi (2013) who also evidenced the need for further research into medicinal benefits (Miroddi et al, 2013).

Free step is a spray on calmer produced in the UK, active ingredients include chloride and magnesium, there is currently no published evidence that supports the use of these ingredients into the treatment of stress in any animals, there is also no published evidence into the efficacy of Free Step.

Pet Remedy is a valerian-based spray on product used to treat stress for a variety of animals currently however, there is no published evidence into the efficacy of Pet Remedy as a treatment for stress in horses. There are currently two published articles on the efficacy of Pet Remedy as a treatment for stress in dogs. Research undertaken by Taylor and Madden (2016) found the product demonstrated no efficacy in a sample of stressed dogs (Taylor & Madden, 2016). Research undertaken by the Unex Designs Ltd found the product was efficient at improving behaviour/reducing excitability in a dog sample, however this research has not been published in a peer-reviewed scientific journal (Buckley, 2019). Whilst there is no published evidence into the beneficial use of pet remedy in the treatment of stress in dogs there is published research into the efficacy of valerian root essential oil as a method of reducing stress related behaviours in dogs (Binks & etal, 2018). This study found stress related vocalisations decreased and rest related behaviours increased with the presence of the product. However, only a small sample of dogs was used and the study used a comparative methodology on the efficacy of multiple products. These papers have been reviewed by Buckley (2019) concluding that currently there is insufficient evidence to show that Pet Remedy is effective in reducing stress in dogs but that there was some evidence into the efficacy of valerian (Buckley, 2019). It is worth noting that these three studies recorded only behavioural indicators and currently there is no published peer reviewed research into the efficacy of Pet Remedy using physiological indicators as a means of measurement, evidencing the need for further research. By comparison there is currently 1 published piece of research into the efficacy of Pet Remedy in the treatment of stress in rabbits. Research undertaken by Unwin (2019) used novel environments when handling rabbits and monitored behavioural indicators as well as heart rate variability (HRV). This study evidenced a significant decrease in HRV during handling when Pet Remedy had been applied as well as significant increase in the number of positive behaviours displayed indicating that Pet Remedy may have potential value for rabbits during periods of acute stress. The study was a double-blind placebo-controlled trial and utilised a large sample to ensure accuracy of evidence. Whilst there is a small amount of published data into the efficacy of Pet Remedy for the treatment of dogs and rabbits the small amount of data available as well as the lack of horse related data evidences the need for further research into the efficacy of this product.

It is apparent that there is a vast array of pharmaceutical interventions available to horse owners to treat stress in their horses. There is, however, little proof of their efficacy evidencing the need for further research. By evidencing the efficacy of these products this will allow owners to make informed choices into the products they use to treat stress in their animals and subsequently improve horse welfare.

3.4 Cortisol analysis

The utilisation of cortisol analysis (CA) for the determination of stress levels in horses is well studied. Whilst some researchers have used plasma secretions others have opted for salivary swab analysis. This method allows for unlimited swabs to be undertaken with minimal welfare considerations by comparison to plasma secretions (Becker-Birck et al, 2013). Research undertaken by Dlugosz (2020) focused on evaluating the cortisol levels of recreational horses using salivary samples at three points, morning, evening and immediately after exercise. This research indicated the type of activity and age of the horse impacted the concentration of cortisol, younger horses having higher concentration than older horses. Concentrations increased after exercise, with dressage horses having the highest concentrations and horses being long reined the lowest. In addition, this research indicated that gender and breed had no influence over cortisol concentration (Dlugosz, 2020). The methodology and results of this study are supported by research undertaken by Aurich (2015) this research focused on the effects of season on cortisol concentrations found that sex and age of horses made no apparent effect on basal cortisol concentrations. This study, however, found that concentrations did change subject to the time of day as well as the season, in addition finding that sexually mature stallions were subject to higher cortisol concentrations (Aurich, (2015)) somewhat contradicting previous research. Both studies found that cortisol concentrations decreased over periods of time with Dlugosz (2020) stating 'this result may suggest that with age and experience, horses acquire the skills to cope with stress caused by working' (Dlugosz, 2020). This statement would be supported by research undertaken by Gehlen (2020) who analysed cortisol concentrations in horses with acute abdominal pain finding that at all horses had high concentrations on arrival at the veterinary clinic but following a period of settling and treatment found that cortisol levels decreased over time (Gehlen & etal, 2020). Research undertaken by Peeters (2013) compared rider and horse cortisol levels, finding that both increased at similar points, with the increase being stronger in the rider. No correlation between rider stress levels and horse stress levels were found (Peeters et al, 2012). This is key as it evidences rider/handler stress levels and its impact on horse stress levels.

There are limitations to ascertaining cortisol concentrations using salivary swabs. Research undertaken by Sauer (2020) found that equine salivary cortisol can be reliably measured using liquid chromatography-tandem-mass spectrometry (LC-MS/MS), immunoassay baseline salivary cortisol measurements correlate poorly with those by LC-MS/MS. This research found consistently higher cortisol levels measured after adrenocorticotrophic hormone (ACTH) stimulation using immunoassay, questioning this method's reliability (Sauer et al, 2020). This is disputed by research undertaken by Peeters (2010), this research compared blood serum results to salivary cortisol concentrations and found the method to be reliable (Peeters et al, 2010). Whilst there are questions into the reliability of salivary cortisol analysis it is a non-invasive method for cortisol level assessment and therefore further research into its accuracy would be beneficial for horse welfare.

3.5 Heart Rate Variability (HRV)

The use of monitoring heart rate variability (HRV) in horses as a stress related indicator is common. The reasons for this is the process is relatively simple either through the use of heart monitoring equipment or through the use of a stethoscope. As well as being simple the process should not be detrimental to an animal's welfare and the method is typically used alongside other physiological and behavioural methods to ensure validity of data. Research undertaken by Lewinski (2013) utilised cortisol analysis as well as monitoring HRV to understand horse and rider responses to training and performance. Finding that HRV for both riders and horses increased during riding which is unsurprising due to physical exertion. However, the HRV in horses didn't significantly increase between rehearsal and public performance whereas it did increase for riders (Lewinski, 2013). This data was used in conjunction with cortisol analysis to ensure accurate outcomes of the research. Research undertaken by Kim (2021) used Heart Rate (HR), Rectal Temperature (RT) and Respiratory Rate (RR) as a method of ensuring validity of Eye Temperature (ET) assessment using Infrared Thermography (IRT) to assess welfare in horses (Kim & Cho, 2021). Data evidenced that HR data is reliable and can be used to comparatively ensure the validity of new technologies such as IRT when making assessments regarding animal welfare. The reliability of HRV has also been identified by Schmidt (2010) who used HRV alongside cortisol analysis to identify whether horses were stressed during transport. Data indicated correlations between HR and cortisol analysis evidencing the accuracy of HR when establishing stress levels in horses (Schmidt et al, 2010).

There are a number of limitations when using heart rate to monitor stress levels in horses, a number of additional variables can impact a horse's heart rate. Exercise levels, fitness, age, housing method (pasture, stabled) can all influence behaviour and heart rate (Manrique et al, 2019) there is also the variety of methods for ascertaining heart rates and their reliability/accuracy. Research undertaken by Frippiat (2021) used a similar heart rate monitor (HRM) as identified in the methodology of this research, albeit using a different brand of device. This research concluded with an 'overall good reliability for both studied HRV-parameters, in rest and during exercise' (Fippiat et al, 2021).

3.6 Eye temperature (ET)

Whilst some methods for ascertaining stress levels in horses are commonly used such as HR and cortisol analysis there are emerging methods that are being used more frequently such as monitoring eye temperature (ET). Research undertaken by Bartolome (2021) utilised IRT to assess effort and recovery in sport horses using data to establish that breed and sex of the horse can impact recovery following physical exertion. This study is significant as a large sample of 495 horses undertook performance related tests alongside IRT data collection, concluding that 'IRT appears as an adequate tool to evaluate the physiological effort and recovery' (Bartolome et al, 2021). This statement is supported by research undertaken by Trindade (2019) who utilised fitness bio markers, respiratory rate, creatine kinase activity and IRT to establish whether ET was an accurate indicator or equine fitness in working ranch horses (Trindade et al, 2019). Concluding that eye surface temperature (EST) has 'potential to be used an indicator of physical fitness in ranch horses' (Trindade et al, 2019). This is supported as a result of research undertaken by de Mira (2021) who utilised IRT in a sample of 61 horses to establish physiological changes during endurance competitions, who used IRT alongside cortisol concentrations relating ET as a stress related indicator. This research indicated that ET increased in younger, less experienced horses supporting evidence by Jansson (2021) and stated that eye temperature was a more immediate indicator than alternative methods (de Mira et al, 2021). Research undertaken by Jansson found standard errors below 0.2 and variance values below 1.0 evidencing reliability of this method (Jansson, 2021). This is also supported in other animal research, such as research undertaken by Scoley (2018). This used IRT in a study on dairy cows, establishing that were low levels of variability in results but suggested accuracy improved with multiple pictures taken and minimal environmental influence that could impact results (Scoley et al, 2018). Environmental influence on results is also referred to in research by Jansson (2021) and de Mira (2021). In all of these studies it has been suggested that a larger sample would improve data accuracy. Whilst these studies are not directly related to stress in horses the accuracy of the results indicate the validity of using IRT in equestrian related research. IRT is a quick method for ascertaining physiological changes in horses, the method is non-invasive and relatively stress free. There is minimal research identifying an increased eye temperature as an indicator of stress, it is presumed that a correlation between these can be made using other physiological and behavioural indicators, evidencing the need for further research.

3.7 Behavioural indicators

Utilising behavioural indicators as a method for ascertaining stress levels in animals is common. The method is typically used alongside physiological indicators to ensure validity of data. In some research behavioural indicators have been used as a sole method for measuring stress levels. Research undertaken by Binks (2018) and Taylor (2016) into the use of olfactory stimulation in dogs, used only behavioural indicators making it difficult to assure data accuracy. In equine related research the use of behavioural indicators as a method for ascertaining stress levels is often supplementary when used alongside physiological indicators. The reason for this is that the majority of equine related research is typically related to horse performance whilst being exercised and horses are usually unable to exhibit natural behaviours freely by comparison to that of a horse on pasture for example (Becker-Birck et al, 2013). Monitoring horse behaviour as part of a wider study was undertaken by Sauer (2019) when understanding the effects of breed, management and personality on cortisol reactivity (Sauer et al, 2019). This method was undertaken to assess associations between questionnaire data and salivary cortisol results. Identifying that 'breed and management parameters, such as number of riders, time spent outside and housing, influence the amount of stress hormones released' (Sauer et al, 2019). Concluding that optimising husbandry conditions rather than changing performance level, may be more important for improving the welfare of horses.

Research evidences that breed, management and personality can affect stress levels of horses. Research undertaken by Sauer (2019) found cortisol was affected by breed and three management factors including number of riders, hours spent outside and group housing (Sauer et al, 2019). This evidences that horses' personality and other factors can impact their behaviour although no significant association or cortisol concentration and personality traits were found.

For research where horses can behave naturally or when undertaking anticipatory behaviour research the study of behavioural indicators alongside physiological indicators can be valuable for data accuracy and correlation.

3.8 Rationale for this study

Research highlighted, found that stress related indicators in equines can be monitored using a variety of techniques. However, there are contradictions in a number of papers that dispute the findings in others evidencing the need for further research. Whilst some methods such as cortisol analysis using salivary swabs and the monitoring of heart rate variability have been used accurately on a number of occasions, new methods such as the use of eye temperature have less supportive data and would therefore benefit from further research. There is also minimal supportive evidence in to the use of Pet Remedy in the management of stress in equines. There is however, some supportive evidence for the use of the product in dogs and rabbits. To prove the products efficacy, further research is required. In addition, there is currently little available research into measuring stress in anticipation of horses being exercised, it is hoped that this research could become an objective tool for the assessment and improvement of horse welfare.

Section 4: Methodology

4.1 Samples and ethical considerations

A total of 30 horses were used for this study, these were housed at equestrian facilities across West Yorkshire and Derbyshire. The mean age of the horses was 13.3, horse age ranged from 3-30 years of age. Of the 30 horses 67% were geldings, the rest were mares (33%). Horses of differing ages, work levels and breeds were used in the study (appendix 1). Control data was taken where the owner thought the horse would be most calm, for some this was in the stable, for others this was on pasture.

Ethical approval for the study was given by the university ethics committee prior to any research being undertaken. Initially a horse personality questionnaire (appendix 2) and consent form (appendix 3) were completed by all horse owners. The majority of horses used in the study were clinically healthy, however there were some horses with medical conditions that were identified on the owner consent form. Medical conditions included Polysaccharide Storage Myopathy (PSSM), paralysed larynx, feather mites, suspensory issues, Pituitary pars Intermedia Dysfunction (PPID) and Equine Metabolic Syndrome. All of these conditions were being managed by the horse owners. Where appropriate physiological data for certain conditions was not included in statistical analysis. All horses were exercise sound and had no ongoing veterinary investigation.

4.2 Apparatus/materials

Various pieces of equipment were used to gather data including and ethogram (appendix 4) and continuous sampling form (appendix 5). Equipment used for physiological data included a heart rate monitor (Polar Equine H10), stethoscope (MDF dual head lightweight MDF747), mobile phone, infrared camera (FLIR E5xt Wi-fi) and swabs with suspensory fluid supplied by Soma Bioscience.

4.3 Study sites

Multiple equestrian facilities in the area were used, these included a riding school and different livery stables. The at rest data was collected either in the stable or in the field, dependant on the owner's recommendation.

4.4 Procedure

Application of Pet Remedy and placebo

When horses were monitored at rest no product was applied, this was to ensure the physiological data collected was accurate baseline data. At this stage a patch test of the horse calming spray was applied to the left shoulder of the horse in a small quantity. This would allow the owner to look for detrimental side effects to the spray. On the second and third visit the horses were treated with product from either bottle 1 or bottle 2. The product was sprayed 6 times onto a clean microfibre cloth, this was then rubbed on the face and chest of each horse prior to any grooming or application of exercise equipment. Horses were not sprayed directly as some horses can have aversions to being sprayed and this could have had an impact on the data gathered (Seaman et al, 2002). To avoid bias the researcher was unaware which bottle contained water and which contained the horse calming spray.

Behavioural observation

Horses were observed at three stages, once at rest either in the field or in the stable. The second and third occasions were prior to exercise. Horses had randomised application of bottle one or two, riders would then groom and tack up their horses. Whilst this was being undertaken any maintenance, bodily function, locomotion, rest, grooming or communicatory behaviours were recorded on the sampling form. For consistency the duration of at rest behaviour monitoring was 30 minutes per horse. The time spent grooming and applying exercise equipment per horse was not stipulated, this was to ensure consistency for horse and rider. The range of time spent preparing horses for exercise was between 10-41 minutes and the mean average time was 20.43 minutes.

Heart Rate (HR) variability

The cardiac beat-to-beat interval was recorded in horses using a mobile recording system where possible (polar equine H10 HR). However, as this research was undertaken in winter some horses were left unclipped and this meant the recording system was unable to get readings for all horses. On these occasions a stethoscope was used. Where the mobile recording system was used the manufacturer's instructions were followed, with the device and horse wettened prior to application with the electrodes panel applied to the chest. This then connected to an application on a mobile phone which allowed the data to be recorded. Where a stethoscope was used the cardiac beat was found by the researcher and a timer of 15 seconds was set, the result was multiplied by 4 to give the data recording. The method of mobile recording of heart rate in horses was performed as described by Lewinski et al (Lewinski, 2013).

Figure 1: Polar Equine H10 (ClockIt, 2021)

Eye temperature

Eye temperature images were taken after each behavioural observation. At rest this was taken after 30 minutes, for the following 2 observations these were taken after the owner had completed grooming and applying exercise equipment. Images were always taken by the same person using a portable infrared thermography (IRT) camera (FLIR E5xt Wi-Fi, FOL7 Lens, 640 X 480 resolution). For accuracy 3-4 pictures were taken per horse and the clearest used. All IRT images of the eye were taken with the camera positioned at an approximate distance of 1m from the side of the horse. Emissivity was 0.98, this was set automatically by the device at calibration (FLIR, 2019). IRT images were analysed using the FLIR Tools software (FlIR Systems, Inc). The maximum eye temperature was taken from the medial canthus region of the eye within an approximate 1cm radius of the palpebral margin as identified in figure 1. Where possible, efforts were made to minimise the impact of environmental conditions on the measured value. Images were collected in enclosed spaces away from wind and significant exposure to sunlight, however due to the nature of each equestrian establishment this was not always possible. The equipment used did take a minute or so to calibrate before use, this was done prior to attempting to use it on any horse. Allowing the camera to calibrate this ensured the camera operated within the specific manufacturer limits and ensured accuracy. This method for ascertaining eye temperature was performed in a similar method to that of Jansson et al (Jansson, 2021).

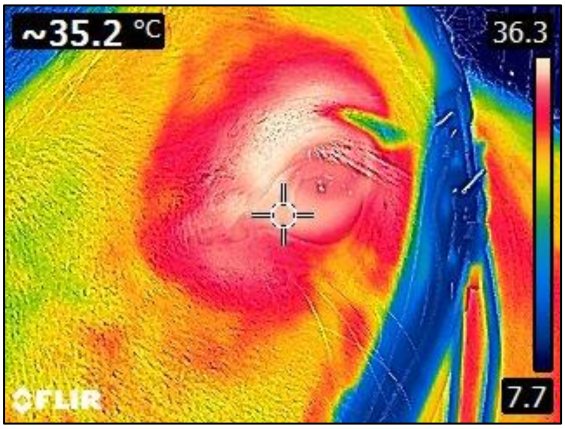


Figure 2: Medial canthus region (Anderson, 2022)

Cortisol analysis

Salivary samples for cortisol concentrations were taken from horses at 3 intervals. One occasion at rest following the completion of the behaviour observation. The second and third intervals were taken following the application of the spray with either bottle 1 or 2 and after grooming/application of exercise equipment. Salivary swabs were placed loosely into the mouths of horses on top of the tongue for approximately one minute, this ensured the swab was acutely soaked. The swabs were then placed into suspensory fluid, labelled and posted to Soma Biosciences laboratory for analysis. The method for saliva collection was done as described by Dlugosz (Dlugosz, 2020) and Lewinski (Lewinski, 2013). 29 horses used within the study had 3 salivary swab samples taken without demonstrating any negative behaviours towards the process, however 1 horse demonstrated stress related behaviours when it was attempted to swab the horse. It was decided not to persevere with the process to ensure the horse's welfare was not compromised.

Saliva samples were collected with an Oral Fluid Collector (OFC) (Soma OFCII, Soma Bioscience, Oxfordshire, UK) consisting of a synthetic polymer-based material on a polypropylene tube. The OFC has a volume adequacy indicator, giving a clear colour change when 0.5mL (± 20%) is collected. Analyte recovery has been demonstrated to be in excess of 85% following 1 minute shaking (Jehanli et al, 2011). Salivary Cortisol levels were determined, in duplicate, using enzyme immunoassay (EIA) test kits (Soma Bioscience Ltd., Oxfordshire, England). In this assay colour intensity is inversely proportional to the concentration of Cortisol. A dose-response curve using standard solutions of Cortisol, was used to determine the concentration of Cortisol (nM) in the saliva samples. The assay range was 0.25-40nM. Intra –assay coefficient of variation was < 7.85% whilst the inter-assay coefficient of variation was, < 5.8%.

4.5 Management procedures

The majority of data was collected by one researcher, however when collecting data from the riding school an assistant was used. To ensure consistency and reduce bias the assistant was trained to collect data by the main researcher. The majority of horse handling, grooming and application of exercise equipment was done so by the horse's owner, this was to ensure the process was controlled for each horse and to avoid handler influence on the data collected. As this research was undertaken during the COVID-19 pandemic additional conditions were in place to ensure the safety for all participants. Where possible social distancing was in place as well as regular hand washing and sanitisation.

4.6 Statistical analysis

Statistical analysis was completed with the SPSS statistics software (version 22.0, SPSS). A variety of statistical tests will be undertaken including tests of association (Pearson and Spearmans Rank) , analysis of variance (two-way ANOVA) and averages.

Section 5: Results and analysis

Horse information

A total of 30 horses were utilised for this study, 20 of which were male and 10 were female. All male horses were geldings. A variety of breeds were used within this study, cross breed (10), Cob (13), Thoroughbred (4), Persia (1), Arab (1) and Shire (1). The average age of all horses was 13.3, the average age of male horses was 12.75 and females was 14.75.

Exercise types

Participants were asked the main type of exercise they undertook with their horse, detail of which can be seen in table 1. In addition, owners were asked to estimate the maximum amount of time spent exercising their horse on a weekly basis, the average amount was 3.9 hours per week.

Table 1: Horse exercise types

Exercise type	n	Percentage of sample
Hacking	14	46.67%
Hacking and schooling	9	30%
Schooling	3	10%
Driving	1	3.33%
In hand	3	10%

Personality questionnaire

Each horse owner was required to complete a personality questionnaire prior to any research being collected. The information held within the questionnaire can be seen in appendix 2.

Horses at rest in the stable or at pasture rarely demonstrated stress/anxiety related behaviours. Only 0.77% of the total time (900 minutes) spent observing horses at rest saw horses demonstrating stress/anxiety related behaviours. After application of bottle 2 and equipment 58 minutes was spent demonstrating these behaviours. Owners spent an average 20.4 minutes preparing their horses for exercise, after application of bottle 1, 7.9%

(47 minutes) of the total time observing, horses demonstrated stress/anxiety related behaviours. After application of bottle 2, 9.1% (58 minutes) of time observed was horses demonstrating stress/anxiety related behaviours. When comparing this data to that of the personality questionnaire it could be suggested that owners perceive their horses demonstrate stress related behaviours more frequently than they do in practice, as on average 8.14% of owners stated that their horses frequently demonstrated stress/anxiety related behaviours whilst being prepared for exercise and 33.33% of owners stated that sometimes their horse can demonstrate these behaviours.

Statistical tests

Heart rate

Mean

The maximum heart rate was 48BPM and the minimum was 16BPM. The mean heart rate in horses at rest (n=30) is 30.46 ± 6.80.

The maximum heart rate was 48BPM and the minimum was 24BPM. The mean heart rate in horses after application of bottle 1 (n=30) is 32.33 ± 5.56.

The maximum heart rate was 48BPM and the minimum was 28BPM. The mean heart rate in horses after application of bottle 2 (n=30) is 35.66 ± 4.70.

Tests of association

There was no statistically significant associations between HR and the age of horses. (Pearson, n=30, HRCont-0.18, P=9.26, HRBot1 – 0.179, p=0.345, HRBot2 0.067, p=0.726).

There was no statistically significant association between heart rate (HR) and sex of horses. (Spearman rank, n=30, HRCont 0.180 p=0.342, HRBot1 0.176, p=0.353, HRBot2 0.186, p=0.324).

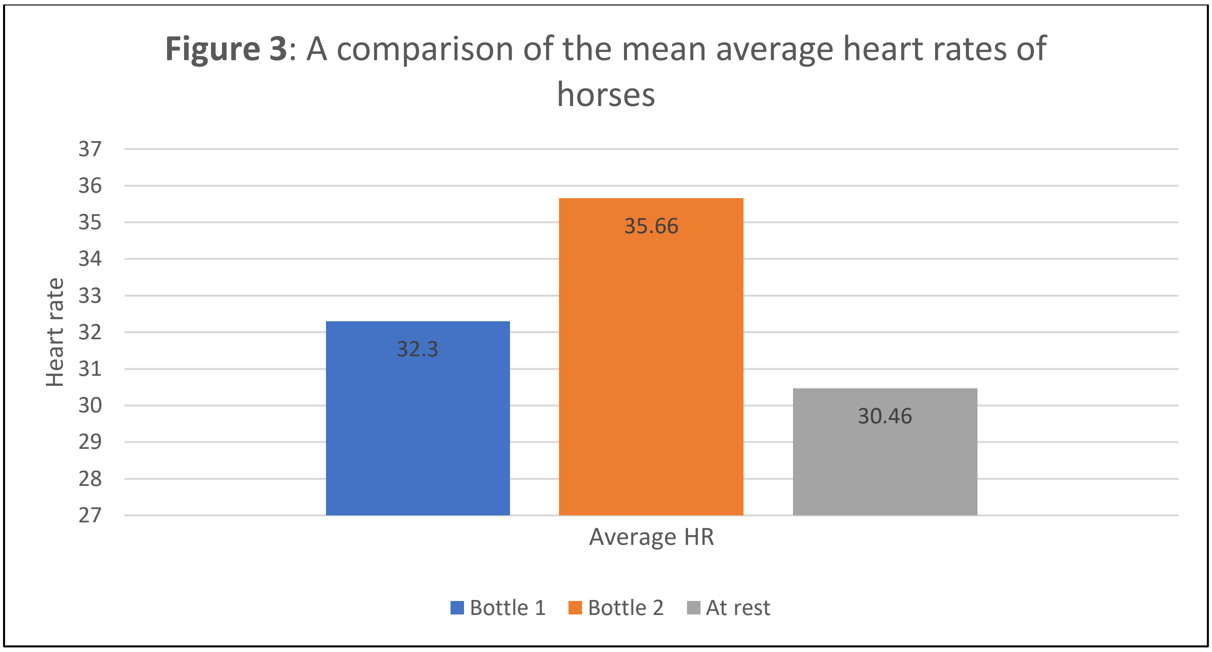
There was no statistically significant association between heart rate and hours exercised. (Spearman’s rank n=30, HRCont 0.123, p=0.518, HRBot1 0.106, p=0.578, HRBot2 0.61, p=0.749).

Analysis of variance

The results of the two-way repeated-measures ANOVA showed that there was a statistically significant difference between the HR of horses between the three categories. Difference is seen between the at rest score, 0.00 and bottle 2, 2.00. However, there was no significant difference seen between bottle 1, 1.00 and bottle 2.00. The result being close to significant at 0.069.

As identified in figure 2, heart rates were typically lower at rest than prior to exercise with the application of bottle 1 or bottle 2. Heart rates were also typically lower after application of bottle 1 prior to exercise when compared to bottle 2.

Figure 3: A comparison of the mean average heart rates of horses



Cortisol concentrations

Mean

The mean cortisol concentration in horses at rest (n=27) is 1.91 ± 1.05. The maximum cortisol concentration was 4.11 and the minimum was 0.25.

The mean cortisol concentration in horses after application of bottle 1 (n=27) is 1.62 ± 1.08. The maximum cortisol concentration was 4.59 and the minimum was 0.25.

The mean cortisol concentration in horses after application of bottle 2 (n=27) is 1.58 ± 1.56. The maximum cortisol concentration was 6.90 and the minimum was 0.26.

Tests of association

There was no statistically significant association between cortisol concentrations and the age of horses (Pearson, n=27, CortisolCont – 0.208, p=0.298, CortisolBot1 0.009, p=0.965, CortisolBot2 0.195, p=0.330).

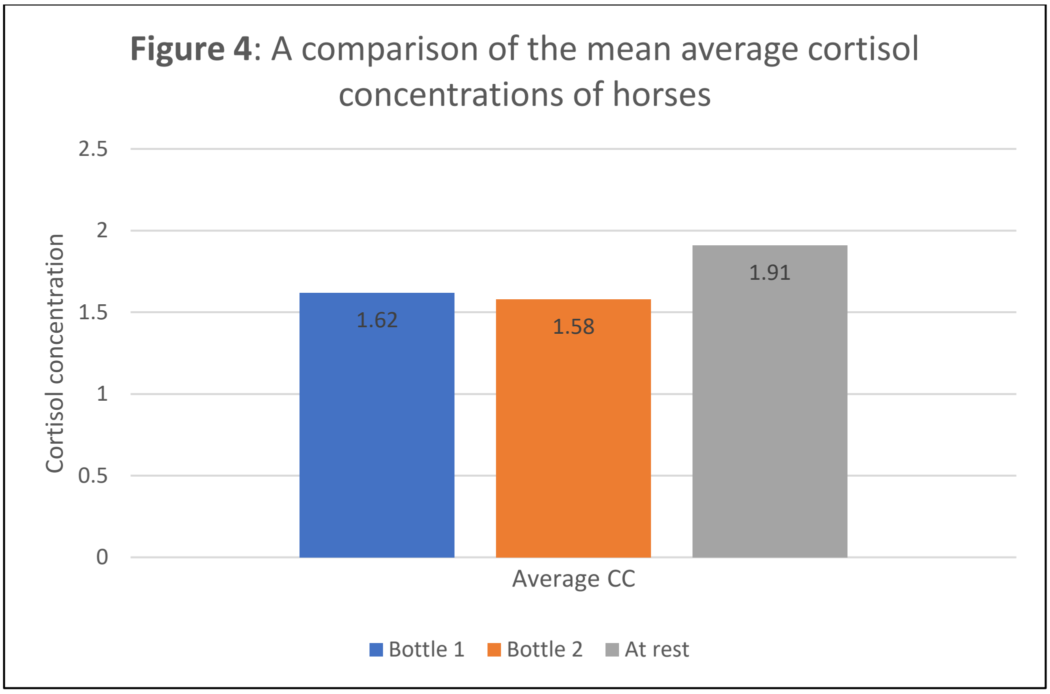
There was a statistically significant association between cortisol concentration and sex of horses. (Spearman rank, n=27, CortisolCont 0.083, p=0.679, CortisolBot1 0.427, p=0.026, CortisolBot2 0.338, p=0.084).

There was no statistically significant association between cortisol concentration and hours exercised (Spearmans rank n=27, CortisolCont 0.007, p=0.971, CortisolBot1 0.087, p=0.668, CortisolBot2 0.114, p=0.573).

Analysis of variance

The results of the two-way repeated-measures ANOVA showed that there was no significant difference between the salivary cortisol of horses between the three categories (ANOVA, between groups, 0.531. Bottle 1 to bottle 2, 0.995. Bottle 2 to bottle 1 0.995).

Figure 4: A comparison of the mean average cortisol concentrations in horses



Eye temperature

Mean

The mean eye temperature in horses at rest (n=30) is 36.30 ± 1.38. The maximum eye temperature was 38.5°C and the minimum was 33.6°C.

The mean eye temperature in horses after application of bottle 1 (n=30) is 37.06 ± 1.19. The maximum eye temperature was 38.90°C and the minimum was 33.20°C.

The mean eye temperature in horses after application of bottle 2 (n=30) is 37.57 ± 1.17. The maximum eye temperature was 39.60°C and the minimum was 34.10°C.

Tests of association

There was a statistically significant association between eye temperature and the age of horses (Pearson, n=30, EyetempCont – 0.365, p=0.47, EyetempBot1 – 0.136, p=0.472, EyetempBot2 0.401, p=0.028.

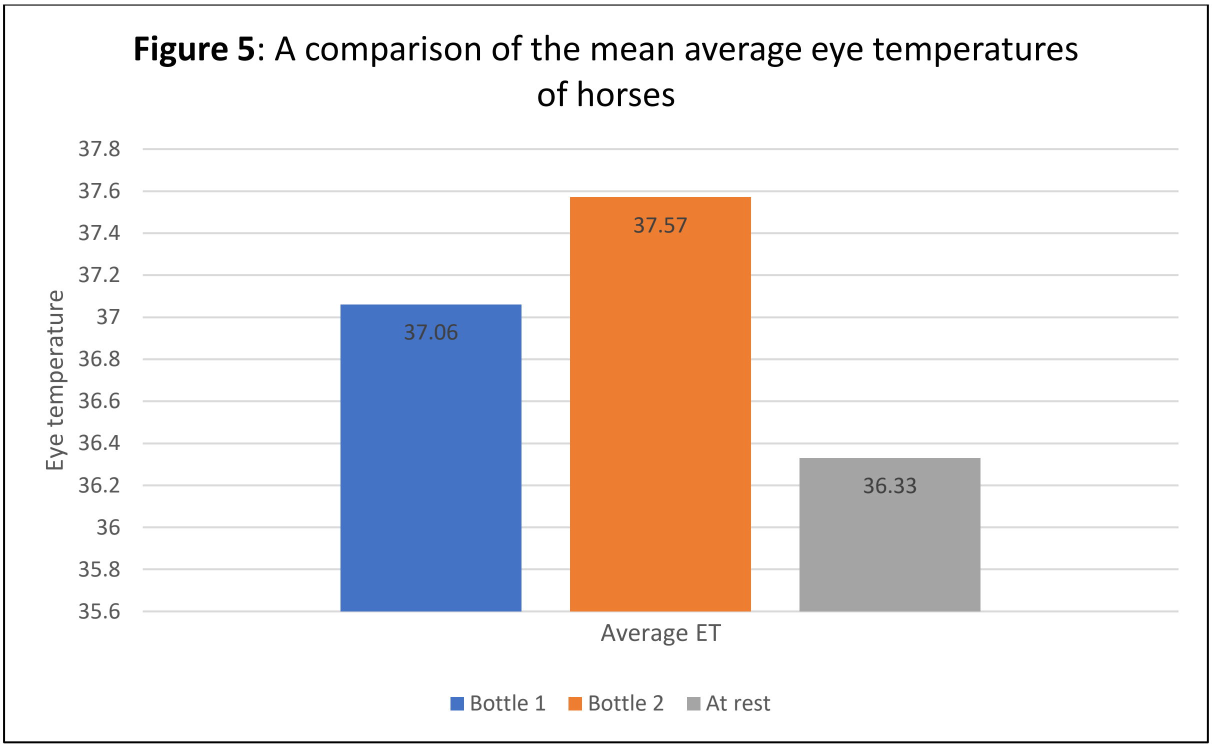
There was no statistically significant association between eye temperature and sex of horses. (Spearmans rank n=30, EyetempCont 0.244, p=0.194, EyetempBot1 0.112, p=0.555, EyetempBot2 0.124, p=0.514).

There was a statistically significant association between eye temperature and hours exercised (Spearman's rank n=3-, EyetempCont 0.247, p=0.189, EyetempBot1 0.220, p=0.242, EyetempBot2 0.508, p=0.004).

Analysis of variance

The results of the two-way repeated-measures ANOVA showed that there was a statistically significant difference of eye temperature between the three categories. The significant difference is seen between the rest score and bottle 1, 1.00. The result for bottle 1 compared to at rest data is also close to significant >0.05. There is no statistically significant difference between bottle 1 (1.00) and bottle 2 (2.00).

Figure 5: A comparison of the mean average eye temperatures of horses

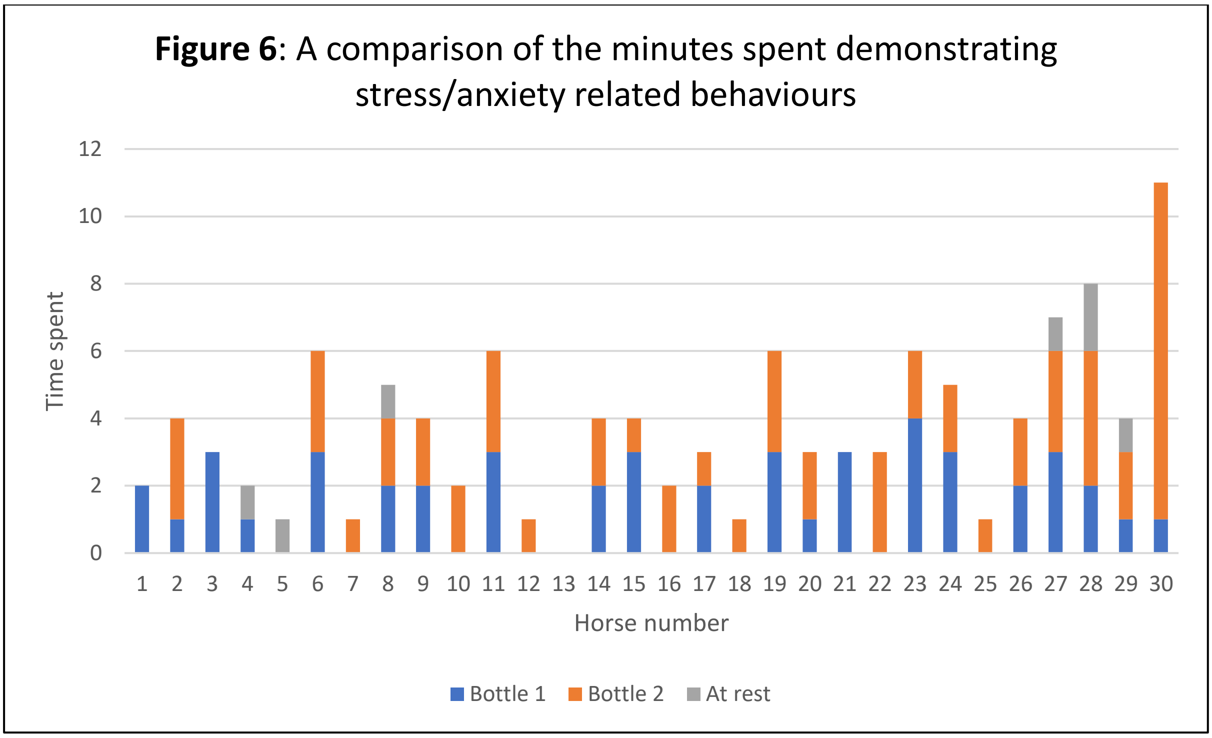


Behavioural data

Horses were observed and data recorded on maintenance, bodily function, locomotion, rest, grooming and communication. As only minimal amount of data related to bodily function, locomotion and grooming was recorded this has not been analysed as part of this study.

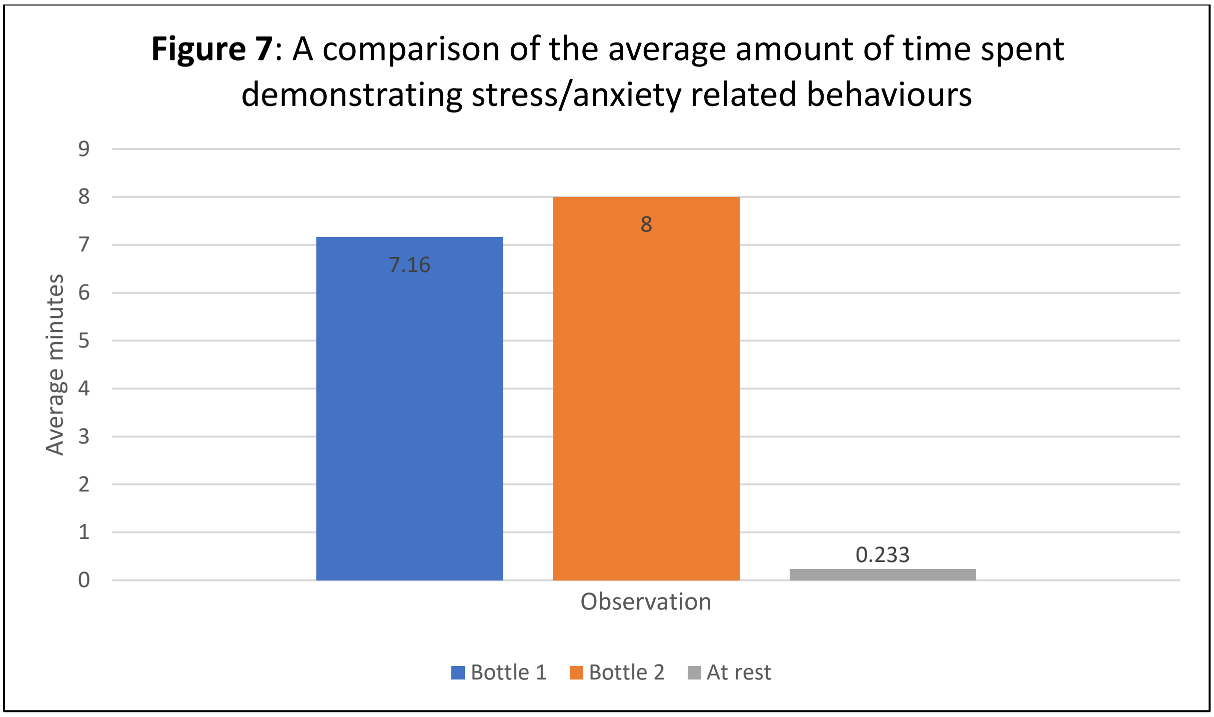
As identified in figure 5 stress related communicatory behaviours were more commonly seen after application of bottle 2 than bottle 1.

Figure 6: A comparison of the minutes spent demonstrating stress/anxiety related behaviours



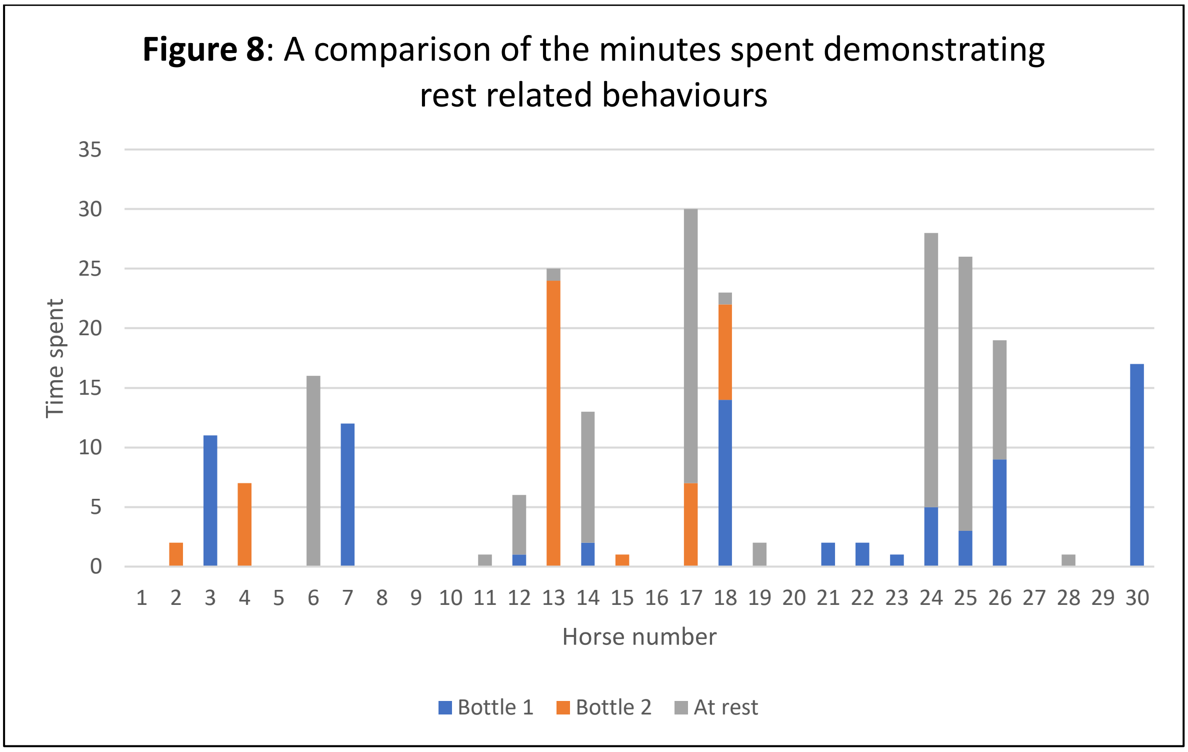
As identified in figure 6 on average stress related behaviours were less frequently seen after application of bottle 1 than bottle 2.

Figure 7: A comparison of the average amount of time spent demonstrating stress/anxiety related behaviours



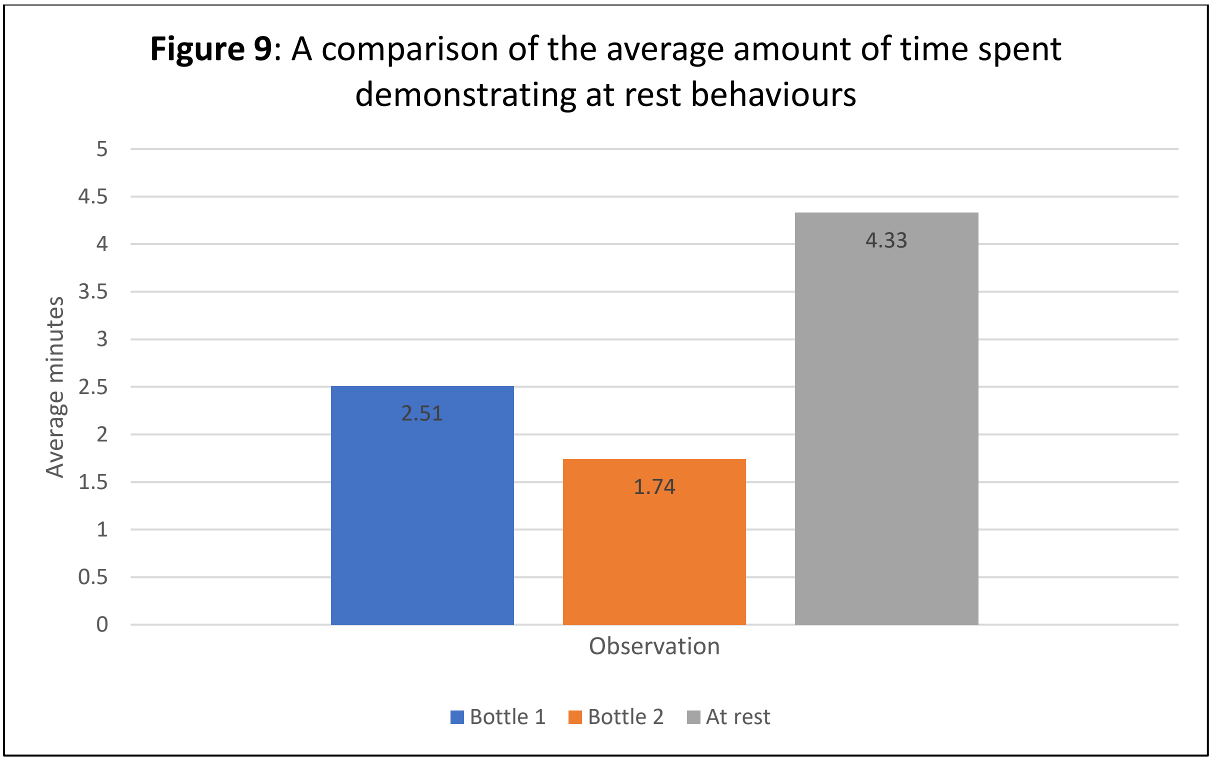
As identified in figure 7, minutes spent demonstrating rest behaviours was more significant after the application of bottle 1 than bottle 2.

Figure 8: A comparison of the minutes spent demonstrating rest related behaviours



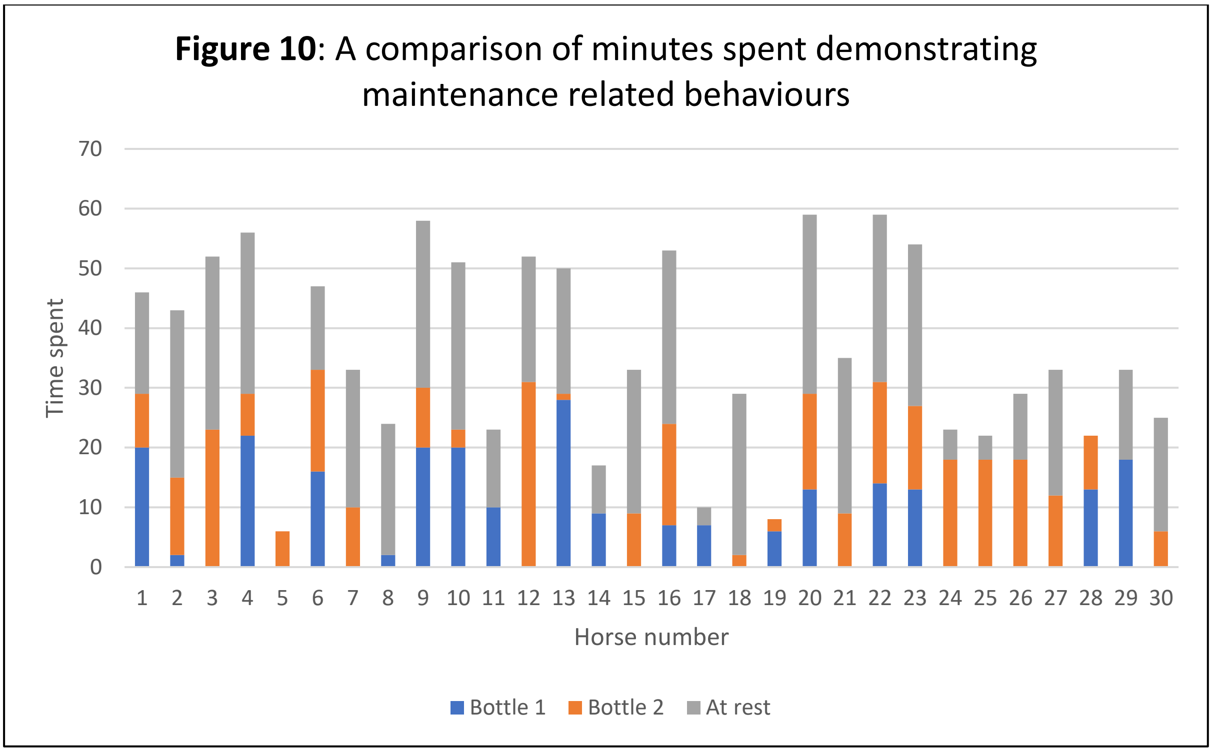
As identified in figure 8 horses spent on average more time demonstrating at rest behaviours after application of bottle 1 in comparison to bottle 2.

Figure 9: A comparison of the average amount of time spent demonstrating at rest behaviours



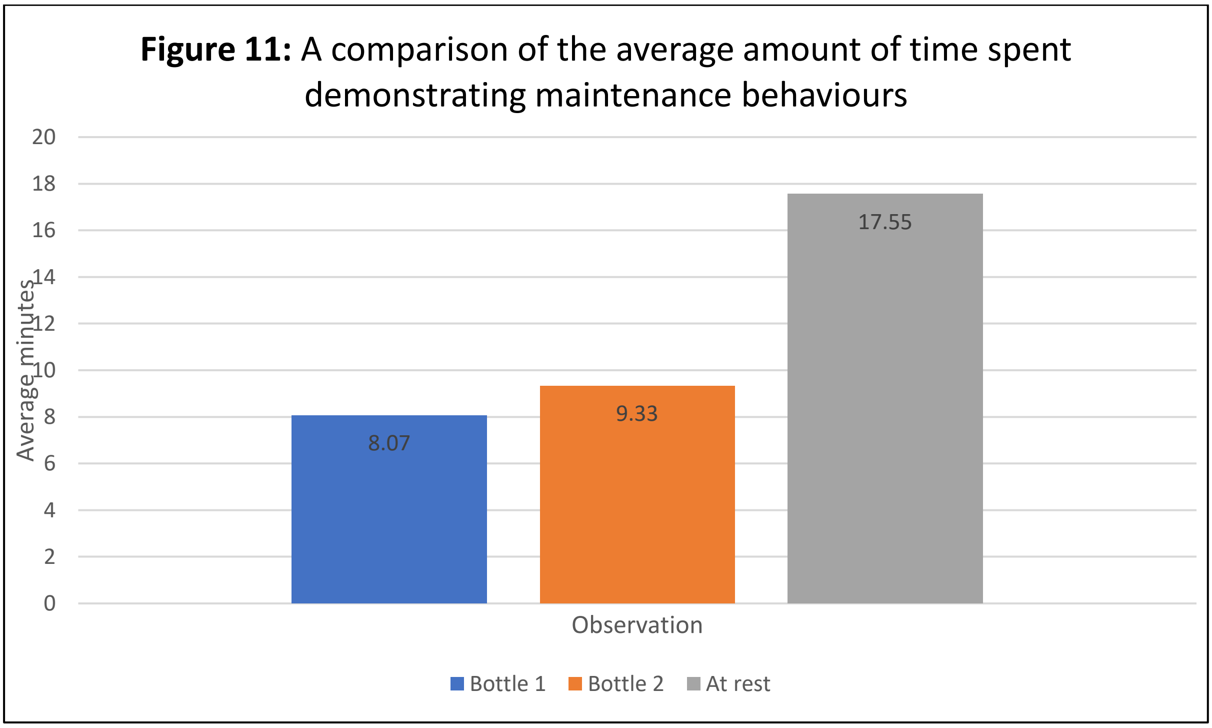
As identified in figure 9, most horses spent more time demonstrating maintenance related behaviours after application of bottle 1 than bottle 2.

Figure 10: A comparison of minutes spent demonstrating maintenance related behaviours



As identified in figure 10 maintenance behaviours were more commonly seen at rest than after application of either bottle.

Figure 11: A comparison of the average amount of time spent demonstrating maintenance behaviours



Chi Square Test for Association

There was a statistically significant association between the bottle being used and the behaviours seen in horses (ChiSquared Association: $X^2_3 = 49.39424186$, P = 1.87726E-11/ P = < 0.05).

Section 6: Discussion

The aim of this study was to test the efficacy of a valerian-based spray for the treatment of stress related indicators in horses prior to exercise, using physiological and behavioural methods. Data suggests that this product is effective at the management of these indicators. Evidence indicates that horses are stressed prior to exercise when compared with baseline data of horses at rest. Average heart rates (HR) and eye temperatures (ET) were considerably lower at rest when compared with prior to exercise with the application of pet remedy or a water-based placebo. In addition, stress/anxiety related behaviours were rarely seen in horses at rest and more commonly seen in horses prior to exercise. Overall results confirm that pet remedy is effective in the management of stress/anxiety in horses prior to exercise.

6.1 Pre-existing medical conditions

Of the 30 horses used in the study, 6 had known health issues. Two horses were diagnosed with Pituitary Pars Intermedia Dysfunction (PPID), on review of research into this condition horses with PPID typically have elevated salivary cortisol concentrations (Morgan et al, 2018) which could be an influence on the data in this study. There is no evidence to indicate that horses with PPID have altered HR or ET as a result of this condition, it is however worth noting that horses with this condition can be lethargic and less active than those without the condition (Getty, 2013). On review of the data recorded these horses have been included in the statistical analysis for behaviour as PPID appears to have had no impact on either, they have been removed from the cortisol data analysis. One horse in the study had polysaccharide storage myopathy (PSSM), research undertaken by Finno (2010) indicates that horses with this condition fed a diet high in non-structural carbohydrates (NSC) typically have a higher cortisol concentration (Finno et al, 2010). The horse used in this study has a managed diet and the cortisol concentrations recorded from it (1.67-3.49) were within the normal expected parameters and therefore have been included in the statistical data accordingly. Research undertaken by Naylor (2012) evidenced that horses with PSSM had no significant difference in HR when compared to horses without the condition (Naylor, 2015). Therefore, the data for this individual has been included in the overall study. One horse included in this study is diagnosed with Equine Metabolic Syndrome (EMS), research undertaken by Walsh (2009) indicates that horses with this condition can have significantly higher concentrations if they are not managed appropriately with exercise and diet (Walsh et al, 2009). The horse included within this study is effectively managed, its concentrations (0.85-2.91) were within the normal expected parameters so it has been included within the statistical data. There is no available research to indicate that this condition would impact any other physiological or behavioural data collected in this study. There is no research related to any of the other known medical conditions that would alter the data collected as a result each horse has had their data included for statistical analysis.

6.2 Eye temperature

There were 30 horses used as part of this study, these included a range of ages, breeds and exercise levels. In addition, there were more castrated male horses used than entire female horses. When undertaking statistical tests for association there was found to be an association between eye temperature and age ($p=0.028$) and hours exercised per week ($p=0.004$). This result is similar to research undertaken by Jansson (2021), who undertook a study with 32 horses finding ET varied subject to breed and sex of the horse as well as other variables including environment and location (Jansson, 2021). Similar studies by Kim (2021) and De Mira (2021) found the environment had a significant impact on the ET results and both studies concluded that a more controlled environment and larger sample would have provided more accurate results to support their hypothesis (Kim & Cho, 2021) (de Mira et al, 2021). Unfortunately due to the time of year that this study was undertaken and the variety of housing methods seen across the sample it was difficult to ensure the ET was taken in a controlled environment each time, this could have had an impact on the results. With the results from this study and the outcomes from previous research this could question the reliability of using eye temperature as a method for ascertaining stress levels.

6.3 Heart rate

When undertaking statistical tests of association there was no significant association found between HR or cortisol concentration when tested for association with age, sex or hours exercised per week. As mentioned, HR was higher in horses prior to exercise than at rest. As identified in the results the mean average HR was lowest at rest (30.46), the mean average HR was at its highest when the horse was treated with a water placebo (35.66), the mean average heart rate after the application of pet remedy was lower than the placebo (32.33). When undertaking the statistical variance test there was no statistical difference seen between the rest score and pet remedy, meaning the product keeps the HR close to at rest levels. There was no statistically significant difference between the water-based placebo and pet remedy, however the result was close to being significant (0.069). If a larger sample had been used the data could have resulted in a significant variance evidencing the efficacy further.

These results are supported by research undertaken by Bohak (2018) who studied anticipatory stress in race horses using HR and cortisol analysis. This research indicated that HR increased in anticipation of a competition (Bohak et al, 2018), however only a small sample of 8 horse were used. Similar research undertaken by Becker-Birck (2013) found that not only did HR increase with physical exertion but also whilst the horses were being groomed and having equipment applied, indicating that horses had anticipatory stress prior to exercise (Becker-Birck et al, 2013), this study had a larger sample than that undertaken by Bohak (2018).

On analysis this indicates that horses have a higher HR in anticipation of being exercised, but pet remedy is effective at reducing the HR by ensuring there is no variance between the heart rate of horses at rest and the heart rate of horses being prepared for exercise.

6.4 Cortisol concentrations

As mentioned, 6 horses in this study had pre-existing medical conditions, 2 of which were diagnosed with PPID which can affect their cortisol concentrations. As a result, the data from these two horses have not been included for analysis. However, the other 4 horses with health conditions have been included in the cortisol concentration data. This statistic is supported by research undertaken by Aurich (2015), who established that cortisol concentrations were not affected by sex and age of horses (Aurich, (2015)). In addition, one horse demonstrated stress related behaviours when it was attempted to collect a saliva sample, it was felt not to continue to pursue collection of this to ensure its welfare wasn't compromised. As a result of this 27 horses' data has been analysed for cortisol concentration.

The cortisol concentration results in this study were surprising as they didn't seem to correlate with any other physiological or behavioural data. The mean average cortisol concentrations were lowest after application of a water placebo (1.58) when compared to the pet remedy (1.62) and at rest (1.91). Results after tests for variance showed no significant difference between the cortisol concentration of horses at rest, after application of pet remedy or the water based-placebo. Despite this the statistical variance result between at rest data and the application of bottle 1 is near to being significant (>0.05).

This statistical result alone would indicate that horses do not have anticipatory stress prior to exercise, this contradicts the other behavioural and physiological methods used in this study suggesting there could be reliability concerns when using this method for ascertaining stress levels in horses, or the need for a larger sample evidencing the need for further research. This outcome is supported by research undertaken by Peeters (2012) who found that morning samples did not differ from baseline samples indicating no anticipation by the horse (Peeters et al, 2012). In this research it was found that the time in which the sample was taken did effect the result, this is also apparent in research undertaken by Aurich (2015) who in addition found seasonal changes (Aurich, (2015)) as well as research undertaken by Dlugosz (2020) who found significant differences in morning and evening samples (Dlugosz, 2020). Due to time constraints and the complexity of using a large sample the time in which samples were collected was not the same for all horses. This will be a recommendation for future studies.

6.5 Behavioural analysis

On analysis of the behavioural data collected it is apparent that horses demonstrate very few stress/anxiety related behaviours such as retreat, bite/kick threats when at rest. Of the 900 minutes spent observing horses at rest only 0.77% of this was spent demonstrating stress/anxiety related behaviours. By comparison when horses were being prepared for exercise these behaviours were seen more frequently. Of the time spent observing after the application of pet remedy, 7.9% of this was seen demonstrating stress/anxiety related behaviours, by comparison to 9.1% of time spent after application of a water-based placebo.

In addition, at rest behaviours such as yawning and stretching were more commonly seen in horses treated with pet remedy than the water-based placebo. Maintenance behaviours such as grazing and drinking were more commonly seen after application of pet remedy by comparison to the water-based placebo.

On analysis of the chi square statistical test this indicates that the bottle of product used had a significant impact on the behaviours displayed by horses, evidencing that pet remedy is effective at reducing behavioural stress related indicators in horses prior to exercise.

6.6 Implications

There are implications for the use of each physiological method used as part of this study. Whilst salivary cortisol concentrations are commonly used in equine related research there are issues with reliability, it is recommended to use this method alongside other behavioural and physiological techniques to ensure accuracy.

Currently there is no published literature into the efficacy of pet remedy in the treatment of stress in horses, this study has evidenced that this product is effective in reducing physiological and behavioural stress/anxiety related indicators in horses prior to exercise. Research undertaken by Taylor (2016) into the efficacy of pet remedy in the treatment of stress in dogs found that the product did not have a discernible effect on the behaviour of dogs (Taylor & Madden, 2016), whilst this was on a different species, horses and dogs are both mammals and subsequently have some similar physiological characteristics. The canine study only utilised behavioural indicators, whereas this study has also used physiological techniques. The sample size was similar across both studies, however in this study each animal was seen three times, compared to two in the canine study. The method of application was also different, in the canine study a diffuser was used whereas in this study the product was applied topically. This study does not support the research undertaken by Taylor (2016), however with a wide range of differences in technique as well as species this does evidence the need for more research to make a more accurate comparison. Research by Unwin (2019) into the efficacy of pet remedy in rabbits has similarities with this study. Unwin's research included a large sample of 50 animals, a placebo was also used. In addition, physiological and behavioural methods were used. Similarly, to this study there was an increase in positive behaviours seen with the product applied alongside a significant decrease in heart rate when compared to the placebo (Unwin et al, 2019). Both of these studies indicate this product can be effective in the management of stress.

There are practical implications following this study. Pet Remedy can be used as an effective method for reducing stress in horses, whilst evidence indicates that stress levels in horses do not impact their performance it does indicate that rider stress can impact the horse's performance. By reducing a horse's stress related indicators this could have a positive influence on rider stress levels which would therefore improve horse performance. It should however be noted that valerianic acid is a controlled medication by the Federation Equestrian International and should therefore not be used prior to competing (FEI, 2022). However, it could have beneficial implications when used in particularly stressful situations such as veterinary consultations or travelling. By reducing stress levels in horses, we can have a positive impact on their overall welfare.

Whilst there are recommendations for future studies due to issues with cortisol and eye temperature data the overall picture of this research is that the product is effective. This is evidenced in the statistical analysis found within the results section of this research.

6.7 Limitations

There have been some limitations to this study. The data has predominantly been collected by one person, however there were occasions when additional people were required help collect data, despite instructions being given this could have resulted in human error.

To reduce the possible amounts of variables within the study it would be preferable to use horses without diagnosed medical conditions. In addition, focusing on either male or female horses could be beneficial, whilst it is believed that none of the female horses in this study were in season this cannot be guaranteed. Female horses in season could have a detrimental impact on results (Aurich, (2015)). Whilst there were minimal statistically significant associations in physiological data there was an association between eye temperature with age of horse and hours worked. On reflection the methodology for taking eye temperature could be amended to ensure accuracy of results, as it has been identified in this research and others that eye temperature is effected by the environment and a more controlled environment would likely see a more accurate data set and therefore reduce significant associations.

6.8 Recommendations

There are a number of recommendations that can be made as a result of this research which will hopefully be beneficial for future equestrian related study. Recommendations include altering the methodology for ascertaining eye temperature in horses, utilising a controlled environment should see a better accuracy in results. In addition, using a consistent time for taking salivary cortisol concentrations would see this no longer being a variable in the data and this would support the findings of previous research. A larger sample of horses would see a more varied data set, it could also be beneficial to focus the study on horses with known behavioural issues or known to suffer from stress. There could also be benefits from using a smaller more specific data set, research undertaken by Sauer (2019) found that breed and housing factors can have significant effects on cortisol concentrations (Sauer et al, 2019). By focusing research on a particular breed or housing method this would potential variables in the data and could therefore have a better understanding of the products efficacy.

Section 7: Conclusion

This research aimed to establish whether pet remedy was effective in the management and treatment of physiological and behavioural stress and anxiety related indicators in horses prior to exercise. Based on the quantitative data of thirty horses both at rest and prior to exercise utilising heart rate, eye temperature, cortisol concentration and behavioural analysis. The data indicates that horses are stressed in anticipation of exercise when compared to that of at rest baseline data. Horses demonstrated higher heart rates, lower eye temperatures and displayed less stress related behaviours at rest when compared to prior to exercise. When comparing pet remedy and a water placebo with baseline data the data evidences that pet remedy is generally effective in the management of stress in horses prior to exercise.

This research clearly states that the product is effective, but it also raises the question as to why the cortisol concentrations were not reflected similarly to other physiological data. There was no significant variance between baseline cortisol concentrations and that of horses treated with pet remedy or a water placebo. To better understand the implications of these results, further studies could test the efficacy of the product further. It is recommended that the product be tested on horses prone to stress prior to exercise, this will hopefully see a greater impact of the product on individuals. In addition, focusing on horses with a smaller age range and only including those with no diagnosed medical conditions. By doing so this would reduce the possibility for data variability. In addition, other recommendations include using a controlled environment when measuring horse eye temperature to reduce environmental impact as well as collecting cortisol salivary swabs at a consistent time as research indicates that the time of day can also impact cortisol concentrations. A larger sample of horses and focusing on a particular housing method or breed could also be beneficial and reduce data variability.

As discussed, horse owners have an obligation to reduce and manage their horses stress, whilst evidence indicates that higher stress levels in horses doesn’t affect horse performance, rider stress can effect it. This evidences the need to manage stress to ensure high levels of performance, in addition by managing stress appropriately owners are ensuring high welfare standards for their horses.

As identified, there is no published literature into the efficacy of pet remedy in the treatment of stress in horses, there is also minimal published literature of its efficacy with other animals. Pet remedy is a valerian-based product and whilst there is some published literature into the efficacy of valerian as a core ingredient in the management of stress in animals, again the research is minimal. This study evidences that pet remedy is effective in the treatment of stress and anxiety related indicators in horses and therefore allows owners to make informed choices when comparing stress treatments available to them. This study has implications for the treatment and management of stress in horses and subsequently could have an impact on the improvement of horse welfare. The hypothesis of this study is therefore supported.

Section 8: Conflict of interest

Pet remedy gave the amount of three hundred pounds to the University Centre North Lincolnshire, this money was used towards the purchase of equipment to gather physiological data as part of this study. The equipment will also be used for future research projects undertaken by students studying at the university.

Section 9: References

Aurich, J. et al. (2015) Effects of season, age, sex and housing on salivary cortisol concentrations in horses. *Domestic Animal Endocrinology,* Volume 52, pp. 11-16.

Bartolome, E. et al. (2021) Genetic Parameters of Effort and Recovery in Sport Horses Assessed with Infrared Thermography. *Animals,* 11(832), pp. 1-11.

Becker-Birck, M. (2013) Cortisol release and heart rate variability in sport horses participating in equestrian competitions. *Journal of Veterinary Behaviour,* Volume 8, pp. 87-94.

Berghold, P. et al. (2007) Effects of reproductive status and management on cortisol secretion and fertility of oestrous horse mares. *Animal Reproduction Science,* 102(3-4), pp. 276-285.

Binks, J. et al. (2018) The behavioural effects of olfactory stimulation on dogs at a rescue shelter. *Applied Animal Behaviour Science,* Volume 22, pp. 69-76.

Bohak, Z. et al. (2018) Anticipatory response before competition in Standardbred racehorses. *PLOS One,* pp. 1-8.

British Equestrian Trade Association, (2022) *Market Information.* [Online]
Available at: https://beta-uk.org/pages/industry-information/market-information.php#:~:text=The%20number%20of%20regular%20riders%20%E2%80%93%20those%20who,The%20average%20age%20of%20a%20horse%20is%2013.
[Accessed 2 March 2022].

British Horse Society, (2021) *Our Horse Welfare and Care Campaigns.* [Online]
Available at: https://www.bhs.org.uk/our-work/welfare/our-campaigns
[Accessed 6 April 2021].

Buckley, L. (2019) Does Pet Remedy Reduce Stress in Dogs? *Veterinary Evidence,* 4(3), pp. 1-18.

Busby, D. (2019). *The horse, a natural history.* 1st ed. Brighton: Ivy Press.

Cook, N. et al. (2001) Adrenocortical and metabolic responses to ACTH injection in horses: an assessment by salivary cortisol and infrared thermography of the eye. *Animal Science,* Volume 81, p. 621.

Costa, E. (2014) Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. *Plos one,* 9(3).

De Mira, M. et al. (2021) Salivary cortisol and eye temperature changes during endurance competitions. *BMC Veterinary Research,* 17(329), pp. 1-12.

Dlugosz, B. et al. (2020) Assessment of changes in the saliva cortisol level of horses during different ways. *Turkish Journal of Veterinary and Animal Science,* Volume 44, pp. 757-762.

Edwards, D & Kurlander, L. (2010) Women's intercollegiate volleyball and tennis: Effects on warm-up, competition, and practice on saliva levles of cortisol and testosterone. *Hormones and Behavior,* Volume 58, pp. 606-613.

Fazio, E. et al. (2008) Effects of competition experience and transportation on the adrenocortical and thyroid responses of horses. *Veterinary Record,* 163(24), pp. 713-716.

FEI, (2022) *FEI Clean Sport.* [Online]
Available at: http://prohibitedsubstancesdatabase.feicleansport.org/search-results/
[Accessed 13 March 2022].

Finno, C. et al. (2010) Effect of fitness on glucose, insulin and cortisol responses to diets varying in starch and fat content in Thoroughbred horses with recurrent exertional rhabdomyolysis. *Equine Veterinary Journal,* 42(38), pp. 323-328.

Fippiat, T. et al. (2021) Accuracy of a heart rate monitor for calculating heart rate variability parameters in exercising horses. *Journal of Equine Veterinary Science,* 104(2021).

FLIR, (2019). *FLIR E5xt (incl. Wi-Fi).* [Online]
Available at: https://docs.rs-online.com/f8fb/0900766b816de263.pdf
[Accessed 15 March 2022].

Gehlen, H. etal. (2020). Association between Disease Severity, Heart Rate Variability (HRV) and Serum Cortisol Concentrations in Horses with Acute Abdominal Pain. *Animals,* Volume 10, pp. 1-14.

Getty, J. (2013) *Equine Cushing's Disease.* Londpm: Create Space Independent Publishing Platform.

Gregic, M. (2020) Variability of stress indicators in jumping horses in parkour due to horse age and competitive season. *Macedonian Veterinary Review,* 43(2), pp. 169-173.

Henshall, C. et al. (2022) The effect of stress and exercise on the learning performance of horses. *Nature portfolio,* 1918(12).

Houghton, J. (2003) *Horse and Stable Management.* 4th ed. Oxford: Blackwell Publishing .

Jansson, A. (2021) An investigation into factors influencing basal eye temperature in the domestic horse (Equus caballus) when measured using infrared thermography in field conditions. *Physiology and Behaviour,* 228(113218).

Jehanli, A. et al. (2011) *International Society of Exercise Synposium: Development and validation of an oral fluid collection device and its use in the immunoassay of salivary steroids and immunoglobulins in sports persons.* s.l.:s.n.

Kamaldeep, D. et al. (2001) Anti-anxiety studies on extracts of Passiflora incarnata Linneaus. *Journal of Ethnopharmacology,* 78(2-3), pp. 165-170.

Kang, O & Yun, Y. (2016) Influence of horse and rider on stress during horse-riding lesson program. *Asian-Australaisan Journal of Animal Science,* 29(6).

Kim, S & Cho, G. (2021) Validation of Eye Temperature Assessed Using Infrared Thermography as an Indicator of Welfare in Horses. *Applied Sciences,* 11(7186), pp. 1-8.

Krueger, K. et al. (2019) Preservation of fecal glucocorticoid metabolites and immunoglobulin A through silica gel drying for field studies in horses. *Conservation Physiology,* 7(1).

Kulkarni, S & Ashish, D. (2008) Withania somnifera: An Indian ginseng. *Progress in Neuro-Psychopharmacology and Biological Psychiatry,* 32(5), pp. 1093-1105.

Lewinski, M. (2013). Cortisol release, heart rate and heart rate variability in the horse and rider: Different responses to training and performance. *The Veterinary Journal,* 197(2), pp. 229-232.

Manrique, L. et al. (2019) Individual differences in behavior and heart rate variability across the preweaning period in the domestic horse in response to an ecologically relevant stressor. *Physiology and Behavior,* 210(2019).

McDonnell, S. (2003) *A Equid Ethogram: A Practical Guide to Horse Behaviour.* 1st ed. London: Eclipse Press.

McGreevy, P. (2012) *Equine Behaviour: A guide for veterinarians and equine scientists.* 2nd ed. London: Elsevier.

Miroddi, M. (2013) Passiflora incarnata L.: Ethnopharmacology, clinical application, safety and evaluation of clinical trials. *Journal of ethnopharmacology,* 150(3), pp. 791-804.

Morgan, R. (2018) Dysregulation of cortisol metabolism in equine pituitary pars intermedia dysfunction. *Endocrinology,* 159(11), pp. 3791-3800.

Mott, R. (2020) Blink rate as a measure of stress and attention in the domestic horse (Equus caballus). *Scientific Reports,* Volume 10.

Murphy, K. (2010) Valeriana officinalis root extracts have potent anxiolytic effects in laboratory rats. *Phytomedicine,* 17(8-9), pp. 674-678.

Naylor, R. (2015) Polysaccharide storage myopathy - the story so far. *Equine Veterinary Education,* 27(8), pp. 414-419.

Peeters, M. et al. (2010). Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotropic hormone challenge. *Equine Veterinary Journal,* 43(4), pp. 487-493.

Peeters, M. (2012) Rider and Horse Salivary Cortisol Levels During Competition and Impact on Performance. *Journal of Equine Veterinary Science,* Volume 33, pp. 155-160.

Sauer, F. et al. (2019) Effects of breed, management and personality on cortisol reactivity in sport horses. *Plos One,* 14(12), pp. 1-19.

Sauer, F. et al. (2020). Salivary cortisol measurement in horses: immunassay or LC-MS/MS?. *Domestic Animal Endocrinology,* Volume 106445.

Schmidt, A. et al. (2010). Changes in cortisol release and heart rate and heart rate variability during the initial training of 3-year-old sport horses. *Hormones and Behaviour,* 58(4), pp. 628-636.

Schmidt, A. (2010) Cortisol release and heart rate variability in horses during transport. *Hormones and behavior,* Volume 57, pp. 209-215.

Scoley, G. (2018) Use of thermal imaging in dairy calves: exploring the repeatability and ccuracy of measures taken from different anatomical regions. *American Society of Animal Science,* Volume 3, pp. 564-576.

Seaman, S. et al. (2002) How reliable is temperament assessment in the domestic horse (Equus caballus)?. *Applied Animal Behaviour Science,* 78(2-4), pp. 175-191.

Starke, K & Oliver, W. (2008) Anticipatory Stress Influences Decision Making Under Explicit Risk Conditions. *The American Physchological Association,* 122(6), pp. 1352-1360.

Taylor, S & Madden, J. (2016) The Effect of Pet Remedy on the Behaviour of the Domestic Dog (Canis familiaris). *Animals,* 6(64), pp. 1-9.

Trindade, P. et al. (2019) Eye Surface Temperature as a Potential Indicator of Physical Fitness in Ranch Horses. *Journal of Equine Veterinary Science,* Volume 75, pp. 1-8.

Unwin, S. et al. (2019) A double-blind, placebo-controlled trial investigating the value of Pet Remedy in ameliorating fear of handling of companion rabbits. *Journal of Veterinary Behaviour,* Volume 36, pp. 54-64.

Walsh, D. et al. (2009) Correlation of plasma insulin concentration with laminitis score in a field study of equine cushing's disease and equine metabolic syndrome. *Journal of Equine Veterinary Science,* 29(2), pp. 87-94.

Zeitler-Feicht, M. (2003) *Horse Behaviour Explained: Origins, Treatment and Prevention of Problems.* 1st ed. London: CRC Press.

Section 10: Appendices

Appendix 1

Horse information

Female horse's - 10

Male horse's – 20

Breeds used : Cross breed (10), Cob (13), Thoroughbred (4), Shire (1), Persia (1), Arab (1)

Average exercise levels – 3.63 hours per week

Exercise types: Hacking, schooling, in hand, driven.

Appendix 2

Personality questionnaire

1. Horse number (given by researcher)
2. Horse name:
3. Sex:
4. Age (in full years):
5. Entire: Y/N
6. Has your horse competed in the last 6 months:
7. Area of competition: Dressage, jumping, schooling, eventing, none of the above (specify)
8. On average how many hours per week do you exercise your horse?

The following questions are in relation to your horses personality in preparation for exercise. Please score your horse accordingly.

How often does your horse display the following behaviours whilst **being prepared** for exercise?

1.	Bite threat	Never	Sometimes	Frequently
2.	Biting	Never	Sometimes	Frequently
3.	Kick threat	Never	Sometimes	Frequently
4.	Kick	Never	Sometimes	Frequently
5.	Stomp	Never	Sometimes	Frequently
6.	Retreat (avoidance)	Never	Sometimes	Frequently
7.	Vocalisation	Never	Sometimes	Frequently
8.	Graze (eg on hay net)	Never	Sometimes	Frequently
9.	Stand alert	Never	Sometimes	Frequently
10.	Moves on the spot	Never	Sometimes	Frequently

Appendix 3

Informed consent form

I hereby consent to my horse being used as part of the research project being undertaken by Jack Anderson. The project, efficacy testing of pet remedy as a treatment for anticipatory stress in horses prior to exercise has been fully explained to me and I am happy for the project to be undertaken using my horse.

________________________________ ________________________________

Signature **Name and Date**

Please note: You will also have the opportunity to ask any questions you may have about the project at any stage. If you decide to take part you are still free to withdraw at any time and without needing to give a reason.

Horse details

Horses name	
Breed	
Age	
Horse gender *If male please note whether it is castrated or not*	
Exercise levels (hours spent per week)	
Exercise type (schooling, hacking etc)	
Any known health issues	

Appendix 4

Ethogram

Domestic Horse *Equus caballus*

Behaviour	**Abbreviation**	**Description**
Maintenance		
Graze	M1	Ingesting grass like vegetation. Using the lips and tongue grass is gathered in the mouth, chewed and swallowed.
Graze recumbent	M2	Grazing (as above) whilst laying down.
Paw	M3	Using a foreleg by dragging across the floor. Can be seen to remove snow or frost from grass.
Browse	M4	Ingesting non grass like vegetation. Using lips and teeth to grasp the vegetation it is then gathered in the mouth, chewed and swallowed.
Pica	M5	Using the lips the horse will draw soil or dirt into the mouth and ingest it.
Drink	M6	Lips are typically below water level, drawing water into the mouth and swallowing.
Bodily function		
Urination	B1	Expelling of urine through the urethra. In males front legs are typically extended forwards with back legs extended backwards. In females the back is arched and tail is typically raised.
Defecation	B2	Expelling of faeces, tail is typically raised as faeces is expelled. Most horses will typically move forward after expulsion, males may turn and paw at the faeces.
Locomotion		
Stand alert	L1	Focusing on something within the area, neck is outstretched with ears pricked forward.
Walk	L2	Slow movement forward, legs moving in opposite pairs.
Trot	L3	Forward movement in a two beat gait. Diagonally paired hooves meet simultaneously.
Canter	L4	Three beat, medium speed gate.
Gallop	L5	Faster run than a canter, 4 beat gait.
Rest		
Rest standing	R1	Head slightly lowered, eyes are partially closed. Often seen weight bearing on 3 legs. Ears may rotate laterally.

Sleep standing	R2	Eyes are closed, the head is lower than the back.
Rest recumbent	R3	Resting on the ground, head might be raised or could be flat to the floor. Legs are outstretched.
Yawn	R4	Deep or long inhalation, eyes partially closed and teeth shown.
Stretch	R5	Extension of either front or back limbs, leaning forward or backwards.
Grooming		
Roll	G1	Dropping to the ground from a standing position, moving from lateral recumbency to sternal recumbency and typically repeated, legs are tucked in.
Rub/Scratch	G2	Rubbing or scratching can be done on a static object, typically the head or backend. The horse might rub its front legs using its head or scratch its head using a hind limb.
Allo groom	G3	Mutual grooming between two horses, using the mouth to lick and lightly chew/bite. Typically this is done around the neck or higher back region.
Communication		
Bite threat	C1	Neck is stretched, the ears typically back with teeth shown. The horse might do swift movements with its neck to the participant.
Kick threat	C2	Hind leg lifts underneath the body, the movement is slow and typically not close enough to touch the participant.
Stomp	C3	Raising and lowering a fore limb abruptly to the ground.
Retreat (avoidance)	C4	Head held low with ears back, the horse is trying to increase the distance between itself and another, typically occurring in trot.
Biting	C5	Neck is stretched, ears back, the horse will turn quickly or lunge at the horse or person with teeth making contact.
Kick	C6	Hind leg lifts underneath the body, quick movement and contact is made
Rear	C7	Fore limbs are raised off the ground whilst the hind limbs remain on the ground, increasing the height of the horse.
Vocalisation	C8	Specify type. The horse might neigh or snort. Neighing is the calling to companions, snort can occur during ingestion.

Appendix 5

Continuous sampling form

Date:	**Study species:**
Start Time: **End Time:**	**Focal animal:**
Heart rate: **Time taken:** **Activity (circle):** Turn out Prep for exercise	**Comments:** **Weather:**

Start time	End time	Behaviour (record abbreviation)	Notes
