



Performance and stress levels of horses in a L1 dressage competition compared for a crossunder bitless bridle and a bridle containing a snaffle bit

Bachelor Thesis



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dressage competition compared for a crossunder bitless
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Summary

Since 2010 the KNHS, the Dutch equestrian association, has allowed horse-rider combinations to use a bitless bridle during official dressage tests. The crossunder bitless bridle is one of the most commonly used bridles during these tests. Research has shown that horses wearing a crossunder bridle exhibit lower heart rates compared to horses wearing a bitted bridle but conflicting results have been found concerning heart rate variability and the frequency of stress related behaviours. No performance related comparison between the crossunder bridle and bit has yet been conducted in the context of a dressage competition. Therefore the aim of this research was to compare performance and stress levels of horses with and without bit. Seven horses, Dutch Warmblood and Andalusian, performed a dressage test at the Dutch L1 level. The test was performed using a crossover design. Every horse performed once with its own bridle containing a snaffle (test 1) and once with a crossunder bridle the horses it been trained with 3 to 5 times before (test 2). Performance was judged by two official judges. A heart rate monitor was installed during the test and salivary cortisol concentration was measured with the use of Salivette rolls. A significant lower cortisol concentration of 6.6 nmol/L (± 1.845 nmol/L) was found for the crossunder bitless bridle compared to the snaffle with 8.41 nmol/L (± 3.017 nmol/L, $P=0.044$). No significant differences were found for mean heart rate ($P=0.744$), heart rate variability ($P=0.128$) and dressage test performance ($P=0.673$). Mouth opening was exhibited significantly more by the horses when wearing a snaffle ($P=0.020$). Independent of the rider's preference for the bridle with snaffle bit or the crossunder bitless bridle, horses were least stressed when riding with the crossunder bridle. It is recommended to riders to take a bitless bridle into consideration as it improves horse welfare and does not interfere with performance.

Samenvatting

Sinds 2010 staat de KNHS (Koninklijke Nederlandse Hippische Sportfederatie) ruiters toe om met een bitloos hoofdstel te starten op dressuurwedstrijden. Een van de bitloze hoofdstellen waar veelvuldig gebruik van wordt gemaakt is het kaakgekruiste hoofdstel. Uit onderzoek is gebleken dat paarden die gereden worden met een kaakgekruist hoofdstel een lagere hartslag hebben dan paarden die met bit worden gereden. Het is echter nog niet duidelijk of er ook een verschil is in de hartslagvariatie en ander stress gerelateerd gedrag. Het doel van dit onderzoek was daarom om prestatie en stress tijdens een dressuurwedstrijd te vergelijken voor paarden met en zonder bit. Zeven paarden, van de rassen KWPN en Andalusiër, namen deel aan het onderzoek. Alle paarden liepen een dressuurproef op L1 niveau. Een crossover studie werd gedaan waarbij elk paard de test één keer met het eigen hoofdstel met een trensbit reed (test 1) en één keer met een kaakgekruist hoofdstel waar van te voren 3 tot 5 keer mee getraind was (test 2). Twee officiële juryleden waren aanwezig om de proeven te beoordelen. Gedurende de proef droeg het paard een hartslagmeter en direct na de test werd een speekselmonster genomen om de concentratie van het stresshormoon cortisol te meten. Een significant verschil in cortisol concentratie werd gevonden met een concentratie van 6.6 nmol/L (± 1.845 nmol/L) voor de proeven die met het kaakgekruiste hoofdstel werden gereden en een concentratie van 8.41 nmol/L (± 3.017 nmol/L, $P=0.044$) voor de proeven met trensbit. Er was geen verschil tussen beide proeven in gemiddelde hartslag ($P=0.744$), variatie in hartslagfrequentie ($P=0.128$) en de behaalde score ($P=0.673$). Paarden die gereden werden met trensbit, sperden hun mond vaker open dan paarden gereden met kaakgekruist hoofdstel ($P=0.020$). Ook bij paarden van ruiters die de voorkeur gaven aan het rijden met trens werd een minder stress gemeten wanneer ze met kaakgekruist hoofdstel gereden werden. Het is aan te bevelen om ruiters een bitloos hoofdstel te laten proberen omdat dit paardenwelzijn verbetert en niet van invloed is op de behaalde score tijdens een dressuurproef.

1. Introduction

This thesis aims to provide more scientific evidence in a subject that has been of increasing interest during the past years: bitless riding. Using a bit to control the horse is a common method employed by practically all people engaged in equestrian activities. However this method has been questioned increasingly in the past years and many riders have begun to believe riding with a bitless bridle is a less stressful method for the horse.

In the past bitless riding was mainly recommended when a horse had mouth problems. However, during the last decade bitless riding has been introduced and accepted by many Dutch riders from another point of view: the bitless bridle is an equivalent method that may improve horse welfare and horse-rider communication (Redactie Horses, 2009a; PaardenSport, 2007). This is mainly due to the work of Dr Cook, a veterinarian and researcher specialized in diseases of the horse's mouth, ears, nose and throat. Dr Cook realized that the mouth is one of the most sensitive anatomical parts of the horse and putting a bit in it might actually be contradictory and stressful for a flight animal. As a consequence he developed a bitless bridle, allowing the rider to steer using pressure on the jaws and nose of the horse to guide it in the right direction and control its speed.

The introduction of this bridle, called the crossunder bitless bridle, caused a lot of discussion and the first riders who tried a bitless bridle were seen as real daredevils. Many people believed that without a bit in its mouth, a horse would be unstoppable and the rider no more than a passenger on its back. People were astonished when they found out a horse without bit was still controllable and could even be steered around quite nicely (van den Enk and Tulp, 2009; Calkoen, 2007; MenSport, 2007). Bitless riding during hacks and dressage training began to gain popularity and more types of bitless bridles were introduced to the market (PaardenSport, 2007).

As a result of many riders' requests to allow bitless bridles in dressage competitions, a unique event was about to happen on the 10th of July 2009. At the grounds of the KNHS (Koninklijke Nederlandse Hippische Sportfederatie, the Dutch national equestrian association) in Ermelo, the Netherlands, a bitless test competition was organized to see whether performing a dressage test in a bitless bridle is comparable to riding with a bit. Internationally, the bitless test competition of the KNHS was the first official event known to allow riders to compete in dressage using a bitless bridle in place of a bridle with a bit. It was concluded that dressage with a bitless bridle is safe and that most bridles do not reduce welfare (KNHS, 2009).

Nevertheless it there were doubts if a bitless bridle would be sufficient to use during dressage tests. Dressage is about harmony between horse and rider and an important factor for this is the horse being 'on the bit'. To allow horses with a bitless bridle in dressage competition, this definition had to be restated into 'the light contact on the reins and the head-neck position of the horse' (KNHS, 2014a).

According to the Dutch instructor and judge Johan Hamminga a horse accepting the bit and gently chewing on it is a very different situation than riding without the presence of a bit in the mouth. He sees the comparison of bitted and bitless bridles as a comparison of apples and oranges (Redactie de Paardenkrant, 2013). Hamminga was one of the attending judges during the bitless test competition of the KNHS. During the event he saw a lot of horse and rider pairs performing with a nice head-neck posture, but without a good balance. Hamminga believes the pressure of a bitless bridle on the nose does not invite the horse to really walk in a collected way like a bit can do (Redactie Horses, 2009b).

Next to this some riders have reported problems attaining the same level of bending and bringing the horse to their hand when riding bitless (Redactie Horses, 2009a).

Another main argument used in the bit vs. bitless discussion is that the type of bridle used is not that important, because it is the rider's hands that decide whether the bridle is a friendly option. A rider can do the horse damage when riding with a rough hand on a bitless bridle as well. Still a bridle with bit and a bitless bridle are two different devices, with one mainly using pressure via a metal object in the mouth and the other using pressure on the nose and jaws. According to Madeleine Calkoen, president of the Dutch association for bitless riding (Nederlandse Vereniging Bitloos Paardrijden), there are indeed differences when riding with a bitless bridle. It can help the horse to achieve smoother gaits and a better collection and the effect of seat aids is stronger (PaardenSport, 2007).

The main goal of a dressage rider should be to work together with the a horse that is a happy athlete. Secondly it is also important to have a good performance at competitions. A bridle should be able to support these goals. Therefor the objective of this research is to compare a bridle with a snaffle bit and the crossunder bitless bridle, two types of bridles that are often used by dressage riders. Firstly it aims to discover if stress levels of a horse wearing a crossunder bitless bridle are lower compared to stress levels of a horse wearing a bridle with snaffle bit. Secondly it will investigate if there are differences in the scores horse and rider receive when performing a dressage test with these bridles. Here it is also important to investigate the scores attained for straightness, relaxation and the horse searching for the rider's hands.

2. Literature review

2.1 Effects of different bits and bridles

Features of the snaffle

The snaffle bit with jointed mouthpiece is frequently used for dressage riding. It has a direct action without leverage and thus the rein pressure executed by the rider is equal to the pressure on the bit (Bennett, 2001). A single jointed mouthpiece consists of two halves or cannons connected by the joint. A double jointed mouthpiece has an intermediate piece in between the cannons and two joints. The ends of the cannons are connected to a bit ring. Common types of bit rings are the loose-ring snaffle (O-shaped) and the eggbutt snaffle (oval shaped, Figure 1).

Figure 1 From left to right: single jointed eggbutt snaffle, single jointed loose-ring snaffle and double jointed loose-ring snaffle (Agradi, 2014).



Clayton et al. (1984) made use of fluoroscopic images to get a picture of what happens in the horse's mouth when applying pressure on a snaffle bit. They demonstrated that a unilateral force applied on one of the reins attached to a snaffle bit causes an inward movement of the other bit ring as well and is unable to cause an independent effect on one side of the mouth. When a bilateral force is applied, the snaffle moves caudally to the tongue. The tongue may then be pressed between bit and bone and as a consequence of this some horses pull their tongue backwards. Therefore Manfredi et al. (2010) suggest a double jointed snaffle may be preferred as it accommodates the shape of the tongue better. Next to pressure on the tongue, McGreevy and McLean (2010) also identify the bars of the mouth and the lips as sites influenced by snaffle bit pressure.

Figure 2 Fluoroscopic images by Clayton et al. (1984) of the position of the snaffle bit in the horse's mouth. Left picture shows a correctly fitted and adjust snaffle bit. Right picture shows that equal force bilateral force deepens indentation of the tongue.



Figure 6. Fluoroscopic photograph of a correctly fitted and adjusted snaffle bit showing the anatomical landmarks.

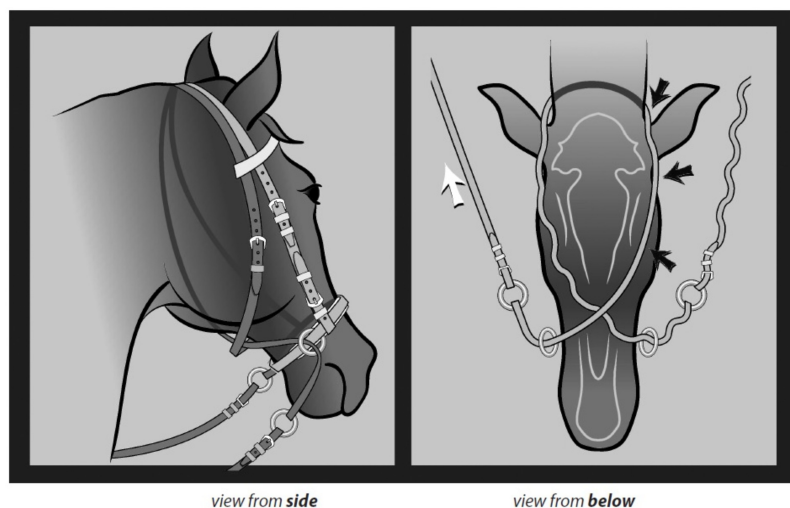
M. edge of mandible	LI. lower corner incisor
P. edge of hard palate	UC. upper canine
CT. cheek teeth	LC. lower canine
UI. upper corner incisor	J. joint of bit
	R. bit ring

Clayton et al. (1984) and Uhlig (2009) have paid attention as to whether the so called 'nut cracker' effect exists. This would happen when an equal bilateral force is applied on the snaffle bit. As a result the mouthpieces are pinching the tongue and bars. Both Clayton et al. (1984) and Uhlig (2009) observed a deepening in the indentation of the tongue, but did not find the bit to contact the hard palate when rein tension was applied (Figure 2).

The crossunder bridle

The crossunder bitless bridle has been developed by a researcher on the mouth, nose, ears and throat of the horse, as a solution to problems which may be connected to the bit (Cook, 2000). The reins of the crossunder bridle are connected to straps. The straps run through a ring on the noseband, cross under the jaw and are connected to the headpiece at the top of the bridle, the place where the throat latch would normally be attached (Figure 3). The noseband is plain and not strengthened with any material that may intensify the pressure on the nose. Sites of the head where pressure is exerted when the reins are used include the ventral mandible, the nose and the poll, and to a lesser extent the cheeks (McGreevy and McLean, 2010). Pulling on one rein will give diffuse pressure on the opposite side of the head, providing a directional aid to the horse. Bilateral rein pressure results in the head being squeezed. This makes the crossunder bridle feel more like a halter for the horse, while the rider may have the feeling of riding with a normal bridle and bit (Cook, 2000).

Figure 3 The crossunder bitless bridle as seen from the side (left picture) and below (right picture) (The Bitless Bridle, 2008).



In his comparison of the crossunder bridle with other bitless bridles Cook (2000) explains why he prefers the crossunder bridle above other types of bitless bridles. Bridles like the sidepull and the hackamore work primarily by pressure on the nose. The crossunder bridle exerts pressure on less sensitive parts of the head as well and this may be more convenient to the horse. In addition, Cook (2008) explains that the design of the crossunder bridle slightly decreases rein pressure because the jaw straps are changing direction due to the metal rings applied to the noseband. Overall the intention of the crossunder bridle design is that the application of rein aids does not depend on infliction of pain. As a result the horse may not express aversive behaviours that are observed to occur when a bit is worn (Manfredi et al., 2010; Heleski et al., 2009; Ashley et al., 2005; Clayton et al., 1984).

2.2 Application of rein aids

McGreevy et al. (2011) point out that both bitted and bitless bridles rely on the use of negative reinforcement. Negative reinforcement is defined by Chance (1993) the subtraction of a stimulus after the desired behavioural change has taken place. According to McLean (2012) dressage is a process of negatively reinforcing correct responses. The bit and bridle are one of the devices the rider can use to apply negative reinforcement by relieving rein pressure when the horse gives a correct response. The goal is to diminish the rider's intervention to avoid a stress response of the horse (McGreevy and McLean, 2010). A horse's performance will improve when it is comfortable with the amount of tension the rider applies on the reins to get the desired response. In addition, this it needs to be comfortable with the mechanical effects of this tension on the bridle and bit (Manfredi et al., 2010).

A decreased responsiveness to rein aids can result when learning theory is not applied correctly by the rider. McGreevy and McLean (2005) see constant maintenance of rein pressure as the most common cause of conflict. The horse gets used to this pressure and learns to ignore it, and thus it will appear to the rider as if the horse has a hard mouth. As a solution the rider will often try to increase the pressure used or use a bit that is more severe (Warren-Smith et al., 2007).

Forces on different bridle types

The amount of pressure applied on the horse by a piece of gear depends on how much surface it is covering. McGreevy et al. (2011) explains that when this area of contact is broader, it can cause less inconvenience and thus its removal will also be less rewarding for the horse. Pesie (2010) did a calculation on the contacting surface of several bits and bridles on a model horse head. When applying a force of 50N on the model head, the amount of surface that applies pressure on the head is larger for the crossunder bitless bridle (97.5 cm²) compared to a bridle with snaffle bit (26.4 cm²). This would indicate that if a rider would apply an equal amount of tension independent of the type of bridle, a crossunder bitless bridle will cause less inconvenience. In reality, the contacting surface for a bridle containing a snaffle will be somewhat larger as Geyer and Weishaupt (2006) mention that both bitted and bitless bridles can affect facial nerves and other sensible areas of the head.

Randle and Wright (2013) did a study on the amount of rein tension applied on a crossunder bridle by riders that were only familiar to bitted bridles. The baseline tension riders applied was significantly lower for the crossunder bitless bridle compared to a jointed eggbutt snaffle. Also the tension applied when going from a walk to halt was significantly less for the crossunder bridle compared to the jointed eggbutt. Warren-Smith et al. (2007) compared rein tension between a rope halter and a bridle with a single jointed eggbutt snaffle without noseband. They did not find a difference in the mean tension applied by riders. Their study indicates a negative correlation between the rider's experience and the amount of rein tension applied.

Uhlig (2009) discovered that rein tensions of 36.8 N and higher on a snaffle bit will compress the tongue between the bit and the lower jaw and between the teeth and the premolar teeth. However the studies by Randle and Wright (2013) and Warren-Smith et al. (2007) who studied rein tensions during normal riding activities in walk and trot only found rein tensions of below 36.8 N.

2.3 Riding issues associated with bit and bridle

When comparing skeletons of domestic horses and feral horses, van Lancker et al. (2007) found that the interdental spaces of domestic horses are more irregular. They conclude that this may be caused by repeatedly induced trauma of the bit on the bars. Tell et al. (2008) found a higher occurrence of buccal ulcers in horses that were currently ridden with bit and bridle compared to horses that were not. They attribute this to poor bit fit, as well as dental procedures that are not adapted to the individual. Engelke and Gasse (2003) found asymmetrical distances between horses' left and right jaws which indicates that every horse has a different mouth shape. Nosebands can also be a source of riding issues. The noseband may cause physiological stress responses (McGreevy et al., 2012) and restrict the horse from chewing on the bit (Kienapfel and Preuschoft, 2010).

Cook (2003) and Hockenhull and Creighton (2012) indicate a difference exists between the kind of behaviour a horse shows when ridden with a bitted bridle as compared to a bitless bridle. In a survey by Cook (2003) among 605 horse owners who switched their horse from a bridle containing a bit (varying from snaffles to leverage bits) to a crossunder bitless bridle, 97% reported positive results. The main behaviours that were eliminated when riding bitless were expression of fear, difficulties slowing or stopping the horse, argumentative or aggressive behaviour, head shaking, teeth grinding, difficulties in bridling the horse, being above the bit and a lack of self-carriage. In total Cook identified 58 behaviours as being adverse effects of wearing a bit. Hockenhull and Creighton (2012) carried out a survey among 791 leisure horses from the UK to evaluate the frequency of 15 behavioural problems in

relation to the type of bridle. Horses wearing a hackamore or another type of bitless bridle were found to show a reduced likelihood of resistance or slowing down problems and discomfort compared to horses wearing a snaffle. Hockenhull and Creighton (2012) did not make a distinction between bitless bridles working with and without a leverage function.

2.4 Stress in horses

A stressor is defined as an external stimulus or environmental condition to which the body gives physical or mental stress responses (Stephens, 1980). These responses are the adaptations the body needs to make in its homeostasis to function well under new conditions. When exercising, a certain amount of physiological stress is a necessary and the body's adaptations are quite similar to those made during the flight and fight response. The intensity of the flight and fight response depends on how used to the amount of exercise the animal is. In relation to animal welfare, Marlin and Nankervis (2002) consider stress being extreme adjustments a horse needs to make in order to cope with adverse effects due to its environment or management.

Competition and moreover the transport towards the competition site can be a potential stressor for the horse (Becker-Birck et al., 2013a) and may affect health, welfare and performance (Peeters et al., 2013). Peeters et al. (2013) hypothesizes that the stress/performance relationship follows an inverted U-shaped curve. A horse may sometimes be on the left side of this curve (having more stress leading to enhanced performance) or on the right side (having more stress leading to reduced performance).

Cortisol

Cortisol is one of the major stress hormones and its concentration increases 2 to 3 times during exercise (Marlin and Nankervis, 2002). Increasing cortisol levels have been found in response to stressors such as competition (Cayado et al., 2006), foundation training to prepare a horse for riding (Schmidt et al., 2010a) and transport (Schmidt et al., 2010b). Becker-Birck et al. (2013) did not find a difference in cortisol level for horses lunged in a hyperflexing and a normal head and neck position.

Blood serum analysis is a common method of measuring cortisol concentration. However, Peeters et al. (2011) and Hughes et al. (2006) validate saliva sampling as a less stressful method with a strong correlation between serum and saliva cortisol concentrations. Cortisol concentration and the period during which differences in baseline values can be detected, will vary depending on stressor intensity and duration (Strzelec et al., 2011; Jimenez et al., 1998). Peeters et al. (2011) stimulated horses with adrenocorticotrophic hormone and concluded that it took 30 minutes for salivary cortisol concentration to become significantly higher than baseline values and 180 minutes to fall back again. Peak times of 10 minutes for light exercise (Hughes et al., 2010), 20 minutes for a jumping course (Peeters et al., 2013) and 30 minutes for riding school horses being exercised (Hughes et al., 2006) have been reported.

Most research on cortisol in equines points at the existence of a circadian rhythm (e.g. Becker-Birck et al., 2013b; Gianetto et al., 2012) with salivary cortisol concentrations being highest from 9 to 11 AM (Bohák et al., 2013) and declining towards the evening (Bohák et al., 2013; Erber et al., 2013). Strzelec et al. (2011) did not find any evidence for circadian rhythm in recreational horses and their control group but did find higher morning values for jumping, dressage and eventing horses.

Peeters et al. (2013) found a positive correlation between competition performance and salivary cortisol

with horses getting more penalties in a jumping course exhibiting a lower cortisol concentration. Research by Cayado et al. (2006) revealed that in contrast to jumping horses, plasma cortisol concentration of dressage horses is already different to baseline value when reaching the arena prior to competition. Cortisol concentration rose to a peak until the end of the test for all levels of dressage horses that participated (moderate, intermediate and most experienced).

Heart rate and heart rate variability

Heart rate (HR) has been found to have a positive correlation to stressors such as being caught (Jeziński et al., 1999), being handled without prior training (Visser et al., 2002) and transport (Schmidt et al., 2010b). Munsters et al. (2012) let horses approach a challenging object and found a lower heart rate when a horse was ridden by a rider that had a good match with the horse compared to a horse that was ridden by a rider that did not have a good match to the horse. Warren-Smith et al. (2007) did not find a correlation between long reining activities and an elevated heart rate frequency.

Heart rate is under control of the autonomic nervous system, with the parasympathetic (vagal) nervous system operating to relax the body and the sympathetic nervous system oppositely reacting to stress situations (Ernst, 2014). An increase in heart rate may be caused by reduced vagal activity, as the vagus nerve stimulates the parasympathetic nervous system, but also by increased sympathetic activity or even simultaneous activity within both systems. Therefore heart rate variability (HRV), the variation in beat-to-beat interval (R-R or NN) is found to be a better indicator of stress than heart rate (Von Borell et al., 2007). This is, for example, shown in research by Mohr et al. (2000) who found a significant difference in HRV but not in HR for horses mentally stressed by a removal compared to a group of control horses.

The heart of an individual with a high HRV seems to have a better ability to adapt to unpredictable stimuli (Acharya et al., 2006). HRV tends to lower, thus being more invariable, when a horse is fearful (McGreevy and McLean, 2010). However this seems to be more complicated as Mohr, Witte and Vos (2000) saw a decrease in SDANN (Standard Deviation of the averages of R-R intervals in all -minute segments of the entire recording) for physical stress while mental stress increased SDANN. Erber et al. (2013) discovered a decreased SDANN during riding for mares stabled individually compared to mares in a group housing system.

Stress related behaviours

Young et al. (2012) found a positive correlation between stress and a raised tail, flared nostrils, repeatedly looking around, pawing at the ground with front legs, stopping eating, approaching and retreating a potential stressor, stereotypic behaviour, snorting and stamping of the hind feet. Rietmann et al. (2004) found head high, explosive behaviour, deviations in speed of pace, stopping, tail swishing and defecation as behaviours indicating stress in warmblood horses during a groundwork task.

In studies concerning ridden horses gaping or chomping at the bit, head lifting, unusually high or low head carriage, head and neck movements, vocalizations, extreme ear positions, mouth opening, general signs of muscle tension, tail position and movement, bucking, rearing and shying are behaviours that have been related to discomfort (Heleski et al., 2009; Hall et al., 2013). Williams and Warren-Smith (2010) observed a competition for dressage horses and concluded that conflict responses were seen in all classes from Preliminary to Grand Prix. Being above the bit and shorting and stiffening of the stride were the responses typically shown by horses competing in the lower levels. Overall tail swishing, ears back, being above the bit and tenseness were observed most frequently.

Horses can show hyper-reactive responses like extreme flexion and tension of the neck towards confusing or strong aids by the riders' hands (McGreevy and McLean, 2005). This can result in a loss of control by the rider. McGreevy and McLean (2005) mention that high rein pressure can result in irregular rhythms and a crooked longitudinal axis.

Manfredi et al. (2010) made use of a fluoroscopic study to view oral behaviours that are not directly visible from the outside. Behaviours that were frequently displayed when rein tension was applied were caudal retraction of the tongue, bulging the tongue between the palate and the bit and lifting the bit between the premolars. Mouthing the bit also increased with the application of rein tension, but this is regarded to be a more positive behaviour indicating acceptance. Other behaviours that were seen less frequently were moving the chin towards the chest, head shaking, flapping of the lips and flicking the tongue out of the mouth. No relationship was found between degree and type of oral behaviour and type of snaffle and behavioural patterns seem to be an individual response.

2.5 Relationship between bridle type, performance and stress levels in dressage tests

A few studies have been done on the performance of horses in a dressage test, comparing the usual bridle to the crossunder bridle. Cook and Mills (2009) found horses performed better and noted an improved horse-rider communication with the crossunder bridle. Scofield and Randle (2013) did not find a significant difference in the behaviours of tail swishing, open mouth, cow kicking, hollowing of the back and pulling down. Warren-Smith and Quick (2009) compared behaviours during foundation training (consisting of getting the horse used to a bridle, long reining and mounting preparation) and found higher frequencies of chewing, mouth opening, pawing and tail swishing in horses wearing a bridle with bit compared to horses wearing a crossunder bridle. Additionally a lower heart rate and heart rate variability was seen when long reining the bitless horses (Warren-Smith and Quick, 2009). Fejsáková et al. (2013) found a lower heart rate, a higher R-R interval and a lower, but not significant, cortisol concentration for bitless bridles compared to other bridle types.

Not only the type of bridle used, but also bit type seems to have an impact on stress. Vanderhorst et al. (2013) found horses wearing a Myler bit (allowing more freedom for the tongue) having a higher R-R interval compared to horses wearing a snaffle bit during a dressage test. Frequency of vertical head-neck fluctuations reduced significantly with the Myler bit and no difference in frequency of mouth opening, off the bit positions and performance was found.

3. Materials and methods

This study aims to draw a comparison between the bridle with snaffle bit and the crossunder bitless bridle in two ways, concerning the horse's stress levels and its performance.

The first main question is: 'Does a horse ridden in a crossunder bitless bridle experience less stress during a competition compared to a horse ridden in a bridle with a snaffle bit?' This has been analysed especially by measuring salivary cortisol concentration. Next to this data concerning heart rate frequency and heart rate were used as well as the frequency of three behaviours (tail swishing, mouth opening and head tossing) that have a relation to stress experienced by the horse when riding (Hall et al., 2013; Williams and Warren-Smith, 2010; Heleski et al, 2009).

The second main question is: 'Does a horse ridden in a crossunder bitless bridle have a higher score in a dressage test compared to a horse ridden in a bridle with a snaffle bit?' To be able to compare performance with both bridles well, the experiment was conducted in the context of a dressage competition at L1 (light) level with horse and rider consequently having experience riding dressage tests.

3.1 Animals

Nine competition horses were available for the experiment. The horses were selected for having a competition level in dressage of at least L1 (novice level). All horses were ridden by their regular rider, either the owner or leaser of the horse. Before the test day two horses (2 and 8, Table 1) dropped out due to health issues. Therefore these horses were not included in any of the analyses.

The remaining group consisted of four Dutch Warmblood horses and three Andalusian horses, all from the same stable. The horses were ranging in age from 6 to 19 (mean 10.7 years, ± 5.09 years). They were fed two meals of concentrates and two meals of roughage a day and were turned out 4 to 8 hours a day. All horses were ridden 5 to 6 times per week. A detailed overview of each horse can be found in Table 1.

Table 1 Horses stabled at the riding school the study took place

Horse number	Name	Gender	Breed	Age	Competition level*	Bit type
1	Zidane	Gelding	Dutch Warmblood	10	L1	Single jointed eggbutt snaffle
2	Excluded					
3	White	Mare	Dutch Warmblood	11	Z1	Single jointed eggbutt snaffle
4	Oficial	Gelding	Andalusian	6	L1	Double jointed loose ring snaffle
5	Calet��o	Gelding	Andalusian	6	L1	Single jointed loose ring snaffle
6	Modoso	Gelding	Andalusian	7	L1	Single jointed eggbutt snaffle
7	Maybe	Mare	Dutch Warmblood	19	M1	Single jointed loose ring snaffle
8	Excluded					
9	Ramaika	Mare	Dutch Warmblood	16	M2	Double jointed loose ring snaffle

* The foundation levels of Dutch dressage competition start with B (beginner) and are followed by L1 and L2 (light), M1 and M2 (medium), Z1 and Z2 (heavy) and ZZL (very heavy, light).

3.2 Experimental design

Twenty two days prior to the test day all horses received a fitting crossunder bitless bridle. Originally this day was also used for data collection on the bridle with snaffle. However, these measurements took place in another riding arena than the other tests and this was found to have a significant effect on the data. Thus it was decided to not to use these data.

Due to the small sample size, only one test group was defined (all animals received a crossunder bridle) instead of one test group wearing a crossunder bridle and one test group only wearing their usual bridle.. A set of 3-5 training sessions was determined to be the minimum number of times to ensure horse and rider are used to the crossunder bridle (M. Calkoen, pers. communication, 2014). These training sessions took place during the twenty two days prior to the dressage tests. Except for the bridle switch, the horse was trained in the usual way. The riders were free to choose when to schedule the training sessions with crossunder bridle.

On the test day, all horses performed a dressage test with their own bridle (test 1) and a dressage test in the crossunder bitless bridle (test 2) in an indoor riding arena of 20 meters by 40 meters (Figure 4, Figure 5). The riding arena was located at the facility where the horses were stabled and all horses were familiar with it. A crossover design was used to prevent influence of the first test on the second test as much as possible (Table 2). During the performance of a test there were no more than two horses in the arena at the same time. All horses were used to be trained with multiple horses being present in the arena and this did not seem to cause extra stress.

Table 2 Crossover schedule

Time	Horse number	Horse	Bridle
08.55	5	Caleteño	Crossunder
09.11	1	Zidane	Crossunder
09.26	9	Ramaika	Crossunder
09.36	9	Ramaika	Snaffle
09.48	3	White	Snaffle
10.03	6	Modoso	Snaffle
10.14	4	Oficial	Snaffle
10.40	1	Zidane	Snaffle
10.42	5	Caleteño	Snaffle
11.22	7	Maybe	Crossunder
11.36	6	Modoso	Crossunder
11.47	4	Oficial	Crossunder
11.57	3	White	Crossunder
12.05	7	Maybe	Snaffle

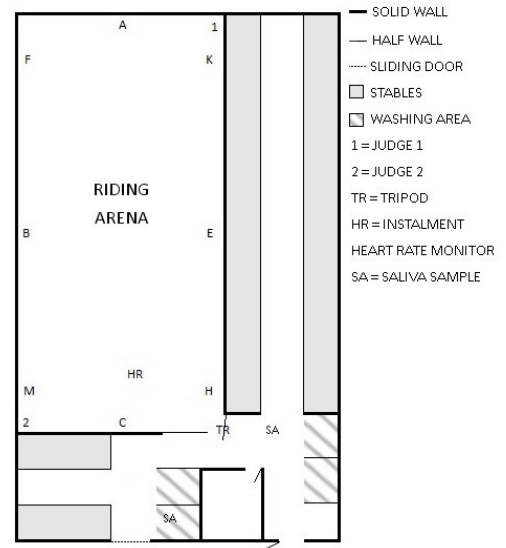
Before the beginning of a test, the next scheduled horse entered the arena for a warm up. Warming up had a duration of about 10 minutes. After the warming up, the heart rate monitor was installed. The horse was ridden for another 1 to 3 minutes in walk and/or trot before starting the test. At the end of the test the heart rate monitor was removed and the horse was cooled down for 3 to 4 minutes. It left the riding arena before the next scheduled horse started the test (Table 3). Upon leaving the horse waited for 5 to 10 minutes either at the washing place or in the hallway (Figure 5) to have a saliva sample taken.

Table 3 Dressage test schedule

Time (minutes)	Action
-10	Warming up horse 1
0	Installment heart rate monitor horse 1
3	Continue warming up horse 1, horse 2 enters arena for warming up
6	Start dressage test horse 1
11	End dressage test horse 1
12	Removal heart rate monitor horse 1
14	Cooling down horse 1, installment heart rate monitor horse 2
17	Horse 1 leaves riding arena
22	Saliva sample horse 1

Figure 4 (Left) The indoor riding arena seen from the viewpoint of the camera (indicated with TR in Figure 5).

Figure 5 (Right) Set up of the experiment. A riding arena of 20 meters by 40 meters was used for performance of the tests. Judges were located in opposite corners of the arena. The tripod was located behind the arena door. Installment of the heart rate monitor was done in the riding arena, saliva samples were obtained at the washing area or in the hallway of the stables.



3.3 Experimental procedures

Saliva sampling

Saliva swabs were collected using cotton rolls (Salivette; Sarstedt, Etten-Leur, Netherlands). This method has been designed for use in human research and is also applied frequently in research on horses (Hall et al., 2014; Becker-Birk et al., 2013a, 2013b; Peeters et al., 2011, 2013; Schmidt et al., 2010b). Through the longitudinal axis a cotton thread was pulled as described by Hall et al. (2014) to be able to hold the roll in place at both sides while it was inserted into the horse's mouth. It was placed on the horse's tongue just in front of the bit's position and was gently chewed on by the horse for one minute. Next the cotton roll was placed in the Salivette tube. All swabs were frozen on -20°C within 12 hours after collection. A chemiluminiscent enzyme immunoassay test was used to measure the concentration of cortisol in nmol/L (Annex 1).

Heart rate monitor

To measure heart rate, a Polar Equine RS800CX heart rate monitor was used. Time-domain measures of heart rate frequency and heart rate variability were obtained. Heart rate frequency was measured in beats per minute (bpm). Heart rate variability was measured using the standard deviation of differences between successive beat-to-beat intervals (RMSSD).

The heart rate sensor and electrodes were secured to the left D-ring at the saddle. The rider was dismounted during the placement of the heart rate monitor. The electrodes were moistened with water and a lubricant. The girth behind the horse's left elbow and a place at the right side of the horse just behind the withers were moistened as well. The electrodes were put at these places with the left electrode being secured behind the girth's straps and the right electrode being secured behind the saddle. A training watch that receives information from the sensors was placed on the rider's wrist.

All tests were filmed using a Canon SX120 IS digital camera standing on a tripod. The recording of film and heart rate monitoring were started simultaneously. The recordings and time display of the camera were used as an indication of when a certain exercise in the dressage test started. All heart rate data were processed and analysed using Polar ProTrainer 5 (Polar Electro, Kempele, Finland).

Behaviour analysis

The camera was standing on a tripod just behind the arena's fence at the flattened corner in between the letters C and H. The camera filmed continually throughout the test. The tests were analysed using an ethogram on tail, head and mouth behaviours as defined by Hall et al. (2014) and Heleski et al. (2009) (Table 4).

Table 4 Ethogram

Behaviour	Definition
<u>Tail</u>	
Swinging	Tail swinging rhythmically with body movement*
Swishing	Purposeful strong lateral and dorsoventral movements of the tail beyond that of simple rhythmic swishing*
<u>Mouth opening</u>	
Mouth still	Mouth still and shut*
Mouth open	Mouth opens and tongue or teeth may be visible*
<u>Being on the bit</u>	
Head toss	Horse moves head quickly out of 'neutral' position (the place where it is positioned for most of the ride)**

* Definitions derived from Hall et al. (2014).

**Definition derived from Heleski et al. (2009).

Performance scoring

Two judges, both registered at the KNHS and certified to judge bitless riding, scored all dressage tests. The judges were located opposite of each other with one judge in the corner in between the riding arena letters K and the A and one judge in the corner between arena letters M and the C (Figure 5). One judge (judge 1) was not familiar with the horses that were performing. The other judge (judge 2) had already judged the horses when they were riding a similar dressage test a few weeks earlier.

For the dressage test ridden with bit a test made by the KNHS was used (test L1 number 7, KNHS 2014b, Annex 2). For the bitless dressage test a similar test was used (test L1 number 7, bitloos rijden, KNHS 2014b, Annex 3). The difference between the tests was the definition used for the horse searching for the rider's hands. When a bridle with bit is used, this is explained as the horse being 'on the bit'. For bitless riding it is explained as 'a light contact on the reins and the head/neck position of the horse'.

The scoring system used for the tests was slightly adapted. Usually one score is given for the horse being straight, relaxed and on the bit. For this research straightness, relaxation and being on the bit were given a separate score.

Questionnaire

Two questionnaires were designed for evaluation of some behavioural issues of the participating horses (Annex 4, Annex 5). The questionnaire was added to the objectives of this study to fully utilize the study opportunity by hearing the experience of riders with bitless riding. To compose the questionnaires, the adverse behavioural effect list conducted by Cook (2003) was used as a guideline. The first questionnaire regarded the usual behaviour of the horse when under saddle and was filled in before the test day. The second questionnaire was specifically about the number of times the riders had been riding with the crossunder bridle and was filled in at the test day.

The questionnaires contained 21 statements about behavioural issues. By giving a score from 1 to 5 on a Likert scale (Likert, 1932) the rider indicated the frequency of the behaviour with 1 implying the horse never shows this behaviour and 5 implying the horse always shows this behaviour. Half of the

questions were stated in a positive way and the other half in a negative way. In the second questionnaire the questions were sorted in a random order. The second questionnaire also contained 8 'true or false' statements concerning the rider's experience with training the horse in the crossunder bitless bridle. Next to this riders had to rate rideability of their horse with the crossunder bridle.

Statistical analysis

Salivary cortisol concentration, heart rate, heart rate variability, behaviour frequency and total performance score were tested for normal distribution with a Kolmogorov Smirnov test. Breed differences for salivary cortisol concentration, heart rate and performance score were tested using the Independent-Samples T-Test. Differences between salivary cortisol concentration, heart rate, heart rate variability and performance score of the two bridles and were tested using a Paired Sample T-Test. For each of the 21 test exercises differences in heart rate were analysed using Kolmogorov Smirnov and a Paired Sample T-Test. The questionnaire statements were tested for differences using the Wilcoxon test. Additionally a Sign Test was used to look for positive and negative differences. For all tests the level of significance was set on $p=0.05$.

4. Results

For the first main question, ‘Does a horse ridden in a crossunder bitless bridle experience less stress during a competition compared to a horse ridden in a bridle with a snaffle bit?’, an analysis of salivary cortisol concentration was conducted. 1 out of 6 samples for test 1 (snaffle bit test) and 3 out of 6 samples for test 2 (crossunder bridle) were not available for analysis as the sample size was too small to conduct the laboratory procedure (Table 5). From one horse (horse 9) no saliva samples were obtained because this horse had to perform two consecutive tests (Table 2). Heart rate frequency and heart rate variability were analysed using the average of the entire dressage test. Heart rate frequency was also analysed using the average frequency of each separate exercise of the test. The behaviours of tail swishing, mouth opening and head tossing were scored for the entire test using an ethogram (Table 4).

Table 5 Overview of salivary cortisol samples that were successfully (✓) or not successfully (-) obtained.

Horse number	Salivary cortisol analysis	
	Test 1	Test 2
1	✓	-
3	✓	✓
4	✓	-
5	✓	-
6	-	✓
7	✓	✓
9	-	-

The second main question was concerning performance: ‘Does a horse ridden in a crossunder bitless bridle have a higher score in a dressage test compared to a horse ridden in a bridle with a snaffle bit?’. For this the total score of all 21 exercises of the dressage and the rating of the criteria for a good dressage performance (numbers 22 to 31 at of the test, Annex 2 and 3) were added up. Three separate scores, those for the criteria of straightness, relaxation and the horse searching for the rider’s hand (being ‘on the bit’), have been evaluated. Next to the riders’ experience of rideability of the horse with crossunder bridle have been compared to the stress level of the horse and the total test score. For one horse (horse 9) the questionnaire was not filled in by the rider who participated during the dressage tests but by another familiar rider.

All data were tested for breed differences between the four Dutch Warmblood horses and the three Andalusian horses. A significant difference was found for the cortisol concentration of with a mean of 10.42 nmol/L for the Dutch Warmblood and a mean of 5.39 nmol/L for the Andalusian horse ($P=0.024$). No other breed differences were discovered.

4.1 Salivary cortisol concentration

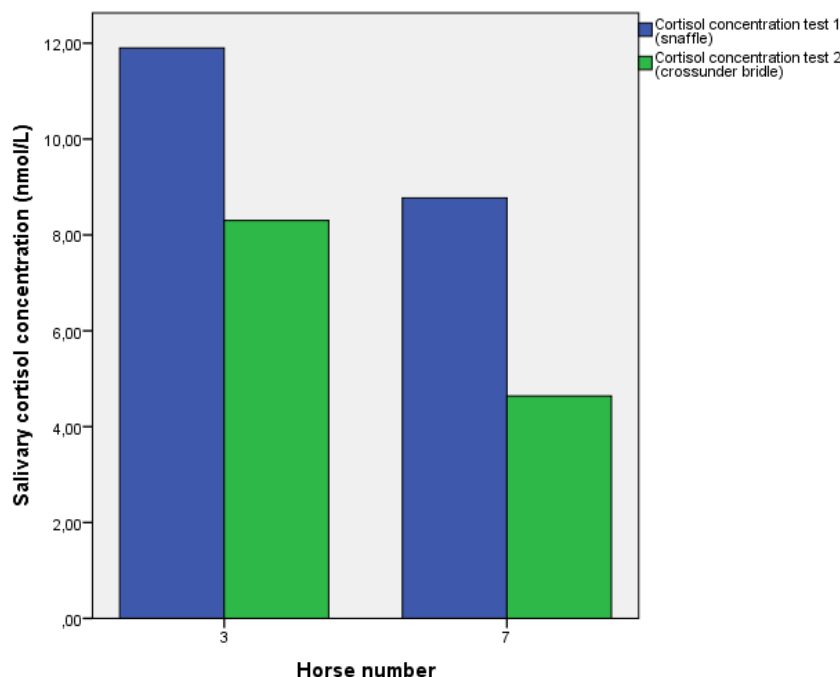
With a Kolmogorov Smirnov test a normal distribution was found for the salivary cortisol concentration of test 1 ($P=0.394$) and test 2 ($P=0.388$). For test 1 an average concentration of 8.41 nmol/L (± 3.017 nmol/L) was found. Test 2 had a mean concentration of 6.6 nmol/L (± 1.845 nmol/L, Table 6). A significant difference ($P=0.044$) was found between the concentrations of test 1 and test 2.

Table 6 Cortisol concentrations found for dressage test 1 (snaffle) and 2 (crossunder bitless bridle).

Horse number	Cortisol concentration (nmol/L)	
	Test 1 (snaffle)	Test 2 (crossunder bridle)
1	10.6	-
3	11.9	8.3
4	7.92	-
5	4.66	-
6	-	6.87
7	8.77	4.64
9	-	-
Average (st. dev.)	8.14 (± 3.017)	6.6 (± 1.845)

For two horses the cortisol concentration of both test 1 and test 2 were successfully analysed. The values found were respectively 11.9 nmol/L (test 1, snaffle) and 8.3 nmol/L (test 2, crossunder bridle) for horse no. 3 and 8.77 nmol/L (test 1) and 4.64 nmol/L (test 2) for horse no. 7 (Figure 6).

Figure 6 Salivary cortisol concentrations for individual horses after dressage test 1 and dressage test 2.



4.2 Heart rate frequency

A Kolmogorov Smirnov tests showed heart rate frequency to be normally distributed for test 1 ($P=0.899$) and test 2 ($P=0.999$) as well as minimum heart rate ($P=0.956$ for test 1, $P=0.864$ for test 2) and maximum heart rate ($P=0.952$ for test 1, $P=0.729$ for test 2). Average heart rates found for the total dressage test were 103.71 bpm (± 6.157 bpm) for test 1 and 104.29 bpm (± 7.365 bpm) for test 2 ($P=0.744$, Table 7). Minimum heart rate for test 1 was 76.71 bpm (± 3.039 bpm) and 76.43 bpm (± 4.276 bpm) for test 2 ($P=0.766$). Maximum heart rate were 132.29 bpm (± 12.737 bpm) for test 1 and 130.86 bpm (± 16.926 bpm) for test 2 ($P=0.716$).

There was no difference in mean heart rate between the 21 parts of test 1 and test 2. Only exercise 20 indicated a difference may exist between both tests. It consisted of a turn, a trot-halt-trot transition and another turn. For this exercise an average heart rate of 109.43 bpm (± 101.96 bpm) was found for test 1 and an average heart rate of 96.14 bpm (± 7.244 bpm) was found for test 2 ($P=0.167$). In total there were 8 out of 21 exercises with a higher mean heart rate of test 1 compared to test 2 and 13 out of 21 exercises with a higher mean heart rate of test 2 compared to test 1 (Annex 6).

Table 7 Individual values of mean heart rate frequency and RMSSD (standard deviation of differences between successive beat-to-beat intervals) for test 1 (snaffle bit) and test 2 (crossunder bridle).

Horse number	Heart rate frequency and st. dev. (bpm)		RMSSD (ms)	
	Test 1 (snaffle)	Test 2 (crossunder bridle)	Test 1 (snaffle)	Test 2 (crossunder bridle)
1	99 (± 9.9)	99 (± 9.8)	11	12.6
3	112 (± 14.6)	109 (± 11.3)	10.6	30.1
4	93 (± 12.4)	92 (± 12.4)	26.3	32.1
5	106 (± 14.9)	103 (± 13.1)	16.9	17.2
6	106 (± 17.7)	115 (± 25.5)	24.6	46.7
7	107 (± 13.1)	105 (± 12.4)	14.2	10.1
9	103 (± 10.5)	107 (± 9.4)	8.2	9.4
Average (st. dev.)	103.71(± 6.157)	104.29 (± 7.365)	15.97 (± 7.06)	22.70 (± 13.96)

4.3 Heart rate variability

A normal distribution was found using the Komogorov Smirnov test for mean beat to beat interval (R-R) ($P=0.997$ for test 1, $P=0.979$ for test 2), average maximum R-R ($P=0.829$ for test 1, $P=0.992$ for test 2) and RMSSD ($P=0.966$ for test 1, $P=0.872$ for test 2). Mean beat to beat (R-R) interval was 588.86 ms (± 34.275 ms) during test 1 and 580.71 ms (± 43.119 ms) during test 2. No significant difference in mean beat-to-beat interval was found between test 1 and test 2 ($P=0.484$, Annex 7). The difference in the average maximum R-R interval was 899.29 ms (± 90.917 ms) for test 1 and 949.57 ms (± 62.846 ms) for test 2 ($P=0.289$). RMSSD (standard deviation of differences between successive beat-to-beat intervals) was 15.97 ms (± 7.06 ms) for test 1 and 22.70 ms (± 13.958 ms) for test 2 ($P=0.128$) (Table 7).

4.4 Behaviour

All behaviours were shown to be normally distributed using Kolmogorov Smirnov (tail swishing $P=0.333$ for test 1, $P=0.129$ for test 2; mouth opening $P=0.939$ for test 1, $P=0.167$ for test 2; head tossing $P=0.892$ for test 1, $P=0.603$ for test 2).

In total the behaviour of tail swishing was counted 149 times for test 1 (snaffle) and 154 times for test 2 (crossunder bridle). Four horses showed less tail swishing during test 2, one horse showed more tail swishing during test 2 and two horses did not show any tail swishing. Horse 4 accounted for the main part of tail swishes by showing 76.51% of the total number of tail swishes during test 1 and 88.96% of the total number of tail swishes during test 2. The average number of tail swishes for test 1 was 21.29 (± 41.65) and 22.00 (± 50.86) for test 2 ($P=0.865$) (Table 8).

Mouth opening was counted 75 times in total for test 1 and 3 times in total for test 2. During test 1 all horses showed mouth opening on average 10.71 times (± 8.73 times). During test 2 mouth opening was only seen in two horses, on average 0.43 times (± 0.79 times) (Table 8). A significant difference ($P=0.020$) was found between the tests.

The number of head tosses was equal for both tests and had a count of 55. Four horses showed a higher frequency of head tossing during test 1 and three horses showed a higher frequency of head tossing during test 2. On average head tossing was displayed 7.86 times (± 6.04 times) during test 1 and 7.86 times (± 9.86 times) during test 2 ($P=1.000$, Table 8).

Table 8 Frequency of the behaviours of tail swishing, mouth opening and head tossing counted per test and on average.

	Number of tail swishes		Number of mouth openings		Number of head tosses	
Horse number	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	23	11	3	0	0	3
3	6	4	16	1	3	2
4	114	137	1	0	19	0
5	2	0	11	2	9	29
6	0	0	9	0	10	6
7	0	0	27	0	6	3
9	4	2	8	0	8	10
Average (st. dev.)	21.29 (± 41.65)	22.00 (± 50.86)	10.71 (± 8.73)	0.43 (± 0.79)	7.86 (± 6.04)	7.86 (± 9.86)

4.5 Performance

Total dressage score for test 1 given by judge 1 ($P=0.635$), judge 2 ($P=0.522$) and the average total score of the judges for test 1 ($P=0.527$) were normally distributed. Total dressage score for test 2 given by judge 1 ($P=0.506$), judge 2 ($P=0.974$) and the average total score of the judges for test 2 ($P=0.598$) were normally distributed as well. No significant difference existed between the average dressage test scores of test 1 and test 2 ($P=0.673$) (Figure 7). Judge 1 gave a mean score of 192.14 (± 9.771 points) for test 1 and a mean score of 190.43 (± 13.939 points) for test 2 ($P=0.579$). Judge 2 gave a mean score of 195.57 (± 11.473 points) for test 1 and a mean score of 200.68 (± 14.133 points) for test 2 ($P=0.252$). Ranking of the horses was comparable for both judges. Horses with a high ranking during test 1, also received a high ranking during test 2 (Table 9).

Figure 7 Error bar presenting the average scores judge 1 and 2 gave for test 1 (snaffle) and test 2 (crossunder bridle) as well as the mean of the marks for both judges combined.

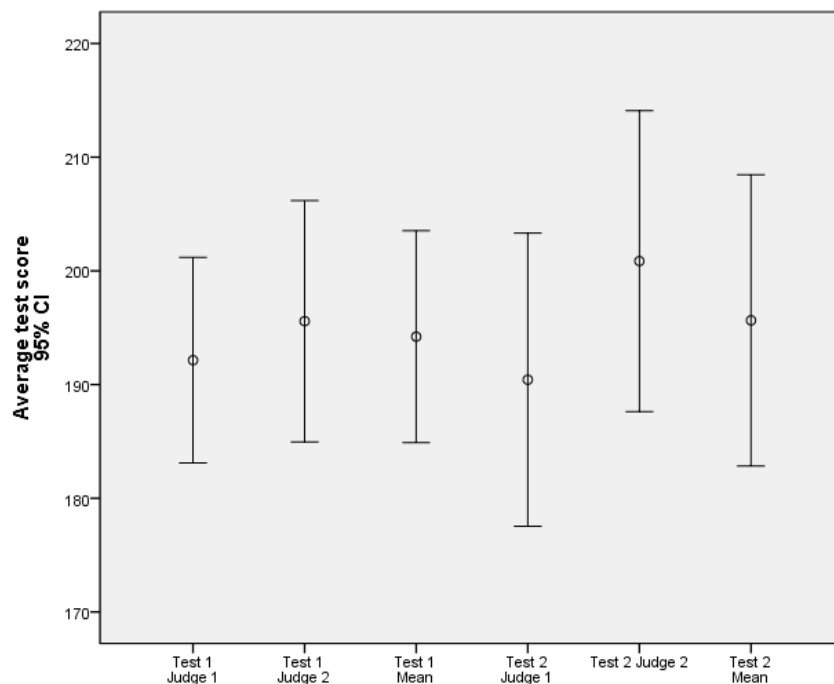


Table 9 Individual marks for each test as scored by judge 1 and judge 2 as well as the average mark and ranking of each combination.

Horse number	Test 1 (snaffle)			Test 2 (crossunder bridle)		
	Mark and ranking judge 1	Mark and ranking judge 2	Average mark and ranking	Mark and ranking judge 1	Mark and ranking judge 2	Average mark and ranking
1	187 (5)	196 (3)	191.5 (4)	184 (4)	202 (3)	193.0 (3)
3	212 (1)	220 (1)	216.0 (1)	217 (1)	222 (1)	219.5 (1)
4	194 (2)	191 (4)	192.5 (3)	202 (2)	218 (2)	210.0 (2)
5	194 (2)	197 (2)	195.5 (2)	183 (5)	188 (6)	185.5 (6)
6	190 (4)	188 (6)	189.0 (5)	180 (6)	193 (5)	186.5 (5)
7	182 (7)	191 (4)	189.0 (5)	187 (3)	198 (4)	192.5 (4)
9	186 (6)	186 (7)	186.0 (7)	180 (6)	185 (7)	182.5 (7)
Average (st. dev.)	192.14 (± 9.771)	195.57 (± 11.473)	194.21 (± 10.070)	190.43 (± 13.939)	200.68 (± 14.133)	195.64 (± 13.853)

4.6 Straightness, relaxation and bringing the horse to the hand

All three criteria were found to be normally distributed for test 1 (straightness $P=0.422$, relaxation $P=0.905$, bringing the horse to the hand $P=0.868$) and test 2 (straightness $P=0.699$, relaxation $P=0.890$, bringing the horse to the hand $P=0.819$) using a Kolmogorov Smirnov test. The average mark horses received for straightness was a 6.14 for test 1 and a 6.07 for test 2 ($P=0.604$). Relaxation was judged with an average mark of 6.0 for test 1 and 6.29 for test 2 ($P=0.321$) and bringing the horse to the hand with 5.86 for test 1 and 6.0 for test 2 ($P=0.703$). Differences in marks given for test 1 and 2 by the individual judges were not significant (Table 10), although judge 2 seemed to perceive the horses performing in the crossunder bridle as being more relaxed ($P=0.094$).

Table 10 Average scores and significance between test 1 and test 2 given for straightness, relaxation and being on the bit.

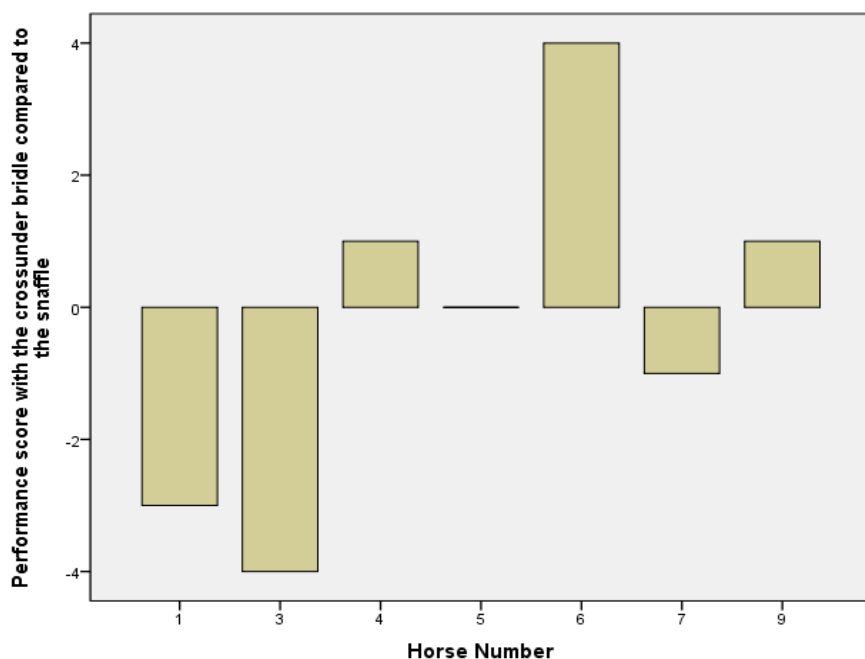
Judge	Criteria	Average mark test 1 (snaffle)	Average mark test 2 (crossunder bridle)	P-value
1	Straightness	6.14 (± 0.38)	6.14 (± 0.38)	1.000
	Relaxation	6.00 (± 0.58)	5.86 (± 0.69)	0.604
	Bringing the horse to the hand	5.86 (± 0.69)	5.71 (± 0.95)	0.604
2	Straightness	6.14 (± 0.69)	6.00 (± 0.82)	0.604
	Relaxation	6.00 (± 0.58)	6.71 (± 1.11)	0.094
	Bringing the horse to the hand	5.86 (± 1.22)	6.29 (± 1.38)	0.407
Average of both judges	Straightness	6.14 (± 0.48)	6.07 (± 0.54)	0.604
	Relaxation	6.00 (± 0.50)	6.29 (± 0.81)	0.321
	Bringing the horse to the hand	5.86 (± 0.90)	6.00 (± 1.16)	0.703

4.7 Rideability

Three riders (of the horses 4, 6 and 9) found it harder to bring their horses in hand when riding bitless. Four riders did not experience a difference ($P=0.083$). Three other riders (of the horses 1, 3 and 5) perceived their horses to be less quickly relaxed, again the other riders did not note a difference ($P=0.083$).

Overall when the riders were asked to compare the rideability of their horse for the crossunder bridle with the rideability of their horse for the bridle with snaffle they rated the rideability of the horse with crossunder bridle slightly worse than rideability with the snaffle bit. A mean score of $-0.29 (\pm 2.690)$ was given. In total three riders think their performance with the crossunder bridle was worse, three think it was better and one rider was neutral (Figure 8).

Figure 8 Performance with the crossunder bridle compared with the bridle with snaffle as perceived by the horses' riders. A score of -5 indicates a very bad rideability for the crossunder bridle compared to the snaffle bit, 0 rideability is rated equal and +5 rideability for the crossunder bridle is much better.



4.8 Overview

For salivary cortisol concentration only for two horses both results were available. For both these horses the lowest concentration was found for the test with crossunder bridle. The mean heart rate was for 3 horses the lowest during test 1, for 3 horses during test 2 and for one horse the values were exactly the same. 6 horses exhibited a higher RMSSD during test 2. Overall four horses show a consistency to have the combination of a lower heart rate and a higher RMSDD during test 2.

Four horses scored higher during the dressage test with the crossunder bridle, three horses scored higher with the snaffle bit. For the variables of straightness and bringing the horse to the hand no difference is seen. For relaxation the horses show a tendency to score higher during test 2. Stress related behaviours are displayed equally between both tests except for mouth opening which is more frequent during test 1. Only one individual horse shows a lower frequency of tail swishing during test 1 and two horses show a lower frequency of head tossing during test 1.

5. Discussion

5.1 Discussion

This study aimed to discover if there is a difference in the stress level and performance of a horse wearing a bridle containing a snaffle bit compared to a crossunder bitless bridle. It was hypothesized that a crossunder bitless bridle would cause less stress in the horse and lead to an improved performance.

An indication for a reduction in stress level of horses wearing a crossunder bridle was found measuring the salivary cortisol concentration. Research by Fejsáková et al. (2013) also found the bitless bridle group inhibited the lowest salivary cortisol concentration, although not significant.

The reduction in stress level when using the crossunder bridle is indicated by a strong tendency of the heart rate variability to increase during the tests ridden with crossunder bridle. As explained by McGreevy and McLean (2010), Borell et al. (2007) and Acharya et al. (2006) this means that when performing with the crossunder bridle the participating horses had a better ability to adapt to stimuli and were less fearful. The only exception was horse no 7 which showed a decrease in RMSSD from test 1 to test 2. This horse performed with crossunder bridle first, but this test was performed after a 40 minute break (Table 1) which may have allowed the rider to perform a longer warm up for the crossunder bridle compared to the snaffle bit. According to Mohr, Witte and Vos (2000) an increase in physical stress may have been responsible for this decrease in heart rate variability.

Mean heart rates of 103.71 bpm for test 1 and 104.29 bpm for test 2 were found. This is comparable to the research of Williams et al. (2008) who found a mean heart rate of 102 bpm (± 13 bpm) when measuring on a British dressage competition of elementary level (comparable to Dutch M2 level). Fejsáková et al. (2013) did find a significant lower heart for horses wearing a bitless bridle and Warren-Smith and Quick (2009) found horses exhibiting lower heart rates when trained with the crossunder bitless bridle.

The dressage exercise with the most difference between the heart rates (exercise 20 in the protocol, Annex 2 and 3) was an exercise with transitions that followed each other relatively quickly. Some of the riders were seen using the reins in a more sawing manner in an attempt to get the horse on the bit during the halt. According to Randle and Wright (2013) riders may have put more tension on the reins during the test with the snaffle bit compared to the test with the crossunder bridle. This may have caused an increase of conflict behaviour as Christensen et al. (2011) found a positive correlation between rein tension and conflict behaviour. Christensen et al. (2011) did not find a correlation between rein tension and heart rate, so it is not clear if there is a connection between the increased heart rate and increased rein tension with the snaffle bit during this dressage exercise.

The scores obtained during the dressage test variables formed a performance-based indication of the difference between both bridles. No significant difference was seen between test 1 and test 2 performance, although the scores for the crossunder bridle were slightly higher comparable to the research of Wittermans-Arnold (2009) and judges of this research indicate some combinations did perform better with the crossunder bridle. Wittermans-Arnold (2009) describes horse-rider combinations achieving a similar ranking independent of which bridle is performed with. No influence of the bridle on ranking can be seen in this research as well. In the research of Cook and Mills (2009) all horses improved performance significantly and no clear rank order can be distinguished. Cook and Mills (2009) rated test exercises on a scale from 0 to 10 similar to the Dutch system used in this

research. Still it appears that the judge of Cook and Mills (2009) actually uses the full scale from 0 to 10 while Dutch judges are mainly used to give a score between a 4 and 7. This may explain the larger difference in performance found by Cook and Mills (2009).

Similar to this research, Wittermans-Arnold (2009) also points out riders reporting difficulties bringing the horse to the hand. According to Cook and Mills (2009), horses with the crossunder bridle were more willing and alert, however this terminology is hard to translate to the measurements of current research.

It is interesting to note that stress level and performance of the horse may not have a relation to rideability. Some horses that were showing a lower salivary cortisol concentration and decreased stress behaviours wearing the crossunder bridle were regarded by their riders to have decreased rideability. This may well be attributed to the fact that they only practiced 3-5 times during the short time frame of 22 days and felt they needed more time to get their horse at the same level of performance with the new bridle.

5.2 Limitations

With seven participants, sample size was limited. To be able to draw far reaching conclusions, a bigger sample size is preferred. As a part of the salivary cortisol samples was not successfully analysed, this especially is a topic in need of more research.

Judging remains a subjective job and this may explain why judge 2 gave significantly higher scores compared to judge 1 when performing bitless. Also this judge had already seen all combinations participate 22 days beforehand. The judges did give similar rankings for each test, however it is better to avoid such a situation.

Because two horses were riding in the arena when a dressage test was performed, the camera was not always able to fully obtain the view of the performing horse-rider combination. Therefore some behaviours may not be noted. All behaviours were counted by hand, which made it harder to decide when a behaviour was displayed intense enough to count.

It is not found in literature whether there is useful to compare mouth opening, a stress related behaviour like related to the bit, with the frequency of mouth opening wearing the crossunder bridle. To make an honest comparison, it should be clear what the stress related signs of too much pressure at the nose are. For example a horse ridden in a bitless bridle suffering from overmuch pressure may not open its mouth but instead show increased frequency of head tossing.

A guidance is existing for correct fitting of the crossunder bridle (The Bitless Bridle, 2008). However a horse may show a preference for a certain noseband height. For example for one horse the noseband was placed at a lower place compared to the other horses. A more thorough guidance of the participating riders on bridle fitting is useful to help them recognize when the horse is comfortable.

6. Conclusion

The crossunder bridle may be an important tool to lower stress levels of dressage horses. Horses performing a dressage test in a crossunder bridle, exhibited a significantly lower salivary concentration of the stress hormone cortisol compared to their performance with a bridle containing a snaffle bit. A higher heart rate variability, indicating less fearfulness (McGreevy and McLean, 2010), was detected in 6 out of 7 horses.

Differences in heart rate were not as pronounced as the differences in heart rate variability. During one out of the 21 exercises performed, a nearly significant difference was found, representing a lower heart rate for the crossunder bridle. The exercise consisted of a trot-halt-trot transition during which some riders used the reins more strongly to bring the horse in the hand. The pressure put on a crossunder bridle may have caused less stress than the pressure on a snaffle bit.

Removal of the bit may enable a horse to be more relaxed. However this does not necessarily include improvements in consistence of head-neck position as no difference was seen in marks for being on the bit and number of head tosses.

Three riders felt rideability of their horse was reduced when riding with the crossunder bridle. This was not reflected by the horses' stress levels which were lower when competing in the crossunder bridle. For these riders their feeling of how they performed may have a stronger connection to riding technique than to the type of bridle used. One of the horses improved test score remarkably when riding with the crossunder bridle, showing that it is possible a horse can be easier to bring to the hand wearing a crossunder bridle. Ultimately, it is the softness of the rider's hands that allow the horse to achieve a connection.

7. Recommendations

Because no significant differences between performances for bridle with snaffle bit and crossunder bridle were observed and horses ridden with the crossunder bridle were even more relaxed, serious consideration should be given to replacing the bitted bridle with the bitless one. Especially for beginning riders it is a big responsibility to be careful with the horse's mouth while they are learning how to use the rein aids.

Some of the horses participating in this research were ridden with a constant rein pressure applied. This is regarded as the most common cause of conflict by McGreevy and McLean (2005). Educating riders in the correct application of learning theory and the anatomical features of dressage may result in a greater difference in performance than using a bitless bridle instead of a snaffle bit. It is important to emphasize the need of good riding education and a proper knowledge of collection. Consequently improvements in riding skills will increase performance for any bridle used.

Next to this it is recommended for riders to compare bridles with and without bit before deciding which one to use when riding dressage. The bridle of their choice may not always be the one their horse says is best.

An issue that still needs to be tackled is the difference in performance of horses participating in higher dressage classes. It is thought by some that steering on the nose may not be sufficient to gain a sufficient level of collection. Because of this riders using a bitless bridle are not yet permitted to compete alongside riders using a bit at M1 level and higher on during official competitions. It would therefore be interesting to assess if the degree of collection using a bitless bridle is different and stays within the definition of an anatomically correctly performing horse.

A long term research, taking into account different types of bitless bridles, can give a better conclusion as to what extent performance improves riding without a bit. Research by Fejsáková et al. (2013) and Hockenhull and Creighton (2012) did not distinct between bridles applying a direct force on the nose and the hackamore applying a leverage action. It is important to make this distinction (Cook, 2000) and have separate groups of horses for each different bridle.

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in vivo). Caution must therefore be exercised with cortisol determinations for patients undergoing therapy with these and structurally related synthetic corticosteroids. Heterophilic antibodies in human serum can react with the immunoglobulin included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Performance Data

See Tables and Graphs for data representative of the assay's performance. Results are expressed in µg/dL. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Conversion Factor:

µg/dL × 27.59 → nmol/L

Calibration Range: 1 – 50 µg/dL (28 – 1,380 nmol/L).

The assay is traceable to an internal standard manufactured using qualified materials and measurement procedures.

Analytical Sensitivity: 0.2 µg/dL (5.5 nmol/L).

Precision: Samples were processed in quadruplicate over the course of 5 days, 4 runs per day, for a total of 20 runs and 80 replicates. (See "Precision" table.)

Linearity: Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Samples spiked 1 to 19 with three cortisol solutions (109, 215 and 349 µg/dL) were assayed. (See "Recovery" table for representative data.)

Specificity: The antibody is highly specific for cortisol. (See "Specificity" table.)

Although some steroids exhibit cross-reactivity, their normal physiological concentrations are low compared to cortisol, hence they will not significantly interfere in the IMMULITE Cortisol procedure. There is, however, an observed cross-reactivity of approximately 49% with prednisone which must be considered for patients undergoing this therapy. Since prednisone is converted to prednisolone *in vivo*, caution must be exercised with cortisol determinations for patients undergoing either therapy.

Bilirubin: Samples spiked with 100 and 200 mg/dL of conjugated and unconjugated bilirubin were analyzed. Bilirubin may interfere with the assay, causing elevation of values. (See "Bilirubin" table.)

Hemolysis: Presence of packed red blood cells in concentrations up to 30 µL/mL has no effect on results, within the precision of the assay.

Lipemia: Presence of triglycerides in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay.

Method Comparison: The assay was compared to Coat-A-Count® Cortisol on 83 patient samples. (Concentration range: approximately 3.3 to 41 µg/dL. See graph.) By linear regression:

(IML) = 0.95 (CAC) + 0.32 µg/dL

$r = 0.986$

Means:
14.6 µg/dL (IMMULITE)
15.0 µg/dL (CAC)

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Technical Assistance

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The Quality System of Siemens Healthcare Diagnostics Products Ltd. is certified to ISO 13485:2003.

Tables and Graphs

Precision (µg/dL)

	Within-Run ¹			Total ²	
	Mean ³	SD ⁴	CV ⁵	SD	CV
1	2.7	0.24	8.6%	0.27	10%
2	4.4	0.33	7.5%	0.37	8.4%
3	11.3	0.88	7.8%	0.87	7.7%
4	19.0	1.1	5.8%	1.2	6.3%
5	29.1	1.8	6.2%	2.2	7.6%
6	35.8	2.4	6.7%	2.6	7.3%

Linearity (µg/dL)

	Dilution ¹		Observed ²		Expected ³		%O/E ⁴
	1	16 in 16 ⁵	31.9	—	—	—	
1	8 in 16	13.3	16.0	83%			
	4 in 16	7.5	8.0	94%			
	2 in 16	3.8	4.0	95%			
	1 in 16	1.9	2.0	95%			
2	16 in 16	34.5	—	—			
	8 in 16	15.8	17.3	91%			
	4 in 16	8.6	8.6	100%			
	2 in 16	4.7	4.3	108%			
3	16 in 16	45.3	—	—			
	8 in 16	20.9	22.7	92%			
	4 in 16	11.2	11.3	99%			
	2 in 16	5.7	5.7	100%			
4	16 in 16	3.1	2.8	111%			

Recovery (µg/dL)

	Solution ¹		Observed ²		Expected ³		%O/E ⁴
	1	—	10.8	—	—	—	
1	A	16.5	15.7	105%			
	B	24.6	21.0	117%			
	C	28.4	27.7	103%			
2	—	8.2	—	—			
	A	13.8	13.2	105%			
	B	20.1	18.5	109%			
3	C	25.9	25.2	103%			
	—	16.7	—	—			
4	A	23.5	21.3	110%			
	B	29.4	26.6	111%			
	C	33.9	33.3	102%			

9.2 Test L1 number 7

Proef 7 Klasse L1

Uitgave 2011

Volgnr.:

Combinatienummer:

Tijdstip:

Datum:

Ruiter:

Paard:

Jury:

Rijtijd: 5.30 minuten Rijbaan: 20x40 meter		Lichtrijden, tenzij doorzitten wordt gevraagd		Cijfer	Corr.	Bemerkingen
1	A-F-B	Binnenkomen in arbeidsdraf op de linkerhand				
2	B E	Afwenden Linkerhand				
3	F-X-M	Gebroken lijn				
4	H-X-F	Van hand veranderen en enkele passen middendraf				
5	K-X-H	Gebroken lijn, daarna doorzitten				
6	Tussen H-C-M	Overgang arbeidsstap				
7	M-E	Van hand veranderen en enkele passen middenstap				
8	Tussen E-K	Overgang arbeidsdraf doorzitten				
9		Afwenden, na enkele paardlengtes mini-maal 5 meter wijken voor het linkerbeen richting B-M, daarna rechtuit en lichtrijden				
10	C-X-C Tussen C-H-E	Grote volte en na enkele drafpassen paard de hals laten strekken Teugels op maat maken				
11	E-F	Van hand veranderen				
12	Voor A A	Doorzitten Afwenden, na enkele paardlengtes minimaal 5 meter wijken voor het rechterbeen richting E-H, daarna rechtuit en doorzitten				
13	Tussen C en M	Arbeidsgalop rechts aanspringen				
14	B-E-B	Grote				

		volte en enkele sprongen middengalop				
15	Tussen F-A-K	Overgang arbeidsdraf				
16	E B	Afwenden Linkerhand				
17	Tussen C en H	Arbeidsgalop links aanspringen				
18	E-B-E	Grote volte en enkele sprongen middengalop				
19	Tussen K-A-F	Overgang arbeidsdraf				
20	Voor B B Op B-X-E lijn E	Doorzitten Afwenden Halthouden Voorwaarts in arbeidsdraf, doorzitten Linkerhand				
21	A Tussen X en G	Afwenden en arbeidsstap Halthouden en groeten Bij A in stap de rijbaan verlaten				
22		Stap				
23		Draf				
24		Galop				
25		De impuls				
26		Rechtgerichtheid				
27		Ontspanning				
28		Aanleuning				
29		De houding en zit van de ruiter/amazone				

30		Rijvaardigheid en effect van de hulpen	2x			
31		Verzorging van het geheel				
		Subtotaal				
		Strafpunten				
		Totaal				

Totaal: 320 punten

Handtekening jury:

9.3 Test L1 number 7 bitless

Proef 7 Klasse L1 Bitloos

Uitgave 2011

Datum:

Volgnr.:

Ruiter:

Combinatienummer:

Paard:

Tijdstip:

Jury:

Rijtijd: 5.30 minuten Rijbaan: 20x40 meter		Lichtrijden, tenzij doorzitten wordt gevraagd		Cijfer	Corr.	Bemerkingen
1	A-F-B	Binnenkomen in arbeidsdraf op de linkerhand				
2	B E	Afwenden Linkerhand				
3	F-X-M	Gebroken lijn				
4	H-X-F	Van hand veranderen en enkele passen middendraf				
5	K-X-H	Gebroken lijn, daarna doorzitten				
6	Tussen H-C-M	Overgang arbeidsstap				
7	M-E	Van hand veranderen en enkele passen middenstap				
8	Tussen E-K	Overgang arbeidsdraf doorzitten				
9		Afwenden, na enkele paardlengtes mini-maal 5 meter wijken voor het linkerbeen richting B-M, daarna rechtuit en lichtrijden				
10	C-X-C Tussen C-H-E	Grote volte en na enkele drafpassen paard de hals laten strekken Teugels op maat maken				
11	E-F	Van hand veranderen				
12	Voor A A	Doorzitten Afwenden, na enkele paardlengtes minimaal 5 meter wijken voor het rechterbeen richting E-H, daarna rechtuit en doorzitten				
13	Tussen C en M	Arbeidsgalop rechts aanspringen				
14	B-E-B	Grote				

		volte en enkele sprongen middengalop				
15	Tussen F-A-K	Overgang arbeidsdraf				
16	E B	Afwenden Linkerhand				
17	Tussen C en H	Arbeidsgalop links aanspringen				
18	E-B-E	Grote volte en enkele sprongen middengalop				
19	Tussen K-A-F	Overgang arbeidsdraf				
20	Voor B B Op B-X-E lijn E	Doorzitten Afwenden Halthouden Voorwaarts in arbeidsdraf, doorzitten Linkerhand				
21	A Tussen X en G	Afwenden en arbeidsstap Halthouden en groeten Bij A in stap de rijbaan verlaten				
22		Stap				
23		Draf				
24		Galop				
25		De impuls				
26		Rechtgerichtheid				
27		Ontspanning				
28		Aanleuning (het lichte contact op de teugels met het paard en de hoofd/halshouding van het paard)				
29		De houding en zit van de ruiter/amazone				

30		Rijvaardigheid en effect van de hulpen	2x			
31		Verzorging van het geheel				
		Subtotaal				
		Strafpunten				
		Totaal				

Totaal: 320 punten

Handtekening jury:

9.4 Questionnaire 1

See attached document “Annex 4 Questionnaire 1”.

9.5 Questionnaire 2

See attached document “Annex 5 Questionnaire 2”.

9.6 Heart rate per exercise

Exercise number	Description	Mean HR (bpm)	Standard deviation (bpm)	Mean HR (bpm)	Standard deviation (bpm)	P-value
1	A-F-B: Enter in working trot on the left lead	101.86	11.082	96.29	12.433	0.422
2	B: turn E: left hand	103.14	13.521	103.14	13.521	0.749
3	F-X-M: loop	101.71	8.939	102.29	7.365	0.832
4	H-X-F: change of hand	104.57	8.997	106.71	7.740	0.475
5	K-X-H: loop, after which sitting trot	105.14	7.151	105.86	6.914	0.751
6	Between H-C-M: transition to working walk	98.00	5.508	99.29	6.448	0.582
7	M-E: change of hand and some strides of extended walk	82.71	5.122	84.29	5.251	0.516
8	Between E-K: transition to working trot, sitting trot	81.29	3.773	82.00	6.245	0.662
9	A: turn, after some strides yield at least 5 meters for the left leg in the direction of B-M. Next go straight on in rising trot	90.71	7.610	89.71	7.825	0.802
10	C-X-C: 20 meter circle, after a few strides full extension of the neck for some strides Between C-H-E: adjust the reins	98.57	6.680	97.57	7.743	0.708
11	E-F: change hand	101.57	5.682	101.43	6.604	0.873
12	Before A: sitting trot A: turn, after some strides yield at least 5 meters for the right leg in the direction of E-H. Next go straight on in rising trot	99.86	8.071	98.57	6.579	0.426
13	Between C and M: transition to working canter on the right lead	99.14	7.647	99.43	6.214	0.912
14	B-E-B: 20 meter circle and some strides in extended canter	113.57	10.502	117.29	11.176	0.524
15	Between F-A-K: transition to working trot	121.29	6.550	122.29	14.151	0.847
16	E: turn B: left hand	118.86	11.142	119.57	17.634	0.845
17	Between C and H: transition to working canter on the left lead	115.57	10.390	116.00	19.468	0.931
18	E-B-E: 20 meter circle and some strides in extended canter	121.71	8.712	125.00	18.547	0.622
19	Between K-A-F: transition to working trot	123.86	8.840	126.57	16.288	0.521

20	Before B: sitting trot B: turn At the B-X-E line: transition to halt. Transition to working trot, sitting trot E: left hand	111.43	11.574	109.43	10.196	0.167
21	A: turn, transition to working walk Between X and G: transition to halt, greet the judge	96.14	7.244	94.86	5.640	0.412

9.7 Mean beat-to-beat intervals

Table 1 Mean length of beat-to-beat intervals for the individual horses in milliseconds.

Horse number	Mean R-R test 1	Mean R-R test 2
1	608 (± 74)	609 (± 73)
3	537 (± 83.1)	558 (± 81)
4	640 (± 97.6)	656 (± 91)
5	574 (± 96.6)	589 (± 95.6)
6	567 (± 98.7)	520 (± 123.9)
7	614 (± 90.5)	569 (± 75.7)
9	582 (± 65.9)	564 (± 63.4)