Forside

Eksamensinformationer
SASK14301E - Animal Science Speciale -
Kontrakt:114453 (Sofja Eklund Koziara)

Besvarelsen afleveres af
Sofja Eklund Koziara
jmh627@alumni.ku.dk

Administration
SUND Studieinformation
eksamen@sund.ku.dk

Bedømmere
Adrian Paul Harrison
Eksaminator
adh@sund.ku.dk
+4535330939

Hanne Justesen
Censor
haju@easj.dk

Besvarelsesinformationer

Titel: Effekten af aromaterapi på hestens ansigtsudtryk, hjerterytme, respiratorisk brystkasseudvidelse og både linjerne og frekvensen af muskelkontraktioner i m. temporalis og m. cleidomastoideus
Titel, engelsk: Effect of aromatherapy on equine facial expression, heart rate, respiratory tidal volume and both the lines and frequency of spontaneous muscle contractures in m. temporalis and m. cleidomastoideus
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Effect of aromatherapy on equine facial expression, heart rate, respiratory tidal volume and both the lines and frequency of spontaneous muscle contractures in m. temporalis and m. cleidomastoideus

Sofja Eklund Koziara, jmh627

Supervisor: Lector Adrian Paul Harrison

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Name of department:	Department of Veterinary and Animal Sciences
Author(s):	Sofja Eklund Koziara
Title and subtitle:	Effect of aromatherapy on equine facial expression, heart rate, respiratory tidal volume and both the lines and frequency of spontaneous muscle contractures in m. temporalis and m. cleidomastoidues
Topic description:	Both qualitative and quantitative methods are included in a trial of whether essential oils can be used as a natural calmative for horses.
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Table of contents

Foreword

This was my final project as candidate in Animal Science from the University of Copenhagen. In addition to my academic background, I am a trained biodynamic craniosacral therapist and acupressor, which is why I am interested in how we humans can help our domestic horses to live a long life with a strong and healthy body and with a high level of welfare, taking into account their anatomical structure in terms of performance, nutrition and housing. I use essential oils privately and for my clients, which is why I had both personal and professional interest in scientifically investigating the effect of using essential oils. It is my opinion that our domestic horses are exposed to a variety of stress factors, which was why I thought it could be of great interest to explore a natural alternative method to calm horses – a method which is easily accessible and with the right guidance can be used by all private horse owners, potentially making the human-horse interaction more free of conflict, also during visits by for example the veterinarian or the farrier.

A huge thank you to Adrian Paul Harrison, who as my supervisor has been an indispensable support throughout the project. Thank you for motivating me, inspiring me and reassuring me when I got nervous or locked. Thank you for not being intimidated by my unusual topic choice, but having the courage and curiosity to learn something new with me.

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Thank you to Dirk Swartz for helping with proofreading the project without having any other connection to the project besides wanting to help me achieve a higher linguistic level when the English language has always been a challenge for me.

Thanks to Jørgen Kruuse A/S, CURO-Diagnostics ApS and LEAP Technology ApS for providing equipment that enabled the quantitative measurements in the trial and thanks to Dr. Rachaël Draaisma for sharing her discoveries on facial expressions and body language in horses which forms the basis for the qualitative measurements in this experiment.

Abstract

Reasons for performing the study: The ability to calm horses affects both the safety and well-being of human-horse relationships. However, not many natural alternatives are known, nor the effectivity of these in supporting a state of calmness in horses.
Objectives: To investigate whether essential oils could be used as a natural calmative for horses.
Null hypotheses: Aromatherapy would neither facilitate greater facial relaxation nor tension, lower nor increase the heart rate, change respiratory tidal volume, nor cause a reduction in spontaneous muscle contractures in the horses tested. Moreover, it was anticipated that there would be no measurable difference between the controls and the essential oils treatments, nor between the essential oils when applied separately.
Methods: Nine horses of different ages, genders and breeds were included in a crossover design with humified essential oils to determine the calming effect of vetiver, spikenard and roman chamomile in relation to water (negative control) and lavender oil (positive control). During the aromatherapy, the horses were videotaped for a later behavioural assessment and the heart rate, respiratory tidal volume, and muscle contractions in m. temporalis and m. cleidomastoideus were measured afterwards. The horse was either tied up with two ropes or held by a handler in front of a diffuser while the breaks were used in its stall with roughage available.
Results: All of the essential oils selected for this trial significantly lowered heart rate and the results indicated that the same was true for respiratory tidal volume, but it could not be documented for roman chamomile. The results for essential oils differed in relation to the reactions in facial expressions and spontaneous muscle contractions. Spikenard was undoubtedly the essential oil best suited to induce relaxed facial expressions: 78% of the horses displayed a relaxed body posture for at least 75% of the recorded time - a result none of the other treatments were close to achieving. In contrast, roman chamomile was significantly more successful at relaxing muscles in comparison to spikenard and the other treatments.
Conclusion and potential relevance: Despite the relatively small test population (n = 9 horses), it was possible to document both qualitatively and quantitatively, that the essential oils calmed the horses. The scientific evidence on the subject is still very limited, which is why more evidence-based literature in general is needed in relation to the application possibilities of essential oils in equine practice.

Resume

Grundene til at udføre forsøget: Muligheden for at berolige heste påvirker både sikkerheden og velfærden i menneske-heste-relationer. Der kendes dog ikke ret mange naturlige alternativer hertil eller effektiviteten af at anvende disse til at understøtte en tilstand af ro blandt heste.

Formål: At undersøge om æteriske olier kunne benyttes som et naturligt berolighedsmiddel for heste.

Nulhypoteser: Aromaterapi vil hverken facilitere større ansigtsafslappelse eller ansigtsspænding, sænke eller øge hjerterytmen, ændre respiratoriske brystkasseudvidelse eller medføre en reduktion i spontane muskelkontraktioner hos hestene, der er blevet testet på. Desuden forventes det at der ikke vil være nogen målbar forskel mellem kontrolbehandlingerne og oliebehandlingerne eller mellem de æteriske olier indbyrdes.

Metode: Ni heste af forskellige aldre, køn og racer indgik i et crossover design med forstøvede æteriske olier, for at bestemme den beroligende effekt af vetiver, spikenard og romansk kamille i forhold til vand (negativ kontrol) og lavendel olie (positiv kontrol). Under aromaterapien blev hestene videooptaget til en senere adfærdsvurdering og bagefter blev hjerterytmen, den respiratoriske brystkasseudvidelse og spontane muskelkontraktioner i m. temporalis og m. cleidomastoideus målt. Hesten var enten bundet op i to reb eller holdt af en assistent foran forstøveren, mens den spenderede pauserne imellem behandlingerne i sin boks med grovfoder tilgængeligt.

Resultater: Alle de æteriske olier udvalgt til dette forsøg sænkede signifikant hjerterytmen og resultaterne pegede på at det samme gjorde sig gældende med hensyn til den respiratoriske brystkasseudvidelse, men det kunne ikke dokumenteres for romansk kamille. De æteriske olier adskilte sig fra hinanden i forhold til reaktionerne i hestens ansigtsudtryk og de spontane muskelkontraktioner. Spikenard var uden tvivl den æteriske olie som var bedst til at indgyde afslappede ansigtsudtryk: 78% af hestene indtog en afslappet kropsposition i minimum 75% af aromaterapien - et resultat ingen af de andre behandlinger var i nærheden af at kunne opnå. Til gengæld var romansk kamille signifikant mere succesfuld i at afspænde musklerne sammenlignet med spikenard og de øvrige behandlinger.

Konklusion og perspektivering: På trods af den relativt lille testpopulation (n = 9 heste) var det muligt både kvalitativt og kvantitativt, at dokumentere, at de æteriske olier virkede beroligende på hestene. Den videnskabelige dokumentation på området er endnu stærkt begrænset, hvorfor der generelt behøves mere evidensbaseret litteratur i relation til anvendelsesmulighederne for æteriske olier i hestehold.

1 Introduction and hypothesis

Aromatherapy, a branch of herbology, dates back over 6,000 years to ancient Egypt, the Far East, and more recently Renaissance Europe. Today, it is one of the fastest growing forms of therapy in the World (Thomas 2002; Moss *et al.* 2006). The practice of aromatherapy can be defined as "the use of organic essences of aromatic plants for healing and the maintenance of vitality" (Moss *et al.* 2006). The essential oils used in aromatherapy are extracted from natural sources such as seeds, bark, stems, roots, flowers and other plant parts. When inhaling the aromatic vapor of an essential oil, the effector molecules bind to receptors in the nasal cavity. Olfactory sensory neurons then transmit signals to the olfactory bulb that filters and processes these signals before transmitting them to the mitral cells. Mitral cells carry the output signals to the olfactory cortex, but some are also connected directly to the amygdala (Baldwin and Chea 2018). The amygdala is a part of the limbic system that regulates memory and primary emotions such as stress and fear (Heitman *et al.* 2018). It is reported that aromatherapy leaves one feeling uplifted, stimulated, invigorated, or reassured, depending on the oil used (Moss *et al.* 2006; Baldwin and Chea 2018).

Aromatherapy may benefit horses due to their high sensitivity, the fact that they are easily stressed and that they have an acute sense of smell (Baldwin and Chea 2018). Horses are obligate nasal breathers and the domesticated horse are exposed to a variety of stressors including adjusting to new environments, high athletic demands, limited turnout, and traveling (Ferguson *et al.* 2013; Heitman *et al.* 2018). Limited research has been performed on the use of aromatherapy to relax horses. Ferguson *et al.* (2013) exposed horses to acute stress by an air horn being blown twice followed by enforced inhalation of either humidified lavender essential oil or humidified air. In both groups, the heart rate of the horses increased after the sound but returned to normal values more quickly in those that inhaled lavender (Ferguson *et al.* 2013). Heitman *et al.* (2018) investigated the effect of horses being hauled in a trailer on salivary cortisol concentrations and heart rate with or without inhalation of lavender essential oil vapor but did not find any significant response in heart rate. Moreover, the cortisol data were unreliable due to significant differences in the baseline cortisol concentrations within the control and treatment groups (Heitman *et al.* 2018). Pouteraud *et al.* (2018) found a lower heart rate, less alert postures and less defecations in horses with lavender in carrier oil applied around the nostrils and exposed to 30 minutes of stress tests. Baldwin and Chea (2018) found that humidified lavender significantly increased the parasympathetic component of heart rate variability in horses that were not subjected to an external stressor while chamomile did not induce the same relaxation effects on the cardiovascular system.

Lavender is the most studied phytotherapeutic for use as an anxiolytic (Lv *et al.* 2013) and has documented calming effects in several other species such as rats (Tsang *et al.* 2012; Coelho *et al.* 2018), sheep (Hawken *et al.* 2012), dogs (Graham *et al.* 2004) and humans (Cruz *et al.* 2011; Liu *et al.* 2013; Kusumawardani *et al.* 2017). Inhalational aromatherapy with lavender essential oil has also been shown to be pain-relieving in human patients in the first days following coronary artery bypass surgery (Seifi *et al.* 2018). Popular anxiolytic essential oils are multifarious, and include much more than lavender; such as rose, orange, bergamot, lemon, sandalwood, clary sage, roman chamomile and rose-scented geranium (Lv *et al.* 2013; Jinyong *et al.* 2015) but the evidence-based documentation of these appear much weaker.

The effect of lavender is the most well documented which makes it suitable as a positive control treatment. Indeed, it could also be interesting to investigate the effect of aromatherapy with plants horses potentially would search for in nature. While grass forms the major proportion in the diet of horses, they also supplement with nutrients, vitamins and minerals from clay, humus, faeces, bark, leaves and twigs (Crowell-Davis *et al.* 1984). Nature is unquestionably the finest resource for diverse medicines (Lv *et al.* 2013) and the feeding behaviour of horses with free access to pasture (Crowell-Davis *et al.* 1984) suggests that horses intuitively understand how to exploit the physiological and psychological benefits of plants. Like lavender, chamomile is considered a powerful sedative as well as being regarded as a useful anti-inflammatory, a diuretic and an analgesic (Moss *et al.* 2006). Baldwin and Chea (2018) did not find consistence in their results with chamomile while Moss *et al.* (2006) revealed significant lower alertness and increased calmness in healthy human adults after being exposed to the scent of roman chamomile. Graham *et al.* (2004) stimulated dogs' olfactory senses, whilst housed in a rescue shelter, and observed activities that indicated relaxation (i.e. less movement, increased resting, less barking) when exposed to either the diffused odour of lavender or chamomile. Based on the promising properties of chamomile, roman chamomile was included in this experiment.

Vetiver is a member of the grass family (Saiyudthong *et al.* 2015). Saiyudthong *et al.* (2015) showed that in the form of vetiver essential oil, it was relaxing, and had anti-anxiety and sedative properties in rats when measured using the elevated plus-maze test. These results make vetiver interesting as a natural sedative for horses and was therefore included in this experiment.

Spikenard is a flowering plant of the Valerian family (Takemoto *et al.* 2015) which is a popular herbal family group often used to treat insomnia, anxiety and related ailments (Dyayiya *et al.* 2016). Takemoto *et al.* (2015) found suppressed stress-induced excitatory behaviours and significantly reduced stress-induced blood corticosterone in mice under stress tests after they had inhaled spikenard. For the same reason as vetiver, spikenard was included in this experiment.

The possibilities of calming horses with essential oils was investigated with an aromatic vapor of either lavender, roman chamomile, vetiver or spikenard. To determine whether the essential oils calm the participating horses or not, video analysis of the horses' facial expressions was be used. Horses naturally live in family groups that ensure safety and survival (Draaisma 2018; Ladewig 2019). Preventing conflicts and attendant injuries or forced isolation through proper communication is of great importance to horses (Ladewig 2019). Communication consists of analysing the face and body posture, and avoid any unpleasantness by using calming signals, displacement behaviour, stress signals or distance increasing signals. Draaisma (2018) analysed 200 video recordings with domesticated horses, which were either self-recorded or donated by private horse owners primarily from the Netherlands. Communication patterns were identified which showed that horses displayed communicative signals towards humans, other animals and inanimate objects such as rocks or trash bins, visual stimuli such as shadows or flickers of light, or sounds such as a cow mooing in the distance or a leaf blower, as if they had communicated with another horse. Thus, horses are able to form relationships outside their own species, just like dogs and humans, and they use the same signals to communicate with these other species that they would use with other horses (Draaisma 2018).

In addition to calming humans, animals, and other environmental stimuli, calming signals have one other important function: They reduce the horse's own tension, and is therefore an essential tool for horses to release tensions that would otherwise accumulate. Another subtlety, calming signals are given both when the horse is relaxed and when it experiences tension. To judge this, the facial expression in the situation is crucial (Draaisma 2018). Analysis of facial expressions according to relaxed, light tension, medium tension and high tension was used to assess how the horses feel about the olfactory stimulation. Measurements of heart rate, respiratory tidal volume and the frequency of spontaneous muscle contractures in m. temporalis and m. cleidomastoideus acted as support to the behavioural assessment.

The objective of this study was to investigate the effect of aromatherapy with either vetiver (*Vetiveria zizanioides),* spikenard (*Nardostachys jatamansi*) or roman chamomile (*Anthemis nobilis*) compared to lavender (*Lavandula angustifolia*) and humified water on equine facial expression, heart rate, respiratory tidal volume and the frequency of spontaneous muscle contractures in m. temporalis and m. cleidomastoideus with the absence of an imposed external stressor. The information will be useful as a natural alternative to reassuring horses that may be prone to nervousness or attenuate stressful situations associated with domesticated horses. The null hypothesis was that aromatherapy would neither facilitate greater facial relaxation or tension, lower nor increase the heart rate, change respiratory tidal volume, nor cause a reduction in spontaneous muscle contractures in the horses tested. Moreover, it was anticipated that there would be no measurable difference between the controls and the essential oils treatments, nor between the essential oils when applied separately.

2 Materials and methods

2.1 Literature

The literature study that preceded this experiment was primarily based on publications found through database searching on ARGICOLA, CAB Abstracts and Web of Science (Table 1). Reference and keyword lists for selected articles were reviewed to find other relevant literature. Articles used as references were selected based on their assessed relevance.

Table 1. Literature search including keywords and publication results in OvidSP search system.

Keywords	**Results**
essential oil* *or* aromatherapy *or* odor* *or* odour*	145,680
⎣ equine* *or* horse* *or* dog* *or* canine* *or* cat* *or* feline* *or* rat* *or* rodents* *or* animal* *or* human*	68,152
⎣ stress* *or* welfare	2,667
⎣ lavender *or* chamomile *or* vetiver *or* spikenard	133
Remove duplicates	122
Limit to English language	101

2.2 Participants

Seven castrated male horses and two female horses were recruited; a total of nine horses from two private farms: Astenslund at Maglebyvej 17, 4672 Klippinge, Magleby and Hyldegården at Hyldegårdsvej 35, 3630 Jægerspris, Overdråby Strand, Denmark, for observation in this experiment. The horses ranged in age from 13 to 25 years and had very little or none previous experience with aromatherapy. The breeds included Arabian, Connemara, Danish Warmblood, Frederiksborg, Haflinger and Northlands. The profile for each horse is listed in Table 2. The sample size was considered sufficient due to a previous equine aromatherapy study performed by Ferguson *et al.* (2013) where a study of seven horses was sufficient to yield statistically significant changes. Each horse was exposed to both control and experimental treatments in a randomized order (Table 3). Randomization was achieved by using a website program (www.random.org).

Table 2. Profile for each participating horse.

Name	Age (yr)	Sex	Breed
Amigo	25	Gelding	Connemara
Cavalos	18	Gelding	Frederiksborg
Copper	17	Mare	Haflinger
Intakt	16	Gelding	Arabian
Max	17	Gelding	Danish Warmblood
Noble	13	Gelding	Danish Warmblood
Siska	18	Mare	Connemara
Ville	18	Gelding	Northlands
Zendi	18	Gelding	Frederiksborg

Table 3. The order of the aromatherapy treatments. Randomized by www.random.org.

Treatment order	Amigo	Cavalos	Copper	Intakt	Max	Noble	Siska	Ville	Zendi
Water	5	4	4	1	5	4	1	3	2
Lavender	1	2	1	2	4	5	3	2	4
Vetiver	3	1	3	3	2	3	5	5	3
Spikenard	2	5	5	4	1	2	4	4	5
Roman Chamomile	4	3	2	5	3	1	2	1	1

This research was performed on domesticated horses. It was an important observation, as the behaviour of domesticated horses may differ greatly from that of horses living in the wild – behaviour being the result of their smaller environment, controlled living conditions, interaction with the world of humans, humans themselves, and the demands people made of domesticated horses.

None of the participating horses had serious behavioural problems. As far as known and assessed, all the horses were healthy. Nor did any of the material collected during the experiment relate to horses who had visible physical injuries requiring veterinary attention. The experiment did not lead to behaviour or situations that resulted in extreme fight or flight responses. All participating horses were, by human standards, able to get along fine in our society. This does not mean that they did not feel tension. These horses experienced mild tension in varying degrees and extent during the experimental period, although they had been asked just to stand still and receive the aromatherapy.

All the horses lived in stables, where they had an individual stall. During the experiment, the horses at Hyldegården stood on pasture all day and night while the horses at Astenslund spend their days at pasture and nights in stalls. Every horse was in contact with people on a daily basis. All the horses are privately owned.

2.3 Experimental design and equipment

The experimental protocol followed a crossover design with an experimental treatment of humidified essential oils, thus allowing each horse to serve as its own control to account for possible confounding variables such as personality, breed, sex and age. The experiment examined the calming properties of humidified vetiver, spikenard and roman chamomile compared to humidified air (water as a negative control) and humidified lavender, which is an essential oil that has documented scientific effects (positive control).

The aromatherapy treatment was performed for 15 minutes while the horse's behaviour was videotaped. After each treatment the heart rate, respiratory tidal volume and spontaneous muscle contractures of the horse were measured for a 10-minute period. These measurements were compared with the average of two 10-minute measurements of the same factors; one measured right after applying the equipment, before any other treatments (cf. Baseline #1), and one measured after all the aromatherapy treatments (cf. Baseline #2). Between each aromatherapy treatment and before the last baseline measurement, the horse had a 20-minute break in its stall, where it was free to eat hay and walk around.

The heart rate of the horse was measured using ECG sensors placed next to the tuberosity of the left and right scapular spine and over the xiphoid. Sensors and related software were provided by Jørgen Kruuse A/S (Havretoften 4, 5550 Langeskov, Denmark). Indications of spontaneous muscle contractures were measured by the AMG technique with sensors located on m. temporalis, just above the masseteric nerve, and on m. cleidomastoideus near the ears. Sensors and related software were provided by CURO-Diagnostics ApS (Tværledet 33, 2880 Bagsværd, Denmark). The respiratory tidal volume was measured by an elastic sensitivity belt around the abdomen of the horse, positioned outward from the third posterior rib. The belt and related software were provided by LEAP Technology ApS (Diplomvej 381, 2800 Kongens Lyngby, Denmark).

2.4 Aromatherapy

The essential oils used in this study came from doTERRA Europe Ltd. (Altius House, 1 North Fourth St, Milton Keynes, MK9 1DG, United Kingdom) who state that their essential oils are gently and carefully distilled from plants that have been patiently harvested at the perfect moment by experienced growers from around the world for ideal extract composition and efficacy. Each batch of essential oils goes through a battery of rigorous and definitive tests to accommodate the CPTG (Certified Pure Therapeutic Grade®) quality protocol.

Lavender essential oil was distilled from *Lavandula angustifolia*. The oil was tapped in 15ml amber glass bottles on 13 January 2018 under lot no. 180133 and indicated to expire in January

2023. The composition of lavender oil is shown in Appendix 1. Vetiver essential oil was distilled from *Vetiveria zizanioides* and tapped into 15ml amber glass bottles on 5 February 2018 under lot no. 180367 with indicated expiry in February 2023. The composition of vetiver oil is shown in Appendix 2. Spikenard essential oil was distilled from *Nardostachys jatamansi*. The oil was tapped in 5ml amber glass bottles on 28 August 2018 with lot no. 182404 and with indicated expiry in August 2023. The composition of spikenard oil is shown in Appendix 3. Finally, roman chamomile essential oil was distilled from *Anthemis nobilis*. The oil was tapped into 5ml amber glass bottles on 1 December 2017 under lot no. 173354 and indicated to expire in December 2022. The composition of roman chamomile oil is shown in Appendix 4. None of the quality control lot analysis performed by Aromatic Plant Research Center (230N 1200E Suite 100, Lehi, UT 84043, USA) revealed contaminants or adulteration and all the samples were assessed to meet the expected chemical profile. Tap water (from 4600 Køge, Denmark) was used for control treatments.

The aromatherapy treatments were placed into a portable ultrasonic essential oil diffuser (doTERRA Iris) that permeates an ultra-fine, yet substantial mist output into the air. The diffuser can contain a maximum of 35ml $\pm$ 2ml of water to which two to three drops of essential oil were added. Excess water and essential oil were discarded after 15-minutes of nebulization and cleaned with water. The diffuser used for the negative control was brand new, had never contained any essential oils and was only used to atomize water throughout the experiment. A second Iris-diffuser was used to nebulize the treatments that included essential oils.

The horse handler sat on a chair in front of the horse and either held the diffuser in their hand while it lay on their thigh or placed the diffuser on the ground between their feet. None of the horses were forced to sniff the vapor but efforts were made to keep the horse nearby during the 15-minute period of exposure. Each horse was either tied using two ropes or held by the handler.

2.5 Data processing and statistical analysis

Raw data of heart rate and respiratory tidal volume were examined for measurement bias and 15 random samples were selected from areas without visible bias for each aromatherapy treatment. Sample results were typed into Microsoft Excel Version 16.27 and calculated for averages, standard deviations (SD) and standard errors of the means (SEM) while also performing analysis to assess whether the data followed a Gaussian distribution (test for normality), after which t-tests were performed.

The data collection of spontaneous muscle contractures was done by counting the number of peaks for each muscle group and calculating for averages, standard deviations (SD) and standard errors of the means (SEM) for each aromatherapy treatment. They were analysed to determine whether they followed a Gaussian distribution or not and if they passed t-tests. The data were typed into and analysed in Microsoft Excel Version 16.27. Data from essential oils that seemed promising in terms of being calming according to the number of muscle contractions were examined for their spatial and temporal summation with the CURO unit; expressed by the S- and T-score of the combined ESTi Score (CURO-Diagnostics ApS, Bagsværd, Denmark). The ESTi score is a mean of the individual scores and gives a relative ranking of fitness. S in terms of the muscle reflects the recruitment of motor units spatially and equates to signal amplitude (a scale of 0 to 0.99, where 0.99 = 1 Volt); which indicates the number of active muscle fibres. T is the motor unit firing rate (temporal summation) or signal frequency; which means how fast the muscle fibres were repeatedly fired. A scale of 0 to 10 was used to assess optimal muscle function, where 0 was considered as 0% optimal and 10 was considered 100% optimal. This means that a very small amplitude and low frequency is an effective utilization of a muscle because few muscle fibres are being used and they are not being repeatedly re-fired at a high frequency, which is energy demanding. Data were collected as one-minute recordings from five to six minutes inside the 10-minute measurement, i.e. approximately in the middle of the total measurement. S-max was equal to 0.05 while threshold was 0.01 due to the fact that the horses stood still, and muscle contractions occurred from a resting tonus state. Data results were typed into Microsoft Excel Version 16.27 and calculated for averages and standard deviations (SD) before t-tests were performed.

InStat Version 3.0b by GraphPad Software (2365 Northside Dr. Suite 560, San Diego, CA 92108) was used to perform the analysis of variance (ANOVA) nonparametric repeated measures, followed by a Wilcoxon matched-pairs signed-ranks comparison for each tested parameter (cf. heart rate, respiratory tidal volume and muscle contractions) to test for statistically significant changes in each treatment compared to that of the average baseline.

Behavioural assessment was *via* the analysis of the video recordings done continuously throughout the measurement; meaning that all behaviour patterns were measured per second when a behaviour started and stopped or counted depending on the type of behaviour. The different types of behaviour were either categorized as states where behaviours occurred for an extended duration, or events where behaviours were short in duration and these were counted rather than timed. All behaviour was recorded according to the ethogram in Table 4. The ethogram was composed of facial expression and behavioural patterns which can be divided into categories of calming signals, displacement activities, stress signals or distance increasing signals according to the definitions by Draaisma (2018). Only signals performed from a position where the horse is standing still was included in this ethogram.

The studies conducted by Draaisma (2018) formed the basis for this ethogram and how the video recordings have been analysed in this experiment. Other scientific studies on the subject was not found in the literature search on the previously mentioned article databases. Dr. Rachaël Draaisma has alone and together with Ristin Olthof filmed domesticated horses in the Netherlands and received private video footage from owners, mostly in the Netherlands but also a few from England and the United States. She analysed 200 video recordings and collected descriptions of the communication signals in her book *Language Signs and Calming Signals of Horses* if the signal was seen at least 35 times to avoid overgeneralisation of a signal seen only once. The footage was based on real-life situations and never staged or manipulated. No equipment to measure for example heart rate, blood pressure or eye temperature of the horses was used to detect possible heightened tension. Conclusions were based solely on analyses of video footage. None of the horses had serious behavioural problems or physical injuries. None of the video footage showed behaviour or situations that led to extreme fight or flight responses. All the horses lived in stables with individual stalls in which they spent the night. All the horses spent their days at pasture for a varied period of time. All the horses were in daily contact with humans. The breeds included Dutch and Belgian purebred horses, Friesians, Lipizzaners, Haflingers, New Forest ponies, Fjords, Tinkers, English Thoroughbreds, Quarter horses, and Welsh Mountain ponies, as well as horses without pedigrees.

Table 4. Ethogram used for continuous sampling on focal observations during aromatherapy. Based on behaviour pattern definitions from Draaisma (2018).

BODY POSTURE			
Low head-neck	Lh	S	The horse's head and neck are kept at a 45- to 20-degree angle below the back line.
Mid-low head-neck	Ml	S	The horse's head and neck are kept at a 20- to 5-degree angle below the back line.
Horizontal head-neck	H	S	The horse's head and neck form a horizontal line in level with the back.
Mid-high head-neck	Mh	S	The horse's head and neck are kept at a 5- to 20-degree angle above the back line.
High head-neck	Hh	S	The horse's head and neck are kept at a 20- to 45-degree angle above the back line.
Carried tail	Lct	E	The tail of the horse is lifted from the hindquarters.
Three legged	Tl	S	A relaxed horse standing with a hind leg raised and rested on the toe of the hoof.
Defecation	D	E	The horse defecates.
Urination	U	E	The horse urinates.

FACIAL EXPRESSION			
Almond-shaped eyes	E	S	The horse's eyes have an almond shape, which means that the eyes are relaxed and without tension.
Rounder eyes	Re	S	The eyes go from being almond shaped to be rounder as an expression of tension.
Visible white of eyes	We	S	The eyes are round in shape and the white in the eye is visible as an expression of greater tension.
Long nostrils	N	S	The horse's nostrils are long in shape, which means the nose is relaxed.
Rounder nostrils	Rn	S	The nostrils become rounder with tension. The nostrils may go from long to round and back again as the horse smells and breathes more heavily, which are not included as rounder nostrils.
Soft, closed lips	L	S	The horse's mouth is closed with soft lips without wrinkles around the muzzle, which means the mouth is relaxed.
Rim of lower lip	Ll	S	The mouth and lower lip are relaxed, allowing the rim of the lower lip to be seen in front of the upper lip.
Clenched lips	Cl	S	The mouth is squeezed together making wrinkles around the mouth and lips, which means a light tension around the mouth.
Extension of the upper lip	Ul	S	There is a light to clearly visible extension of the upper lip as an expression of greater tension.
Actively moving ears	Ae	E	The ears often move back and forth without pauses with a standstill.
CALMING SIGNALS			
Blinking	B	E	The horse makes a marked blink where the eyelids are clearly pinched. It does not include fast, not clearly defined blinking.
Half closing eyes	Hc	E	The eyes are kept half closed for between 2 and 10 seconds.
Looking away	La	E	The horse changes his viewing direction. It differs from head turn and neck turn by both ears are directed in the viewing direction.
Chewing	C	E	Without food in the mount, the horse is doing the same rotating movement of the lower jaw and possibly also producing sound. The tongue is not visible.
Tongue out chewing	Ct	E	The movements made with tongue out chewing are the same as with the chewing described above. The horse makes a rotating motion with the lower jaw, possibly also producing sound. However, as

			he rotates his lower jaw, the tongue is pushed straight forward, out of the mouth, and then retracted again. This can happen several times in succession.
Yawning	Y	E	When yawning, a horse opens his mouth for several seconds. The upper lip is retracted, and the upper and lower teeth are visible. Sometimes the eyes roll back slightly or are half closed. The ears turn sideways during the yawn.
Head turn	Ht	E	The horse turns his head to the side while the neck and body remain stationary. The head turns can be to both sides. One ear points forwards and the other ear points in the direction of the diffuser/handler.
Neck turn	Nt	E	The horse turns his head and neck to the side while the torso and feet remain in the same fixed position. The neck turn can be to both sides. One ear points forwards and the other ear points in the direction of the diffuser/handler.
Neck shake	Ns	E	The horse's head and neck are between the horizontal and mid-high positions. The horse pushes his nose forwards. The head and neck of the horse moves in a short, side-to-side shaking motion a number of times while the horse' torso stays still. The eyes are closed.
Body shake	Bs	E	The horse lowers his head and neck to a horizontal or mid-low position. The nose is extended forwards slightly. He makes a sideways shaking motion with his body, starting at his head, then moving to his neck, his torso, and finally flowing into his tail. Sometimes the mouth is slightly open during this shaking.
See-saw lowering	Ss	E	In 1 second, the head goes from a high, mid-high, or horizontal position to a mid- low to a low position. Then, within 1 to 5 seconds, the head is lifted back up to a mid-high or high position.
Sustained lowering	Sl	E	The horse moves his head and neck from a high, mid-high, or horizontal position to a mid-low to low position in a single second. This pose is held for 5-25 seconds, after which the head is lifted to a mid-high to high position again.

DISPLACEMENT ACTIVITIES			
Sniffing/stirring up the ground or the diffuser without eating	S	E	The horse extends the neck to the ground or down to the diffuser, where the nose and mouth are kept within a few centimetres from the ground/diffuser for several seconds.
Self-biting	Sb	E	The horse bites himself on the forelegs, shoulder or torso.
Rubbing the head along own leg	Rl	E	The horse scratches the head along his on forelegs.
Rubbing the head or neck on objects	Ro	E	The horse scratches the head or neck against other objects. Objects include walls, chairs and handler.
Licking objects	Lo	E	The horse used the tongue to touch/investigate an object. Object includes walls, chairs and handler.
Pawing	P	E	The horse is scraping the foreleg on the ground. Count as one every time the horse hit the ground.
DISTANCE INCREASING SIGNALS			
Moving away	Ma	E	The horse is either moving backwards or to the sides in an attempt to move away from the diffuser/handler.

Horses do not lie. Their posture and body language show what they feel and what their intentions are. Emotions and signals come and go according to the horse's feelings (Draaisma 2018). Thus, how a horse experiences a stimulus, how they respond and communicate can be deduced from their body language.

Calming signals are observed both when the horse is relaxed and when it experiences tension. This can be expressed in three different situations: 1) When a horse expresses its relaxation with a relaxed posture and facial expression. In a very brief moment before the horse gives the calming signal, it does not produce added tension. Therefore, the horse is relaxed, there is a stimulus, the horse registers this in a moment of light tension, gives a calming signal, and then relaxes again. When a horse is calm, they are often seen giving calming signals without making direct contact with their environment. 2) When a horse experiences tension and the tension either stays level or increases, a horse may give calming signals during or right after the stimulus or situation that is causing the tension. Instead of relaxing after having given the calming signal, the horse stays tense for a period of time or the tension may even increase. 3) When a horse experiences tension but the severe tension decreases, they may be very tense despite the fact that the stimulus or situation that caused it has stopped and the horse is recovering from the elevated tension. On the descent into relaxation, when the tension is decreasing, the horse can give calming signals (Draaisma 2018).

Calming signals can be seen in all possible head-neck positions, but most calming signals are seen in the mid-high and horizontal positions. Any position of the ear is possible. The more calming signals a horse gives over a short period of time, and the faster they succeed one another, the sooner the horse will show displacement behaviour, stress signals or both (Draaisma 2018).

Displacement behaviours can follow and alternate with every calming signal. They can be shown from every head–neck position and afterwards the horse may return to every possible head–neck position. During displacement activities, the horse's mouth is usually active and an integral part of the behaviour in question. Examples are licking, biting, or sniffing the ground. The horse can show displacement behaviours with a face that carries light, medium, or high tension (Table 5), according to the level of tension the horse is feeling at that moment. All displacement behaviours also have a self-contained biological function. A horse can sniff the ground and stir it up in order to smell. It can grind and bite because of an itch. It can lick to taste. It is up to the observer to tell the difference between when a horse is performing these behaviours to fulfil a biological function or when the behaviour is part of a communicative sequence (Draaisma 2018).

The mouth of a horse is a good measure of tension. A relaxed mouth has a relaxed lower lip. The corner of the lower lip may protrude slightly in front of the upper lip or the rim of the lower lip may be visible. The lower lip may also be slightly longer than the upper lip. When a horse is becoming slightly tense, it will tighten its lips. When this happens, one lip is no longer more extended than the other, but they connect seamlessly and may even be clenched. If the tension then rises further, the upper lip can assume different shapes (Draaisma 2018).

Facial features such as the nose and eyes also reflect tension. The higher the tension, the rounder the nose and eyes become simultaneously. Sometimes even the white of the eyes are visible. The nostrils may contract from round to long and back, or nostrils that stay round. A horse who has been running fast or working hard, or as in this experiment, had been doing scent work, can also have round nostrils, which indicates that the horse needs a greater intake of air, and this does not apply as a sign of tension. When tension is rising the tail dock will often range between a lightly carried tail to a highly carried tail, but the tail also has the task of balancing the body and is used to swipe away insects too (Draaisma 2018). The camera angle in this experiment was not optimal for assessing tail placement and is, therefore, only noted when a horse clearly carried its tail, and is not divided into groups of lightly to highly carried tail.

In summary, the more elements observed which reflect tension simultaneously, the higher the tension level of the horse (Table 5) with emphasis to pay close attention to the entire situation and context because many of the signals also have a biological function.

Table 5. State of mind and physical features of the horse (Draaisma 2018).

State	Head position	Nose, eyes, mouth	Tail
Relaxed	All head-neck positions are possible	Nostrils long, eyes almond-shaped and mouth relaxed. A rim or corner of the lower lip may be visible	Tail is relaxed, hangs loosely
Light tension	All head-neck positions are possible	Nose and/or eyes round, mouth relaxed and loosely closed	Tail is relaxed or carried lightly
Medium tension	Mid-high to high head-neck positions	Nose and/or eyes round, mouth clenched	Tail is carried lightly to moderate
High tension	High head-neck position	Nose and eyes round, mouth clenched and possible a differently shaped upper lip	Tail is carried lightly to high

3 Results

3.1 Physiological measurements

3.1.1 Heart rate

Of the nine horses, seven demonstrated lower or unchanged heart beats per minute after lavender aromatherapy; the heart rate of Intakt and Noble increased slightly (respectively 2.9% and 6.5%). Eight out of nine with vetiver; Copper was the only horse in which the heart rate increased a little (6.6%). Seven out of nine with spikenard; Copper and Intakt showed a small increase (respectively 13.1% and 10.7%) in heart rate after aromatherapy. All nine horses had either lower or unchanged (only Cavalos) heart beats per minute after roman chamomile aromatherapy. These measurements were based on a comparison between the individual's own baseline and their biological reaction to the aromatherapy.

Based on the mean-values across the participating horses, the initial t-tests only found a significant difference between baseline and spikenard. When baseline and the five aromatherapy treatments were statistically compared (ANOVA), no significant differences appeared. A Wilcoxon test revealed significant differences between baseline and each of the four aromatherapy treatments with about five percent lower heart rate (Table 6). Lavender, vetiver and roman chamomile were considered very significant.

Table 6. Results of statistical analysis on equine heart rate (bpm) performed using InStat.

	Baseline	Water	Lavender	Vetiver	Spikenard	Roman Chamomile
Mean	37.56	37.56	35.67	34.78	36.00	35.89
SEM	2.18	2.14	1.56	1.04	1.61	2.16
SD	6.54	6.42	2.69	3.11	4.82	6.47
Normality	No	No	Yes	Yes	No	Yes
ANOVA	p = 0.0569 considered not quite significant					
One-tailed			p = 0.0015	p = 0.0004	p = 0.0429	p = 0.0010
Decreased			5% ↓	7% ↓	5% ↓	5% ↓

3.1.2 Respiratory tidal volume

For all four essential oils, approximately half of the participating horses had a greater respiratory band capacitance (larger tidal volume) after aromatherapy compared to their individual baseline. This response differed between the horses, as to whether they had a higher capacitance: Max, Noble and Siska reacted on three out of four essential oils and were thus the ones who responded to most oils by expanding their chest and thereby, their tidal volume. It was different oils that provoked this physical reaction in the horses. The number of breaths showed a slightly different picture. Two to three horses breathed more frequently (per minute) after aromatherapy: Cavalos and Intakt responded to all four essential oils while Copper reacted only in this way to vetiver and spikenard.

Based on the mean-values for all participating horses, the initial t-tests indicated only a significant difference in capacitance between the baseline and the negative control group (water) and no differences for the respiratory rate (breaths per minute). When baseline and the five aromatherapy treatments were statistically compared (ANOVA), no significant differences appeared either. A Wilcoxon test on the capacitance revealed significant differences between baseline and three of the aromatherapy treatments with about five percent shallower breathing (Table 7). The Wilcoxon test was not performed on roman chamomile due to the mean being higher than that of the baseline. Lavender and spikenard were considered very significantly different from baseline. A Wilcoxon test was not performed on the number of breaths because the initial t-test did not reveal any significant differences, and neither did the nonparametric repeated measures (ANOVA).

Table 7. Results of statistical analysis on equine respiratory band capacitance (pF) and breaths per minute; separated by bold line. Performed using InStat.

	Baseline	Water	Lavender	Vetiver	Spikenard	Roman Chamomile
Mean	351.22	380.89	334.56	331.22	337.67	353.11
SEM	36.37	43.98	33.25	48.15	50.97	47.12
SD	109.12	131.93	99.75	144.45	152.91	141.36
Normality	No	Yes	Yes	No	Yes	Yes
ANOVA	p = 0.6078 considered not significant					
One-tailed			p = 0.0007	p = 0.0252	p = 0.0054	
Decreased			5% ↓	6% ↓	4% ↓	
Mean	12.67	15.44	14.11	13.67	12.56	13.56
SEM	2.17	1.83	1.61	1.61	1.58	1.95
SD	6.52	5.50	4.83	4.82	4.75	5.86
Normality	Yes	Yes	Yes	Yes	Yes	Yes
ANOVA	p = 0.5417 considered not significant					

3.1.3 Muscle contractures

In general, the aromatherapy treatments appear to result in fewer spontaneous muscle contractions in the horses for both muscle groups. Four horses had one or two oils that triggered more muscle contractions compared to their individual baseline, but it was not the same oils that triggered these responses in the two muscle groups. Three out of four horses responded to vetiver and/or spikenard with multiple muscle contractions: Ville showed multiple contractions in both m. temporalis and m. cleidomastoideus after spikenard aromatherapy. The same was true for Zendi, just after aromatherapy with vetiver.

Based on the mean-values for all participating horses (Table 8), the initial t-tests found significant differences for lavender, spikenard and roman chamomile in m. temporalis, and for lavender and roman chamomile in m. cleidomastoideus. When baseline and the five aromatherapy treatments were statistically compared (ANOVA), a significant difference was found for roman chamomile in m. temporalis, and almost but not quite a significant effect for any of the aromatherapy treatments in m. cleidomastoideus.

Data for m. temporalis passed normality for all treatments, therefore a Wilcoxon test was performed both as a one-tailed and a two-tailed test. Two-tailed analyses require both groups to be normally distributed while a one-tailed test is only interested in a difference in one direction and therefore can be used for groups which are both normally distributed, or where one of the groups failed the test for normality. In this case, a two-tailed test was normally used, but one should also bear in mind that this test was performed on a small test population of nine horses, which is why it can be argued that a one-tailed test was perhaps more appropriate. The results revealed a sensitivity for lavender aromatherapy, which was significantly different using a two-tailed test, but not with a one-tailed test. Data for m. cleidomastoideus did not all pass the test for normality and therefore, a one-tailed Wilcoxon tests was used (Table 8).

All four essential oils decreased the number of spontaneous muscle contractures in m. temporalis over a response range of 17% to 39% fewer responses which was significant for both a one-tailed and a two-tailed test for spikenard and roman chamomile (Table 8). Both were considered very significant. Again, all four essential oils decreased the number of spontaneous muscle contractures in m. cleidomastoideus over a response range of 20% to 34% fewer responses which was significant for lavender and roman chamomile.

Table 8. Results of statistical analysis on equine spontaneous muscle contractures (number of peaks) in m. temporalis and m. cleidomastoideus; separated by bold line. Performed using InStat.

	Baseline	Water	Lavender	Vetiver	Spikenard	Roman Chamomile
Mean	7.33	6.33	5.33	6.00	4.89	4.44
SEM	0.78	1.08	0.50	0.99	0.59	0.82
SD	2.35	3.20	1.50	2.96	1.76	2.46
Normality	Yes	Yes	Yes	Yes	Yes	Yes
ANOVA	p = 0.0158 considered significant for Baseline vs. Roman Chamomile					
One-tailed			p = 0.4216	p = 0.1914	p = 0.0098	p = 0.0010
Two-tailed			p = 0.0313	p = 0.3828	p = 0.0195	p = 0.0039
Decreased			27% ↓	17% ↓	33% ↓	39% ↓
Mean	8.22	8.00	5.67	6.22	6.56	5.44
SEM	0.64	0.62	0.55	1.70	0.82	1.07
SD	1.92	1.87	1.66	5.10	2.46	3.21
Normality	No	No	Yes	Yes	No	Yes
ANOVA	p = 0.0553 considered not quite significant					
One-tailed			p = 0.0391	p = 0.1797	p = 0.0977	p = 0.0484
Decreased			30% ↓	24% ↓	20% ↓	34% ↓

Lavender, spikenard and roman chamomile showed promising results in terms of reducing the number of spontaneous muscle contractions. Therefore, their S- and T-scores were compared for both muscle groups (Table 9 and Table 10).

Only the T-score for spikenard were found to be significantly different from baseline values for m. cleidomastoideus. Neither lavender nor roman chamomile differed significantly from the baseline, but lavender showed a trend on the T-score for m. cleidomastoideus. Finally, spikenard and roman chamomile were also significantly different on the T-score for m. cleidomastoideus. In relation to the T-score for m. temporalis, a tendency was found between lavender and roman chamomile as well as roman chamomile and spikenard (Table 10).

As for the S-score, significant difference was found between lavender and roman chamomile, and between roman chamomile and spikenard (Table 10). Both differences were to be found in m. cleidomastoideus. Neither significant differences nor trends were found in m. temporalis.

No statistical differences were found for m. temporalis; therefore, the focus was directed towards m. cleidomastoideus: Lavender and spikenard had both a higher amplitude and higher frequency while roman chamomile had a lower amplitude and only a slightly higher frequency (Table 9). Spikenard had a significantly higher frequency than both the baseline and roman chamomile, which can be interpreted as meaning that the muscle fibres were re-fired faster during aromatherapy with spikenard which is more energy intensive. In contrast, roman chamomile gave a significantly lower amplitude than both lavender and spikenard, which can be interpreted as meaning that the muscle fibres were less active, suggesting that roman had a more relaxing effect on muscles measured compared to lavender and spikenard.

Table 9. Mean values on S- and T-scores for baseline, lavender, spikenard and roman chamomile.

ESTi Score	S-score		T-score	
Muscle group	Temporalis	Cleidomastoideus	Temporalis	Cleidomastoideus
Baseline	6.45	6.53	6.81	7.69
Lavender	6.19	6.17	7.34	6.71
Spikenard	6.79	6.21	7.37	6.31
Roman Chamomile	6.05	6.81	6.53	7.46

Table 10. T-test values on S- and T-scores for lavender, spikenard and roman chamomile.

ESTi Score	S-score		T-score	
Muscle group	Temporalis	Cleidomastoideus	Temporalis	Cleidomastoideus
Baseline vs Lavender	p = 0.3079	p = 0.4492	p = 0.2114	p = 0.0771
Baseline vs Spikenard	p = 0.2777	p = 0.4519	p = 0.2102	p = 0.0069
Baseline vs Roman Chamomile	p = 0.2480	p = 0.1018	p = 0.3467	p = 0.3278
Lavender vs Roman Chamomile	p = 0.4152	p = 0.0491	p = 0.0704	p = 0.1084
Lavender vs Spikenard	p = 0.1382	p = 0.4930	p = 0.4772	p = 0.2623
Roman Chamomile vs Spikenard	p = 0.1162	p = 0.0342	p = 0.0691	p = 0.0069

3.1.4 Summary

All four essential oils had equally calming effects on the equine heart rate and the same seemed to be the case for equine respiratory tidal volume, but this was not evident for roman chamomile. In contrast, it was roman chamomile that resulted in the fewest spontaneous muscle contractions

(Table 11) and the failure to find a significantly lower capacitance from the respiratory band after roman chamomile aromatherapy may be due to the low number of horses used in this experiment (n = 9 horses).

Table 11. Comparison of one-tailed p-values and the percentage change across equine heart rate, respiratory tidal volume and muscle contractures in relation to the essential oils. One-tailed p-values were selected for muscle contractures in m. temporalis although data have been tested to be normally distributed because the test population was small: Lavender goes from being significantly different from the baseline (two-tailed) to not being significant (one-tailed). No changes were observed for the other essential oils.

n = 9 horses	Heart rate (bpm)		Tidal volume (pF)		Muscle contractures (no. of peaks)			
					Temporalis		Cleidomastoideus	
Lavender	p = 0.0015	5% ↓	p = 0.0007	5% ↓	p = 0.4216	27% ↓	p = 0.0391	30% ↓
Vetiver	p = 0.0004	7% ↓	p = 0.0252	6% ↓	p = 0.1914	17% ↓	p = 0.1797	24% ↓
Spikenard	p = 0.0429	5% ↓	p = 0.0054	4% ↓	p = 0.0098	33% ↓	p = 0.0977	20% ↓
Roman Chamomile	p = 0.0010	5% ↓			p = 0.0010	39% ↓	p = 0.0484	34% ↓

As expected, the results indicated that lavender oil seemed to have relaxing effect on all three parameters and was a good choice for a positive control group, although the number of spontaneous muscle contractions was lower for spikenard and roman chamomile. Therefore, the S- and T-scores for lavender, spikenard and roman chamomile were examined further. Spikenard was the only essential oil that differed significantly from the baseline by having a significantly higher frequency, but roman chamomile had both a significantly lower amplitude and lower frequency than spikenard along with a significantly lower amplitude than lavender, suggesting roman chamomile had a better calming effect on muscle contractions than lavender and spikenard. Considering the physical parameters, roman chamomile oil apparently had the potential to be a better natural calmative than lavender oil.

3.2 Behavioural assessment

The videotapes range between 852 seconds to 903 seconds per aromatherapy treatment with an average time period of approximately 896 seconds for most of the treatments (Table 12). With the goal of recording the behaviour for 900 seconds per treatment, the average is considered approved.

Table 12. Time in seconds each horse was exposed to aromatherapy and videotaped.

	Water	Lavender	Vetiver	Spikenard	Roman Chamomile
Amigo	897	901	887	898	902
Cavalos	898	899	899	899	900
Copper	900	898	900	901	895
Intakt	898	900	896	901	901
Max	897	897	895	900	903
Noble	898	899	899	893	893
Siska	870	885	895	887	889
Ville	900	889	852	896	899
Zendi	903	896	896	893	896
Average	**896 s**	**896 s**	**891 s**	**896 s**	**898 s**

Within the group of calming signals, displacement activities, stress signals and distance increasing signals, only the most common are documented by Draaisma (2018) and a further reduction has occurred because all those signals that cannot be made whilst a horse stands, have also been excluded from this experiment. The participating horses did not show every one of the signals included in the ethogram (Table 4). Horses vary when it comes to body language, and they also seem to develop personal habits in their way of using the body signals (Draaisma 2018). This experiment supports that allegation by observing that some horses for example blinked a lot to calm themselves and/or environmental stimuli, while others were found to chew more. Differences in signalling within the same treatment for different horses was compared in Table 13-17.

The facial expression was in the ethogram (Table 4) listed as states which were timed per second a horse showed a given combination of eye, nose and mouth signals. These combinations were according to Table 5 divided into groups of a relaxed face, light tension, medium tension and high tension. The total time spent in each facial group was converted to a percentage relative to the full length of the video recording (shown in Table 12). In this way, it was known what percentage of time the horse spends within each facial expression and thus, what percentage of the time the horse exhibited relaxation and tension, respectively. The percentage distribution of facial expressions is illustrated in Figures 1-7.

3.2.1 Water

General overview

With the exception of one horse (Noble), attention was in general paid to what was going on outside the stable during aromatherapy with water (Figure 1 and Table 13). Impatience and irritation were noted several times for these recordings. This was expressed as more movement, more frequent calming signals with none or only short breaks in between, which more frequently escalated into displacement activities or distance increasing signals. Noble was the only one who was extremely interested in the diffuser while it released water. His ears were turned forward and he spent a long time smelling the vapor and using his upper lip to move the diffuser.

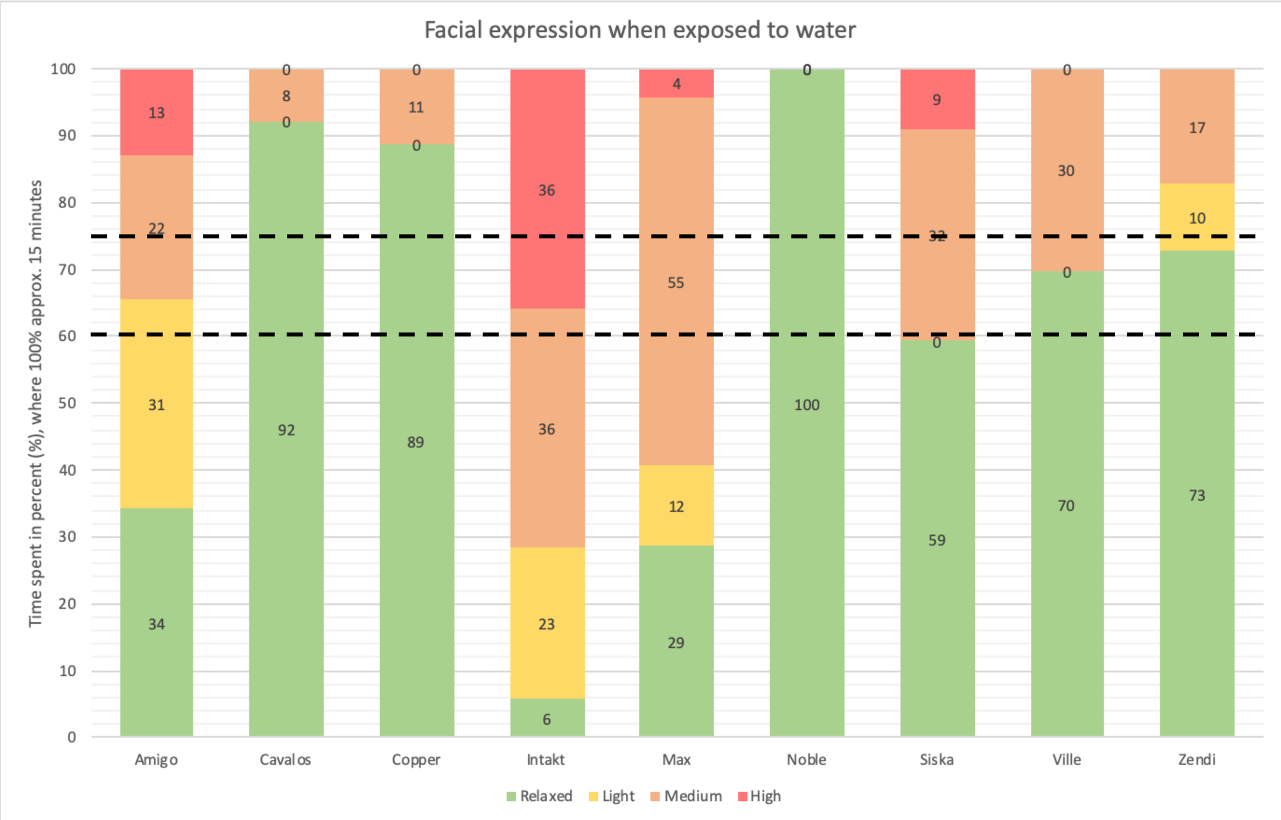

Figure 1. Summary of observed facial expressions for the horses when exposed to humified water. Time spent in seconds is converted to percent with 100% being approx. 15 minutes, cf. the aromatherapy protocol. The facial expressions are divided into relaxed (green), light tension (yellow), medium tension (orange) and high tension (red) according to the criteria in Table 5.

Facial expression

Based on the facial expression, nebulizing with water did not cause significant tension among the majority of the horses: 56% of the horses had a relaxed facial expression for 60% of the recorded time while it was down to 33% of the horses who were relaxed for 75% of the recorded time (Figure 1).

Amigo, Intakt, Max and Siska exhibited medium tension for a large proportion of the time and also had periods of high tension. Cavalos and Copper showed medium tension but was relaxed almost the entire time period. Ville and Zendi came just within a relaxation marker of 70% but distributed the remaining time on either medium tension only or both light tension and medium tension. Noble was the only horse who did not show any signs of facial tension.

Table 13. Summary of observed events (no.) for the horses when exposed to humified water.

Events	**Amigo**	**Cavalos**	**Copper**	**Intakt**	**Max**	**Noble**	**Siska**	**Ville**	**Zendi**
Ae	11	3	1	12		5	6		3
B	61	47	2	26	6	1	2	8	20
Lct		1							1
D									
U									
Hc			10		2			2	9
La	6	25	2	22	15	3	17	20	14
C	1	2	2		2		5	3	1
Ct	8	6	5	8	13	4	3	5	4
Y					4				
Ht	7	2			6	8			9
Nt	3				5	1			
Ns		7			1		10	1	3
Bs									
Ss	3			1	4	3	2	1	3
Sl	1	1		1	3	7	9		
S	1		1		3	15	6		
Sb			1	2	1		3		
Rl		1	2			1	1		
Ro					11	11	16	6	
Lo							1		
P	4						4		14
Ma	2	3		1	10	3	4	5	3

Signals

All the horses use blinking as a calming signal, but it was especially used by Amigo, Cavalos, Intakt and Zendi. Noble blinked the fewest times and he was at the same time the horse with the most relaxed facial expression, cf. Figure 1. All the horses looked away several times and turned their attention to what was going on outside the stable instead of concentrating on the vapor or handler. Cavalos, Intakt, Max, Siska, Ville and Zendi looked away for more than 10x during 15 minutes. All the horses chewed with their tongue out, but Max did it most times and he was the only horse to do it more than 10x in 15 minutes. Max, Noble and Siska rubbed their head several times against objects or the handler while Zendi preferred to paw. Other than that, the majority of horses moved their ears frequently, chewed with their mouth shut and did see-saw lowering (Table 13).

3.2.2 Lavender oil

General overview

Lavender oil was given a mixed welcome by the horses (Figure 2 and Table 14). Copper, Noble and Zendi seemed to find the scent pleasant and calming. Zendi did not show a lot of signals during the recordings but was observed to yawn for seven to eight times successively after the diffuser with lavender oil was turned off. Copper and Noble were interested in smelling the vapor. Both stood still with a relaxed lower lip, long periods of half-closed eyes and rested on three legs. Amigo, Cavalos and Siska were distracted by their surroundings, either in the form of people coming in and out of the stable or by flies. However, they seemed to calm down faster with lavender aromatherapy than with humified water. Intakt sniffed the vapor several times but the scent seemed a bit overwhelming for him and therefore, he moved around a lot. Neither Max nor Ville seemed interested in the scent of lavender and appeared impatient to the handler's desire for them to stand still.

Facial expression

During aromatherapy with lavender, 67% of the horses had a relaxed facial expression for 60% of the recorded time while it was 56% of the horses who were relaxed for at least 75% of the recorded time.

Copper, Noble and Siska had a perfectly relaxed facial expression during aromatherapy with lavender oil (Figure 2). The scent of lavender did not prevent Intakt from showing medium tension for most of the recorded time. Amigo and Ville showed light tension but was relaxed about 80% of the time. Max had a relaxed facial expression for around 60% of the time while Cavalos and Zendi was down to 52-55% of the time. Thus, they showed some degree of tension for almost half the time.

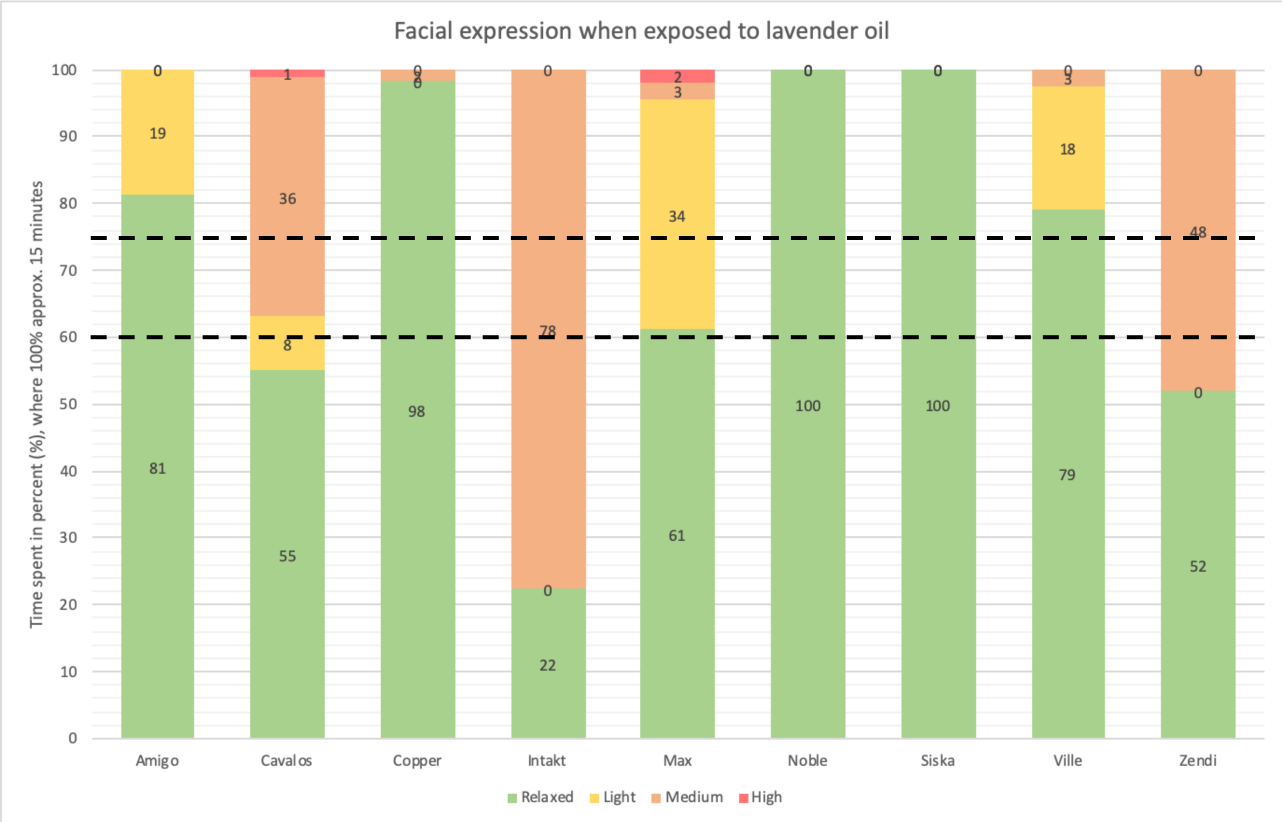

Figure 2. Summary of observed facial expressions for the horses when exposed to lavender oil. Time spent in seconds is converted to percent with 100% being approx. 15 minutes, cf. the aromatherapy protocol. The facial expressions are divided into relaxed (green), light tension (yellow), medium tension (orange) and high tension (red) according to the criteria in Table 5.

Signals

Again, all the horses blinked as a calming signal, but this time it was only Max and Siska that did not blink more than 10x. Amigo was again the horse that blinked most times. In contrast, Amigo was the only horse not observed to look away or sniff the ground or diffuser. All the other horses did. Intakt and Siska looked away the most times. Siska was (like the aromatherapy with water) the horse who rubbed her head against objects or the handler. Amigo was the only horse to yawn while the lavender aromatherapy was on. Again, all the horses were chewing with their tongue out as a calming signal where Intakt was the only horse to do it more than 10x in 15 minutes. Intakt was the horse that moved away from the diffuser most frequently, closely followed by Max and Siska (Table 14).

Table 14. Summary of observed events (no.) for the horses when exposed to lavender oil.

Events	Amigo	Cavalos	Copper	Intakt	Max	Noble	Siska	Ville	Zendi
Ae		2		11		2	4		1
B	58	19	17	20	2	15	14	3	20
Lct									5
D				1					
U									
Hc		13	7			13	15		10
La		3	3	24	17	8	20	14	13
C		1	4	2	1	1	5	1	
Ct	5	2	3	14	6	2	4	6	3
Y	4								
Ht	12	5		5	6	1	2	5	
Nt	4			4	1				
Ns	1	6	3	1			7	4	
Bs		1							
Ss	2			6	1		3	3	1
Sl	1			1	5	1	5	2	
S		1	1	5	3	3	3	1	2
Sb		1		1	1		1		
Rl							1		
Ro		3			4		17	11	
Lo									
P	1				2		2		4
Ma	1	1		15	13		13	6	4

3.2.3 Vetiver oil

General overview

Vetiver oil also gave different reactions among the horses (Figure 3 and Table 15). Max and Noble both spent a long time smelling the oil. They seemed calm as they stood still. Noble became a little impatient near the end, a bit like a child who does not want to fall asleep. Zendi appeared annoyed at first but calmed down and stood still during the rest of the aromatherapy protocol. Amigo and Intakt seemed nervous and were more attentive to what was happening outside the stable. Some large and noisy machines interrupted the recordings with Intakt when he was exposed to vetiver oil, which was why this treatment was repeated after a break. Unfortunately, the same machines were also active during the second attempt. Cavalos and Siska were greatly annoyed by flies and therefore, it was difficult to assess whether the tension was due to insects or the scent and whether the signals were used communicatively or had a biological function. Copper and Ville did not seem interested and therefore, became impatient. Copper calmed down but it took a longer time than that observed for lavender and spikenard.

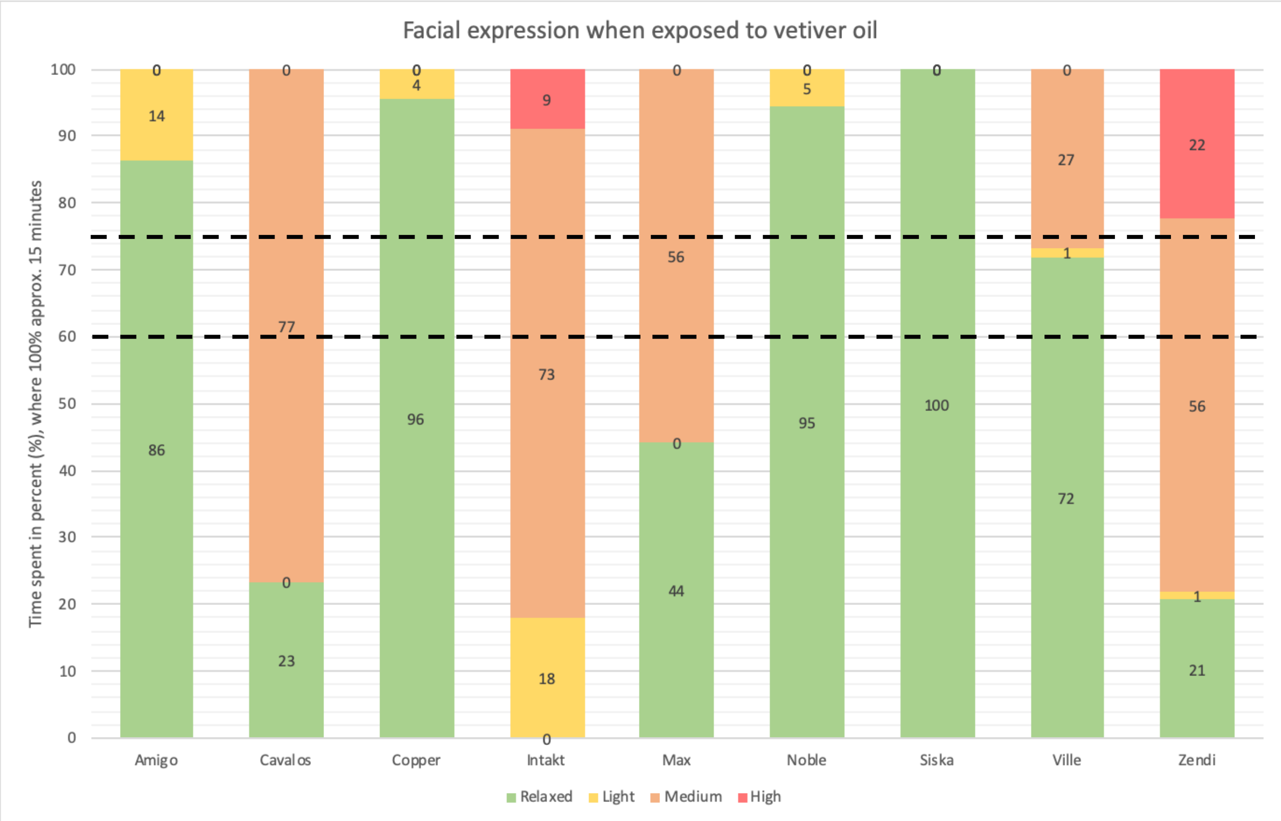

Figure 3. Summary of observed facial expressions for the horses when exposed to vetiver oil. Time spent in seconds is converted to percent with 100% being approx. 15 minutes, cf. the aromatherapy protocol. The facial expressions are divided into relaxed (green), light tension (yellow), medium tension (orange) and high tension (red) according to the criteria in Table 5.

Facial expression

When zooming in on the facial expression (Figure 3), the picture was a little different than what was noted as a general overview when going through the videos. During aromatherapy with vetiver, 56% of the horses had a relaxed facial expression for 60% of the recorded time while 44% of the horses were relaxed for 75% of the recorded time.

The facial expression of Siska was 100% of the time categorised as relaxed while Cavalos' irritation with flies expressed as a medium tension on his face. Zendi's irritation was also transmitted to his facial expression as longer periods of tension compared to relaxation. Amigo, Copper and Noble showed only light tension but were mostly relaxed. Although Max stood still and spent a long time smelling the oil, half the time he showed a medium tense facial expression. Ville did not seem interested in the scent of vetiver, but most of the time he had a relaxed expression. He was more tense than Copper who also showed no interest in the oil. As expected, due to the noise from the large machines, Intakt showed only tension - primarily medium tension - but together with Zendi he was the only other horse exhibiting high tension during vetiver aromatherapy.

Table 15. Summary of observed events (no.) for the horses when exposed to vetiver oil.

Events	Amigo	Cavalos	Copper	Intakt	Max	Noble	Siska	Ville	Zendi
Ae	4	1		5		1		1	5
B	49	39	9	20	10	16	1	2	20
Lct				14					2
D						1			
U									
Hc		11	10		6	15	5	1	11
La			11	16	13	12	11	19	13
C	1		2			4	6	2	
Ct	7	8	4	24	5	3	6	5	6
Y					2				
Ht	3	2		6	1	5			
Nt	5			5	1				
Ns		6	3	1		1	9	11	
Bs									
Ss	1		3	3		4	1	1	
Sl	2	1		2		3	6		
S		2	1	1	5	6		1	1
Sb				1	1		2		
Rl		6					6		
Ro		1		1		2	13	25	
Lo									
P	12					13	6		7
Ma	4	2	4	22	9	5	11	8	2

Signals

As seen before, blinking was a calming signal displayed by all the horses. Amigo, Cavalos, Intakt and Zendi used it 20x or more during the 15 minutes of aromatherapy. All the horses needed to chew with their tongue out and move away from the diffuser or handler. Intakt was both the horse that chewed the most and moved around the most. Otherwise, there were no other signals commonly displayed by all the horses. Ville preferred to look around and rub against objects or the handler while Noble spent more time with half-closed eyes and scratching the ground (Table 15).

3.2.4 Spikenard oil

General overview

Spikenard seemed to be well liked by all the horses (Figure 4 and Table 16). Cavalos and Copper were both very interested in the scent and relaxed on three legs for most of the recorded time. Max had problems with cramps in his hindlegs and probably therefore he did not stand for long resting on three legs, but showed his well-being by keeping his head lowered. Cramps in his hindlegs is an old problem, according to the owner, of unknown origin and will not be discussed further. Amigo, Noble, Ville and Zendi moved impatiently around to begin with, but calmed down in response to the aromatherapy and eventually stood completely relaxed. Although Siska continued to be irritated by flies, she was also noticeably calmer. Only Intakt did not really seem calmed down by the nebulization and was both nervous and tense, which resulted in him moving around a lot.

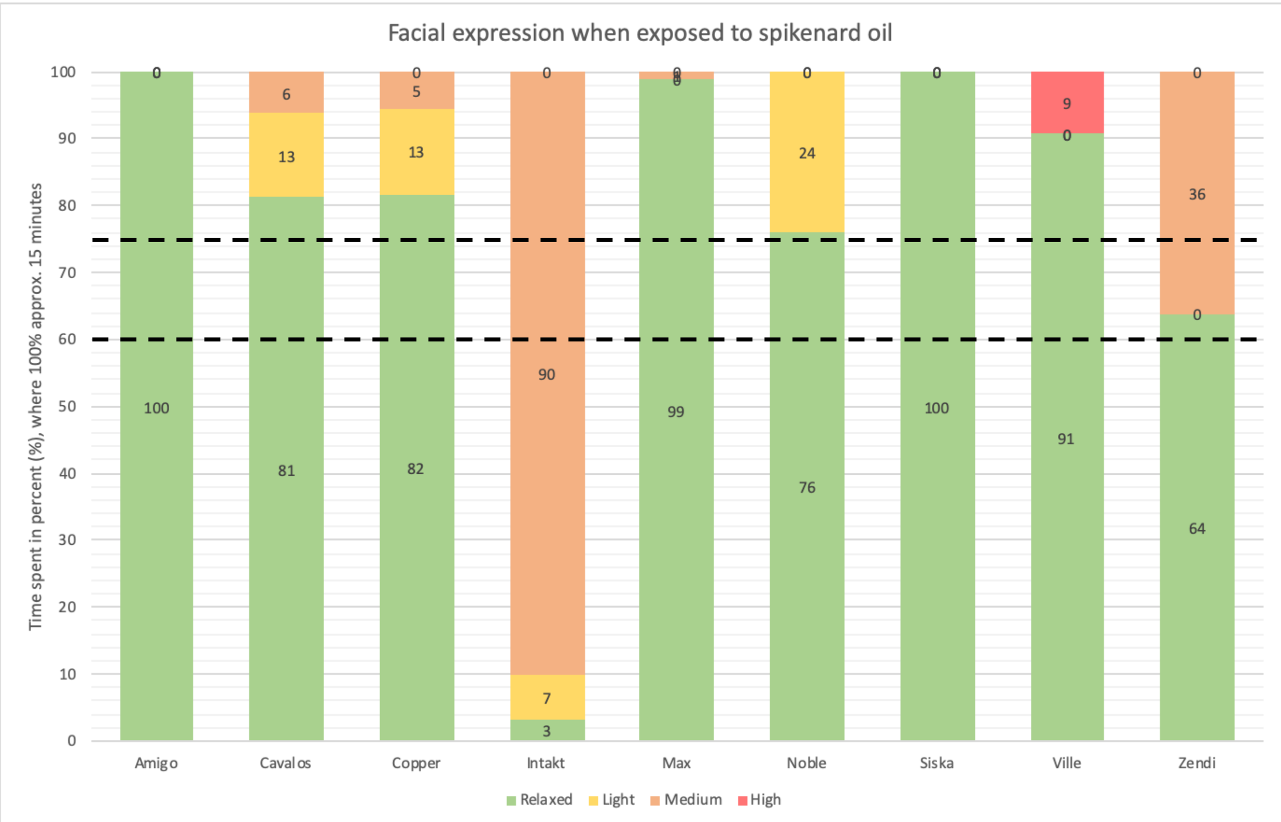

Figure 4. Summary of observed facial expressions for the horses when exposed to spikenard oil. Time spent in seconds is converted to percent with 100% being approx. 15 minutes, cf. the aromatherapy protocol. The facial expressions are divided into relaxed (green), light tension (yellow), medium tension (orange) and high tension (red) according to the criteria in Table 5.

Facial expression

The graph supports what was observed under the general overview (Figure 4). During aromatherapy with spikenard, 89% of the horses had a relaxed facial expression for 60% of the recorded time while as many as 78% of the horses were relaxed for at least 75% of the recorded time.

With the exception of Intakt which primarily showed medium tension, the horses were mostly relaxed during spikenard aromatherapy. Amigo and Siska had a perfectly relaxed facial expression. Although Max had cramps in his hindlegs, it did not result in tension in his face. Cavalos and Copper reacted almost identically to the scent with relaxation for about 80% of the recorded time. Ville had a relaxed facial expression for 91% of the time but was the only horse to show high tension during vetiver aromatherapy. Noble and Zendi were relaxed for more than 60% of the time, but Zendi's tension was categorized as medium while Noble showed only light tension. Intakt was again tense most of the time but did not show signs of high-tension during aromatherapy with spikenard.

Table 16. Summary of observed events (no.) for the horses when exposed to spikenard oil.

Events	Amigo	Cavalos	Copper	Intakt	Max	Noble	Siska	Ville	Zendi
Ae		1		3		3	3		2
B	59	12	9	25	22	13	15	4	9
Lct				7	2				
D			1			1			
U									
Hc		8	10		10	7	11	7	15
La		8	11	25	6	18	13	7	9
C	1	1	2	4	2	4	6		
Ct	6	3	4		9	3	3	2	5
Y					2				
Ht	4			2	5	9	1	1	3
Nt	5	1				4	1		
Ns	4	3	3	1	4	1	9	5	3
Bs									
Ss	1		3	2		6	1	2	4
Sl	1	1		2		3	12	4	
S			1	2	2	2	1	6	1
Sb	1					1	1		
Rl		1					2		
Ro	2	1		1	1	1	20	9	3
Lo									
P						25	1		22
Ma	2	1	4	12	2	8	8	8	5

Signals

Again, the horses similarly used blinking as a calming signal and needed to use distance increasing signals in the form of moving away from the diffuser or handler. Amigo was the one who blinked the most, while Intakt was the one who moved the most. Otherwise, this was the first oil that all the horses displayed neck shakes. Siska shook her neck most and also spent most of her time rubbing against objects or the handler, although it was probably due to the flies. Noble and Zendi scraped the ground many times with a front leg. Where Noble often looked away, Zendi often stood with half-closed eyes (Table 16).

3.2.5 Roman chamomile oil

General overview

Roman chamomile seemed to appeal to many of the horses (Figure 5 and Table 17). Cavalos and Copper took a bit longer time to get used to the scent but ended up becoming calm. Siska continued to suffer with flies in the stable on what was a warm summer day and as a result had difficulty in becoming relaxed and calm. Max did not seem to dislike the scent, but he was annoyed by the handler holding him too tightly. The rest of the horses simply enjoyed the scent of roman chamomile as they stood relaxed and calm. Several of them spent a long time smelling the diffuser up close.

Facial expression

During aromatherapy with roman chamomile, 56% of the horses had a relaxed facial expression for 60% of the recorded time while it was down to 44% of the horses who were relaxed for 75% of the recorded time (Figure 5).

Copper seemed to spend a little longer time to get comfortable with the scent of roman chamomile, but her facial expression was perfectly relaxed just like Amigo and Noble. Intakt was the only horse to show high tension, but he appeared relaxed for 40% of the time which was roughly on a par with Cavalos and Siska. Max and Zendi showed about 60% relaxation with Max having an even distribution between light and medium tension, while Zendi was mainly categorized as medium tension in the remaining time. Ville looked relaxed for 86% of the time and was only lightly tense for the remainder of the time.

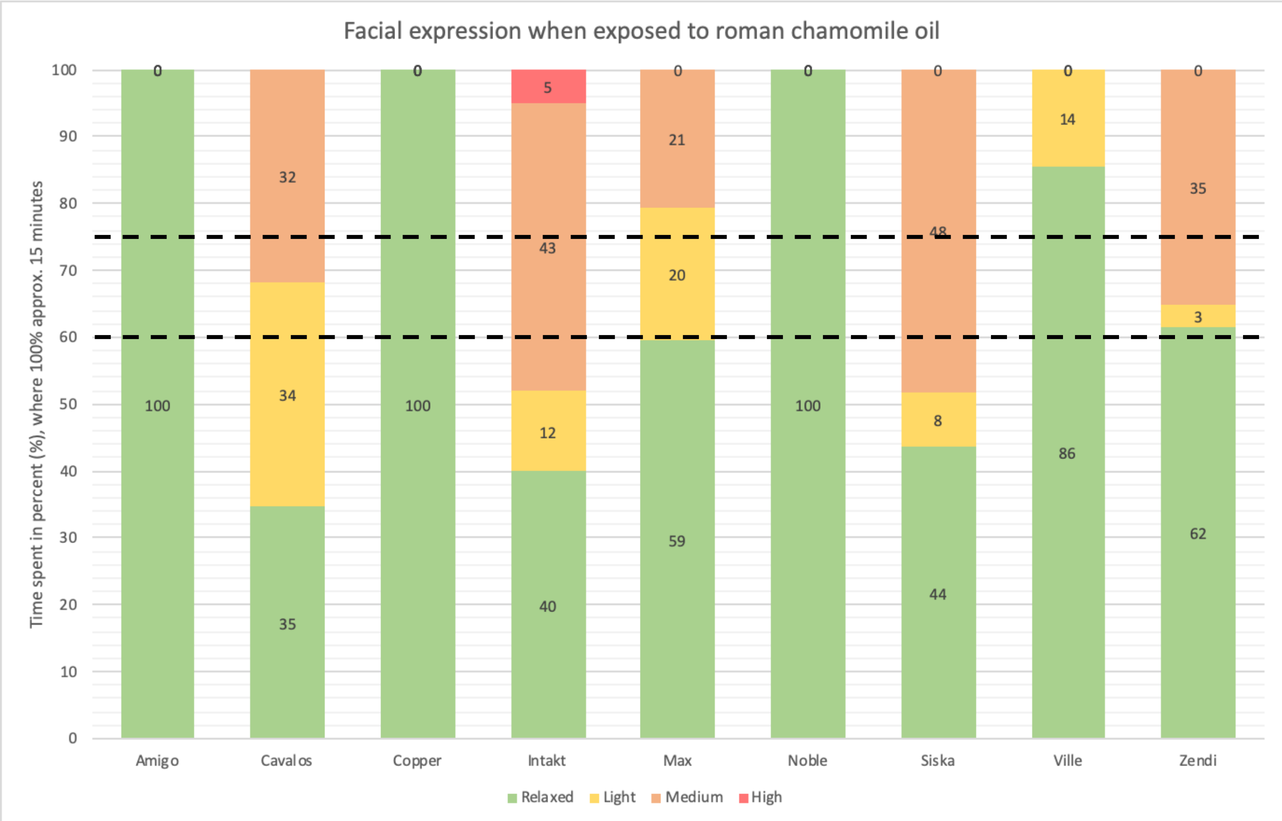

Figure 5. Summary of observed facial expressions for the horses when exposed to roman chamomile oil. Time spent in seconds is converted to percent with 100% being approx. 15 minutes, cf. the aromatherapy protocol. The facial expressions are divided into relaxed (green), light tension (yellow), medium tension (orange) and high tension (red) according to the criteria in Table 5.

Signals

This time, all the horses displayed blinking and Cavalos, Intakt and Zendi blinked more than Amigo. Flies were probably the reason Siska displayed the most neck shakes. Despite these flies, Siska was actually also the horse most often recorded to stand with half-closed eyes. Zendi did not show quite a lot of signals during the aromatherapy, which has been common to all the recordings, but with roman chamomile oil he was the horse that looked away most times. Max turned his head several times - many times looking away, but also several times where his attention remained on the diffuser or handler (Table 17). The distinction between these two signals is whether both ears faced forward or in another direction, cf. looking away, or one ear still facing the direction of the diffuser even though the head was turned away, cf. head turn (Table 4).

Table 17. Summary of observed events (no.) for the horses when exposed to chamomile oil.

Events	Amigo	Cavalos	Copper	Intakt	Max	Noble	Siska	Ville	Zendi
Ae		3	1	4		4		4	4
B	25	39	2	32	14	22	15	7	30
Lct		2		9					
D		1			1				
U									
Hc		13	6	1	7		25	6	5
La		10	5	17	16	6	10	7	20
C	2	5	1	1		2	8	2	
Ct	2	2	6	19	10		1	5	
Y									
Ht	2	2		2	12	8		10	1
Nt	3	5		2	1	3	2	1	
Ns		8	1				18	1	
Bs									
Ss	2				2	7	4		1
Sl					1	14	2		
S	4	3	2	2	6	3	1	5	
Sb			1		1	1	2	1	
Rl							1		
Ro	2	2			8	1	7	1	
Lo							1		
P	1		1				3	1	
Ma	2	3		20	8	11		4	

3.2.6 Summary

To sum up the previous sections, the relaxation markers on 60% and 75%, respectively have been grouped for comparison in Table 18. Vetiver and roman chamomile did just a little better than humified water by causing more horses to display relaxed facial expressions for 75% of the recorded time. Lavender oil did it better for both relaxation markers but spikenard did noticeably better than all the other treatments in terms of causing most horses to display relaxed facial expressions for both relaxation markers.

Table 18. How many percent of the horses had a relaxed facial expression for 60% and 75% of the recorded time, respectively.

Time	Water	Lavender	Vetiver	Spikenard	Roman chamomile
60%	56%	67%	56%	89%	56%
75%	33%	56%	44%	78%	44%

The events were found to be far more driven by personal preferences of the horses, as prescribed by literature (Draaisma 2018), and it is therefore unclear whether anything can be concluded in general. The observations supported the literature that the signals are used both when the horse is perfectly relaxed or tense (Draaisma 2018). It should also be noted that the horses may have completely relaxed facial expressions, but still exhibit displacement activities for example. In several cases, these were listed as communicative signals, but probably could have a biological function. Due to the problem of comparing the results of personal preferences and the fact that these appeared to be associated with relevant validity problems, focus was shifted to the results from the facial expressions.

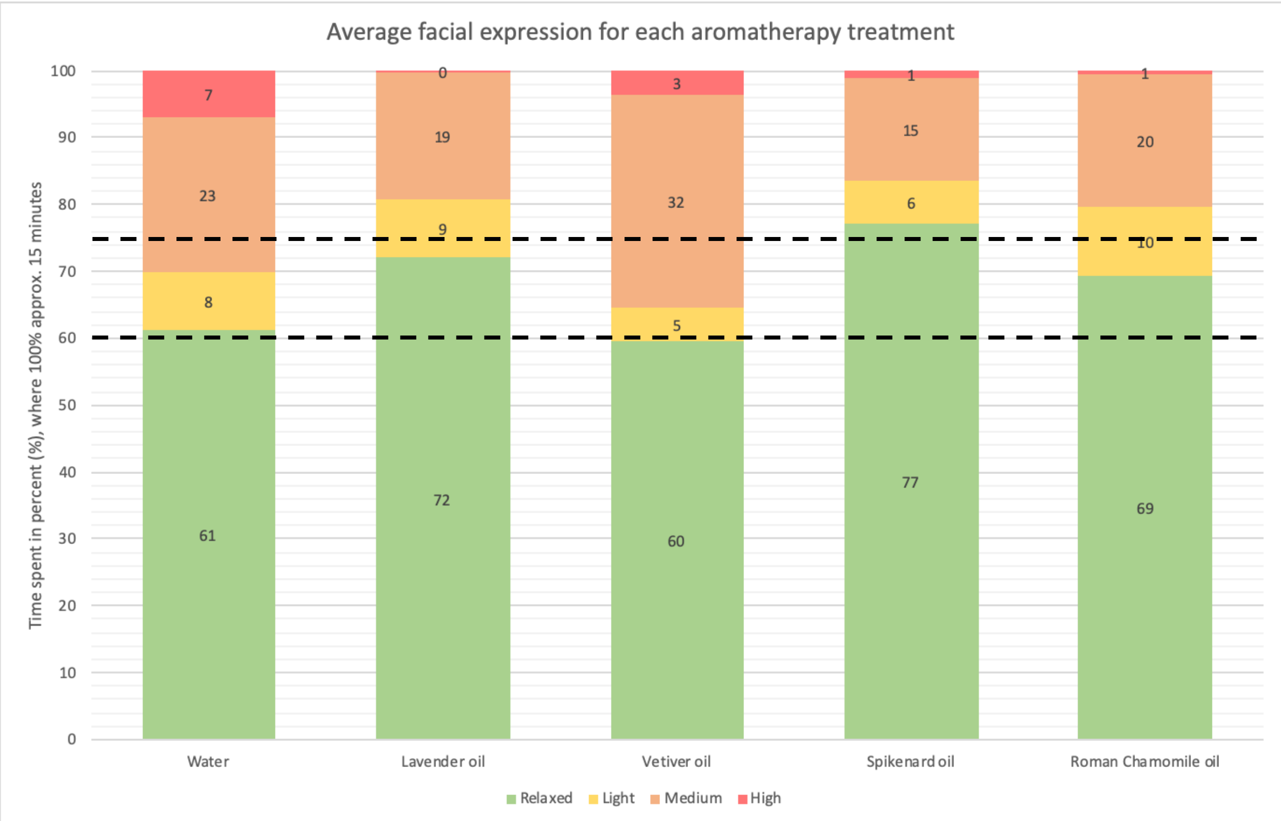

Figure 6. Average of observed facial expressions divided into aromatherapy treatments. Time spent in seconds is converted to percent cf. averages listed in Table 11. The facial expressions are divided into relaxed (green), light tension (yellow), medium tension (orange) and high tension (red) according to the criteria in Table 5.

All treatments gave the horses an average relaxed facial expression for 60% of the recorded time, but only spikenard made the horses relax for at least 75% of the time (Figure 6). With the exception of vetiver oil, all the oils managed to create longer periods of average facial relaxation compared to humified water. Aromatherapy with water triggered the most high-tension periods of all the treatments. Lavender aromatherapy triggered less than one percent of high-tension periods. Spikenard and roman chamomile resulted in high tension on average only one percent of the recorded time. Spikenard resulted in the highest percentage of relaxation by 77% and thus, had shorter periods of light and medium tension than lavender oil.

The treatments can be visually ranked according to the purpose of choice (Figure 7). Spikenard was the essential oil of the four selected, which facilitated most recorded time displaying relaxed facial expressions among the participating horses. At the opposite end of the scale, vetiver oil proved to be no better than water to inspire facial relaxation. When the goal is to reduce light tension, it was aromatherapy with vetiver or spikenard that gave rise to the least light tension periods, while roman chamomile triggered twice as much light tension as vetiver. On the other hand, it was also vetiver oil that definitely had the longest periods of medium tension which may be the reason why there was little time for light tension. Spikenard was again the essential oil that triggered the shortest time period with medium tension. Aromatherapy with water gave most cases with high tension, while lavender oil showed the fewest incidences. Looking in isolation at the treatments with essential oils, it was aromatherapy with vetiver oil that caused the longest periods of high tension. All in all, it means that spikenard was the essential oil in this experiment which facilitated the longest time of relaxation, the shortest time with light and medium tension, and was very close to also being the essential oil associated with the shortest time period with high tension. Based on the behavioural assessment, spikenard was the best essential oil to calm the participating horses.

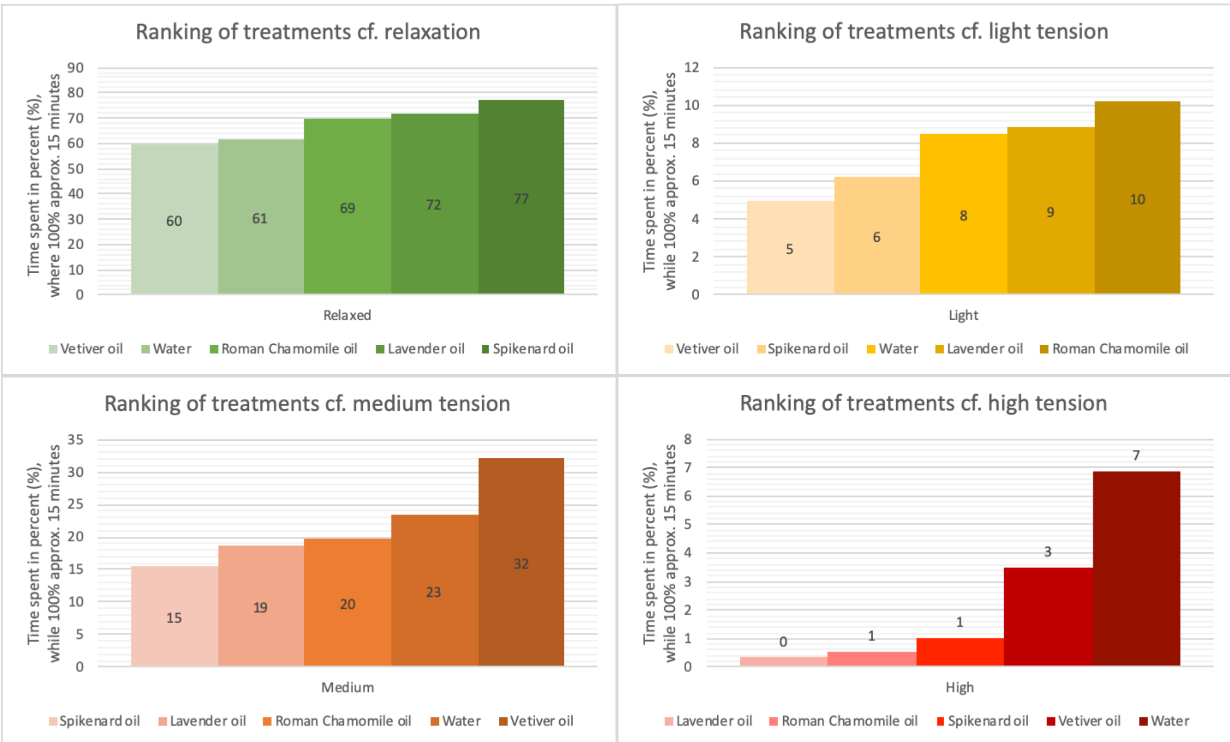

Figure 7. Ranking of treatments in relation to average facial expressions. From left to right, the treatments were ranked from lowest to highest in terms of relaxation (green tones), light tension (yellow tones), medium tension (orange tones) and high tension (red tones) in percentage.

4 Discussion

4.1 Evaluation of the results

It was not entirely clear what to expect from the results, as there was only very limited scientific literature on the subject, and it was necessary to make assumptions based on studies done on other animal species. To my knowledge, this was the only experiment that included both qualitative and quantitative data collection methods to investigate the effect of aromatherapy on horses, based on following null hypothesis: *"Aromatherapy would neither facilitates greater facial relaxation nor tension, lower nor increase the heart rate, change respiratory tidal volume nor cause a reduction in spontaneous muscle contractures in the horses tested. Moreover, it was anticipated that there would be no measurable difference between the controls and the essential oils treatments nor between the essential oils when applied separately"*.

The horses were filmed during the aromatherapy treatments. The facial expression and body language were subsequently assessed according to the definitions of body posture, calming signals, displacement signals and distance increasing signals by Draaisma (2018). The null hypothesis was rejected as spikenard facilitated close to 20% more time with a relaxed facial expression compared to humified water. Spikenard proved to be better than lavender oil in facilitating the longest time period of relaxation and the shortest time period with light tension and medium tension. Lavender was only slightly better than spikenard to avoid high tension, but within such small margins that it was reasonable to assume that spikenard was as good as lavender to avoid high tension. On the other hand, it was surprising that in terms of facilitating relaxation or avoiding medium tension, vetiver oil did worse than humified water. This could indicate that horses did not find the scent of vetiver oil calming in this unfamiliar situation of being asked to stand still with various equipment attached. Roman chamomile was just as good at avoiding high tension as lavender and spikenard, but performed slightly worse than lavender compared to the other three categories.

It is possible that the different reactions to the essential oils are due to stimulation of the relaxation response by different molecular mechanisms. Baldwin and Chea (2018) suggested that the effectiveness of the essential oil might change depending on the emotion state of the animal. The antianxiolytic effect of lavender is potentially mediated by stimulation of serotonin receptors (Chioca *et al.* 2013) while it is suggested that aristolen-1(10)-en-9-ol identified in spikenard results in a sedative effect, expressed *via* the GABAergic system (Takemoto *et al.* 2015). Roman chamomile has demonstrated to induce a vasorelaxant effect through the NO-cGMP pathway or possibly through a combination of Ca^{2+} channel inhibition plus NO-modulating and phosphodiesterase inhibitory mechanisms (Sándor *et al.* 2018).

Ladewig (2019) mentioned the importance of the handler's body language for the outcome of scientific studies. To take an example, it is proven that placebo can also be effective if the individual knows it is receiving an ineffectual treatment (Ladewig 2019). Placebo was initially defined as a so-called ineffective treatment, intended to deceive the recipient, but if the body language of the handler reveals the presence of a placebo treatment, it can potentially change the horse's response to the control treatment. This may be a possible reason why the control treatment with humified water performed relatively well and, in several cases, better than vetiver essential oil. The importance of the handler will be discussed further in the next section.

The procedure of this trial was to complete all aromatherapy treatments on one day per horse, separated by a minor break. The order of treatments had been randomized (Table 3) but there is always the risk of an effect being "carried over" from one experimental condition to another. Therefore, carry-over effect can affect outcomes and results of research, and are important to consider. Spikenard performed really well in facilitating a long period of facial relaxation, except in the case of Intakt. Prior to aromatherapy with spikenard, Intakt had been presented with vetiver oil. During the treatment with vetiver, Intakt was disturbed by large and noisy machines outside the stable. Because it was assessed that the noise potentially affected these measurements, the treatment with vetiver oil was repeated. Unfortunately, the machines continued during the second attempt. Vetiver oil was found to do poorly in facilitating facial relaxation, and along with the potential of a carry-over effect, the measurements of Intakt's reaction to spikenard may have been influenced by the elevated stress level of the previous treatment.

In the context of the physiological measurements, the null hypothesis must also be rejected. With ECG sensors, the heart rate of the horses was measured. All four essential oils facilitated a significantly lower heart rate, with lavender, vetiver and roman chamomile being considered to be very significant. On average, the heart rate was lowered by 5% compared to the baseline and humified water with the potential of vetiver oil actually being able to lower the heart rate slightly more than the other tested essential oils. With an elastic sensitivity belt, the respiratory tidal volume of the horses was measured. Lavender, vetiver and spikenard resulted in a significant 5% smaller expansion of the ribcage, i.e. the horses breathed shallower, which may be a sign of relaxation. Again, vetiver had the potential to decrease the respiratory tidal volume more than lavender and spikenard. The mean was higher for roman chamomile than it was for the baseline measurement and therefore, it was not possible in this trial to detect any significant differences towards increased relaxation for this essential oil. No significant differences were identified for the number of breaths because the initial t-tests revealed no differences among the essential oils. With AMG technique and sensors located on m. temporalis and m. cleidomastoideus, the number of spontaneous muscle contractures was measured. All the essential oils decreased the number of spontaneous muscle contractions, but the difference was only significant for spikenard (cf. 33% reduction) and roman chamomile (cf. 39% reduction) in m. temporalis, and significant for lavender (cf. 30% reduction) and roman chamomile (cf. 34% reduction) in m. cleidomastoidues. No significant differences were measured on the S- and T-scores for lavender, spikenard and chamomile at m. temporalis. However, spikenard had a significantly higher frequency than both the baseline and roman chamomile at m. cleidomastoideus while roman chamomile had a significantly lower amplitude than both lavender and spikenard. Thus, this study supported the other literature (Sándor *et al.* 2018) about roman chamomile having a remarkably smooth muscle relaxant effect and was better than the other essential oils for this purpose.

This study was limited by having only nine horses to conduct the trial, but still managed to document significant differences in several parameters. The results indicated that the selected essential oils all had in common the ability to lower the heart rate and that the same was true for the respiratory tidal volume, except that was not conclusive for roman chamomile. Therefore, the major differences between the essential oils were in terms of facial expression and spontaneous muscle contractions. Although vetiver was potentially better at lowering the heart rate and respiratory tidal volume, it did not perform very impressively in terms of muscle relaxation and poorly in providing a relaxed facial expression. It appeared that spikenard excelled in facilitating relaxed facial expression, while chamomile proved to be the best of the selected essential oils to soothe the muscles. Therefore, the purpose of the essential oil can be important for choosing the right one for a horse. For this study, it was decided to test the oils separately due to the very limited literature on the subject. It could be interesting to explore the possibilities of combining several essential oils with each other, whether it would provide a corresponding improvement of the relaxing effect according to the number of combined essential oils. A combination of essential oils would perhaps appeal to a wider group of horses, because there would be a greater chance that at least one of the scents would appeal to the horse, or it would repel more horses, because the combination could potentially end up smelling strange. This area certainly needs evidence.

4.2 Evaluation of the method

In line with nebulization being a non-invasive treatment, the measurement methods that were selected were similarly non-invasive. Analysis of the horses' facial expressions was supported by physiological measurements of heart rate, tidal volume and the frequency of spontaneous muscle contractures in m. temporalis and m. cleidomastoideus. Unlike similar scientific studies, this experiment was conducted with the intention of no imposed external stressors.

The fact that the participating horses had no or only limited previous experience with essential oils gave a true-to-life picture of domestic horses in Denmark. Thus, the results of this trial can be transferred directly to private horse stables. However, some bias was potentially caused due to the horses not being trained to stand completely still without expecting any action or entertainment. The horses were not used to one or more people just watching them without doing anything. It could potentially have stressed the horses and it can therefore be difficult to distinguish whether tension was due to the scent, to stand still, that people looked at them, that they were inside without the herd or whether they sought entertainment because they were bored. Should the experiment be repeated, it might be interesting to examine the effect of the essential oils when the horses were trained to stand still while tied up without the entertainment of an active handler, available roughage or anything else.

As for the measuring equipment, it could also have been an advantage if the horses were trained to wear it. Some of the horses were bothered by the stickers that held the sensors in place and the wires running from a sensor to the computer device. This was especially true for Cavalos, who regularly shook his skin as if he was trying to remove a fly. More effort could have been put into attaching the wires so that they did not hang freely around and from the neck of the horse, although the stickers were cut as small as possible to bother as little as possible. If the horses were accustomed to the equipment, it probably would have been less of a problem. The respiration belt was fixed as securely as possible in its location by attaching it to an elastic girth with Velcro straps, but it still moved down the abdomen as the horse breathed. When the respiration belt moved, it was no longer stretched and therefore, it could not measure the breathing of the horse because the elastic effect was reduced. Thus, the belt was regularly moved back into place between measurements. It was not moved while measuring so as not to interfere further with the measurements. This could potentially make the horse appear to have a shallower breathing than it actually had because the belt was not stretched as much as it was initially and therefore, the belt became less sensitive. It is recommended to investigate ways of making sure that the respiration belt remains at its chosen location. However, when reviewing and sampling the measurements from the respiration belt, it was considered not to have a significant impact on the results that the belt moved and needed to be adjusted on a regular basis. Yet, there was always the risk that the belt was not moved back to its original position or the need for readjustment was overlooked. There were some problems with the diffuser being turned on but not producing a visual mist of vapor. It was unknown whether the horses could smell the oil when these periods occurred. It was something that the handler became more aware of as the experiment progressed and it is therefore not known how pronounced this problem was for the first horses being measured. Thus, it is suggested to investigate possibilities and the effect of other ways to allow the horse to inhale the essential oils.

As discussed in the previous section, there was a risk of carry-over effect, mentally and physiologically, because the treatments were taken one by one with only a short break in between. It would probably have been more ideal to measure one treatment per day per horse. With a minimum of 24 hours break from the previous treatment, it must be assumed that at least the physiological effect of the essential oil from the previous treatment had been released from the body, i.e. it was not the previous treatment that caused the reaction in the horse during the next treatment. It was unclear whether this could change the mental carry-over effect because horses have proven several times that they have an excellent memory and recall ability (Murphy 2009) - though it was not prioritized in this experiment for it would have taken too long to complete these measurements for a master thesis.

As Forkman *et al.* (2007) rightly point out, animal testing almost always, if not always, involves the presence of humans to a greater or lesser extent. The type of handling and the human involved in the handling procedures is crucial to the interaction between the confidence level of the tested horse towards the handler and the responsiveness to the treatment itself (Forkman *et al.* 2007). Ladewig (2019) noted that it cannot be certain that the decision of a horse who have been handled by people right before a trial is not somehow influenced by the body language of the experimenters. While most research in equitation science has focussed on maximising performance using intentional signal through training, the effects of unintentional signals have been underestimated (Keeling *et al.* 2009; Ladewig 2019). These unconscious signals are especially important because a person may communicate for example her or his anxiety about a particular object or situation to the horse. Keeling *et al.* (2009) investigated the heart rates of horses and the people when leading or riding them four times past an assistant at a distance of 30 meters in an indoor arena. They found that the heart rate significantly increased for both the person and the horse when the participants were told that an umbrella would be opened as they rode or led the horse past the assistant. The umbrella was never opened, however; so there was no difference to the previous control occasions. Thus, the heart rate of the horse increased when the person 'thought' the horse might be frightened by the umbrella. Semin *et al.* (2019) discussed the possibility of emotion transfer from human to other species *via* chemosignals. The general argument about the function of body odours is that they constitute chemical signals that have evolved for species-specific communication. Chemoreception is the oldest sense that precedes the evolution of specialized sensory systems and is universal. Therefore, it is possible that chemosignals in general and emotion chemosignals, in particular, may not only be a species-specific medium for communication but also play a role as an interspecies signal. Synanthropic species, such as dogs and horses, seem to be particularly good at dealing with interspecies chemosignaling. This probably comes from a long domestication process in close proximity with humans where odours could be considered as a silent and invisible warning signal of the probable intention of behaviours of others and thereby, make it possible for them to regulate their social interaction (Semin *et al.* 2019). Lanata *et al.* (2018) examined the autonomic nervous system activity of horses in response to human body odours produced under happy and fearful states. Collected as heart rate variability of the tested horses, the results showed that human body odours induced sympathetic and parasympathetic changes and stimulated horses emotionally. This suggested that emotion transfer happened interspecies *via* body odours (Lanata *et al.* 2018). Although, it is still primarily on a conspiratorial level, according to Semin *et al.* (2019), it has been suggested that this synchronicity between humans and horses in for example heart rates may be due to emotion chemosignals from the human that unintentionally start a similar physical and emotional reaction in the horse.

For this experiment, there were two or three people to help holding the horses during the trials at Astenslund and Hyldegården, respectively. All these people were used to socialize with the horses in the respective stables but were not necessarily the owner of the horse in question. This aspect of emotion chemosignals may have been applicable to one or more of the tested horses. The Northlands horse, Ville, could be an example of this. He had been moved to Hyldegården only a few weeks before joining the experiment and was therefore not very familiar with other people in the stable besides the farm owner who was responsible for the everyday handling of the horses. The owner of Amigo and Zendi assisted in holding Ville during aromatherapy with spikenard. Although, he had a relaxed facial expression for the vast majority of the time, he was easily distracted and not interested in the diffuser. He instead spent a lot of time rubbing his hindquarter against the wall. He moved a lot and moved further away than when the farm owner held him. He first calmed down and stood still without scratching when the farm owner returned. His reaction could potentially be due to an unfamiliar person who was potentially uncomfortable about holding him which may have made it difficult for him to anticipate how to respond in the situation.

Dogs have been shown to discriminate between human facial expressions, and they seem to use human emotional communication to regulate their behaviour towards an external object or situation (Merola *et al.* 2013). Similarly, Smith *et al.* (2015) has proved that horses have the ability to spontaneously discriminate, both behaviourally and physiologically, between positive and negative human facial expressions. Each horse was shown two photographs - one happy and one angry - across two trials, while its behavioural and physiological (i.e. heart rate) responses were measured. Horses demonstrated right-hemispheric biases towards angry stimuli (preferentially viewing images with the left eye), which was positively correlated with both avoidance duration and a mean increase in heart rate (Smith *et al.* 2015). Interestingly enough, Proops and McComb (2012) has been investigating cross-modal discrimination of familiar human handlers from strangers. Cross-modal matching was once thought to be unique to humans (Sankey *et al.* 2011) but horses have been shown to have cross-modal perception of both conspecifics (Proops *et al.* 2009) and humans (Proops and McComb 2012) that involve auditory and visual sensory systems. Although the horses were free to look at the people with both eyes, the horses were overall significantly better at matching the familiar person with the sound of their voice when the correct person stood on their right side. When the horses heard the voices of their owners without the owner being present, the horses actually tended to look more to their left, suggesting marked hemispheric specialization (left hemisphere bias) in this ability, i.e. horses appear to be better at identifying people initially with their right eye (Proops and McComb 2012). Familiarity is found to be a significant factor in dogs' recognition of human expressions (Merola *et al.* 2013): Dogs find it easier to discriminate their owners compared to a stranger's emotional expressions which opens up the possibility that the same may be the case for horses. Smith *et al.* (2015) suggested their lack of tracking a response to the positive human expression could be due to recognition of a negative stimulus having a particular functional relevance for the horse in the form of avoiding negative consequences (e.g. rough treatment or punishment) or due to the unfamiliarity of the photographed humans, i.e. it could simply be more difficult for the horse to interpret the facial expression of strangers.

Putting everything together, it could be of great interest to investigate the effect of humans being present when the essential oils are presented to the horses. How important is it to the horse that the human has 'approved' the object or scent? Will the horse react differently to the same essential oil depending on whether it is presented by a familiar person or a stranger? Will the horse react differently with no humans around? Does the horse have a different reaction pattern if the oil was presented only with a picture of a familiar person? Would there be a difference in the horse's reaction whether it was alone or could examine the scent with one or more conspecifics, presuming that the horse was trained to be alone without anxiety? Ideally, the horse should be trained to be led into a reassuring, but confined space where the air was filled with the scent of a given essential oil without interference from humans or an object such as a diffuser. The importance of a familiar person for the horse's response to the scent could then be tested by hanging a picture of a person who usually handled the horse. In doing so, the interference from the chemosignals, emotion transfer or body odour of the human was avoided by making use of the fact that horses have a full range of stereoscopic skills (Murphy *et al.* 2009). The same measurement parameters could be used for this trial or, for example, an attempt could be made to use thermal imaging to test the body temperature of the horse. Increases in body surface temperature are related to increased blood flow associated with increased metabolic activity and altered local circulation while decreases in body surface temperature are a result of reduced tissue perfusion (Tunley and Henson 2004). Thereby, an alternative way to achieve a potential target for tense and relaxed muscles. These findings could both open the way to better understanding the behaviour of horses with and without interaction with humans in relation to the application possibilities of essential oils. They remain highly speculative at this stage and invite replication with an elaborate sample size.

4.3 Application possibilities and limitations

Uncooperative horses can both be a challenge and a threat to equine practitioners such as veterinarians and farriers. They are often the recipients of bites, strikes, kicks and other physical injuries from their patients. The horse's motivation for its misbehaviour is mostly fear (Houpt and Mills 2006) and fearful reactions in horses are also a major cause of accidents where the rider is injured (Keeling *et al.* 2009). Fear is a negative emotion and as such often included in assessments and recommendations of animal welfare (Forkman *et al.* 2007), e.g. 'freedom from fear' is included in the five freedoms that define ideal states of welfare (Christensen *et al.* 2008). High levels of fear can have negative consequences in performance, health, reproduction and as previously mentioned; welfare. Therefore, it is highly relevant to scientifically explore the possibilities for reducing fearfulness in horses.

Tryptophan supplements has been marketed worldwide as a calmative for 'excitable' horses due to tryptophan being an essential amino acid and the precursor of serotonin but few studies on tryptophan have actually been conducted in horses, and none of them has produced direct evidence of calmative efficacy in this species (Grimmett and Sillence 2004). Based on a facial expression assessment and several physical measurements in the present experiment, essential oils have been proven to calm horses. The advantage of essential oils is that they can be used non-invasively and without a prescription from a veterinarian. They are derived from plant parts, and with their natural origin, they usually come with minimal toxic side effects (Srivastava *et al.* 2010) and it must be relevant to assume that there is no risk of developing resistance as seen in the pharmaceutical industry. This form of treatment provides horse owners with a natural alternative to sedative medicine if the horse for example is afraid of standing in a horse trailer, of being examined by a veterinarian or of being shod by a farrier. Additionally, when the essential oil is tested and certified for therapeutic use (as for doTERRA Europe Ltd.), it can also be used internally and externally. As part of a well-balanced diet, essential oils have the potential to also help improve cardiovascular conditions, stimulate immune system, reduce pain, improve skin quality and hair growth, and support healthy digestion (Srivastaba *et al.* 2010; Lv *et al.* 2013; Seifi *et al.* 2018). Further evidence is needed to understand the full potential of combining feed with essential oils.

The disadvantage of essential oils is that it can be difficult to determine the most effective single oil or combination of oils because no connection has yet been found to why an essential oil seems more calming than another to a horse. As mentioned before, it is possible that the essential oils stimulate the relaxation response by different molecular mechanisms which can change the effectiveness of the essential oil depending on the emotional state of the animal (Baldwin and Chea 2018). Evidence is needed for how to predict the emotional state of a horse along with knowledge of which essential oils are most effective for different emotional states. It will require a better understanding of which components of the essential oils appear calming to horses. It might be easier to find the most effective essential oil if one addressed the underlying cause of excitability in horses, such as altering diets, management and husbandry procedures. Keeling *et al.* (2009) found that the heart rate of the horse increased when the person 'thought' the horse might be frightened by the opening of an umbrella. Keeling *et al.* (2009) did not investigate the effect of chemosignals or body odours, but Lanata *et al.* (2018) found a coactivation of sympathetic and parasympathetic subsystems when the human odour of fear was presented to horses. It opens the opportunity to address the human-horse interaction by addressing the human. By finding the essential oils that make the human calm in various stressful situations with horses, the chemosignals of the human will potentially induce the same state of mind in the horse and thereby, result in calming the horse.

There are many different manufacturers of essential oils, which can make it difficult to find a high-quality brand. The quality is very much related to the choice of plants, cultivation methods and post-harvest handling not to mention the purity testing and detail price. As with so many other products, quality and price are inextricably linked. A low price can lure consumers to buy the product with the risk of getting a product that is either diluted or manipulated to keep the price low. A beneficial effect of this type of essential oil cannot be guaranteed. That leads to the next major problem in the essential oil industry: There exists large amounts of unsavory sources that both claim miracles or warn against poisoning when using essential oils. The articles usually do not have any scientific basis for their statements. The articles are often published without an author being mentioned and without reference to a manufacturer, brand or even what amount of the essential oil should be used in a given situation. It can be difficult to separate evidence from empty claims and it requires tremendous source criticism to navigate through it.

5 Conclusion

Scientifically investigating the possibilities of reducing fearfulness in horses was highly relevant because fearful reactions in horses were a major cause of misbehaviour that results in injuries among all types of equine practitioners and freedom from fear was considered essential to the performance, health, reproduction and welfare of horses.

Based on both qualitative and quantitative methods in the present trial, it can be concluded that aromatherapy facilitated greater facial relaxation, significantly lower heart rate and respiratory tidal volume along with significantly fewer spontaneous muscle contractures in horses. The biggest differences between the selected essential oils in this experiment appeared to be in terms of facial expression and spontaneous muscle contractions. Spikenard oil was best at achieving the longest possible time with a relaxed facial expression and that for the largest percentage of the participating horses, i.e. 78% of the horses tested apparently agreed that spikenard inspired to show a relaxed body posture for 75% of the recorded time, while roman chamomile was better than the other tested essential oils to achieve muscle relaxation. Thus, spikenard and roman chamomile could potentially be better at calming horses than lavender oil, which was otherwise the best-documented essential oil within the highly limited literature on this research subject.

This experiment was conducted under some limitations in terms of available time, untrained horses and the number of participating horses, but the greatest uncertainty was how to determine the most effective calmative essential oil for the individual horse and in whether the handler affected the reaction of the horse. Literature suggested the risk of human emotion transfer *via* body odours and body language affecting the physical and emotional reactions of horses. Therefore, evidence is now needed to clarify how the most effective essential oil or combination of oils can be determined and how it is influenced by a handler. At present, the effect of combining several essential oils is not known, but this study has confirmed that essential oils are good for different purposes and it would therefore be obvious to continue investigating how combinations of essential oils calm horses.

6 References

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7 Appendices

A1: Composition of lavender oil

Composition of *Lavandula angustifolia* essential oil. Lot number: 180133. Date filled: 1/13/2018.

Compound name	%	Compound name	%
Linalool	31,74	Butyl butanoate	0,14
Linalyl acetate	28,48	Terpinolene	0,14
Lavandulyl acetate	6,09	trans-alpha-Bergamotene	0,13
Terpinen-4-ol	4,21	Hotrienol	0,11
cis-beta-Ocimene	4,02	delta-Amorphene	0,11
trans-beta-Farnesene	3,75	alpha-Thujene	0,1
beta-Caryophyllene	3,57	Camphene	0,1
trans-beta-Ocimene	2,13	para-Cymene	0,1
3-Octanone	1,39	trans-Linalool oxide	0,1
1-Octen-3-yl acetate	1,24	Hexyl tiglate	0,08
alpha-Terpineol	1,23	7-epi-Sesquithujene	0,08
Geranyl acetate	1,09	n-Hexanol	0,07
Lavandulol	1,07	Hexyl isobutanoate	0,07
1,8-Cineole	1,02	Bornyl acetate	0,07
Hexyl acetate	0,84		
Myrcene	0,73		
Borneol	0,72		
Hexyl butanoate	0,58		
Neryl acetate	0,56		
Limonene	0,5		
Germacrene D	0,44		
beta-Phellandrene	0,33		
3-Octanol	0,31		
1-Octen-3-ol	0,29		
Nerol	0,27		
cis-Linalool oxide (furanoid)	0,22		
Cryptone	0,22		
Caryophyllene oxide	0,2		
Camphor	0,19		
allo-Ocimene	0,18		
delta-3-Carene	0,17		
gamma-Terpinene	0,17		
3-Octyl acetate	0,17		
1-methoxy Hexane	0,16		
alpha-Pinene	0,16		
Hexyl hexanoate	0,16		

A2: Composition of vetiver oil

Composition of *Vetiveria zizanioides* essential oil. Lot number: 180367. Date filled: 2/5/2018.

Compound name	%	Compound name	%
trans-Isovalencenol	16,72	cis-Cadin-4-en-7-ol	0,53
Khusimol	13,86	Prezizaene	0,5
alpha-Vetivone	5,7	Vetiver Sesquiterpenoid 9	0,5
Vetiselinenol	5,19	Nootkatone isomer	0,47
Zizanol	4,88	Gurjunene isomer	0,41
beta-Vetivone	4,42	Khusimone	0,41
alpha-Cadinol	3,96	Hinesol	0,38
Vetiver Sesquiterpenoid 7	3,27	Amorphadienol isomer	0,37
Selina-6-en-4-ol isomer	3,25	Vetiver Sesquiterpenoid 1	0,35
Vetiver Sesquiterpenoid 2	2,68	dehydro-Isolongifolenene isomer	0,34
Vetiver Sesquiterpenoid 11	2,66	Vetiver Sesquiterpenoid 6	0,32
Eudesmol epi isomer 1	2,41	nor Eudesmenone	0,28
beta-Vetivenene	2,36	alpha-Cuprenene	0,24
Vetivenic acid	2,26		
14-hydroxy-9-epi-(trans)-Caryophyllene	1,63		
Vetiver Sesquiterpenoid 10	1,61		
Eudesmol epi isomer 2	1,58		
alpha-Amorphene	1,49		
Eudesm-7(11)-en-4-ol	1,35		
Vetiver Sesquiterpenoid 12	1,13		
Spiro isomer of vetiver	1,12		
beta-Vetispirene	1,09		
2-epi-Ziza-6(13)-en-3-alpha-ol	1,02		
Nootkatol isomer	1,01		
beta-Guaine	0,92		
Vetiver Sesquiterpenoid 4	0,86		
Vetiver Sesquiterpenoid 3	0,78		
Vetiver Sesquiterpenoid 5	0,78		
Rosifoliol isomer	0,71		
Vetiver Sesquiterpenoid 8	0,69		
delta-Amorphene	0,67		
cis-beta-Guaiene	0,64		
alpha-Elemol	0,57		
Khusimene	0,56		
gamma-Amorphene	0,55		
Zizanal	0,54		

A3: Composition of spikenard oil

Composition of *Nardostachys jatamansi* essential oil. Lot number: 182404. Date filled: 8/28/2018.

Compound name	%	Compound name	%
beta-Gurjunene	13,28	gamma-Patchoulene	0,48
Spirojatamol	10,57	Ledol	0,48
Valeranal	8,75	Spikenard Sesquiterpenoid 4	0,47
6,9-Guaiadiene	6,54	alpha-Bulnesene	0,46
Velerana-7,11-diene	6,34	beta-Selinene	0,45
Jatamansone	4,83	Spikenard Sesquiterpenoid 1	0,43
Nardol isomer A	4,54	Viridiflorol	0,43
Nardol isomer B	3,75	Sequiterpene alcohol	0,42
alpha-Gurjunene	3,3	Viridifloral	0,41
1(10)-Aristolen-9-beta-ol	3,18	beta-Patchoulene	0,39
Valencene	2,43	Spikenard Sesquiterpenoid 3	0,37
Patchouli alcohol	2,4	Spikenard Sesquiterpenoid 5	0,37
Valerinic acid	1,8	Bicyclogermacrene	0,33
trans-Muurola-4(14),5-diene	1,79	delta-Selinene	0,27
Cyclocolorenone	1,57		
7-epi-alpha-Selinene	1,54		
Aristolene	1,45		
Alloaromadendrene	1,39		
Spikenard Sesquiterpenoid 6	1,27		
beta-Vatirenene	1,26		
Ledol isomer	1,16		
Aristolone	0,98		
alpha-Cadinol	0,95		
delta-Cadinene	0,92		
Aromadendrene dehydro isomer	0,88		
alpha-Maaliene	0,88		
Nordosina-7,9,11-triene	0,84		
Sesquisabinene	0,84		
alpha-Selinene	0,78		
Spikenard Sesquiterpenoid 7	0,71		
Pacifigorgia-1(6),10-diene	0,63		
Gurjunene isomer	0,61		
beta-Elemene	0,55		
beta-Pinene	0,53		
Spikenard Sesquiterpenoid 2	0,52		
epi-Eudesmol isomer	0,5		

A4: Composition of roman chamomile oil

Composition of *Anthemis nobilis* essential oil. Lot number: 173354. Date filled: 12/1/2017.

Compound name	%	Compound name	%
3-Methylpentyl angelate	24,27	2-methyl Butanol	0,13
Methallyl angelate	15,11	Dimethyl Heptadienone	0,12
Hexyl isobutyrate	10,69	RC Sesquiterpenoid 3	0,11
Isoamyl angelate	8,96	1,8-Cineole	0,11
Isoamyl tiglate	6,87	Prenyl isobutyrate	0,11
trans-Pinocarveol	6,39	3-cis-Hexenol	0,1
Isobutyl angelate	3,7	RC Sesquiterpenoid 1	0,09
alpha-Pinene	3,1	trans, trans-alpha-Farnesene	0,09
Hexyl 2-methylacrylate	2,24	2-Methyl butyl acetate	0,08
2-Methylbutyl isobutyrate	1,98	2-methyl-2-butene Angelate	0,08
Pinocarvone	1,66	Thuja-2,4(10)-diene	0,07
Isoamyl isobutyrate	1,43	Hexyl cyclobutanecarboxylate	0,07
3-methylpentyl, 3-methyl butanoate	1,25	Methyl tiglate	0,06
Myrtenal	1,1	1-Octene	0,04
3-methyl-Pentanol	1,07		
Isopentul methacrylate	0,95		
Amyl angelate isomer	0,94		
Ethyl tiglate isomer	0,75		
3-methyl-Cyclohexanone	0,72		
RC Sesquiterpenoid 2	0,58		
Isobutyl isobutyrate	0,54		
Camphene	0,49		
Prenyl isomer	0,47		
Trimethyl nonane	0,43		
Propyl hexanoate	0,38		
beta-Pinene	0,34		
Isopentyl acetate	0,33		
Methacrylic acid, isobutyl ester	0,33		
Borneol	0,32		
Butyl angelate	0,28		
Hexyl 3-methyl hexyl ether	0,23		
Isopentyl 2-methyl butanoate	0,21		
1-Octen-3-ol butanoate	0,18		
2-methyl-1-Butyl tiglate	0,16		
Isopentyl alcohol	0,14		
2-Methyl butyl-2-methyl butyrate	0,14		