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1. Abstract

'Bits' are common devices to control and steer horses during riding. The effects of the bit have been subject of an ongoing debate in the rider's community. Up to the present, a lot of studies have been conducted on this topic. However, physiological data regarding bit-induced stress have been largely neglected. Our aim was to investigate whether horses experience more stress when wearing a bit than without it. On a larger perspective we wanted to know if mouthpieces constitute a welfare reduction for horses. Based on former studies we expected to see more conflict behavior and also higher stress level in the horses when wearing a bit than without it. For our study, we observed 8 bit-naïve and 11 bit-familiar horses lunged once with a bit and once with a bitless alternative. To quantify their stress levels we conducted behavioral observations and salivary Cortisol concentration measurements. We compared the frequency of conflict behavior and the rise in the Cortisol level between the two groups.

All horses, of both groups, showed a higher frequency of 'mouth opening' with the bit. Four horses opened their mouth constantly with a mouthpiece. 'Chewing' was observed significantly more often with the bit. The difference to the bitless trial was stronger in bit-familiar horses. This group also showed a higher prevalence of saliva production ('visible frothing') and 'headshaking' with the bit indicating that working horses regularly with a mouthpiece induces behaviors absent in horses ridden with bitless alternatives.

We couldn't find any differences in the Cortisol concentrations. Consequently, we can't definitely say that bits lead to an endocrine stress responses in horses. Anyhow, our study shows that bits do induce digestive system responses and headshaking, strongly suggesting that bits not only impair concentration and high performance by distracting the horse, but also represent a welfare subject by triggering conflict behavior.

‘Gebisse’ werden zum Kontrollieren und Lenken des Pferdes während des Reitens verwendet. Die Effekte der Gebisse auf das Pferd sind seit Längerem Grund für Debatten in der Reitergemeinschaft. Es gibt bisher schon einige Studien, die sich mit diesem Thema beschäftigen. Physiologische Daten zu Gebiss-induziertem Stress wurden bis jetzt jedoch weitestgehend vernachlässigt. Unser Ziel war es zu untersuchen ob Pferde gestresster sind, wenn sie ein Gebiss tragen als mit einem gebisslosen Zaum. Allgemeiner betrachtet wollten wir herausfinden ob Gebisse eine Reduktion des Wohlbefindens des Pferdes zur Folge haben. Basierend auf bisher durchgeführten Studien erwarteten wir, dass Pferde mehr aversives Verhalten und, damit zusammenhängend, einen höheren Stresslevel mit Gebiss zeigen als ohne. In unserer Studie beobachteten wir 8 ‚Gebiss-naive’ und 11 ‚Gebiss-gewöhnte’ Pferde beim Longieren, einmal mit Gebiss und einmal mit einer gebisslosen Alternative. Um den Stresslevel zu messen nutzten wir Verhaltensbeobachtungen und Kortisol-Messungen im Speichel. Wir verglichen die Frequenz von auftretendem Konfliktverhalten und den Anstieg im Kortisol Level. Zusätzlich untersuchten wir Unterschiede zwischen den beiden Gruppen.
Alle Pferden öffneten ihr Maul öfter mit Gebiss. Vier Pferde hatten es durchgehend geöffnet als sie ein Mundstück trugen. ‘Kauen’ wurde signifikant öfter mit Gebiss beobachtet. Der Unterschied zum Durchgang ohne Gebiss war stärker in der ‚Gebiss-gewöhnten’ Gruppe. Diese Gruppe zeigte auch eine höhere Prävalenz an Speichelproduktion (‚sichtbares Speicheln’) und ‘Kopfschütteln’ mit Gebiss. Dies weist darauf hin, dass regelmäßiges Arbeiten mit Gebiss Verhalten induziert, dass in Pferden, die sonst ohne Gebiss geritten werden weniger häufig auftritt.
Wir konnten keinen Unterschied in den Kortisol-Konzentrationen finden. Dadurch können wir nicht mit Sicherheit sagen, ob Gebisse eine Stressreaktion im Pferd auslösen. Trotzdem zeigt unsere Studie, dass Gebisse ‚Fressens-assoziierte’ Verhaltensweisen und ‚Kopfschütteln' induzieren. Dies suggeriert, dass ein Gebiss nicht nur Konzentration und Leistung beeinträchtigt, indem es das Pferd ablenkt, sondern auch ein Tierschutzthema ist, indem es aversives Verhalten hervorruft.

2. Introduction

Since their domestication 3.500 years BC (INT. MUSEUM OF THE HORSE) horses have been used for human purposes, including farm work and more recently competitive riding or recreational pursuits (COOK, 2006). Humans have invented different devices such as bridles to control them. '*Bridles*' comprise all forms of headgear used for horse riding and handling. They enable communication between rider and horse through the reins attached to it.

Today the most common devices are bridles with a mouthpiece that is attached to the reins leading to the rider's hands. The market of alternatives without a mouthpiece is increasing as more and more studies on the effects of the bit linked to the horse's welfare are published (E.G. GEYER & WEISHAUPT, 2006; TREMAINE 1998; COOK, 2003). Authors from previous studies describe drawbacks that range from interference with locomotion (GEYER & WEISHAUPT, 2006), to injuries amongst others in the horse's bars, tongue and hard palate (E.G. TREMAINE, 1998; COOK, 2003; JOHNSON, 2002; BENNETT, 2005) and conflict behavior due to aversion to the bit (E.G. LYNCH & BENNETT, 2000; COOK, 2003). In comparison to bridles with a mouthpiece the reins of bitless bridles are attached to a noseband and thus exert pressure mostly on the nose (LYNCH & BENNETT, 2000). Data on negative effects of bitless bridles are rare.

Although numerous studies have been conducted on behavioral effects induced by the bit, physiological data underlining the observed effects are missing.

In this study we want to investigate whether the bit leads to stress in horses. Therefore we combined measurement of Stress hormone levels (*Cortisol*) and behavioral observations. All horses were tested twice- once wearing a bridle with a mouthpiece and once wearing a bitless alternative- and the results were compared. The reins were tightened both times, so that the horses experience pressure comparable to the pull of a rider's hands. We await to observe aversive behavior when the reins are tightened and thus pressure is exerted either in the mouth (with bit) or on the nose (without bit). We expect to see more aversive behavior and a higher increase in the Cortisol level with the bit.

Additionally, we subdivided the horses into a bit-naïve (horses used to bitless bridles) and a bit-familiar group (horses used to bridles with a mouthpiece). We hypothesize that the amount of aversive behavior is higher in the bit-naïve group. They may react stronger to the bit than horses usually worked with these devices. Consequently, we also expect a higher increase in the Cortisol level in the bit-naïve group.

Our aim is to explore whether or not the use of a bit contributes to stress in horses. We want to investigate if the use of a bitless bridles leads to less stress-signs and therefore constitutes a softer alternative to the common bridles with a mouthpiece.

2.1. Bits and Bridles

It is easy to forget that riding without a bit is a much older technique than riding with a bit, as the use of a bit to control a horse has become so generally accepted in the last centuries. However, bitless riding was widespread around the world since the horse's domestication (INT. MUSEUM OF THE HORSE). Back then, riders probably used nothing more than a cord around the horse's neck and maneuvered the horse with a stick. Scientists assume that Native Americans have invented the precursor of today's bits by using a loop of horsehair, rope or rawhide around the horse's lower jaw (RUSSELL ET AL, 1996). Figure 1 shows a variation of this form. Bits similar to those we know today were introduced quite rapid after that. The first curb bits recorded were used by the Celts of Gaul in the fourth century BC (ANDERSON, 1961). Figure 2 shows a Curb Bit from the Bronze-age.

Fig. 1. '*War bridle*' used by the Native Americans[1].

Fig. 2. Bit from the Bronze-age[2].

[1] https://www.westernhorseman.com/wh-blogs/neu-perspectives/2807-riding-in-a-war-bridle
[2] http://worksofchivalry.com/bronze-age-bits/

The idea of controlling a horse by putting pressure on the nose region didn't disappear and was reapplied in the Western horsemanship in America (COOK, 2006).

2.1.1. Bits

A *bit* is defined as the part of the bridle lying in the horse's mouth. It is placed in the intermandibular space, called the *bars*, between the molars and the front teeth. Figure 3 and 4 show the exact position of the bit in a horse's mouth.

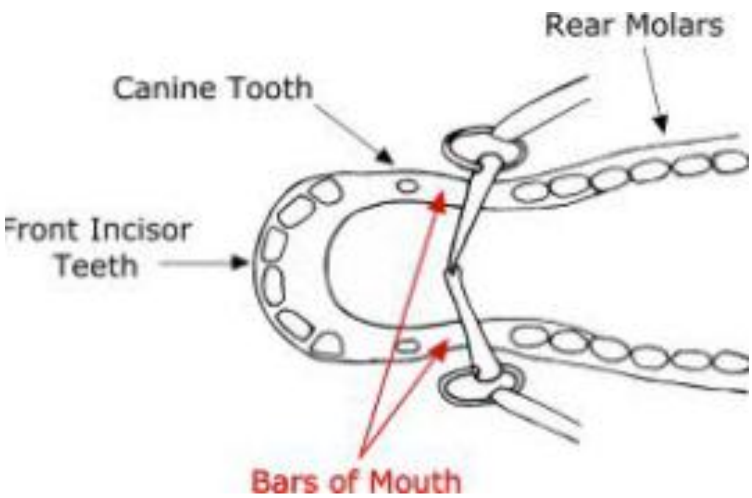


Fig. 3. **Fig. 4.**

Position of the mouthpiece. Fig. 3. Outside view on the position of the mouthpiece (blue) within the horse's mouth[3]. Fig. 4. Inside view on the exact position of the bit between the horse's teeth[4].

Nowadays, a great variety of bits exist exerting pressure on different parts of the horse's mouth including tongue, bars, hard palate and lips (BENNETT, 2005). They can be divided into *snaffle bits* and *leverage/curb bits* (see Figure 5 and 6). Snaffle bits have rings that attach to the cheeks of the bridle and the reins (LYNCH & BENNETT, 2000; BENNETT, 2001). They lead to a direct line of pull from the rider's hands to the horse's mouth. In leverage/curb bits on the other hand the bridle attaches to a ring above the mouthpiece whereas the reins attach to a ring below the mouthpiece leading to an amplification of the rider's pull (LYNCH & BENNETT, 2000; BENNETT, 2005).

[3] http://www.sustainabledressage.net/tack/bridle.php#placement
[4] https://tackandtalk.wordpress.com/2011/08/23/a-bit-of-advice/

Fig. 5.

Fig. 6.

Snaffle Bit vs. Curb Bit. Fig. 5. Western horse wearing a snaffle bit[5]. The reins and the bridle attach to the same ring. Fig 6. Western horse wearing a curb bit[6]. The reins attach to the end of the shanks whereas the bridle attaches to the top part.

The severity of bits is amongst others determined by their size and shape (LYNCH & BENNETT, 2000; ENGELKE & GASSE, 2002). If the mouthpiece is too short it pinches the corners of the lips against the cheek teeth. If it is too long it may move sideways, putting the port or joint out of position leading to possibly painful pressure points (BENNETT, 2006). Moreover the ideal position for the bit in the interdental space varies from horse to horse and bit to bit. The common rule to adjust the bit so that the commissures of the horse's lips are pulled into one or two wrinkles impedes a relief at the corners of the mouth when the reins are released (LYNCH & BENNETT, 2000; BENNETT, 2001, 2005) leading to permanent pressure in the mouth region. Another point to consider is that the oral cavity changes as the teeth continuatively erupt (SCOGGINS, 2001). Consequently, a once comfortable bit may become uncomfortable or even painful (BENNETT, 2006). Generally, dental abnormalities like shed premolar caps or sharp enamel points, can lead to bit-induced pain (SCOGGINS, 2001).

This short overview of different kinds of bits and the different needs of horses indicate how difficult it is to find and fit the perfect bit.

[5] http://www.horsechannel.com/horse-experts/western-horse-training-advice/snaffle-or-curb.aspx

[6] http://www.horsechannel.com/horse-exclusives/online-bit-guide.aspx

2.1.2. Bitless Bridles

Bitless bridles don't have a mouthpiece. The signal from the rider's hand is mainly transferred to the horse's nose bridge. The noseband should be placed on the dorsal surface of the nose above the rostral extremity of the nasal bone (Bennett, 2005). If it is placed too low it puts immense pressure on the nasal cartilages leading to interference with breathing. Depending on the model they can also exert pressure on the lower jaw, cheeks and neck (Geyer & Weishaupt, 2006). Bennett (2006) describes the following four types of bitless bridles.

The *Traditional Hackamore* consists of a *bosal*, which is a rawhide or leather noseband around a rawhide core, a headpiece and the *mecate* (reins) (see Figure 7) (Lynch & Bennett, 2000; Bennett, 2005). The *Mechanical Hackamore* consists of a noseband and a curb chain attached to metal shanks (see Figure 8) amplifying the pull on the reins (Bennett, 2005; Bennett, 2006; Cook & Strasser, 2003). The *Side Pull* is comparable with a halter and can be described as the bitless variation of a snaffle bit (Cook & Strasser, 2003). It consists of a noseband with rings on both sides of the mouth, where the reins are attached (see Figure 9) (Bennett, 2005). Cook's *Bitless Bridle 2000* consists of two loops. Those loops cross under the horse's chin and form a figure-eight configuration (see Figure 10). If a rider pulls on the reins the pressure is distributed on poll, cheeks, chin, nose and behind the ears (Cook, 1999).




Fig. 7. . **Fig. 8.** **Fig. 9.** **Fig. 10.**

Different forms of bitless bridles. Fig. 7. Traditional Hackamore[7]. Fig. 8. Mechanical Hackamore[8]. Fig. 9. Sidepull[9]. Fig. 10. Dr. Cook's Bitless Bridle[10].

[7] https://www.dmtack.com/products/hu4-bosal-1-inch/
[8] https://www.thespruce.com/all-about-mechanical-hackamores-1886064
[9] https://www.pferdefluesterei.de/produkt/sidepull-amber-von-barefoot/
[10] https://www.actionridertack.com/Bitless-Bridles-by-Dr-Cook-Leather-p/105.htm

The main advantage of riding bitless is that interference with the horse's sensitive mouth is avoided (BENNETT, 2006). Nevertheless immense pressure can be applied with these advices as well. However, no studies exist describing negative effects.

2.1.3. Effects of the bit

Several studies showed that bits can cause discomfort for horses in different ways. The following chapters are to summarize those findings.

Effects on behavior

MANNING (1979) already described the similarities between reflexes triggered by stimuli and complex behavior. A possible stimulus caused by the bit is assumed to be 'pain' because the mouthpiece is placed in the interdental space where the teeth ridge is badly padded and subsequently extremely sensitive to mechanical stimulation. The sensory pathway is the *Trigeminal Nerve*, which is associated with sensations in the face and controls activities including biting and chewing, as its branches amongst others are located underneath the interdental space. In many cases pain sensations are expressed through complex behavior (CAREY ET AL., 2016), also referred to as *conflict* or *aversive behavior*. GÓRECKA-BRUZDA AND COLLEAGUES (2015) describe conflict behavior to be '*a response exhibited by animals that experience difficulty coping with mental or physical discomfort and is most often demonstrated as some form of resistance to handling or training cues and/or equipment*.'

COOK (2003) lists conflict behavior eliminated by removal of the bit and use of the bitless bridle observed in a study published in 2003. His paper is based on reports from 605 users switching to his bitless bridle between 1997 and 2002. Table 1 lists the most frequently observed behaviors.

Table 1. Cited adverse behaviors in 605 reports of riders showing also the main bodily systems affected (N = nervous system, R = respiratory system, M/S = musculoskeletal system). Copied from COOK (2003).

Behaviors	**System**		
	N	**R**	**M/S**
Headshaking, head tossing or flipping	+	+	+
Chomping, teeth grinding, fussing with the bit, evading contact	+	+	+
Difficult to bridle, holds head high, panics at sight of bridle	+		
Above the Bit (poking nose in the air), high-headed, avoiding The bit	+		+
Difficult to steer, inability to travel straight	+		+
Anxious expression, 'unhappiness' when exercised	+		
Stiff-necked and locked-jaw, reluctant to flex at the poll	+		+
Lack of self-carriage, absence of 'collection', poor balance	+		+
Heavy on the forehand, leaning on the bit, tongue over bit, low-headed	+		+
Tongue behind the bit, roaring, DDSP, gurgling, laryngeal stridor	+	+	
Incoordination, stiff or choppy stride ('bridle lameness'), short stride giving slower speed	+		+
Gaping of the mouth/open mouth, constant jaw and tongue movement	+	+	
Pulling on the bit	+		

Other authors have also described some of these behaviors in horses ridden with bits. JOHNSON (2002) describes behavioral signs including going behind the vertical, head flipping and tongue hanging. CAREY AND COLLEAGUES (2016) name head shaking/tossing and tail swishing.

In addition, the pressure on the interdental space irritates the mandible. COOK (1999) proposes that pressure on the mandibular branch is referred to other branches (especially the second and third trigeminal nerve) leading to facial, eye and/or ear pain (see Figure 11). This may lead to the *head-shaking syndrome* but can also peak in other neurological signs including head rubbing, blepharospasm and photophobia.

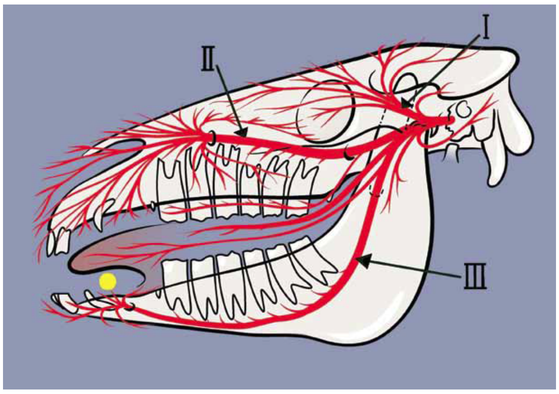
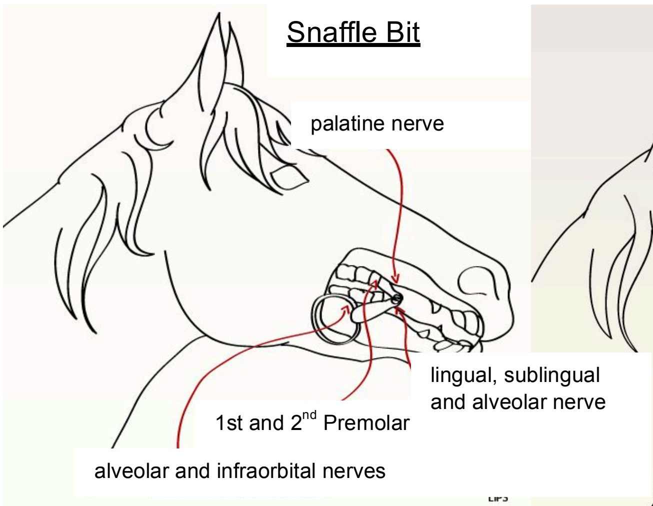


Fig. 11. Distribution of the Trigeminal Nerve and Position of the Bit (yellow). Facial Neuralgia develops when pain signals caused by the Bit are transferred along the II (causing facial pain) and III (causing eye and/or ear pain) nerve[11].

Pressure points

By pulling on the reins the rider can exert immense pressure in the horse's mouth, especially the interdental space. The underlying nerves transmit these impressions to the brain. Figure 12 and 13 demonstrate the different pressure points of snaffle and curb bits.



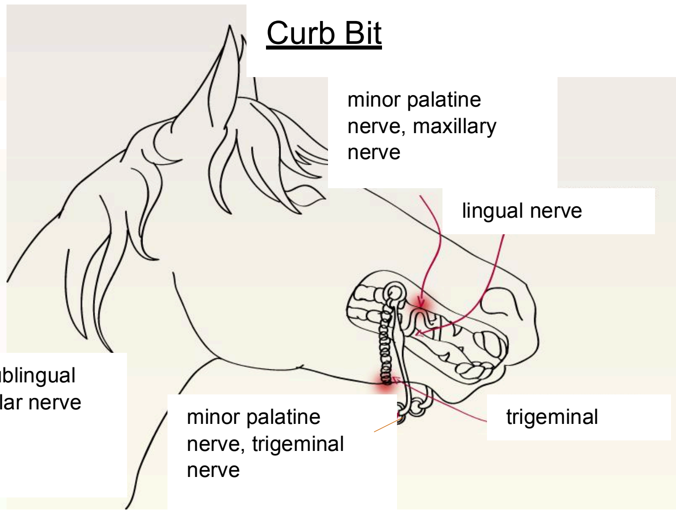


Fig. 12. **Fig. 13.**

Drawings indicating the pressure points caused by the snaffle and curb bit. Fig. 12. Pressure points caused by the snaffle bit[12]. The snaffle joint digs into the palate and tongue. The mouthpiece presses against the first and second premolar as well as on the interdental space and the lips. Notice the impact on the underlying nerves. Fig. 13. Pressure points caused by the curb bit[13]. The mouthpiece's port presses against the palate. The bit squeezes the tongue and presses against the interdental

[11] Carley Sparks: Are Bits "Bronze Age" Technology? in Horse Sport June 2012. p. 54.
[12] http://horseconscience.blogspot.co.at/2012/08/the-principles-of-snaflle-and.html
[13] http://horseconscience.blogspot.co.at/2012/08/the-principles-of-snaflle-and.html

space. The chain presses against the lower chin. The shanks (arms) multiply the effects. Notice the impact on the underlying nerves.

Punctual pressure points can lead to various injuries. The most obvious injuries associated with the improper use of bits are lacerations of the tongue (BENNETT, 2005). Tissues can moreover become trapped between the bit and the first lower cheek teeth, where it gets pinched and cut leading to painful ulcers. By continuing bit use the tissue gets irritated each time the bit moves (SCOGGINS, 2001).

Most bit-induced injuries are superficial and heal quickly because of the high blood supply in the mouth and the antibacterial action of saliva (JANSSON ET AL., 1998; SMITH, 1993). Still, severe injuries can leave permanent defects. Persistent trauma in the interdental space can lead to penetration of the mandible. This, in the worst case, can result in *mandibular periostitis (bone spurs)*, which can be described as additional bone formation (JOHNSON, 2002) (see Figure 14). Most horses suffering from bone spurs have been reported to be high performance horses ridden with a lot of bit contact (SMITH, 1993).

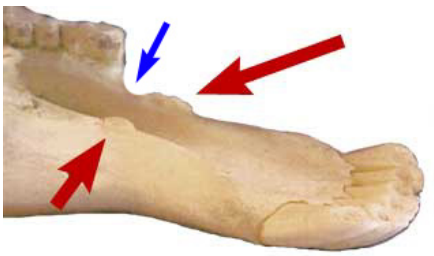


Fig. 14. Mandibular periostitis[14]. Bone growth on the bars caused by the wear of a bit (indicated by the red arrows).

COOK (2011) examined 66 jawbone specimens from domestic horses in three Natural History museums and found out that 62% of the jawbones showed bone spurs in the interdental bars. Other authors also showed skeletal evidence for bit-induced mouth injuries that were absent or rare in feral equidae (VAN LANCKER ET AL., 2007; MATA ET AL., 2015).

[14] http://www.sustainabledressage.net/tack/bridle.php#placement

Effects on physiology

Evolutionary, horses are obligate nasal breathers (HINCHCLIFF ET AL., 2014) as naso- (respiratory system) and oropharynx (digestive system) are separated by the *soft palate (*see Figure 15). MELLOR AND BEAUSOLEIL (2017) observed 150 feral horses filmed during roundups in Australia, France, New Zealand and USA. Free running horses have a closed mouth, sealed lips, an immobile tongue and jaw and an empty, relatively dry oral cavity. Horses ridden with bitless bridles keep their mouths also closed when exercising (COOK, 2002; QUICK & WARREN-SMITH, 2009; HANSON & COOK, 2015). COOK (2002) predicts that when horses start walking or moving at higher paces they swallow. In combination with an airtight lip-seal this causes a negative pressure in the oropharynx, holding the soft palate against the root of the tongue and thereby widening the nasopharyngeal airway (HINCHLIFF ET AL., 2014; COOK, 1981).

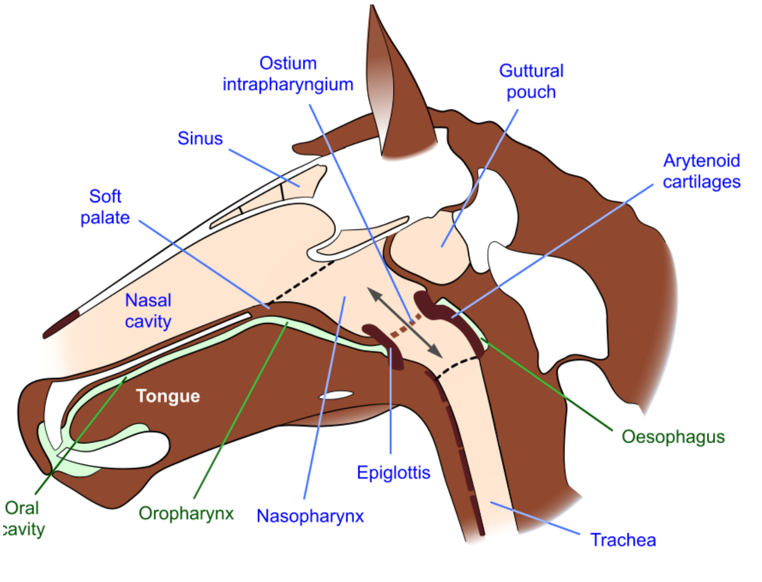


Fig. 15. Drawing indicating the position of the soft palate and larynx of a horse while breathing with the mouth closed. The soft palate presses against the Oropharynx enlarging the Nasopharynx. [15]

The bit being a foreign body in the horse's mouth initiates digestive system responses including salivation, chewing, swallowing, tongue and palate movement. The presence of the bit itself and especially the mentioned bit-induced behaviors break the airtight lip-seal. This may dissipate the negative pressure in the airway. Consequently, this destabilizes the soft palate and dorsally displaces it into the nasopharynx during inspiration. The cross-sectional area decreases leading to an

[15] Mellor, D.J., Beausoleil, N.J.: Equine Welfare during Exercise: An Evaluation of Breathing, Breathlessness and Bridles. Animals 2017; 7 (6); p. 41.

increase in airflow resistance (Hinchcliff et al., 2014). In summary, this makes breathing harder for the horse.

A galloping horse takes one stride for every breath (Ducharme & Cheetham, 2014). Consequently, the bit interfering with breathing- by inducing swallowing and thereby breaking the breathing rhythm- also interferes with striding. Described effects include stumbling and a loss of the normal fluidity of gait (Ahern, 1999). As swallowing or attempting to swallow leads to saliva production this may lead to cough reflexes when saliva contacts the larynx (Hinchcliff et al., 2014). The saliva produced during exercise has to either be swallowed or it floods the lungs. Repeated inhalation of saliva can irritate the lungs and lead to inflammation (Cook, 2002).

When we put a bit into the horse's mouth the digestive system is activated and the horse thinks 'eat'. Then we want the horse to move making it think 'exercise'. This confuses a horse cognitively as we expect it to do two things at one time it naturally never would (Hinchcliff et al., 2014). This can be further detailed when we look at the neurophysiological and hormonal processes. During eating, horses are in a relaxed state of mind, controlled by *cholinergic responses*, essential to produce saliva. During exercising *adrenergic responses* dominate enabling the horse to flee efficiently, for example from predators (Hinchcliff et al., 2014).

Together all the described physiological, neurological and musculo-skeletal signs lead to a horse's inability to focus on the work and unable for high performance (Cook, 2003).

2.1.4. With bit vs. bitless

There are only a few studies on the effect of bitless bridles. Most of them compare bitted and bitless bridles in respect to behavior and performance and suggest a change for the better (Cook, 1999, 2003; Cook & Strasser, 2003). Quick & Warren-Smith (2009) tested 4 horses in foundational training and detected that horses wearing a bitless bridle performed at least as well, if not better, than horses wearing a snaffle bridle. Cook (2013) investigated the before-and-after behavior in 56 horses that were changed from a bitted to a bitless bridle. His study shows a significant reduction of conflict behavior. Carey and colleagues (2016) examined the effect of bitted and bitless bridles on 8 horses that were changed from bitted to bitless within therapeutic riding sessions. They found out that negative/aversive behaviors were higher in horses wearing a bridle with a mouthpiece.

Although there are numerous studies indicating a behavioral change when switching from a bitted to a bitless bridle some studies also found counter-indicating results. SCOFIELD & RANDLE (2013) compared the performance of 20 horses when ridden with a bit versus bitless and found no significant difference in the horse's behavior. Still, no further details are described in their paper. A study from MANFREDI AND COLLEAGUES (2005) compared the effects of different bits and bridles on the frequency of induced swallowing. They couldn't detect a difference between bitless and bitted although it was suggested that the presence of a bit increases salivation and interferes with swallowing. BENNETT (2006) states that riding problems and mouth injuries are caused by the wrong bit in combination with the wrong hands controlling the reins.

We can see that previous studies indicate that the bit causes diverse problems for horses. Still, the counter-indicating results propose further research on this topic. The described effects of the bit raise the question if wearing a bit is stressful for the horse.

2.2. Stress

The main hypothesis of this work is that the bit is painful and thus induce a physiological stress in horses. The following chapters are to describe what 'Stress' is and how it affects the body and the behavior.

Stress, as originally described by SELYE (1946), is a very complex concept investigated by many authors in different ways. For the maintenance of life it is critically important to keep the internal milieu constant in a changing environment (BERNARD, C. 1865). CANNON (1929) called this process *homeostasis*. Mainly, stress is the physiological response to reestablish this stable internal environment in the body. ROMERO (2004) describes a *Stressor* as any 'noxious stimulus' and the reaction to such a stressor- called *Stress response*- as 'a suite of physiological and behavioral coping mechanisms'. First, a stressful event is registered by the sensory system. The gathered information is transmitted to the central nervous system (brain) where it is processed. The resulting stress response is performed by two endocrine systems mediated through different hormones (see Figure 16).

The *sympatho-medullary system* works fast and triggers a so called *fight-or-flight* reaction by affecting behavior, metabolism and the cardiovascular system. It enables short-term physical and mental maximum performance enabling the animal to react appropriately to sudden dangers such as predators or storms (Romero, 2002) and ensure survival. Typically, it doesn't impose a health burden (Schneiderman et al. 2005). This system is mediated by the *Catecholamines Adrenaline* and *Noradrenaline*.

The *pituitary-adrenal system* on the other hand affects gene transcription and has delayed, but more sustained effects. The involved hormones, the *Glucocorticoids*, modulate behavior, metabolism, immune system and energy production (Romero, 2007).

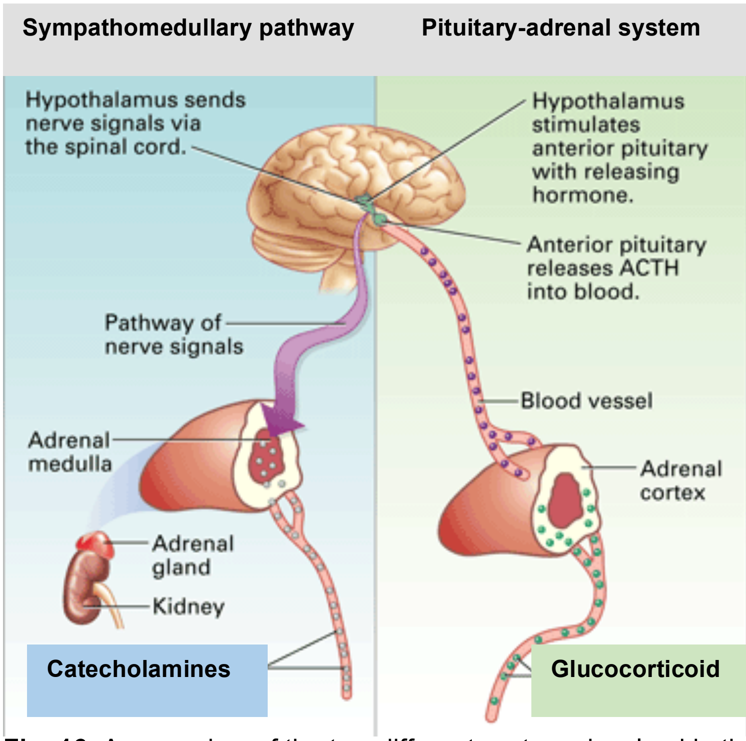


Fig. 16. An overview of the two different systems involved in the stress response[16].

To summarize, both systems have the aim to help individuals to survive a stressful event. In an evolutionary context, the goal of responses to short term stress is adaption to changing environmental conditions. The elicited stress responses differ according to the situation and context. In addition, individual differences in stress responses to the same situation have been observed (e.g. Lacey & Lacey 1958; Ebner & Singewald, 2017). Some tend to show stress responses associated with active coping;

[16] modified from http://www.glutenfreehomestead.com/2015/04/soooo-fatigued/

others tend to show stress responses associated with aversive behavior (KASPROWICZ ET AL. 1990, LLABRE ET AL. 1998).

Any reaction to Stressors is accomplished through the two following features. First, stress hormones are released for energy mobilization. Second, energy distribution is altered. More precisely, energy is distributed to the skeletal muscles and the brain, as they are crucial for fight-or-flight behaviors. Additionally, cells of the immune system are activated. These cells are in position to fight microbes entering the body through wounds. Their goal is to facilitate and fast track healing (DHABAR & MCEWEN, 1997). On the other hand, less important activities, in other words processes being detrimental to physical activity and even survival, become deferred (SCHNEIDERMAN ET AL., 2005).

In this case Stress is beneficial for the individual's adaption and survival. However, if a stressor becomes constant it may lead to severe, prolonged stress responses in the animal's system, which can have negative health impacts triggered by dysfunction of the HPA-axis including tissue damage and disease (MCEWEN, 2017). Stress-elicited chronic stimulation of the cardiovascular system, for example, leads to sustained increases in blood pressure culminating in vascular hypertension. Long-term high blood pressure is known as a major risk factor for coronary artery disease, stroke, heart failure, vision loss and dementia (LACKLAND ET AL. 2015; MENDIS ET AL. 2011). Moreover, high Glucocorticoid levels suppresses immunity (SEGERSTROM & MILLER, 2004). KIECOLT-GLASER AND COLLEAGUES (2002) name effects including slower wound healing and recovery from surgery, poorer antibody responses to vaccination and higher vulnerability to viral infections. GCs can moreover suppress the gonadal axis (FREE & TILLSON 1973; MOBERG 1985) which is essential for successful reproduction. Long-term exposure to GCs can lead to deleterious effects, for example neuron death. (SAPOLSKY, 1992). This suggests that a balance between GC-concentration to survive noxious stimuli and the concentration to prevent deleterious exposure is important (ROMERO 2002).

Animals are also known to adapt their stress responses when a certain stressor occurs repeatedly. STONE (1979) was the first to investigate the adaptation to chronic (footshock) stress in rats. He found out that adaptation to chronic stress leads to a loss of depressive-like symptoms after about 2 weeks by increasing resistance to stress due to development of subsensitivity of noradrenergic receptors. A work from DAL-ZOTTO AND COLLEAGUES (2000) on rats in a repeated forced swimming test (FS) showed that repeated experiences with FS reduced struggling (fight) behavior and a faster recovery of plasma corticosterone compared to FS-naïve rats. The researchers suggest that the observed differences in behavior and physiology are a consequence to the repetition of the stressful situation. JONES AND COLLEAGUES (2016) found out that physiological adaptation to chronic stress in humans differ individually.

Habituation is a very important compound in the process of domestication. Animals had to adapt to the environments humans kept them in (PRICE, 1999). This lead to changes in morphology, behavior and physiology, including increased stress tolerance. Research has shown that domesticated species, including horses, show attenuated behavioral and physiological stress responses compared to their wild counterparts (E.G. TRUT ET AL., 1999; KÜNZL & SACHSER, 1999). Scientists compared the stress responses of the domesticated White Leghorn chickens and the *Red Junglefowl*- the wild ancestor of all domesticated breeds, and found out that the expression of stress-related genes in the brain, the pituitary and the adrenals changed during chicken domestication. These changes cause a reduction of fear related behavior (CAMPLER ET AL., 2009) and a delayed return to baseline in the domesticated breed (ERICSSON ET AL., 2014). Similar patterns between wild and domestic counterparts have been observed in other species.

2.2.1. Quantification of stress

Stress level can be examined in different ways. Quantification of GC concentrations are often used as a parameter indicating stress. We know two subclasses of GCs: *Corticosterone* and *Cortisol*. Cortisol is the predominant Glucocorticoid in horses. It is formed in the adrenal gland and then released into the blood stream. In horses and other mammals, 67 to 80 % of the cortisol is bound to *corticosteroid-binding globulins (CBG*) (GAYRARD ET AL. 1996). Nonetheless, only free cortisol can penetrate target tissue, bind to intracellular receptors and trigger a biological response (PEROGAMVROS ET AL., 2012).

A lot of research has been conducted on the role of GCs. We do know that GCs are crucial for survival. An experiment on adrenalectomized rats (adrenalectomy completely removes GCs) showed that even mild noxious stimuli can lead to death in those rats (Darlington, 1990).

Research on the magnitude of GC release has shown that the amount of GC release to the identical stimuli is remarkably consistent throughout an animal's life. Exceptions have been found depending on the animal's degree of control over the noxious stimulus (reviewed by Levine et al. 1989), pregnancy (Smith & Thomson 1991), early development (Sapolsky & Meaney 1986), changes in social rank (Sapolsky, 1987) and some pathological changes during aging (Sapolsky, 1992).
Recent research has shown that GC concentrations are modulated seasonally in some free-living species (e.g. Gesquiere et al., 2011). This concerns both basal and stress-induced GC concentrations. Anyhow, it is still unknown which factors trigger these variations, such as photoperiod, temperature or food availability. (Romero, 2002).

Because the brain controls the GC release, the rise of GC concentrations from baseline levels are considered an indicator for an individual's psychological condition in different situations (Thun & Schwarz-Porsche, 1994).Janssens and colleagues (1995) for instance found an increase in plasma GC in pigs in bad husbandry. In horses, acute illnesses lead to an increase in plasma GC (Hoffsis et al., 1970).Hellhammer and colleagues (2009) describe changes is Cortisol level in horses caused by age, gender, oral contraceptive and medical conditions. Moreover, increased Cortisol levels in exercising horses can be partly caused by the performed physical activity (Schmidt et al., 2010).

Cortisol secretion follows a circadian rhythm. Edwards and colleagues (2001) describe a rise in the morning with a subsequent decline throughout the rest of the day with a nadir in the evening. The degradation of Cortisol is carried out in the liver, the kidneys and the salivary glands, enabling measurement in blood, saliva, escreta (feces and urine) and integumentary structures (hair and feathers). Which to choose depends amongst others on the research question, (e.g. whether the effect of acute or chronic stressors is investigated) and the species (e.g. which degree of invasiveness is possible and/or desired). All four materials demand a certain degree of expertise. First of all in obtaining the sample and second in extracting and analyzing the GCs (SHERIFF ET AL., 2011).

So far most studies investigating stress in horses, have measured GC concentrations using plasma samples but recently, a non-invasive technique by quantifying GC concentrations in saliva has been put forward with the advantage to avoid venipuncture (SCHMIDT ET AL., 2010). A comparison of serum and salivary GC concentrations underlines the advantage of this noninvasive technique for GC level assessment in horses (PEETERS ET AL., 2010). By using this novel technique several stressful situations for horses have been studied, including new environment (PEETERS ET AL., 2013), transport (SCHMIDT ET AL., 2010) and competition (PEETERS ET AL., 2010).

To enable comparing the rise of GC levels, SCHMIDT ET AL. (2010) suggest measuring basal values in addition to measuring concentrations during stressful events. Previous studies proved that salivary Cortisol concentration in horses differed largely before and after a stressful event. PEETERS AND COLLEAGUES (2010) showed that Cortisol levels after a cross-country event were 340% higher than before starting. A following study of PEETERS ET AL. (2013) measured rider and horse salivary Cortisol levels during competition with regard to their impact on performance. This study showed a rise of salivary Cortisol concentrations during competition of 190% in relation to the basal levels. SCHMIDT AND COLLEAGUES (2010) tested the salivary Cortisol levels during trailer rides and found an increase of 600%.

After obtaining the sample, GCs can be measured with different biochemical techniques. *Immunoassays* for instance are tests to investigate if and how much of a specific molecule is present in a sample (e.g. a saliva sample). In Immunoassays we make use of antigen-antibody specificity by labeling the antibody with specific structures. Consequently, the formed antigen-antibody complex produces signals that are detectable (e.g. because they produce a color change in the solution, fluoresce under light). In our work we used an *Enzyme-linked immunosorbent assay (ELISA)*. It uses color change to identify a substance (see Figure 17) (*http://www.elisa-antibody.com/ELISA-Introduction*).

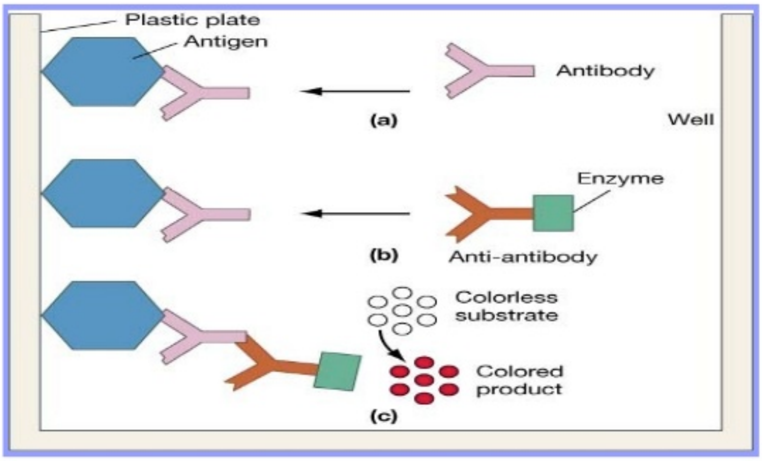


Fig. 17. An overview of ELISA showing the different steps[17]. An Antigen is fixed to a surface (Plastic plate) (a) Antibody binds to Antigen (b) Antibody labeled with an Enzyme binds to Antibody (c) this complex alters the substrate to a colored product that can be detected.

2.2.2. Stress in Horses

Signs of stress in a horse can be subtle. This bears the risk of overlooking or misinterpreting them. Not recognizing and addressing stress can affect a horse's performance and health in a negative way. Often stress signs are simply termed *behavioral problems* because of a lack of understanding.

There are many potential causes for stress in horses. Many of them can be found in a change of routine or a change in the environment (e.g. feed, pasture, isolation from herd, unfamiliar environments). Researchers name various situations domestic horses have to face regularly that may be stressful for them, including physical training (SNOW & ROSE, 1981), equestrian competitions (LANGE ET AL., 1997; CAYADO ET AL., 2006), transport (SCHMIDT ET AL., 2010), veterinary examinations (BERGHOLD ET AL., 2007) and exposure to a new group (ALEXANDER & IRVINE, 1998). After ÖDBERG (1987) inappropriate training is the most important factor for chronic stress in horses. FOREMAN AND FERLAZZO (1996) name transport, workload, lameness and changes in ambient temperature and air humidity as the most common stressors.

[17] https://image.slidesharecdn.com/antibodystructureandthegenerationofb-cell-130320092901-phpapp01/95/antibody-structure-and-the-generation-of-b-cell-74-638.jpg?cb=1363771864

3. Hypothesis and Aim

In this study we investigated whether a *bit* induces a stress response in horses. We quantified the stress response in two different ways. First, we used behavioral observations to document aversive behavior to the bit. Second, we applied Cortisol measurements to see if the observed behavioral effects show a physiological stress response.

Our hypothesis is that due to the effects of the *bit*, described in previous studies, the bit induces a stress response in horses. We expect to observe aversive behavior to the bit, conferming results of other studies. Additionally, we expect an increase in the Cortisol levels based on the bit eliciting a stress response.
This study is carried out with two groups of horses. One group includes horses that are used to wearing a bit when being ridden or lunged (bit-familiar). The other group is bit-naïve, meaning that they don't experience a bit in their mouth very often. Our hypothesis is that the bit-naïve horses show a higher stress response when a bit is applied. We expect to see more behaviors to avoid the pressure in the bars and a higher rise in the Cortisol levels.

The aim of this study is to see if there is a higher stress response to the bit than to a bitless bridle. This work is to investigate whether working horses with bitless alternatives may reduce stress for the horse and thus, increase their welfare.

4. Material and Methods

To test whether horses experience bit-induced stress we compared 19 horses in their behavior and Cortisol levels when wearing a bit vs. without a bit. To do so every horse was lunged twice for 15 minutes- one time with the bit and another time with the bitless alternative. We observed whether horses showed more aversive behavior when wearing a bit. To test for a physiological response we used saliva samples to compare the rise in the Cortisol levels.

Results from former studies, eg. (COOK, 2003), are based on observing ridden horses. Due to the fact that a rider may influence the horse's behavior subconsciously we decided to test the horses without a rider by lunging them. This avoids influence of the horse through other aids from the rider, including leg and seat.

4.1. Frame conditions

4.1.1. Horses

19 horses from three different stables participated in this experiment. The experiment included horses from 13 different breeds from the age of 4 to 20 years. 13 of them belong to recreational riders. 6 of them work in a therapeutic facility. All horses were used to bits or/and bitless bridles and to wearing a lunging girth. 7 horses are used to be trained with a bitless bridle (bit-naïve group). The other 12 horses are more often trained with a bit (bit-familiar group).
Horses were used when the horse's owners signed a letter of agreement. In addition, an application for the ethic committee was penned.

4.1.2. Equipment

We chose two bridles for this experiment. For the bitless trial, a padded *sidepull* was applied. In the other test phase, a *Dee snaffle bit* with one joint was combined with a straight western bridle. The reins were adjusted to a lunging girth.

4.2. Experimental procedure

The tests took place between 10 a.m. in the morning and 2 p.m. in the afternoon. This period was chosen to avoid increased Cortisol levels because of the horses' excitement of feeding before and after this time. Horses didn't eat or drink for at least half an hour before the test trials to assure correct saliva sampling.

Every horse was tested once in two consecutive days wearing two different bridles:– Depending on whether they are more used to wearing a bit (bit-familiar group) or wearing a bitless bridle (bit-naïve group) the familiar bridle type was used first (see Table 2).

Table 2. Experimental setup according to familiar bridle type.

	1st round	**2nd round**
Bit-naive	Bitless	With Bit
Bit-habituated	With Bit	Bitless

First, the horse was brought into the round pen. The first saliva sample was taken and stored in a cool box at about 4°C. All horses except one allowed saliva taking.

Next, the horse was bridled using either the bitted or the bitless variation. The bridles were individually fitted to the horse's head with the following rules: Two fingers between chin and chin strap. One fist between throat and throat strap. Two wrinkles in the corner of the mouth.

The reins were attached to the lunging girth to simulate light rein tension in a way that the horse's neck topline was positioned in one line with the back topline and the horse's mouth was positioned in a 90° angle to the neck topline.

The horses were then moved 15 minutes in the round pen according to a standardized program, including walk and trot.

After 15 minutes the horse was released from girth, reins and bridle. Immediately then, the second saliva sample was taken and again stored in the cool box at about 4°C.

After the test was finished all the samples taken were stored at -20°C.

When all the trials were completed the samples were transported to the University of Vienna using dry ice and stored in a freezer at – 80°C until further tests were applied.

4.3. Data collection

4.3.1. Behavioral observation

Our first parameter to test for bit-induced stress was behavioral observation. For this we chose the continuous sampling method. MILLS & NANKERVIS (1999) among others point out that it is the most accurate method, if behavior is to be registered in its frequency, length and characteristics.
The chosen behaviors chosen behaviors are listed in Table 3.

Table 3. Behavioral categories.

Behavior	Description
Head shaking/tossing	Shaking of head from side to side.
Open mouth	Opening mouth without closing it immediately.
Tail swishing	Tail moving from side to side.
Chewing	Biting with the teeth/Opening mouth and closing it immediately.
Licking	Shortly protruding tongue out of mouth.
Snorting	Breathing loudly through nostrils.
Visible frothing	Production of Saliva.
Pulling down	Moving head up and down with force.

In addition, any striking behavior shown by individual horses was noted.

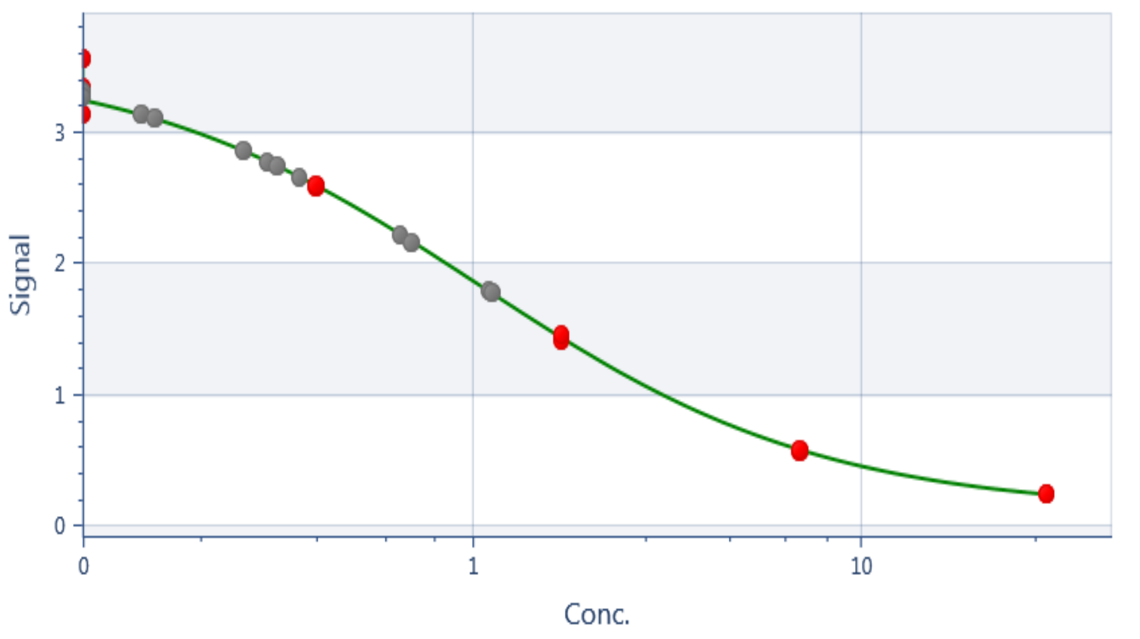
4.3.2. Saliva cortisol

To quantify the stress levels we measured the Cortisol levels twice. Saliva samples were taken before and after the trials using *Salivettes* (Salivette® Cortisol, Code blau from Sarstedt). Therefore the wad of cotton was held into the horse's mouth. After about 40 seconds the wad was soaked with saliva and was inserted into the Salivette.
The samples were then stored at -20°C.

Enzyme-Immunoassay

For this experiment the '*DEMEDITEC Cortisol free in Saliva ELISA DES6611'* Kit was used.

In a first step, a *Parallelism* was conducted to validate the assay for our species. Therefore, 4 additional saliva samples were used to create a pool. Out of this pool a serial dilution was created to test for Parallelism (see Graph 1).



Graph 1. The result of the conducted Parallelism. The red dots show the basal point and the four created Pools. The grey dots indicate additional samples.

Before starting the ELISA all saliva samples were centrifuged at 1692 x g for 20 minutes. After that 50 µl of each *Calibrator*, *Control* and saliva sample were pipetted into wells in duplication. In addition, 50 µl of *Enzyme Conjugate* was added to each well. After that the samples were incubated for 60 minutes at room temperature on a horizontal shaker. Next, the contents of the wells were emptied and rinsed 4 times using diluted *Wash solution* provided by the company (300 µl/well). The washed wells were then filled with 200 µl *Substrate Solution*. After incubating for 30 minutes in the dark at room temperature 50 µl of *Stop Solution* was added to each well to stop the enzymatic reaction. Immediately afterwards, the absorbance of each well was determined using a wavelength of 450 +/- 10 nm.

4.4. Statistical analyzes

The results were analyzed using *IBM SPSS Statistics 24*.
Non parametric tests were applied because the data doesn't follow a normal distribution. As we tested two different groups- one group new to the bit and another group used to the bit- we decided to statistically test these groups separately.

We used the *Wilcoxon signed rank test* to test whether horses showed more aversive behavior and a difference in the Cortisol levels between the trial with the bit and the trial without the bit.

5. Results

The following chapters list the results conducted in this study. All data is demonstrated with *standard values (SD)*.

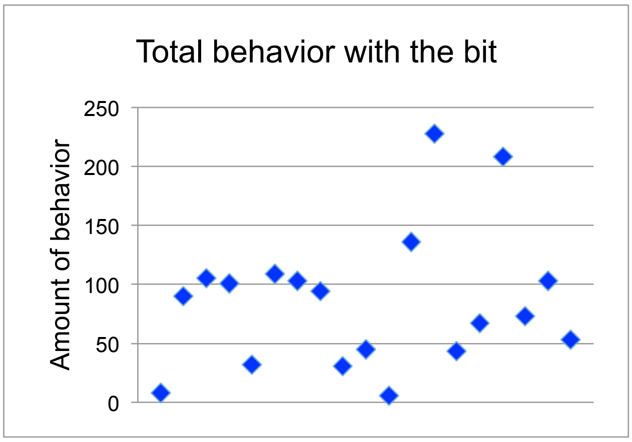
5.1. Behavioral observations

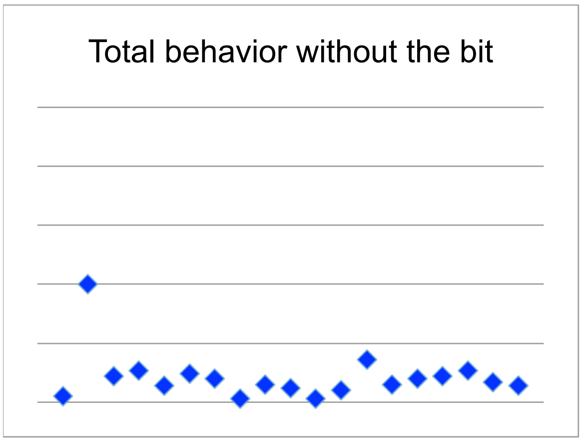
Differences could be observed in four behavioral categories: open mouth, headshaking, chewing and visible frothing. Table 4 shows an overview of the obtained results.

Table 4. Differences in behavior between bit and bitless trial. Wilcoxon paired rank test. Data is statistically significant with a p<0.05. Significant results in bold.

	Bit-naïve group (N = 7)		**Bit-familiar group (N = 12)**	
Behavior	p-value	Z	p-value	Z
Tail swishing	n.s.	1.000	n.s.	0.962
Open mouth	0.066	1.841	**<0.05**	2.673
Headshaking	n.s.	0.813	0.068	1.825
Snorting	n.s.	0.248	n.s.	-0.316
Licking	n.s.	0.593	n.s.	0.983
Chewing	**<0.05**	2.371	**<0.05**	3.059
Pulling down	n.s.	1.289	n.s.	1.442
Visible frothing	n.s.	1.414	**<0.05**	2.449

We found a lot of variation in our results. Some horses showed a lot of behavior, whereas others showed less. This pattern existed in the trial with the bit (see Graph 2) as well as in the bitless trial (see Graph 3).

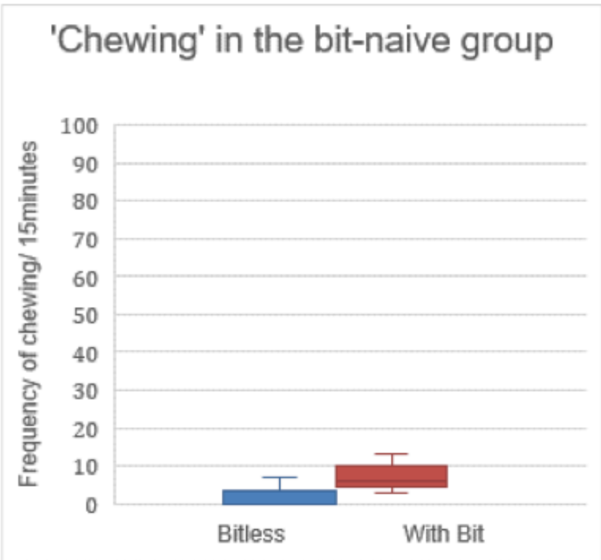


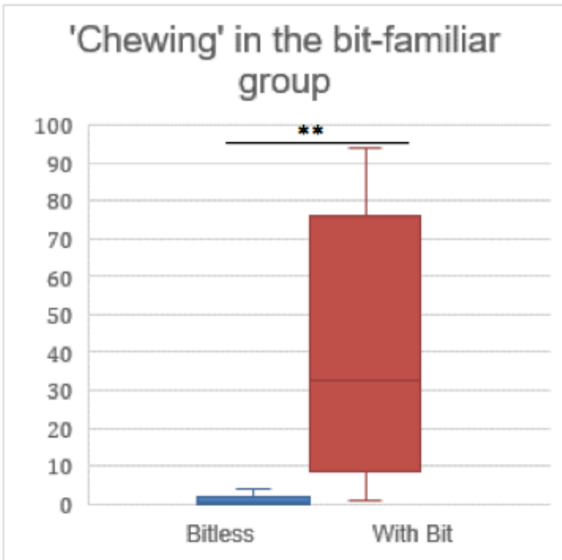


Graph 2. Variation in 'total behavior' during the trial with the bit. One dot represents one horse.

Graph 3. Variation in 'total behavior' during the trial without the bit. One dot represents one horse.

We observed a significant difference in the behavioral category *chewing*. Horses chewed significantly more when wearing a bit. The effect could be measured in both, bit-naïve ($p = 0.018$, $N = 7$, $Z = 2.371$; see Graph 4) and bit-familiar horses ($p = 0.002$; $N = 12$, $Z = 3.059$; see Graph 5). Moreover bit-familiar horses showed a higher rate of chewing when wearing a bit than bit naïve horses.

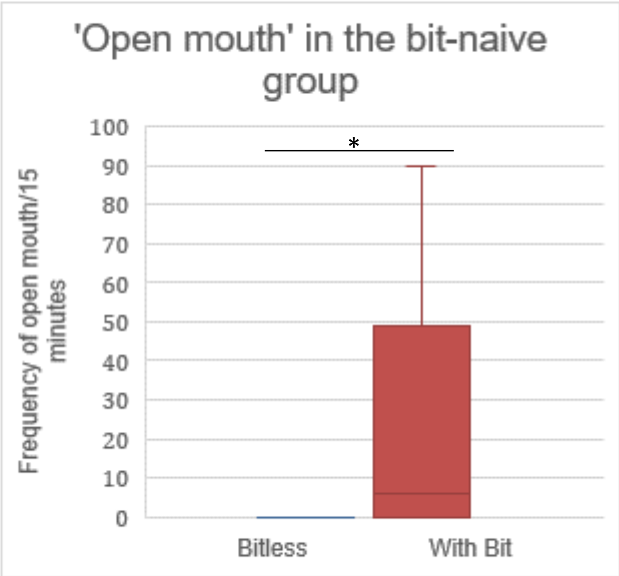


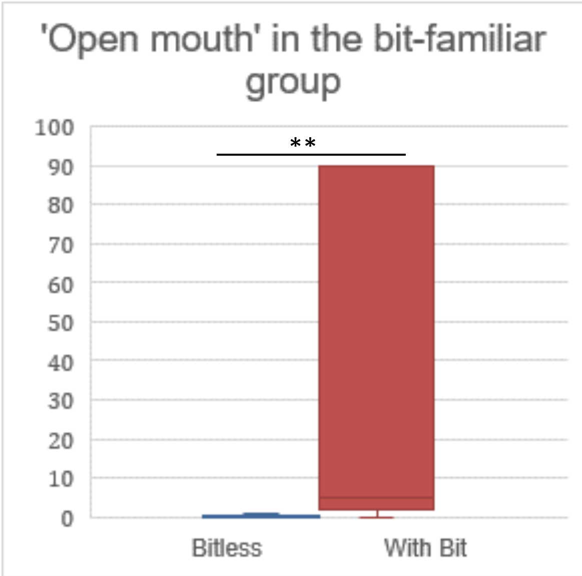


Graph 4. Frequency of 'chewing' in the bit naïve Group.

Graph 5. Frequency of 'chewing' in the bit-familiar group.

A significant difference between the two test series could be found in the behavioral category *open mouth*. Horses had their mouth open more often when wearing a bit. Whereas the effect is highly significant in the bit-familiar group ($p = 0.008$, $N = 12$, $Z = 2.673$; see Graph 7), we noted a strong tendency in the bit-naïve group ($p = 0.066$, N = 7, Z = 1.841; see Graph 6). Overall, 6 horses had their mouth open constantly during the trial. This behavior was only observed in the trial with bit. Still, it was shown by bit-familiar and bit-naïve horses.

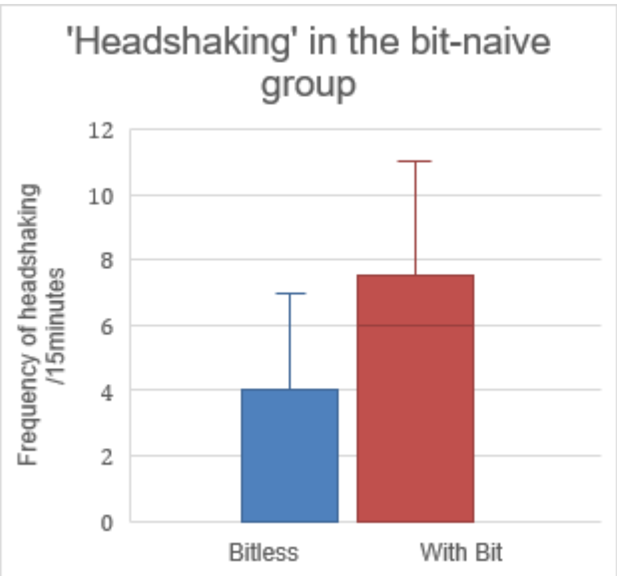


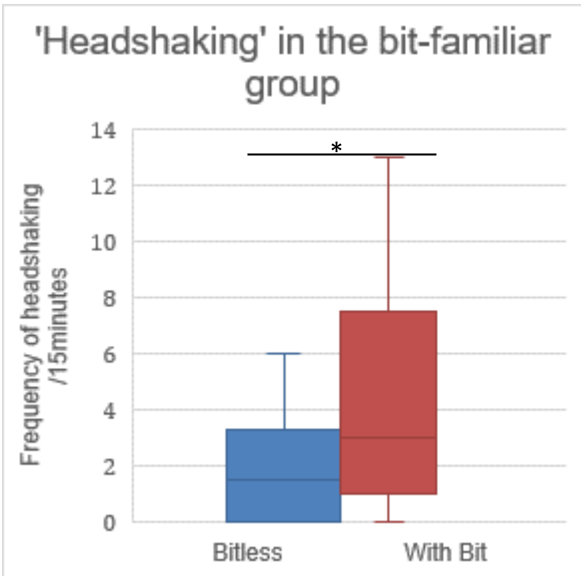


Graph 6. Frequency of 'open mouth' in the bit naïve group.

Graph 7. Frequency of 'open mouth' in the bit-familiar group.

A difference could also be found in the frequency of *headshaking*. Whereas the bit-familiar group showed a tendency of more headshaking when wearing a bit (p = 0.068, N = 12, Z = 1.825; see Graph 9), this effect couldn't be observed in the bit-naïve group (p = 0.416, N = 7, Z = 0.813; see Graph 8).





Graph 8. Frequency of 'headshaking' in the bit naïve group.

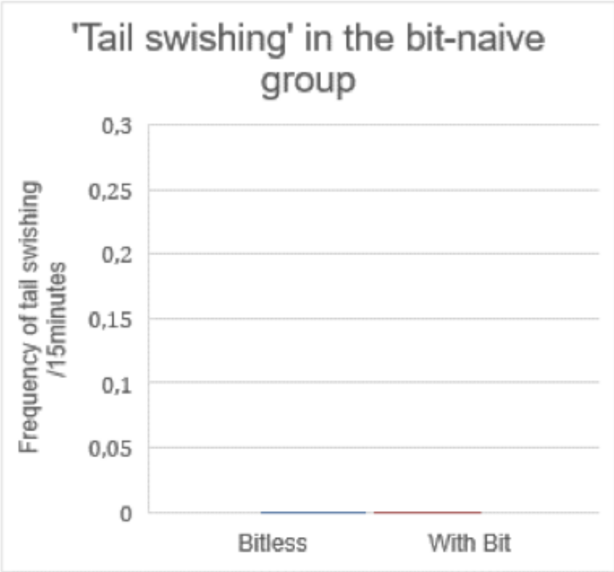
Graph 9. Frequency of 'headshaking' in the bit-familiar group.

Visible frothing during the bitless trial was shown by one horse, whereas half of the participating horses showed visible frothing during the trial with the bit. Whereas the observed difference is significant in the bit-familiar group (p = 0.014, N = 12, Z = 2.449), the observation couldn't be supported statistically in the bit-naïve group (p = 0.157, N = 7, Z = 1.414; see Table 4).

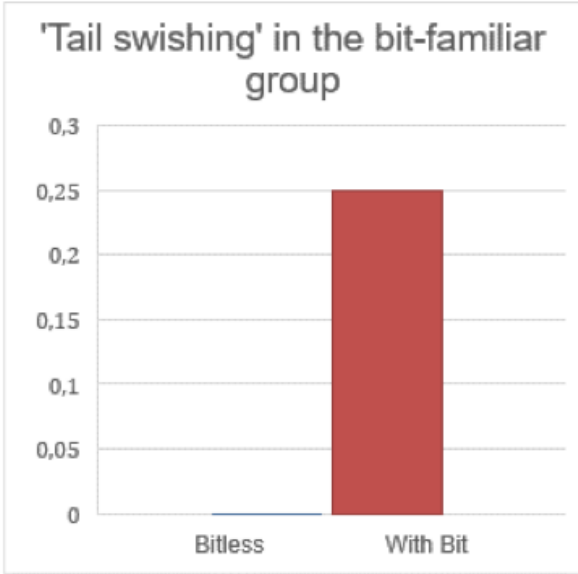
Table 5. Frequency of ‚visible frothing' with and without the bit.

	With Bit	**Without Bit**
Horse 1	**yes**	no
Horse 2	**yes**	no
Horse 3	no	no
Horse 4	no	no
Horse 5	**yes**	no
Horse 6	no	no
Horse 7	**yes**	no
Horse 8	no	no
Horse 9	no	no
Horse 10	no	no
Horse 11	**yes**	**yes**
Horse 12	**yes**	no
Horse 13	no	no
Horse 14	no	no
Horse 15	**yes**	no
Horse 16	**yes**	no
Horse 17	no	no
Horse 18	**yes**	no
Horse 19	no	no

We didn't find any significance in the frequency of *tail swishing*, not in the bit-naïve (p = 0.317, N = 7, Z = 1.000; see Graph 10) nor in the bit-familiar group (p = 0.336, N = 12, Z = 0.962; see Graph 11).

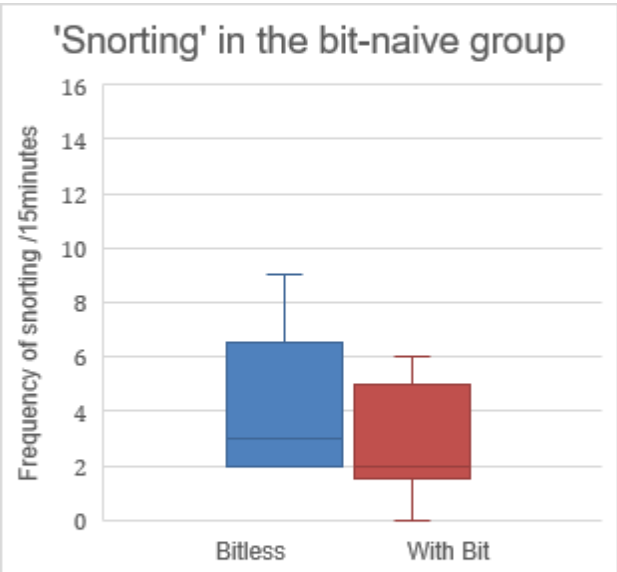


Graph 10 Frequency of 'tail swishing' in the bit-naïve Group

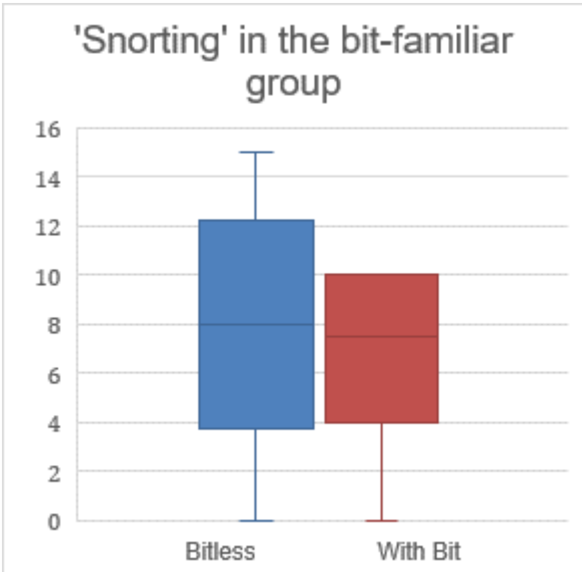


Graph 11 Frequency of 'tail swishing' in the bit familiar Group

We also couldn't detect a significant difference in the behavioral paramater *snorting* in the bit-naïve (p = 0.248, N = 7, Z = -1.156, see Graph 12) and the bit-familiar group (p = 0.752, N = 12, Z = -0.316; see Graph 13).

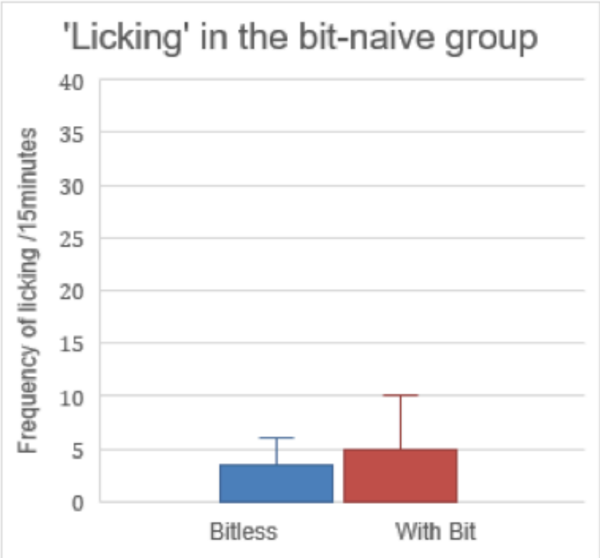


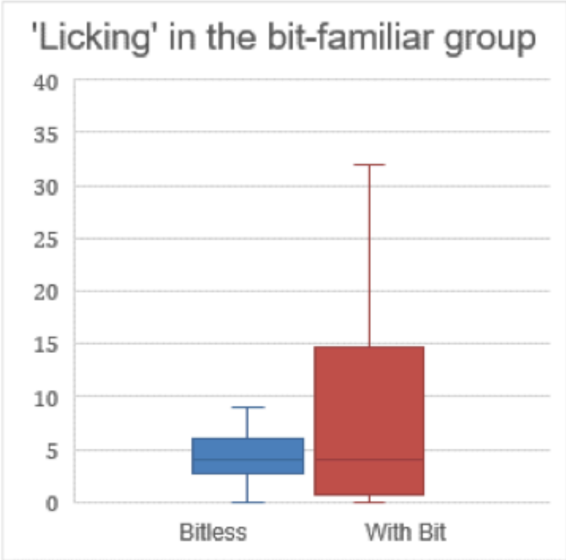
Graph 12. Frequency of 'snorting' in the bit naïve Group.



Graph 13. Frequency of 'snorting' in the bit-familiar group.

No significance was found in *licking* between the bitted and the bitless trial, not in the bit-naïve (p = 0.593, N = 7, Z = -0.535; see Graph 14) nor in the bit-familiar horses (p = 0.326, N = 12, Z = 0.983; see Graph 15).

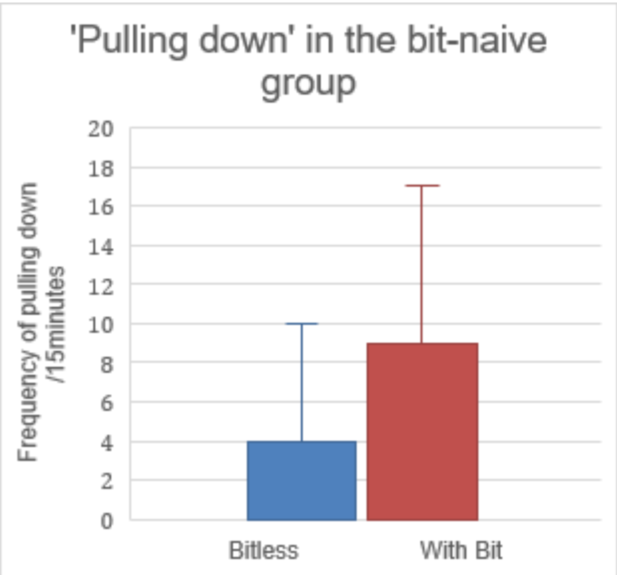


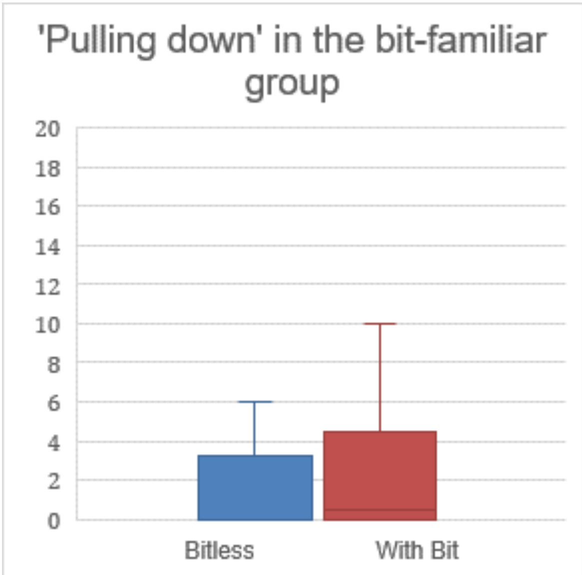


Graph 14. Frequency of 'licking' in the bit naïve Group.

Graph 15. Frequency of 'licking' in the bit-familiar group.

Pulling down was also an element with no significant difference between the two trials, neither in the bit-naïve (p = 0.197, N = 7, Z = 0.197; see Graph 16) nor in the bit-familiar group (p = 0.149, N = 12, Z = 1.442; see Graph 17).





Graph 16. Frequency of 'pulling down' in the bit-naïve group.

Graph 17. Frequency of 'pulling down' in the familiar group.

Three horses showed individual aversive behavior when wearing a bit including *running off, teeth grinding* and *tongue out*. No additional striking behaviour was noticed during the bitless trials.

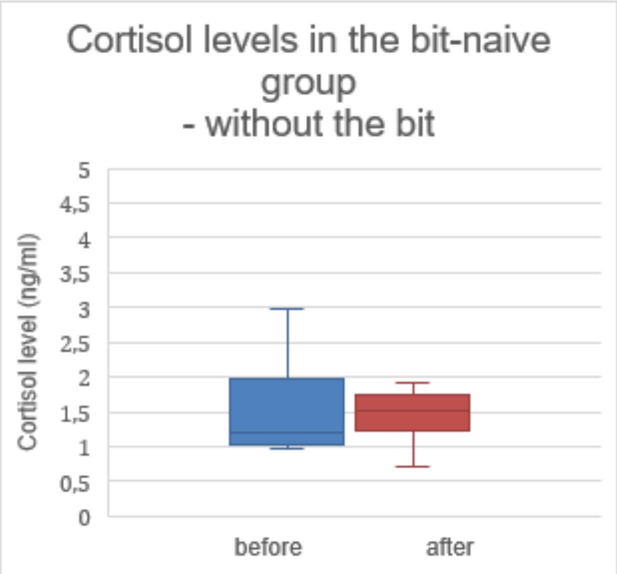
5.2. Saliva Cortisol

No correlation between Cortisol level and bit-type could be detected. Table 6 shows the obtained statistical results.

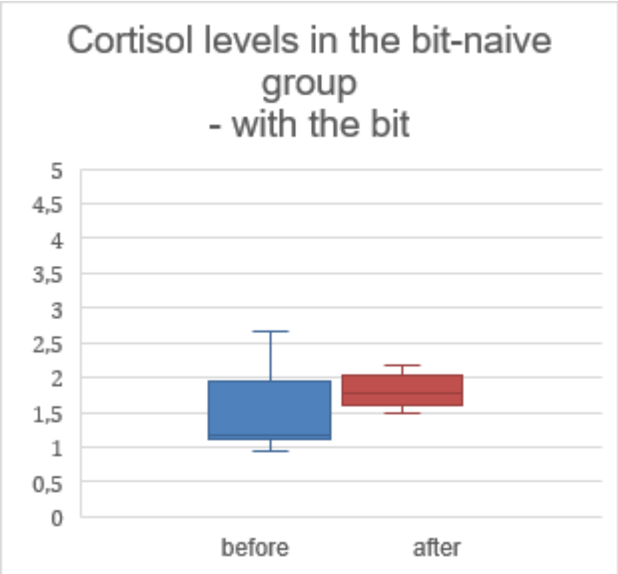
Table 6. Difference in the Cortisol levels.

	Bit-naïve group (N = 7)		Bit-familiar group (N = 11)	
	p-Value	Z	p-Value	Z
Cortisol level before and after – bitless	0.612	-0.507	0.424	0.800
Cortisol level before and after – with bit	0.176	1.352	0.594	0.533
Difference Cortisol increase between trials	0.176	1.352	0.722	-0.356

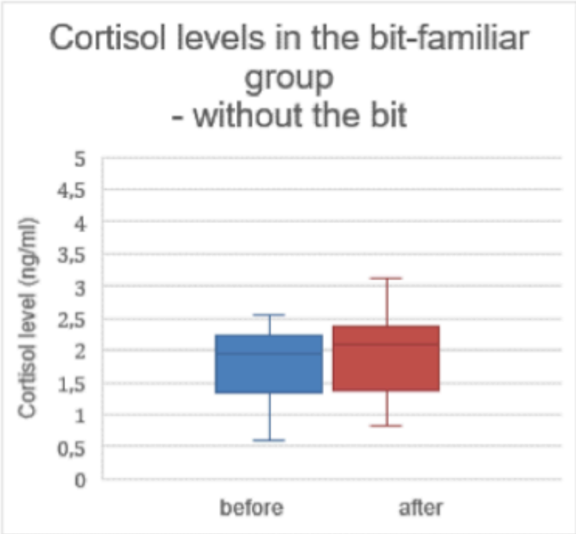
We couldn't detect significant differences in the Cortisol levels before and after the trials- neither in the bit-naive nor in the bit-familiar group (see Graphs 20-24)

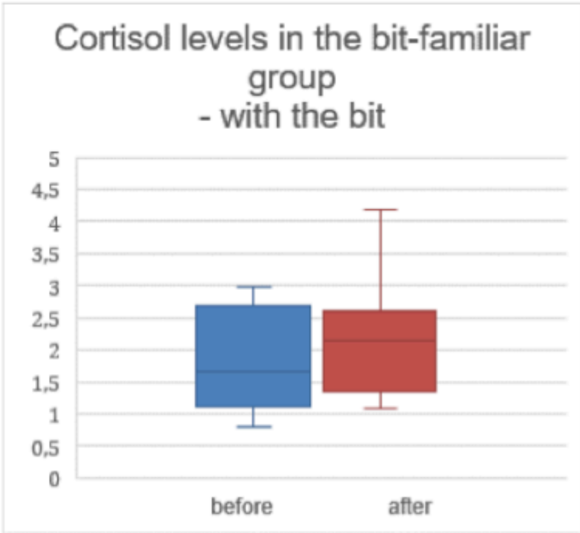


Graph 20. Cortisol levels before and after trial without a bit in the bit-naïve group



Graph 21. Cortisol levels before and after trial with the bit in the bit-naïve group





Graph 22. Cortisol levels before and after trial without a bit in the bit-familiar group

Graph 23. Cortisol levels before and after trial with the bit in the bit-familiar group

d

We couldn't find any correlation between the Cortisol levels and the observed behavior.

6. Discussion

The aim of this experiment is to test whether wearing a bit impairs welfare in horses. More precisely, we wanted to test whether a bit induces a stress response in horses. From our data we cannot conclude that mouthpieces trigger a physiological stress response in horses. Anyhow, our work strongly suggests that bits induce discomfort and lead to behavioral changes.

To answer our research question we compared the stress response between horses wearing a bit versus horses without a bit. For this purpose we tested 19 horses. 12 of these horses are usually ridden with a bit (bit-familiar group). 7 horses are normally worked without it (bit-naïve group). Every horse was tested twice: one time with a bridle including a mouthpiece and the other time with a bitless alternative. According to their previous experiences the familiar bridle type was used first. During the trials we conducted behavioral observations. Additionally, we measured Salivary Cortisol concentrations before and after the test to compare the increase.

Based on previous studies, our hypothesis is that bit (the presence of a mouthpiece) induce a stress response in horses. If so, this stress response should be measurable through the performed behavior and the Cortisol levels.

We asked if bits represent a welfare issue for horses. Contemporary animal welfare science deals with what animals experience subjectively. As we can't read the animal's thoughts we can only infer their emotions through their behavior. Horses exhibiting diverse conflict behaviors definitely don't feel entirely positive about their situation. In this study, we observed various conflict behavior. These behaviors were more often shown when horses were wearing bits. So in summary, bits do induce conflict behavior and thus have an effect on the horse's well-being. This leads to our conclusion of the bit impairing welfare in horses.

Whether or not bits induce negative effects in horses is a controversial topic and subject of an ongoing debate in the riders community. Previous research showed detrimental effects of bits to the horse's health and psyche. An increased risk of injuries especially in the oral part compared to bitless bridles has been shown by

numerous researchers (e.g. TREMAINE, 1998; HAGUE, 1998; COOK, 2003; BENNETT, 2005). Other studies point out that bits induce various conflict behaviors (e.g. BENNETT & LYNCH, 2000; COOK, 2003 & 2013).
Additionally, the bit's effect on the horse's physiology has been investigated from different perspectives. COOK (2002) and HINCHCLIFF AND COLLEAGUES (2014) name impacts on the respiratory system. AHERN (1999) described effects on locomotion. Several authors emphasize consequences regarding the nervous system (e.g. HINCHCLIFF ET AL., 2014) and Trigeminal Neuralgia (COOK, 1999).
Moreover, the use of bits in riding has been associated with several medical conditions including Arthrosis (GEYER AND WEISHAUPT, 2006), Dorsal Displacement of the Soft Palate (COOK, 1999; ALLEN ET AL., 2012), Inflammatory Airway Disease (COOK, 2002) and Head Shaking Syndrome (COOK, 1999)

Nonetheless, research on bit-induced stress responses supported by physiological data do not exist. We want to fill this gap by not also observing behavior but additionally measuring stress hormone levels.

1. Behaviour:

We found some overall significant differences in four behavioral categories.

The most striking parameter showing significant disparities is the frequency of chewing. All horses, regardless of being naïve or familiar to a mouthpiece, showed a significant higher rate of chewing when wearing a bit than without it. This underlines COOK's hypothesis (2002) that bits induce 'eating-like' behavior resulting in chewing, tongue and palate movement and saliva production.

One interesting result that we obtained is that 'bit-familiar' horses showed higher frequencies in chewing than 'bit-naïve' horses. One possible explanation could be that 'bit-familiar' horses already know the feeling of a mouthpiece and start to play with it. A more worrying statement could be that they try to evade the pain. In the worst case scenario they already have injuries in their oral cavity from being ridden with a bit and therefore fight the mouthpiece. Previous authors described bits leading

to injuries on the tongue (BENNETT, 2005), ulcers on lips and tongue (SCOGGINS, 2001) and persistent trauma in the interdental space (JOHNSON, 2002). It would be very interesting to examine their mouths to look for existent injuries. This might bring an insight to this question.
Independently if pain or play – both will have an effect on the concentration of horses. As they focus on the bit, even if they do so in a playful way, they can't fully concentrate on the aids given by the rider let alone high performance (e.g to express the maximum of their performance for wining a horse competition). The difference in the performance outcome between horses ridden with and without a bit might be a very interesting research question for future projects. Studies on this topic are absent so far and as the majority of horses wear a bit during competitions we don't know if they would show better results without a bit.

A consequence of chewing when we look at the horse's physiology is saliva production. Beforehand, I already mentioned COOK's idea (2002) that bits induce 'eating-like' behavior including saliva production. In our study we investigated if the presence of a mouthpiece induces visible frothing resulting from saliva production. We observed that visible frothing was shown by 9 horses when wearing a bit and only one during the bitless trial. This together with our observations of significantly more horses chewing with the bit strongly support COOK's work. Frothing may be important for the horse to diminish bit-induced pain as foam lubricates it. Without a bit no foam would be produced but also wouldn't be needed to lubricate it in the first place. COOK (1999) explained why chewing and saliva production (visible frothing) necessarily lead to swallowing and may cause Dorsal Displacement of the Soft Palate. If so, our data support that a bit is a risk for this clinical condition.
Interestingly, the difference in visible frothing wasn't significant in the bit-naïve group whereas it turned out that bit-familiar horses showed visible frothing significantly more often when wearing a bit. As bit-familiar horses showed a higher rate of chewing in the bit-trial and saliva production and chewing is coupled in horses this might explain why, as a consequence, they also showed more visible frothing.
In this context, it has to be added that in equestrian sports chewing on the bit and frothing are desired behaviors because they are understood to show a relaxed horse. This idea could be explained by the fact that when a horse chews it also slackens the neck muscles. We can observe chewing in bitless riding and groundwork as well, but

less frequently. Possibly, we have to distinguish between chewing sometimes and chewing a lot as this might indicate different mental states. No studies have been conducted on this topic so far.

Overall, all horses opened their mouths more often with the bit than without it. Six horses showed a constant open mouth when wearing a bit. Out of these six horses four were familiar with wearing mouthpieces, two were normally worked with a bitless bridle.

COOK (2002) states that the bit is interfering with breathing by breaking the airtight lip seal. For instance, MELLOR AND BEAUSOLEIL (2017) observed horses in the wild running with their mouth closed. This supports the fact that horses are originally nasal breathers. Oral breathing has only been observed in horses with anatomical abnormalities or disruptive conditions (CHEETHAM ET AL., 2013). Our observations during this study showed that horses ran with an open mouth only when a mouthpiece was present. Similar effects were described by other authors as well (COOK, 2002, 2009; QUICK & WARREN-SMITH, 2009; HANSON & COOK, 2015). Keeping the mentioned facts in mind this strongly suggests that a bit affects the horse's breathing behavior leading to an unnatural open mouth during movement.

It may also be possible that horses open their mouth to evade potential pain caused by the bit as described by other authors (e.g. BENNETT, 2005, SCOGGINS, 2001, JOHNSON, 2002) which suggests detrimental effects coming from the bit. Those theories are further supported by the fact that mouth opening was hardly ever observed during the bitless trials. We couldn't find a significant difference between bit-familiar and bit-naïve horses. This might add up to the fact that a bit is uncomfortable for all horses.

In this context it has to be added that six horses had their mouth open during the whole time of the trial which can be considered as abnormal behavior as it isn't part of the behavioral repertoire of horses running free (MELLOR AND BEAUSOLEIL, 2017).

Another parameter that showed interesting results is 'head shaking'. In our context, every obvious head motion (from side to side or up and down) was named 'head shaking'. In our study we detected a tendency of more head-shaking with the bit only in the bit-familiar group. Maybe bit-familiar horses are already more sensitive to pressure on the nerves underneath the interdental space and thus show a higher difference in this behavioral category.

COOK (1999) described the bit to be the major cause of Trigeminal Neuralgia which favors the Headshaking Syndrome. Trigeminal neuralgia is described as hypersensitivity of the facial nerve. The induced neurotic habit is described as stereotypic head motion triggered by irritation of different origins. As the bit comes to rest in the bars above the facial nerve (see Figure 3A and B, and Figure 11) it might be possible that the bit is the immediate cause for headshaking during bitted riding.

An interesting fact is that three horses, all of them from the bit-familiar group, showed additional behavior with the bit, which they didn’t show during the bitless trial. One horse started teeth grinding. Another horse ran off. A third horse put his tongue out of the side of his mouth. All of those behaviors have been described in former studies from COOK (2003) as aversive behaviors to the bit. Our results suggest that pressure on the nose doesn’t promote a specific form of aversive behavior. Even the bit-familiar horses that are not used to pressure on the nose didn’t show significant rejection to the pull and no striking individual behaviors could be observed during the bitless trials. Former studies support these findings by postulating that switching to a bitless alternative reduces conflict behavior (e.g. QUICK & WARREN-SMITH; COOK, 2013; CAREY ET AL., 2016).

Overall, the behavioral results show that horses exhibit more aversive behavior with the bit than without it. We could see this effect in several behavioral categories including ‘chewing’, ‘mouth opening’, ‘visible frothing’ and ‘head shaking’. This supports our starting hypothesis that bits induce discomfort in horses.

Anyhow, behavioral data alone don’t reveal whether bits induce a stress response in horses. Until now, papers conducting investigations on physiological stress responses in horses caused by the bit are missing. For this reason, we wanted to support our behavioral data by additionally measuring Stress hormone levels.

2. Physiological Stress Response

Our aim was to test whether horses experience stress when wearing a bit and consequently answer if bits impair welfare. To test for this we took saliva samples from all horses in both trials and compared the difference in the Cortisol concentrations. Unfortunately, we couldn't support our hypothesis with our Cortisol measurements.

In stressful situations, Stress Hormones (including Cortisol) are released into the blood stream. This increase culminates in behavioral and physiological effects (KORTE, 2001) to prepare the animal for fight-or-flight behavior. The elicited stress response differs according to situation and context and also individually. Some individuals show active coping while others tend to perform aversive behavior (KASPROWICZ ET AL. 1990, LLABRE ET AL. 1998). The aversive behaviors triggered by the bit have been described above. Now we want to look at the consequences from a physiological perspective.

For this purpose, we used Cortisol measurements. This method is a standard way of measuring stress and considered to be quite reliable when it comes to quantifying stress levels. Considering the aim of our study it was crucial to use an non-invasive method via salivary sampling.
In our work, we couldn't find differences in Cortisol levels between the trials with and without the bit. Many reasons may add up to this outcome. The first suggestion is that wearing a bit does not induce any stress response at all. Either because it is not perceived as a stressor or because the horses are already habituated to mouthpieces. Another explanation could be that experimental constraints altered our results.

As we observed horses living in herds we had to take into account that we couldn't control what the horses were doing before the experiment. Some slept, some were playing with others. As exercise (including running, playing, ..) increases the Cortisol level and sleeping on the other hand decreases it this surely has an influence on their basal Cortisol levels (e.g. KUOPPASALMI ET AL., 1979; DEL CORRAL ET AL., 1994)
Another point to mention is that we observed horses during exercise. A review from KÖNIG AND COLLEAGUES (2017) showed that Cortisol levels may not be the most accurate method to measure stress to a specific stimulus in horses during motion because in exercising horses the Cortisol release is linked closely to the activation and exercise levels. They emphasize that behavioral parameters may be of more valence when

assessing psychological stress in exercising horses than physiological parameters due to high susceptibility of physiological parameters to errors at different experimental stages.

Additionally, we don't know much about the horses' previous experiences with riding. Horses that have been ridden with a lot of rein tension before, might show aversive behavior in our experiment that they wouldn't have shown if they had been ridden with loose reins all their life.

Also, all the horses we studied are used to being handled, lunged and all of them have been wearing a bit at some point in their lives. Thus, although some horses are bit-naïve, more precisely they aren't ridden with a bit anymore, they still had experience with something in their mouth at one point of their live. It is possible that the horses are so habituated to the test situation that their stress levels didn't rise as much.

JANZEKOVIC AND PRISENK (2017) didn't find a significant rise in Salivary Cortisol Concentration when they assessed stress in horses after lunging. This on one hand supports our chosen method as we explicitly only wanted to measure the influence of the bit. Anyhow, the test situation may have altered our results by not stressing the horses enough.

Many studies exist that found a Cortisol level increase in ridden horses (e.g. PEETERS ET AL., 2010; SCHMIDT ET AL., 2010). These findings may indicate that lunging isn't as stressful for horses as riding. This might also imply that the rider plays an important role in eliciting stress in horses. We purposely excluded the rider from our experiment to avoid their influence on the horses. Still, a soft hand operating reins attached to a bit might not cause the described effects when on the other hand a hard rein guidance with a bitless bridle might lead to massive aversive behavior. It is important in this context to note that in our experiment the reins were always under tension because we fixed them on a lunging girth. The horses didn't have any opportunity to evade the pressure. Some riding disciplines, Western riding for instance, are based on a loose rein and aids given mostly with the seat and legs. It is possible that a bit with loose or flexible reins doesn't induce the same amount of aversive behavior we detected in our work.

Nevertheless, this study shows that if we compare bridles with and without a bit under the same circumstances bits induce more rejection behavior.

It could also be that the difference in the stress level between bitless and bitted might be so small that the Cortisol release is too low to be compared. As explained before the test situation wasn't new to the horses. The type of bridle might not have such an influence on the horse's stress level. The effect might be increased by putting more tension on the reins. This has been tested in studies regarding Rollkur, which is low and deep riding often seen in high performance Dressage Horses. SLOET VAN OLDRUITENBORGH-OOSTERBAAN AND COLLEAGUES (2006) showed that riding horses with a lot of rein tension didn't increase their Cortisol level significantly though. VAN BREDA (2006) found similar results suggesting that unnatural head-neck positions per se don't lead to an increased Cortisol Concentration.

Another argument might be that the test time of 15 minutes was too short. Anyhow, former papers working with Saliva Cortisol levels in horses chose similar time frames and found significant results. CHRISTENSEN AND COLLEAGUES (2014) for example investigated effects of hyperflexion on acute stress responses in riding horses. They chose a 10 minutes enduring test period and measured the Salivary Cortisol levels 0, 5, 15 and 30 minutes after the test. In their work, the highest Cortisol levels were measured immediately after the trial. Another study from JASTRZEBSKA AND COLLEAGUES (2017) on Conflict Behavior in Show Jumping Horses detected the peak in Salivary Cortisol evolution curves 20 minutes after the course.

Overall we also to have to take in account that only 19 horses were used in this study. This made a statistical correction for age and sex, which might influence the results, not possible. Anyhow, former studies used a similar sample size and acquired significant results (e.g. JASTRZEBSKA AND COLLEAGUES (2017))

One problem in the statistical analyzes was that we had some horses that showed constant behavior, for instance in the categories 'mouth open' and 'chewing'. These outliers might have biased the results.

Overall, we found a lot of variation in our results. Some horses were very expressive and showed a lot of behavior, whereas others didn't do as much. The high variation in our data made it difficult to compare the horses statistically. One reason for the high variability may be the individual previous experiences. Another explanation may be that horses do react in very different ways to discomfort.

After KÖNIG AND COLLEAGUES (2017) especially the frequency of performed behaviors may indicate stress. Anyhow, we know from former studies on personality that individuals deal with stressful situations in different ways. SUWALA AND COLLEAGUES (2016) for instance describe different personality types in horses. LLYOD AND COLLEAGUES (2008) identified *Anxiousness* and *Excitability* to differ most between horses and even breeds. Some show active coping while others tend to perform aversive behavior (KASPROWICZ ET AL. 1990, LLABRE ET AL. 1998).

It doesn't mean that less expressive horses feel indifferent about a mouthpiece, but might not show their discomfort in a unique or uniform behavioural pattern. Maybe they express themselves through more subtle behaviors that haven't been measured in this work. We selected the chosen behavioral parameters based on COOK's study (2003) on bit-induced Conflict behavior. Various studies picked similar parameters (e.g. JOHNSON, 2002; GÓRECKA-BRUZDA ET AL., 2015; CAREY ET AL., 2016). Other authors indicate that horses show stress amongst others with their ear position (KAISER ET AL., 2006) and through yawning (FUREIX ET AL., 2011). Different sources (e.g. TAFE NSW Online Project on Body Language of stressed Horses) describe differences in ear, eye and nose expression to indicate the horse's mental state. As scientific work on this is rare we didn't look at these signs and thus may have missed some information.

It is also questionable if Conflict Behavior and Salivary Cortisol Measurements are sufficient to test stress in horses. JASTRZEBSKA AND COLLEAGUES (2017) for instance investigated if there was a relationship between behavioral and physiological stress measures in sport horses by observing 19 Show Jumping horses. They found no correlation between conflict behavior and Salivary Cortisol Concentrations.

To recapitulate, an increase in the Cortisol level leads to the individual preparing for fight-or-flight responses. Consequently, blood pressure and heart rate increase to enable fast reactions to possible dangers (LLABRE ET AL. 1998, SCHNEIDERMAN & MCCABE 1989). Former studies used related parameters to assess stress in horses, including heart rate and heart rate variability (KÖNIG ET AL., 2017, VON LEWINSKI ET AL., 2013, SMIET ET AL., 2014, CHRISTENSEN ET AL., 2014). Including those parameters in further tests might deliver more data about the horses' stress level and might uncover more differences in the stress response.

For future projects on this topic, we think it might be interesting to have a bigger sample size. That would allow correction for sex, age and previous experiences for instance. Additionally, it would be relevant to examine the horses' mouths before and after the experiment. First, of all existing dental abnormalities could bias the results. Second, it would be interesting if horses that are often ridden with a bit show any irritated structures. moreover it would be helpful to test stress including other methods suas a measuring heart rate and heart rate variability.

Today, bits are so widely-accepted that it appears 'normal' to us to control a horse with a mouthpiece. When we look at the history horse riding actually started with bitless inventions (e.g. RUSSELL ET AL, 1996). Bits were introduced later (EDWARDS, 1994) Nowadays, we mostly use horses for recreational purposes.
The main argument used by people in favor of bit-use is that bits allow more control on the horse. First of all, we should ask critically why horses react stronger to a pull on the bit than on a noseband. One suggestion is that bits allow better control because they elicit pain in the horse's mouth. Studies showed on the other hand that horses tend to run into the pain (e.g. SCOGGINS, 2001; LYNCH & BENNETT, 2000) questioning this argument.
We should also consider that a stressed horse, regardless where this stress comes from- may it be from within the horse, induced by the rider, the equipment or environmental factors- , is a possible danger for the rider. If the horse's stress level is low, chances are higher that the ride will be a positive and safe one. So, if bits induce stress in horses they are, in fact, a possible danger for the horse-rider team.

Moreover, horses in stress or under pain may not feel that riding is a positive experience. This must have an effect on the horse-rider relationship and their time together. Also, it may impair cooperation and high performance.

The market is full of variations of bits. They range from solid, to two or more jointed mouthpieces and Curb bits (see Figure 4, 5A and 5B). They exist in different sizes and shapes. Every bit type acts a little bit different in the horse's mouth and pushes against different areas (BENNETT, 2005). Depending on the horse's discipline –dressage riding, racing, jumping, etc. – the position of the horse's head varies influencing the action of the bit (BENNETT, 2006). Additionally, a horse's mouth cavity changes during his life as it gets older which necessarily requires adaption of the bit from time to time (SCOGGINS, 2001). The combination of all the different variations of bits, the use of the horse and the individuality of the mouth anatomy make it very difficult to find and fit the right bit for one horse.

In Western horse riding it is still quite common to start a horse with a bosal- a bitless bridle (COOK, 2006). The explanation is to protect the horse's mouth at least while the horse's teeth are erupting. Still, we know that the horse's teeth are constantly growing and the mouth cavity changes throughout their whole lives. This doesn't only complicate finding and fitting of the bit, as described before, but also questions why the use of bitless alternatives is limited to a specific time period.

We also can't ignore the fact that sometimes horses already have dental abnormalities that can promote bit-induced pain. SCOGGINS (2001) for instance names shed premolar caps.

In our study we used the simplest type of bitless bridles and bits. As described in the first chapter of this study horses are also ridden with curb/leverage bits. They exist in many variations with different shank lengths. We do know that the shanks lead to an amplification of the riders pull and thus a much higher pressure on the horses' mouth/nose (LYNCH & BENNETT, 2000; BENNETT, 2005). If we look at the aversive behavior found in our study it would be very interesting to look at the effects with curb bits. In modern horse riding they are normally used for older horses that already have experience with bridles and pressure on facial parts and are supposed to be ridden with less rein tension. Anyhow, a lot of riders lack this knowledge and the danger is quite high that inexperienced riders use these devices in the wrong way. This does apply to bitless bridles as well as bridles with bits.

To summarize our findings we postulate that bits do influence a horse. They lead to more aversive behavior, especially chewing and mouth opening. We can't say from our data if this induces a physiological stress response in horses. Anyhow, it is obvious that horses are more concentrated on chewing on the bit, independently whether it is to play or due to pain, but does nott focus on the rider and movement. This impairs high performance and may also represent a possible danger for the rider in critical situations, which might be avoidable by changing to a bitless alternative.

To conclude, I want to come back to our research questions.
Does the bit induce stress in horses? We can't answer this entirely with our study.
Do bits lead to welfare reduction? We did find that horses reacted with more aversive behavior to the bit than to a bitless bridle. This indicates that horses ridden with a bit experience discomfort in some way. Although it is not measurable through stress hormones it is still observable when we look at the horse's behavior. So yes, bits lead, in our opinion, to welfare reduction in horses.

A lot is already known about the effects of the bit. Many research questions remain unanswered. That's why I, personally, wouldn't risk my horse's well-being with a device whose effects are not completely understood – especially if alternatives exist.

7. Literature

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Ich habe mich bemüht, sämtliche Inhaber der Bildrechte ausfindig zu machen und ihre Zustimmung zur Verwendung der Bilder in dieser Arbeit eingeholt. Sollte dennoch eine Urheberrechtsverletzung bekannt werden, ersuche ich um Meldung bei mir.

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