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The effect of aromatherapy on the sleeping patterns in horses (*Equus caballus)*

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “The effect of aromatherapy on the sleeping patterns in horses (*Equus caballus*)” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague April 25, 2024

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Bc. Karolína Svobodová

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Abstract

Domestic horses are often exposed to high levels of stress, for example during competitions, during transport, or as a result of inappropriate stabling management in captivity. Unfortunately, many horse owners prioritize the performance of their animals over their well-being, often neglecting their need for adequate rest. Aromatherapies in horses are not uncommon, and over the past decade, numerous effects of various essential oils have been discovered. Many of these effects demonstrate positive outcomes in stress reduction, heart rate modulation, and muscle relaxation. However, one relatively unexplored area pertains to the impact of lavender essential oil on equine sleep patterns. Therefore, this study aimed to investigate the potential influence of lavender essential oil on the sleep patterns of horses. Specifically, it focused on evaluating the impact of lavender essential oil on rest length, sleep length involving three distinct states (sternal recumbency NREM, sternal recumbency REM, and lateral recumbency REM), the length of sleep devoted to these three states, and sleep interruptions. Lavender was administered to the horses through vapour. Of these seven horses, four were hobby horses (all geldings), and three were sport horses (two mares and one gelding). The results for all variables suggest that lavender essential oil does not have a significant effect on sleep patterns and thus does not affect the length of rest, length of sleep, or sleep interruptions. However, the study revealed that various factors, including working time, work type, age, and sex, have a significant impact on the length of rest and its constituent sleep components. Although lavender essential oil did not significantly affect sleep length or sleep interruptions, findings from other studies suggest its potential efficacy in increasing the length of sleep and reducing sleep interruptions, albeit with possible differences due to the administration method, concentrations of lavender essential oil, and species-specific sensitivities. Additionally, the findings that geldings and young horses tend to sleep longer align with findings from numerous studies. The findings presented herein provide valuable insights into the intricate sleep patterns exhibited by horses, broaden the understanding of aromatherapy performed on horses, and offer an opportunity for further study.

Key words: Perissodactyla, horse, lavender aroma, sleep, NREM phase, REM phase

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List of the abbreviations used in the thesis

CNS – Central Nervous System

NREM – Non-Rapid Eye Movement

REM – Rapid Eye Movement

TST – Total Sleep Time

WOAH – World Organisation for Animal Health

1. Introduction

Horses play a pivotal role in various sectors around the globe, contributing significantly to sports (Bartolomé & Cockram 2016; Campbell 2021), healthcare (Lönker et al. 2020; White-Lewis 2020), agriculture (Rzekęć et al. 2020), and forestry (Malatinszky et al. 2022). Within the healthcare domain, horses are employed in hippotherapy (Wood & Fields 2021; Maresca et al. 2022), antivenom production (Miranda et al. 2021; Miranda et al. 2023), and even psychotherapy for addressing psychological distress (Kelly et al. 2023; Friend et al. 2023). In agriculture, horses serve as draft animals (Mota-Rojas et al. 2021; Malatinszky et al. 2022), navigating challenging terrains and slopes with gradients reaching up to 50 % (Ezzati et al. 2015). The realm of sports showcases diverse disciplines, including dressage (Dyson & Pollard 2021), parkour (Gregić et al. 2020), horse racing (Ryan et al. 2020), cross-country (Tuomola et al. 2021), horse-drawn carriage events (Taş Gürsoy 2020), and even vaulting, which involves gymnastics and dance on horseback (Bochiş 2021). Regardless of the sector in which horses are utilized, their lifestyle and needs are intricately shaped by their roles (Furtado et al. 2021; Tavanaeimanesh et al. 2022).

Primarily within the sports industry, there exists a notable emphasis on horse performance, evident in ligament and tendon injuries (Ortved 2018), heightened stress levels (Bartolomé & Cockram 2016), and overall compromised welfare, such as the stress associated with transporting horses to competitions (Padalino & Riley 2020). This culminates in a deterioration of both the physical and mental well-being of the individual horse (Campbell 2021). Unfortunately, the importance of adequate rest is often overlooked, even though it plays a vital role in maintaining a horse's health, as insufficient rest not only contributes to stress but is also reciprocally influenced by it (Aleman et al. 2008; Greening & McBride 2022). Moreover, high-quality rest is essential for the efficient conservation of energy across all animal species (Kelly et al. 2022) and facilitates tissue regeneration (Han et al. 2022; Stich et al. 2022). In artificial environments where horses are kept under human supervision, it is crucial to prioritize the provision of high-quality rest (Hausberger et al. 2008; Merkies & Franzin 2021). An effective strategy for achieving this is through the application of aromatherapy (Thomson 2020; Bini de Lima et al. 2023).

2. Literature Review

2.1. Social behaviour

Horses are social animals, preferring to live in groups or herds (Hartmann et al. 2012; Linnartz & Linnartz-Nieuwdorp 2017; Wolter et al. 2018). While the term "herd" refers to horses living in more natural settings (Go et al. 2020; Hunold & Britton 2022), the term "group" is predominantly used in association with domesticated horses (Giles et al. 2020; Stachurska et al. 2021). Groups are formed artificially to form groups of grazing horses that have visual contact with each other (Hartmann 2010; Ruet et al. 2020). In a herd, there is a constant system of hierarchy, which can be linear or nonlinear, and is designed to prevent aggression and establish an arrangement of dominant and subdominant individuals (Awadallah et al. 2022; Krishna & Ramanajneyulu 2022; Mavi et al. 2024).

Domestic horses are commonly seen kept in individual boxes (Ruet et al. 2019) with a minimum amount of social contact (Søndergaard et al. 2011). Even in tropical countries such as Malaysia, individual horse housing has become more common over the past decades (Chung et al. 2018). Behavioural and physical needs cannot be fulfilled by keeping horses in individual boxes (Hartman et al. 2012). Horses are animals that need strong interaction and social contact with conspecifics (Reid et al. 2017). Social bond in horses is represented by three parameters determining social relationships. These are spatial proximity measurement (the horses are in direct contact with each other or are two horse lengths apart), affiliative approaches (the horses go towards each other and are one horse length apart) and mutual grooming (the horses are side by side rubbing, nipping and nuzzling each other) (Wolter et al. 2018).

Social behaviours such as aggression (Inoue et al. 2019), altruism (Mills & Nankervis 2013), cooperation (Schork et al. 2018), mutualism (Kavesh 2023) and parental care (Nuñez et al. 2015) can occur among horses. Aggressive behaviour involves chasing, biting, kicking (Inoue et al. 2019) and many others such as ears back towards the head, very fast tail wagging, lips pulled up, so the teeth are seen, nostrils dilated, skeletal muscles tensed, and the subject is turned towards the object (Olczak & Klocek 2014).

Even though the horse is a domesticated animal, its ethological needs have not changed with domestication. It is therefore very important to ensure that horses in human management are allowed to establish solid affiliative connections (Torres Borda et al. 2023). Although the husbandry conditions have improved in recent years, unfortunately, horses are not always allowed to participate in affiliative bonds. Reduced affiliative bonds are observed in horses that are individually stabled and thus have limited physical contact with other individuals (Costa et al. 2019). This particularly affects horses in tropical countries, as they are housed individually in stalls and, due to the high temperatures, have very short periods in a shared paddock during the day and therefore have reduced affiliative bonds (Chung et al. 2018). These signs of individual breeding are most evident in adult stallions (Costa et al. 2019).

2.2. Feeding behaviour

The natural diet of feral horses is influenced by where the horses live and therefore it is difficult to say exactly what horses eat in the wild. For example, feral horses in northwest Colorado (more specifically, the Little Book Cliffs Wild Horse Herd Management Area) underwent a faecal analysis which revealed that the diet of feral horses consisted of nearly 79 % graminoids, 16 % shrubs/trees, and only 5 % forbs. The sixteen percent shrubs and trees portion are because even though the horses are grazers, during the winter the grasses are covered with a layer of snow or are not nutritionally rich (King & Schoenecker 2019). On the other hand, feral horses in Southern India (more specifically in Point Calimere Wildlife Sanctuary) were found to have a diet of nearly 70 % graminoids, 17 % herbs and 1 % climbers using Jacob's Preference Index (Baskaran et al. 2016).

Domestic horses are herbivores (Fujimori 2021) that eat roughage such as hay and grasses for about 14 hours per day (Krueger et al. 2021). Hay is an all-purpose feed that retains its nutritional properties over a long period with low nutrient loss (Sharpe 2018). The consumption of hay is in most cases *ad libitum* (Mueller et al. 2022; Kranenburg et al. 2023), in some circumstances hay restriction is set up to treat metabolic syndrome in horses (McGowan et al. 2013), or the amount of hay that is consumed is limited using hay-bags, which are designed to increase the consumption time of roughage (Rochais et al. 2018).

Two types of hay are commonly found, the first is grass hay and the second is legume hay. Horses living in the tropical climate in Florida (USA) tend to prefer legume hay over grass hay as it contains less fibre and on the other side has a higher energy and protein content (Vasco et al. 2022). Despite the significant differences in fibre content among hay types, the amount of fibre content is still important to maintain a favourable gut environment, because a fibre-rich diet causes a decrease in valeric acid in all intestinal compartments and a decrease in the dry matter content in the right dorsal colon (Raspa et al. 2022). What's more, high-fibre diets improve immune function, digestion, fitness, and sports performance and minimise the risk of gastrointestinal disease (Ermers et al. 2023).

The nutritional needs of sport horses can be quite different from non-sport or feral horses, as they need a higher amount of cereal grain to maintain higher energy needs, and the high fibre forage content is reduced as it has low energy content (Richardson & Murray 2016). Common cereal grains include barley, oats, and corn. The higher starch content is also closely linked to the digestion process. Even though cereals contain different amounts of starch (corn has the highest starch content), their processing should occur through enzymes in the small intestine. If starch is not processed properly in the anterior digestive system, microbial fermentation can occur in the caecum, which can lead to acidosis, laminitis, or even colic (Potter et al. 2022).

The regularity of feeding is also an important factor in ensuring the correct behaviour of horses. With timely and accurate feeding at specific times, horses generally show less time spent eating hay and more time resting. Horses that are fed later than the usual feeding time exhibit aggressive behaviour, such as kicking and pawing the ground (Zupan et al. 2020).

2.3. Senses

Most vertebrates have five senses, which are olfaction, vision, hearing, touch, and taste, through which the animal receives awareness and information about its environment. Understanding the sensory abilities of animals (e.g. colour spectrum, sensitivity to sounds, use of artificial light, use of scents etc.) is of fundamental importance to mankind, as humans can improve the welfare of animals kept in unnatural conditions and thus understand the ethology of animal behaviour (Rørvang et al. 2020).

2.3.1. Olfactory senses

The horse's sense of smell is much better developed compared to the human sense of smell. Since horses are obligate nasal breathers (they do not use their mouths to breathe), they have nostrils adapted for this purpose, making them very flexible and mobile. They also use their sense of smell to detect scents from their surroundings by opening their nostrils widely and allowing air from the environment to flow directly into their olfactory apparatus. It is also worth noting that the nostrils are deviated outwards, which allows horses to pick up scent marks from their bodies. The nasal passages are covered with a very vascular and moist mucous membrane from which the cilia emerge. The cilia are nerve cells that contain sensory cells that assist in the recognition of scents (McBane 2012). The longer the horse takes in odours from the environment, the more chance they give the receptors to analyse the odours and once the receptors identify the odour, nerve impulses are sent (Wilkinson 2016). The nerve cells send scent information to the central nervous system (CNS) (McBane 2012). Nerve impulses travel along nerves to the olfactory bulbs, which are located in the front of the cerebrum and are responsible for identifying smells. There are two olfactory bulbs in total - left and right - each of which is associated with olfactory receptors in one of the two nostrils, i.e. the left bulb with receptors in the left nostril and the right bulb with receptors in the right nostril (Wilkinson 2016). Unlike the sense of smell, all other senses are processed in the thalamus before entering the cerebral cortex. Thus, the sense of smell skips the thalamus and transmits information directly to the amygdala (processing emotions) and then to the hippocampus (memory formation and learning) (Shwetz 2021). The amygdala and hippocampus are a significant and very important part of the limbic system, as the limbic system influences the parasympathetic and synaptic divisions and communicates with both branches of the autonomic nervous system (Cook & Lynch 2008). When essential oil enters the limbic system, mental and emotional responses are affected (Cook & Lynch 2008; Shwetz 2021). It is also worth mentioning that each molecule, for example of different essential oils, has a specific frequency of vibration. Different frequencies are perceived by the olfactory receptors based on specific chemical bonds and this information is stored in the brain, forming a so-called sensory map (Cook & Linch 2008).

Horses have a so-called vomeronasal organ, receiving low-volatile and non-volatile molecules of body secretions. As soon as the horses smell the substance of interest, they initiate a flehmen reaction. In this reaction, the horse lifts and curls its upper lip, allowing the substance of interest to pass into the vomeronasal organ. During the flehmen the nostrils are closed, which subsequently increases the air pressure in the horse's nasal cavity, allowing the detection of the molecules of the compounds by the vomeronasal organ. The entrance to the vomeronasal organ opens behind the front teeth and the organ itself is located in the upper jaw, more precisely between the nasal cavity and the upper palate of the mouth. The sense of smell, and especially the reception of smells, helps horses to recognize individuals (Rørvang et al. 2020).

Horses live in complex social arrangements that require horses to have sufficiently good cognitive and perceptual skills to recognize group members (Krueger & Flauger 2011). It is for this reason that the olfactory sense is very important for horses as it helps with social discrimination (Hothersall et al. 2010). Based on faeces and urine samples, horses can distinguish whether an individual is conspecific or not (Hothersall et al. 2010; Krueger & Flauger 2011).

Thus, it is clear that horses can recognize their conspecifics based on an odour profile that is unique to each individual. Horses obtain information about the odour profile of a given individual by coming into close contact with the odour profile (distance up to 10 cm) and during this contact, they move their nostrils rapidly and sniff the odour profile (Péron et al. 2014). Closely related to this is scent marking, which is a standard form of intra-species communication. In males, scent marking is carried out by defecation, where males place dung on the territory of the routes they use and inside core parts. Before and after each defecation, males sniff their excrement piles. Mares, on the other hand, sniff the excrement piles of males only before marking. Mares use urine for marking. The reason both sexes do this marking is that this information lets rival males know that the harem is already dominated by male and therefore the intruder should not attempt to mate with the females of that harem (King & Gurnell 2007).

Olfaction, of course, also plays a role in courtship, during which the male and female make oral-nasal contact (Guarneros et al. 2020). The protein lipocalin, which is abundant in horse sweat of the parotid glands, is associated with reproduction in terms of mating attractiveness. Molecules of this odorant are gradually released into the environment where they can be captured by females and where they serve as an important marker for mate selection (D'Innocenzo et al. 2006; Guarneros et al. 2020). To maintain the exclusive privilege of breeding with females, males disguise the smell of oestrous female faeces and urine by relabelling it with their urine (Jezierski et al. 2018).

The sense of smell is also an important factor in distinguishing between the dam and the foal. It was found that foals that were separated from their breeding dams for five months (at the age of seven months) were able to recognize them after this time (at the age of one year) and showed stronger preferences to their dam than with well-known mares from the natal group. Fillies even showed stronger attachment (looked at the mother more times, sniffed her more times and overall contact was longer) to the breeding mother than colts (Lansade et al. 2022).

2.3.2. Vision senses

Among terrestrial mammals, the horse's eye belongs to one of the largest. The horse's eye provides a relative magnification of image that is up to half bigger than the eye of a human. The shape of the eyeball is ovoid, and eyes are placed laterally on the head which makes the horse's view panoramic. The uniocular field of view of the horse has a maximum range between 228° and 215° (Timney & Macuda 2001). Even though horses are mostly diurnal animals, they have evolved a form of night vision called scotopic vision. Scotopic vision allows them to see objects even in low light (Timney & Macuda 2001; Rørvang et al. 2020). Scotopic vision is due to a higher ratio of rods to cones, approximately 9:1 (Wouters & De Moor 1979; Timney & Macuda 2001). And thanks to the reflective tapetum lucidum, located at the back of the eye, the horse's eye can better stimulate photoreceptors and photons, capture light, and at the same time increase sensitivity to light (Ollivier et al. 2004).

In the animal kingdom, the visual abilities of individual species vary depending on the number of cones responsible for colour discrimination and are therefore divided into five groups: monochromats such are cetaceans (Springer et al. 2016), dichromats as dogs (Valenta et al. 2021), trichromats like primates (Onstein et al. 2020), tetrachromats for example reptiles (Jiang et al. 2023) and pentachromats such are pigeons (Reichenbach & Bringmann 2022). Colour discrimination is an important part of information transmission. In humans, colour vision is composed of three types of cones in the retina. These three cones are sensitive to blue, green, and red colours, which implies that humans are classified as trichromats (Mathebula 2022). Horses, unlike humans, are dichromatic individuals with two types of cones that differentiate the colour spectrum at short wavelengths and at middle to long wavelengths (Carroll et al. 2001) which corresponds to the perception of a continuous range of colours from blue to yellow (Roth et al. 2007). Understanding the differences in the perception of the colour spectrum will help us to improve rider safety and animal welfare in horseracing, as horses cannot recognise some of the colours used by humans on obstacles, such as orange, and often overlook obstacles, resulting in injury to both horse and rider (Paul & Stevens 2020).

2.3.3. Auditory senses

Horses as prey species have developed a good auditory sense. This sensitivity to sound is closely associated with their tendency to exhibit anxiety behaviour when exposed to noisy environments. Anxiety behaviour is expressed by trembling, sweating and attempts to escape, which could cause health problems for the horse and rider. Again, maintaining an optimal noise environment is an important factor in achieving sufficient welfare for horses (Riva et al. 2022).

The horse's ear consists of three parts - the external ear, the middle ear, and the inner ear. The external ear is further composed of two parts namely the auricle and the external ear canal (both cartilaginous and osseous) (Blanke et al. 2015). The tympanic membrane is a very thin membrane connected to the external ear canal (osseous part). The tympanic membrane consists of three layers – lamina propria, epidermal layer and mucosal layer (Sommerauer et al. 2012; Blanke et al. 2015). The tympanic membrane also known as the eardrum splits the external ear from the middle ear, which is the second part of the ear. The middle ear, in most mammals, consists of the anvil (*incus*), hammer (*malleus*) and stirrup (*stapes*) (Nitovski et al. 2014). The space inside the middle ear is filled with air and lined with epithelium of cuboidal and flattened cells (Blanke et al. 2015). The third, and final part, forms the inner ear, which consists of the cochlea, semi-circular canals, and vestibule system (Ekdale 2016).

The range of frequencies that can be detected by horses is different from humans. The lowest detectable limit is a few tens higher in horses than in humans and is around 50 Hz in horses, while in humans it is 20 Hz. The highest detectable frequency is then higher compared to humans and is 33,000 Hz in horses and only 20,000 Hz in humans (Heffner & Heffner 1986; Saslow 2002; Rørvang et al. 2020). These differences imply that humans may be resistant to some sounds because they either do not hear them or easily overhear them. Therefore, when on horseback, the rider must be vigilant to any sounds such as the snapping of a branch, the hissing of air brakes, or any high frequency sounds that are made by, for example, machinery (Saslow 2002).

Closely related to the auditory organ is so-called auditory laterality, whereby horses prefer one ear associated with a given hemisphere over the other (Basile et al. 2009), as is the case, for example, with right-handed people who involve the left hemisphere (Pujol et al. 1999). Horses primarily respond to sounds by turning their ear/ears. After that, they turn their heads to identify the visual aspect of the individual emitting the sound. Sounds that come from an individual from a different (but familiar) group are received by the horse using the right ear connected to the left hemisphere. While completely foreign sounds were more likely to be received by the left ear (Basile et al. 2009).

2.3.4. Tactile senses

Just like the other senses, the tactile sense is a very important sense involved in the survival of an individual (Gueguen et al. 2022). The tactile senses themselves have many uses such as social interaction, communication, maternal care, sexual context, and many more (Dezfouli et al. 2014; Zimmerman et al. 2014; Ringhofer & Yamamoto 2017). The skin is one of the largest sensory organs and helps transmit information about temperature, pain, touch and itching to the CNS. Thermoreceptors are responsible for the transmission of temperature information, nociceptors are responsible for pain information, low threshold mechanoreceptors respond to touch and pruriceptors are present in the skin to register itching (Zimmerman et al. 2014).

Within equine tactile reactivity, there are many differences in reactivity to filament size, site of touch and sex. Horses generally respond better to larger von Frey filaments than smaller ones. The location of irritation is generally most sensitive in the withers because there are many touch receptors (Gourlay 2014; Gueguen et al. 2022). The facial area around the nose, mouth, and eyes, where a thin layer of epidermis is located, is also considered to be a very sensitive and tactile area. In this facial area, there are stiff, long, and never moulting vibrissae (simply called whiskers), which have very good enervation, and when they are removed, the horse loses an important sensory organ and the horse's welfare is impaired (Mills & Redgate 2017). Depending on sex, geldings show greater sensitivity around the muzzle than mares (Gueguen et al. 2022).

The sense of touch is also very important in horses from the riders' perspective, as horses use intensive human touch to evaluate and determine actions (Ladewig 2019). Of course, touch is even more important for mares and foals or other closely related horses because they use touch for grooming (Ekesbo & Gunnarsson 2018). During mutual grooming, horses groom each other on certain parts of the coat that are difficult for individuals to access (Costa et al. 2019). Mutual grooming, as well as overall affiliative behaviour, is observed in all ages and sexes of horses (Heitor et al. 2006) but its frequency varies within seasons (Wells & von Goldschmidt-Rothschild 1979) but also with reproductive status (Heitor & Vicente 2010).

Horses have developed specific responses to defend themselves against unpleasant tactile situations. Unpleasant tactile situations include blood-sucking and biting horseflies and flies. Typical reactions against these flies are tail swishes, foot stomps, ear flicks, skin rippling, direct bites, and head shakes (Saslow 2002). Another example of an unpleasant tactile situation is electric fencing (Rørvang et al. 2020), which results in serious soft tissue injuries to the front and hind pairs of limbs (Tunakova et al. 2023) and through these negative experiences, horses learn to avoid these unpleasant situations and this avoidance is referred to as conditioned avoidance (McKillop & Sibly 1988).

2.3.5. Gustatory senses

The tongue is an important muscular organ in the process of distinguishing tastes. The tongue consists of the apex, body, and root. The surface of the tongue is covered with stratified squamous epithelium. On the dorsal side of the tongue, there are the lingual papillae on which the taste buds are located (Rezaian 2006). Horses can discriminate between four basic tastes (salty, sweet, sour, and bitter) but how they distinguish between tastes and odours is still not fully understood (Rørvang et al. 2020). It is uncertain whether horses, like humans, can link a scent with a taste and vice versa. Unlike humans, who can breathe through both their nose and mouth to combine taste and scent components, horses are obligate nasal breathers, which leaves the connection between scent and taste unresolved (Holcombe & Ducharme 2004). Very often horses acquire information about the digestibility of food through orosensory mechanisms and especially post-ingestive mechanisms. Positive feedback from the gut and brain will increase the horse's preference for the food because it contains sufficient nutrients to satisfy the horse nutritionally. More accurate horses have a greater preference for feeds that are rich in hydrolysable carbohydrates or protein and for this reason, they prefer them in their diet (Van Den Berg et al. 2016a). The preference for sweetened food increases with moisture and horses tend to prefer wet-sweetened diets more than dry ones (Stachurska et al. 2022). Van Den Berg et al. (2016b) specifically tested four food types and found that horses preferred sweet scents such as coconut and banana over spearmint and cinnamon. On the other hand, horses do not look for a bitter taste in their food, which can be represented by the group of B vitamins.

2.4. Sleep patterns

Sleep is one of the most fundamental phenomena in the entire animal kingdom (Keene & Duboue 2018). It is an important biological requirement through which the body acquires regeneration and the ability to solve cognitive tasks and store the outcomes in memory (Greening & McBride 2022). Originally, sleep was thought to play a primarily passive role in strengthening memories by protecting them from distracting stimuli. Now the active role of sleep is being promoted, whereby memories in sleep are subjected to a systemic consolidation process (Rasch & Born 2013). During sleep, sensorimotor interactions with the environment are interrupted and accompanied by immobility (Greening & McBride 2022). Once the body reaches sleep, it maintains a state of quiescence, which is also associated with loss of consciousness and reduced reactivity to stimuli (Rasch & Born 2013; Keene & Duboue 2018).

Depending on the movement of the eyes, there are two phases of sleep in horses and all mammals, the first one is REM (Rapid-Eye-Movement) also known as the paradoxical phase and the second phase is NREM (Non-Rapid-Eye-Movement) (Greening et al. 2021; Mukai & Yamanaka 2022; Oliveira et al. 2022). A total of two NREM phases (Hartman & Greening 2019) and two REM phases (Greening et al. 2021) are known in horses, which are described in more detail in the methodology. Both REM and NREM phases play a role in proper glucose metabolism and neuroendocrine function (Beccuti & Pannain 2011). Total sleep time (TST) in horses varies between studies depending on whether sleep was measured using an observational cohort method (based on mere observation) (Chung et al. 2018), or whether sleep was measured using electroencephalography (based on the recording of brain activity) (Williams et al. 2008). Using the above-mentioned observational cohort method, it was found that thoroughbred horses in three different stalls, with three different stall sizes, different sawdust heights and the same daily activity did not exceed the TST threshold of 100 minutes (Chung et al. 2018). Similar studies observing TST in horses based on behavioural observations report sleep ranging from one to six hours per day (Greening & McBride 2022). And that total sleep that is measured by electrocorticography or electroencephalogram is more accurate, and its range is lower ranging from three to four and a half hours (Greening et al. 2021; Greening & McBride 2022). Total sleep time is divided into several NREM and REM periods, which in this study varied over five consecutive 24-hour periods (Oliveira et al. 2022). According to the results of Williams et al. (2008), the NREM phase is divided into 52 periods, and the REM phase has a total of 15 periods during the night. The inconsistency in these two studies in the different numbers of phases may be attributed, for example, to the fact that Oliveira et al. (2022) looked at the sleep of hospitalized Lusitano horses (all mares aged from 5 to 8 years) after severe joint injuries and thus showed problems in rising from lying to standing. Williams et al. (2008) examined sleep in normal horses (Standardbred mares, Thoroughbred mares, Quarter Horse geldings, Paint mares aged from 4 to 13 years) without serious illness. Thus, it can be seen that there is a wide variation in results between TST and sleep periods depending on many parameters such as housing conditions, physical health and the methods by which these results were obtained.

The term wakefulness in sleep refers to the time occurring between REM and NREM sleep phases that lasts longer than three minutes, during which the sleep cycle is modified (Greening & McBride 2022). Periodic jaw movements are a specific sign of wakefulness, and wakefulness is followed by drowsiness, which is characterized by periods of lying, standing, or resting (Williams et al. 2008). In total, there are three types of drowsiness, namely, excessive drowsiness induced by monotony, excessive drowsiness associated with an uncertain environment, and excessive drowsiness associated with pain (Bertone 2006).

Increased daily physical activity plays an important role in sleep, and the effects of exercise on sleep have largely been conducted in humans (Kubitz et al. 1996; Youngstedt et al. 1997; Kredlow et al. 2015), with only a few studies in laboratory rodents (Blanco-Centurion & Shiromani 2006) or dogs (Kis et al. 2014; Bunford et al. 2018). Based on the information from these studies, it was found that older laboratory rats aged 22 months that were exposed to daily exercise on a rotating wheel for 50 minutes for eight weeks had less sleep fragmentation and, at the same time, the total number of NREM and REM transitions was reduced and they spent more time in single NREM and REM phases (Blanco-Centurion & Shiromani 2006). A study conducted by Bunford et al. (2018) focused on sleep in dogs and found that after an active day, they spent more time in REM and NREM and less time in drowsiness. And since dogs also sleep during the day, after an active day there was a significant increase in REM sleep at night compared to during the day. On the other hand, a study conducted by Kis et al. (2014) found that sleep latency (time from transition from full wakefulness to sleep) decreased after an active day, but that REM sleep duration did not differ between passive and active days. The difference within REM may be because, in the study by Kis et al. (2014), the active day was such that dogs were required to walk for six to eight hours during the day, be active, and not fall asleep during transport on public transport. Whereas an active day in the study by Bunford et al. (2018) meant that the dog participated in advanced training or competition. These variations in different activities could be the reason for the disparity in REM sleep duration. However, there is a study conducted by Jones et al. (2016) that focused on how ridden exercise affects nocturnal resting behaviour in horses. The results of this study suggested that horses showed less total rest time after ridden exercise than horses without ridden exercise. But for a change, horses with ridden exercise spent more time in sternal recumbency and horses without ridden exercise in turn spent more time in resting standing. This study attributes the difference in findings to the fact that horses during physical load change their resting pattern, while horses without workload are resting more in the sternal recumbency during the day.

Sleep is affected by ubiquitous light, which by its light intensity in the evening and morning hours disrupts circadian rhythms and thus affects sleep (Chellappa 2021). In general, for all horses, sleep is further influenced by stall type, feeding, fasting, social grouping and weather (Belling 1990). For individuals, sleep varies depending on age (Auer et al. 2021), and sex (Stachurska et al. 2016). Unlike stallions and geldings, mares spend twice as much time lying down, and although stallions and geldings spend time lying down exclusively at night, mares spend 10 % of their total time lying down during the morning and afternoon (Stachurska et al. 2016). In young foals, sleep takes up about half of the entire 24-hour day, most of which is spent napping between feedings, horses up to two years of age spend more time lying down than adult horses and, last but not least, it is clear that sleep increases with age and therefore older horses nap more often (Watson 2017). It is also interesting to note that horses spend more time in standing NREM with 5 cm high bedding than on bedding with 15 cm but prefer higher bedding when lying down (Greening et al. 2021). This claim is contrary to Watson (2017), who argues that horses prefer to lie down on harder, dry, and clean bedding.

Poor quality sleep has a negative impact on longevity, cognitive abilities, and development of an individual (Keene & Duboue 2018). There exists a close relationship between sleep and the immune system, establishing a reciprocal connection (Gamaldo et al. 2012; Irwin 2019). Prolonged sleep deprivation, characterized by sleep disturbances or short sleep duration, can heighten the susceptibility to infections, consequently diminishing immune defences (Besedovsky et al. 2019; Irwin 2019), and ultimately contribute to the development of chronic diseases with adverse effects on longevity (Mazzotti et al. 2014; Klein et al. 2017). Conversely, microbial infections trigger an immune system response marked by inflammation, leading to an extended period of sleep (Besedovsky et al. 2019). Sleep also forms a close bidirectional relationship with emotions, where sleep deprivation induces an emotional imbalance (higher sensitivity and arousal to stressful events and stimuli) (Vandekerckhove & Cluydts 2010; Vandekerckhove & Wang 2018). Even in this case sleep is a necessary factor in helping to cope with emotional stress (Vandekerckhove & Wang 2018). Among other aspects, the lack of sleep also affects the development of the individual during adolescence, primarily due to the secretion of growth hormones being predominantly active during sleep (Chennaoui et al. 2020).

Within this diploma thesis, a special focus has been given to the REM phase of sleep, because it assists in developmental functions and regulates psychological and physical functions (Mukai & Yamanaka 2022). The REM phase is very important for brain development (Knoop et al. 2021) and for proper memory function because it regulates neural circuits, thereby strengthening recent memories and weakening older ones (Poe et al. 2000). At the same time, the amount of REM sleep correlates with the consolidation of high emotional arousal (Wiesner et al. 2015). Insufficient amounts of REM sleep are reflected in sleep deprivation (Watson 2017). With more than ninety hours of REM deprivation, lipid peroxidation is induced in the brain of rats (Mathangi et al. 2012), and during this process, biological micromolecules, macromolecules and membranes are irreversibly degraded (Ahmed et al. 2023). When the REM phase is maintained for a sufficient duration, several effects are exerted, including improved cognitive function (Jozwiak et al. 2017), motor skill memory consolidation (Pereira & Lewis 2020), reduced stress levels (Suchecki et al. 2012), and even remodelling of dendritic spines (Pereira & Lewis 2020). REM phase is a critical stage of sleep (McDevitt et al. 2015), so it is crucial for the physical and mental health of the individual that this phase is not omitted (Fraigne et al. 2014; Cho et al. 2016).

2.5. Stress factors

This section provides a synopsis of the key discoveries related to the stress factors encountered by sport horses (Witkowska-Piłaszewicz et al. 2021; Henshall et al. 2022). In the context of effectively breeding sports horses, it is crucial to emphasize increased flexibility in management practices to ensure the welfare of the animals (Krupa et al. 2022). Animal Welfare is defined by the World Organisation for Animal Health (WOAH) as "the physical and mental state of an animal in relation to the conditions in which it lives and dies". Despite efforts to provide horses with appropriate conditions, horses are often subjected to stressful situations due to the domestication process (Henderson & Waran 2001; Yarnell et al. 2015). One of the most stressful elements horses face is the transportation of horses in horse trailers, which is associated with the occurrence of problem behaviours and the development of injuries (York et al. 2017). Horse trailers are largely used to move horses to races and competitions. The intense performances that horses exhibit at races result in higher stress reactions and an increase in cortisol concentration in both endurance horses and racehorses (Witkowska-Piłaszewicz et al. 2021).

Welfare concerns are associated with the presence of noise in the environment in which the horses are kept. Noise can cause shaking, sweating and a tendency for horses to flee. The anxiety that noise induces in the horse results in a more frequent recurrence of noise reactivity and anxiety-related behaviours (Riva et al. 2022). Horses encounter loud noises at races and competitions where the audience is noisy (Medica et al. 2020). Horses are exposed to noisy environments, for example when they are near wind farms (e.g., in Ireland, Australia and New Zealand), which have an aversive effect on horses (Rørvang et al. 2020).

The change in behaviour is influenced by lighting conditions, as the confinement of horses to stalls with artificial lighting, which is far from matching the spectral composition of natural light, results in changes in circannual and circadian rhythms. Artificial light then affects the breeding efficiency of the horses, the development of the young and reproductive behaviour (Murphy 2019).

They face stressful situations when exposed to unnatural situations such as individual box housing instead of group housing (Yarnell et al. 2015). It is certainly important to mention that although individual housing is unnatural for horses, horses born into these conditions show fewer stress reactions than horses that were used to outside housing and then moved to individual stalls (Marr et al. 2020). Not to mention that stall housing has an impact on several abnormal behaviours that help horses cope with captivity (Cooper & Mason 1998) and that can help keepers identify environmental deficiencies (Cooper & Albentosa 2005).

Stress caused by jumping competitions is reflected in increased heart rate (Bartolomé et al. 2013). The fact that horses are stressed during racing is mirrored in increased acidity in the stomach, which promotes the formation of gastric ulcers, thus reducing the horse's appetite (Sasaki et al. 2021) and also reducing the weight of horse (Padalino et al. 2017). Whether the stress is either caused by the environment (Yarnell et al. 2015; Murphy 2019; Riva et al. 2022) or by transporting horses to competitions (York et al. 2017; Sasaki et al. 2021) there are many ways how to reduce stress and its associated factors. McBride et al. (2004) claim that the stress caused by transporting horses can be alleviated through systematic horse loading training, pre-handling training, selecting appropriate handling methods and adequate driving experience. In stabling, changes may involve, for example, the choice of suitable management techniques (Park et al. 2013), providing horses with massage to reduce stress and associated heart rate (York et al. 2017), or allowing horses to show signs of stereotypic behaviour, which according to

Freymond et al. (2020) reduces stress in comparison to a control group of horses, who do not exhibit stereotypic behaviour.

However, stereotypical behaviour can be perceived negatively in terms of performance crib-biting and windsucking. Both stereotypical behaviours can cause colic in horses (Archer et al. 2008; Malamed et al. 2010; Wickens & Heleski 2010). Therefore, there is the possibility of reducing stereotypic behaviour with equiball (Henderson & Waran 2001) or reducing stereotypic stall walking with aromatherapy (Muñoz et al. 2018).

2.6. Aromatherapy and its effect

Aromatherapy is a complementary or practical health therapy that uses volatile concentrates extracted from plants, known as essential oils, for its therapeutic use (Gnatta et al. 2016). Essential oils are absorbed through the olfactory apparatus or the skin (Marzouk et al. 2013). There are many forms in which aromatherapy can be provided such as bathing, inhalation, or massage (Gnatta et al. 2016; Sánchez-Vidaña et al. 2017; Farahani et al. 2019). While during massage the plant essential oils are gradually absorbed into the skin and have a physical and physiological effect, during inhalation the volatile oils stimulate the olfactory cells and soon enter the limbic system and thereby have a positive effect on the recipient (Farahani et al. 2019).

The use of aromatherapy is broad-spectrum and is used to treat depression, as it relieves depressive symptoms (Sánchez-Vidaña et al. 2017), cures and alleviates temporary anxiety (Gong et al. 2020), reduces stress (Li et al. 2019), and helps with insomnia (Tang et al. 2021). The effects of aromatherapy have been studied in many animal species of different sizes, from mice (Wang et al. 2018), to dogs (Wells 2006), and giraffes (Fay & Miller 2015), but also in farm animals such as ruminants (Nehme et al. 2021). In mice, the effects of spearmint essential oil were investigated by inhalation and oral intake, and it was found that the mice exhibited fewer behavioural elements that were associated with pain (Wang et al. 2018). In dogs, the effect of lavender on travel-related excitement has been observed, and there was a significant increase in time spent resting and sitting when lavender was provided (Wells 2006). The effect of six scent enrichment was tested in zoo-bred giraffes, and giraffes showed much higher activity associated with moving around the exposure (Fay & Miller 2015). Plant essential oils in ruminants help during fermentation, inhibit proteolytic bacteria, reduce ammonia production, and inhibit the development of the microorganisms of methanogens (Nehme et al. 2021).

The impacts of lavender on humans are extensively documented, demonstrating augmentation in the proportion of deep or slow-wave sleep (Goel et al. 2005), substantial enhancement in well-being, encompassing increased energy, vibrancy, and improved sleep (Lillehei et al. 2016), enhancements in quality of sleep, sexual desire, anxiety, depression, physical and psychological symptoms in menopausal and older women (Roozbeh et al. 2019), betterment of sleep quality are also observed in women both during pregnancy and postpartum (Effati-Daryani et al. 2018), elderly individuals also encounter improvements in various sleep quality aspects, encompassing duration, latency, disturbances, efficiency, and daytime dysfunction (Maharianingsih et al. 2020). Lavender has a profound effect on human sleep, whereby the effect of this essential oil on naps administered through an aroma diffuser is reflected in reduced production of α-amylase, which is associated with a lower stress response of the sympathetic nervous system (Yogi et al. 2021). Another way in which lavender can be provided to people is by using lavender pillows and wipes, which increase the quality of sleep (Demirbağ & Çalik 2019). Overall, it can therefore be argued that in humans, lavender is involved in improving sleep quality (Cavanagh & Wilkinson 2002; Afshar et al. 2015; Samadi et al. 2021), but also in improving the overall quality of life (Lucena et al. 2023). In a way, lavender is also involved in sleep in animals such as mice, where it causes a reduction in sleep latency and a reduction in the number of wake episodes (Manor et al. 2021) and helps to extend sleep duration (Hajhashemi & Safaei 2015).

The use of all kinds of oils (spikenard - *Nardostachys jatamansi*, roman chamomile - *Anthemis nobilis*, vetiver - *Vetiveria zizanioides*, lavender - *Lavandula angustifolia*) in horses can reduce respiratory tidal volume, heart rate and spontaneous muscle contractions in horses. The respiratory tidal volume and heart rate were always lowered by at least 4 % using all four oils (spikenard, roman chamomile, vetiver, lavender). Spontaneous muscle contractions were measured for *Musculus cleidomastoideus* and *Musculus temporalis* and in this case, the incidence results were significantly decreased by all four oils (Kosiara & Harrison 2021). It is crucial to acknowledge that many studies are currently investigating the effectiveness of aromatherapy utilizing a diverse range of essential oils. Among the most common is the use of lavender essential oil (Ferguson et al. 2013; Adkins et al. 2016; Baldwin & Chea 2018; Heitman et al. 2018; Muñoz et al. 2018; Poutaraud et al. 2018). The influence of spikenard (*Nardostachys jatamansi*), roman chamomile (*Anthemis nobilis*) and vetiver (*Vetiveria zizanioides*) has also been studied (Kosiara & Harrison 2021) together with true chamomile (*Matricaria recutita*) (Baldwin & Chea 2018) or chamomile (*Chamaemelum nobile*) (Chea 2016). Other essential oils include commonly available herbs such as oregano (*Origanum vulgare*) (Simirgiotis et al. 2020), coriander (*Coriandrum sativum L.*) (Kačániová et al. 2020), rosemary (*Rosmarinus officinalis L.*) (Bozin et al. 2007) or black cumin (*Nigella sativa*) (Kazemi 2014).

The correlation between a high-stress level and a high salivary cortisol level in horses was investigated by Chea (2016), who found that during lavender aromatherapy, salivary cortisol levels always decrease after a given period from baseline, resulting in lower stress levels. Almost the same was investigated in a study conducted by Heitman et al. (2018), which also monitored cortisol levels in horses exposed to high levels of stress in the form of horse trailers. Horses that were treated with lavender showed lower levels of cortisol compared to control horses treated with water. The effects of lavender have been shown to increase heart rate variability, positively influencing the heart's adaptability and its responsiveness to the body's moment-to-moment requirements (Baldwin & Chea 2018), which helps to adapt to environmental and psychological challenges (Shaffer & Ginsberg 2017). Lavender aromatherapy also has a positive effect on reducing the respiratory rate after an unnaturally induced acute man-made stress response (Ferguson et al. 2013). In the study of Poutaraud et al. (2018), authors point out that lavender essential oil reduces alert postures and heart rate. The authors also state the fact that lavender essential oil therefore reduces overall stress. However, none of these studies and other studies have looked at the effect of lavender essential oil on equine sleep, and thus the effect of lavender essential oil in this regard is unknown.

3. Aims of the Thesis

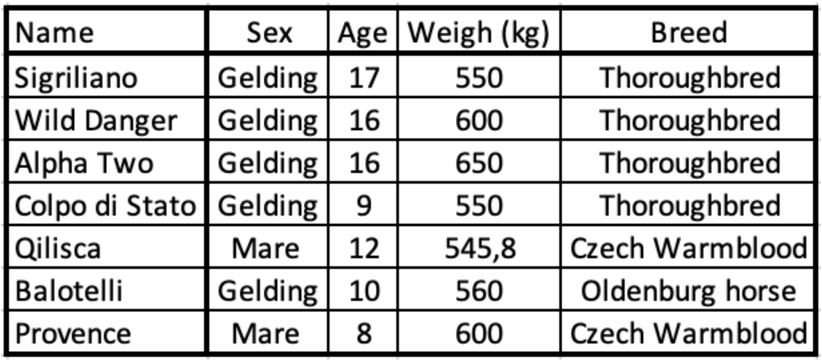
The primary aim of this study was to determine the nocturnal sleep patterns exhibited by seven horses. Secondly, the study sought to explore the potential impact of aromatherapy, specifically focusing on the lavender essential oil, on the length of sleep across three distinct states: sternal recumbency NREM, sternal recumbency REM, and lateral recumbency REM, including the total length of sleep in these three positions together. Additionally, the research aimed to evaluate whether the application of lavender essential oil influenced the overall length of rest, encompassing periods spent in the lying position, and if lavender essential oil influenced sleep interruptions. Finally, the study aimed to investigate whether other variables such as age, sex, work type, and working time also contribute to the change in sleep.

4. Methods

4.1. Animals

A total of seven horses were included in the experimental part. These horses were members of three breeds of different sexes with different weights and ages (see Table 1). All seven horses had the same housing conditions, i.e., on pasture during the day and in a box during the night. The horses were taken out to pasture each morning by the staff at 7:00 a.m. and were also penned up by the staff at 4:00 p.m. The indoor housing for all horses was identical. The size of the box was 3 × 3 metres, and the bedding consisted of sawdust with a height of 15 cm. Water was supplied to the box by an automatic waterer. The size of the grazing area outside the paddock was different depending on whether the horse was in a group of horses or alone. In the pasture, water was provided in large containers with a capacity of 60 litres and changed by the horse's owner/s every other day. Feeding was done every morning before the horses were brought out of the box at 6:30 a.m. and every evening at 6:30 p.m. Each horse had its own feeding ration, which was the same throughout the duration of the experiment and is listed in the description of each horse below. The feed is stored in the back of the stable, more precisely in the feed room. The horses received hay in both the stable and the pasture. In the pasture, the hay was available ad libitum. In the morning, the horses were given one kilo of hay in the box to be consumed before being put out to pasture. In the evening, the hay ration in the box was higher and amounted to 10 kg.

Table 1: Information about horses



Name	Sex	Age	Weigh (kg)	Breed
Sigriliano	Gelding	17	550	Thoroughbred
Wild Danger	Gelding	16	600	Thoroughbred
Alpha Two	Gelding	16	650	Thoroughbred
Colpo di Stato	Gelding	9	550	Thoroughbred
Qilisca	Mare	12	545,8	Czech Warmblood
Balotelli	Gelding	10	560	Oldenburg horse
Provence	Mare	8	600	Czech Warmblood

Sigriliano

The owner of this horse is a twenty-eight-year-old lady who has been riding since 2009. Sigriliano, or Sigi for short, has been owned by her since 2015. Health-wise, the horse is problem-free and takes no medication. Sigi has a pasture size of 67 × 36 meters and shares it with two other geldings (Wild Danger and Alpha Two). Sigi wears a fly mask and halter to pasture. He has no enrichment on the pasture. He has a salt lick in his box. Sigi's morning ration consists of 0,5 l of muesli + 0,5 l of chaff, and his evening ration consists of 0,5 l of muesli + 0,3 l of warm alfalfa. Sigi's work type is a hobby with a mid-level of performance. He is ridden without a whip and spurs. He trains three times a week, and the total time for each training session is approximately 50 minutes. Sigi has had two riders in the last year, but currently there is only one rider, and that is the horse's owner.

Wild Danger

Wild Danger is owned by a young, fourteen-year-old lady who has been riding since 2019. Wild Danger has been owned by this owner since 2019. Wild Danger is health-wise, the horse is problem-free and takes no medication. He is out on pasture with Sigi and Alpha Two. Their pasture measures 67 × 36 meters. Wild Danger wears a fly mask to pasture. There is no enrichment on the pasture. He has a salt lick in his box. Wild's morning ration consists of 0,5 l of muesli + 0,5 l of chaff, and his evening ration consists of 1 l of muesli + 0,5 l of chaff + 0,5 l of warm alfalfa. The work type is hobby with a mid-level of performance. The frequency of training is 3 to 4 per week, and the time spent in training is approximately 50 to 60 minutes. Wild Danger is ridden by two riders: the owner of Wild Danger and the lady who owns Alpha Two. Only the whip is used while riding the horse.

Alpha Two

Alpha Two belongs to a lady who is twenty-three years old and has been actively riding since 2009. Alpha Two has been owned by the current owner since 2006. Health-wise, the horse is problem-free and takes no medication. Alpha Two shares a 67 × 36 metres pasture paddock with Wild Danger and Sigi. He wears only a fly mask to pasture.

As an enrichment, Alpha Two has a teddy bear and is also given a mineral lick. Alpha Two is fed as follows: morning feeding 0,5 l of muesli + 0,5 l of chaff; evening feeding 1 l of muesli + 0,5 l of chaff + 0,5 l of warm alfalfa. The work type is a hobby with a medium level of performance. He trains 3 to 4 times a week for 50-60 minutes. Alpha Two is ridden only by the owner. A whip is used on the horse while riding, and spurs are used when the horse has jump training.

Colpo di Stato

The owner of this horse is a twenty-seven-year-old woman who has been riding horses since 2005. She has owned Colpo di Stato since 2014. Even though the horse is not taking any medication, he has health problems that are linked to poorly healed Achilles tendons. This health problem has not been sufficiently treated by the previous owner. Colpo di Stato is ridden by only one rider, and the work type is hobby with a light performance level. Training is twice a week for 35 minutes at most. The owner does not use a whip or spurs when riding. This gelding is on his own in the pasture, and the size of his outdoor paddock is approximately 50 × 50 metres. He is given a fly mask for pasture. The box has an enrichment in the form of a red rubber ball. His ration consists of 0,5 l of muesli + 0,5 l of chaff in the morning and 1 l of muesli + 0,5 l of chaff in the evening.

Quilisca

Together with Balotelli, she belongs to a nineteen-year-old owner who has been riding since 2012. Quilisca has been owned by her owner since 2019. The mare is health-wise, the horse is problem-free and takes no medication. She is alone in the outdoor enclosure, and the enclosure is 60 × 60 meters. Like most of the other horses in this experiment, Quilisca wears a fly mask in the enclosure. Quilisca's work type is sport horse with a heavy performance level of parkour. She works a total of six times a week, and of those, she has one to two workouts a week of either jumping or dressage. The average time spent working under the saddle is around 45 minutes. For this reason, Quilisca is fed three times a day, in the morning, at noon, and in the evening. In the morning, she receives 1 l of pure oats, at noon she receives 0.5 l of Condition Mix from AgramM + 0.5 l of Structure E from Hippolyt + 0.5 l of Reformer from Fitmin, and in the evening she receives 1 l of pure oats. The total number of riders for this horse is only one and that is the owner. When riding the horse, the owner uses a whip for jumping and dressage competitions and spurs only for jumping training.

Balotelli

Balotelli, together with Quilisca, are owned by a nineteen-year-old lady. The owner has had this horse since 2020. Health-wise, the horse is problem-free and takes no medication. He is alone in the 80 × 80 metres outdoor pasture and wears a fly mask to the pasture. Inside the box, he has enrichment in the form of a blue, deflated rubber ball. This horse is also ridden only by the owner, and the work type is a sport horse with a heavy performance level of parkour. Even Balotelli has training sessions six times a week (including one or two jumping or dressage sessions) for an average of 45 minutes. The owner does not use a whip when riding and only uses the spurs when Balotelli has jump training. He is fed the same as Quilisca three times a day, 1 l of oats in the morning, 0,33 l of Condition by AgramM + 0,5 l of Stuktur E by Hippolyt at noon, and 1 l of oats in the evening.

Provence

Provence is the second of the mares in this experiment. The owner of Provence is an eighteen-year-old lady who has been riding since 2013. Provence has been in her ownership since 2020. Provence is also free health-wise, the horse is problem-free and takes no medication. She goes to pasture with a mask, and her pasture is 40 × 40 meters. She has enrichment in the form of a plush strawberry. Provence is fed twice a day. In the morning and evening, her ration consists of 0,5 l of pellets + 0,75 l of muesli. Provence's work type is sport horse with a medium-heavy to heavy performance level of parkour. Provence is worked only by the owner, and four to five times a week (including one jump training session per week) for 45 minutes. The owner uses the whip and spurs on the Provence only for competitions.

4.2. Data collection

Data collection took place at the Farm U Bílého domu (49°36'46.514"N 13°34'37.146"E). This horse stable is in the Czech Republic in the village of Spálené Poříčí, which is located 25 kilometres south of the larger statutory town of Pilsen. Farm U Bílého domu is equipped with two stables, offering a total of twenty-six boxes (14 boxes in the newer stable + 12 boxes in the older stable). The premise is equipped with countless places for riding training such as an indoor riding arena of 25 × 60 metres, an outdoor riding arena of 35 × 60 metres and an outdoor circular riding arena with a diameter of 18 metres. All these places have sand on the surface with geotextile underneath. The surrounding fields, which are owned by this stable, are divided into several paddocks, which serve as an enclosure for the horses during the day.

The data collection took place in the summer, more precisely from the 10th of July 2023 to the 30th of July 2023. The first seven days of observation (i.e., from July 10th to July 16th) were used to get a closer insight into how the horses sleep in this stable. The following fourteen days (i.e., from July 17th to July 30th), distilled water control and lavender essential oil samples were placed in the stable. During the summer period, many equestrian competitions take place in the Czech Republic, in which more than half of the horses in this stable participated. For the horses to be rested, an evening quiet time, i.e., the time when no one is present in the stable, is set in this stable during the summer. Quiet time is respected by all riders and staff and is every day from 8:00 p.m. to 6:00 a.m. Even though there was no close contact with the horses, this work fulfilled the etic requirements.

4.3. Equipment

Camera system

To make the conditions as natural as possible and not affect the horses during their night-time rest, a camera system was installed. To meet all the requirements for a good quality camera system with sufficient storage and continuous recording, a total of seven cameras including a recorder were installed in the stable. The cameras, from Hikvision company, had a pixel resolution of 2 mpx and a Full HD standard definition of 1920×1080 with a colour sensitivity of 0.003 lux. The cameras had night-time infrared illumination with a range of up to 30 meters, which was essential for this work. The exact camera model is Turbo camera Hikvision DS-2CE16D8T-ITF(2.8mm). A video/utp converter was connected to each of these cameras, and the cameras shared a single power supply. At the same time, a UTP cable was connected to all cameras, and a total of 80 meters of this UTP cable was required. All recordings produced were stored on a Hikvision digital video recorder model DS-7216HGHI-SH including a 6TB hard disk. The cameras were also connected via a Wi-Fi router, and therefore the recordings could be viewed both online and browsed backwards using the Hik-Connect mobile app. It must be said that this option was only possible if the cameras were connected to the network. Once the cameras were disconnected, all recordings remained on the recording device only.

The camera system was set for a nine and a half-hour night period during which the recording was stored on a recording device. This period was from 9:00 p.m. to 6:30 a.m. with continuous recording. During the rest of the time, from 6:30 a.m. to 9:00 p.m., no recording was stored to save space for the recordings that were crucial to this work. Each of the seven cameras was attached above to the box of an individual horse. Due to the varying roof pitches and wall heights in the boxes, there were a total of two types of camera placement. The first type of placement was to place the cameras on the wall (Figure 1), and the second type was to place the cameras on wooden beams on the ceiling (Figure 2). This placement did not affect the evaluation of the data, only the angle at which the horses were observed varied. The camera system was installed for three weeks during the entire observation period, i.e., from the 10th of July 2023 to the 30th of July 2023.

Figure 1: Placement of cameras on the wall

Figure 2: Placement of cameras on the ceiling

Diffusers and bowls

The ETA Airco 1629 90000 humidifier and diffuser in one were used to gradually release lavender particles into the environment of the stable. The humidifier was used from the eighth to the eleventh day of this research (17th to the 20th of July). There were always two containers in total. This was to avoid mixing the "contaminated" container of lavender with the "clean" container of distilled water, which was the control sample, during the experiment. The humidifier was connected to the mains using a 25-metre extension lead reel and an EMOS socket. The humidifier had a built-in timer for up to 12 hours and had three stages of steam generation. The timer was set to 12 hours for all four days and ran from 7 p.m. to 7 a.m. on the highest steam generation stage. Due to a technical failure and damage to the diffuser from the factory, the experiment had to have modifications during the experiment, and instead of the humidifier, glass bowls were placed in the stall. The diameter of the bowls was 28 centimetres with a capacity of 5 litres. To maintain the same conditions, on the twelfth through twenty-first days, from the 21st to the 30th of July, glass bowls were placed in the exact location as the humidifier at 7:00 p.m. each day. At 7:00 a.m., the container was taken outside the stable. From the eighth day to the twenty-first day of this work, the containers were rotated day by day, beginning with water on the 17th of July, followed by lavender on the 18th, followed by distilled water, and so on (see Figure 3).

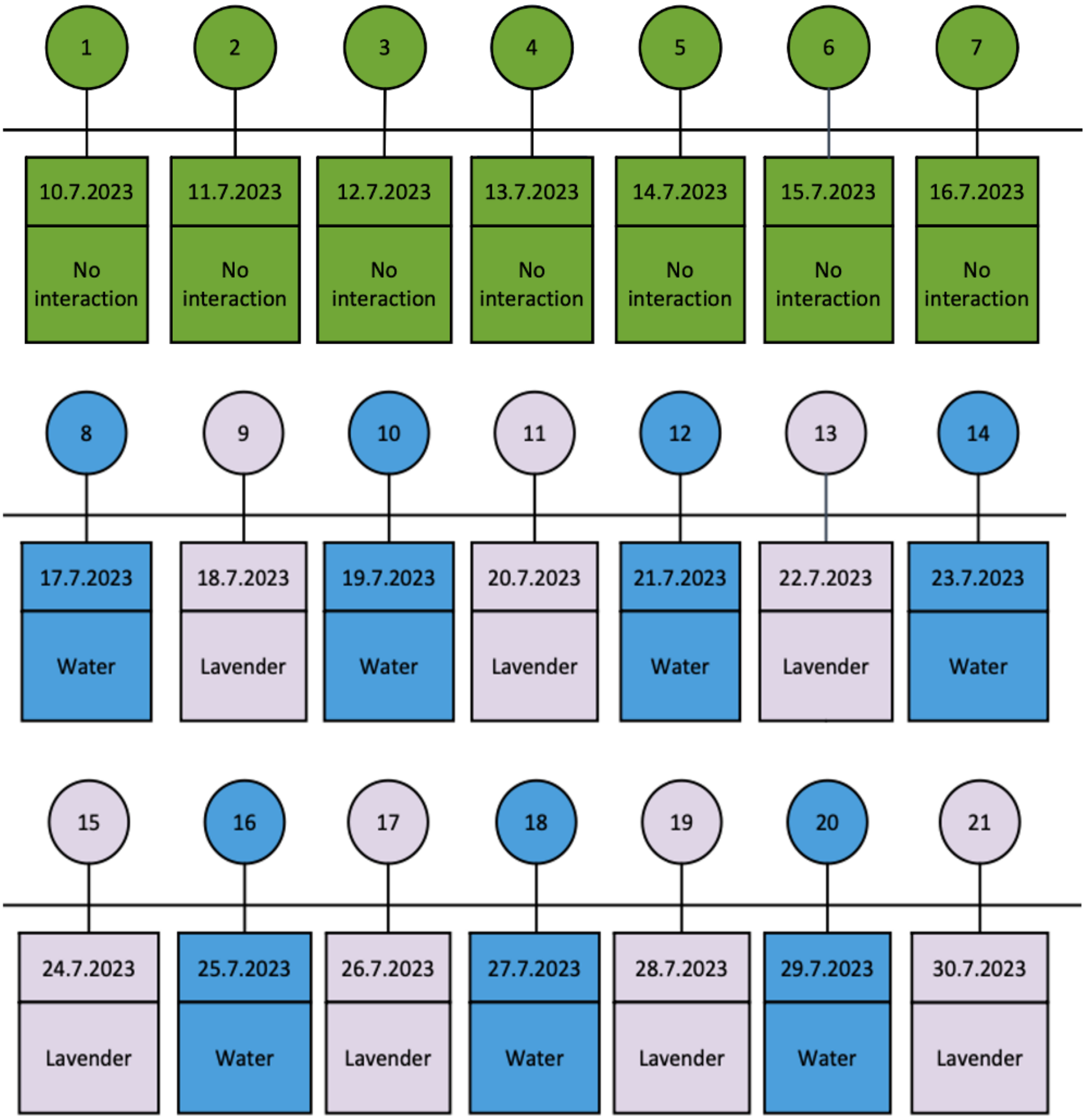


Figure 3: Timeline

Essential oil

Lavender (*Lavandula officinalis*) essential oil distilled with steam was used. The flash point of this lavender essential oil is 74°C. The ratio at which the essential oil was diluted in distilled water was adapted from a paper by Fay & Miller (2015) who used a range of scents at a ratio of 1 ml of essential oil to 31 ml of distilled water. This thesis used 1 drop of lavender essential oil to 30 ml of distilled water. Because more distilled water was needed to create the vapour during the first four days that the humidifiers were in the stable, 133 drops of lavender oil were dripped into a 4-litre container. On subsequent days, when glass bowls were used, the ratio was half that, 2 litres of distilled water to 67 drops of lavender essential oil.

4.4. Data recognition

Recent studies on equine sleep have divided sleep into two phases, which are REM and NREM (Hartman & Greening 2019; Greening et al. 2021). The first division of the sleep phases in horses into three states was introduced by Hartman & Greening (2019), who defined standing sleep (NREM), and “lying” sleep i.e., sternal recumbency (REM) and lateral recumbency (REM). The fourth state was added by the authors Greening et al. (2021), who defined sternal recumbency (NREM). All these four states have been clearly defined and delineated, and it is possible to determine which of these sleep states the horse is in.

1) Standing sleep (NREM) is recognized as the following - a horse is standing immobile with the articulation between the C0 and C1 vertebrae below the level of the withers and with the hind leg resting or not.
2) Sternal recumbency (NREM) is described as - a horse is lying down, the sternum is in contact with the ground, all four limbs are folded under the body, and the muzzle is not in contact with the ground (Figure 4).
3) Sternal recumbency (REM) - in this state, the horse shows almost identical signs as in sternal recumbency (NREM) with the difference that the muzzle is in contact with the ground (Figure 5).
4) Lateral recumbency (REM) - in this condition, the horse is lying on one side (left or right) in a lateral recumbent position, and the lateral thoracic area is

in contact with the ground, all four limbs are extended, the head is touching the ground, and the ears and eyes are immobile (not moving) (Figure 6).

This complex recognition of the four sleep states was used in the primary study created, defined, and adapted by (Greening et al. 2021) and in the study by (Greening et al. 2023). However, due to the time-intensive nature of assessing and evaluating all four states of sleep, this thesis concentrates solely on both REM sleep states and sternal recumbency NREM state. Moreover, REM sleep states are important because they prevent the development of deprivation (Mathangi et al. 2012; Watson 2017) and thanks to these states, a complete sleep cycle can be successfully achieved (Aleman et al. 2008).

Figure 4: Sternal recumbency NREM position

Figure 5: Sternal recumbency REM position

Figure 6: Lateral recumbency REM position

4.5. Analysis of data

Before the data could be evaluated, it was necessary to download the recordings from the recorder, convert them, and merge them into one complete recording. For capacity reasons, the individual nights were automatically split by the recorder into approximately thirty-minute recordings. To simplify the evaluation and consolidate the recordings, these thirty-minute segments were merged to form a record of nine and a half hours in a row (i.e., one night). To convert the videos to the correct MP4 format supported by the Noldus Observer XT software, and to merge the videos into one night, the Free Video Converter by Freemake was used.

The whole recordings were then analyzed using the Noldus Observer XT software which is used to evaluate behavioural data. In this software, a template was created and used to record all the sleep activities of the horses during the night. The template contained a mutually exclusive group called locomotion, where a total of five factors (other behaviour, lying, sternal recumbency NREM, sternal recumbency REM, lateral recumbency REM) were present and could not occur simultaneously. It is important to note that more factors were included in the other behaviour group, such as walking, standing, standing sleep NREM, drinking, mineral licking, and defecating.

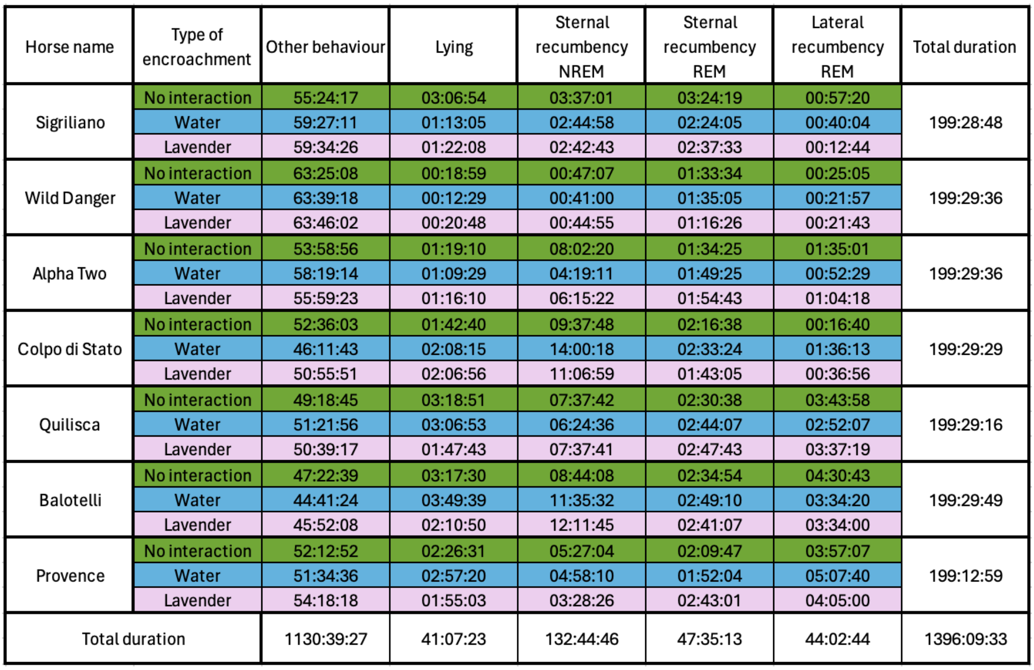
The lying factor included only horses lying down but showing no signs of sleep. The predefined template also focused on user-defined variables, which were also crucial for the subsequent statistics. The user-defined variables included sex (gelding or mare), age (from 8 to 17 years), the lowest temperature during the night (from 8°C to 18°C), work type (hobby horse vs. sport horse), and working time (measured in minutes). The data obtained using the Noldus observer XT software were converted into Excel spreadsheets. All statistical analyses were conducted in IBM SPSS Statistics 28 (IBM, Armonk, New York). Data inspection (Q-Q plots) and normality tests for the dependent variables (length of rest, length of sleep, sleep interruptions, sleeping positions: sternal recumbency NREM, sternal recumbency REM, lateral recumbency REM) were conducted, and further analyses were designed according to the characteristics of the studied variable. The dependent variables sternal recumbency NREM and lateral recumbency REM were not normally distributed. As a next step, sets of generalized linear mixed models (GLMMs) were designed for each of the previous dependent variables. Group structure based on ID and day served as the repeated measure. All models had the linear distribution and identity function, except the two for not normally distributed dependent variables, where the gamma distribution and log function were used. Independent variables were the following: encroachment (no/water/lavender), sex (gelding/mare), age (years), work type (hobby/sport), working time (hours), and temperature (degrees). The total sample size was 147 for all the analyses.

5. Results

5.1. Fundamental sleep characteristics

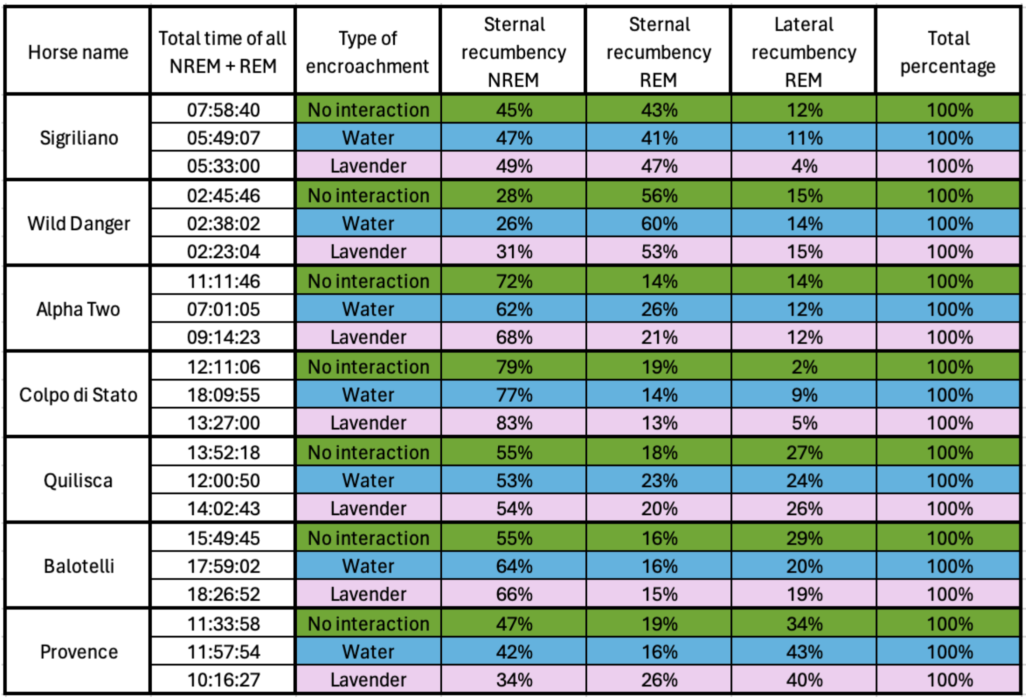
Each horse was observed for 199 hours and 30 minutes over three weeks (weekly time allocation of 66 hours and 30 minutes), of which the total observation time for all seven horses was almost 1400 hours. Of this total observational time, all horses spent 1130 hours in the other behaviour position during all three weeks (total variability for individuals ranged from 138 hours to 191 hours (mean 164.5 ± 26.5) in the other behaviour position over three weeks). The sternal recumbency NREM position was observed for a duration eight times less than that of the other behaviour position, yet it still accounted for a substantial amount of time. All individuals spent a total of 132 hours in the sternal recumbency NREM position over three weeks, with individual durations ranging from 2 hours 13 minutes 2 seconds to 34 hours 45 minutes 5 seconds (mean18 hours 57 minutes 49 seconds ± 10 hours 58 minutes 59 seconds) during these three weeks. All individuals collectively spent nearly 48 hours in the sternal recumbency REM position over the three weeks, with individual variability ranging from 4 hours 25 minutes 5 seconds to 8 hours 25 minutes 57 seconds (mean 6 hours 47 minutes 53 seconds ± 1 hour 24 minutes 18 seconds) over these three weeks. Slightly less total time over the three weeks was observed for all horses in the lateral recumbency REM position, with this value being 44 hours and highly variable among horses, ranging from 1 hour 8 minutes 45 seconds to 13 hours 9 minutes 47 seconds (mean 6 hours 17 minutes 32 seconds ± 4 hours 46 minutes 38 seconds). The least time was spent by all horses in the lying position, with a total duration of 41 hours over the three weeks and individual variability among horses ranging from 52 minutes 16 seconds to 9 hours 17 minutes 59 seconds (mean 5 hours 52 minutes 29 seconds ± 2 hours 38 minutes 38 seconds). All above-mentioned information can be found in Table 2. The total weekly sleep duration (hh:mm:ss), encompassing positions such as sternal recumbency NREM, sternal recumbency REM, and lateral recumbency REM, which are converted from total weekly times to percentages can be seen in Table 3. This table serves as the basis for the pie charts presented for each horse in the subsequent chapters. The average daily sleep values of each individual for all phases for the entire observed period are depicted in Table 4.

Table 2: All documented time measurements during sleep



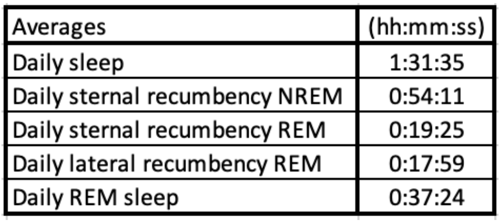
Horse name	Type of encroachment	Other behaviour	Lying	Sternal recumbency NREM	Sternal recumbency REM	Lateral recumbency REM	Total duration
Sigriliano	No interaction	55:24:17	03:06:54	03:37:01	03:24:19	00:57:20	199:28:48
	Water	59:27:11	01:13:05	02:44:58	02:24:05	00:40:04	
	Lavender	59:34:26	01:22:08	02:42:43	02:37:33	00:12:44	
Wild Danger	No interaction	63:25:08	00:18:59	00:47:07	01:33:34	00:25:05	199:29:36
	Water	63:39:18	00:12:29	00:41:00	01:35:05	00:21:57	
	Lavender	63:46:02	00:20:48	00:44:55	01:16:26	00:21:43	
Alpha Two	No interaction	53:58:56	01:19:10	08:02:20	01:34:25	01:35:01	199:29:36
	Water	58:19:14	01:09:29	04:19:11	01:49:25	00:52:29	
	Lavender	55:59:23	01:16:10	06:15:22	01:54:43	01:04:18	
Colpo di Stato	No interaction	52:36:03	01:42:40	09:37:48	02:16:38	00:16:40	199:29:29
	Water	46:11:43	02:08:15	14:00:18	02:33:24	01:36:13	
	Lavender	50:55:51	02:06:56	11:06:59	01:43:05	00:36:56	
Quilisca	No interaction	49:18:45	03:18:51	07:37:42	02:30:38	03:43:58	199:29:16
	Water	51:21:56	03:06:53	06:24:36	02:44:07	02:52:07	
	Lavender	50:39:17	01:47:43	07:37:41	02:47:43	03:37:19	
Balotelli	No interaction	47:22:39	03:17:30	08:44:08	02:34:54	04:30:43	199:29:49
	Water	44:41:24	03:49:39	11:35:32	02:49:10	03:34:20	
	Lavender	45:52:08	02:10:50	12:11:45	02:41:07	03:34:00	
Provence	No interaction	52:12:52	02:26:31	05:27:04	02:09:47	03:57:07	199:12:59
	Water	51:34:36	02:57:20	04:58:10	01:52:04	05:07:40	
	Lavender	54:18:18	01:55:03	03:28:26	02:43:01	04:05:00	
Total duration		1130:39:27	41:07:23	132:44:46	47:35:13	44:02:44	1396:09:33

Table 3: Total weekly sleep for each encroachment



Horse name	Total time of all NREM + REM	Type of encroachment	Sternal recumbency NREM	Sternal recumbency REM	Lateral recumbency REM	Total percentage
Sigriliano	07:58:40	No interaction	45%	43%	12%	100%
	05:49:07	Water	47%	41%	11%	100%
	05:33:00	Lavender	49%	47%	4%	100%
Wild Danger	02:45:46	No interaction	28%	56%	15%	100%
	02:38:02	Water	26%	60%	14%	100%
	02:23:04	Lavender	31%	53%	15%	100%
Alpha Two	11:11:46	No interaction	72%	14%	14%	100%
	07:01:05	Water	62%	26%	12%	100%
	09:14:23	Lavender	68%	21%	12%	100%
Colpo di Stato	12:11:06	No interaction	79%	19%	2%	100%
	18:09:55	Water	77%	14%	9%	100%
	13:27:00	Lavender	83%	13%	5%	100%
Quilisca	13:52:18	No interaction	55%	18%	27%	100%
	12:00:50	Water	53%	23%	24%	100%
	14:02:43	Lavender	54%	20%	26%	100%
Balotelli	15:49:45	No interaction	55%	16%	29%	100%
	17:59:02	Water	64%	16%	20%	100%
	18:26:52	Lavender	66%	15%	19%	100%
Provence	11:33:58	No interaction	47%	19%	34%	100%
	11:57:54	Water	42%	16%	43%	100%
	10:16:27	Lavender	34%	26%	40%	100%

Table 4: Average daily sleep values



Averages	(hh:mm:ss)
Daily sleep	1:31:35
Daily sternal recumbency NREM	0:54:11
Daily sternal recumbency REM	0:19:25
Daily lateral recumbency REM	0:17:59
Daily REM sleep	0:37:24

5.1.1. Sigriliano

Sigriliano consistently spent most of the time in the sternal recumbency NREM position during the first week (no interaction), and he preferred this position in the subsequent weeks (water and lavender treatment) (see Figure 7). The average sleep duration of this horse each night over the entire three-week period was 55 minutes 17 seconds.

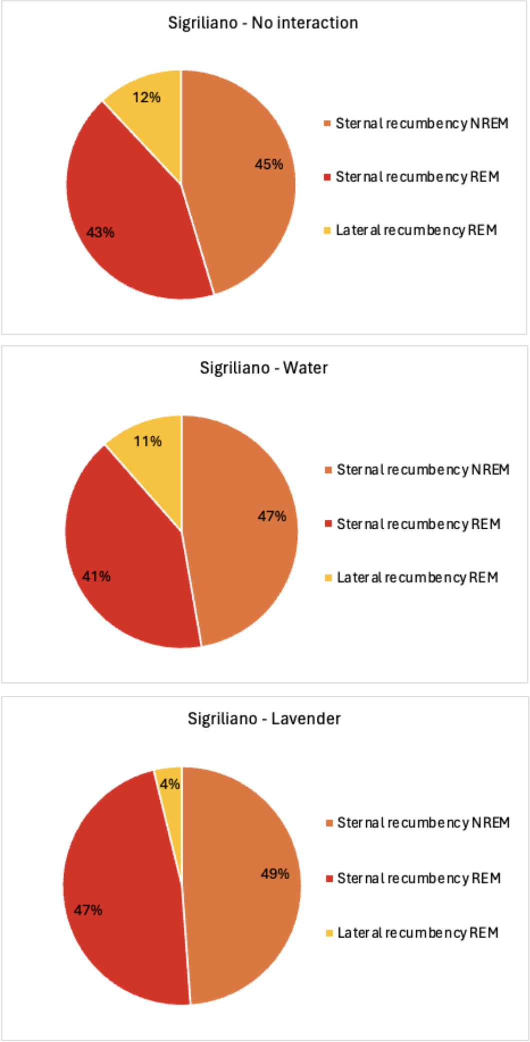


Figure 7: The total weekly sleep time for Sigriliano during different encroachments

5.1.2. Wild Danger

Wild Danger mostly slept in the lateral recumbency REM position, and it remained consistent throughout the observation period, with negligible variation (± 1 %) (Figure 8). The total time spent in the sternal recumbency NREM position increased by 3 % over the seven days when using lavender, while the total time spent in the sternal recumbency REM position decreased by 3 % over those seven days. The average sleep duration of this horse each night over the entire three-week period was 22 minutes 14 seconds.

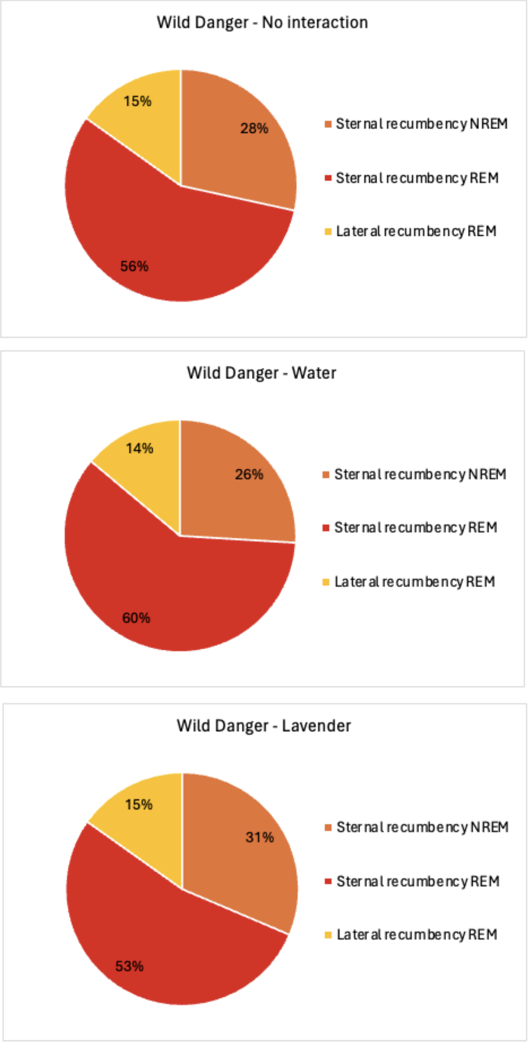


Figure 8: The total weekly sleep time for Wild Danger during different encroachments

5.1.3. Alpha Two

In the case of Alpha Two, the lateral recumbency REM position remained consistent throughout the observation period with a percentage deviation of ± 2 %. The position sternal recumbency REM was 7 % higher during the week of lavender application compared to the week with no interaction (Figure 9). The average sleep duration of this horse each night over the entire three-week period was 1 hour 18 minutes 26 seconds.

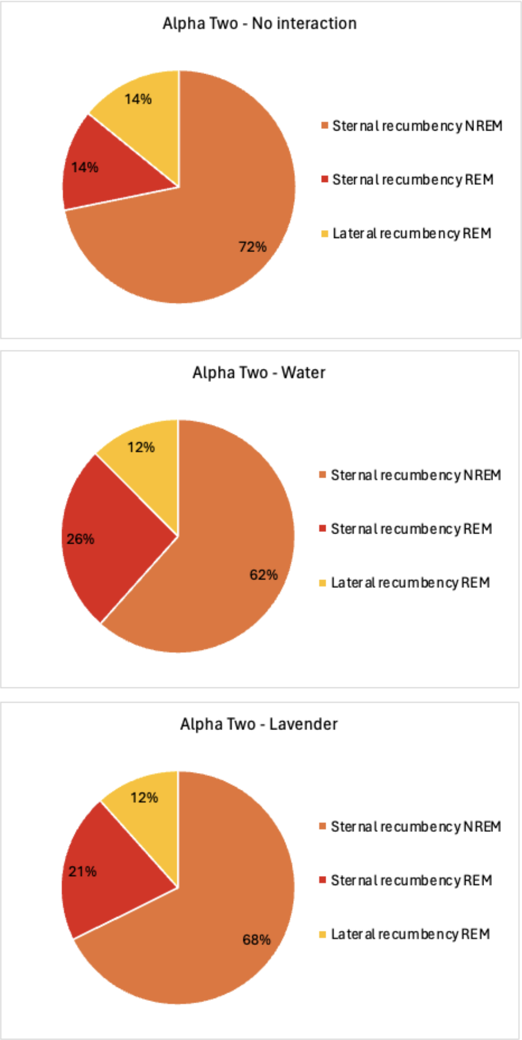


Figure 9: The total weekly sleep time for Alpha Two during different encroachments

5.1.4. Colpo di Stato

The positions of sternal recumbency NREM and lateral recumbency REM were most affected in the case of Colpo di Stato, as both experienced an increase of 3-4 % during lavender application compared to the first week with no interaction (Figure 10). The average sleep duration of this horse each night over the entire three-week period was 2 hours 5 minutes 9 seconds.

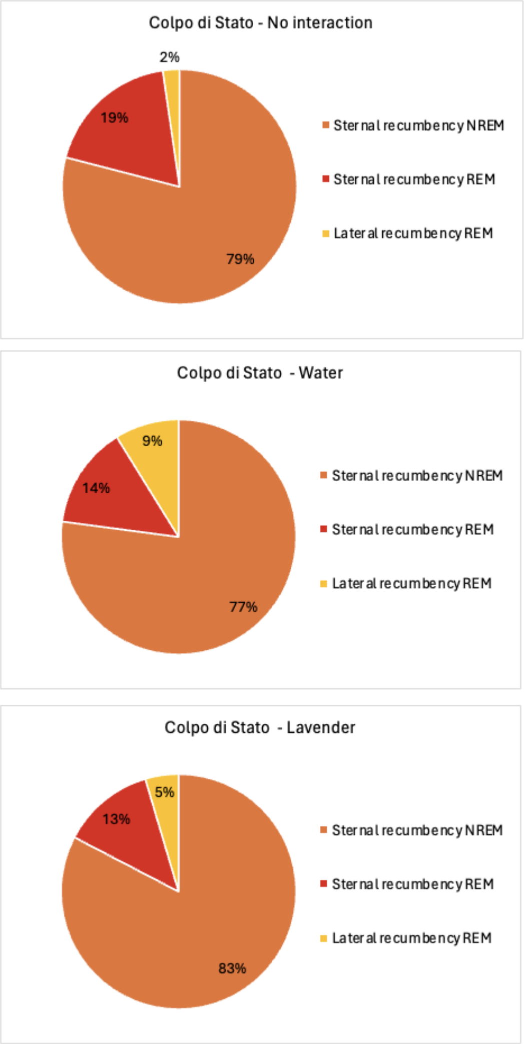


Figure 10: The total weekly sleep time for Colpo di Stato during different encroachments

5.1.5. Quilisca

In the case of Quilisca, the times for all three positions remained almost consistent throughout the entire observation period (± 3 %). However, there is a slight increase in the time spent in the sternal recumbency REM position by 2 % (Figure 11). The average sleep duration of this horse each night over the entire three-week period was 1 hour 54 minutes 5 seconds.

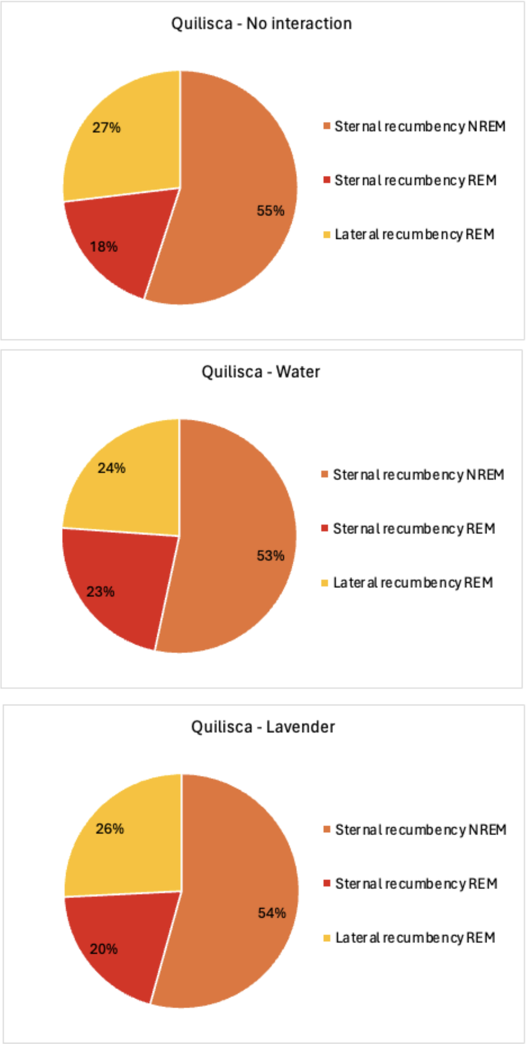


Figure 11: The total weekly sleep time for Quilisca during different encroachments

5.1.6. Balotelli

In the case of Balotelli, there was a linear increase in sleep by up to 11 % in the sternal recumbency NREM position during the week with lavender application compared to the week with no interaction. The sternal recumbency REM position remained unchanged for three weeks (± 1 %) (Figure 12). The average sleep duration of this horse each night over the entire three-week period was 2 hours 29 minutes 19 seconds.

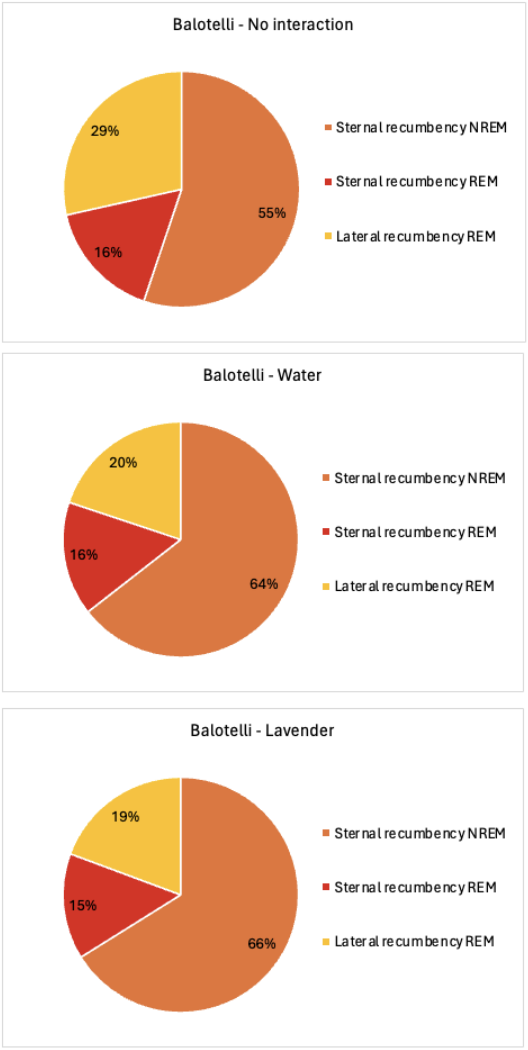


Figure 12: The total weekly sleep time for Balotelli during different encroachments

5.1.7. Provence

Provence is a horse where, during the week of lavender application, there was an increase in sleep in both REM positions, namely sternal recumbency REM and lateral recumbency REM, compared to the week with no interaction. In the case of the sternal recumbency REM position, there was an increase of 7 %, and in the case of the lateral recumbency REM position, there was an increase of 6 % (Figure 13). The average sleep duration of this horse each night over the entire three-week period was 1 hour 36 minutes 35 seconds.

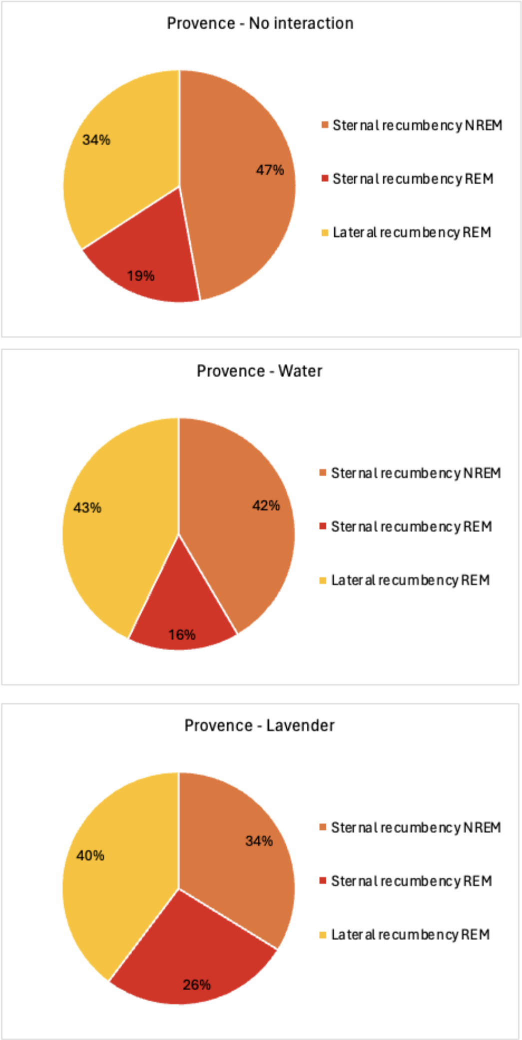


Figure 13: The total weekly sleep time for Provence during different encroachments

5.2. Length of rest

The lavender treatment had no significant effect on the length of rest (F = 1.416, P = 0.246). However, there was a significant effect of the age (F = 36.502, P = 0.001), for older horses resting less, work type (F = 31.913, P = 0.001), hobby horses resting more than sport horses, sex (F = 15.242, P = 0.001), for geldings resting more than mares, and working time (F = 7.949, P = 0.006), for horses working more and resting less. Figure 14 shows the estimated means of a three-way comparison for length of rest, and although at first sight lavender appears to have higher mean values than control, these results are not significant (P = 0.311).

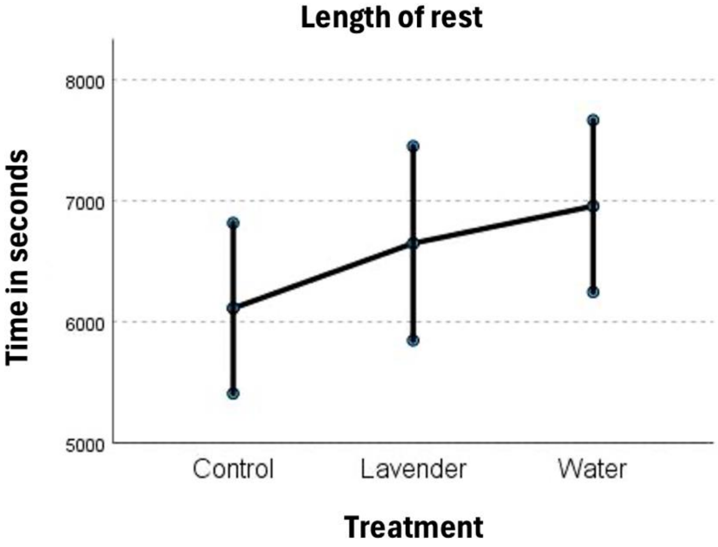


Figure 14: Length of rest for all three encroachments

5.3. Length of sleep

Neither the length of sleep was affected by lavender (F = 1.669, P = 0.192). However, some other tested variables were significant: age (F = 43.744, P = 0.001, β = -440.977), work type (F = 24.214, P = 0.001), sex (F = 15.242, P = 0.001), and working time (F = 5.688, P = 0.018). With increasing age, horses slept less in the sternal recumbency NREM position (β = -440.977). In the case of the comparison of sleep length between geldings and mares, it was found that geldings had statistically significantly longer lengths of sleep than mares (β = 2131.183). Additionally, statistically significant differences were observed between work types, indicating that hobby horses slept less than sport horses (β = -2853.927). In addition, when working time increases by one second, sleep length decreases by twenty seconds (β = -20.577). The estimated means of a three-way comparison for the length of rest are shown in Figure 15. Although lavender may appear to affect sleep duration compared to control and lavender, these results are not statistically significant (lavender-control P = 0.110, lavender-water P = 0.840).

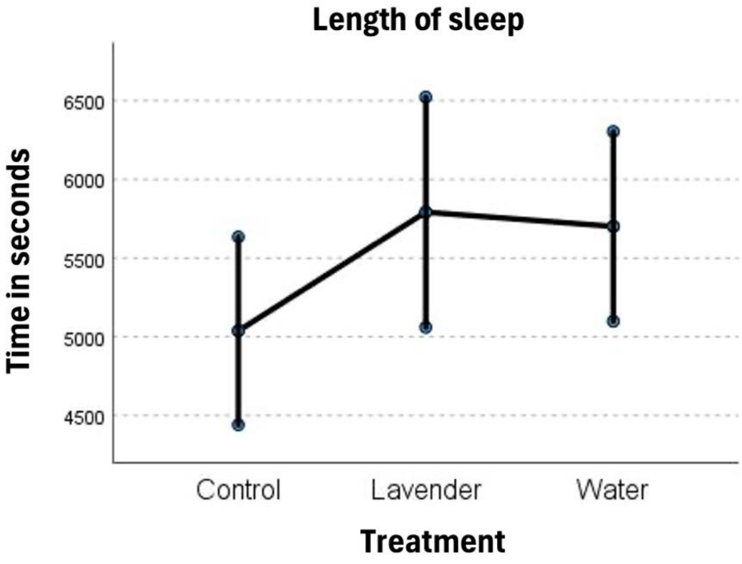


Figure 15: Length of sleep for all three encroachments

5.4. Length of sternal recumbency NREM

It was found that treatment had no significant effect on the length of the sternal recumbency NREM (F = 0.752, P = 0.473). However, there was an effect of age (F = 39.248, P = 0.001), sex (F = 10.108, P = 0.002), working time (F = 8.989, P = 0.003), and work type of horse (F = 6.232, P = 0.014). With increasing age, horses slept less in the sternal recumbency NREM position (β = -0.118). In the case of the comparison of sleep length between geldings and mares, it was found that geldings had a statistically significantly longer sleep duration of sternal recumbency NREM than mares (β = 0.484). Additionally, statistically significant differences were observed between work types, indicating that hobby horses slept less in sternal recumbency NREM than sport horses (β = -0.402). Although lavender may appear to affect sleep duration compared to control and lavender, these results were not statistically significant (lavender-control P = 0.229, lavender-water P = 0.450). See this estimated means of three-way comparison for the length of sternal recumbency NREM in Figure 16. None of the variables are significant (P = 0.229, P = 0.450, P = 0.553).

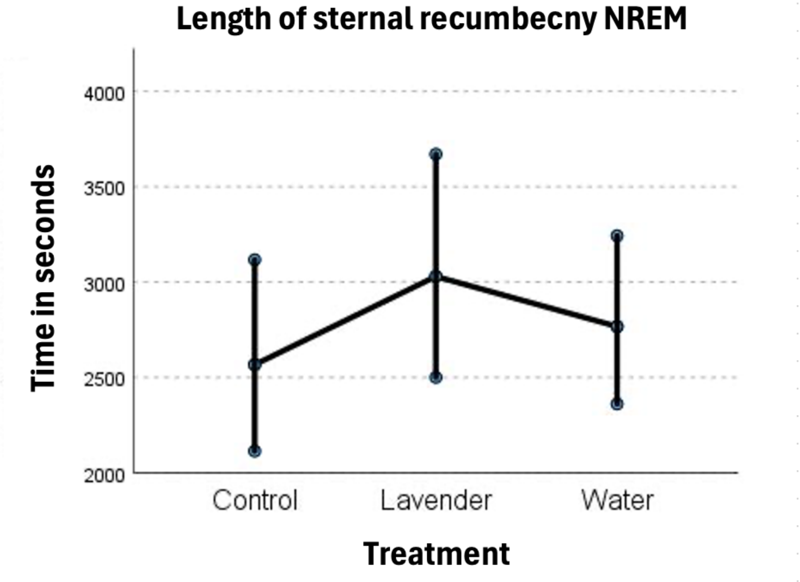


Figure 16: Length of sternal recumbency NREM for all three encroachments

5.5. Length of sternal recumbency REM

From these data, it was found that treatment had no significant effect on the length of sternal recumbency REM (F = 1.578, P = 0.210). However, there was an effect of two variables: working time (F = 12.386, P = 0.001) and work type of horse (F =7.744, P = 0.006). Statistically significant differences between work types, indicated that hobby horses slept less in sternal recumbency REM than sport horses (β = -318.118). Significant statistical differences are linked to working hours, indicating that with a unit increase in working hours, the length of sternal recumbency REM decreases by five units (β = -5.368). The three-way comparison for the length of sternal recumbency REM (Figure 17) indicates that although the length of sternal recumbency REM was greater with lavender compared to the control, these differences did not reach statistical significance (P = 0.099). Similarly, the comparison between lavender and water also yielded statistically insignificant results (P = 0.725).

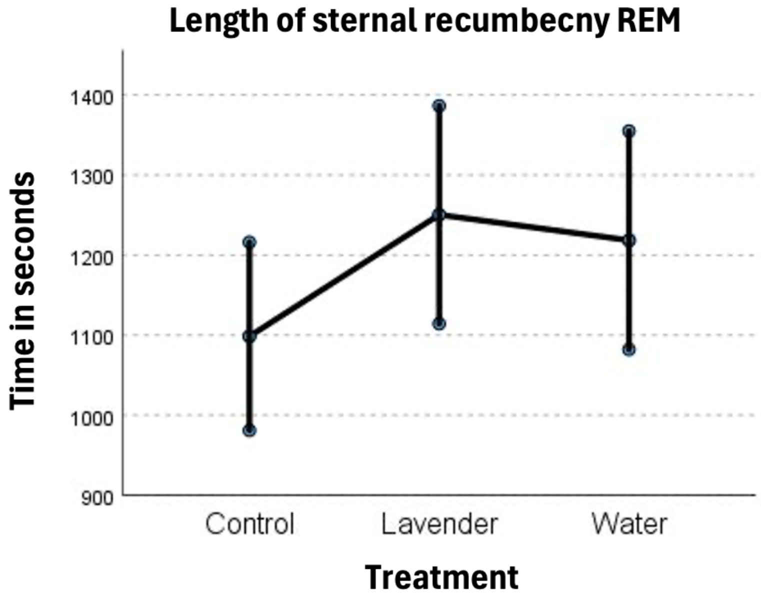


Figure 17: Length of sternal recumbency REM for all three encroachments

5.6. Length of lateral recumbency REM

It was found that treatment had no significant effect on the length of lateral recumbency REM (F = 0.064, P = 0.938). Statistically significant differences affecting length of the lateral recumbency REM were found for work type (F = 74.369, P = 0.001) and age (F = 6.357, P = 0.013). It was found that with increasing age, horses slept less in the lateral recumbency REM position (β = -0.044) and that hobby horses slept less in this position than sport horses (β = -1.198). The three-way comparison for the length of lateral recumbency REM (Figure 18) indicates that lavender had no statistically significant results (control-lavender P = 0.891, control-water P = 0.861, lavender-water P = 0.720).

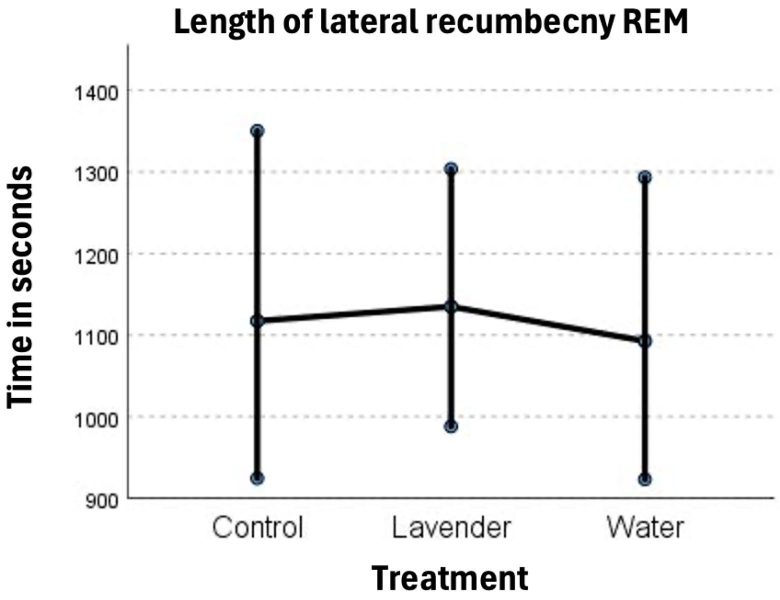


Figure 18: Length of lateral recumbency REM for all three encroachments

5.7. Sleep interruptions

Sleep interruptions are defined as the total number of lying down episodes observed during a period in which a horse accumulates a certain number of hours of sleep. It does not account for the number of transitions between sleep phases. The three-way comparison for the sleep interruptions (Figure 19) indicated that there are statistically significant differences between water-control P = 0.019, and water-lavender P = 0.035 but that there are no statistically significant differences for lavender-control P = 0.550.

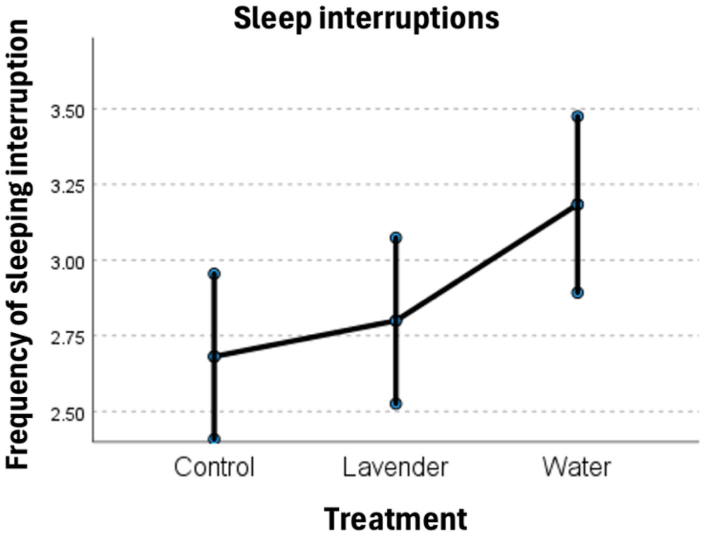


Figure 19: Sleep interruptions

6. Discussion

The results of the sleep pattern analysis of seven horses did not show statistically significant differences between individual encroachments but identified statistically significant variables influencing sleep and its individual phases, which are supported by a series of studies (Koehl et al., 2006; Andersen et al., 2008; Ehlen et al., 2013; Mander et al., 2017; Chaput et al., 2018; Kocevska et al., 2021; Li et al., 2022). The range of sleep length, whether in sternal recumbency NREM, sternal recumbency REM, or lateral recumbency REM phases, exhibited relatively high variability among individual horses. The duration of individual phases varied among horses, leading to different overall sleep distributions. It is worth noting that sleep monitoring occurred only at night, thus lacking data on daytime sleep, which could affect the overall sleep duration and distribution of sleep phases.

Most of the authors claim that horses sleep an average of 3.5 hours per day (Wöhr et al. 2016; Fuchs et al. 2018; Greening & McBride 2022). However, some authors do not align with this view, such as Greening et al. (2021), who stated that horses sleep an average of 5.18 ± 0.88 hours (more precisely, 3.94 ±0.85 in the NREM phase and 1.4 ±0.13 hrs in the REM phase). Conversely, there are contrasting extremes, as observed by Fuchs et al. (2018), who documented a notably low average sleep duration of 24.5 ± 3.5 minutes in horses experiencing collapses. All these inconsistencies can be attributed to a range of factors, including adaptation to the surrounding environment (Williams et al. 2008; Greening et al. 2021; Kelemen et al. 2021; Greening et al. 2023), age (Auer et al. 2021; Zanker et al. 2021), individual health (Kelemen et al. 2021; Oliveira et al. 2022), bedding type (Pedersen et al. 2004; Greening et al. 2021), and environmental factors such as food availability, social interaction, and temperature (Keene & Duboue 2018). The results of this study indicate an overall average daily sleep of all horses to be 1 hour 31 minutes 35 seconds, which is below the average sleep value of 3.5 hours found by the vast majority of authors (Wöhr et al. 2016; Fuchs et al. 2018; Greening & McBride 2022). This lower average sleep value is because standing sleep was not included in the data analysis, which would significantly increase the overall average sleep. Ambiguities in this regard may also be attributed to the fact that each study conducts sleep measurements for a different duration.

Overall, horses devote 67.4 minutes to REM sleep phases throughout the day (a twenty-four-hour period) (Kelemen et al. 2021). Of course, there is inconsistency in this regard compared to many other publications, as authors Aleman et al. (2008) report an average REM sleep value twice as low (30 minutes/day) for the same time frame (i.e., twenty-four hours). The same figure, 30 minutes of REM sleep, is reported by authors Fuchs et al. (2018), but with the difference that this time was measured in horses only during night-time sleep, thus within a demonstrably shorter observation interval. The results of this study cannot definitively lean towards any of these publications because the overall average time of all seven horses over three weeks of night-time sleep observation (a period of 9.5 hours) is 37 minutes 24 seconds. Nevertheless, these values most closely align with the findings conducted by authors Fuchs et al. (2018), who also observed night-time sleep in horses. These shortcomings may be attributed to the inconsistent length of observation, as well as to the fact that the results of this study were obtained based on behavioural observation, whereas the results from Aleman et al. (2008), Fuchs et al. (2018), and Keleman et al. (2018) were obtained using wearable monitoring devices, with the possibility of differences arising between these distinct devices.

However, based on statistical analysis, this paper concluded that the total length of sleep was statistically insignificant between treatments. The statistical results further suggest that the total length of rest did not differ statistically between treatments. In animal models, there is no clear answer regarding whether or not lavender statistically affects the length of sleep. According to authors Xu et al. (2023) and Manor et al. (2021), there was no increase in sleep length after administration of lavender essential oil, whereas according to authors Linck et al. (2009) and Xu et al. (2021), there was an increase in total sleep length after administration of lavender essential oil. It should be mentioned that these results were observed in rat and mouse models. In addition, the studies by Linck et al. (2009) and Xu et al. (2021) looked at a specific component of lavender essential oil, specifically linalool, which may be present in lavender essential oil at different concentrations, and thus higher concentrations of linalool may result in longer sleep length. While animal models are poorly studied in this regard, there is a large body of literature on the effect of lavender essential oil on sleep length in humans. Although almost identical in their results, there are interesting findings that are worth noting. For example, a study conducted by Kawai et al. (2018), while demonstrating that lavender essential oil increases the length of sleep, adds that the increase in length of sleep only occurs in students subjected to poor sleep quality. Interestingly, a study conducted by Sentürk & Kartin (2018) also found that there was an increase in sleep length in the intervention group after the administration of lavender essential oil, but this intervention group was compared to a control group, which contained different individuals who may have responded differently to lavender essential oil administration. Therefore, from this point of view, it is better to adhere to the study conducted by Ko et al. (2021), who tested the effects of lavender essential oil between stimulus and control nights in the same subjects and found that the effects were identical before and after administration of lavender essential oil. After all, it is possible to state the fact that in most cases, the length of sleep is increased in humans by the effect of lavender, as evidenced by other publications such as (Kasper et al. 2010; Yang et al. 2023). The conflicting results are due to the different models (mouse, rat, and human) that are compared with the horse model. However, as mentioned, there may be more than one reason, with the most likely reason relating to the concentration of the active ingredient linalool. An interesting finding was mentioned by Kasper et al. (2010), who mentioned that the effect of lavender essential oil was evident after two weeks, with statistically significant results from week six onwards. Since in this study, lavender essential oil was administered for seven days, the inconsistent results can be attributed to this fact as well.

A more precise division of time devoted to specific REM phases is addressed by Chung et al. (2018), who found that horses spend 57.00 ± 25.00 min/24 h in sternal recumbency, while they spend much less time in lateral recumbency, only 8.00 ± 6.00 min/24 hours. Lower results for the 48-hour time interval were obtained by Barbosa et al. (2024), who measured sternal recumbency (132 minutes/48 hours) and lateral recumbency (22 minutes/48 hours). Opposite results to these two studies were obtained by Pedersen et al. (2004), who reported average times spent in sternal recumbency (5.6 minutes) and lateral recumbency (57.5 minutes). The results from this study suggest that the daily mean time per horse during the entire observation period was 19 minutes 25 seconds for sternal recumbency REM, while it was 17 minutes 59 seconds for lateral recumbency REM. Statistical analyses found that there were no statistically significant differences between treatments for any of the phases. In this case, there is diversity in the results for several reasons, one of which is the type of bedding material. Pedersen et al.

(2004) used straw bedding in their study, Barbosa et al. (2024) used wood shavings, and Chung et al. (2018) used sawdust. There is no clear preference for bedding type among horses, as individual preferences may vary. In this case, we could only speculate the possibility that Pedersen et al. (2004) used a suitable bedding type with a sufficiently high layer of straw, which is reflected in the very high number of minutes spent in lateral recumbency. However, among the important factors influencing such differences could also be the location of the studies, as one of them was conducted in the temperate climate of Denmark, the second was conducted in the humid subtropical climate of Brazil, and the third in the tropical climate of Malaysia. The geographical location of countries is associated with temperature, which was not found to have a significant effect on any sleep phase in this study. However, direct comparisons with other studies cannot be made as they did not investigate this issue. The differences between this study and the results from other publications ((Pedersen et al. 2004), (Chung et al. 2018)) can also be seen in the fact that in other publications, the division of REM phases was different compared to this study, which includes in sternal recumbency only horses lying in a sternal position with their muzzle resting on the ground, not horses in a sternal position with the muzzle not resting on the ground (this position was considered sternal recumbency NREM in this study).

It is evident that, in many cases, age is a significant variable affecting various aspects of individual sleep fractions. There is a noticeable decline in sleep with advancing age, a trend that is consistent with findings from many studies in humans (Mander et al. 2017; Chaput et al. 2018; Kocevska et al. 2021; Li et al. 2022), but not in mice (Hasan et al. 2012; Panagiotou et al. 2017; McKillop & Vyazovskiy 2020; Wintler et al. 2020; Panagiotou et al. 2021). Genetic adaptations of laboratory mice may be a significant reason, as local sleep homeostatic mechanisms and local cortical neuronal dynamics do not change with age in mice and maintain stable neuronal function across the lifespan, thus there is no reduction in sleep (McKillop et al. 2018; Panagiotou et al. 2021). The obtained statistics revealed that males allocated significantly more time to sleep across nearly all sleep stages compared to females. These findings are supported by a plethora of literature, encompassing studies conducted in humans (Paul et al. 2008; Mong et al. 2011; Mong & Cusmano 2016; Bonsignore et al. 2019) as well as demonstrated in mouse and rat models (Koehl et al. 2006; Andersen et al. 2008; Ehlen et al. 2013), corroborating the observed results. The work type of the horse emerged as a significant factor influencing sleep across all stages. Analysis revealed that hobby horses exhibited shorter sleep lengths compared to sport horses. This trend is primarily attributed to the age composition within each group, as sport horses typically consist of young and promising individuals geared towards competitive performance (Neumann et al. 2021), while hobby horses, serving riders in recreational riding, are often older. Finally, working time was also a significant variable affecting sleep. Increased working time resulted in decreased sleep, which is highly unusual in many human studies (Youngstedt 2005; Jurado-Fasoli et al. 2020; Tse et al. 2020). On the other hand, physical activity leads to an increase in lactic acid production and a decrease in pH in muscle tissues (Rusdiawan et al. 2020), which may cause discomfort for some individuals, resulting in reduced ability to fall asleep. But even in this case, it is important to note that this variable is also highly dependent on age or sex (Chennaoui et al. 2015).

It is worth noting that previous studies looking at horse sleep have not looked at the number of sleep interruptions, which are closely linked to sleep quality, thereby determining how well horse’s sleep. Even though this paper concluded that there was no statistically significant reduction in sleep interruption counts after administration of lavender essential oil, it is important to note that many other publications observing sleep interruption counts in mouse, rat, and human models disagree with this statement. In mice and rats, there was a reduction in sleep interruptions during the night using lavender essential oil, and it is therefore clear that the effect is positive in these rodents (Manor et al. 2021; Xu et al. 2023). Lavender essential oil has the same effect on humans as well, which is the conclusion of many studies (Hudson 1996; Chien et al. 2012; Lillehei et al. 2016; Faydali & Çetinkaya 2018; Arbianingsih et al. 2020). Even so, there is one study conducted in humans that dismisses the positive results of lavender essential oil on sleep interruptions (Lee 2004). But in this case, it was a study that sought to determine the effects of not only lavender essential oil, but also eucalyptus essential oil, and so a one-to-one mixture of the two oils was inhaled, and thus the effect of lavender essential oil may have taken a back seat. However, one of the reasons that may be attributed to the different results of this study compared to other studies is the distribution of the lavender aroma (drops of oil on the pillow, through massage, to vapour to inhalation). This is related to the actual concentration of lavender essential oil administered. In addition, horses are physiologically different compared to humans, mice, and rats and the horse's sensitivity to scent is very delicate and therefore may react differently to lavender essential oil than the human or mouse/rat model.

7. Conclusions

Lavender aromatherapy had no significant effect on the resting and sleeping behaviour of the horses in this study. There were significant differences in sleep between horses when age, sex, and work type were taken into account. The total number of hours observed (almost 1400 hours) was long enough to determine the sleep patterns of the horses. The present work provides insights into the sleep patterns of horses that can help keepers to understand the patterns of equine sleep. In addition, this work can serve as a pilot study for further research.

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