The effect of an abrupt change in functional surface properties on equine kinematics and neuromuscular activity

Volume 1 of 1

by

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Arena surfaces used for training and competition are influenced by factors such as weather and maintenance, which can lead to spatial variations in functional surface properties. The ability of the horse to adapt to such changes may have implications for injury prevention. The aim of the PhD was to quantify kinematic and neuromuscular responses of horses to a camouflaged abrupt change in functional surface properties.

Horses (*n*=7) were trotted in hand at a consistent speed across an arena surface that had been prepared in four ways: continuous firm; continuous soft and when the surface presented a camouflaged, abrupt change from firm to soft and soft to firm. Kinematic data (232Hz) synchronised with surface electromyography (sEMG) (1926Hz) from selected forelimb muscles were recorded. The first trial (no awareness of change) was categorised separately to the subsequent trials (2-8; aware of change). A General Linear Model was used to assess the effect of horse, stride location and awareness on kinematics and sEMG.

There were limited stride to stride changes on the continuous surfaces. When travelling from firm to soft, fore **F (2, 125) = 11.55, *P* <0.0001** and hind **F (2, 116) = 12.47, *P* <0.0001** limb retraction significantly reduced as the horses stepped onto the soft surface. Awareness of the abrupt change also significantly reduced fore **F (1, 125) = 7.28, *P* =0.008** and hind **F (1, 116) = 10.16, *P* =0.002** limb retraction. When travelling from soft to firm, hindlimb stance duration **F (1, 99) = 7.3, *P* =0.008** and duty factor **F (1, 61) = 7.82, *P* =0.007** significantly increased and peak metacarpophalangeal extension significantly **F (1, 93) = 7.85, *P* =0.006** reduced as the horses stepped onto the firm surface. Awareness of the abrupt change significantly increased stance duration **F (1, 99) = 14.92, *P* <0.0001,** duty factor **F (1, 61) = 8.18, *P* =0.006** and peak metacarpophalangeal extension **F (1, 93) = 3.98, *P* =0.049**. There was some evidence of neuromuscular contributions that helped to stabilise the forelimb and control posture immediately before hoof impact and during stance.

The gait modifications observed demonstrated horses can alter their balancing strategy to cope with a change in surface condition. Reduced limb retraction shifted the COM position relative to the hoof position at lift off more caudal and reduced a falling forward posture as the horses stepped down onto the soft surface and with awareness. When the horses travelled from soft to firm, vertical impulses increased in the hindlimb, which was thought to maintain pitch stability. Vertical impulses showed a more even distribution between the fore and hind limbs with awareness suggesting the fore limbs played a larger role raising the forehand.

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1.0 Introduction

Performance and soundness are key factors to the success and longevity of a horse's career, however this will be strongly influenced by the suitability of the environment under which the horse is managed. Equine surfaces and the influence they have on performance and welfare is an increasingly popular research topic. It has long been established that the surface a horse works on is a risk factor for injury (Barrey *et al.*, 1991; Chateau *et al.*, 2009; Crevier-Denoix *et al.*, 2009; Murray *et al.*, 2010b; Peterson *et al.*, 2012; Symons *et al.*, 2013; Hobbs *et al.*, 2014a) and the last decade has seen more collaborations and research groups develop and focus on this area. Awareness of the importance of providing a surface that prioritises the orthopaedic health of the horse yet supports an optimal performance has dramatically increased, especially since the London 2012 Olympics. This is further reflected by the recent publication of two White Papers, which have collated current scientific research and knowledge on racing (Peterson *et al.*, 2012) and arena surfaces (Hobbs *et al.*, 2014a).

The physical components used to create an arena, for example washed silica sand and fibre or rubber, will influence the functional surface properties such as cushioning and grip to some extent. It is well documented that the biomechanical response of the horse alters according to surface type and preparation, which is reflected by changes in variables such as ground reaction forces (Gustås et al, 2006), hoof deceleration patterns (Barrey *et al.*, 1991; Gustås et al, 2006; Chateau *et al.*, 2009; Kruse *et al.*, 2012) and other kinematic parameters such as metacarpophalangeal extension (Walker *et al.*, 2012; Northrop *et al.*, 2013; Crevier-Denoix *et al.*, 2013; Symons *et al.*, 2013). Some physical components used to create an arena surface may be more stable than others under a variety of conditions, however, all functional surface properties are changeable according to the environmental conditions they are exposed to.

The weather is one of the greatest influences, especially if the arena is outdoors. Variations in moisture content for example have previously shown to cause large fluctuations in the properties of a dirt race track (Ratzlaff *et al.*, 1997) and a synthetic equestrian surface (Holt *et al.*, 2014). The discipline a surface is used for can also alter the functional surface properties. For example, arenas commonly used for dressage or flatwork may have a greater amount of traffic around the outer track where most exercises are executed from, whereas a surface used for show jumping may have perturbed areas around a fence where horses have continually taken off and landed.

Spatial variations in the functional surface properties can be reduced with the use of a suitable maintenance programme, which improves the uniformity of the surface however they cannot be completely eradicated. A study by Northrop *et al*. (2016) has revealed that a large degree of variation in peak load and moisture content was still evident within an arena that was routinely maintained. A lack of uniformity in the functional surface properties has been identified as a risk factor for injury in dressage horses previously (Murray *et al.*, 2010). The complex interactions that the horse makes with such variations within the same arena is yet to be investigated and this has developed the focus for this PhD project.

Horses are expected to perform consistently during training and competition regardless of the surrounding influences including the surface conditions. It has been suggested previously that horses have limited capacity to adapt to surface changes, which is largely due to the passive properties of the distal limbs. Human athletes have demonstrated the ability to adjust limb stiffness according to surface stiffness (Ferris and Farley, 1997; Ferris *et al.*, 1998, 1999). Limb stiffness represents the stiffness of the integrated musculoskeletal system during locomotion and this dictates the interaction between the musculoskeletal system and the external environment during the ground-contact phase of locomotion. Horses have evolved in order to optimise speed and economy, however it is thought that this is at the cost of adaptability (Parkes and Witte, 2015) and it has been postulated that it is unlikely horses can tune limb stiffness as a function of surface (McGuigan and Wilson, 2003). The majority of the joints are restricted to movement in the sagittal plane and this, in conjunction with the small muscle mass within the distal limbs and limited muscle shortening ability, suggests there is little potential to adapt to different surfaces (Parkes and Witte, 2015).

It is generally agreed that horses cannot respond to variations in the functional surface properties like humans can (Ferris and Farley, 1997; McGuigan and Wilson, 2003; Parkes and Witte, 2015). This may be so, but other mechanisms must exist to prevent a horse from tripping or even falling on a surface with known variations. There may be subtle, yet measurable changes present that enable the horse to prevent disruptions to locomotion and sustain a performance. It is well established that the same horse can respond differently to various surface types (Chateau *et al.*, 2009; Kruse *et al.*, 2012; Symons *et al.*, 2013), which suggests that there is scope for horses to adapt. It is still unknown however, whether horses can adapt to more abrupt changes in the functional surface properties of the same surface. Research in this area would make an important contribution to current literature, considering horses train and compete worldwide on various surfaces. It is important to further our understanding on how the horse responds to this extrinsic factor and how this may have implications in injury prevention.

2.0 Synthetic Equine Surfaces: A risk factor for injury

The concern that a surface may be a source of injury in humans arose in the late 1960s when the use of artificial playing surfaces constructed using synthetic or manufactured materials became more popular (Nigg and Yeadon, 1987). The synthetic surfaces were associated with a higher injury rate and negative effects on the locomotor system in comparison with naturally occurring surfaces. Human surfaces have been researched extensively since the work published by Nigg and Yeadon (1987) and a more recent study has shown that injury risks in humans can be reduced and performance enhanced, if training and competition is performed on a suitable surface that meets safety requirements (Swan *et al.*, 2009).

There are currently no safety guidelines specified for equestrian surfaces. Research however, has helped to identify surfaces that would be considered favourable in terms of prioritising equine orthopaedic health (Barrey *et al.*, 1991) or that can support an optimal performance (Chateau *et al.*, 2009, 2010; Crevier Denoix *et al.*, 2009, 2010). The major challenge is supplying a surface that addresses both criteria because they generally have an inverse relationship. Functional testing of elite surfaces including those used for racing (Peterson and McIlwraith, 2008) and disciplines such as Dressage and Show Jumping (Holt *et al.*, 2014) has become more popular over the last 5-10 years. Such testing has improved understanding on the impact of the functional surface properties on the equine athlete and has subsequently helped to identify surface components that can meet the respective criteria under certain conditions. The negative response of riders to the 2011 Olympic test event appeared to create a major milestone, whereby industry professionals really began to realise the value of functional testing and its use as a tool to determine if a surface was fit for purpose (Gibson, 2011; Hart, 2011).

2.1 Functional map of the equine trot

Footfall sequence and timing

Many studies have investigated the equine trot, which is considered to be the preferred gait for evaluation including lameness detection (Clayton, 2004). It is a two-beat running gait performed by the majority of quadrupeds and is characterised by the more or less synchronous movements of the diagonal limb pairs (left diagonal: left fore and right hind; right diagonal: right fore and left hind) (Muybridge, 1887) where the diagonal stance phases are separated by a moment of suspension (Clayton, 1989). The inherent symmetry associated with the trot enables asymmetric movement patterns within the gait to be detected more easily than in walk or canter which are four-beat and three-beat gaits respectively (Clayton, 2004).

Slow motion video analysis of horses has more recently revealed (Clayton, 1994; Holmstrom *et al.*, 1994) that the timing of hoof impact of a diagonal limb pair can be asynchronous where one of the limbs contacts the ground first. In sports horses, it is preferable for contact of the hindlimb to precede the front limb. This is known as positive diagonal dissociation, positive diagonal advanced placement or hind-first dissociation (Hobbs *et al.*, 2016) and is a trait commonly looked for in training and competition (Holmstrom *et al.*, 1994). An example of this can be seen in Plate 2.1.1 where the left hindlimb will contact the ground prior to the right forelimb. Dressage horses perform four different types of trot depending on the training level including; collected, working, medium and extended trot with average speeds varying from 3.2 m/s for collected trot to 4.93 m/s for extended trot (Clayton, 1994). The progression from one type of trot to the next is mainly achieved by increasing stride length with only small changes in stride rate (Clayton, 2004).

Plate 2.1.1: an example of positive diagonal dissociation

Approximately 60% of the horse’s body weight is carried by the forelimbs and 40% by the hindlimbs (Witte *et al.*, 2004) and so positive diagonal dissociation of the hindlimbs must be encouraged through correct training and conditioning. Alterations in contact times can result from changes in mass distribution where horses alter the vertical force distribution as a strategy to maintain balance during trotting at a constant speed (Hobbs *et al.*, 2016). During training, horses are encouraged to take more of the load into the hindlimbs to ‘engage’ the hindquarters and develop self-carriage in order to generate a more uphill appearance. This improves the aesthetics of a performance (Holmstrom *et al.*, 1994) and can also assist in maintaining balance when executing more demanding movements (Clayton *et al.*, 2017; Weishaupt *et al.* 2009).

A hind-first dissociation may also help to improve locomotion efficiency. Hobbs *et al.* (2016) have identified that collisional or energy losses experienced at trot can be mitigated to some degree by diagonal dissociation. A hind-first dissociation was associated with significantly lower collisional losses in comparison to synchronous footfalls of the diagonal limb pairs at the same speed (Hobbs *et al.*, 2016). At a moderate relative velocity, Hobbs *et al.* (2016) has revealed that the footfall pattern can be selected, but above and below that velocity range, a need to stabilize the trunk is a priority.

Dissociation pattern may also vary according to the breeding and conformation of the horse. Hobbs *et al*. (2016) suggested that dynamic rather than static posture is a more important determinant Dynamic posture can be defined as the position of body segments during movement. Changes in dynamic posture have been observed on different surface types and preparations previously (Northrop *et al.*, 2013; Symons *et al.*, 2013), which has possibly been a strategy adopted to help re-balance the horse. Alexander (2002) suggested that dynamic stability in quadrupeds may be achieved by altering the timing of peak force production within limbs. This has been shown to alter by selecting a hind-first dissociation where peak forelimb force occurs later during diagonal stance (Hobbs *et al.*, 2016).

Diagonal dissociation has shown to have important consequences on trotting dynamics. Due to the experimental set up proposed for this study, it will not be possible to quantify the contact timing of the diagonal limb pairs. The study therefore aims to measure the contact timing of the ipsilateral limb pair, that is, right forelimb and right hind limb. Alterations in timing of the ipsilateral limb pair may constitute changes in the inter-limb vertical force distribution throughout an entire stride (Lee *et al.*, 1999). An earlier contact time of the hind limb may be implicated in reducing collisional losses as with a hind-first dissociation of the diagonal limb pair and may also prevent large fluctuations in the COM trajectory.

Contributions of the axial skeleton to the equine trot

The axial skeleton can also provide dynamic stability (Dunbar *et al.*, 2008). During trot, vertical movements of the head, neck and back have a sinusoidal pattern, the magnitude of which is dependent on speed (Robert *et al.*, 2002). The head and trunk of the horse are also rotationally stabilized, where pitching moments about the centre of mass (COM) are ≤ 20º (Dunbar *et al.*, 2008). This is thought to help determine and maintain whole-body spatial orientation within the horse's surroundings (Dunbar *et al.*, 2008).

The viscoelastic properties of the nuchal ligament and other tissues within the equine neck are thought to provide a passive, energy efficient mechanism during lengthening and shortening for maintaining head orientation. The *splenius* also plays a role in postural control where it has been identified as a functional stabiliser of the head andneck against gravitational forces (Tokuriki and Aoki, 1991; Robert *et al.*, 2002; Zsoldos *et al.*, 2010). An increase in activity of the other axial muscles including the *longissimus dorsi* and *rectus abdominus* provides trunk stability and increase spinal rigidity (Robert *et al.*, 2002), from which the limbs can articulate.

Contributions of the appendicular skeleton to the equine trot: The passive and active properties

During bouncing gaits such as the trot, muscles, tendons and ligaments can all behave like springs, where elastic energy is stored when they are stretched and returned when they recoil (Alexander, 1988). This has been modelled as a simple spring mass model previously, which consists of a single linear leg spring and a mass equivalent to the animal's mass (Mcmahon and Cheng, 1990; Farley *et al.*, 1993) (Figure 2.1.1). In reality, the musculoskeletal system is complex but the simple spring mass model has been shown to describe and predict the mechanics of bouncing gaits such as running remarkably well (Mcmahon and Cheng, 1990; Farley *et al.*, 1993; Ferris *et al.*, 1998, 1999).

During the stance phase of the stride, the structures within the limbs support the COM of the body against gravity and propel the body forward. The limbs can move the body forward at a steady speed or the structures can act to accelerate the limb in early swing in order to reposition it in preparation for ground contact. The equine distal limb has significant passive elastic properties where the long tendons and muscle aponeurosis of the equine limbs are used to store elastic energy during the stance phase and return approximately 93% during propulsion, which reduces the energetic cost of locomotion (Ker, 1981; Biewener, 1998; McGuigan and Wilson, 2003; Payne *et al.*, 2004; Harrison *et al.*, 2010). The remaining work must be performed by active contributions from the muscles.

Even at a constant velocity, it is not possible to achieve 100% energy return and ultimately some mechanical work must be performed (Pfau *et al.*, 2006). Energy is lost from the system as heat, due to aerodynamic drag, surface deformation and because there may not be a complete interchange between potential, kinetic and elastic strain energy (Pfau *et al.*, 2006). During the trot, which is categorised as a running gait, the fluctuations of kinetic and potential energies are in phase, which is thought to help maintain the efficiency of the gait during the stance phase and can be compared to the action of a pogo stick (Pfau *et al.*, 2006). Harrison *et al.* (2012) demonstrated that the passive stay apparatus generated a large proportion of joint torques developed during stance, which mainly consists of the superficial digital flexor, deep digital flexor and suspensory apparatus (Harrison *et al.*, 2010; Swanstrom *et al.*, 2005). Stretching of these structures increases tendon force and helps to reduce the amount of active muscle contraction required to maintain locomotion.

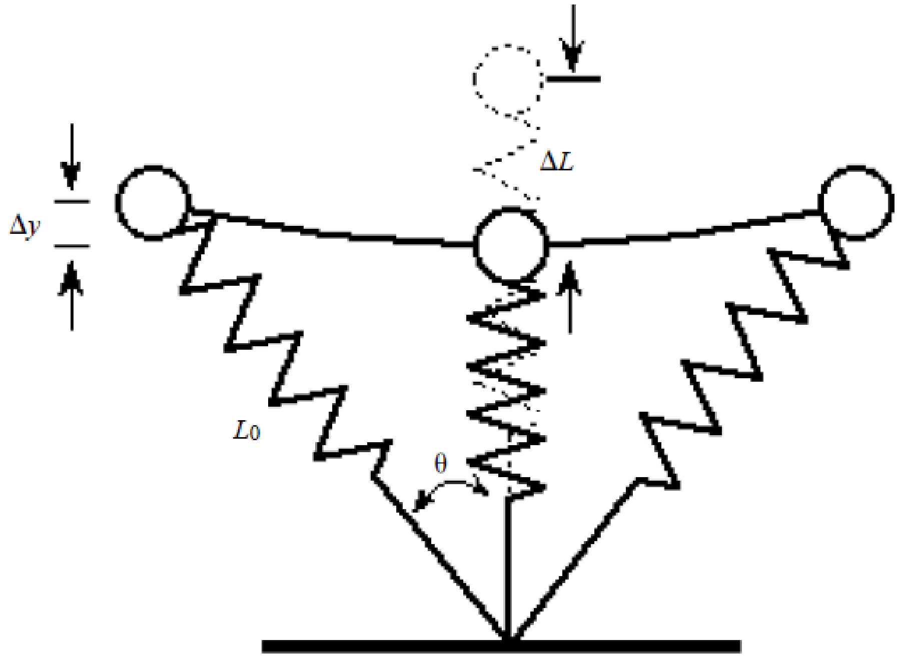


Figure 2.1.1: A simple spring mass system consisting of a mass and a single leg spring (joins the foot and COM of the animal). The figure illustrates the model at the start (left spring), middle (middle spring) and end of the stance phase (right spring). The leg spring has an initial length (L0) at the beginning of the stance phase, and its maximal compression is represented by ΔL. The dashed spring-mass model shows the length of the uncompressed leg spring. Thus, the difference between the length of the dashed leg spring and the maximally compressed leg spring represents the maximum compression of the leg spring (ΔL). The downward vertical displacement of the mass during the stance phase is represented by Δy and is substantially smaller than ΔL. Half of the angle swept by the leg spring during the ground contact time is denoted by θ. Extracted from Farley et al., (1993).

Steady state locomotion requires a small amount of muscular work however, if the horse is required to elevate or accelerate the COM during various equestrian disciplines such as jumping or during the piaffe in Dressage, then increased work production is required from the muscles (Payne *et al.*, 2004). Many of the muscular and tendinous structures responsible for moving and stabilising the equine limb segments cross more than one joint (Clayton *et al.*, 1998). This creates complex relationships between the movements and moments at different joints that interact to meet the demands of an increased work load.

The swing phase of the forelimb can be represented by the action of a pendulum rotating around the proximal scapula (Kruger, 1938). The shoulder joint is extended at initial hoof impact before flexing during loading, which allows the storage of elastic energy (Clayton *et al.*, 2013). The elbow joint extends throughout stance and undergoes maximal flexion during swing, which has the effect of retracting and protracting the distal limb respectively (Clayton *et al.*, 2013). After hoof impact, the carpal joint is over extended, which helps to stabilise the distal limb segments during stance. The carpus begins to flex towards the end of stance before reaching maximal flexion at mid-swing. The MCP or fetlock joint is the main site of elastic energy storage (Clayton *et al.*, 2013) as it extends through to mid-stance before flexing and releasing energy as the limb moves into the swing phase of the stride (Figure 2.1.2). During primary impact, there is an inflection in the joint angle-time curves of the MCP and coffin joints (Back *et al.*, 1995), which is thought to be in response to the increasing ground reaction force (Merkens and Schamhardt, 1994). This is more pronounced in the forelimb in comparison to the hindlimb, possibly because of the larger distribution of body weight to the forehand of the horse.

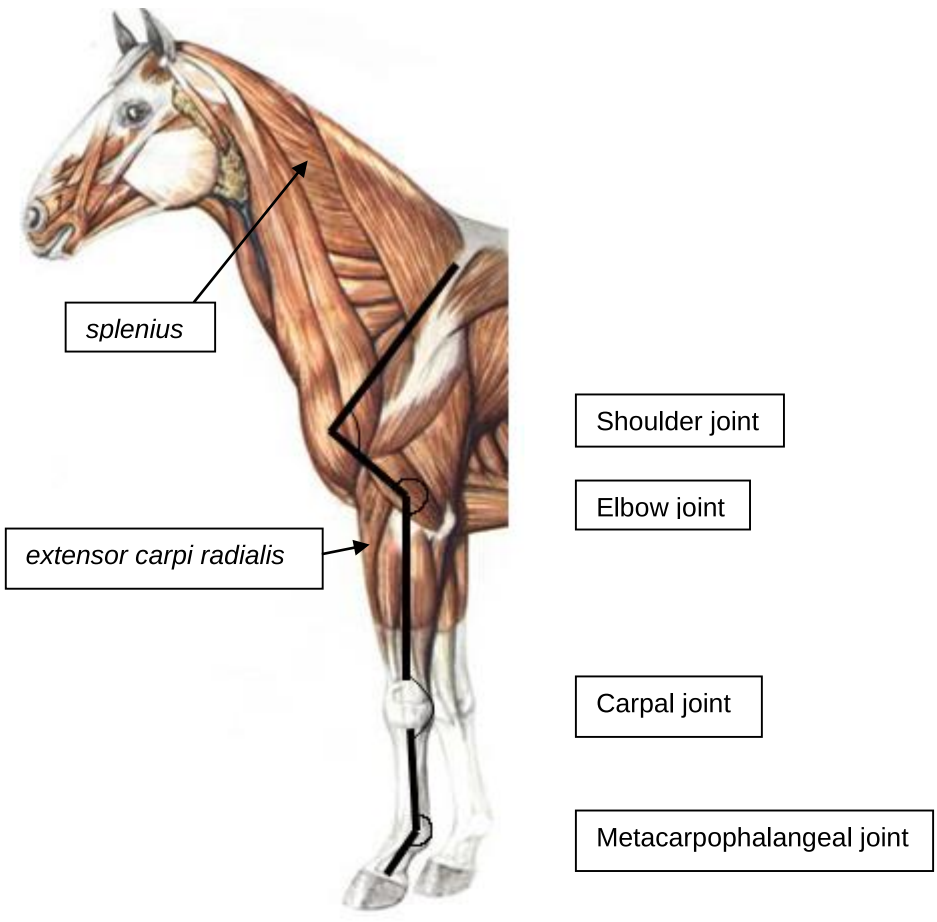


Figure 2.1.2: An illustration demonstrating selected musculoskeletal structures within the equine forelimb. Image taken from i.pinimg.com (2015)

It has been suggested by Harrison *et al.* (2012) that the proximal muscles (inserting proximal to the carpus) perform most of the work done by the forelimb during locomotion whereas the distal muscles (distal to the elbow) play a supporting role, possibly through helping to stabilise the joints (Brown *et al.*, 2003). The lack of contribution of the distal muscles to gross movement could be explained by the limited fibre length and muscle shortening ability (Biewener, 2006). Instead, the distal forelimb muscles are thought to assist with dampening high frequency vibrations generated at the primary impact of stance (Wilson *et al.*, 2001), which could help to further justify the supporting role proposed by Harrison *et al.* (2012). A study by Brown *et al.* (2003) claimed that these muscles can also create rapid flexion and extension of the limb during the swing phase, reflecting the importance of measuring the contributions of these muscles to locomotion.

Harrison *et al.* (2012) measured the patterns of activity in selected equine forelimb muscles (n=15) in order to correlate the data with the net torques developed about the joints during walk, trot and canter. All of the muscles were active at trot mainly from late swing through to early stance before deactivating in late stance, with the exception of the *extensor carpi radialis*, which was only active during early swing. This was also observed by Jansen *et al.* (1992) when analysing ponies at walk. A second burst of activity was also recorded from late swing until early stance, which was thought to aid carpal extension. The second burst was not observed by Harrison *et al.* (2012), however the *biceps* muscle was found to be active during late swing at walk, which has an insertion *via* the *lacertus fibrosus* on the external fascia of the *extensor carpi radialis*. There is potential for cross talk between these muscles due to their close proximity and EMG sensor location used. The presence of muscle activity during early swing supports the distal limb function claimed by Brown *et al.* (2003) where the *extensor carpi radialis* acts to extend the carpus and flex the elbow joint, suggesting there is a small degree of muscular control required to protract the limb. The inactive period of the other muscles during early swing (Harrison *et al.*, 2012) suggests that limb protraction is generally initiated by a largely passive process.

Wilson *et al.* (2003) suggested that horses use the elastic biceps brachii muscle to store and release bursts of energy required for rapid limb protraction creating a catapult action. The forward movement of the trunk in conjunction with the orientation of the ground reaction force generated during stance stretches the biceps muscle whilst the carpus is extended. In late stance, the carpus buckles and the stored energy helps to release the limb into swing (Wilson *et al.*, 2003). The *biceps brachii* was in fact active during the first 10% of the swing phase at trot as well as the majority of stance in the study by Harrison *et al.* (2012), which supports the findings of Wilson *et al.* (2003).

The flexor and extensor muscles of the forelimb were found to co-contract during the stance phase of the stride, which is the simultaneous activation of the agonist and antagonist muscle pair (Harrison *et al.*, 2012). Co-contraction provides added stability to the surrounding joints. When antagonistic muscles develop forces simultaneously, the contribution to the joint torque that is generated by one muscle is mitigated by the torque of its paired antagonist (Harrison *et al.*, 2012). It is thought that the muscle forces pull the bones together at a joint, which increases the compressive joint force (Harrison *et al.*, 2012). This can reduce the net shear or tensile loads on the joint that contribute to injury as observed by Ackland and Pandy (2009) in the human shoulder. The results from Harrison *et al.* (2012) showed that most co-contraction occurs at impact prior to the development of large joint torques, which may be a protective effect during limb loading. Muscle co-contraction during late swing and early stance was also thought to promote positional control of the limb (Harrison *et al.*, 2012). This is supported by Burn and Usmar (2005) who state that the limb is accelerated in retraction after limb protraction, which is thought to decrease the velocity of the hoof relative to the ground prior to ground contact.

During trotting motion of the hindlimb can also be considered as pendular with a rotation point in the acetabulum. This is because the movement trajectory of the coxofemoral joint and the cranio-caudal movement of the distal metatarsus are similar (Back *et al.*, 1995). The coxofemoral joint shows a general sinusoidal flexion and extension pattern where maximal flexion occurs prior to hoof impact (Back *et al.*, 1995) (Figure 2.1.3). During primary impact, the stifle, tarsal and distal interphalangeal joints are rapidly flexed in response to limb loading, which is thought to contribute to the shock absorbing properties of the limb (Back *et al.*, 1995). The metatarsophalangeal (MTP) joint continues to extend from late swing into early stance before being maximally extended at midstance (Back *et al.*, 1995). Just before the end of stance the coxofemoral and tarsal joints are maximally extended and the coffin joint is rapidly flexed to aid push off. At the beginning of swing, the MTP joint reaches maximal flexion followed by maximal flexion of the stifle and tarsal joints during mid-swing (Back *et al.*, 1995), which is all supported by the release of elastic strain energy.

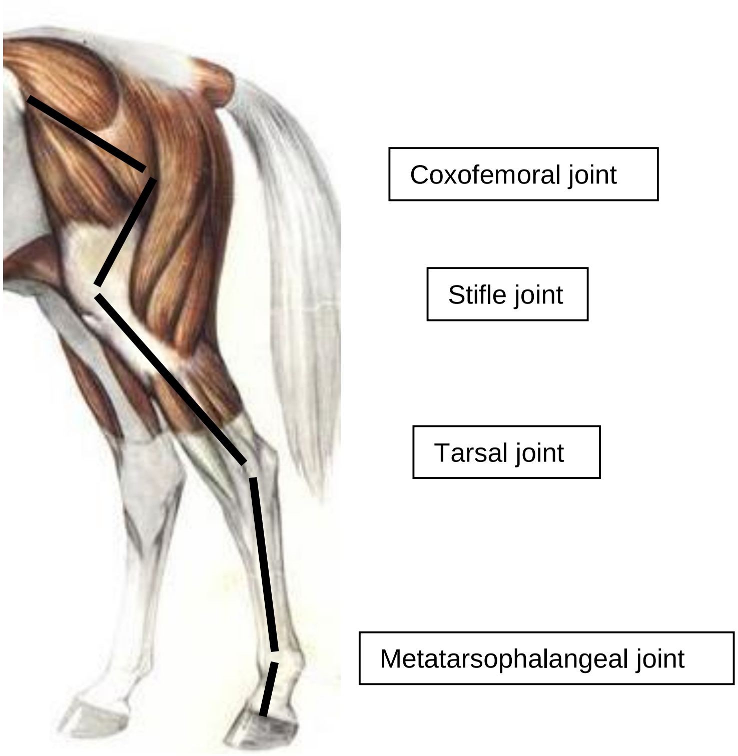


Figure 2.1.3: An illustration demonstrating the musculoskeletal structures within the equine hindlimb. Image taken from i.pinimg.com (2015)

The swing phase of the hindlimb is largely passive, which is reflected by a lower amount of muscle activity present at this time (Jansen *et al.*, 1992; Robert *et al.*, 1999). The superficial muscles of the hindlimb analysed by Robert *et al.* (1999), including the *gluteus medius, vastus lateralis, biceps femoris* and *semitendinosus,* were generally active from late swing through to mid-stance. The *tensor fasciae latae* appeared to be active from late stance through to early swing where it contributes to stifle extension and subsequent protraction of the hindlimb (Robert *et al.*, 1999). The *extensor digitorum longus* was the only muscle that demonstrated constant electrical activity throughout the swing phase, possibly because it is thought to be responsible for tarsal flexion and stabilisation of the stifle joint (Robert *et al.*, 1999). A similar activation pattern was also observed by Jansen *et al.* (1992) in ponies at walk.

It is clear that equine locomotion results from a complex co-ordination of forces generated by active muscle contraction and passive stretching of tendons and ligaments (Harrison *et al.*, 2012). The muscle activity evident during the swing phases of the fore and hind limbs reinforces that gait efficiency is far from 100%. Active contraction must occur to aid limb placement, provide joint stability and sustain locomotion. The presence of muscle activity at this time suggests there is potential for the magnitude of active control of the limb to alter according to external factors that the horse is exposed to.

2.2 Hoof-surface interaction and functional surface properties

The hoof-surface interaction is influenced by the functional surface properties or the physical characteristics of the surface and will determine the kinematic response of the horse. The properties are influenced by surface material, arena design, maintenance methods, geographical location and the resulting environmental conditions the surface is exposed to (Hobbs *et al.*, 2014a). The combination of these factors affect how a surface performs and how it feels to a horse and rider (Hobbs *et al.*, 2014a). The Equine Surfaces White Paper by Hobbs *et al.* (2014a) has described the functional surface properties in detail and also defined rider terms that can all be objectively measured and are commonly used in industry to describe a surface. These include cushioning, impact firmness, responsiveness, grip and uniformity.

The stride cycle of one limb consists of a swing and stance phase. The stance phase has been sub-divided by Peterson *et al.* (2012) into the following stages: primary impact, secondary impact, support (midstance) and rollover (breakover) (Figure 2.2.1). The swing phase can be sub-divided into post breakover, midswing and pre- impact (or terminal swing). The stance phase appears to be the focus in current research because it is associated with accelerations and forces that are responsible for potentially increasing the risk of injury (Barrey *et al.*, 1991; Drevemo and Hjertén, 1991; Gustås *et al.*, 2006a; Reiser *et al.,* 2000; Peterson *et al.*, 2008, 2012).

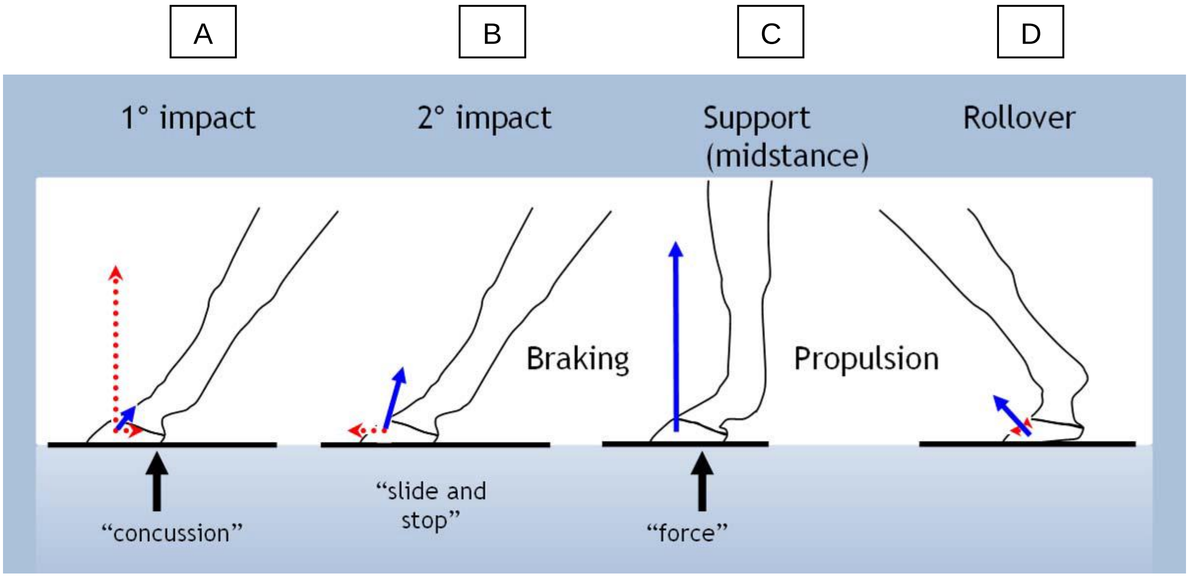


Figure 2.2.1 Stages of stance showing the differences in acceleration (red) and ground reaction force (blue) among the stages. When the blue arrow is tilted, it indicates that both vertical and horizontal components of the ground reaction force are present. The arrow shows the direction in which the ground is pushing the horse. Adapted from Peterson et al. (2012).

Primary and secondary impact

Preimpact is the phase immediately before the hoof hits the ground and precedes the primary (Figure 2.1.1 A) and secondary impacts (Figure 2.1.1 B), which create the first third of stance. This can also be referred to as the hoof braking period, which lasts approximately 30-50 ms and is independent of speed (Gustås *et al.*, 2006b). It is characterised by a small ground reaction force, prominent peak decelerations and high loading rates (Gustås *et al.*, 2006b). The magnitude of hoof deceleration and ground reaction forces on impact were found to be significantly affected by the speed at which the horse is travelling (Gustås *et al.*, 2006b; Thomason and Peterson, 2008) and also by the type of surface (Barrey *et al.*, 1991; Gustås *et al.*, 2006a). The impacts are further categorised according to how much the limb is loaded.

Primary impact

The primary impact (Figure 2.1.1 A) occurs when the hoof collides with the surface and is associated with high accelerations (Thomason and Peterson, 2008). The vertical acceleration is higher than the horizontal acceleration due to the ratio of the forward and downward hoof movement (Gustås *et al.*, 2006b). The ground reaction forces are small at this stage due to the low mass of approximately 5 kg involved with the collision (Chateau *et al.*, 2010).

Impact Firmness

The peak vertical accelerations generated during the primary impact are determined by the impact firmness of the surface, which relates to the hardness of the top surface layer and influences the amount of shock experienced by the horse. Peak vertical acceleration, which is converted into gravities is used to quantify the amount of impact firmness a surface provides. The hoof hits the ground approximately 47 times per minute at walk (Hodson *et al.*, 2000) and up to 150 times a minute at gallop (Peterson *et al.*, 2012), therefore identifying surface properties that reduce impact shock yet enable the horse to sustain its performance has been of great research interest.

A maximum impact firmness of 150 gravities is generally recommended for arena surfaces when testing using the OBST. A surface that generates a maximal vertical acceleration beyond this limit will increase vibrations and oscillatory movement of the hoof and surrounding tissue (Setterbo *et al.*, 2009), which will ultimately contribute to subchondral bone damage (Johnston and Back, 2006; Radin 1973; Parkin *et al.*, 2004). Intense exercise on a surface so hard has the potential to inhibit bone remodelling within the distal metacarpus based on the findings from Holmes *et al.* (2014) and exposes the area to high risk of fatigue damage. A link between shock and vibration and subchondral bone damage was established by Radin *et al.* (1973) where the knee joints of rabbits stiffened after impulsive loading, which represented changes consistent with degenerative joint disease (DJD), a disorder that is also a major cause of reduced athletic function and retirement in the performance horse (Dyson, 2000).

If the surface presents a low impact firmness however, the musculoskeletal system may not be conditioned properly for sufficient bone adaptation (Firth, 2006). Tissue strength and the ability to resist deforming forces will only improve after a suitable number of cycles on an adequate surface has occurred (Firth, 2006). If training is performed on a surface that is too soft and the horse is later exposed to a harder surface during competition, the tissue strength may not be sufficient to cope with the increase in impact shock.

Asphalt has generated an impact shock nearly three times greater than a firm wet beach sand track commonly used to train trotters (Chateau *et al.*, 2010), which would most certainly contribute to subchondral bone damage if sufficient time was not left for adaptive hypertrophy. The horses were travelling much faster (7.2 vs. 4.23 m/s) on the sand track (Chateau *et al.*, 2010), which will have magnified the vertical acceleration (Gustås *et al.*, 2006b). The different surface types were therefore not completely comparable and suggests that the effect of asphalt may be even more damaging at similar speeds achieved on the sand track.

Surfaces that offer more structural damping such as woodchip and sawdust are considered to be more efficient at reducing impact shock (Barrey *et al.*, 1991; Kruse *et al.*, 2012). An all-weather waxed track has also shown to reduce impact shock and the vibrations associated with this collision in comparison to a crushed sand track (Chateau *et al.*, 2009). Both surfaces were considered to be good training tracks for trotters but the findings suggest that the all-weather waxed track had better damping capabilities and could reduce the risk of injury.

A study by Drevemo and Hjerten (1991) studied the effect of installing a woodchip layer under a conventional sand harness race track. Impact firmness was significantly reduced (Drevemo and Hjerten, 1991), which supports the findings of other research (Barrey *et al.*, 1991; Kruse *et al.*, 2012). This was at the cost of maintaining surface consistency however and was therefore considered unsuitable because of the negative influence it may have on gait stability (Drevemo and Hjerten, 1991). Surfaces with better damping properties generally offer more cushioning but this can be associated with greater energy loss (Drevemo and Hjerten, 1991). This has reduced stride length and increased stride frequency previously (Chateau *et al.*, 2010), which is considered to be undesirable in terms of performance quality (Holmstrom *et al.*, 1994).

Secondary impact

The secondary impact (Figure 2.1.1 B) is characterised by increasing forces and greater horizontal accelerations. The body of the horse, which is still travelling forwards whilst the hoof is in contact with the ground, pushes the limb into the ground and creates a 'slide and stop' action (Pardoe *et al.*, 2001; Ruina *et al.*, 2005). The horizontal deceleration represents the braking forces of the hoof in order to resist sliding (Reiser *et al.*, 2000).

Grip

The frictional forces experienced during the secondary impact are determined by the amount of surface grip and will influence how much the hoof slides during landing, turning and pushing off (Hobbs *et al.*, 2014a). Friction has been described by Medoff (1995) as a combination of mechanical interlocking and adhesion between two interfaces, which can be generated between the hoof and surface but also between the surface particles (Hobbs *et al.*, 2014a). Initial estimates of grip suggest 15-25 Nm would fall within the optimal range when measured using a Glen Withy Torque Tester (Lewis *et al.*, 2015) but further work is still required to validate this claim.

Higher friction between the hoof and ground will reduce the amount of hoof slide during the secondary impact phase, which has shown to increase impact shock, resulting in higher mechanical stress and risk of injury (Gustås *et al.*, 2006a). A study by Orlande *et al.* (2012) found a reduction in hoof slip during jump landing on an arena surface with 10% wax content when compared to a similar surface with 3% wax. Hoof slip showed a relatively weak correlation with surface traction, however it was also influenced by other properties such as impact firmness and surface penetrability. A significant negative correlation was found between hoof slip and hardness on the 10% wax surface, which suggests this surface should be avoided based on the claims made by Gustås *et al.* (2006a). A surface with insufficient grip on the other hand, may prolong hoof slide into the support phase of the stride when the limb must be stationary to support the weight of the horse, which also increases the risk of sprain related injuries.

Support

The support phase of the stride (Figure 2.1.1 C) is initiated when the weight of the body is evenly applied to the leg and the hoof flattens out before continuing to rotate through to the next part of the stance phase (Reiser *et al.*, 2000; Thomason and Peterson, 2008). The vertical and horizontal accelerations generated during the initial impact diminish and peak vertical forces are experienced, which represents mid-stance (Thomason and Peterson, 2008). This can be further characterised by the transition from braking (negative) to propulsive (positive) longitudinal forces (Drevemo *et al.*, 1980). Peak forces may reach 2.4 times the body weight of the horse at a racing gallop (Witte *et al.*, 2004). The distal limb is structured in such a way so the peak forces exerted with the ground during natural movement do not exceed tolerable limits. The physical demands placed on the domesticated horse however, may surpass acceptable loads, causing micro-trauma and eventually leading to equine musculoskeletal and orthopaedic disorders (van Weeren, 2010).

Cushioning

The peak forces experienced during the support phase are determined by the surface cushioning. A surface that offers more cushioning generates lower peak forces or offers more force reduction in comparison to a reference surface (Hobbs *et al.*, 2014a). Ideally, a surface should be able to support peak loads of 12-15 kN where deviations from this will ultimately reduce the performance quality of the equine athlete. An average 500 kg horse will experience the equivalent of one body weight of force through its limb at trot during the support phase of the stride, which equates to approximately 5 kN (Force =500*9.81). This can increase up to twice the horse's body weight in the trailing forelimb during landing from a 1.30 m jump (Schamhardt *et al.*, 1993), which means the surface must be able to support peak loads of at least 10 kN. Sports horses are generally heavier than 500 kg and fence height at grand prix level can reach up to 1.60 m, which will undoubtedly increase the peak forces experienced and therefore a surface that can support slightly greater loads is desirable.

If the surface does not offer sufficient cushioning however, concussive forces will increase thereby increasing the peak stress experienced by the horse (Hobbs *et al.*, 2014a) and subsequently the risk of injury. This has been implicated in increasing the risk of fractures on dirt racetracks previously (Hill, 2003). A surface that generates low peak loads will similarly increase the risk of injury due to the lack of support from the surface (Hobbs *et al.*, 2014a). The propulsive muscular effort must be increased to achieve the same movement, which will ultimately hasten the onset of fatigue (Crevier-Denoix *et al.*, 2010).

It is possible to alter the amount of cushioning a surface provides without changing the surface components. Heavy maintenance has significantly reduced the average peak loads on a dirt training track from 13.8 to 9.11 kN (Peterson and McIlwraith, 2008).

This was measured using an Orono Biomechanical Surface Tester (OBST), which simulates the forelimb of a horse impacting and loading the ground at gallop (Peterson *et al.*, 2008). The OBST was also used in a study by Tranquille *et al.* (2015), which revealed that a more superficial surface preparation could significantly increase the cushioning of waxed sand and fibre surfaces (*n* = 6). The sand and rubber surfaces (*n* = 5) that were also tested showed no significant change in peak load however, which was attributed to the same loads being generated regardless of the distribution of rubber throughout the surface (Tranquille *et al.*, 2015).

Increasing surface density has shown to have the opposite effect to maintenance on the cushioning values where peak loads can be significantly increased by compacting the surface components (Holt *et al.*, 2014). The sub-base used beneath the surface can also affect the ability of the surface to compact and therefore its load bearing capacity (Holt *et al.*, 2014). Permavoid™ units, which are more deformable than traditional gravel sub-bases have significantly reduced peak loads (Holt *et al.*, 2014), which is an important factor to consider when a surface may be required to support greater loads during jumping for example.

Moisture content, which is considered to be one of the most influential variables on functional surface properties (Peterson *et al.*, 2012; Hobbs *et al.*, 2014a), has previously shown to influence the forces exerted by galloping horses, where lower forces were measured around 8% (2 to approximately 16%) surface moisture content (Ratzlaff *et al.*, 1997). The speed at which the horses were galloping at also determined the hoof-surface interaction where a slightly higher moisture content of 12% reduced the forces exerted at faster speeds. This demonstrates the sensitivity of the cushioning experienced by the horse in relation to the activity being performed.

The peak load measured on an arena surface using an OBST appeared to be less responsive to changes in moisture (11.96 to 19.08%) (Holt *et al.,* 2014). Crevier-Denoix *et al.* (2010) also found no significant difference in the peak forces experienced by trotters on beach sand training tracks (13.5 to 19% moisture). The lack of change could be explained by the much smaller range in moisture contents tested, the nature of the activities performed and different surface material used. The surface used by Ratzlaff *et al.* (1997) had also been harrowed, which reduces compaction by loosening the surface material and increases the pore space between the surface particles. Moisture will distribute more easily throughout the surface potentially making the material more sensitive to changes in the amount of cushioning it provides.

Breakover

Breakover or roll over (Figure 2.1.1 D) occurs when the hoof lifts at the heels and rolls from the ground which causes propulsive forces in the cranial direction as the horse moves forward (Reiser *et al.*, 2000; Thomason and Peterson, 2008). The forces are steadily decreasing at this stage. The vertical accelerations are low whereas the horizontal accelerations may reach greater values than during the secondary impact (Thomason and Peterson, 2008). Post breakover immediately follows where the hoof and digit flex rapidly and forms the start of the swing phase (Thomason and Peterson, 2008).

Responsiveness

Responsiveness relates to the tuning of the surface or how 'active' it feels to the rider. The surface will deform during the loading phase of the stride, some of which is elastic and therefore has the ability to recover. If the surface recovers at the correct time during the breakover phase of the stride, it has potential to return energy to the horse and will feel active or springy to ride on. This is the ideal condition, however if the surface recovers too quickly or too slowly, it will feel 'dead' to ride on (Figure 2.2.2).

A very compacted surface that generates high peak forces or has a low amount of cushioning may rebound too quickly to return energy to the horse (Hobbs *et al.*, 2014a). The testing device used by Ratzlaff *et al.* (1997) to measure the properties of a dirt racing track measured energy return within 0.06 seconds after the initial impact. This timing was considered too fast and would occur whilst the MCP joint was maximally extended during the support phase of the stride (Ratzlaff *et al.*, 1997). This would not only provide a 'dead' feel to the surface based on the illustration in Figure 2.4.1 but it would also represent additional forces that must be dissipated by the limbs and increase the risk of injury (Ratzlaff *et al.*, 1997). A surface that rebounds too slowly such as a deep surface will not aid the propulsive phase of the gait in any way and equally feel dead to ride on.

The response time is related to the natural frequency of the surface, the limb and gait frequency (Hobbs *et al.*, 2014a), which suggests that the ideal responsiveness is dependent upon the activity being performed. The ideal surface stiffness for creating an optimal response time has been identified for human running tracks (McMahon and Greene, 1979). This has helped to improve running performance and is also associated with low injury rates (McMahon and Greene, 1979). This would make an extremely valuable contribution to the equine industry but it is yet to be investigated further.

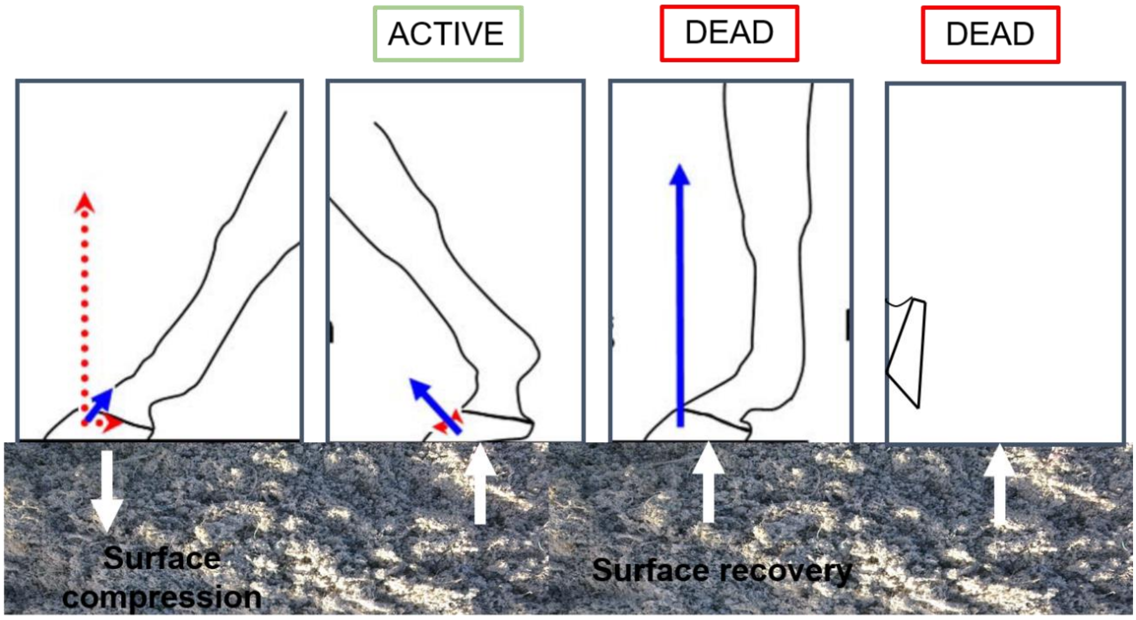


Figure 2.2.2: An illustration representing how active a surface can feel if the surface recovers during the breakover phase of the stride. If the surface recovers before or after this time, it will provide a dead feel to the rider.

Uniformity

Uniformity relates to the consistency of the functional surface properties throughout the whole arena. It is possible to spatially map each of the properties of the arena, which provides an excellent visual on how uniform a surface is (Figure 2.2.3). This can also be repeated to reveal changes over time. A surface should ideally be as consistent as possible and therefore show no spatial or temporal variations. This is difficult to achieve due to the changeable nature of a granular surface, which alters according to sub-base, the amount and type of use and the environmental conditions it is exposed to (Holt *et al.*, 2014). Such factors are responsible for creating inconsistencies in the functional surface properties and ultimately what is experienced by the horse during the hoof-surface interaction.

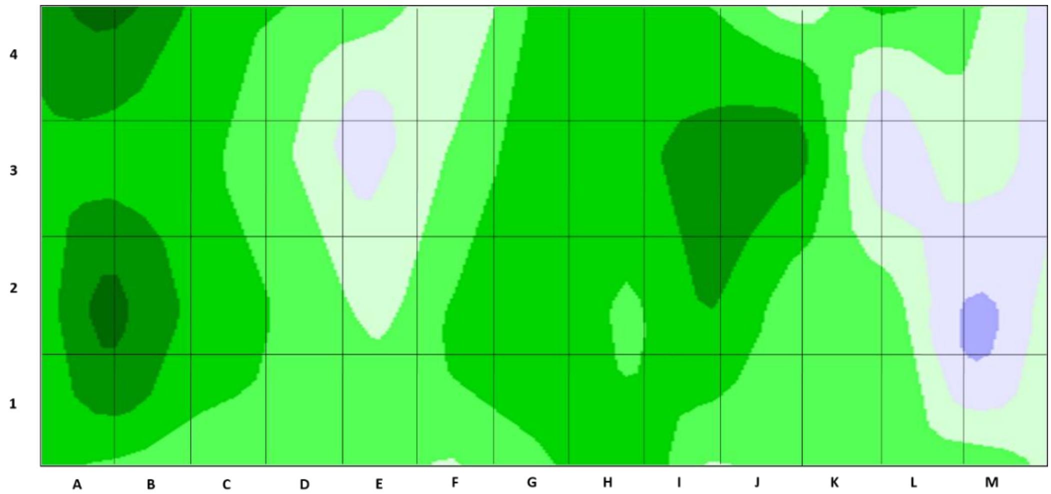


Figure 2.2.3: A contour plot revealing spatial variation in the amount of surface cushioning in a 20 x 60m indoor arena.

Spatial and temporal variations in the functional surface properties will mean that the horse is commonly presented with gradual or sudden changes in surface conditions during training and competition. Surface deformation during stance can vary from a few millimeters to several centimeters (Burn and Usmar, 2005) where changes in track compliance of this order have potential to cause large disruptions to locomotion and gait efficiency (Dyson *et al.*, 2002). An uneven surface has been identified as a risk factor for lameness in dressage horses (Murray et al, 2010b), which demonstrates the importance of maintaining uniformity.

Surface type, maintenance methods used and arena use can have a large impact on the magnitude of variations observed in the functional properties. Sand used without any additives and woodchip surfaces are more likely to become uneven under various conditions whereas wax-coated or sand and rubber are less likely under normal conditions (Murray *et al.*, 2010a). Sand and woodchip have been associated with tripping and slipping respectively and ultimately loss of balance in the horse (Murray *et al.*, 2010a), which could be explained by the lack of uniformity in functional properties.

A combination of poor arena maintenance and a high degree of usage is very common within the industry, which can be responsible for making the surface more uneven and increasing the risk of lameness (Murray *et al.*, 2010a). An arena that is predominantly used for Dressage for example may have been ridden on more frequently on the outer track whereas an arena used for jumping may have disturbed areas where the horses have repeatedly taken off and landed, especially if the jumps are not moved very often. This will undoubtedly increase the spatial variability of the functional surface properties within the arena. The images in plates 2.2.1, 2 and 3 have been taken from 3 different training arenas across the North West of England, which clearly demonstrate the lack of uniformity horses are commonly exposed to.

The influence of a woodchip and dirt surface before (rough track) and after maintenance (smooth track) on vertical hoof forces in the race horse has been investigated by Kai *et al.* (1999). The trajectory of the resultant forces were much more irregular on the rough tracks, which was thought to change the balance of the resultant hoof forces. It was postulated that these changes may be highly destructive to the joints, tendons and ligaments within the distal limb if the hoof is forced to move horizontally or diagonally on a rough surface. Such irregularities are also expected to affect gait stability (Drevemo and Hjerten, 1991), which increases the risk of injury.

Plate 2.2.1.:
***Surface**: Waxed Sand and Fibre surface in an indoor arena.*
***Usage**: Used for flatwork and jumping up to 25 times per day.*
***Visual inconsistencies**:*
A combination of poor maintenance and a high amount of traffic by the mounting block has moved the fibre away from this area. The exposed sand is highly compacted.

Plate 2.2.2.:
***Surface**: Un-waxed Sand and Rubber surface in an outdoor.*
***Usage**: Used for flatwork, jumping and turn out up to 8 times per day.*
***Visual inconsistencies**:*
Drainage issues mean that moisture content throughout the arena is highly variable after periods of heavy rainfall.

Plate 2.2.3.:
***Surface**: Waxed Sand and Fibre surface in an outdoor.*
***Usage**: Used for flatwork once a day.*
***Visual inconsistencies**:*
Poor maintenance has made the surface extremely compact and fibres only remain on the top of the surface.

Superficial harrowing has been shown to improve uniformity of a waxed sand and fibre surface by reducing the standard deviation of maximum vertical acceleration and peak load values, measured using an OBST (Tranquille *et al.*, 2015). The variation increased with subsequent drops of the OBST, which suggests that uniformity would reduce with arena use. A sand and rubber surface, which is one of the most popular surface types used for dressage horses (Murray *et al.*, 2010a) showed little response to superficial harrowing in terms of improving uniformity (Tranquille *et al.*, 2015). This demonstrates that maintenance equipment should be tailored according to the surface components used in order to manipulate the functional properties.

Even with regular maintenance, spatial variations in the functional surface properties are still evident (Northrop *et al.*, 2016), which poses a challenge in providing a completely uniform surface for training and competition. The arena in Plate 2.2.4, is a good example of this. The arena appears visually uniform after routine maintenance, however after testing the functional surface properties, the results reveal that variations still exist.

Plate 2.2.4: A maintained arena surface that visually appears uniform, yet still has variations within the functional surface properties.

It is well documented that different surface types and preparations can influence equine kinetics and kinematics. Variables such as ground reaction force (Kai *et al.*, 1999; Gustås *et al.*, 2006a; Robin *et al.*, 2009; Chateau *et al.*, 2010; Crevier Denoix *et al.*, 2010; Chateau *et al.*, 2013), hoof and fetlock accelerations (Barrey *et al.*, 1991; Burn, 1997; Chateau *et al.*, 2009; Kruse *et al.*, 2012), temporal stride parameters such as stance duration and stride frequency (Robin *et al.*, 2009), kinematic parameters such as limb inclination (Burn and Usmar, 2005; Northrop *et al.*, 2013) and hoof slide during landing from a jump (Orlande *et al.*, 2012) have all demonstrated significant changes according to the surface conditions.

One may expect differences on surfaces with properties that are so obviously different, such as asphalt compared to a fibre sand surface (Chateau *et al.*, 2013). The spatial variations that exist within the same arena surface are much more subtle, however there is evidence to show that even changes of this magnitude can elicit changes in stride parameters of the equine athlete (Burn and Usmar, 2005; Chateau *et al.*, 2009; Kruse *et al.*, 2012; Northrop *et al.*, 2013). It is still unknown whether horses can make rapid stride to stride adjustments to adapt to sudden changes in the functional properties within the same arena. There must be mechanisms present to enable quick adaptations due to the low incidence of tripping and falling in comparison to the number of surfaces used throughout the industry for training and competition. Investigating how horses encounter changes in surface properties would be extremely beneficial and may improve understanding on the degree of variability that is deemed acceptable in terms of reducing injury risk.

2.3 Gait modifications

Gait modifications in humans

The environment humans ambulate through is complex and different ground terrain is continually encountered, which must be safely negotiated to maintain balance (Marigold and Patla, 2007). Human runners have demonstrated their ability to actively modify their gait in response to changes in surface conditions. When encountering sudden changes in substrate stiffness or damping (Farley *et al.*, 1998; Ferris *et al.*, 1999; Kerdok *et al.*, 2002; Moritz *et al.*, 2004), or uneven ground with changes in terrain height (Grimmer *et al.*, 2008; Müller and Blickhan, 2010), humans can adapt their limb properties (leg stiffness, orientation and length) to the altered situation. This adaptation appears to help maintain a consistent COM trajectory and avoid disruptions to locomotion (Ferris and Farley, 1997; Ferris *et al.*, 1998, 1999; Kerdok *et al.*, 2002). An increase in limb stiffness has frequently been observed to offset reductions in surface stiffness (Farley and González, 1996; Ferris *et al.*, 1998), which has been achieved by increasing peak forces (Ferris and Farley, 1997), reducing the angle swept by the limb during stance (Farley and González, 1996) or altering the amount of knee flexion (Dixon *et al.*, 2000). This adjustment offsets the increased surface compression and enables subjects to maintain a linear COM trajectory regardless of surface stiffness (Ferris *et al.*, 1998, 1999). It is important to note that these findings were obtained during steady-state running on a continuous surface.

Ferris *et al.* (1999) aimed to establish how quickly runners could respond to an abrupt change in surface stiffness. The subjects were able to completely adjust their limb stiffness when running from a soft to a hard surface by their first step (Figure 2.3.1). By rapidly reducing leg stiffness from 10.7 to 7.6 kN /m, subjects made a smooth transition with no disruptions to their running pattern. A similar finding was also observed when the subjects ran from the hard onto the soft surface. There did appear to be an anticipatory increase in limb stiffness immediately prior to the surface transition however, although this was not significant. It seems as though a conscious decision was made by the runner to prepare themselves for the transition.

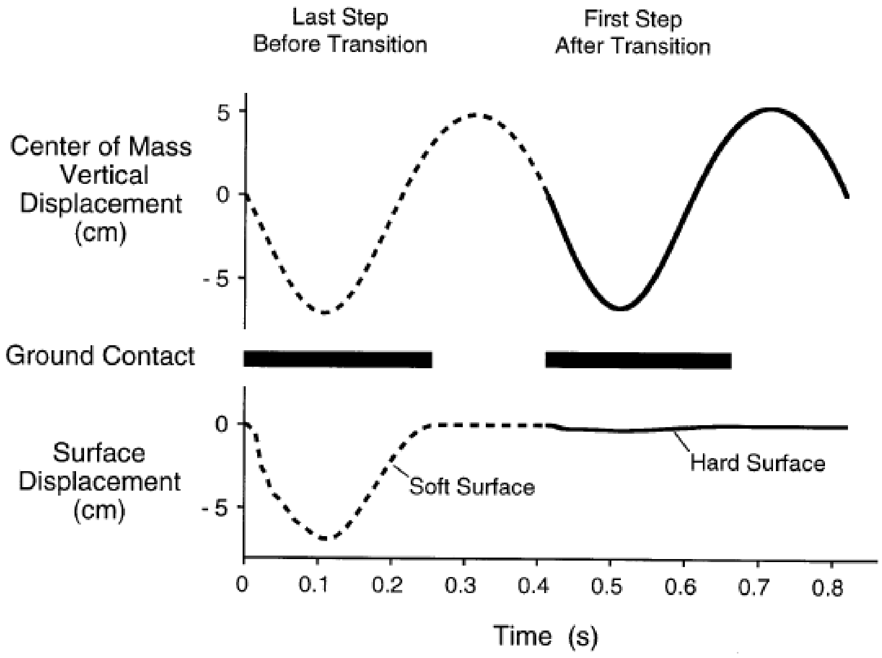


Figure 2.3.1: COM displacement and surface displacement during a transition from soft to hard surfaces. Because the runner decreased leg stiffness to offset the increase in surface stiffness, the trajectory of the COM was the same despite a change in surface compression from 6.9 to 0.3 cm. Extracted from Ferris et al. (1999).

Computer simulations were also constructed by Ferris *et al.* (1999) in order to determine how the running mechanics would be affected if limb stiffness was unadjusted immediately after the transition from one surface to another. The running mechanics were substantially altered where an asymmetrical COM trajectory and velocity were observed (Ferris *et al.*, 1999). A change in peak ground reaction force was also recorded, which may be responsible for increasing the risk of injury (Ferris *et al.*, 1999). Based on these findings, if horses were unable to make any gait adaptations to variations in the surface condition, then surely the quality of performance would undoubtedly be affected and the risk of injury increased.

It is well documented that humans can modify their gait. All of the respective studies provided visual information to the subjects so they were aware of the changes in surface condition. The best means of preventing a fall is to process the visual information about the eliciting hazard ahead of time. Vision provides information about the body relative to the environment, which is referred to as visual kinaesthesis or visual exproprioception (Patla, 1997). This is in contrast to visual exteroception, which affords information on environmental characteristics such as obstacle height (Patla, 1997). These types of visual information are critical for implementing avoidance strategies, for making proactive adjustments to accommodate different ground terrain and for navigation (Patla, 1997).

The visual system is unique in that it is the only sensory system that can provide information to the central nervous system about features of the environment at a distance (Marigold and Patla, 2007) and so it may be used to preplan the path to a specific goal. Such feedforward visual control is important so that the locomotor pattern may be modified before balance is disrupted by a hazardous surface or to ensure that an obstacle can be avoided (Marigold and Patla, 2007). Visual information gained as a person moves through and interacts with the environment has both a feedforward (planning) and a feedback (correction) component (Marigold, 2008). Although feedforward planning may dominate in situations where visual information obtained several steps in advance determines a gait modification is necessary, when there is limited time before a perturbation, visual feedback mechanisms may play a larger role (Marigold, 2008). This visual feedback may in fact serve to update (or fine-tune) the visual information gained in a feedforward manner depending on the situation. Patla (1997) has demonstrated that unexpected changes in step width and length require visual information one step ahead, whereas sudden changes in direction require visual information two steps in advance. Muller *et al.* (2014) also found that gait and posture adjustments occurred one step before (preparatory contact) a visible change in ground level where adjustments increased with increasing drop height. Preparatory adaptations are made to lift or lower the COM (Müller *et al.*, 2012), which is considered to smooth the COM trajectory (Blickhan *et al.*, 2007).

Can the equine athlete modify its gait?

Equine limb stiffness does not appear to change as a result of alterations to the surface properties (McGuigan and Wilson, 2003) like in humans. The human (McMahon and Cheng, 1990; Farley and Gonzalez, 1996; Müller *et al.*, 2010) and equine (McGuigan and Wilson, 2003) limbs have been modelled as springs previously however the components responsible for controlling the stiffness of this spring are quite different between the two species. Alterations in human limb stiffness are mainly initiated by changes in the activity of the long muscle fibres, which is energetically expensive whereas the large passive properties of the equine distal limbs (Biewener, 1998) suggests that the mechanisms behind possible gait adaptations will differ.

The equine athlete has evolved to optimise speed and economy, which is thought to be at the cost of adaptability and makes the horse vulnerable to changes in its external environment (Parkes and Witte, 2015). Peterson and Mcilwraith (2008) also state that it is unlikely a horse can adapt each stride to an inconsistent surface. Equine limb compliance appears to be constrained (McGuigan and Wilson, 2003), but this does not necessarily mean that other adaptations are not possible, which would allow the horse to modify its gait in response to abrupt changes in the surface conditions.

More recent work has suggested that limb posture can be altered in response to different surface properties (Northrop *et al.*, 2013; Symons *et al.*, 2013). Different surface preparations (rolled vs harrowed), which created only slight differences in the hardness of the cushion layer, had a significant effect on the third metacarpal inclination in roll at impact (Northrop *et al.*, 2013). This was thought to be a proprioceptive alteration (Northrop *et al.* (2013) and suggests that horses can actively adjust their gait. Horses have also shown to alter hindlimb posture at heel-strike in a different study by Symons *et al.* (2013) with respect to surface type. The authors could not conclude whether the change was a function of active neuromuscular control or passive tissue properties (Symons *et al.*, 2013). Burn and Usmar (2005) stated that a change in track compliance might cause a more general change in locomotion dynamics, either directly or indirectly through proprioceptive feedback, which may help to explain the findings of Synmons *et al.* (2013). The changes observed in relation to limb posture appear promising that it is possible for horses to modify their gait, however current conclusions are still lacking support, which necessitates further work. The speed at which these changes can be made has also not been investigated and so it is still unknown whether the equine athlete can make rapid stride to stride modifications in limb posture.

Active control of possible gait modifications

Conflict exists regarding the ability of the horse to actively control the properties of its limbs. It has been argued that the hoof braking phase after initial hoof impact is largely passive. The hoof braking period has been reported to be 30-50 milliseconds (Gustås *et al.*, 2006b; Chateau *et al.*, 2010) and is independent of speed Gustås *et al.* (2006b). This period of time corresponds to the period of muscle latency or the period elapsing between the application of a stimulus such as a change in surface condition and the response. During the latent period, excitation-contraction coupling takes place. This is the physiological process of converting an electrical stimulus, when the action potential is generated in the sarcolemma, to a mechanical response or the start of a muscle contraction. This implies that the events at the primary impact phase cannot be modulated actively by muscle contraction (Johnston and Back, 2006). Subsequently, it has been stated that the stability of the structures within the distal limb during this part of stance can only be controlled by passive mechanisms (Hjerten and Drevemo, 1994; Clayton *et al.*, 1998; Gustås *et al.*, 2006b).

Such claims are based on the latent period recorded in humans (Latash, 2008). Nollet *et al.* (2004) measured a mean onset latency of 19.32 milliseconds in the *extensor carpi radialis* in the forelimb of 84 healthy horses. The horses were also sedated in this study, which is well correlated with muscle relaxation and possibly a longer muscle latency (Figueiredo *et al.*, 2005). The shorter latency period recorded in horses in comparison to humans, which may be even shorter in non-sedated horses, suggests that there is potential for more active control of the limb during the braking phase of stance than current thinking (Johnston and Back, 2006; Gustås *et al.*, 2006b).

There is scope for the latent period to be altered by factors such as training, nutrition, fatigue and temperature (Latash, 2008). Training has shown to shorten the latent period of the hamstrings and gastrocnemius in humans (Fong and Ng, 2006), possibly by making the conversion of an electrical stimulus into a mechanical response more effective. It may be postulated that a well-trained horse that has undergone appropriate conditioning for its discipline may also have reduced muscle latency and possibly more active control of its limbs during stance.

After the latent period, there is a group of semi-automatic reactions known as pre-programmed reactions, which can range from 50-100ms and are followed by voluntary reactions (Latash, 2008). It is possible to modulate the pre-programmed reactions in humans by prior instruction (Latash, 2008). If humans are instructed to maintain a joint position against a load, the subject must generate a voluntary command to the muscles controlling the joint. If the instructions are given prior to applying the load, then the commands can be prepared in advance and be ready to be triggered by an appropriate peripheral response. If a rider is effective in applying aids to the horse by producing a peripheral signal and encouraging it to maintain a regular rhythm, then perhaps the voluntary commands controlling the locomotor muscles are prepared in advance.

The processes that occur prior to hoof impact are therefore important to consider and may also contribute towards limb placement and stability during stance. Chateau *et al.* (2010) also identified that anticipation and changes in the locomotor pattern can modify the hoof-surface interaction at the beginning of the stance phase. This has been studied extensively in humans (Marshall and Murphy, 2003; Hobara *et al.*, 2007; Müller *et al.*, 2010) and the presence of muscle activity immediately before impact is known as pre-activation. This allows the contribution of muscle activity responsible for aiding limb placement and posture prior to ground contact to be established. Pre-activation control in humans is a key factor in altering leg posture in preparation for altered ground conditions such as a change in step height (Müller *et al.*, 2010). The presence of muscle activity in 14 out of the 15 equine forelimb muscles prior to hoof impact in the study by Harrison *et al.* (2012) suggests there is an active contribution of the muscles before the primary impact. This further reinforces the notion that it is possible for horses to actively modify their gait but this may arise from events that occur prior to hoof impact through pre-activation.

If a horse encounters irregular surface properties within an arena, then rapid changes in the movement trajectory will be required to sustain locomotion. The pre-programmed reactions are unlikely to be able to fully compensate for the effects of the perturbation. As such, later voluntary corrections are likely needed and the risk of sustaining an injury increases. Chateau *et al.* (2010) also stated that on irregular surfaces, the mechanism of anticipation may also be affected, which may lead to high injurious or stress levels. Horses competing in Dressage and Show Jumping are commonly transported to different venues where there may be no prior experience of irregularities that may exist within the different surfaces. Based on the claims made by Chateau *et al.* (2010), it is surprising that the effects of such irregularities has not already been researched in detail.

Contributions of awareness to gait modifications

Visual information is extremely effective at informing the necessary gait modifications but what mechanisms are present to prevent a loss of balance when visually guided pre-adaptations are not possible? Every day, individuals must also react to unexpected perturbations to maintain balance and continue locomotion (Shinya *et al.*, 2009). Human research has shown that corrective postural responses are possible to prevent a loss of balance when there are no visual cues but this comes at the cost of reduced performance and increased risk of injury (Shinya *et al.*, 2009; Muller *et al.*, 2012; 2014). Evidence has shown that when greater precision is required during a step, foot placement is less accurate when vision is occluded at the point of foot lift off (Reynolds and Day, 2005). Muller *et al.* (2014) have studied the effect of camouflaged changes in ground level on human walkers. The results suggested that the subjects lowered their centre of mass in preparation for the camouflaged step (Muller *et al.*, 2014) in order to maintain balance. A different response has also been recorded when subjects were or were not made aware of a potentially slippery surface. With prior knowledge, subjects have shown to adopt a more cautious gait (Cham and Redfern, 2002; Heiden *et al.*, 2006), which successfully resulted in a decrease of slip probability. Without knowledge however, it was not possible to make adjustments until slipping had already commenced, which increased the risk of injury.

If the horse is aware of inconsistencies in functional surface properties, possibly within an arena that is repeatedly used for training, a different response may be detected. The effect of awareness or habituation to external stimuli on equine behaviour has been investigated previously (Hall and Cassaday, 2006), however awareness of a change in surface conditions has not been quantified. Horses are said to have excellent memories (Nicol, 2002; Murphy and Arkins, 2007), but the limits of this ability in terms of previous hoof-surface interactions are yet to be studied. Awareness of sudden changes in surface conditions could alter the magnitude of pre-programmed reactions (Latash, 2008), which suggests the horse may be able to produce an adequate response more quickly after repeated exposure to similar perturbations. Horses may be able to employ feed-forward mechanisms like humans can and make suitable modifications during the stride preceding the change in ground conditions (Müller *et al.*, 2014). It is envisaged that this would only play a role with visual changes in the surface condition however and repeated exposure to a camouflaged change may elicit a different or more delayed reaction.

2.4 Measurement techniques

To analyse the hoof-surface interaction, accurate quantitative data describing the associated movements is required. Kinematic analysis measures the geometry of movement without taking into account the forces that cause the movement (Clayton and Schamhardt, 2013). Electromyography (EMG) detects the electrical activity created during skeletal muscular contraction (Clayton and Schamhardt, 2013) and enables the active contribution of muscles to certain movement patterns observed to be quantified.

Kinematic Analysis

Kinematic analysis measures the geometry of movement without taking into account the associated forces and enables the quantification of specific gait characteristics that are assessed qualitatively during a visual examination (Clayton and Schamhardt, 2013). The output is in the form of temporal (timing), linear (distance) and angular measurements that describe the movements of the body segments and joint angles. The analysis of movement is by no means a new research interest. Locomotion patterns of humans and other animals have been recorded using motion picture cameras as early as the 19th century. Eadweard Muybridge used photographs in 1877 to demonstrate that a moment of suspension exists at a fast trot. By the 20th Century, automated and semi-automated computer-aided motion analysis was made possible using both manual and automatic marker identification techniques (Hobbs *et al.*, 2010). The hardware and software that these systems use has developed rapidly in the last

10-15 years and a large variety of different methods can now be used to track movement in two (2-D) or three-dimensions (3-D) (Hobbs *et al.*, 2010).

Three-dimensional motion analysis is the "gold standard" for quantifying kinematic parameters however, its use can be limited by financial, spatial and temporal restraints (Maykut, 2015), which explains why 2D videographic analysis is still commonly used throughout the industry. Collecting data in 3D overcomes the issues associated with 2D data but the procedures involved are much more complex. The markers used can be automatically identified and tracked by software such as Qualisys Track Manager (QTM), which removes the element of human error. Processing the data and extracting the relevant parameters in software such as Visual 3D or Codamotion ODIN can be a lengthy process. It is possible to save models and pipelines that are used to derive specific parameters, which can later be applied to unlimited dynamic trials and improves analysis efficiency. The 3D software generally has the option to synchronise other forms of data such as ground reaction force and electromyography data. A full locomotory profile can be created for each subject, which has the potential to address more complex research questions.

Three dimensional motion analysis can be performed in a laboratory setting or outdoors under field conditions. The improved accuracy in comparison to 2D has potentially enabled more subtle changes in response to various factors such as surface to be quantified. The accuracy of data collected outside can be affected by the presence of background light but it is possible to use active filtering to address this issue (QTM, 2011). This dramatically increases the ability to capture passive markers by capturing extra images without the IR flash, which are then used to remove background light from the images used by the cameras (QTM, 2011). This reduces the normal maximum frequency at which data can usually be collected at in a laboratory. Nevertheless, it is essential to collect data under field conditions to understand the true effect of certain factors on the horse and several publications have been generated using such methods (Burn and Usmar, 2005; Gustås *et al.*, 2006a; Northrop *et al.*, 2013).

Electromyography Analysis

The relationship between voluntary muscular contractions and the production of an electrical signal was identified as early as the 17th Century by Francesco Redi when studying the electric ray fish (Richards, 2016). Methods to detect the electromyographic signal in humans slowly developed during the 19th Century, which helped to investigate muscular function (Richards, 2016). Skeletal muscles consist of thousands of muscle fibres that contract when stimulated by a nerve impulse generated in the motoneuron (Richards, 2016). A single motoneuron and all the fibres innervated by it creates a functional unit called a motor unit (Richards, 2016). The number of fibres per motor unit will vary within and between muscles where the size of the muscle generally corresponds with the size of the motor unit (Richards, 2016). To sustain a muscular contraction, the motor units must be repeatedly activated by the continuous depolarisation and repolarisation of the muscle fibre membrane potential, which creates motor unit action potential trains (MUAPTs) (Richards, 2016). The summation of electrical activity from the MUAPTs can be detected with an EMG sensor positioned inside a muscle (intramuscular EMG) or on the skin on top of the muscle (surface EMG).

Surface electromyography (sEMG) is a more practical, cost effective method (De Luca, 1997) and may be a more suitable alternative for assessing superficial muscle activity during locomotion (Farina, 2006). This is further supported by the numerous equine publications that have used sEMG to measure muscle activity during dynamic movements (Robert *et al.*, 2002; Hodson-Tole, 2006; Licka *et al.*, 2009; Zsoldos *et al.*, 2010; Harrison *et al.*, 2012; Kienapfel, 2015). sEMG is non-invasive and therefore should not affect normal muscle function but this measurement technique is accompanied with other limitations.

Activity from adjacent muscles can cause interference within the EMG signal, which is known as cross talk. This can cause problems when recording from small muscles in close proximity to bigger muscles that demonstrate the same activation patterns. Cross-talk EMG signals have a similar frequency bandwidth to the target muscle, which makes it difficult to eliminate during processing and can mislead the interpretation of the EMG signal (De Luca *et al.*, 2012; Richards, 2016). Cross talk contamination has been highlighted as a serious concern in gait studies by De Luca *et al.* (2012) who identified an optimal inter-electrode distance of 1 cm to help reduce interference from adjacent muscles. This improved signal reliability by reducing the pick-up area of the signal (De Luca *et al.*, 2012). A small inter-electrode distance in conjunction with the large mass of equine superficial locomotory muscles should reduce the likelihood of detecting electrical activity from adjacent muscles.

2.5 Summary

The equine athlete is expected to perform consistently regardless of whether it is aware of the surface irregularities or not. Surface maintenance can improve surface uniformity to some extent but the highly sensitive nature of the functional properties to the surrounding environment means that variations are impossible to eradicate. Understanding the mechanisms behind possible gait modifications that occur as a result of such variations in order for the horse to sustain locomotion would undoubtedly provide an essential contribution to current surface and biomechanical research. Based on previous work, it was hypothesised that gait modifications: 1) may occur with an earlier contact timing of the ipsilateral hind limb, 2) may occur by altering dynamic posture i.e. limb position, 3) may occur by increasing the active contribution of muscles which would alter ground reaction forces. All of these modifications have been found in trotting horses to assist in managing balance (Hobbs *et al.*, 2016). In addition, gait modifications: 4) may occur by increasing the magnitude of pre-activation control of limb muscles in order to stabilise the limb prior to ground contact, 5) may represent a more cautious gait when horses are aware of an abrupt change in surface condition and 6) will not occur through changes in equine limb stiffness as a function of surface stiffness. There are currently no guidelines on what variation is acceptable within an arena and so the findings of this work may help to answer some fundamental questions in relation to how much horses need to modify their gait to cope with an abrupt change in surface condition.

MPhil Phase

Gait event study

Aim:

- To authenticate a method of identifying kinematic gait events in the fore and hind limbs.

Objective:

- To use ground reaction force data to authenticate an original, straightforward method for the identification of: hoof-on, mid-stance and hoof-off using kinematic data, which can be universally applied to all limbs.
- To use a prototype force shoe to determine whether the method can be applied to data collected on a compliant surface with the same degree of accuracy and precision.

Variability Study

Aim:

- To quantify gait variability when moving over a uniform surface.

Objective:

- To investigate gait variability within horses and between days using surface electromyography and kinematic measurements.
- To develop techniques for collecting, processing and analysing kinematic and EMG data for measuring the response to a change in surface conditions.

PhD Phase

PhD study: Equine response to an abrupt change in surface conditions

Aim:

- To quantify the kinematic and electromyographic response of a sample of horses to a camouflaged, abrupt change in the functional surface properties.

Objectives:

- To create kinematic gait events using the method identified in the ‘gait event study’ to calculate kinematic and surface electromyographic parameters.
- To investigate stride to stride kinematic and electromyographic changes on a continuous surface and on a surface that presented an abrupt change in properties.

- To investigate the effect of awareness (no awareness vs. awareness) of the abrupt change on equine locomotion on kinematic and electromyographic parameters.
- To use the Orono Biomechanical Surface Tester to measure functional surface properties and quantify the magnitude of the abrupt change in surface conditions that the sample of horses are exposed to.
- To evaluate possible implications of the findings in terms of performance and risk of injury.

4.0 Ethical Considerations and Health and Safety

The study was approved by the ethics committee (project reference number: RE/13/01/SH) at University of Central Lancashire (Appendix I). Risk assessments (Appendix II) were formulated and approved for the use of horses and all the equipment. The procedures in place to keep the working environment safe and the equipment protected from the elements was thoroughly discussed prior to commencing data collection.

4.1 Development of Measurement Techniques

4.1.1 Development of a kinematic model

Motion analysis systems generally track retro-reflective markers fixed to the skin or bones where the marker locations are chosen in accordance with the purpose of analysis (Back and Clayton, 2013). Calculation of a joint angle for example, requires a minimum of three markers; one over the centre of joint rotation and one on each segment (Back and Clayton, 2013). A three dimensional model, consisting of 30 retro-reflective markers, has been used to study the effects of a flat and banked curve on forelimb inclination and relative body inclination (Hobbs *et al.*, 2011). The marker set included anatomical markers, which define the joints and segment orientation and tracking markers that track movement of a particular segment through 3D space (Hobbs *et al.*, 2011).

It was necessary for Hobbs *et al.* (2011) to track movement in more than one plane in order to address the research aims. A basic two dimensional model has been used previously to investigate the effect of two different beach tracks on hoof landing (Chateau *et al.*, 2010) and limb loading parameters in the sagittal plane (Crevier-Denoix *et al.*, 2010). A two-dimensional high speed camera (600 Hz) was used to track circular, reflective markers applied to the main forelimb joints (shoulder, elbow, carpus, fetlock and coffin) of the horses (Chateau *et al.*, 2010; Crevier-Denoix *et al.*, 2010). Hindlimb kinematics have been studied on two race track surfaces by Symons *et al.* (2013). Two-dimensional high speed video analysis (500 Hz) was sufficient to quantify hoof orientation and displacement. A simple 2D model was also used by Burn and Usmar (2005) to investigate the effect of three surfaces of varying density on limb landing angle. Markers were applied to the lateral aspect of the forelimb and tracked using a three dimensional motion capture system (200 Hz).

The use of 2D models in other, relevant literature, even when there is the ability to capture data in 3D, suggests that applying markers laterally to the fore and hind limbs will be appropriate to address the research aims of this PhD. Movement of the equine limb segments and joints also occurs predominantly in the sagittal plane. It is envisaged that possible adaptations to surface conditions would be observed in this plane, which further supports the use of a 2D model. Using a 3D motion analysis system to track movement of a 2D model does have the advantage of being able to quantify the true angle more accurately due to using a 3D capture volume.

4.1.2 Selecting muscles for surface electromyographic analysis

The superficial muscles chosen for this work were selected based on their associated function with limb placement and because of their suitability and reliability for taking surface EMG measurements (Jansen *et al.*, 1992; Hodson-Tole, 2006; Harrison *et al.*, 2012; Kienapfel, 2015).

splenius

Function: Elevates the head and neck

Origin: Spinous processes of T3-T5

Insertion: Nuchal crest, mastoid process, atlas and transverse processes of C3-C5

Sensor location: A tape measure was placed from the poll to withers. The midpoint was located and the muscle belly identified 12-18cm ventral to this point depending on the height and breed of horse. To confirm that the muscle belly had been correctly identified, the horses were gently asked to flex at the poll, in order to emphasise the profile of the muscle (Plate 4.1.1).

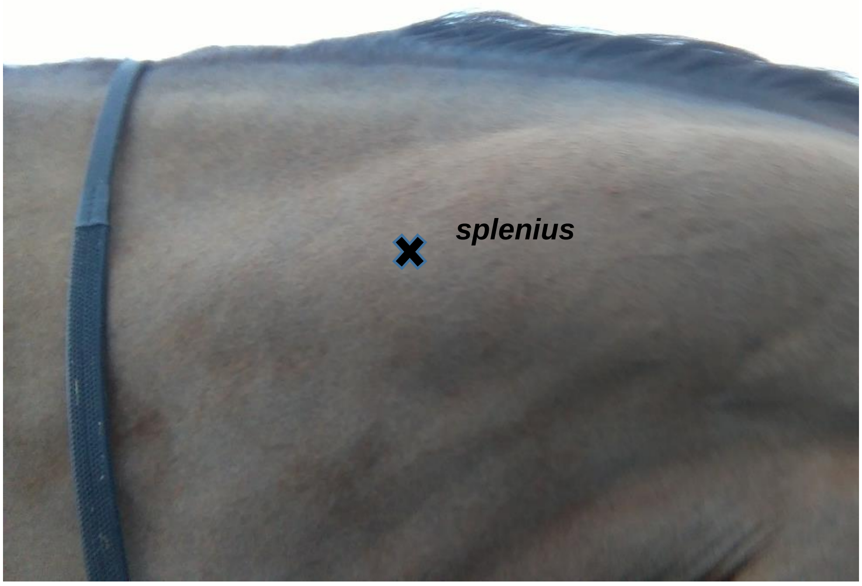


Plate 4.1.1: Flexing the poll to highlight the profile of the splenius muscle.

trapezius cervicis

Function: Elevates shoulder; draws the scapula cranial and dorsal (forward and upward).

Origin: Nuchal ligament funicular portion (cord) from C2-T3 (Pattillo, 2007)

Insertion: Entire cranial surface of the scapula spine. Blends with the shoulder and arm fascia

Sensor location: The detection surface area of the electrodes used for this work was relatively small in relation to the size of the *trapezius cervicis* muscle. A larger inter-electrode distance can increase the pick-up area of the sensor and subsequently increases the amplitude of the EMG signal (Richards, 2016). This may be considered more appropriate for an equine based application, however this also increases the risk of cross talk (Richards, 2016). To minimise this risk and account for variations that may be observed in different areas of the muscle, two EMG sensor locations were used.

Site 1: A tape measure was placed from the poll to withers. The ¾ point was located and the muscle belly identified approximately 10 cm ventral to this point depending on the height and breed of horse; Site 2: 4.5-7cm cranial to site 1. To confirm that the muscle belly had been correctly identified, the horses were gently asked to flex at the poll again, which helped to make the *splenius* more prominent and differentiate between the muscles of the neck (Plate 4.1.2).

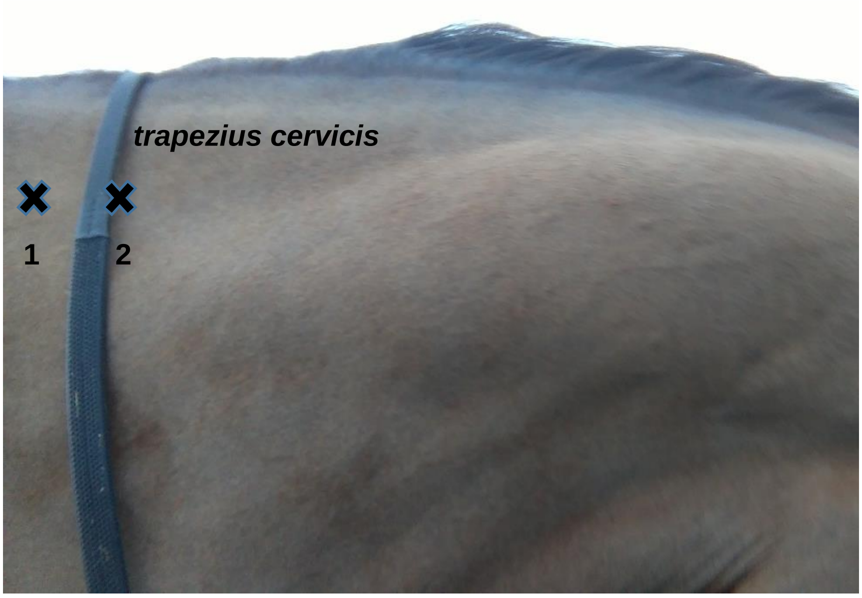


Plate 4.1.2: Flexing the poll to differentiate between the muscles in the neck.

<u>The long head of the triceps brachii</u>

Function: Flexes the shoulder joint and extends the elbow.

Origin: Caudal border of scapula (Watson and Wilson, 2007).

Insertion: Olecranon (Watson and Wilson, 2007).

Sensor location: Due to the size of the muscle and the justification used for the *trapezius cervicis* muscle, two sensor locations were used. Site 1: Midway between the olecranon and the proximal point of the scapular spine, measured at an angle of 50° from a line drawn between the olecranon and the intermediate tubercle of the humerus. The position of the lateral head was determined to be under a point 40% along a line joining the greater tubercle and the olecranon, measured at a 50° angle from a line between the intermediate tubercle of the humerus and the olecranon (Hodson-Tole, 2006) from J. Watson personal communication. Site 2: 6cm caudal to site 1 (Plate 4.1.3)

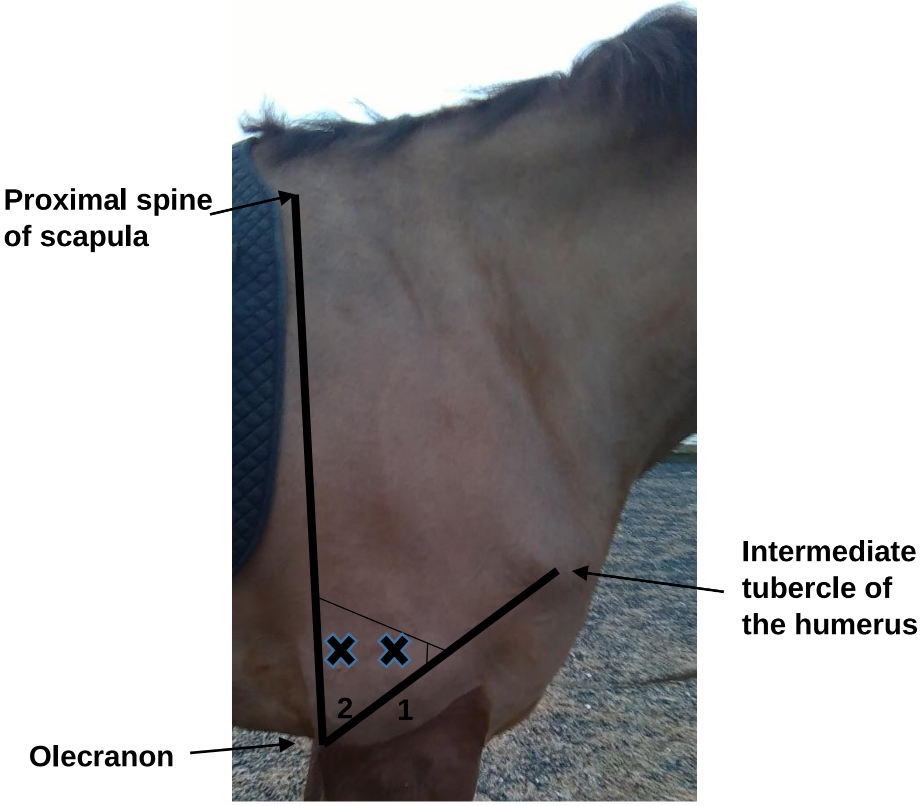


Plate 4.1.3: The two sensor locations on the muscle belly of the triceps brachii

extensor carpi radialis

Function: Extends the knee and flexes the elbow joint.

Origin: Lateral epicondyle and crest of humerus.

Insertion: Crosses the carpal joint and inserts on the metacarpal (cannon) tuberosity.

Sensor location: The lateral tuberosity of the radius was located before moving cranial from this location to the *extensor carpi radialis*, a narrow yet quite prominent muscle at the most anterior and lateral portion of the forelimb. The muscle belly was relatively easy to palpate and the sensor was located approximately 10cm ventral to the level of the lateral tuberosity of the radius (Plate 4.1.4).

ulnaris lateralis

Function: Flexes carpal joint and extends elbow.

Origin: Lateral epicondyle of the humerus, caudal and distal to the lateral collateral ligament of the elbow joint.

Insertion: 1)accessory carpal bone 2)Proximal to fourth metacarpal bone

Sensor location: This was located after the *extensor carpi radialis* muscle. The lateral tuberosity of the radius was located before moving caudal from this location to the *ulnaris lateralis*. The distance moved caudally was approximately half that moved cranially to the *extensor carpi radialis* muscle. The muscle belly was identified approximately 14-17cm ventral to the level of the lateral tuberosity of the radius (Plate 4.1.4).

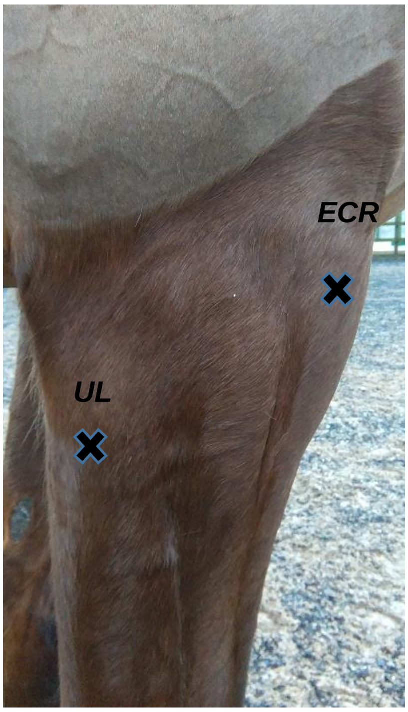


Plate 4.1.4: Sensor locations for the extensor carpi radialis and ulnaris lateralis

4.2 General Methods: Experimental Set Up

The set up identified in this section was used for data collection throughout the project unless stated otherwise in the relevant chapters. Kinematic data were recorded at 232 Hz using an eight camera (Qualisys Oqus) motion analysis system with Qualisys Track Manager Software. Surface electromyography (EMG) measurements were taken at a sampling rate of 2088 Hz using a Delsys® base station. The EMG and kinematic data were synchronised in Qualisys Track Manager (QTM) using a trigger and Delsys® Trigno™ Control Utility. The motion analysis system was set up to perform an extended calibration (Plate 4.2.1).

Plate 4.2.1: An eight camera motion capture system set up for an extended calibration.

This set up does have limitations because data can only be collected in the sagittal plane and from one side of the horse. It does increase the length of the recording volume however, which makes it possible to collect data from consecutive strides. This is extremely beneficial for the current application where the effect of a change in surface condition on the kinematic response of the horse during successive strides must be quantified.

The illustration in Figure 4.2.1 was used to inform the experimental set up and to ensure it was the same on different data collection days because the equipment was collapsed and erected again each day. The poles were used as a guide to help maintain straightness when leading the horses through the recording zone. A new project was opened in QTM and the project options tab was used to ensure that the ‘use external trigger’ option was selected. Active filtering under the camera system tab in project options was set to continuous mode, which was recommended for data collection outdoors (QTM, 2011).

Retro-reflective markers were placed on the surface 0.5 m away from the poles at approximately 1.0 m increments along the recording area before starting a new measurement in the QTM software (Figure 4.2.1). The camera position was then altered accordingly so the markers were visible at the bottom of each screen. It was possible to adjust the marker threshold at this stage if necessary by opening or closing the aperture on each camera, which altered the amount of light entering the camera and the size of the markers visible in QTM.

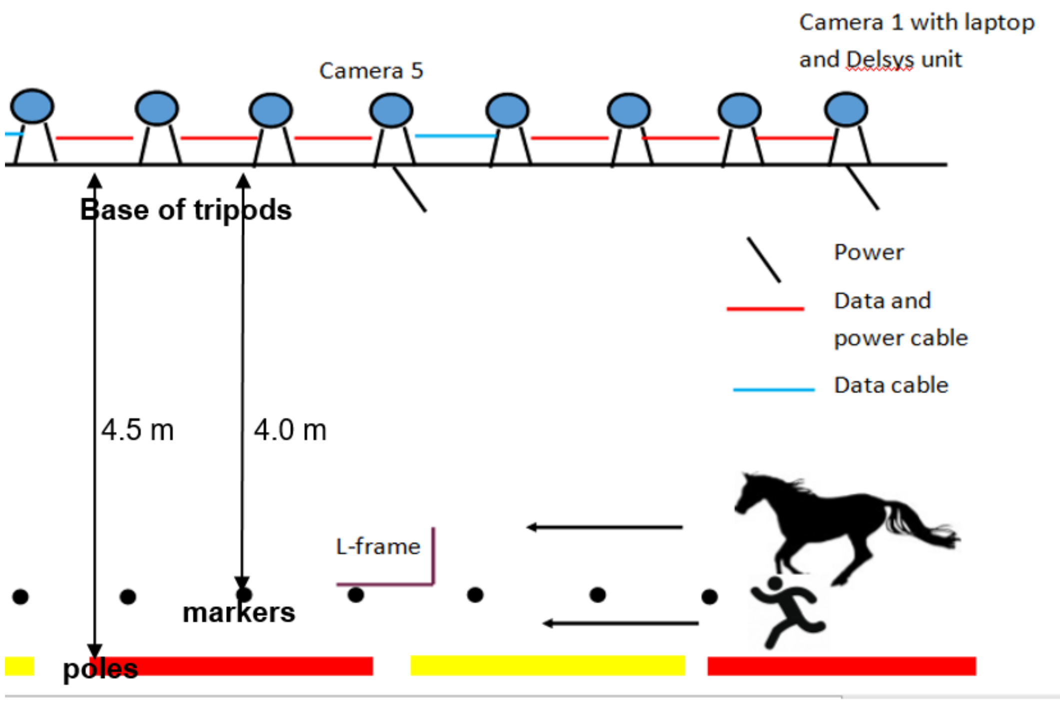


Figure 4.2.1: An illustration of the experimental set up

Calibration

The recording area was calibrated in order to scale the linear measurements (Back and Clayton, 2013). This enabled the movement of the markers within the 3D space to be translated into measurable parameters. It was necessary to repeat the calibration at the start of each day when the camera system had been re assembled and several times during the day to maintain the accuracy of the data collected. The wand calibration method is commonly used with Qualisys Track Manager for sport specific applications and uses a calibration kit that consists of two parts: an L-shaped reference structure and a calibration wand (Qualisys Track Manager (QTM), 2011). In a standard calibration, all of the cameras can see the reference L-frame whereas only 3 to 4 cameras can see the frame in an extended calibration (QTM, 2011). The remaining cameras are calibrated by the overlap with the reference cameras field of view, which reduces the accuracy of the three dimensional data (QTM, 2011).

The exact dimensions of the wand kit were entered in the calibration tab of the project options in QTM prior to calibrating the system. The L- frame was placed so it was visible by cameras four, five and six with the long arm in alignment with the markers used to position the cameras (Figure 4.2.1). These markers were removed from the recording zone before performing a calibration for 180 seconds by moving the wand along the intended recording area. This created an approximate calibrated volume of **L** 7.3 m x **H** 1.8 m x **W** 1.0 m.

Calibration accuracy

Once a calibration had been completed, the results window revealed the quality of the calibration and whether it had passed. It was also possible to visually assess the calibrated volume to identify any areas that may have been missed with the wand (Plate 4.2.2, 3 and 4).

Plate 4.2.2: (above): Lateral view of a 'passed' calibrated volume demonstrating no gaps.

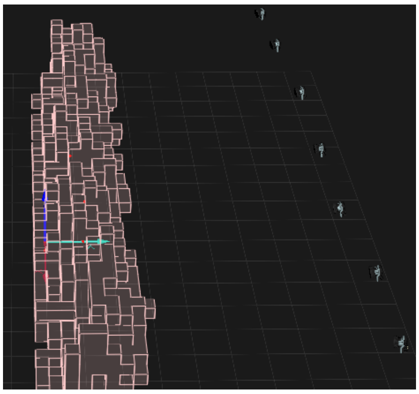
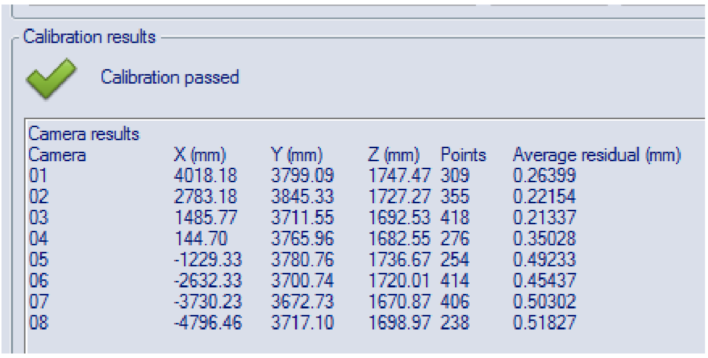


Plate 4.2.3: (right): Aerial view of a 'passed' calibrated volume demonstrating no gaps.

Plate 4.2.4: Lateral view of a 'failed' calibrated volume, which is much shorter in length and demonstrating gaps.

The average residual values from each camera were displayed, which provides an indicator of the amount of error of the marker position within a linear measurement of 1000mm (Figure 4.2.2). Factors such as sunlight and marker masking where immovable reflective objects have been covered up in QTM, have a large effect on the residual values. Data is commonly collected outdoors for equine type applications and therefore the respective factors do pose a limitation. Active filtering reduces this problem because it helps to filter out background light (QTM, 2011) and possibly prevents the residual values from exceeding an acceptable range. The user manual developed by QTM (2011) suggests that the average residual can differ between 0.5 to 1.5mm depending on the measurement volume. It was therefore important to get the average residual values of a calibration within this range during data collection.


Calibration results

Calibration passed

Camera results

Camera	X (mm)	Y (mm)	Z (mm)	Points	Average residual (mm)
01	4018.18	3799.09	1747.47	309	0.26399
02	2783.18	3845.33	1727.27	355	0.22154
03	1485.77	3711.55	1692.53	418	0.21337
04	144.70	3765.96	1682.55	276	0.35028
05	-1229.33	3780.76	1736.67	254	0.49233
06	-2632.33	3700.74	1720.01	414	0.45437
07	-3730.23	3672.73	1670.87	406	0.50302
08	-4796.46	3717.10	1698.97	238	0.51827

Figure 4.2.2: A successful calibration results window displaying the average residual values

In order to account for this inherent variation that may influence the processed data, the effect of two different successful calibration files on the same dynamic files was quantified. Temporal variables are not expected to change according to the calibration file, however linear and angular parameters may be affected. Stride length and peak MCP extension at the palmar aspect of the joint were therefore calculated from each trial according to the calibration file.

The average residual values varied from 0.213-0.518 mm and 0.758-1.466 mm for calibration 1 and 2 respectively and therefore were deemed acceptable to use according to the guidelines created by QTM (2011). There was no significant difference (*P* >0.05) found between the calibration trials for the two parameters. Mean fetlock angle varied up to 0.19% for Horse 1 and up to 0.1% for Horse 2 between the different calibration files (Figure 4.2.3). Mean stride length varied up to 0.08% for Horse 1 and up to 0.003% for Horse 2 between the different calibration files (Figure 4.2.4).

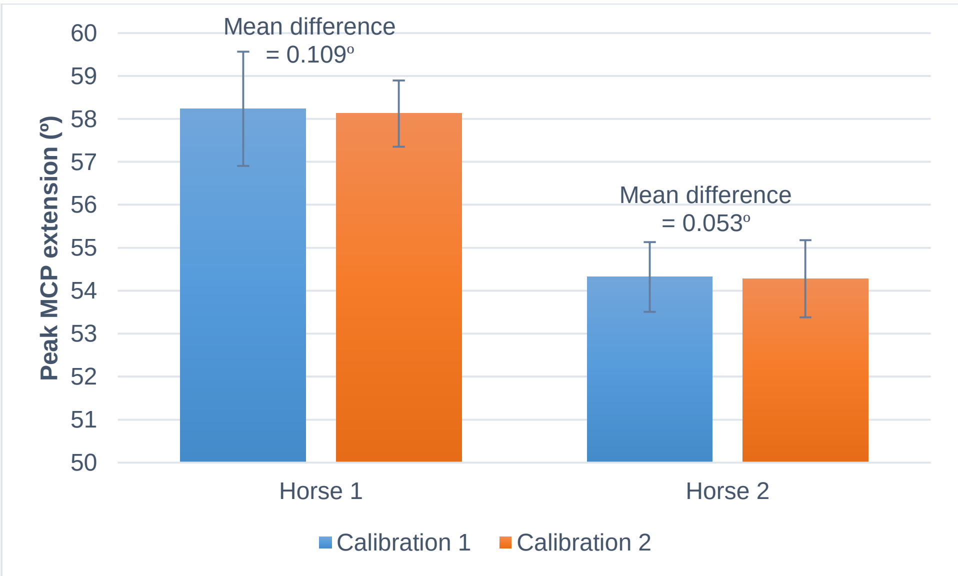


Figure 4.2.3: Mean (±SD) fetlock angle for each horse and calibration file.

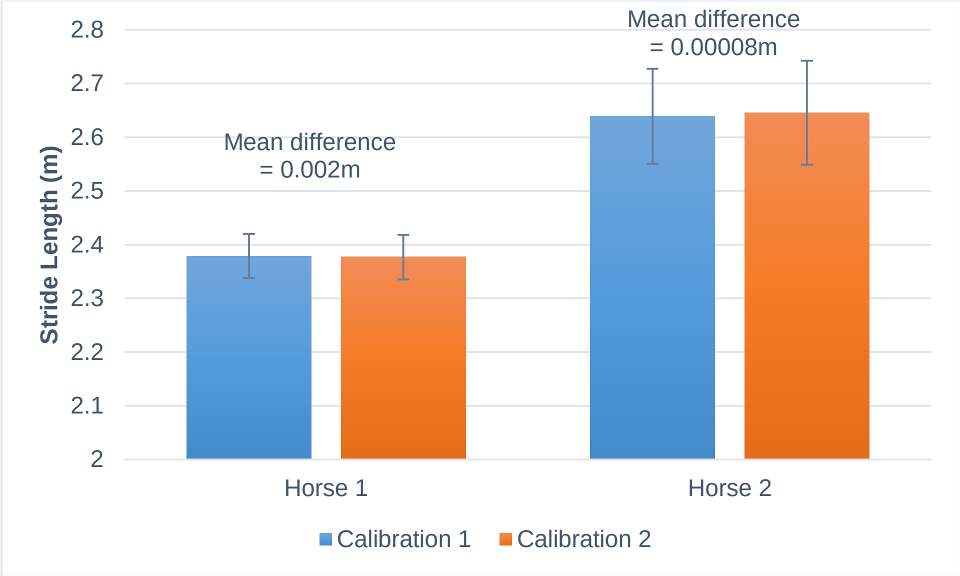


Figure 4.2.4: Mean (±SD) stride length for each horse and calibration file.

The main study may establish extremely subtle changes in kinematic parameters and therefore any variations that are not initiated by the actual response of the horse must be accounted for. The differences reported here are negligible and as long as the residual values are within a suitable range, it can be suggested that different calibration files can successfully be applied with limited effect on the accuracy between files.

4.3 General Methods: 3D kinematic marker application

Eighteen spherical retro-reflective markers, 25 mm in diameter were secured to the horse using a premium double-sided tape on the following anatomical locations on the right side of all subjects: Axial locations included: projecting cheek bone; wing of the atlas and T6 (withers) to measure neck inclination; and croup in order to track speed. The appendicular locations selected enabled joint angles and segment inclinations to be quantified, which enabled comparisons between studies that investigated equine locomotion on a uniform surface. Appendicular locations on the forelimb were informed by the marker set used by Hjertén and Drevemo (1994) and included: the proximal end of the spine of the scapula; the greater tubercle of the humerus (centre of rotation of the shoulder joint); the lateral epicondyle of the humerus (centre of rotation of the elbow joint); the lateral tuberosity of the radius; the lateral styloid process of the radius; the proximal end of metacarpal IV; centre of rotation of the MCP joint and the lateral hoof wall, approximately over the centre of rotation of the DIP joint. Appendicular locations on the hind limb were informed by the marker set used by Hodson *et al*. (2001) and included: the most ventral part of the tuber coxae; greater trochanter; lateral epicondyle of the femur; talus; and the centre of rotation of the MTP joint and the lateral hoof wall, approximately over the centre of rotation of the hind DIP joint (Plate 4.3.1).

Plate 4.3.1: Horse 6 from the main study with the 3D markers and EMG sensors applied ready to collect data.

4.4 General Methods: EMG sensor application

Prior to data collection, the sensor locations were shaved with a razor. It was important to remove all hair to ensure there was a consistent contact between the electrodes and skin. The shaved sensor locations were thoroughly cleaned with an alcohol wipe to remove dirt and dead skin cells before applying the EMG sensor. A Delsys® Trigno™ wireless system with standard sensors was used, which have patented parallel bar technology (Delsys®, 2016) (Plate 4.4.1). This uses two EMG inputs with proprietary stabilising references, which enables the sensors to react instantaneously to disturbances detected at the electrode-skin interface (Delsys®, 2016). This reduces the impact of the noise components on the EMG signal quality and was therefore considered ideal for an equine based application. A custom-made adhesive label was applied to each sensor before swabbing each electrode with salt water to improve the conduction between the skin and electrode interface. The sensor was then applied to the prepared muscle belly with the arrow running in alignment with the muscle fibres (Plate 4.4.1 and Figure 4.4.1).

Plate 4.4.1*: Delsys® Trigno™ sEMG sensor.*

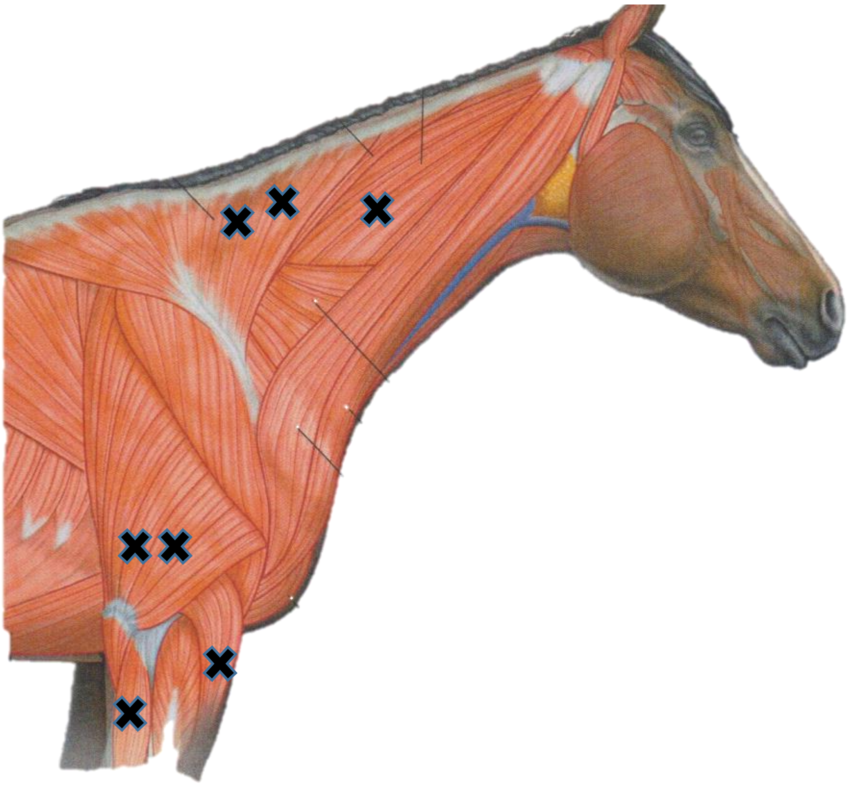


Figure 4.4.1*: The illustration used to aid correct sensor placement in alignment with muscle fibres.*

5.0 Surface construction and preparation

A purpose built research test track, which was installed in 2011 parallel to the outdoor arena at Myerscough College, was used for the main study (Plate 5.0.1). The test track was constructed over Permavoid 85 units (Permavoid Limited™, Warrington), which were also used under the 2012 Olympic equestrian footing. Permavoid 85 is a modular interlocking plastic storage system with a thickness of 85 mm and creates an innovative sub-base replacement system.

Plate 5.0.1: The purpose built research test track, which is accessed from the main outdoor arena at Myerscough College.

The pre-existing surface on the test track (waxed sand and fibre), which was laid to a depth of 150mm (Plate 5.0.2) was used to create the following surface conditions;

1. Firm
2. Firm to Soft
3. Soft to Firm
4. Soft

Condition one and four were included in order to generate baseline data for each horse and quantify the resultant effect of an abrupt change in the functional surface properties on the kinematic and neuromuscular response of the horse. The test track had not been used prior to data collection for approximately 1 year and was considered to be well compacted, which made it ideal to simulate a firm preparation. Development work was carried out to determine the most suitable piece of equipment to create a soft surface.

Plate 5.0.2: Synthetic equestrian surface laid to a depth of 150mm over a Permavoid Limited™ 85 sub-base, separated by a non-woven geotextile membrane.

5.1 Surface development work

The surface on the test track was well compacted, similar to a well-established arena and considered appropriate to use for the firm surface condition. The aim of the development work was to identify the most suitable piece of equipment to use to create the soft surface condition and to ensure that the two surface conditions presented a significant difference in functional surface properties. A small area of the test track that was not being used for the main study was designated for surface development work and split into three strips. A tiller and rotovater were used on two of the sections to create the soft surface condition (Plate 5.1.1 and 5.1.2). A hand rake was used on the third section to gently level the top cushion layer and create the firm surface condition. An Orono Biomechanical Surface Tester was used to quantify the response of the surface to the different preparation methods.

Plate 5.1.1: Tiller

Plate 5.1.2: Rotovator

The Orono Biomechanical Surface Tester (OBST)

The OBST was originally developed and constructed by Professor Mick Peterson at the University of Maine approximately 10-15 years ago. The OBST enabled race track surface properties to be objectively quantified and helped to inform track management protocols with an aim of improving consistency and surface safety (Peterson *et al.*, 2008). The mechanical equipment was designed to simulate the speed, directions and loads of the leading forelimb of a Thoroughbred horse impacting and loading the surface at gallop. The OBST was later replicated by Glen Crook at the University of Central Lancashire in 2010 with the predominant focus of testing equestrian arena surface properties including the surface used for the 2012 Olympics (Plate 5.1.3).

The device is a two axis drop tower type apparatus that impacts an aluminium hoof into the surface from a height of 0.86 m at an off set angle of 8˚ from the vertical, which is measured with the use of an inclinometer. The two non-orthogonal axes of motion allows acceleration and force to be calculated in three dimensions when the hoof impacts the surface (Plate 5.1.4). Gravity acts on the first axes, the long rails on which the hoof and instrumentation slides and generates a force by accelerating a mass of 33kg down the rails (Peterson *et al.*, 2008). This provides approximately 278 Joules of energy at impact, which accounts for the energy of the hoof impacting the surface including the partial weight of the animal and associated musculature (Peterson *et al.*, 2008). A second set of shorter linear rails, which is preloaded by a spring moves down as a part of the mass attached to the slide and only moves once the hoof is in contact with the surface (Peterson *et al.*, 2008). The difference in the angle between the first and second axes of 8˚ forces the hoof to slide forward towards the toe as it impacts the ground and the second preloaded axis is compressed. The angle of hoof impact has been selected to match the published biomechanical data for initial impact of the hoof (Ratzlaff *et al.*, 1993). The instrumentation on the OBST comprises a triaxial load cell (Kistler Instruments Ltd. Hook, Hampshire, UK, type 9347C), single axis load cell, triaxial accelerometer (Kistler Instruments Ltd. Hook, Hampshire, UK, type 8793A), linear potentiometer (Novotechnik, Ostfildern, Germany, model TRS 100) and string potentiometer (Celesco, Chatsworth, CA, USA, model PT5A). Together, this provides a detailed, objective data set on the functional surface properties as discussed in chapter 2.0.

Plate 5.1.3: The OBST mounted onto a tractor

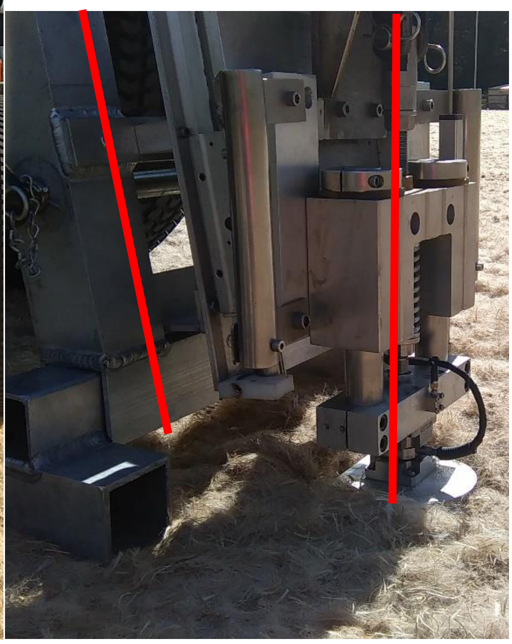


Plate 5.1.4: A lateral view of the OBST demonstrating two non-orthogonal axes of motion.

The OBST was mounted onto the back of a tractor using a three point linkage to provide stability during testing and in order to move to different drop locations. The equipment was powered by two 12 volt batteries and connected to a laptop via Ethernet. The data from the instrumentation were recorded simultaneously (National Instruments UK, Newbury, Berkshire, UK, A/D converter model NI USB-6210) for two seconds at 2000 Hertz (Hz) using LabVIEW 2010 software (National Instruments UK, Newbury, Berkshire, UK). The testing device has demonstrated the ability to distinguish between different surfaces and preparations previously (Tranquille *et al.*, 2015) and has also been used to quantify the effects of different moisture contents and rates of compaction on the surface properties (Holt *et al.*, 2014). The OBST was therefore deemed suitable for use to measure the effect of the different preparations on the functional surface properties for this project.

Development work protocol

A single drop was made with the OBST in five locations on each of the three test strips. The signals were processed using the method shown in section 6.3. A one way ANOVA was used to investigate the effect of surface preparation on the cushioning (Figure 5.1.1) and impact firmness (Figure 5.1.2) of the surface in order to select the most suitable piece of equipment to create the soft surface condition.

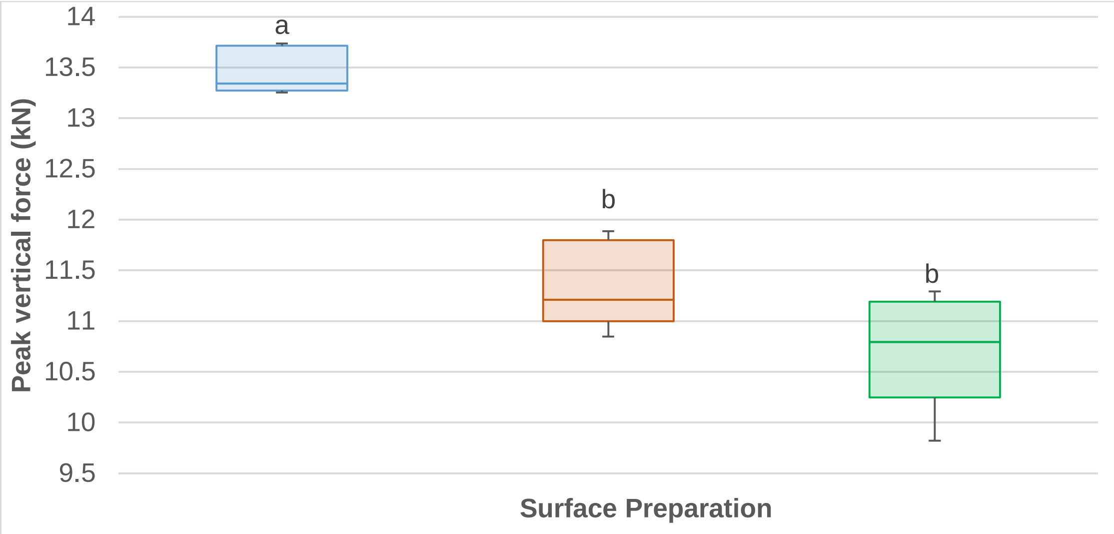


Figure 5.1.1: A comparison between the cushioning on the different preparations. Different letters (a, b) denote significant differences (P < 0.0001)

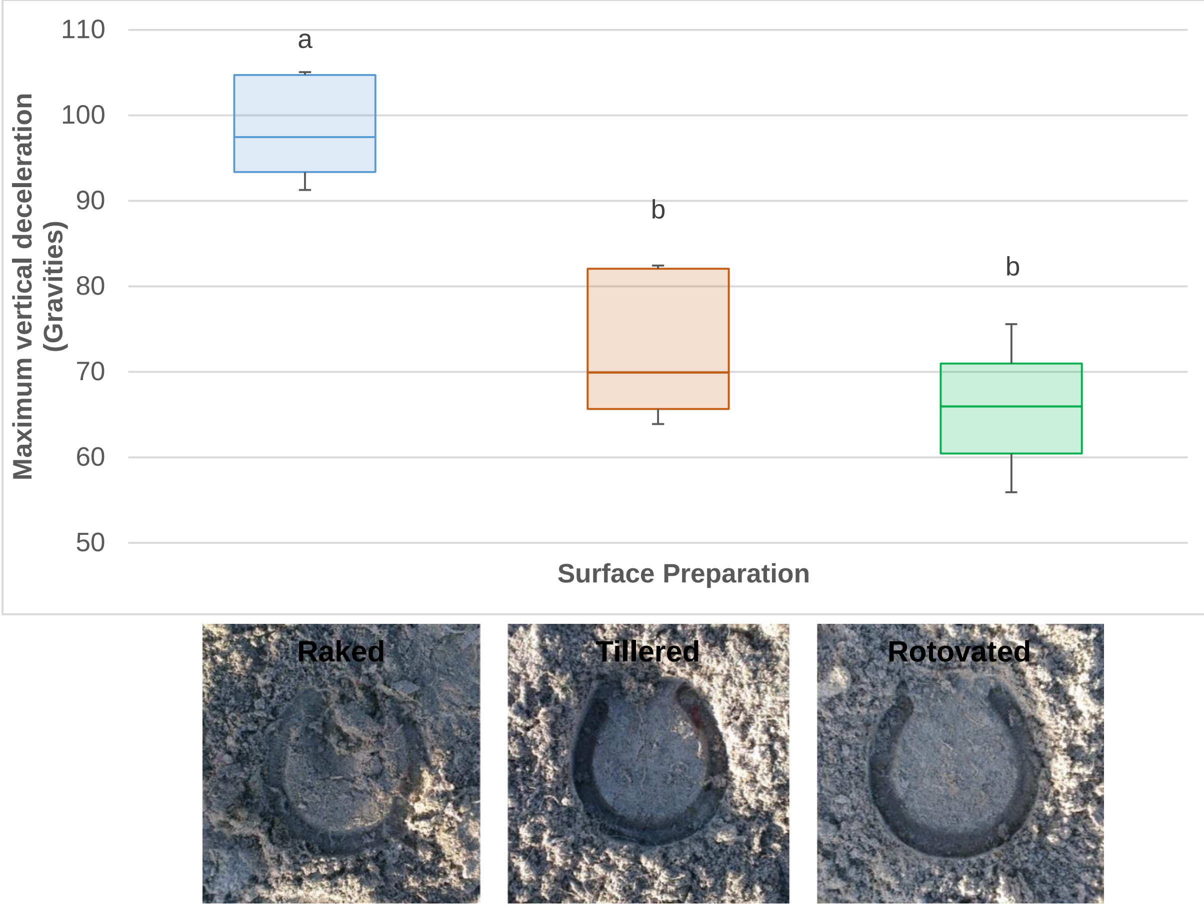


Figure 5.1.2: A comparison between the impact firmness on the different preparations. Different letters (a, b) denote significant differences (P < 0.0001).

The findings suggest that a sufficient change in the functional surface properties has been made between the two surface conditions. The tillered and rotovated preparation significantly reduced peak vertical load **F (2, 12) = 55.22, *P* <0.0001** and maximum vertical deceleration **F (2, 12) = 28.9, *P* <0.0001**, which represents an increase in cushioning and a reduction in impact firmness respectively in comparison to the firm, raked preparation. The rotovated preparation created the most cushioning, however the data was more variable between the five drops. A slightly higher range in impact firmness was also observed on the rotovated preparation. The rotovater was a much heavier piece of equipment and was more difficult to control the speed and depth at which it moved across the surface, which could explain the lack of consistency. This variation alone may be sufficient to initiate measurable changes in the kinematics and neuromuscular activity of the horse and therefore the tiller was deemed more appropriate for this project.

5.2 Main Study

5.2.1 Study design: Surface preparation

The study design has been informed by the protocol used by Ferris *et al.* (1999) where the authors investigated how quickly runners could adjust leg stiffness when they encountered an abrupt change in surface stiffness (hard vs. soft). The study for this project took place over four consecutive days. The data from day 1 and 4 were used to generate baseline data from each horse and establish the amount of variability that may be expected on a surface without induced changes in functional properties.

On day 1, the top cushion layer of the surface was gently raked to create a continuous firm surface condition (Plate 5.2.1). On day 2 and 3, half of the test track was prepared with the tiller, which was used to a depth of 100mm in order to measure the effect of an abrupt change in surface condition on the kinematic and neuromuscular response of the horse. On day 2, the horses were led from the firm to soft surface condition (Plate 5.2.2) and on day 3, the horses were led from soft to firm (Plate 5.2.3). In order to measure the same side (offside) of the horse each day, the camera system was set up on the opposite side of the test track for day 3. It could be argued that the 3D markers and EMG sensors should have been applied to the left hand side of the horse. The horses will have required habituation to being led from the right hand side (horses are used to being led from the left hand side from a young age) and slight differences in the marker and sensor locations, which is inevitable, could have influenced the results. On day 4, the whole test track was prepared with the tiller (100mm depth) to create a continuous soft surface condition (Plate 5.2.4). The entire test track was raked gently each day and between horses in order to camouflage the abrupt change on day 2 and 3 and remove any visual cues of a change in surface condition (Plate 5.2.5).

Plate 5.2.1: Continuous firm surface condition used on day 1

Plate 5.2.2: Firm to soft surface condition used on day 2 prior to raking and camouflaging the change

Plate 5.2.3: Soft to firm surface condition used on day 3 prior to raking and camouflaging the change

Plate 5.2.4: Continuous soft surface condition used on day 4 prior to raking the surface

Plate 5.2.5: The surface was raked each day so it was visually the same regardless of the surface condition.

5.2.2 Subjects

Seven horses of varying breeds from the Myerscough College Equine unit were used for the main study (Table 5.2.1). The horses were considered to be sound and free from any ailments such as a skin condition that would cause them discomfort whilst markers and sensors were applied during data collection.

Table 5.2.1: Profile of horses used for the study

Horse	Breed	Height	Age	Weight (kg)
1 (Polly)	TB x	15.3 hh	18	516
2 (Ben)	Connemara	14.2 hh	10	540
3 (Theo)	Anglo Arab	15.2 hh	16	520
4 (Ruby)	Welsh Section D	14.3 hh	6	619
5 (Fly)	Welsh x TB	14.3 hh	6	515
6 (Raison)	TB	15.2 hh	18	470
7 (Terry)	Irish Cob x	15.2 hh	14	728

5.2.3 Experimental Protocol: Equine response

The camera system was set up on the test track following the same method as discussed in section 4.2. The 3D marker set and EMG sensors were applied to the same anatomical locations as shown in section 4.3 and 4.4. One of the Delsys® sensors, which also have inbuilt tri-axial accelerometers was also secured to the head piece of each horse's bridle to monitor possible changes in poll acceleration (Plate 5.2.6). The accelerometer had a range of 9 gravities and a sampling rate of 148 Hz. The equine head and trunk are thought to remain rotationally stabilised during trot, however variations in head pitch rotations can be caused by surface characteristics and other environmental stimuli (Dunbar *et al.*, 2008). The accelerometer used on the poll in this study in conjunction with the 3D markers secured to the head enabled any variations in head and neck movements initiated by the change in surface preparation to be quantified.

Plate 5.2.6: Accelerometer being secured to the poll of Horse 7.

After a successful calibration had been performed, the horses were led in hand at trot past the camera system at a consistent speed and rhythm (Plate 5.2.7). The handler maintained a loose contact with the horse during each trial to ensure that there was no interference with the horses' way of going. On day 1 and 4, five successful trials were performed on the continuous surface conditions and on day 2 and 3 with the abrupt change in surface condition, eight successful trials were performed. Trials were immediately rejected and repeated if a marker fell off, if a consistent speed was not maintained or if there was interference from the handler. A video of one successful trial can be seen by following this link: https://www.youtube.com/watch?v=ru1WWRBIEgw

Plate 5.2.7: Horse 6 being led in trot through the recording zone.

A static trial of each horse stood square was recorded each day so a model could be created during data processing and applied to the relevant dynamic trials (see section 6.0.1). The static trials were recorded after the dynamic trials on each day so the horse had no awareness of the change in surface condition during the first dynamic trial on day 2 and 3. This enabled the first trial to be categorised as 'not aware of change' and the second and subsequent trials to be categorised as 'aware of change'. Figure 5.2.1 outlines the structure of the dynamic trials performed with each horse throughout the study. In some of the trials it was possible to collect data from 2 strides after the change in surface condition depending on how early the 1st stride before the change was captured within the calibrated volume.

Day	Surface preparation	No. of trials	Awareness?	Stride classification
1	Just firm	5	n/a continuous surface	2 consecutive strides
2	Firm → Soft	8	Trial 1: No Awareness Trial 2-8: Awareness	1 stride before the change 1 stride after the change 2 strides after the change (only in some trials)
3	Soft→ Firm	8	Trial 1: No Awareness Trial 2-8: Awareness	1 stride before the change 1 stride after the change 2 strides after the change (only in some trials)
4	Just	5	n/a continuous surface	2 consecutive strides

Figure 5.2.1: Structure of the dynamic trials performed throughout the study with each horse

The number of trials selected for this study was based on other literature. Studies investigating the kinematic response of horses to a continuous surface have collected between two to 30 strides from each subject previously (Barrey *et al.*, 1991; Robin *et al.*, 2009; Chateau *et al.*, 2009, 2010, 2013), which enabled the authors to address their relevant research questions. Studies investigating the effect of a change in surface stiffness or a change in surface height on human limb adjustments have collected from three to 15 successful trials per condition from each subject (Ferris *et al.*, 1998, 1999; Grimmer *et al.*, 2008; Müller *et al.*, 2010, 2012). A study by Daley *et al.* (2006) studied the dynamic stability of guinea fowl during an unexpected drop in surface height and carried out 15-20 trials on a level surface and two to three of which contained unexpected drops.

A total of five successful trials on the continuous preparations (day 1 and 4) was deemed appropriate for this work and would generate at least 10 strides per horse (2 strides per trial) for each surface condition. A greater number of trials (n = 8) was used when there was a change in surface preparation (days 2 and 3) due to the strides in each trial being categorised further according to stride location. It was not possible to increase the number of successful trials further without increasing the number of data collection days, which would undoubtedly increase external factors that could influence the results.

5.2.4 Experimental Protocol: Surface response

The Orono Biomechanical Surface Tester (OBST) was used each day after data had been collected from the seven subjects in order to quantify the response of the surface to the different preparations. Ten single drops were made at 1 m increments along the recording zone on the same track used by the horses earlier on in the day (‘horse track’). It was envisaged that the surface properties may have altered throughout each day due to the number of horses being led on the same track. A strip of test track running parallel to the ‘horse track’ that had received the same preparation treatments, yet had not been exposed to any traffic was subsequently tested (‘fresh track’) (Plate 5.2.8). Researchers were informed to keep off the designated ‘fresh track’ during data collection so a data set could be generated from the functional surface properties that may have been experienced by the first horse at the start of the day.

A small surface sample was also taken at each drop location (approximately 100 grams) on the ‘horse track’ to calculate moisture content. The samples were dried in the oven for 48 hours at 40^{o}C, which is the protocol outlined by the Racing Surfaces Testing Laboratory (2015). The moisture content was calculated using the following equation (Rowell, 1994):

$$\boldsymbol{Moisture\ Content} = \left(\frac{\boldsymbol{Moist\ mass} - \boldsymbol{Dry\ mass}}{\boldsymbol{Dry\ mass}}\right) \times \mathbf{100}$$

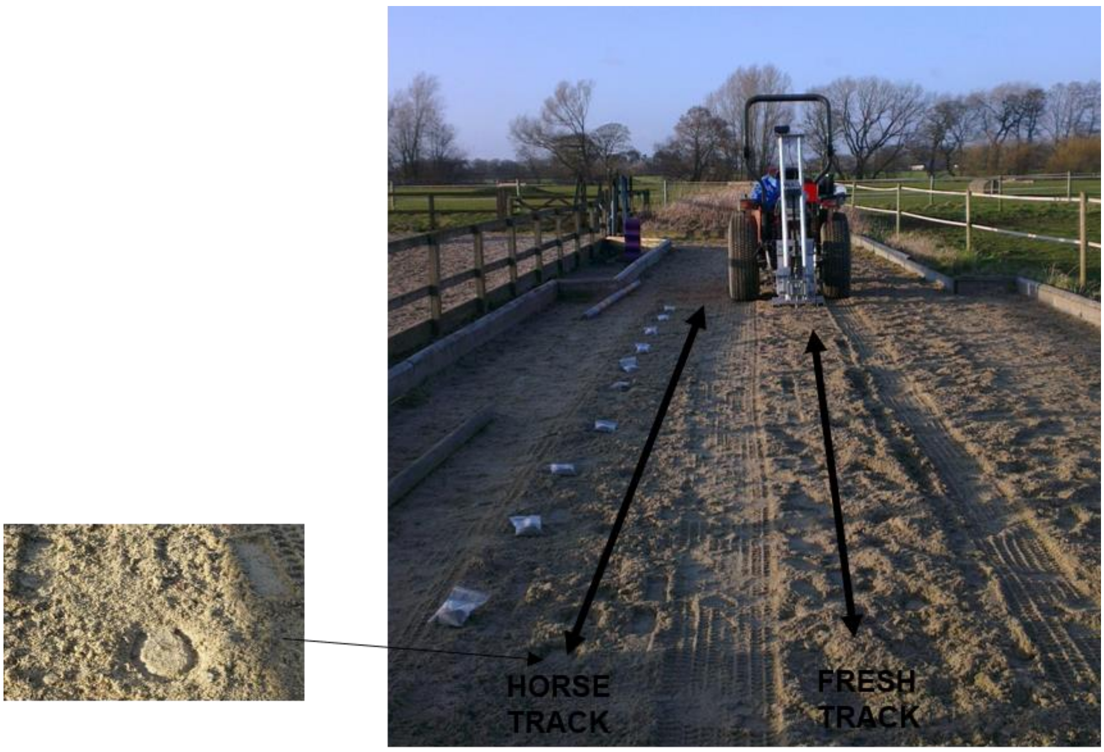


Plate 5.2.8: The OBST being used to test the ‘horse track’ and ‘fresh track’. The small bags (n=10) contain the surface sample used to calculate moisture content.

Other parameters

In addition to using the OBST and measuring surface moisture content, temperature, humidity and rainfall measurements were recorded during data collection. Tinytag© temperature and humidity dataloggers were used and have been considered useful in other studies where the loggers recorded one of the most comprehensive sets of dwelling-related temperature data for English homes (Oreszczyn *et al.*, 2006). The dataloggers were sealed within waterproof containers and were programmed to take temperature (n=1 dataloggers on the surface, n=1 dataloggers 10cm beneath the surface) and humidity (n=1 datalogger on the surface) readings every ten minutes. Rainwise rain gauges (n=2) were placed next to the test track in order to measure the amount of rainfall whilst data was not being collected.

6.0 Optimisation of methods for processing kinematic data

6.0.1 Raw data files

Qualisys Track Manager was used to ensure the correct calibration file was applied to all raw data files. An AIM (Automatic Identification of Markers) model was generated from one trial, which was subsequently applied to the rest of the raw data files from all subjects (Figure 6.0.1). Marker trajectories that were not continuous throughout a trial were interpolated with a maximum gap of ten frames so a complete set of kinematic parameters could be calculated at a later stage. It was not always possible to do this however, because some of the files were contaminated with a large number of unidentified trajectories, created by reflections from the sunlight during data collection, which made it difficult to identify the true marker trajectories. These files were subsequently not included for further analysis. At times, the AIM model did not correctly identify some of the markers, especially if they were located close together. Each marker trajectory in every file was visually checked and altered accordingly before saving and exporting the file to C3D format.

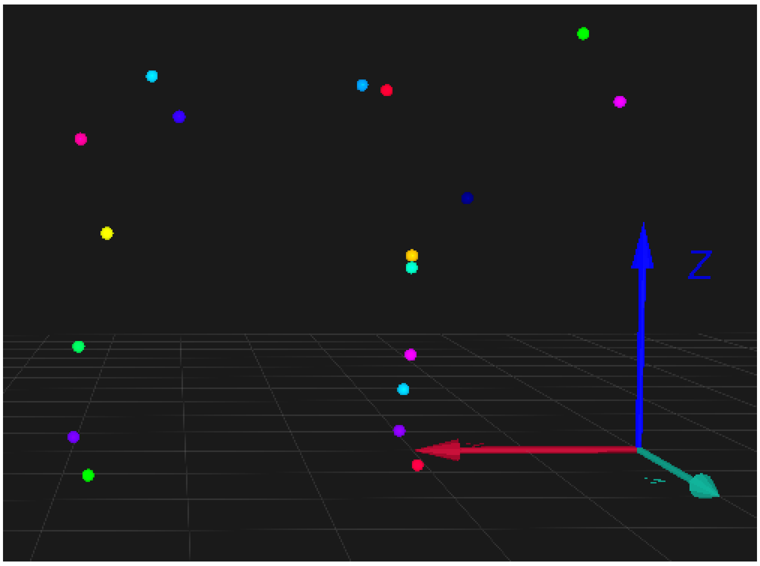


Figure 6.0.1: The AIM model applied to a static trial

The C3D files from the dynamic trials were uploaded into a Visual 3D workspace, which was the software used to derive the kinematic parameters from. The static trials were uploaded separately using the model tab and assigned to the relevant dynamic trials. Virtual landmarks were created next to all of the actual markers with a medial offset of 0.02 m. The landmarks provided medial locations in order to create a 17 segment model from which selected parameters could be derived from (Figure 6.0.2). It was important to apply appropriate filtering techniques before any parameters could be calculated and extracted for analysis.

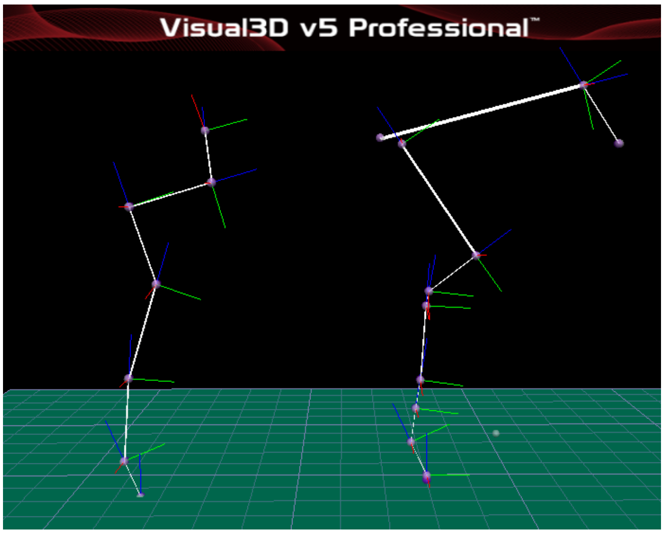


Figure 6.0.2: The segments created in Visual 3D using actual markers and virtual landmarks.

6.0.2 Kinematic Data Processing

It is necessary to filter kinematic data in order to remove noise from the signal (Sinclair *et al.*, 2013). Noise can result from factors such as soft tissue artefact, improper digitisation and electrical interference (Winter *et al.*, 1974). A low pass filter is generally applied to kinematic data (Clayton *et al.*, 2004; Hobbs *et al.*, 2006; Hobbs and Clayton, 2013; Northrop *et al.*, 2013; Hobbs *et al.*, 2014b), which will attenuate higher frequencies yet keep data with a frequency below the specified cut off frequency. As the cut-off frequency increases, the influence of the filter on the data is reduced and the data will be similar to the raw signal, including some of the high frequency noise (Robertson and Dowling, 2003).

The fourth-order zero-lag is frequently utilised in biomechanical analyses (Hobbs *et al.*, 2006; Hobbs and Clayton, 2013). Butterworth filters yield a weighted average of data points across the kinematic waveform. A low pass fourth order Butterworth digital filter with a cut off frequency of 10 Hz has been used when studying the equine walk (Hobbs *et al.*, 2006) and trot (Hobbs and Clayton, 2013; Hobbs *et al.*, 2014b) previously. Clayton *et al.* (2004) used a cut-off frequency of 12 Hz to investigate distal limb kinematics at the trot and Hobbs *et al.* (2011) used an even higher cut- off frequency of 30Hz when studying the canter. Previous literature is an important start point to inform the cut-off frequency to use for this work, however it is essential to inspect the signal characteristics of the current work to determine the most appropriate cut-off frequency (Sinclair *et al.*, 2013).

Residual analysis, which is deemed appropriate for low frequency kinematic data (Sinclair *et al.*, 2013), was performed on the original target data from selected marker locations. Data from the croup, proximal scapula and fore distal interphalangeal (DIP) joint markers from all subjects were analysed (*z* and *x* axis) due to the variation in noise contribution expected from the different locations. The croup marker is generally associated with a small amount of vertical displacement, suggesting there may be a low amount of noise contamination within the true signal. In contrast, the proximal scapula and fore DIP joint targets may contain a large degree of noise due to the effects of skin displacement (van Weeren *et al.*, 1990) and vibrations generated during the hoof surface interaction on the respective markers. This would provide information on the two extremes of data and enable the most appropriate cut off frequency for the low pass filter to be identified.

Figure 6.0.3 has been adapted from Sinclair *et al.* (2013) and demonstrates the graph used to perform residual analysis on the data. The red line represents the best estimate of the noise residual and is positioned so that it follows the linear portion of the residual plot (black line) and intercepts the Y axis at X (0 Hz) (Sinclair *et al.*, 2013). The decision regarding the cut-off requires a compromise between the extent of signal attenuation and the amount of noise allowed to pass through (Sinclair *et al.*, 2013). The yellow horizontal line is projected from X to intersect the residual plot at Y and the cut-off is selected at this frequency (blue line).

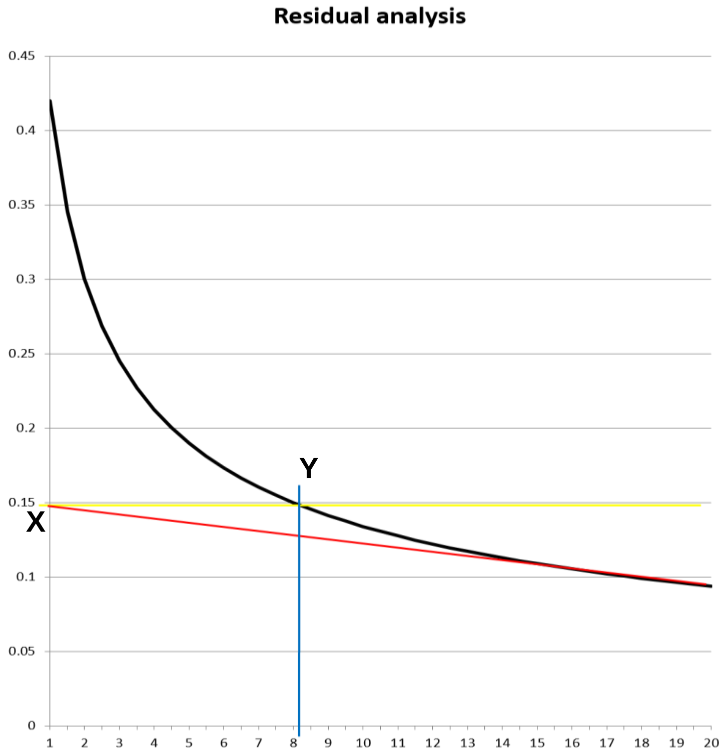


Figure 6.0.3: Residual Analysis used to identify the cut off frequency for the low pass filter. This particular example would have a cut off frequency of 9 Hz. Adapted from Sinclair et al. (2013).

The mean cut-off frequencies for the croup, proximal scapula and fore DIP joint targets were 9, 8 and 7 Hz respectively with a maximum cut-off of 9 Hz between the three targets. This was not expected and demonstrates the importance of investigating the signal characteristics further. The cut-off frequency used previously (Hobbs and Clayton, 2013; Hobbs *et al.*, 2014b) therefore appears to be sufficiently reliable to inform the cut-off frequency used for this study. A low pass fourth order Butterworth digital filter with a cut off frequency of 10 Hz was subsequently used on all kinematic targets for this work.

6.0.3 Kinematic parameters

After the targets had been filtered and interpolated, it was possible to extract the kinematic parameters of interest, which were categorised according to temporal, linear and angular variables. Gait events including hoof impact, mid-stance and hoof lift off, were applied to the fore and hind limbs in all files using the method described in Holt *et al.* (2017) (Appendix III). This was a simple method that was authenticated using gold standard force data and subsequently published as a technical note in Equine Veterinary Journal. One of the limitations of the paper included further testing being required before applying it to data that had been collected on a compliant surface. This was to ensure the accuracy and precision of the kinematic gait event detection methods were not significantly altered on a surface that presented different functional properties than the one used for the initial authentication. Further testing was subsequently carried out with one horse on an arena surface using a prototype force shoe that was designed and developed at the start of the PhD. The design and calibration of the force shoe has been described further in the MPhil to PhD transfer document in appendix IV The experimental protocol and results from testing on a compliant surface can be found in chapter 7.0.

The hoof impact and lift off gait events for the fore and hind limbs enabled temporal parameters to be calculated including stance duration and duty factor. Duty factor, as described by Witte et al (2004) is the proportion of the stride for which the limb is in stance and is expressed as a percentage. It was also possible to calculate the contact timing of the right ipsilateral limb pair where the timing of the right hind limb hoof impact was negated from the timing of right forelimb hoof lift off. The mid-stance gait event was calculated as the timing of peak MCP and MTP extension. This has shown to be a precise method of representing the timing of peak vertical force during the support phase of the stride (Holt *et al.*, 2017).

To calculate stride length, a fore and hind hoof segment was created using the distal interphalangeal markers and virtual landmarks. The hoof position with respect to the lab was calculated at each impact so the distance travelled in the sagittal plane during each stride could be calculated. Joint angles were computed using the pipeline in Visual 3D.

The MCP and MTP were constructed using the third metacarpal and metatarsal bone as reference segments with the fore and hind pastern segments respectively. This enabled peak extension of the palmar aspect during stance to be calculated, which has shown to have a very strong positive correlation with peak vertical force in the forelimb (McGuigan and Wilson, 2003) The shoulder angle was constructed using the scapula as a reference segment and the humerus. Shoulder angle at hoof impact and hoof lift off was calculated to determine possible changes in limb posture on the different surface conditions. The absolute joint angles for the MCP, MTP and shoulder were calculated with respect to the more distal segments, which is illustrated in figure 6.0.4

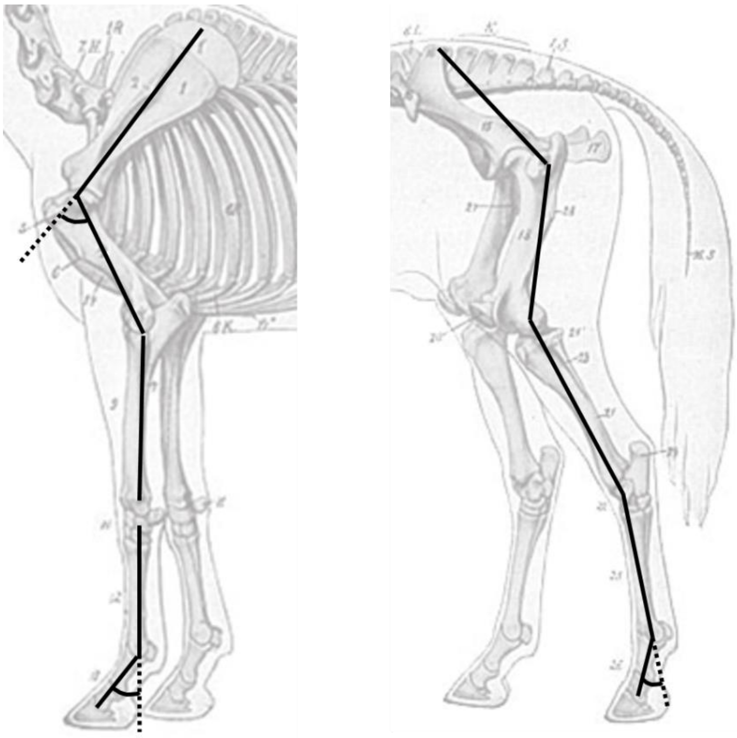


Figure 6.0.4: An illustration showing where the joint angles were derived from. Equine skeleton taken from Veterinary Medicine (2016).

The inclination of selected segments within the 3D space including the neck, scapula and fore and hindlimbs at hoof impact and lift off were also calculated to establish variations in body posture on the different surface conditions. Due to the fore and hind limbs consisting of several segments, a new segment was created to specifically measure limb inclination. Forelimb inclination has been measured previously by Crevier-Denoix *et al.* (2010) where the authors used the elbow-fetlock axis. The forelimb segment for this project was therefore created using the lateral epicondyle of the humerus and the distal end of the third metacarpus, which are the centres of rotation for the joints used by Crevier-Denoix *et al.* (2010). The hind limb segment was created using equivalent locations; the lateral epicondyle of the femur and distal end of the metatarsus. The inclination angles were calculated with respect to the lab and therefore computed differently to the joint angles (Figure 6.0.5).

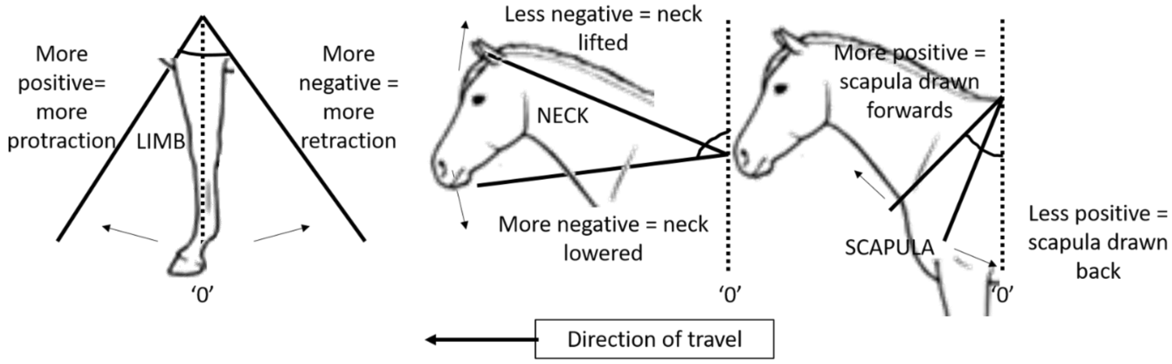


Figure 6.0.5: An illustration demonstrating where the inclination angles were derived from for the limbs, neck and scapula. When the segment in the sagittal plane is at the vertical, the angle is '0'.

To determine if equine limb stiffness altered as a function of surface stiffness, the following equation as presented by McMahon and Cheng (1990) was used:

$$\frac{Maximum\ vertical\ force\ (kN)}{Change\ in\ vertical\ leg\ length\ or\ surface\ deformation\ (m)}$$

Ground reaction forces were not measured for this project, however it was possible to predict peak vertical ground reaction force using peak MCP extension (McGuigan and Wilson, 2003). This has shown to have a relatively strong positive correlation (r^2= 0.70) previously (McGuigan and Wilson, 2003). Effective stiffness of the fore and hind limbs was therefore calculated to explore possible changes in response to the change in surface stiffness. Peak vertical GRF (N/kg) was estimated using the following equation reported by McGuigan and Wilson (2003):

$$y = 0.2113\text{x} - 38.68$$

y = N/kg

x = peak MCP extension from the posterior aspect of the 3rd metacarpal bone to the ventral part of the long pastern.

The body weight of each horse was subsequently used to calculate the total estimated peak vGRF for each stride. The equation above was also used to calculate peak vGRF in the hind limbs using peak MTP extension. This does pose a limitation due to anatomical differences between the fore and hind limbs and peak force production in the hind limbs contributing to 46% of total peak vGRF during diagonal stance at over-ground trot (Hobbs and Clayton, 2013). This was factored in to the equation above where total peak vGRF in the hind limbs was reduced to 84% of the original value to account for the lower forces occurring in the hind limb.

The change in vertical leg length for the fore and hind limbs was computed using the pipeline in Visual 3D. Limb length at peak MCP and MTP extension was negated from limb length at hoof impact. The equine forelimb has been modelled by two compression springs previously (McGuigan and Wilson, 2003) and so length change in the forelimb was categorised according to the distal (lateral epicondyle of the humerus to the distal interphalangeal joint) and proximal springs (proximal spine of the scapula to the lateral epicondyle of the humerus) (Figure 6.0.6). The length change of the hind limb was derived from the distance between the greater trochanter and hind DIPJ. This was based on the method of measuring limb length in humans although this has been calculated as the distance from the greater trochanter to the ground (Ferris *et al.*, 1999). The hind DIPJ was used instead of the ground in this project however, due to the compliant surface being used. Effective limb stiffness was subsequently calculated for each stride. The method for calculating surface stiffness is presented in section 6.3.2.

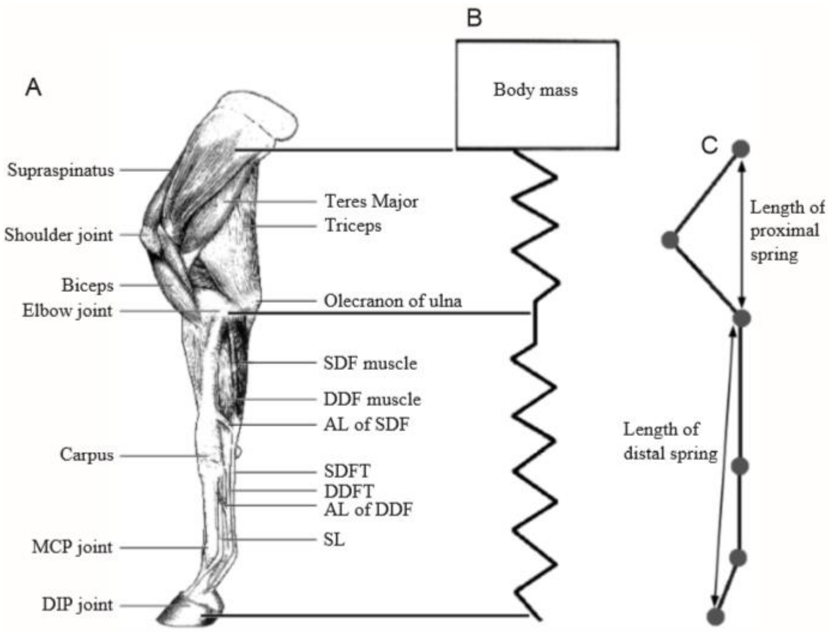


Figure 6.0.6: The distal and proximal springs; (A) The equine forelimb, showing the muscles associated with weight bearing; (B) The limb can be conceptually divided into two springs, representing the proximal part of the limb (shoulder to elbow) and the distal part of the limb (elbow to fore DIPJ); (C) The length of the springs. Taken from McGuigan and Wilson (2003).

Speed was derived from the first derivative (velocity) of the croup target path. On day one and four, the mean speed from the entire length of each trial was calculated. On day two and three the mean speed before and after the change in surface condition was calculated separately. The statistical analysis methods used for the kinematic parameters are presented in section 6.2.

6.1 Optimisation of methods for processing EMG

6.1.1 Raw EMG files

The raw EMG data were exported to C3D format with the corresponding kinematic data before being uploaded to a Visual 3D workspace as analogue data. The raw surface electromyographic (sEMG) signal contains the signal that originates from within the muscle and various noise components, which are unavoidable (De Luca *et al.*, 2010). These noise components contaminate the sEMG data like the kinematic data and may lead to an inaccurate interpretation of the signal (De Luca *et al.*, 2010). The signal must therefore be processed using suitable techniques to minimise the noise components, yet retain as much of the true sEMG signal as possible (Wang *et al.*, 2013).

Noise sources can originate from the electronics of the amplification system and the skin-electrode interface, which are referred to as thermal and electro-chemical noise respectively (Huigen *et al.*, 2002; De Luca *et al.*, 2010; Richards, 2016). These sources form the baseline noise, which is detected when a sensor is secured to the skin (De Luca *et al.*, 2010). Movement artefact noise also originates at the electrode-skin interface and usually contaminates the EMG signal at low frequencies. This type of noise is influenced by factors such as muscle movement under the skin, impulses travelling through the muscle and skin displacement (De Luca *et al.*, 2010).

Movement artefact is expected to contaminate the equine sEMG signal to an even greater extent in comparison to humans due to the much larger muscle mass and skin displacement over anatomical landmarks (van Weeren *et al.*, 1990). The vibrations generated during the hoof-surface interaction, which are impossible to completely dampen, may also be responsible for creating an inconsistent skin-electrode interface and generating movement artefact within the EMG trace, especially in more distal locations. This demonstrates the need for a suitable, robust signal processing technique to attenuate the noise components and reveal the true EMG signal.

6.1.2 EMG Data Processing

The frequency spectrum of the sEMG signal with commonly used sensors ranges from 0 to 400 Hz in humans but this is influenced by the electrode spacing, the amount of fatty tissue between the skin and the muscle tissue, the shapes of the action potentials, and muscle type (De Luca *et al.*, 2010). The exact frequency spectrum for horses is unknown, however it appears that similar cut off frequencies used to process human EMG data previously have been successfully applied to equine data (Harrison *et al.*, 2012). At the high frequency end of the spectrum, a low-pass filter cut-off frequency should be set so the noise components that exceed the sEMG signal are attenuated and therefore it is preferable to have a low-pass cut-off frequency in the range of 400–450 Hz (De Luca *et al.*, 2010). At the low-frequency end of the spectrum, the choice of the location of the high-pass filter cut-off frequency is more variable because the low frequency spectra of several noise sources overlap with that of the sEMG signal, which has generated research interest previously (De Luca *et al.*, 2010).

Some of the more recent EMG systems (De Luca *et al.*, 2010) have an in built band pass filter. The Delsys® system used for this project has an in-built band pass filter between 20-450Hz, which helps to truncate the contribution of some of the noise, yet minimise the attenuation of the low frequency components of the EMG signal. The sEMG signal brought into the analysis software still requires processing in order to further reduce the noise contamination. The extent to which the signal is filtered will vary according to how dynamic the trials are where a more aggressive filter will be required for more vigorous movements. It is common to use a high pass filter to reduce baseline noise and suppress movement artefact. The figure (Figure 6.1.1) below demonstrates a good example of movement artefact recorded from the *long head of the triceps* muscle during trot, which is later attenuated with the use of a suitable high pass filter (Figure 6.1.5). Figure 6.1.2 also shows an example of the baseline noise, which is characterised by a much smaller amplitude between bursts of muscle activity.

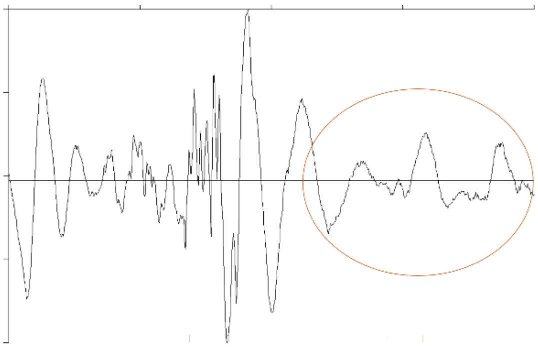


Figure 6.1.1:: An example of movement artefact

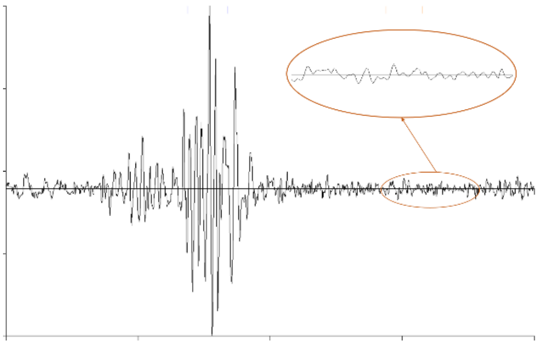


Figure 6.1.2: An example of baseline noise

Recommendations for EMG signal filtering have been proposed for human research previously and the suggested cut off frequency for a high pass filter usually varies from 5Hz (Merletti, 1999) to 15-28 Hz (Van Boxtel, 2001). The Journal of Electromyography and Kinesiology also require a high pass cut-off frequency of 10 Hz for a report to be published, demonstrating more stringent guidelines exist for human studies in comparison to equine. There does not appear to be a standardised method for processing equine EMG data and the techniques used previously vary greatly.

EMG data has frequently been collected with kinematic data and it has been common practice to rectify and reduce the sampling rate of the EMG data in order to make it comparable to the motion data (Peham *et al.*, 2001a; Licka *et al.*, 2009; Groesel *et al.*, 2010; Zsoldos *et al.*, 2010). A Butterworth low pass filter with a cut off frequency of 10Hz has then usually been applied to the down-sampled EMG data in order to assess the envelope curve of the signal (Peham *et al.*, 2001a; Licka *et al.*, 2009; Groesel *et al.*, 2010; Zsoldos *et al.*, 2010) (Figure 6.1.3). This filtering technique was deemed acceptable for the analysis of repeated movement cycles and enabled the authors to calculate the mean amplitude during each motion cycle amongst other parameters such as maximum (Peham *et al.*, 2001a), minimum and the relative amplitude (Licka *et al.*, 2009) of muscle activity. The paper used to justify this filtering technique (Peham *et al.*, 2001b) however, did not conclude that a low pass filter was the best processing method. It was in fact a signal adapted filter that generated a significantly greater signal to noise ratio (Peham *et al.*, 2001b).

Applying a low pass filter with a cut off frequency of 10Hz to the rectified signal mitigates any frequencies above 10Hz, which suggests that a considerable amount of the true EMG signal has been attenuated if horses have a similar EMG frequency spectrum to humans. Zsoldos *et al.* (2010) even acknowledged that low-pass filters have the disadvantage that some movement and cross-talk artefacts may still be present, which will make the subsequent signal analysis difficult (Peham *et al.*, 2001b). It is interesting to note that no reference was made to the use of a high pass filter on the raw EMG data in any of these studies, which again suggests that the signal was still contaminated with low frequency noise. This component is considered to be significant and has the potential to influence the readings obtained (Huigen *et al.*, 2002; De Luca *et al.*, 2010), which may affect the validity of previous work that only used a low pass filter. In order to reveal the true sEMG signal, this low frequency noise must be attenuated with the use of a high pass filter that has a suitable cut-off frequency.

There are more recent equine papers that have applied a high pass filter to sEMG signals. Harrison *et al.* (2012) used a high pass filter with a 20Hz cut off frequency prior to calculating the root mean square amplitude of a 40ms window passed across the time series, which enabled the authors to determine the mean signal strength within each onset-offset period. A different technique was also used to estimate the amplitude of muscle activation, which involved removing the mean from the data, low pass filtering at 40Hz, rectifying the data and high pass filtering at 10 Hz (Harrison *et al.*, 2012). This method assumes that the frequency spectrum of the selected muscles is relatively small and may still have low frequency noise or artefact retained within the sEMG signal due to the dynamic nature of the walk, trot and canter trials performed (Harrison *et al.*, 2012). The sEMG signals recorded in a different study by Kienapfel (2015) were filtered with a butterworth 4thorder high pass filter with a cut off frequency of 40Hz before being rectified and smoothed with a moving average window of 40 frames.

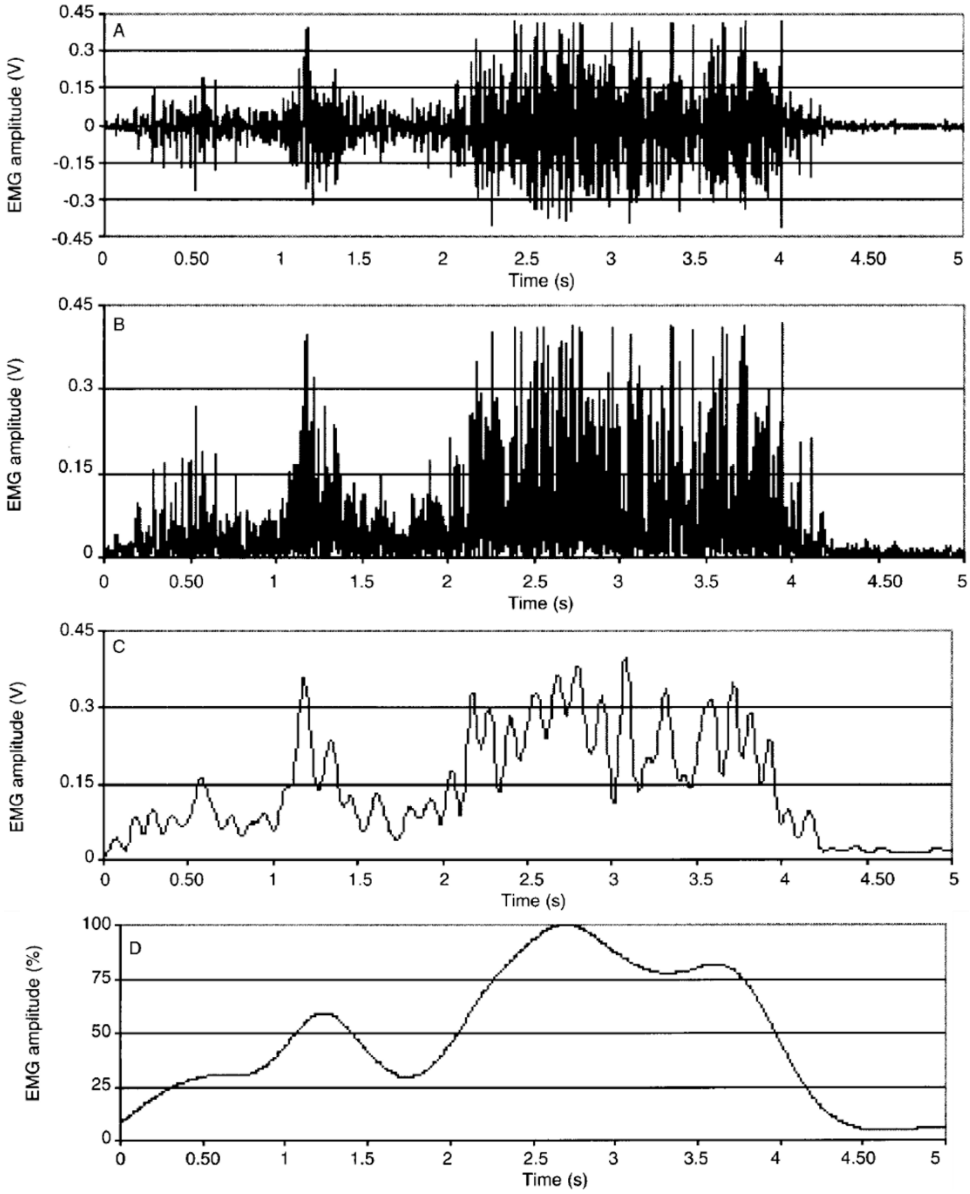


Figure 6.1.3: EMG signal-processing method used previously; A) The raw EMG signal sampled with 1.2 kH; B) The rectified EMG signal; C) The down-sampled EMG signal where the sampling rate has been reduced to 120 Hz; D) The EMG signal with a 7th order Butterworth low pass filter applied with a cut off frequency of 10 Hz. Extracted from Peham et al. (2001a).

It has been noted by Peham *et al.* (2001b) that difficulties can arise during EMG signal processing due to the variation of the signal, which is influenced by factors such as the random firing rates of the selected muscles (Peham *et al.*, 2001b), skin preparation and the dynamic nature of trials, which will influence the amount of noise observed within the signal. This variation is responsible for the low reliability of applying similar processing techniques to all sEMG signals and therefore it is essential to investigate the effects of certain filtering and smoothing techniques on the signal in order to identify an optimal filtering method for a particular activity. This has been done previously on human sEMG data where the effect of several different cut-off frequencies for a high pass filter on signal quality has been examined (Potvin and Brown, 2004; De Luca *et al.*, 2010). A study by De Luca *et al.* (2010) quantified the effect of a 10, 20 and 30 Hz cut off frequency on noise reduction within the sEMG signal whereas Potvin and Brown (2004) investigated the effect of a much larger range of high pass filter cut off frequencies (20-440 Hz) on the ability to estimate muscle force from the EMG signal.

The raw sEMG data recorded for this project (Figure 6.1.4 a) had the DC offset removed before being shifted by 5 frames to account for a synchronisation delay between the kinematic and EMG data (Figure 6.1.4 b). It was then possible to apply a high pass filter with several different cut off frequencies.

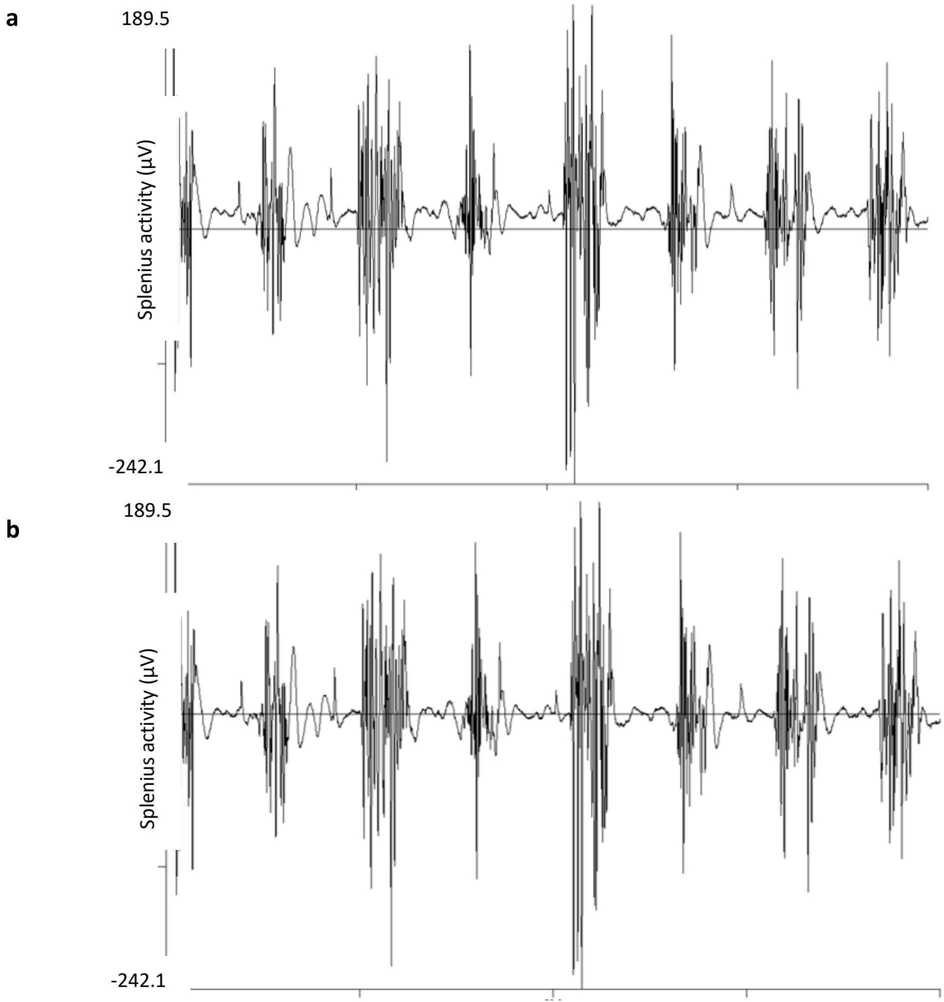
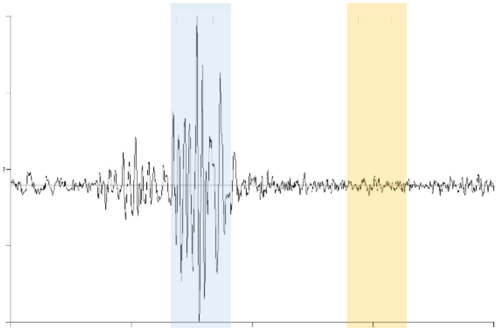


Figure 6.1.4: a) Raw sEMG data from the splenius muscle recorded during one trial of trot; b) The signal with a DC offset and shifted by 5 frames.

Several high pass filters (cut off frequencies: 20, 40, 60 and 80Hz) were applied to a small sample of EMG data from each muscle in order to select a filter that generated the best signal to noise ratio. The signal-to-noise ratio (SNR) is an accepted measure of the quality of the EMG signal (Richards, 2016). It reflects the measure of the wanted EMG signal versus the unwanted signal (baseline noise and movement artefact) and is calculated as follows:

$$\text{Signal-to-noise ratio (SNR)} = \frac{EMG\ Signal}{Baseline\ Noise}$$



The effect of the different cut off frequencies on the SNR and sEMG signal quality of two selected muscles is presented in Figure 4a and 4b. The activity from the *splenius* muscle was considered generally quiet whereas the activity recorded from the *long head of the triceps* muscle was contaminated with a substantial amount of noise.

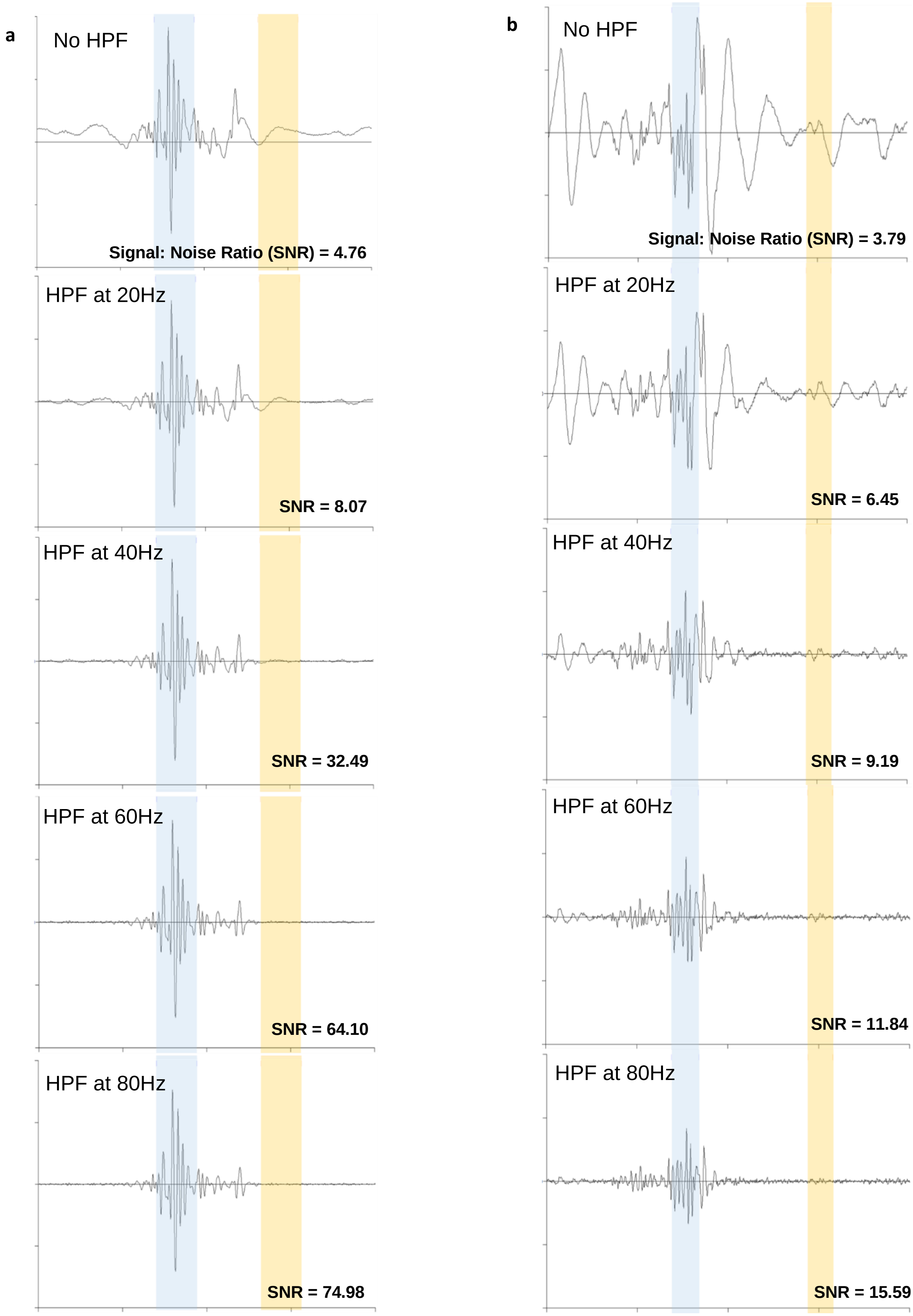


Figure 6.1.5: a) The effect of applying a high pass filter with different cut off frequencies to the signal-to-noise ratio of the splenius muscle. b) The effect of applying high pass filters with different cut off frequencies to the signal-to-noise ratio of the long head of the triceps muscle. To calculate the signal to noise ratio (SNR), the mean value from the

area highlighted in blue (signal) was divided by the mean value from the area highlighted in orange (baseline noise).

The choice of cut-off frequency was informed by what Harrison *et al.* (2012) and Kienepfel (2015) had used previously (20 and 40 Hz) however it was evident that the SNR continued to increase with a cut-off of 60 and 80Hz, demonstrating the importance of identifying an optimal cut-off frequency that is specific to the activity being performed. The high pass filter with a cut off frequency of 80Hz generated the greatest SNR for the selected muscles. A study by Potvin and Brown (2004) used a Butterworth high pass filter with a cut- off frequency of up to 410Hz to remove approximately 99% of the sEMG signal power. The authors acknowledged that this is extremely high, however it significantly improved the accuracy of estimating muscle force within the *biceps brachii* of humans. Potvin and Brown (2004) were interested in the true myoelectric processes occurring across the muscle fibre, which were considered to be within the high band of sEMG frequencies. It was believed that the low frequency signals were not a true representation of what was occurring at the muscle fibre level, which again demonstrates the need for an EMG signal processing method that will address the research aim.

The focus of this project differs to Potvin and Brown (2004) where the magnitude of muscle activity between specific gait events is of interest. In this situation, it is important to keep as much of the true sEMG signal as possible whilst reducing the amount of noise contamination. Selecting a cut off frequency for the high pass filter based on the most suitable SNR has been deemed an acceptable approach for this work and based on the values presented in Figure 4a and b, the optimal cut off frequency to be used is 80 Hz. It is important to note that the SNR continued to increase for the *long head of the triceps* when a cut of frequency of 100Hz was used however, it decreased for the *splenius* muscle, which suggests that a much larger proportion of the true sEMG signal may be lost. The SNR may continue to increase for some muscles according to De Luca *et al.* (2010) however, the rate of sEMG signal loss will also increase. The optimal cut off frequency may also be determined by considering the percentage of noise reduction and percentage of sEMG signal loss as a function of frequency increment. Table 6.1.1 shows the percentage signal and noise reduction at different cut off frequencies for the data presented in Figure 6.1.5 a and b.

Table 6.1.1: The % signal loss and noise reduction from the original signal when using a high pass filter with different cut off frequencies.

	Splenius		***Long head of the Triceps***	
Cut off frequency (Hz)	**Signal loss (%)**	**Noise reduction (%)**	**Signal loss (%)**	**Noise reduction (%)**
20	0.55	41.30	16.47	50.91
40	1.05	85.50	39.87	75.19
60	3.66	92.84	50.78	84.23
80	8.62	94.20	57.44	89.65

The rate of noise reduction is greatest at 40Hz and 20Hz for the *splenius* and *long head of the triceps* respectively. This continues to decrease to a lesser extent between subsequent increments whilst the rate of signal loss continues to increase, which is a similar finding to De Luca *et al.* (2010) when investigating the effect of different cut off frequencies of a high pass filter on the sEMG signal recorded from two muscles in humans. The 40Hz cut off frequency is potentially an optimal compromise based on the results from table 6.1.1 however, the noise contamination is still clearly evident in the signal recorded from the *long head of the triceps* (Figure 6.1.5 b). The *splenius* muscle appears to have little noise contamination upon visual observation, however it can be seen in table 6.1.1 that the high pass filter incurs minimal signal loss whilst attenuating most of the noise. By taking the SNR, data from table 6.1.1 and visual observations into account, a high pass Butterworth filter with a cut off frequency of 80 Hz was consequently applied to the EMG data.

6.1.3 EMG trace accept and reject criteria

After a suitable processing technique had been identified and applied, every trace from each muscle and horse was also visually assessed for its quality before being analysed further. The signal to noise ratio was generally optimised, however some of the signals were still contaminated with a large degree of noise, which made it necessary to discard the respective files. The EMG trace in figure 6.1.6 is an example of a good, clean signal that would be accepted. The EMG traces in the other figures (6.1.7, 8, 9 and 10) show examples of noise still present and whether that particular file would be accepted or rejected from further analysis.

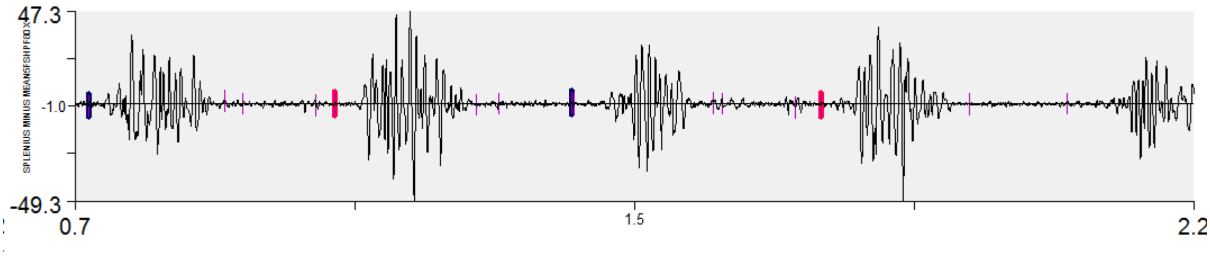


Figure 6.1.6: An example of a clean sEMG trace from the splenius muscle that would be accepted for processing. Blue marker = Hoof impact; Red marker: Hoof lift-off.

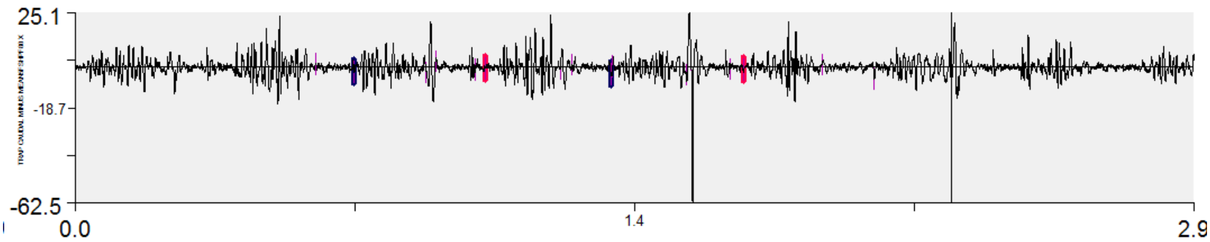


Figure 6.1.7: An EMG trace from the caudal site of the trapezius cervicis muscle. Blue marker = Hoof impact; Red marker: Hoof lift-off. The data from the second stance phase was discarded due to the spike in the amplitude caused by noise. It was not necessary to discard the first stance phase due to the signal being unaffected.

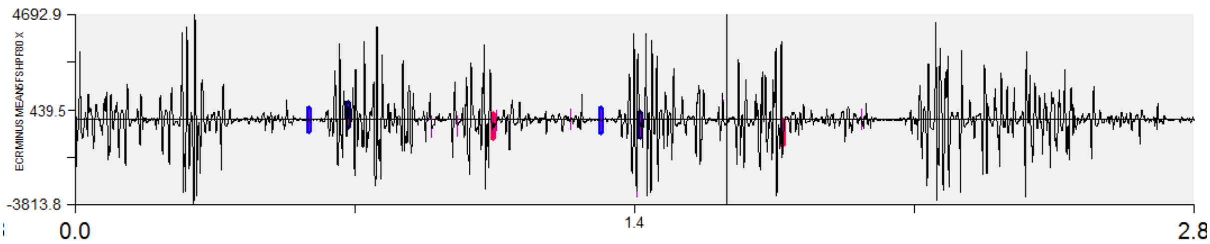


Figure 6.1.8: A signal from the extensor carpi radialis containing noise. It was possible to accept this signal due to the phasic pattern observed within the trace. If the amplitude from a particular horse did not vary greatly between trials, it was possible to subsequently calculate the relevant EMG parameters.

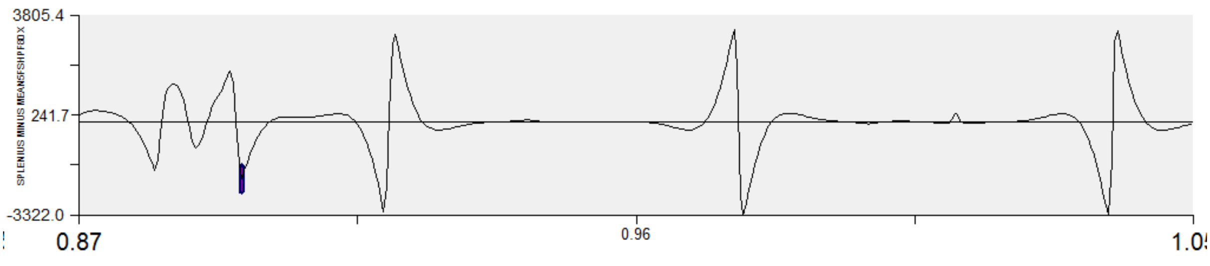


Figure 6.1.9: An example of movement artefact present within the splenius EMG signal, which was subsequently rejected. The presence of the characteristic fluctuations in the EMG amplitude after the blue marker (hoof impact) suggests that the skin- electrode interface was no longer secure. It is important to note that these could represent individual motor unit action potentials, however in this particular example noise was evident throughout the whole signal upon zooming out.

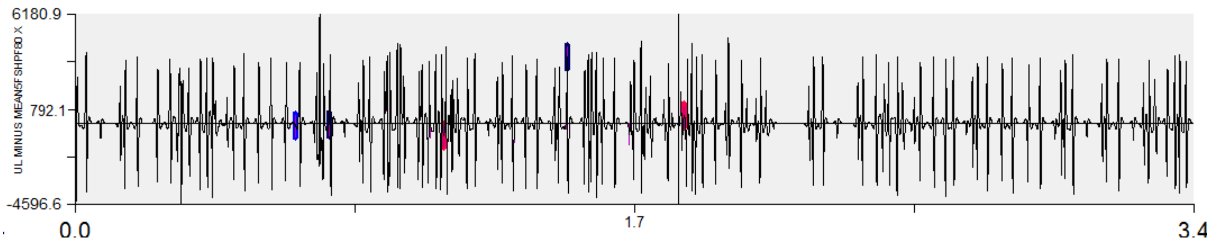


Figure 6.1.10: This signal from the ulnaris lateralis is heavily contaminated with noise and was rejected immediately.

6.1.4 EMG Signal Analysis

The EMG signal analysis method employed will depend on the area of muscular control and activity to be investigated. Research on EMG data that has been conducted previously has presented the length of muscle activation (Harrison *et al.*, 2012), maximum (Peham *et al.*, 2001a) and relative amplitude (Licka *et al.*, 2009) to determine the contributions of specific muscles to various activities. The EMG data has also been enveloped with a low pass filter, which has enabled the motor unit activity at one specific event to be quantified (Richards *et al.*, 2008) and the onset and offset of muscle activation. The accepted EMG signals for this work were investigated using integrated EMG (iEMG), which provides information about the amount of muscle activity during a specific task (Richards *et al.*, 2008).

The iEMG has been calculated previously to help identify rehabilitation exercises for humans that may be of more benefit due to an increase in muscle activity (Richards *et al.*, 2008). Integrated EMG was used by Robert *et al.* (2002) to investigate the level of activity in equine muscles at different trotting speeds. It was possible to identify that variations in velocity as small as 0.5 m/s resulted in significant changes in the active contribution of the muscles to equine locomotion (Robert *et al.*, 2002). EMG data has also been integrated in order to be correlated with the length of the *longissimus dorsi* muscle of the equine back (Groesel *et al.*, 2010). This method enabled the authors to establish a linear relationship between the respective variables and begin to validate a preliminary biomechanical model of the equine spine (Groesel *et al.*, 2010).

Integrated EMG has been measured during a specific task (Richards *et al.*, 2008; Groesel *et al.*, 2010) and during consecutive strides (Tokuriki *et al.*, 1999; Robert *et al.*, 2002), which demonstrates it is possible to measure iEMG between two events in the time domain. This is considered to be ideal for investigating the effect of different surface conditions on muscle activity for this research. The iEMG is derived from the area under the curve and therefore must be calculated based on rectified data (Figure 6.1.11 a and b). The iEMG was calculated during stance (hoof impact to hoof lift off or between the blue and red lines in figure 6.1.11 b) and also during the pre-activation period preceding hoof impact (between the green and blue lines in figure 6.1.11 b) to determine if the active contributions of the muscles altered according to the surface conditions.

The hoof impact and lift off gait events had already been applied for the kinematic parameters and therefore it was possible to calculate the iEMG during stance. To calculate the iEMG value during pre-activation however, another gait event was added to each file 100 milliseconds prior to hoof impact (Müller *et al.*, 2010). Muscle activity observed in humans during this time has been identified as a key factor for altering leg posture in preparation for altered surface properties (Müller *et al.*, 2010). This is extremely relevant to this PhD project where horses have encountered perturbations in the functional surface properties. If the muscle activity alters during the pre-activation window, this may help to explain why certain kinematic changes are observed.

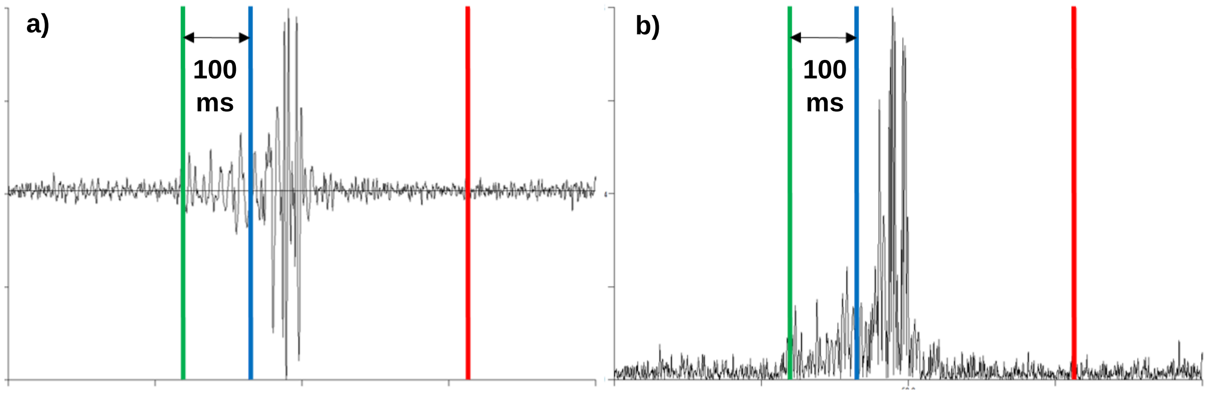


Figure 6.1.11: a) A processed EMG signal from the cranial site of the triceps brachii. The raw signal with the DC offset removed, shifted five frames and high pass filtered with a cut off frequency of 80 Hz; b) The signal presented in a) full wave rectified. The iEMG is calculated from the rectified signal between specific gait events. Blue vertical line: hoof impact; Green vertical line: 100 milliseconds preceding hoof impact in order to measure pre-activation; Red vertical line: hoof lift-off.

The integral values were categorised according to 'iEMG pre-activation' and 'iEMG stance'. It was obvious however that noise contamination was still evident within some of the signals, which was reflected by greater and more variable iEMG values within individual horses. This made the iEMG analysis challenging and therefore it was necessary to discard some of the values to quantify the true effect of the surface conditions on muscle activity. To the researcher's knowledge, there is no published literature outlining a robust method to inform removal of contaminated iEMG values. The method outlined below in conjunction with the provisional accept and reject criteria has proved to be the most reliable and repeatable and has enabled the research aims of this work to be addressed.

The rectified EMG signal was low pass filtered using a fourth order Butterworth digital filter with a cut off frequency of 25 Hz. The peak magnitude of the low pass filtered signal during pre-activation and stance was calculated. Some iEMG values may be larger in magnitude but in order to determine if this is due to noise, the peak magnitude must also be assessed. Upon visual observation of these values, it was possible to discard blocks of data as shown in the print screen below. It was clear from the maximum values that the signal was still contaminated with noise and therefore the iEMG values were subsequently removed from further analysis.

A	B	C	D
File name	Stride no.	ECR iEMG stance	ECR max stance
FlyMarch240006.c3d	1	61.046841	612.783691
-	2	58.833351	662.254456
-	3	61.568497	607.498535
FlyMarch240008.c3d	1	172.241882	1419.238281
-	2	154.844131	1075.644287
-	3	136.297485	1109.756592
FlyMarch240009.c3d	1	117.436653	1127.828125
-	2	143.732758	1315.362549
-	3	148.481705	1547.979614

The remaining iEMG values were assessed vigilantly and values that added to more than 30-40% variation within a horse were also discarded. An example can be seen in the print screen below where the highlighted value was removed from further analysis. It is important to note that the iEMG values may have varied more on day 2 and 3 due to the change in surface conditions. This method was therefore only applied to the first stride in each trial on these days to prevent removal of data that may have been a true response to the surface conditions.

File name	Stride no.	Splenius iEMG stance	Splenius max stance
BenMarch240001.c3d	1	4.555411	75.887535
-	2	3.477343	70.970535
-	3	3.914675	60.44173
BenMarch240002.c3d	1	11.820275	340.787872
-	2	3.477609	49.67701

To investigate the EMG activity between horses and on different surface conditions, it is necessary to normalise the data (DeLuca, 1997; Halaki and Ginn, 2012). The EMG signal can vary between individuals and also within repeated contractions of the same subject and therefore normalising the iEMG values makes it possible to compare data collected under various conditions (DeLuca, 1997). Normalising is usually performed by dividing the EMG signals during a task by a reference EMG value obtained from the same muscle so a relative measure of the muscle activity can be obtained (Halaki and Ginn, 2012).

The maximum iEMG value recorded during stance and pre-activation within each horse and for each muscle on each data collection day during this study was selected as the reference value to normalise the remaining iEMG values to and expressed as a percentage. The print screen below demonstrates how the values were normalised. The normalised values were subsequently grouped according to surface condition and horse for analysis purposes.

	A	B	C	D	E	F
12	**File name**	**Stride no.**	**Triceps iEMG pre-activa**	**Triceps max pre-acti**	**Normalised eq.**	**Normalised iEMG**
13	BenMarch24	1	0.315089	5.94938	(D13/$D$16)*100	71.71659235
14	-	2	0.359639	6.09667	(D14/$D$16)*100	81.85650263
15	-	3	0.424338	6.032363	(D15/$D$16)*100	96.58247468
16	BenMarch24	1	0.439353	9.941103	(D16/$D$16)*100	100
17	-	2	0.365649	8.164068	(D17/$D$16)*100	83.22442319
18	-	3	0.28107	4.08401	(D18/$D$16)*100	63.97361575
19	BenMarch24	1	0.279245	5.084565	(D19/$D$16)*100	63.55823222
20	-	2	0.249508	2.939848	(D20/$D$16)*100	56.78987056

This has been done previously where iEMG data has been normalised to the maximal dynamic contraction in humans (Richards *et al.*, 2008). The maximum value from each human subject was considered to be 100% and the other data were normalised based on this. Achieving a maximal voluntary contraction on command in the equine athlete is simply not possible, however using the maximum value recorded was still considered to be a meaningful reference value (Halaki and Ginn, 2012).

This revealed invaluable information on how the contribution of muscle activity possibly altered on different surface conditions. It was unfortunately not possible to use the absolute maximum value recorded over the four days due to the magnitude of muscle activity showing too much variation between days. Every effort was made to secure the EMG sensors in exactly the same place each day however due to the intricate structure of the muscle fibres, a sensor placed slightly differently on the muscle belly will collect firing patterns from different motor units.

6.2 Statistical Analysis for kinematic and EMG data

Speed effect

The mean speed calculated from each trial was grouped according to horse and surface condition. A general linear model (Figure 6.2.1) was used to determine whether speed altered according to surface condition. It was envisaged that the speed may alter throughout the trial when the horses encountered the change in surface condition and so speed from the first step and second step was categorised separately. The speed for '1 firm' and '1 soft' was taken from the start of the trial to the timing of the first step onto the change in surface condition whereas '2 soft' and '2 firm' were taken from the timing of the respective hoof impact to the end of the trial.

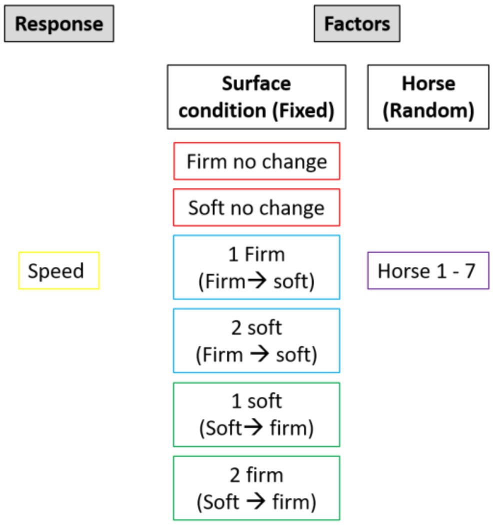


Figure 6.2.1: General linear model used to assess changes in speed on different surface conditions. Surface condition was a fixed factor whereas horse was imported as a random factor.

The effect of an abrupt change in surface condition

In order to investigate the effect of an abrupt change in surface condition on the measured parameters a general linear model was used. Data from day two (Firm → Soft) and day three (Soft → Firm) was categorised according to surface condition or stride number, whether the horses were aware of the surface condition and horse number and analysed separately (Figure 6.2.2 and 6.2.3). The data was also analysed to determine any interactions between surface condition and awareness. It was possible to collect data from three consecutive strides during some of the trials on day two and therefore this was included in the general linear model.

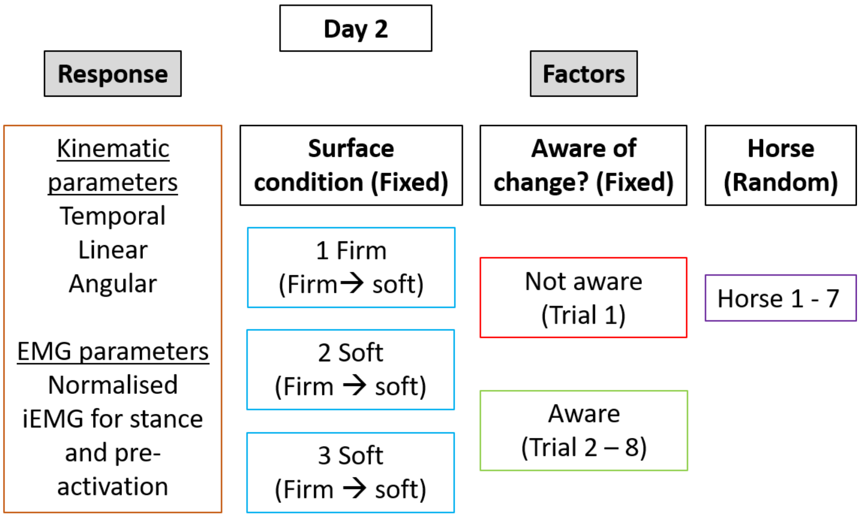


Figure 6.2.2: General linear model used to assess changes in the kinematic and EMG parameters on day two. Surface condition and awareness were fixed factors whereas horse was entered as a random factor.

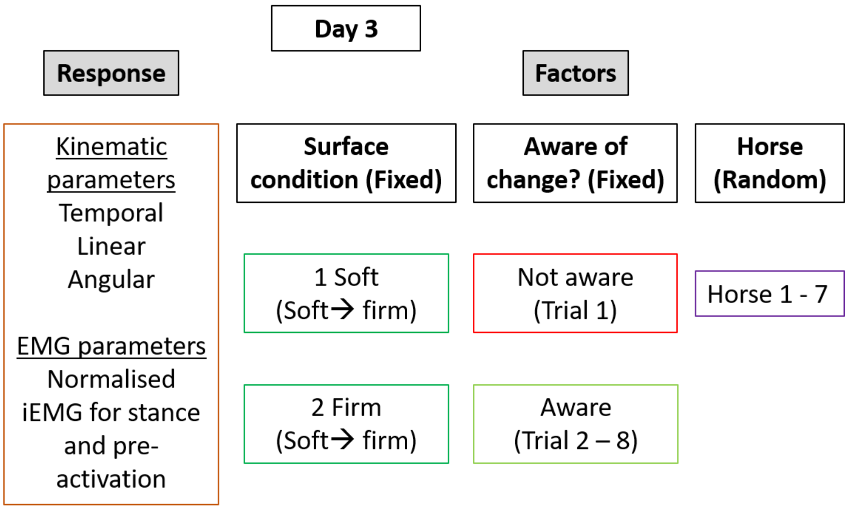


Figure 6.2.3: General linear model used to assess changes in the kinematic and EMG parameters on day three. Surface condition and awareness were fixed factors whereas horse was imported as a random factor.

The effect of a continuous surface on stride to stride changes

The data collected on the continuous surfaces was categorised according to stride number and horse before using a general linear model (Figure 6.2.4) to establish if stride to stride changes were evident. This enabled differences expected on a continuous surface to be accounted for and the resultant effects of the abrupt change on the same parameters to be established.

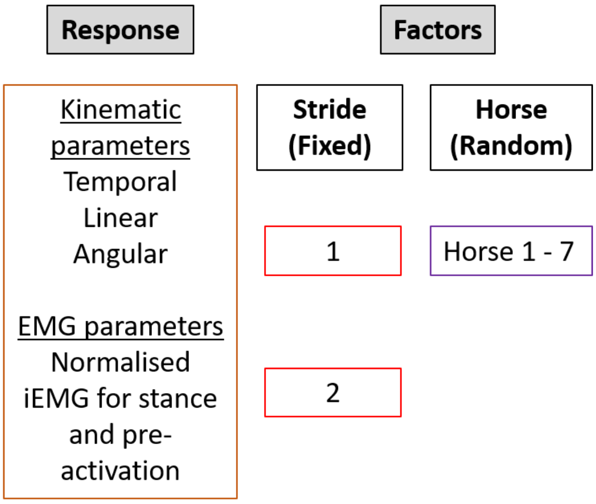


Figure 6.2.4: General Linear model used to assess stride to stride changes in the kinematic and EMG parameters on the continuous surfaces. Stride was a fixed factor whereas horse was entered as a random factor.

Minitab 17 Software was used to analyse all of the data for the PhD project. Post hoc analysis was carried out using a Tukey test to determine where the significant differences lied. Values of P <0.05 were considered statistically significant.

6.3 Optimisation of methods for processing surface data

6.3.1 Surface data processing

The raw data files (Figure 6.3.1) were converted into an ASCII format before being uploaded into Visual 3D software. This enabled the signals to be converted from volts to the relevant units before extracting the parameters of interest. The signals from the linear and string potentiometer on the OBST, used to derive responsiveness and grip, were also filtered with a low pass Butterworth filter with a cut off frequency of 100 Hz before further analysis.

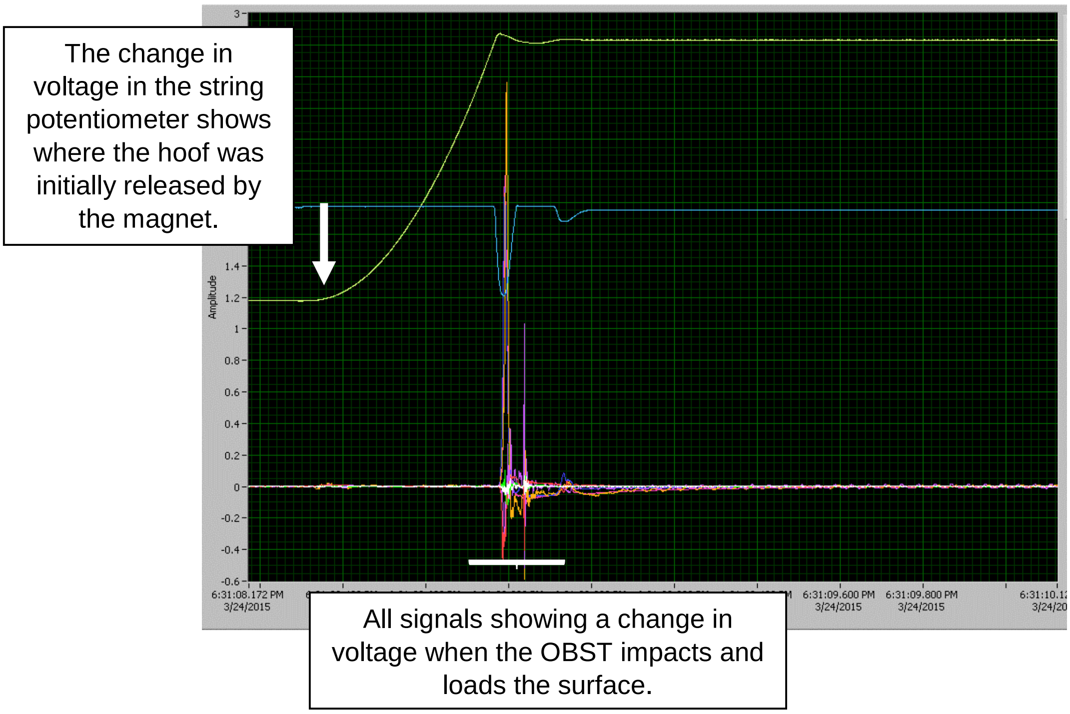


Figure 6.3.1: An example raw data file collected using LabVIEW 2010 software. There are 9 channels (3 from the triaxial load cell, 3 from the triaxial accelerometer, 1 from the single axis load cell and 2 from the string and linear potentiometer), each identified with a different colour.

6.3.2 Surface parameters

Cushioning

This was derived from the maximum vertical force recorded by the tri-axial load cell (Figure 6.3.2).

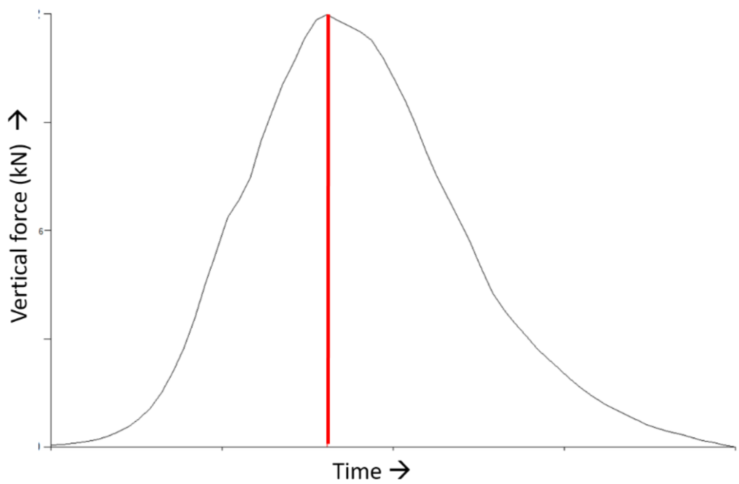


Figure 6.3.2: Change in vertical force during impact and loading of the OBST. Red vertical line represents peak vertical force.

Impact Firmness

This was derived from the maximum vertical acceleration recorded by the tri-axial accelerometer (Figure 6.3.3).

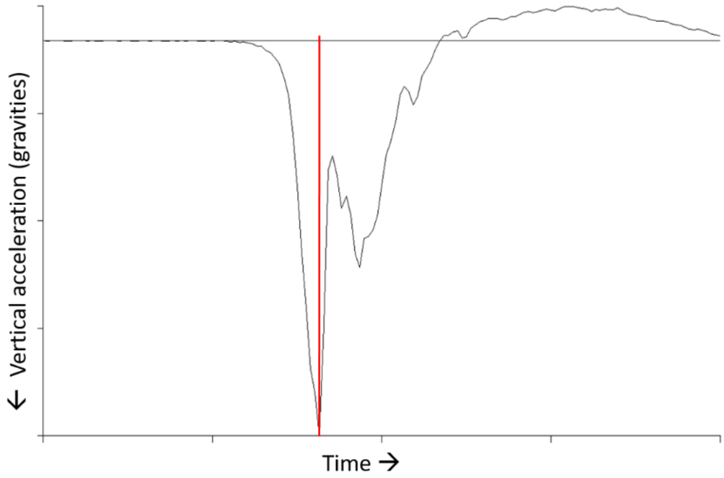


Figure 6.3.3: Change in vertical acceleration during impact and loading of the OBST. Red vertical line represents maximum acceleration.

Responsiveness

Responsiveness was calculated using the first derivative (velocity) of the linear potentiometer signal. Specific events were identified on the displacement velocity signal including minimum velocity, maximum compression and maximum velocity (Figure 6.3.4), which provided the timings required for the equation below.

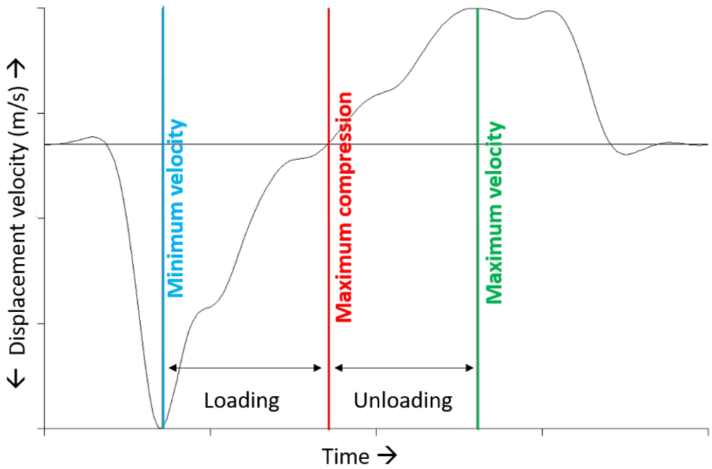


Figure 6.3.4: Displacement velocity during impact, loading and unloading of the OBST. Blue vertical line: minimum displacement velocity; Red line: the timing of maximum compression as taken from the original displacement signal and also represents '0' displacement velocity; Green line: maximum displacement velocity.

Responsiveness was calculated as follows:

$$\frac{Timing\ of\ \max compression\ \ -Timing\ of\ minimum\ velocity}{\text{Timing of max}\ compression\ \ -Timing\ of\ maximum\ velocity}$$

Grip

As described by Hernlund (2016), grip was calculated by determining the amount of forward slide of the hoof (mm) from impact of the OBST to peak vertical force. The speed at impact was calculated using the first derivative of the string potentiometer signal. The horizontal component of the landing speed was subsequently determined using the angle of the guide rails. Horizontal acceleration from impact was calculated by using the y-axis force signal from the tri-axial load cell and dividing it by the mass of the sliding components. The indefinite integral of the acceleration was calculated using a constant of integration of zero to obtain horizontal velocity. The indefinite integral of this result was calculated using the horizontal speed at impact for the constant of integration which then gives the horizontal displacement.

Uniformity

In order to assess uniformity of surface cushioning and impact firmness, a contour plot was presented for each surface condition and the fresh and horse track. The range in values was reported on each plot.

Surface stiffness

Surface stiffness was calculated using the same method for calculating effective limb stiffness as presented by McMahon and Cheng (1990):

$$\frac{Maximum\ vertical\ force\ (kN)}{Change\ in\ vertical\ leg\ length\ or\ surface\ deformation\ (m)}$$

The cushioning values, which represented the maximum vertical force, were divided by surface deformation instead of change in leg length. Maximum deformation was derived from the linear potentiometer signal.

6.3.3 Statistical analysis for the surface data

Raw OBST data were stacked according to the factors outlined in Figure 6.3.5 before analysing the data with a general linear model. The ‘fresh’ and ‘horse’ track represented how the functional surface properties changed from the start to the end of each day respectively. Post hoc analysis was carried out using a Tukey test to determine where the significant differences lied. Values of P <0.05 were considered statistically significant.

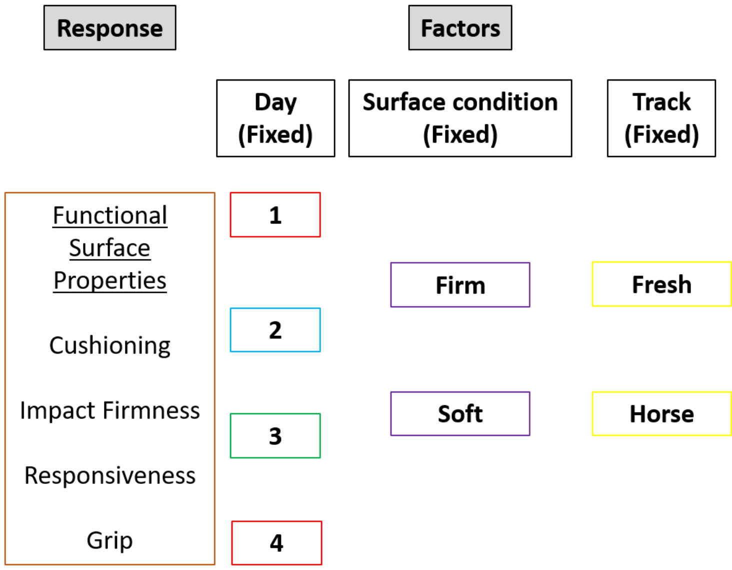


Figure 6.3.5: General Linear Model used to assess changes in the functional surface properties according to day, surface condition and track. All factors were fixed in the model.

7.0 Introduction

It was not possible to collect force data for this project and so gait events for hoof impact and lift off were identified using kinematic data. A simple kinematic gait event detection method has been identified and published by Holt *et al.* (2017), which was subsequently used for this project (Appendix III). The study authenticates the detection method on a rubberised track surface and therefore it was unknown if the method could be applied to kinematic data collected on a compliant surface with the same degree of accuracy and precision. It was thought that the forelimb landing angle used to calculate the gait events may be affected by a compliant surface based on the results reported by Burn and Usmar (2005).

The MPhil part of the PhD project involved designing and validating a force shoe that was capable of measuring vertical ground reaction force during stance. The force shoe was unfortunately not suitable for use during the main study due to inconsistencies in the calibration values during peak loads. The signal still clearly revealed the onset and offset of hoof impact and lift off however and was deemed appropriate for use during this small study. The aim of this study therefore was to use the force shoe to authenticate the kinematic gait event detection method identified by Holt *et al.* (2017) for use on compliant surfaces.

7.1 Methods

Subject

One horse was used (height at withers: 1.60m; weight approximately 450 kg) without any gait asymmetries to address the aims of the study.

Data Acquisition

An 8 camera 3D motion analysis system (Qualisys Oqus) was used and set up as shown in section 4.2 and the same marker set was used as shown in section 4.3. There were also two extra markers applied to the dorsal hoof wall in order to measure hoof orientation at impact. One marker was positioned at the coronet band and the second marker was positioned 50 mm distally on the dorsal hoof wall. A prototype force shoe was applied to the right fore hoof, the design, data acquisition and validation of which can be seen in detail in the MPhil to PhD transfer report (Appendix IV). The subject was habituated to the additional height and weight of the force shoe prior to data collection.

The force shoe was secured to a modified standard steel shoe, which had been drilled with 5mm holes at three locations before being fitted by the farrier during the shoeing preceding data collection (Plate 7.1.1 and 7.1.2). The height of the force shoe was 10mm and weighed 0.6 kg including the standard steel shoe and Delsys® load cell adaptor, which transmitted the data wirelessly to the base station (range 30 m) (Plate 7.1.3 a and b). Compensatory height and weight was added to the left fore shoe in the form of a 'dummy' force shoe using the same attachment method in order to prevent asymmetrical movement. The load cell wire ran towards the heels before being directed up the lateral aspect of the distal limb and secured above the MCP joint to a tendon boot. It was possible to use the same trigger system described in section 4.2 because the Delsys® base station was used to collect the force data and so this was synchronised with the kinematic data.

Plate 7.1.1: Modified steel shoe with three points of attachment prior to being fitted by the farrier

Plate 7.1.2: Force shoe secured ready for data collection

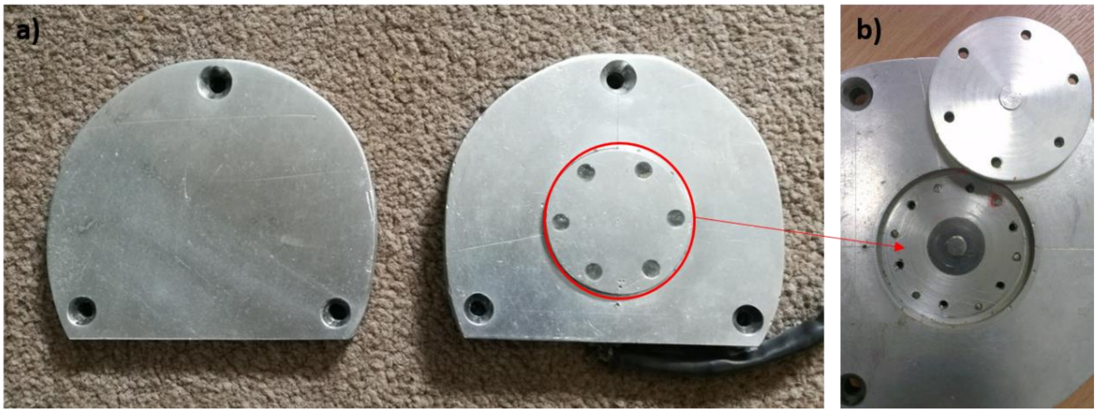


Plate 7.1.3 a: 'Dummy' force shoe (left) and prototype force shoe (right);b: The single axis button load cell (Interface LBS miniature compression load) secured beneath the plate.

A synthetic waxed sand and fibre indoor arena surface was used for the study. The arena was maintained using the standard protocol prior to data collection to improve surface uniformity. A total of eight successful trials were performed with the force shoe on during in-hand trot (mean speed: 3.63 m/s ± 0.03) to simulate the activity being performed in the main study.

Data Processing

Kinematic and kinetic data were analysed using Visual 3D (C-Motion Inc.). Kinematic data were interpolated (maximum gap 10 frames) and then filtered with a low pass zero lag 4th order Butterworth digital filter (cut off frequency of 10 Hz), which was the same method discussed in section 6.02. The same filter was also applied to the kinetic data with a cut off frequency of 100 Hz in accordance with Hobbs and Clayton (2013). The timing of hoof impact and lift off was calculated using GRF and kinematic data.

Gait event detection using GRF data

The vertical ground reaction force (GRFz) data was used to determine gait events using a 50N threshold. The data was converted from microvolts to Newtons using the calibration equation in the transfer document (Appendix IV). Hoof impact was identified as the signal increased above 50 N and hoof lift off was identified as the signal reduced below 50N.

Gait event detection using kinematic data

As described in Holt *et al.* (2017), to determine the kinematic hoof-on and hoof-off events for the fore limbs, a sagittal plane angle was computed using the following markers: 1) centre of rotation of the MCP joint; 2) centre of rotation of the distal interphalangeal (DIP) joint; 3) the lateral epicondyle of the humerus (Plate 7.1.4). A planar angle-time curve was plotted for each trial. A threshold of 0 degrees was used to define events when the two segments were aligned, with hoof-on coinciding with descent through 0 degrees and hoof-off on ascent through 0 degrees (Figure 7.1.1).

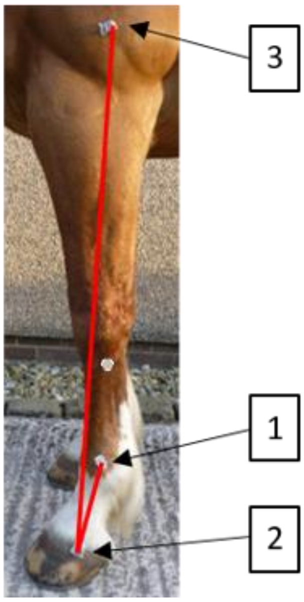


Plate 7.1.4: The two segments (red lines) used to compute the sagittal plane angle that enabled the identification of hoof-on and hoof-off events for the forelimbs; 1) MCP joint; 2) fore DIP joint; 3) lateral epicondyle of the humerus.

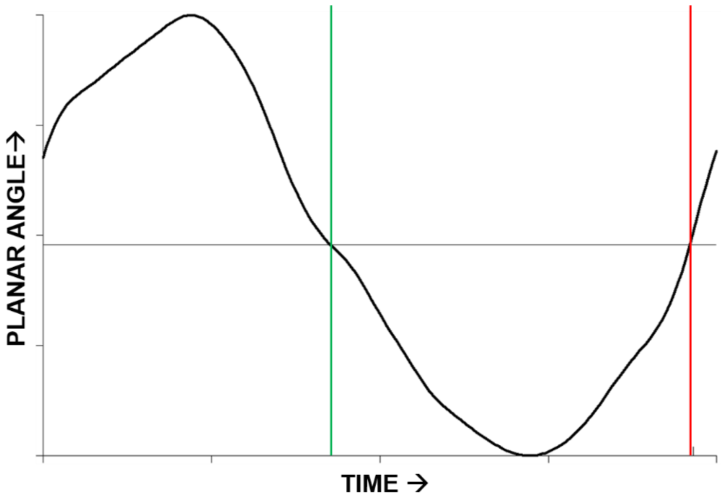


Figure 7.1.1: Planar angle-time curve for one stride used to identify the kinematic gait events for hoof impact when the planar angle descended through '0' on the y axis (green line) and hoof lift off when the planar angle ascended through '0' on the y axis (red line).

Data Analysis

Gait event timings were derived using the GRF and kinematic methods. The accuracy and precision of the kinematic gait events at representing the GRF events were calculated for the forelimbs in accordance with Boye *et al.* (2014) and Holt *et al.* (2017). Accuracy was defined as the mean difference between kinematic and GRF events (bias) and precision as the standard deviation (SD) of the mean difference (Boye *et al.*, 2014). A consistently smaller difference was interpreted as improved accuracy and precision. Hoof orientation was also calculated to account for variations during impact that may have influenced the identification of the gait events.

7.2 Results

A total of 30 stance phases were analysed (2 trials: 3 strides each; 6 trials: 4 strides each). Upon observing the data, it was clear that the first three trials induced more variation throughout the dataset. Every effort was made to habituate the horse to the additional height and weight of the force shoe however, it appears that there was a habituation effect as the trials progressed. The data from the first three trials were subsequently removed. Accuracy and precision of the kinematic gait events for the right forelimb (Table 7.2.1) showed that hoof impact was identified more accurately and hoof lift off more precisely, as shown by a smaller deviation from the GRF event (Figure 7.2.1). The hoof consistently landed on the lateral toe at impact (standard deviation medio-lateral: 2.03º; standard deviation dorso-palmar: 1.80º).

Table 7.2.1: Accuracy (mean) and precision (standard deviation) of the kinematic gait event detection methods. Positive values indicate that the kinematic event occurred before the GRF event and vice versa for negative values. n = 20 stance phases

Gait event	Accuracy (ms)	Precision (ms)
Hoof impact	31.25	31.11
Hoof lift off	-34.7	5.7

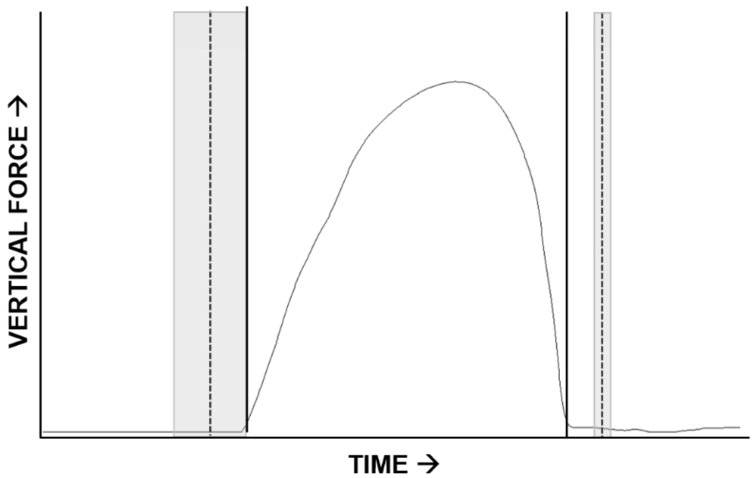


Figure 7.2.1: The accuracy (dashed line) and precision (shaded area) of the kinematic gait events on a GRF trace. The solid black lines represent the GRF events on a mean vertical GRF trace from all 20 stance phases.

7.3 Discussion

The aim of this study was to use the force shoe to authenticate the kinematic gait event detection method identified by Holt *et al.* (2017) for use on compliant surfaces. The results of this study were comparable to that reported in Holt *et al.* (2017). Hoof impact identification was more accurate on the compliant surface but with a slightly lower degree of precision. The identification of the hoof lift off event was not as accurate on the compliant surface but the precision was still excellent nonetheless. The lower accuracy suggests that the kinematic hoof-off event was consistently identified later on the compliant surface in this particular horse. This represents a longer time before the planar angle was in alignment, which may have been caused by the surface components moving as the toe penetrated the surface during breakover.

The accuracy and precision found in this study was of a similar magnitude to that reported by Boye *et al.* (2014) who investigated the accuracy and precision of several algorithms that had been published previously. Hoof impact was generally identified with greater accuracy in this study and hoof lift off had greater precision than all of the methods explored by Boye *et al.* (2014). The algorithms used by Boye *et al.* (2014) use velocity thresholds or horizontal displacements of the hoof segment to detect the kinematic gait events, which are influenced by surface type (Barrey *et al.*, 1991; Orlande *et al.*, 2012). A lack of documentation of the effect of different surfaces on the accuracy and precision of the kinematic gait events was identified as a limitation of the study (Boye *et al.*, 2014). The surface properties have shown to influence forelimb landing angle (Burn and Usmar, 2005), which will have influenced the detection method used in this study. The results presented here suggest that the kinematic gait event detection method can be translated for use on compliant surfaces with similar accuracy and precision to what has been reported previously. The mean speed and stance duration were also comparable to that found for the main study of the project, suggesting good repeatability would be expected between this small study and the main study.

The kinematic hoof impact and lift off events were consistently identified earlier and later than their respective GRF events. This may mean a prolonged stance duration is recorded in the absence of force data but this will be consistent throughout all trials, which will still allow for comparisons between treatments. It is also important to acknowledge hoof orientation at impact, which was consistently on the lateral toe throughout data collection. This will have caused a delay in the GRF hoof impact event because the plate covering the load cell was centrally located. The plate was positioned as close to the centre of pressure (COP) as possible but this design may mean that the primary impact and the end of the stance phase is not detected and so the kinematic event may in fact be more accurate than the data set currently allows. The force shoe was not applied to the hind limb, which does pose a limitation. The hind limb gait events demonstrated a much better accuracy and precision when compared to the fore limb in Holt *et al.* (2017) and so it can only be assumed that a similar accuracy and precision can be translated based on the results reported for the fore limb.

8.0 Introduction

Pilot work was performed as part of the MPhil phase of the project to determine the amount of inherent intra-horse variability expected during data collection. The effect of different surface types and preparation methods on certain kinematic parameters of the horse has been researched extensively to determine how the substrate affects performance and potentially poses a risk factor for injury. The effect of surface type on variables such as ground reaction force (Ratzlaff *et al.*, 1997; Kai *et al.*, 1999; Gustås *et al.*, 2006; Robin *et al.*, 2009; Chateau *et al.*, 2010, 2013; Crevier Denoix *et al.*, 2010), hoof and fetlock accelerations (Barrey *et al.*, 1991; Burn and Usmar, 2005; Gustås *et al.*, 2006; Chateau *et al.*, 2009; Kruse *et al.*, 2012) and stride parameters such as stance duration, duty factor, stride frequency (Robin *et al.*, 2009; Chateau *et al.*, 2013) and hoof slide during landing from a jump (Orlande *et al.*, 2012) have been quantified. The effect of preparation techniques on limb kinematics at stance have also been investigated (Northrop *et al.*, 2013).

It is well documented that surface type influences kinematic variables, however it is important to consider the amount of inherent variation contributing to the changes in each parameter including neuromuscular contributions that would be expected under the same conditions. Inter-horse variability and inter-stride variability of horses trotting on a circle with a 4m radius on fibre sand and asphalt has been recorded by Chateau *et al.* (2013). Inter-horse variability was greater for all of the measured kinetic and kinematic variables in comparison to inter-stride variability (Chateau *et al.*, 2013), which has also been found previously (Faber *et al.*, 2002; Heaps *et al.*, 2011). The aim of this work was to quantify gait variability in two horses on consecutive days whilst performing in hand trot on a uniform surface.

8.1 Methods

The experimental protocol is described in detail in the transfer document (Appendix IV). The set up was similar to the general methods described in section 4.2 and the raw data files were processed using the same methods described in Chapter 6.0 before extracting all the relevant parameters that were explored during the main study. The coefficient of variation (CV)) was used to demonstrate intra-horse variability for all of the parameters presented throughout sections 9.1 to 9.3. Between day variability (BDV) was calculated for each horse by averaging the CV values from each day in accordance with Faber *et al.* (2002). BDV values were subsequently averaged to obtain a mean BDV for both horses (Faber *et al.*, 2002). Horse 2 was removed from the study on day 3 due to appearing asymmetrical.

8.2 Results and Discussion

Speed

Speed was slightly slower than that recorded for the main study. For the pilot work, there was no significant change in speed between days for horse 1 **$F_{(2, 18)}$ = 1.38, *P* =0.276** or 2 **$t_{(8)}$ = -0.23, *P* =0.823** (Table 8.2.1).

Table 8.2.1: The mean (±SE) speed for each horse on each day of data collection. P<0.05 denotes a significant difference.

	Horse 1					Horse 2		
Parameter	**Day 1**	**Day 2**	**Day 3**	**P-value**		**Day 1**	**Day 2**	**P-value**
Speed (m/s)	3.23 ± 0.08	3.13 ± 0.02	3.13 ± 0.03	0.276		3.21 ± 0.05	3.20 ± 0.02	0.823

Kinematic and Neuromuscular variability

The between day variability is presented in table 8.2.2, 3 and 4 for the temporal, linear and angular parameters respectively. The total number of fore and hind strides that were grouped for analysis is presented at the bottom of table… It was not possible to report neck inclination at hoof impact and lift off because the marker set used for pilot work did not extend to the head and neck. Mean BDV was below 5 for all parameters except the ipsilateral contact timing, which showed a great amount of variation. The contact timings were both positive and negative for horse one on day three suggesting that the right hind hoof impact did not always occur before the right fore hoof lift off. This lowered the mean closer to 0 when all days were grouped and created a standard deviation higher than the mean, which meant the resultant CV was greater than 100%. If the CV value from horse one on day three was removed from the BDV calculation, the mean BDV is lowered to 6.58.

The between day variability for the different muscle contributions during pre-activation and stance is presented in table 8.2.5. The variability is relatively consistent with the mean BDV ranging from 16.88 to 20.32 across all muscles except the *trapezius cervicis* muscle during pre-activation, which had a mean BDV of 28.59. The *splenius* muscle was yet to be identified for further investigation at this stage in the project and so no data has been presented for this muscle. Data was also only collected from one site on the *trapezius cervicis* and *triceps brachii* (labelled accordingly). It is envisaged that variation exceeding the mean BDV values reported here during the main study would be a direct result of the change in surface condition.

Table 8.2.2: The mean ±SE (coefficient of variation) temporal parameters for each horse on each day of data collection. The between day variability (BDV) was calculated by averaging the CV values from each horse. The BDV values from horse one and two were subsequently averaged to calculate mean BDV.

	Horse 1					Horse 2			Mean BDV
Parameter	**Day 1**	**Day 2**	**Day 3**	**BDV**		**Day 1**	**Day 2**	**BDV**	
Right fore stance duration (seconds)	0.337 ± 0.003 (3.94)	0.382 ± 0.002 (2.33)	0.375 ± 0.002 (2.53)	2.93		0.348 ± 0.002 (3.64)	0.345 ± 0.001 (2.27)	2.96	2.95
Right fore duty factor (%)	46.15 ± 0.28 (2.69)	51.08 ± 0.22 (1.64)	50.39 ± 0.17 (1.5)	1.94		51.46 ± 0.2 (1.87)	50.35 ± 0.08 (0.85)	1.36	1.65
Timing of peak MCP ext.* (% of stance)	51.99 ± 0.23 (2.25)	54.28 ± 0.15 (1.37)	53.87 ± 0.27 (2.6)	2.07		55.86 ± 0.3 (3.07)	55.89 ± 0.25 (2.63)	2.85	2.46
Right hind stance duration (seconds)	0.343 ± 0.003 (3.65)	0.425 ± 0.002 (2.53)	0.366 ± 0.002 (2.7)	2.96		0.372 ± 0.005 (7.2)	0.375 ± 0.001 (1.66)	4.43	3.7
Right hind duty factor (%)	46.97 ± 0.24 (2.14)	56.96 ± 0.2 (1.64)	49.29 ± 0.2 (1.68)	1.82		55.01 ± 0.77 (6.74)	54.71 ± 0.16 (1.27)	4.01	2.92
Timing of peak MTP ext.* (% of stance)	54.68 ± 0.81 (7.21)	59.4 ± 0.51 (4.57)	57.36 ± 0.42 (3.73)	5.17		56.54 ± 0.3 (2.98)	59.24 ± 0.35 (3.31)	3.15	4.16
Ipsilateral contact timing (seconds)	-0.017 ± 0.002 (-51.10)	0.039 ± 0.002 (20.39)	0.0005 ± 0.002 (1564.95)	511.41		0.04 ± 0.003 (47.05)	0.037 ± 0.001 (9.98)	28.52	269.97
Number of strides fore (*n*)	27	24	27			32	35		
Number of strides hind (*n*)	24	28	26			31	28		

**MCP ext.:* metacarpophalangeal extension; *MTP ext.:* metatarsophalangeal extension

Table 8.2.3: The mean ±SE (coefficient of variation) linear parameters for each horse on each day of data collection. The between day variability (BDV) was calculated by averaging the CV values from each horse. The BDV values from horse one and two were subsequently averaged to calculate mean BDV.

	Horse 1					Horse 2			Mean BDV
Parameter	**Day 1**	**Day 2**	**Day 3**	**BDV**		**Day 1**	**Day 2**	**BDV**	
Right fore stride length (m)	2.35 ± 0.03 (5.18)	2.36 ± 0.02 (2.52)	2.33 ± 0.01 (2.46)	3.39		2.17 ± 0.01 (3.17)	2.20 ± 0.007 (1.66)	2.42	2.91
Right hind stride length (m)	2.32 ± 0.03 (4.87)	2.34 ± 0.01 (2.3)	2.32 ± 0.01 (2.3)	3.16		2.16 ± 0.02 (3.36)	2.20 ± 0.009 (1.86)	2.61	2.89

Table 8.2.4: The mean ±SE (coefficient of variation) angular parameters for each horse on each day of data collection. The between day variability (BDV) was calculated by averaging the CV values from each horse. The BDV values from horse one and two were subsequently averaged to calculate mean BDV.

	Horse 1				Horse 2			Mean BDV
Parameter	**Day 1**	**Day 2**	**Day 3**	**BDV**	**Day 1**	**Day 2**	**BDV**	
Peak MCP ext. (°)	43.60 ± 0.22 (2.58)	52.05 ± 0.16 (1.54)	52.02 ± 0.19 (1.86)	1.99	53.54 ± 0.17 (1.75)	54.31 ± 0.12 (1.27)	1.51	1.75
Peak MTP ext. (°)	42.42 ± 0.18 (2.06)	55.32 ± 0.19 (1.8)	46.04 ± 0.25 (2.72)	2.19	54.01 ± 0.84 (8.62)	50.74 ± 0.15 (1.6)	5.11	3.65
Forelimb inclination at hoof impact (°)	26.69 ± 0.15 (2.99)	29.93 ± 0.11 (1.75)	29.67 ± 0.12 (2.01)	2.25	29.05 ± 0.13 (2.48)	29.15 ± 0.09 (1.83)	2.16	2.21
Forelimb inclination at hoof lift off (°)	-24.45 ± 0.14 (-2.96)	-24.17 ± 0.16 (-3.31)	-23.67 ± 0.12 (-2.66)	-2.98	-22.23 ± 0.12 (-3.15)	-22.13 ± 0.12 (-3.12)	-3.14	-3.06
Scapula inclination at hoof impact (°)	29.97 ± 0.18 (3.09)	30.46 ± 0.14 (2.24)	29.43 ± 0.19 (3.39)	2.91	32.0 ± 0.14 (2.43)	35.49 ± 0.11 (1.88)	2.16	2.54
Scapula inclination at hoof lift off (°)	14.73 ± 0.26 (9.11)	17.35 ± 0.19 (5.43)	16.56 ± 0.2 (6.19)	6.91	19.51 ± 0.15 (4.46)	23.91 ± 0.14 (3.4)	3.93	5.42
Shoulder angle at hoof impact (°)	-82.52 ± 0.08 (-0.52)	-74.94 ± 0.11 (-0.7)	-72.1 ± 0.06 (-0.46)	-0.56	-83.13 ± 0.08 (-0.54)	-91.61 ± 0.09 (-0.56)	-0.55	-0.56
Shoulder angle at hoof lift off (°)	-81.69 ± 0.09 (-0.59)	-73.17 ± 0.06 (-0.39)	-72.13 ± 0.07 (-0.47)	-0.48	-80.11 ± 0.08 (-0.59)	-87.56 ± 0.08 (-0.55)	-0.57	-0.53
Hindlimb inclination at hoof impact (°)	20.07 ± 0.17 (4.04)	18.73 ± 0.15 (4.23)	19.96 ± 0.17 (4.36)	4.21	18.26 ± 0.2 (6.03)	19.27 ± 0.18 (5.15)	5.59	4.9
Hindlimb inclination at hoof lift off (°)	-25.75 ± 0.24 (-4.58)	-25.98 ± 0.2 (-4.1)	-25.03 ± 0.2 (-4.07)	-4.25	-25.03 ± 0.19 (-4.15)	-24.43 ± 0.21 (-4.75)	-4.45	-4.35

Table 8.2.5: The mean ±SE (coefficient of variation) normalised integrated EMG (iEMG) values (%) during pre-activation (100 ms preceding stance) and stance for each horse on each day of data collection. The between day variability (BDV) was calculated by averaging the CV values from each horse. The BDV values from horse one and two were subsequently averaged to calculate mean BDV.

	Horse 1					Horse 2			Mean BDV
Parameter	**Day 1**	**Day 2**	**Day 3**	**BDV**		**Day 1**	**Day 2**	**BDV**	
Trapezius (caudal site) pre-activation	71.11 ± 4.12 (26.54)	63.68 ± 7.73 (38.4)	70.99 ± 2.75 (17.34)	27.43		68.4 ± 9.16 (29.93)	58.59 ± 3.11 (29.57)	29.75	28.59
Trapezius (caudal site) stance	75.92 ± 2.55 (15.03)	71.22 ± 3.35 (23.01)	77.21 ± 2.62 (15.54)	17.86		72.95 ± 5.89 (21.37)	61.27 ± 2.20 (18.31)	19.84	18.85
Triceps (cranial site) pre-activation	76.9 ± 2.73 (16.62)	65.44 ± 2.36 (17.28)	67.25 ± 2.77 (21.4)	18.43		72.0 ± 2.01 (14.52)	77.78 ± 2.12 (16.13)	15.33	16.88
Triceps (cranial site) stance	82.89 ± 2.24 (12.96)	82.8 ± 4.33 (14.79)	76.46 ± 2.57 (17.49)	15.08		83.7 ± 1.48 (9.38)	62.12 ± 3.14 (29.5)	19.44	17.26
Extensor Carpi Radialis pre-activation	77.51 ± 4.07 (19.67)	78.03 ± 2.66 (16.33)	62.19 ± 2.75 (22.94)	19.65		81.79 ± 2.26 (14.07)	66.49 ± 2.91 (25.55)	19.81	19.73
Extensor Carpi Radialis Stance	72.88 ± 3.79 (24.94)	72.46 ± 2.97 (19.64)	72.77 ± 2.18 (15.57)	20.05		71.26 ± 3.54 (21.08)	69.19 ± 2.35 (20.07)	20.58	20.32
Ulnaris Lateralis pre-activation	63.05 ± 4.3 (32.72)	72.67 ± 2.78 (17.53)	83.7 ± 2.29 (11.26)	20.5		68.17 ± 2.09 (15.95)	70.4 ± 2.17 (18.21)	17.08	18.79
Ulnaris Lateralis stance	82.87 ± 3.93 (14.23)	82.16 ± 2.42 (13.84)	86.72 ± 2.59 (8.43)	12.17		73.59 ± 3.06 (21.97)	64.82 ± 2.95 (24.94)	23.46	17.82

9.0 Results from the main study

The kinematic and electromyographic results have been presented in a format to address the hypotheses of the project: 1) To present stride to stride temporal changes on the different surface conditions (Section 9.1.); 2) To present stride to stride linear and angular changes on the different surface conditions (Section 9.2); 3) To present stride to stride changes in the muscle contributions during pre-activation and stance on the different surface conditions (Section 9.3); 4) To present the effects of awareness of the abrupt change on the kinematic and sEMG data (Section 9.4); 5) To present stride to stride changes and the effect of awareness on fore and hind effective limb stiffness on the different surface conditions (Section 9.5). All of the significant kinematic and sEMG data has been summarised in Section 9.6. A post hoc observational analysis section has been included to aid with the discussion (Section 9.7). The surface data has also been reported in section 9.8. This demonstrates how the functional surface properties changed according to surface condition and also how they altered in response to equine traffic.

9.1. Stride to stride temporal changes

9.1.1 The effect of an abrupt change in surface condition

There was no significant **F (5, 273) = 9.6, P >0.05** change in speed when the horses made the transition from one surface condition to another (Table 9.1.1). This is important due to some of the measured parameters being speed dependent (Dutto *et al*., 2004; Gustås *et al.*, 2006b).

Table 9.1.1: Mean ± SE speed recorded on the different surface conditions for all horses.

	Firm → Soft		**Soft → Firm**	
Speed (m/s)	3.90 ± 0.03	3.79 ± 0.04	3.85 ± 0.03	3.77 ± 0.04

When the horses moved from the firm to soft surface condition, it was possible to collect three consecutive strides during most of the trials and therefore the data for the second stride on the soft surface condition was also used in the statistical model. When the horses moved from the soft to firm surface condition however, there were only two trials where it was possible to collect data from three complete stance phases due to the positioning of the cameras. Data from the second stride on the firm surface condition was therefore excluded from the statistical model and not presented for any of the parameters.

When the horses moved from the firm to soft surface condition, stride location did not have a significant (*P* >0.05) effect on any of the temporal parameters (Table 9.1.2). When the horses travelled from the soft to firm surface condition however, stance duration **F (1, 99) = 7.3, *P* =0.008** and duty factor **F (1, 61) = 7.82, *P* =0.007** within the hind limb significantly increased. A higher value for the ipsilateral contact times suggests that the right hind was contacting the ground earlier prior to right fore hoof lift. The parameters that showed significant stride to stride changes in response to the abrupt change were also presented in graph format in order to observe the amount of variation between horses (Figure 9.1.1 and 9.1.2).

Table 9.1.2: Mean ± SE (coefficient of variation) temporal parameters according to stride location. Values of P <0.05 denote a significant difference between the strides on the corresponding surface condition. It was not possible to report duty factor for stride two on the soft surface condition due to data only being collected for two swing phases during each trial.

	Firm → Soft				**Soft → Firm**		
Parameter	Firm	Soft (stride 1)	Soft (stride 2)	***P* - value**	Soft	Firm (stride 1)	***P* - value**
Right fore stance duration (seconds)	0.349 ± 0.003 (5.97)	0.349 ± 0.003 (5.67)	0.346 ± 0.004 (5.44)	0.755	0.346 ± 0.003 (6.94)	0.347 ± 0.004 (7.37)	0.944
Right fore duty factor (%)	49.96 ± 0.30 (4.44)	49.71 ± 0.36 (4.27)		0.773	49.71 ± 0.35 (5.09)	50.09 ± 0.96 (6.37)	0.260
Timing of peak MCP ext.* (% of stance)	57.06 ± 0.36 (4.46)	57.33 ± 0.31 (4.09)	56.90 ± 0.53 (4.83)	0.419	57.38 ± 0.49 (6.16)	56.70 ± 0.61 (7.7)	0.953
Right hind stance duration (seconds)	0.399 ± 0.005 (8.9)	0.394 ± 0.005 (9.31)	0.40 ± 0.007 (8.9)	0.061	0.376 ± 0.003 (6.78)	0.389 ± 0.004 (7.69)	**0.008 (**Fig 9.2.10**)**
Right hind duty factor (%)	56.67 ± 0.66 (8.47)	56.73 ± 0.80 (8.95)		0.105	54.25 ± 0.67 (9.2)	56.16 ± 1.06 (7.57)	**0.007 (**Fig 9.2.11**)**
Timing of peak MTP ext.* (% of stance)	57.92 ± 0.96 (11.55)	58.28 ± 0.84 (10.54)	58.82 ± 0.96 (8.17)	0.932	59.7 ± 0.59 (7.09)	58.44 ± 0.46 (5.84)	0.052
Ipsilateral contact timing (seconds)	0.040 ± 0.005 (87.72)	0.034 ± 0.005 (101.31)	0.030 ± 0.005 (87.17)	0.408	0.023 ± 0.004 (139.91)	0.026 ± 0.004 (126.48)	0.369

**MCP ext.:* metacarpophalangeal extension; *MTP ext.:* metatarsophalangeal extension

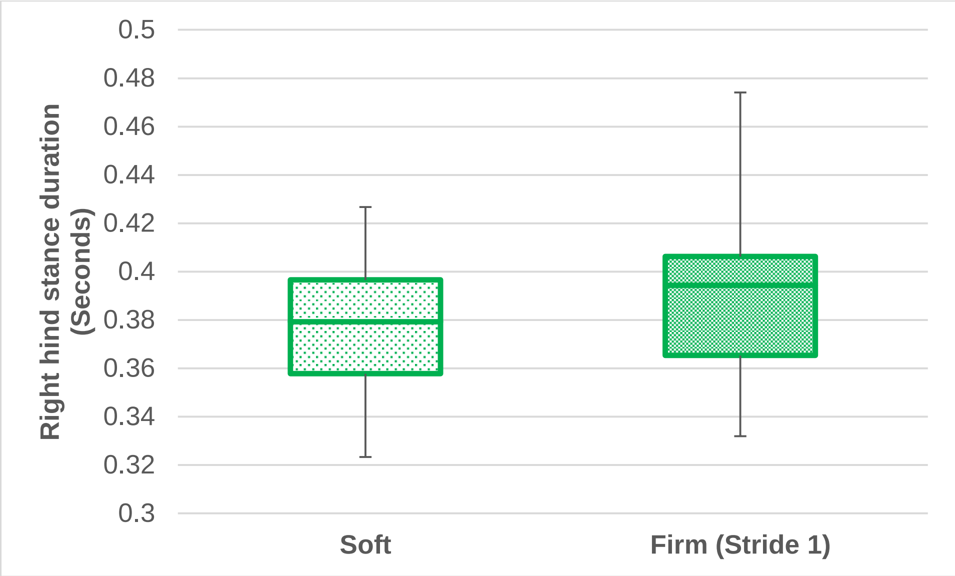


Figure 9.1.1: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in stance duration for the right hindlimb.

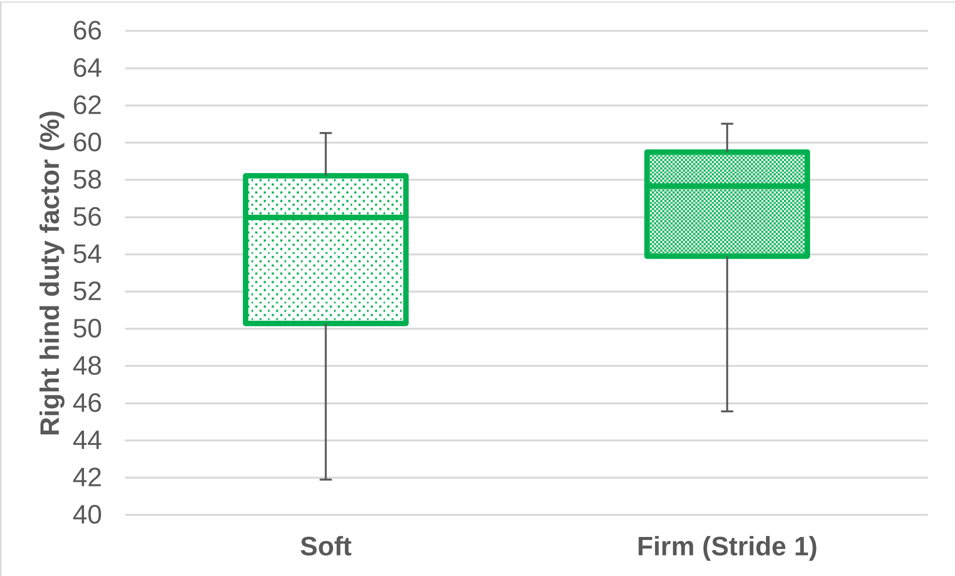


Figure 9.1.2: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in duty factor for the right hindlimb.

9.1.2 The effect of continuous surfaces

There were no significant (P>0.05) temporal stride to stride changes recorded in the forelimb and most of the hind limb parameters on either continuous surface condition (Table 9.1.3). The timing of peak MTP extension during stance on the continuous firm surface occurred significantly **F (1, 47) = 5.35, *P* =0.025** earlier during the second stride. Hind limb stance duration also significantly **F (1, 53) = 8.69, *P* =0.005** increased during the second stride on the continuous soft surface condition. It was not possible to report duty factor for stride one and two due to data only being collected for one swing phase (between the two consecutive stance phases) during each trial.

Table 9.1.3: Mean ± SE (coefficient of variation) temporal parameters according to stride number on the continuous surface conditions. Values of P <0.05 denote significant differences between stride one and two on the corresponding surface condition. The data has been grouped from all horses.

	Continuous Firm Surface				Continuous Soft Surface		
Parameter	Stride 1	Stride 2	***P* - value**		Stride 1	Stride 2	***P* - value**
Speed (m/s)	3.8 ± 0.05				3.57 ± 0.04		
Right fore stance duration (seconds)	0.363 ± 0.004 (6.29)	0.364 ± 0.005 (8.19)	0.922		0.352 ± 0.003 (5.23)	0.357 ± 0.004 (6.36)	0.094
Right fore duty factor (%)	52.43 ± 0.44 (4.74)				49.38 ± 0.29 (3.37)		
Timing of peak MCP ext. (% of stance)	58.62 ± 0.56 (5.29)	58.57 ± 0.51 (5.07)	0.719		55.94 ± 0.43 (4.54)	56.79 ± 0.45 (4.52)	0.087
Right hind stance duration (seconds)	0.387 ± 0.006 (7.54)	0.393 ± 0.005 (6.01)	0.364		0.371 ± 0.004 (6.55)	0.384 ± 0.005 (7.21)	**0.005**
Right hind duty factor (%)	56.22 ± 0.69 (6.33)				52.22 ± 0.70 (7.13)		
Timing of peak MTP ext. (% of stance)	59.61 ± 0.48 (4.16)	58.35 ± 0.56 (4.97)	**0.025**		59.17 ± 0.58 (5.65)	58.68 ± 0.71 (6.16)	0.284
Ipsilateral contact timing (seconds)	0.034 ± 0.006 (91.01)	0.04 ± 0.006 (76.53)	0.136		0.013 ± 0.004 (154.62)	0.019 ± 0.004 (125.88)	0.064

9.2 Stride to stride linear and angular changes

9.2.1 The effect of an abrupt change in surface condition

Linear Parameters

When the horses moved from the firm to soft surface condition, stride length in the hind limb significantly **F (1, 82) = 6.59, *P* =0.012** reduced (Table 9.2.1). The variation in the hind limb stride length between strides can also be seen in figure 9.2.1. When the horses moved from the soft to firm surface condition, stride length in both the fore **F (1, 55) = 6.04, *P* =0.017** and hind **F (1, 60) = 5.06, *P* =0.028** limbs significantly reduced (Table 9.2.1). The stride to stride variations between the two surface conditions can be seen in figures 9.2.2 and 9.2.3.

Table 9.2.1: Mean ± SE (coefficient of variation) linear parameters according to stride location. Values of P <0.05 denote a significant difference between the strides on the corresponding surface condition. There was no data for the second stride on the soft surface condition due to four hoof impacts required to calculate this.

	Firm → Soft				**Soft → Firm**		
Parameter	Firm	Soft (stride 1)	Soft (stride 2)	***P* - value**	Soft	Firm (stride 1)	***P* - value**
Right fore stride length (m)	2.57 ± 0.03 (7.21)	2.56 ± 0.03 (7.38)		0.950	2.53 ± 0.03 (7.54)	2.44 ± 0.05 (7.28)	**0.017 (Fig 9.2.12)**
Right hind stride length (m)	2.59 ± 0.03 (7.77)	2.52 ± 0.03 (7.17)		**0.012** (Fig 9.2.1)	2.50 ± 0.03 (8.09)	2.43 ± 0.05 (8.08)	**0.028 (Fig 9.2.13)**

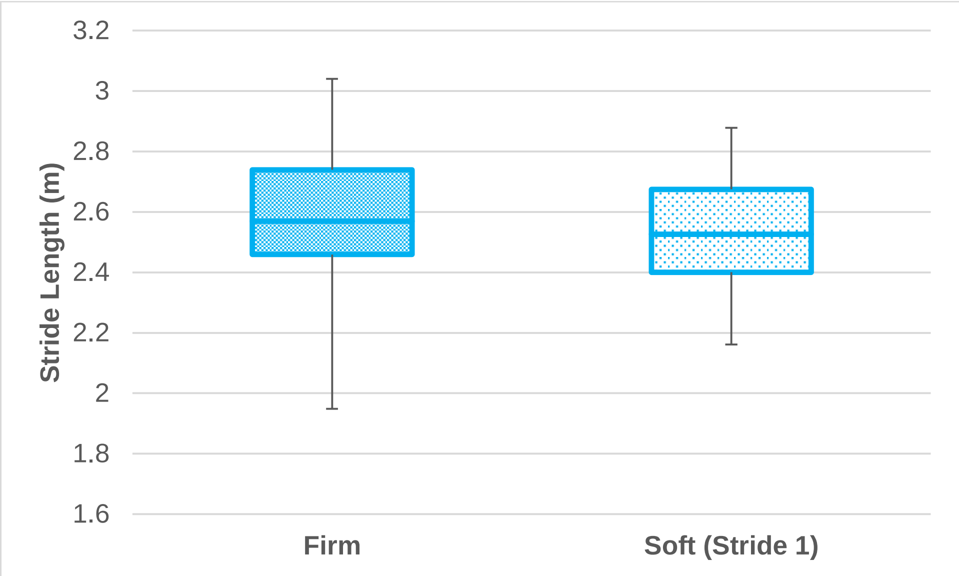


Figure 9.2.1: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in stride length within the right hindlimb.

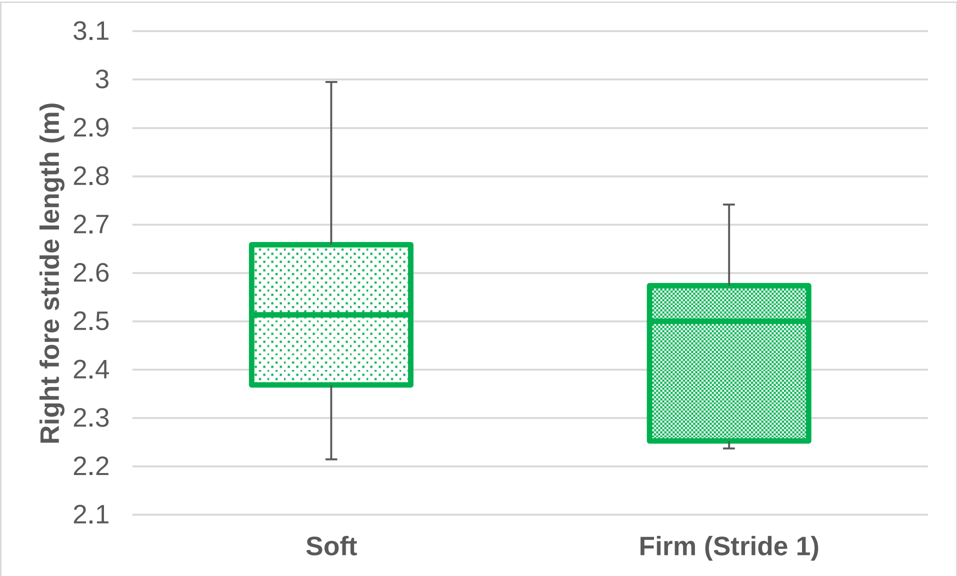


Figure 9.2.2: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in right fore stride length.

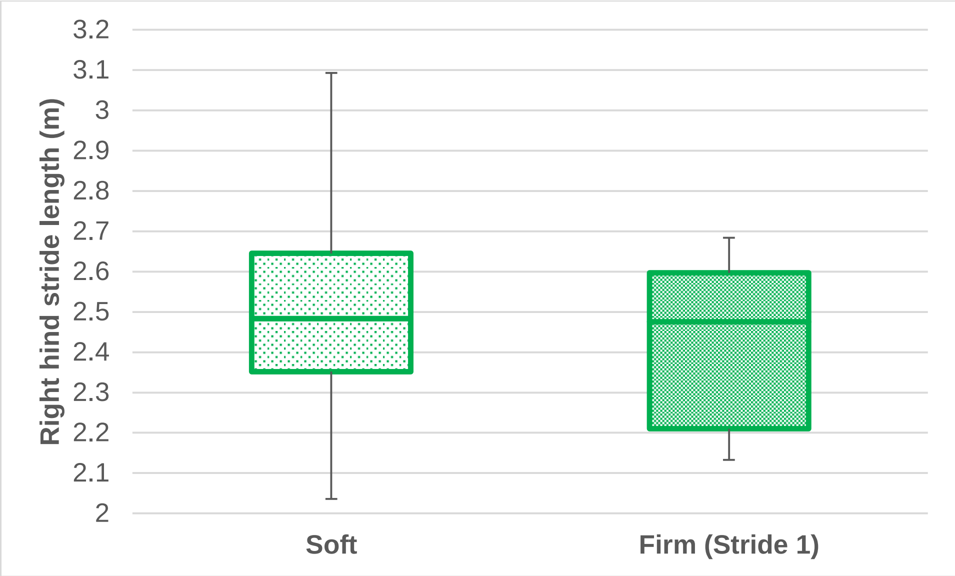


Figure 9.2.3: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in right hind stride length.

Angular Parameters

As illustrated in section 6.03, a greater MCP and MTP angle represents greater peak extension. A more positive fore and hind limb inclination at hoof impact represents more protraction whereas a more negative value represents more retraction at hoof lift off. A more negative value for neck inclination suggests the neck is inclined lower to the ground. A greater scapula inclination value suggests the scapula is positioned closer towards the horizontal and a more negative shoulder angle suggests the joint has more flexion.

When the horses moved from the firm to soft surface condition, stride location had a significant effect on fore and hind limb inclination at hoof lift off and also shoulder angle at hoof lift off (Table 9.2.2). All parameters reduced in absolute magnitude with each stride suggesting less limb retraction and less shoulder flexion. This change was significant **$F_{(2, 116)} = 12.47$, $P < 0.0001$** with each stride for hind limb retraction. The changes observed in forelimb retraction **$F_{(2, 125)} = 11.55$, $P < 0.0001$** and shoulder angle **$F_{(2, 117)} = 3.46$, $P = 0.035$** however, were only significant between the stride on the firm surface before the change and the second stride after the change on the soft surface. The stride to stride variations observed in the respective parameters can also be seen in figures 9.2.4, 5 and 6.

When the horses moved from the soft to firm surface condition, stride location had a significant effect on peak MCP extension **$F_{(1, 93)} = 7.85$, $P = 0.006$** and scapula inclination at hoof impact **$F_{(1, 88)} = 4.54$, $P = 0.036$**. Each parameter showed a decrease and increase by the first stride on the firm surface condition respectively (Table 9.2.2).

The stride to stride variations between the two surface conditions can also be seen in figures 9.2.7 and 9.2.8.

Table 9.2.2: Mean ± SE (coefficient of variation) angular parameters according to stride location. Values of P <0.05 denote a significant difference between the strides on the corresponding surface condition. There was only one data point for neck inclination at hoof lift off during the second stride on the soft surface so this was removed from the analysis. There was also no data for neck inclination at hoof impact and lift off when the horses moved from the soft to firm surface condition.

	Firm → Soft				**Soft → Firm**		
Parameter	Firm	Soft (stride 1)	Soft (stride 2)	***P* - value**	Soft	Firm (stride 1)	***P* - value**
Peak MCP ext. (°)	56.63 ± 0.53 (6.57)	57.02 ± 0.49 (6.41)	56.79 ± 0.69 (6.34)	0.397	56.24 ± 0.38 (4.89)	55.53 ± 0.38 (4.84)	**0.006 (Fig 9.2.14)**
Peak MTP ext. (°)	61.32 ± 0.59	61.54 ± 0.62	60.33 ± 0.92	0.253	59.26 ± 0.6 (7.28)	59.19 ± 0.52 (6.48)	0.488
Forelimb inclination at hoof impact (°)	31.54 ± 0.33 (7.42)	32.19 ± 0.3 (6.92)	31.66 ± 0.39 (7.25)	0.662	28.90 ± 0.34 (8.49)	28.51 ± 0.37 (9.43)	0.067
Forelimb inclination at hoof lift off (°)	-23.83 [a] ± 0.23 (-7.16)	-23.11 [a] ± 0.24 (--7.68)	-22.22 [b] ± 0.32 (-7.57)	**<0.0001** (Fig 9.2.2)	-26.0 ± 0.23 (-6.37)	-25.39 ± 0.31 (-8.78)	0.183
Neck inclination at hoof impact (°)	-79.93 ± 0.85 (-6.98)	-79.50 ± 0.73 (-6.77)	-80.27 ± 0.86 (-5.48)	0.899			
Neck inclination at hoof lift off (°)	-79.13 ± 0.70 (-6.45)	-79.55 ± 0.74 (-6.5)		0.861			
Scapula inclination at hoof impact (°)	36.57 ± 0.50 (9.43)	36.02 ± 0.42 (8.47)	36.38 ± 0.56 (9.03)	0.106	35.46 ± 0.54 (10.82)	36.63 ± 0.57 (10.66)	**0.036 (Fig 9.2.15)**
Scapula inclination at hoof lift off (°)	22.96 ± 0.50 (15.98)	22.62 ± 0.50 (16.18)	23.27 ± 0.93 (18.27)	0.551	22.64 ± 0.52 (16.62)	24.13 ± 0.71 (18.3)	0.673
Shoulder angle at hoof impact (°)	-82.99 ± 0.80 (-6.67)	-82.53 ± 0.74 (-6.51)	-82.84 ± 0.91 (-6.51)	0.543	-79.01 ± 0.55 (-4.93)	-79.88 ± 0.55 (-4.68)	0.122
Shoulder angle at hoof lift off (°)	-81.30 [a] ± 0.55 (-4.93)	-81.22 [ab] ± 0.54 (-4.91)	-80.19 [b] ± 1.01 (-5.78)	**0.035** (Fig 9.2.3)	-78.59 ± 0.52 (-4.91)	-79.2 ± 0.60 (-4.72)	0.452
Hindlimb inclination at hoof impact (°)	12.05 ± 0.58 (33.34)	12.65 ± 0.56 (32.03)	13.34 ± 0.60 (28.51)	0.428	9.39 ± 0.54 (41.93)	8.54 ± 0.55 (47.33)	0.056
Hindlimb inclination at hoof lift off (°)	-31.52 [a] ± 0.31 (-7.22)	-30.45 [b] ± 0.32 (-7.74)	-29.50 [c] ± 0.62 (-9.14)	**<0.0001** (Fig 9.2.4)	-33.88 ± 0.34 (-7.38)	-32.80 ± 0.29 (-6.24)	0.317

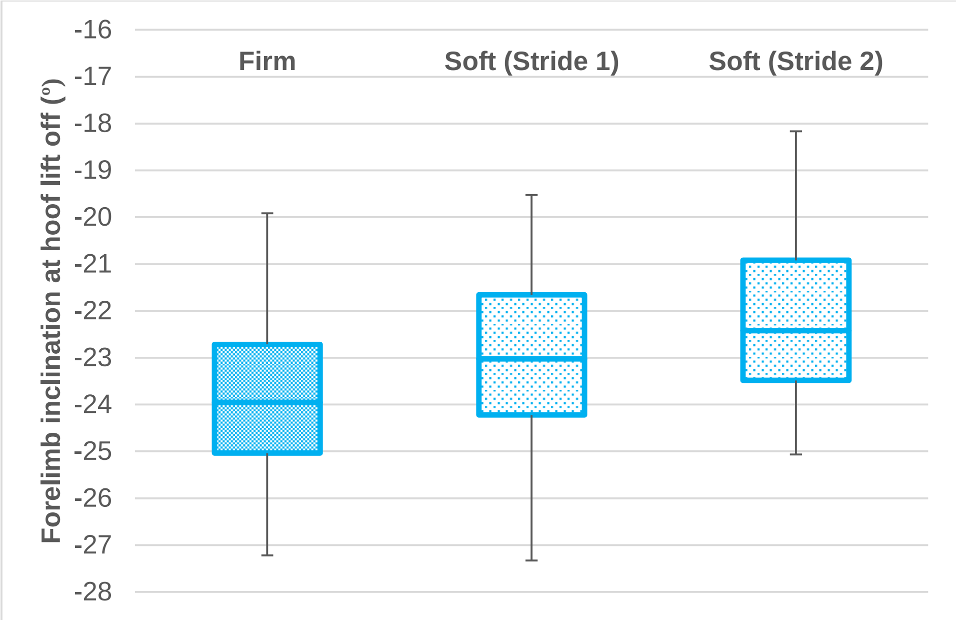


Figure 9.2.4: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in forelimb inclination at hoof lift off.

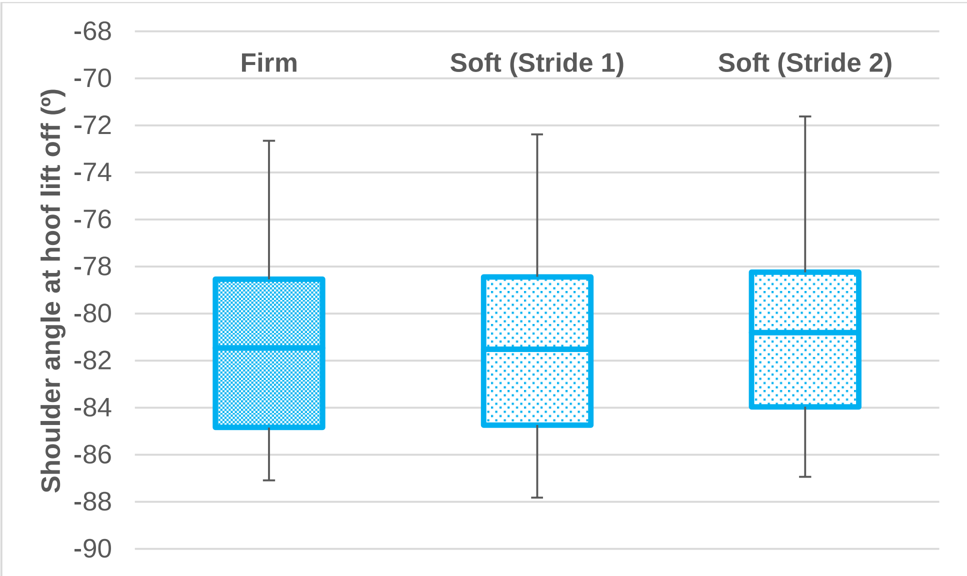


Figure 9.2.5: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in shoulder angle at hoof lift off.

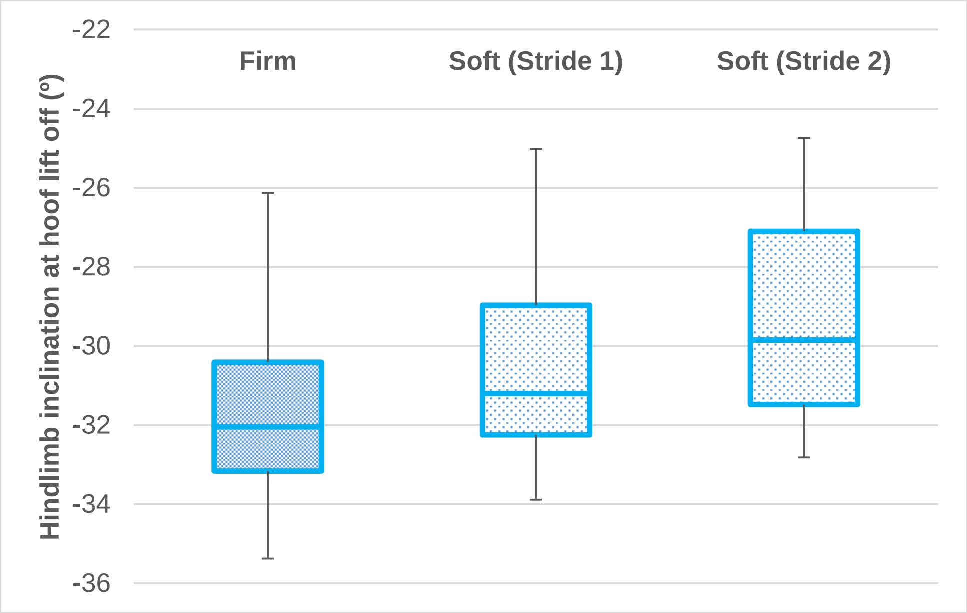


Figure 9.2.6: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in hindlimb inclination at hoof lift off.

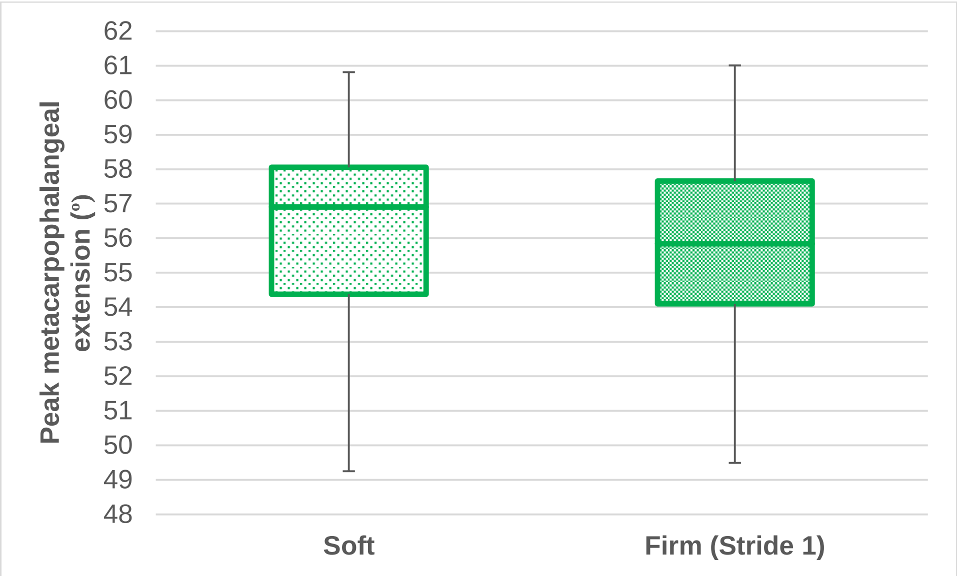


Figure 9.2.7: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in peak MCP extension.

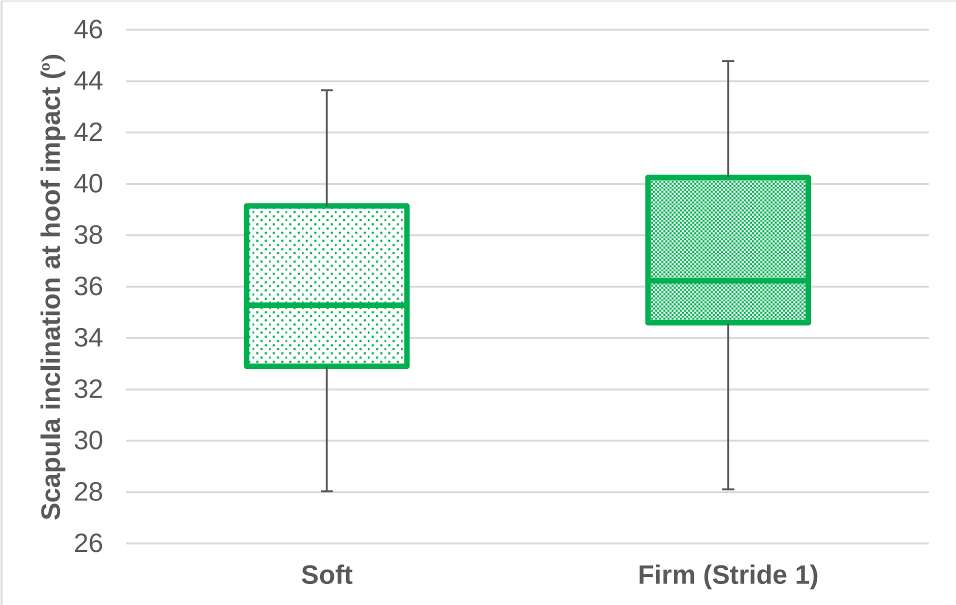


Figure 9.2.8: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in scapula inclination at hoof impact.

9.2.2 The effect of continuous surfaces

Linear parameters

The average stride length recorded on each surface condition is reported in table 9.2.3. It was not possible to report stride length for stride one and two due to data only being collected for one swing phase (between the two consecutive stance phases) during each trial.

Table 9.2.3: Mean ± SE (coefficient of variation) stride length on the continuous surface conditions. The data has been grouped from all horses.

	Continuous Firm Surface			**Continuous Soft Surface**		
Parameter	Stride 1	Stride 2	***P*- value**	Stride 1	Stride 2	***P*- value**
Right fore stride length (m)	2.44 ± 0.04 (8.7)			2.45 ± 0.03 (6.64)		
Right hind stride length (m)	2.46 ± 0.03 (6.4)			2.43 ± 0.04 (8.39)		

Angular parameters

No significant (P>0.05) angular stride to stride changes were recorded in the fore and hind limbs on either continuous surface condition (Table 9.2.4).

Table 9.2.4: Mean ± SE (coefficient of variation) angular parameters according to stride number on the continuous surface conditions. Values of P <0.05 denote significant differences between stride one and two on the corresponding surface condition. The data has been grouped from all horses.

	Continuous Firm Surface				Continuous Soft Surface		
Parameter	Stride 1	Stride 2	***P* - value**		Stride 1	Stride 2	***P* - value**
Peak MCP ext. (°)	57.52 ± 0.7 (6.97)	58.42 ± 0.74 (7.41)	0.113		55.65 ± 0.72 (7.64)	56.32 ± 0.72 (7.3)	0.113
Peak MTP ext. (°)	59.57 ± 1.35 (11.78)	60.70 ± 1.35 (11.51)	0.082		54.52 ± 0.99 (10.42)	54.36 ± 1.03 (9.8)	0.832
Forelimb inclination at hoof impact (°)	31.39 ± 0.35 (6.57)	31.37 ± 0.36 (6.75)	0.945		31.77 ± 0.37 (6.8)	31.69 ± 0.34 (6.14)	0.454
Forelimb inclination at hoof lift off (°)	-24.75 ± 0.36 (-8.3)	-24.45 ± 0.37 (-8.92)	0.764		-23.28 ± 0.31 (-7.80)	-22.88 ± 0.41 (-10.0)	0.091
Neck inclination at hoof impact (°)	-77.81 ± 1.10 (-6.46)	-78.01 ± 1.14 (-6.72)	0.756		-79.19 ± 0.85 (-6.26)	-80.26 ± 1.09 (6.54)	0.523
Neck inclination at hoof lift off (°)	-77.84 ± 1.23 (-7.71)	-79.33 ± 1.12 (-6.01)	0.3		-78.75 ± 1.01 (-7.37)	-80.16 ± 1.17 (-6.52)	0.374
Scapula inclination at hoof impact (°)	36.38 ± 0.62 (9.78)	36.12 ± 0.63 (10.04)	0.228		35.21 ± 0.34 (5.66)	34.63 ± 0.3 (4.8)	0.191
Scapula inclination at hoof lift off (°)	23.5 ± 0.64 (15.53)	23.05 ± 0.67 (16.28)	0.225		22.22 ± 0.39 (10.25)	22.1 ± 0.41 (10.42)	0.970
Shoulder angle at hoof impact (°)	-82.35 ± 1.06 (-7.38)	-81.67 ± 1.11 (-7.81)	0.756		-79.35 ± 0.55 (-4.12)	-79.02 ± 0.6 (-4.24)	0.386
Shoulder angle at hoof lift off (°)	-79.66 ± 1.01 (-7.25)	-79.83 ± 1.04 (-7.24)	0.930		-78.55 ± 0.6 (-4.55)	-78.47 ± 0.64 (-4.56)	0.886
Hindlimb inclination at hoof impact (°)	14.36 ± 0.92 (33.71)	14.11 ± 1.05 (39.36)	0.538		14.24 ± 0.61 (25.35)	14.09 ±0.72 (26.39)	0.509
Hindlimb inclination at hoof lift off (°)	-29.70 ± 0.75 (-13.11)	-29.88 ± 0.73 (-12.53)	0.777		-30.30 ± 0.49 (-9.53)	-29.91 ± 0.5 (-8.41)	0.103

9.3 Stride to stride changes in neuromuscular activity during pre-activation and stance

Prior to analysis, muscle activity was visually assessed for not only signal quality but also to establish periods of activation.

Splenius

The *splenius* was predominantly active during the first half of the stance phase and also around mid-swing. Activity was minimal during the pre-activation window (Figure 9.3.1).

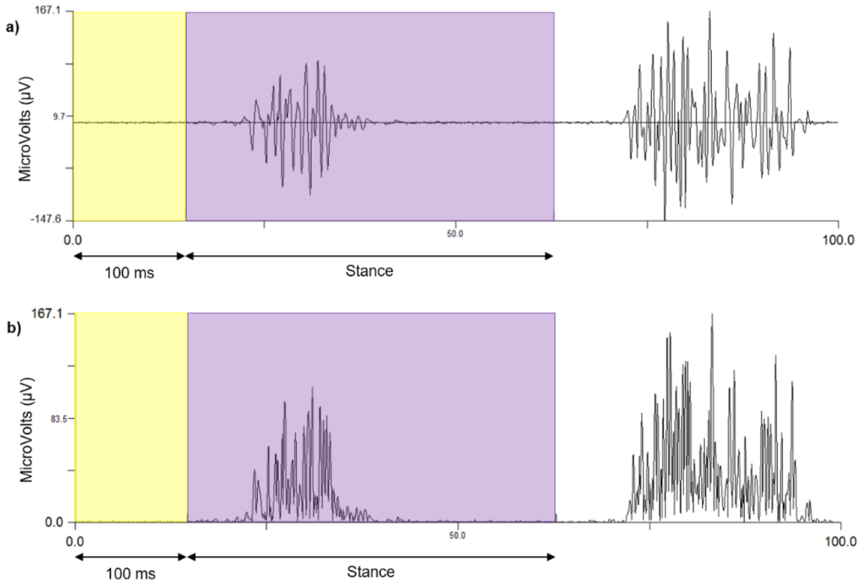


Figure 9.3.1: a) High pass filtered EMG signal (µV) from one stride (% stride) recorded from the splenius muscle. The '0%' represents the start of pre-activation and '100%' represents the start of pre-activation of the subsequent stride. The pre-activation window is highlighted in yellow and stance highlighted in purple;b) The rectified signal, which was used to calculate the integral during pre-activation (iEMG of this particular signal = 0.045) and stance (iEMG of this particular signal = 2.910).

Trapezius Cervicis

The *trapezius cervicis* was mainly active during the first half of stance and at times was active prior to hoof impact during the pre-activation window, although this was at a relatively low magnitude. A second burst of activity was also seen in some trials during late stance at the cranial site (Figure 9.3.2 a and b). The caudal site demonstrated a more continuous burst of activity throughout stance but this was at a much lower magnitude (Figure 9.3.2 c and d). The muscle was also active around mid-swing until just before the start of the pre-activation window.

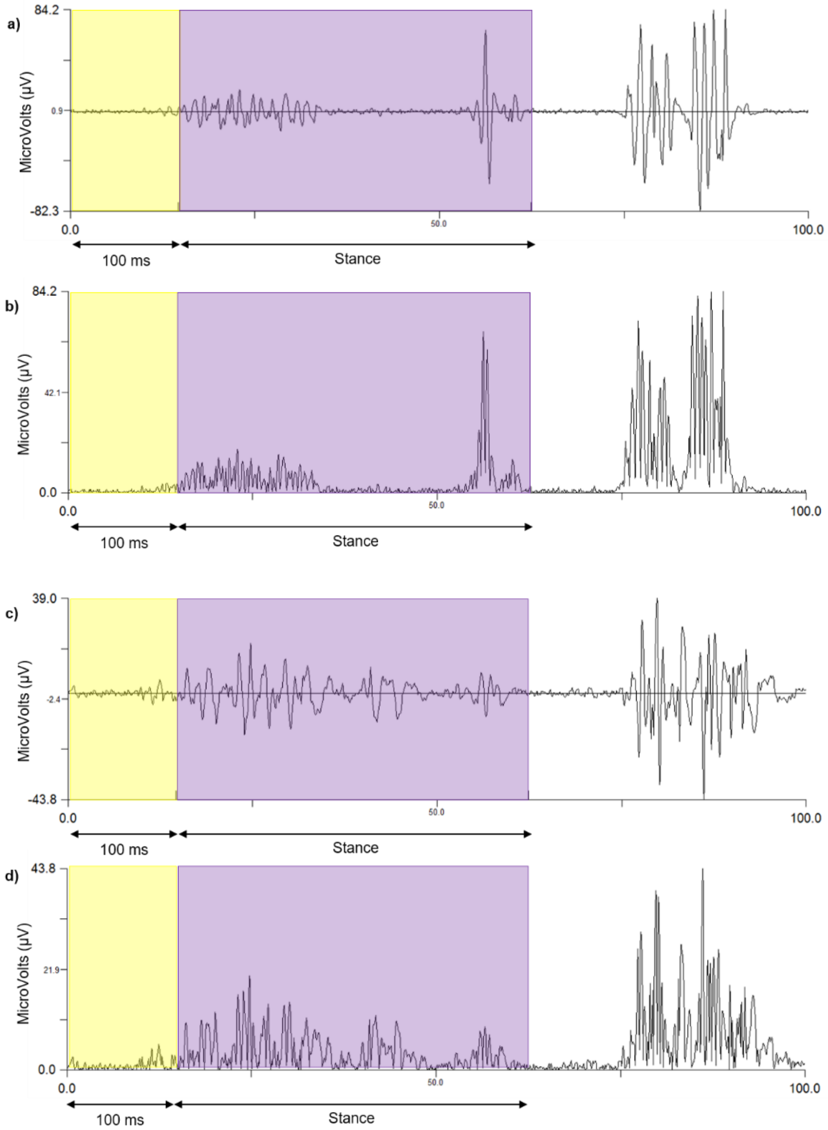


Figure 9.3.2 a) High pass filtered EMG signal (µV) from one stride (% stride) recorded from the cranial site of the trapezius muscle. The '0%' represents the start of pre-activation and '100%' represents the start of pre-activation of the subsequent stride. The pre-activation window is highlighted in yellow and stance highlighted in purple. b) The rectified signal from figure 9.3.2 a, which was used to calculate the integral during pre-activation (iEMG = 0.087) and stance (iEMG = 1.537). c) High pass filtered EMG signal (µV) from one stride (% stride) recorded from the caudal site of the trapezius muscle. d) The rectified signal from figure 9.3.2 c, which was used to calculate the integral during pre-activation (iEMG = 0.103) and stance (iEMG = 1.241).

Long head of the Triceps

The *long head of the triceps* was generally active from the start of pre-activation through to mid-stance at both the cranial (Figure 9.3.3 a and b) and caudal sites (Figure 9.3.3 c and d), although not all horses used for this study demonstrated evidence of pre-activation upon visual assessment of the signal. Very little activity was recorded during swing.

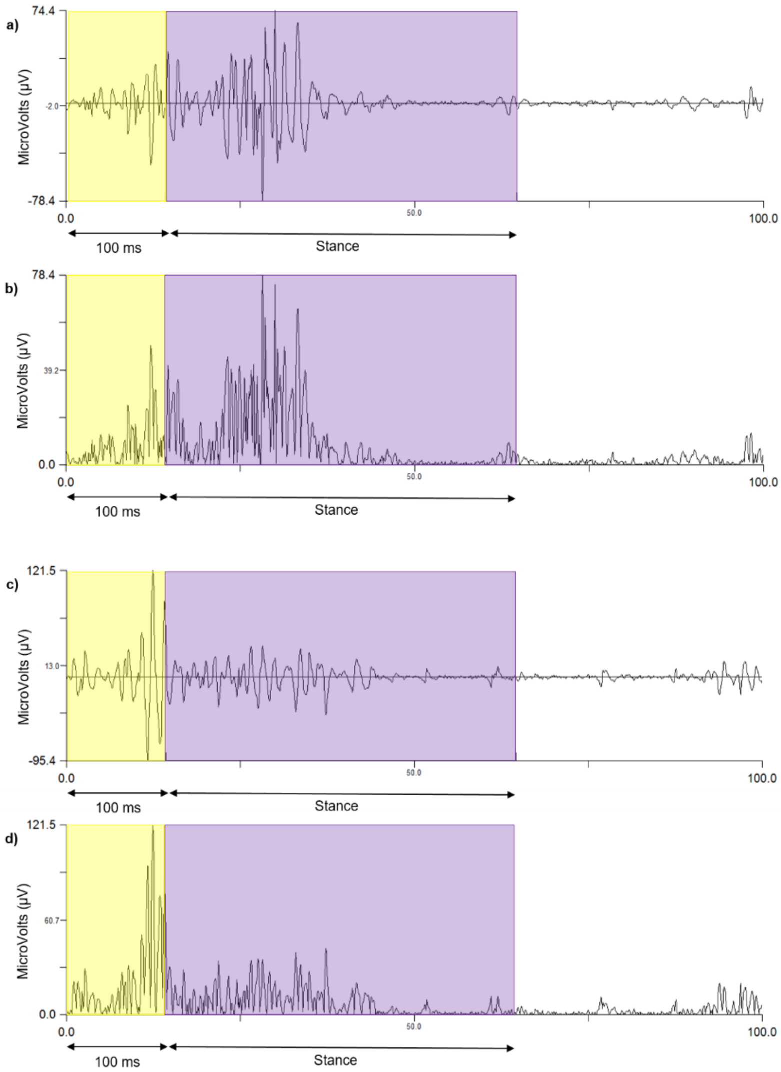


Figure 9.3.3 a) High pass filtered EMG signal (µV) from one stride (% stride) recorded from the cranial site of the triceps muscle. The '0%' represents the start of pre-activation and '100%' represents the start of pre-activation of the subsequent stride. The pre-activation window is highlighted in yellow and stance highlighted in purple. b) The rectified signal from figure 9.3.3 a, which was used to calculate the integral during pre-activation (iEMG = 0.805) and stance (iEMG = 3.463). c) High pass filtered EMG signal (µV) from one stride (% stride) recorded from the caudal site of the triceps muscle. d) The rectified signal from figure 9.3.3 c, which was used to calculate the integral during pre-activation (iEMG = 2.053) and stance (iEMG = 2.711).

Extensor Carpi Radialis

The *extensor carpi radialis* was mainly active during stance with a larger burst of activity observed around hoof impact and late stance during some trials. At times, the burst of activity around hoof impact encroached into the end of the pre-activation window (Figure 9.3.4 a and b).

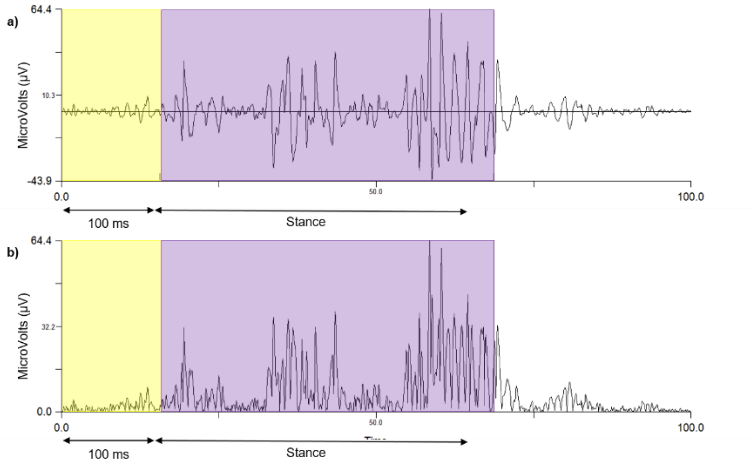


Figure 9.3.4 a) High pass filtered EMG signal (µV) from one stride (% stride) recorded from the extensor carpi radialis muscle. The '0%' represents the start of pre-activation and '100%' represents the start of pre-activation of the subsequent stride. The pre-activation window is highlighted in yellow and stance highlighted in purple. b) The rectified signal, which was used to calculate the integral during pre-activation (iEMG = 0.190) and stance (iEMG = 3.455).

Ulnaris Lateralis

The *ulnaris lateralis* was generally active from the start of the pre-activation window through to mid-stance and in some horses this was until the end of stance (Figure 9.3.5 a and b). Little activity was observed from hoof lift off through till the start of the pre-activation window.

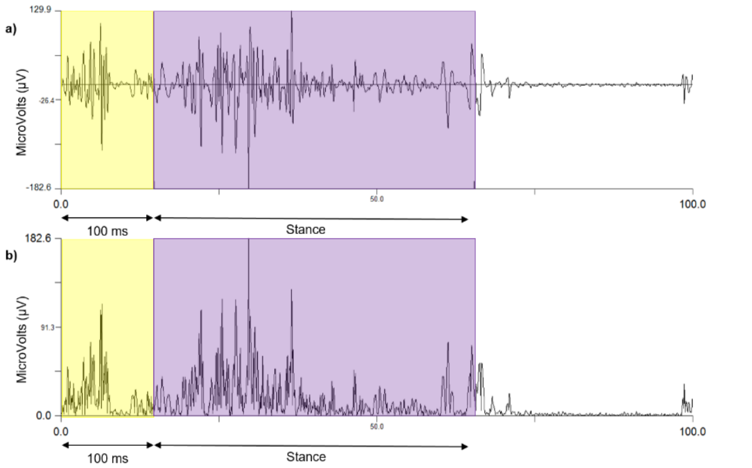


Figure 9.3.5 a) High pass filtered EMG signal (µV) from one stride (% stride) recorded from the ulnaris lateralis muscle. The '0%' represents the start of pre-activation and '100%' represents the start of pre-activation of the subsequent stride. The pre-activation window is highlighted in yellow and stance highlighted in purple. b) The rectified signal, which was used to calculate the integral during pre-activation (iEMG = 0.172) and stance (iEMG = 2.352).

9.3.1 The effect of an abrupt change in surface condition

The normalised integrated EMG according to stride location is presented in table 9.3.1. When the horses moved from the firm to soft surface condition, stride to stride changes were observed in the *splenius* muscle during pre-activation and also within the *long head of the triceps* muscle (cranial site) during both pre-activation and stance. The contribution of the *splenius* during pre-activation and the *long head of the triceps* during stance reduced with each stride whereas the *long head of the triceps* demonstrated a gradual increase during pre-activation.

Although a change was observed with each stride, this was only significant between stride one and two on the soft surface condition for the *splenius* **F (2, 72) = 4.9, *P* =0.01** and *long head of the triceps* **F (2, 110) = 4.0, *P* =0.021** during stance. The significant change **F (2, 114) = 3.52, *P* =0.033** in the contribution of the *triceps* during pre-activation was between the stride on the firm surface and the second stride on the soft surface. The stride to stride variations between the two surface conditions can also be seen in figures 9.3.6, 7 and 8. When the horses moved from the soft to firm surface condition, no significant (P > 0.05) changes in the contribution of the muscle during pre-activation or stance were observed with each stride.

*Table 9.3.1: Mean ± SE (coefficient of variation) normalised integrated EMG (iEMG) values (%) during pre-activation (100 ms preceding stance) and stance according to stride location on the different surface conditions. A * represents data that was transformed due to the residual values generated from the GLM not being normally distributed. Different superscripts denote significant P <0.05 differences between the strides on the corresponding surface condition.*

	Firm → Soft				**Soft → Firm**			
Parameter	Firm	Soft (stride 1)	Soft (stride 2)	***P* - value**	Soft	Firm (stride 1)	Firm (stride 2)	***P* - value**
Splenius								
Pre-activation	65.95 ^{ab} ± 2.62 (21.78)	65.42 ^{a} ± 2.89 (24.98)	61.62 ^{b} ± 3.83 (27.78)	**0.010 (Fig 9.2.5)**	66.68 ± 4.8 (40.09)	65.99 ± 4.63 (39.06)	62.7 ± 13.4 (52.42)	0.580
Stance	70.03 ± 3.82 (29.86)	61.76 ± 2.91 (27.08)	57.49 ± 5.09 (35.42)	0.427	65.13 ± 4.07 (36.48)	60.60 ± 4.10 (35.82)		0.626 *
Trapezius Cervicis								
Cranial site pre-activation	49.47 ± 4.9 (52.37)	48.94 ± 3.99 (42.36)	50.17 ± 5.77 (51.4)	0.075*	56.47 ± 3.76 (36.46)	62.14 ± 3.97 (36.66)	48.47 ± 7.56 (41.28)	0.389 *
Cranial site stance	68.03 ± 3.07 (23.89)	61.93 ± 3.90 (32.72)	60.86 ± 4.45 (29.28)	0.752	72.62 ± 4.24 (28.59)	68.78 ± 3.66 (23.81)		0.297
Caudal site pre-activation	51.10 ± 4.89 (40.56)	57.74 ± 5.04 (38.02)	54.77 ± 7.35 (51.99)	0.803	54.94 ± 5.58 (49.72)	52.73 ± 5.25 (45.59)	51.1 ± 14.1 (61.46)	0.954
Caudal site stance	75.61 ± 3.25 (18.72)	66.21 ± 4.97 (33.57)	69.21 ± 5.49 (28.61)	0.183	75.01 ± 4.81 (23.97)	76.80 ± 5.40 (26.33)		0.549
Long head of the Triceps								
Cranial site pre-activation	70.50 ^{b} ± 2.5 (24.33)	74.13 ^{ab} ± 2.19 (20.66)	77.19 ^{a} ± 2.65 (18.8)	**0.033 (Fig 9.2.6)**	66.61 ± 3.08 (31.32)	68.53 ± 2.76 (28.24)	62.9 ± 4.18 (21.02)	0.534
Cranial site stance	76.41 ^{a} ± 2.19 (20.07)	67.84 ^{a} ± 2.33 (24.24)	63.05 ^{b} ± 3.24 (24.64)	**0.015* (Fig 9.2.7)**	74.06 ± 2.87 (25.43)	71.54 ± 2.31 (21.44)		0.380
Caudal site pre-activation	69.46 ± 2.3 (22.97)	72.09 ± 2.01 (19.48)	72.49 ± 2.73 (20.98)	0.254	68.15 ± 3.13 (29.38)	70.83 ± 3.16 (28.94)	65.52 ± 3.79 (18.27)	0.352
Caudal site stance	72.01 ± 2.47 (23.24)	66.71 ± 2.55 (25.94)	60.31 ± 4.12 (33.46)	0.497	71.50 ± 3.14 (28.15)	68.49 ± 2.76 (25.12)		0.911 *
Extensor Carpi Radialis								
Pre-activation	55.78 ± 5.28 (48.28)	52.09 ± 5.34 (50.21)	51.39 ± 5.81 (46.63)	0.912	63.25 ± 5.14 (38.95)	56.27 ± 5.97 (48.59)	53.1 ± 11.8 (54.67)	0.743
Stance	69.33 ± 4.20 (28.41)	69.81 ± 4.03 (27.65)	65.79 ± 6.90 (37.82)	0.750*	77.28 ± 3.05 (21.22)	75.94 ± 3.05 (21.26)		0.855
Ulnaris Lateralis								
Pre-activation	75.05 ± 3.85 (21.77)	72.34 ± 4.08 (25.82)	68.97 ± 4.54 (24.61)	0.486 *	74.07 ± 3.03 (21.22)	73.72 ± 3.30 (23.69)	72.74 ± 7.45 (25.09)	0.606 *
Stance	81.89 ± 2.76 (17.21)	77.99 ± 3.26 (22.49)	67.72 ± 4.05 (22.35)	0.053	79.70 ± 2.67 (18.65)	76.63 ± 3.33 (21.3)		0.710

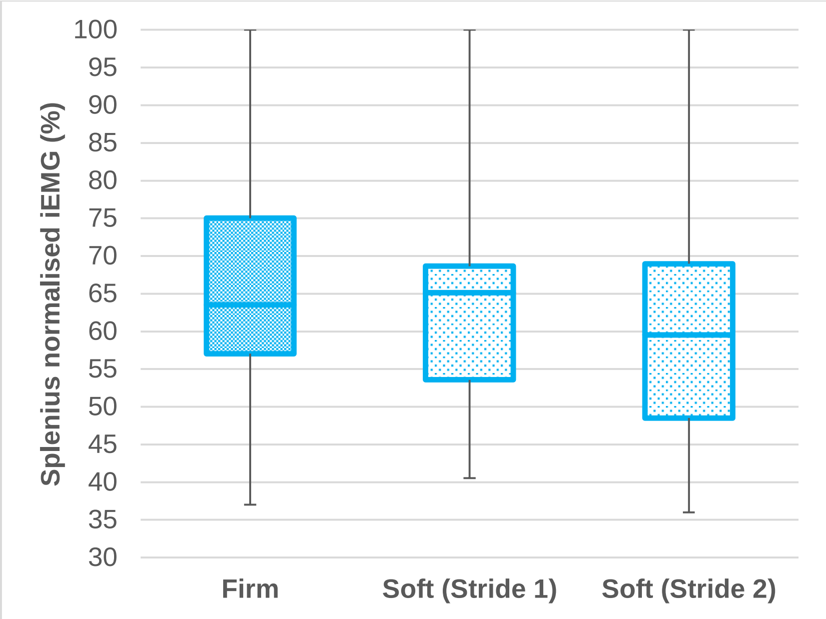


Figure 9.3.6: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in the normalised iEMG values for the splenius during pre-activation.

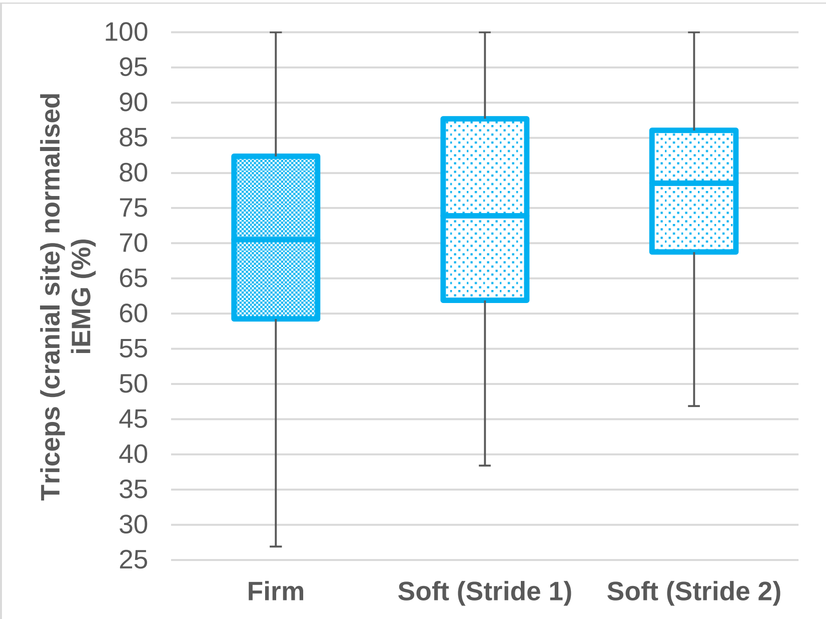


Figure 9.3.7: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in the normalised iEMG values for the triceps cranial site during pre-activation.

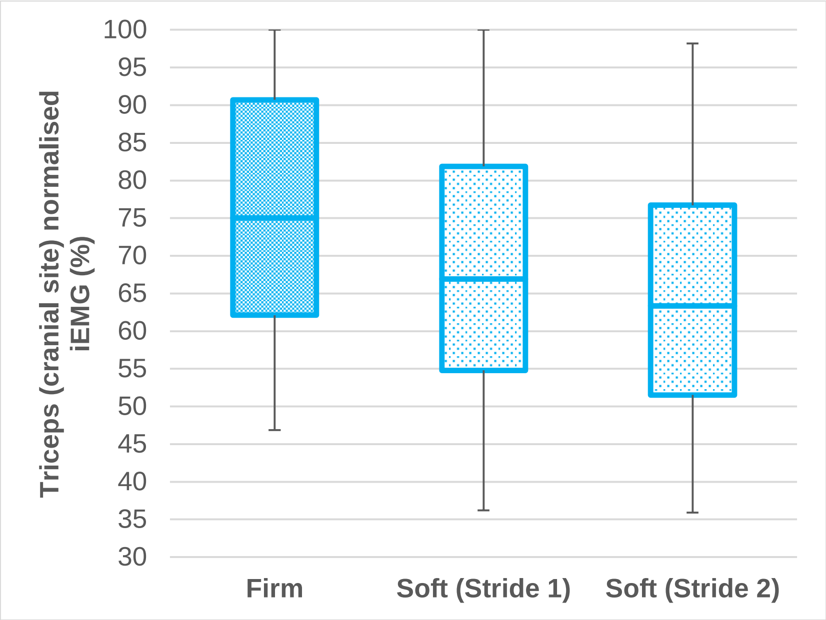


Figure 9.3.8: Minimum, median, interquartile range and maximum values demonstrating the stride to stride variations in the normalised iEMG values for the triceps cranial site during stance.

<u>9.3.2 The effect of continuous surfaces</u>

There were no significant (*P* >0.05) stride to stride changes in the contribution of any of the muscles during pre-activation or stance on the continuous firm surface condition (Table 9.3.2). Some changes were recorded on the continuous soft surface however. The contribution of the *trapezius cervicis* (caudal site) **F (1, 40) = 5.6, *P* =0.023** during stance and the *long head of the triceps* **F (1, 50) = 6.11, *P* =0.017** during pre-activation significantly reduced during the second stride. The contribution of the *ulnaris lateralis* during stance also significantly **F (1, 11) = 7.09, *P* =0.022** increased during the second stride.

Table 9.3.2: Mean ± SE (coefficient of variation) normalised integrated EMG (iEMG) values (%) during pre-activation (100 ms preceding stance) and stance according to stride number on the continuous surface conditions. Values of P <0.05 denote significant differences between stride one and two on the corresponding surface condition. The data has been grouped from all horses.

	Continuous Firm Surface			Continuous Soft Surface		
Parameter	Stride 1	Stride 2	***P* - value**	Stride 1	Stride 2	***P* - value**
Splenius						
Pre-activation	70.63 ± 6.64 (35.17)	77.79 ± 5.76 (31.43)	0.233	80.20 ± 2.76 (17.86)	84.93 ± 2.75 (15.85)	0.151
Stance	75.93 ± 3.78 (21.14)	72.28 ± 4.59 (26.18)	0.578	76.27 ± 3.03 (21.75)	70.68 ± 4.04 (29.14)	0.206
Trapezius Cervicis						
Cranial site pre-activation	66.02 ± 6.38 (32.06)	73.08 ± 7.47 (32.34)	0.504	63.15 ± 4.41 (38.24)	65.17 ± 4.36 (35.41)	0.860
Cranial site stance	80.56 ± 4.46 (16.61)	81.84 ± 4.28 (18.13)	0.889	82.21 ± 2.88 (19.2)	75.94 ± 3.17 (20.86)	0.054
Caudal site pre-activation	68.15 ± 5.36 (38.53)	64.49 ± 4.52 (32.85)	0.607	72.90 ± 3.38 (23.17)	74.47 ± 3.65 (23.98)	0.832
Caudal site stance	81.37 ± 3.95 (18.14)	81.33 ± 4.67 (21.48)	0.832	82.72 ± 2.77 (16.73)	74.42 ± 2.9 (18.27)	**0.023**
Long head of the Triceps						
Cranial site pre-activation	73.46 ± 4.08 (28.34)	75.67 ± 3.88 (25.65)	0.597	76.06 ± 3.26 (23.09)	71.33 ± 3.35 (24.37)	0.290
Cranial site stance	75.29 ± 3.15 (21.35)	82.95 ± 2.90 (17.5)	0.063	77.07 ± 3.61 (23.43)	70.34 ± 3.32 (23.12)	0.182
Caudal site pre-activation	73.71 ± 4.58 (32.28)	66.51 ± 4.58 (34.45)	0.331	79.97 ± 2.86 (19.23)	70.22 ± 3.04 (22.89)	**0.017**
Caudal site stance	74.99 ± 3.35 (18.39)	82.08 ± 3.77 (21.06)	0.248	75.94 ± 3.06 (20.54)	77.01 ± 3.11 (20.96)	0.748
Extensor Carpi Radialis						
Pre-activation	72.26 ± 5.43 (30.06)	68.33 ± 5.5 (32.19)	0.832	77.71 ± 4.45 (25.63)	72.73 ± 5.39 (32.29)	0.585
Stance	71.62 ± 4.92 (26.59)	80.66 ± 5.24 (25.16)	0.150	71.97 ± 4.66 (28.96)	75.27 ± 4.29 (25.51)	0.448
Ulnaris Lateralis						
Pre-activation	76.66 ± 5.84 (28.52)	79.64 ± 4.34 (20.41)	0.824	73.14 ± 6.62 (32.63)	73.67 ± 6.54 (32.02)	0.964
Stance	85.99 ± 3.8 (14.65)	93.91 ± 2.77 (8.35)	0.211	78.51 ± 4.39 (17.67)	93.17 ± 3.42 (10.37)	**0.022**

9.4 The effect of awareness of an abrupt change on kinematics and neuromuscular activity

To determine whether horses adopt a more cautious gait when they are aware of an abrupt change in surface condition, the kinematic and sEMG data has been presented according to ‘not aware’ (trial 1) vs. ‘aware’ (trial 2-8) with *P*-values presented in relation to the corresponding surface condition. The *P*-value for the interaction between awareness and stride location has also been presented.

9.4.1 Kinematics

Temporal parameters

When the horses were aware of the change from the firm to soft surface condition, there was a significant increase in the fore and hind stance durations and duty factor (Table 9.4.1). Stance duration and duty factor in the hind limb also showed a significant increase **$F_{(1, 99)} = 14.92$, $P < 0.0001$** and **$F_{(1, 61)} = 8.18$, $P = 0.006$** when the horses were aware of the change from the soft to firm surface condition. The ipsilateral contact timing significantly increased with awareness on both surface conditions, which would suggest that the right hind was contacting the ground earlier prior to right fore hoof lift off in comparison to when the horses were not aware of the change. There were no significant ($P > 0.05$) interactions between stride location and awareness on either day for any of the temporal parameters (table 9.4.1).

Table 9.4.1: Mean ± SE (coefficient of variation) temporal parameters according to awareness. The P-value for the interaction (Int.) between stride location and awareness has also been reported. Values of P <0.05 denote a significant difference between 'not aware' and 'aware' on the corresponding surface condition.

	Firm → Soft			**Int.**	**Soft → Firm**			**Int.**
Parameter	Not aware	Aware	***P*- value**	***P*- value**	Not aware	Aware	***P*- value**	***P*- value**
Right fore stance duration (seconds)	0.338 ± 0.004 (5.11)	0.350 ± 0.002 (5.66)	**<0.0001**	0.248	0.340 ± 0.007 (7.45)	0.348 ± 0.003 (7.07)	0.146	0.658
Right fore duty factor (%)	49.24 ±0.57 (3.99)	49.96 ± 0.25 (4.4)	**0.039**	0.706	49.56 ± 1.04 (6.67)	49.81 ± 0.34 (5.07)	0.651	0.411
Timing of peak MCP ext. (% of stance)	57.50 ± 0.45 (3.44)	57.08 ± 0.24 (4.5)	0.413	0.395	57.57 ± 1.30 (8.46)	56.96 ± 0.41 (6.72)	0.399	0.204
Right hind stance duration (seconds)	0.376 ± 0.008 (9.02)	0.401 ± 0.003 (8.73)	**<0.0001**	0.258	0.365 ± 0.006 (6.15)	0.385 ± 0.003 (7.34)	**<0.0001**	0.611
Right hind duty factor (%)	54.24 ± 1.51 (10.04)	57.09 ± 0.53 (8.25)	**<0.0001**	0.959	53.43 ± 1.71 (10.15)	54.89 ± 0.61 (8.73)	**0.006**	0.156
Timing of peak MTP ext. (% of stance)	59.25 ± 0.91 (6.67)	58.08 ± 0.61 (11.01)	0.308	0.848	58.56 ± 0.92 (5.93)	59.13 ± 0.41 (6.66)	0.375	0.623
Ipsilateral contact timing (seconds)	0.025 ± 0.008 (137.41)	0.052 ± 0.015 (303.59)	**<0.0001**	0.631	0.012 ± 0.01 (308.15)	0.026 ± 0.003 (118.7)	**0.002**	0.794

Linear Parameters

When the horses were aware of the change (trial 2-8) from the firm to soft surface condition, there was a significant reduction in stride length in both the fore **F (1, 79) = 15.64, *P* <0.0001** and hind limbs **F (1, 82) = 15.27, *P* <0.0001** (Table 9.4.2). Stride length in the fore limb also significantly reduced **F (1, 55) = 4.94, *P* =0.03** when the horses were aware of the change from the soft to firm surface condition. There were no significant (*P* >0.05) interactions between stride location and awareness on either day for stride length (Table 9.4.2).

Table 9.4.2: Mean ± SE (coefficient of variation) linear parameters according to awareness. The P-value for the interaction (Int.) between stride location and awareness has also been reported. Values of P <0.05 denote a significant difference between 'not aware' and 'aware' on the corresponding surface condition.

	Firm → Soft			**Int.**	**Soft → Firm**			**Int.**
Parameter	Not aware	Aware	***P* - value**	***P* - value**	Not aware	Aware	***P* - value**	***P* - value**
Right fore stride length (m)	2.65 ± 0.05 (6.69)	2.55 ± 0.02 (7.23)	**<0.0001**	0.631	2.61 ± 0.08 (9.77)	2.50 ± 0.02 (6.94)	**0.03**	0.184
Right hind stride length (m)	2.64 ± 0.58 (7.88)	2.55 ± 0.02 (7.45)	**<0.0001**	0.397	2.54 ± 0.08 (9.84)	2.47 ± 0.02 (7.86)	0.203	0.417

Angular Parameters

When the horses were aware of the change from the firm to soft surface condition, awareness did not have such a large effect on the angular parameters as with the temporal and linear parameters. Fore **$F(1, 125) = 7.28$, $P = 0.008$** and hind limb **$F(1, 116) = 10.16$, $P = 0.002$** retraction at hoof lift off were the only two parameters sensitive to change and significantly reduced with awareness (Table 9.4.3). When the horses were aware of the change from the soft to firm surface condition, peak MCP extension significantly **$F(1, 93) = 3.98$, $P = 0.049$** increased, scapula inclination significantly **$F(1, 88) = 10.02$, $P = 0.002$** reduced and hind limb inclination at hoof impact significantly **$F(1, 98) = 8.99$, $P = 0.003$** reduced. There were no significant ($P > 0.05$) interactions between stride location and awareness on either day for any of the angular parameters (Table 9.4.3).

Table 9.4.3: Mean ± SE (coefficient of variation) angular parameters according to awareness. The P-value for the interaction (Int.) between stride location and awareness has also been reported. Values of P <0.05 denote a significant difference between 'not aware' and 'aware' on the corresponding surface condition. There was no data for neck inclination at hoof impact and lift off when the horses travelled from soft to firm.

	Firm → Soft			**Int.**	**Soft → Firm**			**Int.**
Parameter	Not aware	Aware	***P*-value**	***P*-value**	Not aware	Aware	***P*-value**	***P*-value**
Peak MCP ext. (°)	57.06 ± 0.85 (6.48)	56.79 ± 0.34 (6.43)	0.138	0.133	55.33 [b] ± 0.73 (4.94)	55.98 [a] ± 0.29 (4.88)	**0.049**	0.269
Peak MTP ext. (°)	61.16 ± 1.12 (7.95)	61.22 ± 0.41 (7.05)	0.941	0.456	59.14 ± 1.08 (6.86)	59.24 ± 0.43 (6.89)	0.483	0.724
Forelimb inclination at hoof impact (°)	31.88 ± 0.57 (7.39)	31.82 ± 0.21 (7.19)	0.9	0.517	28.61 ± 0.68 (8.51)	28.72 ± 0.27 (9.06)	0.396	0.521
Forelimb inclination at hoof lift off (°)	-23.67 ± 0.44 (-8.01)	-23.15 ± 0.17 (-7.79)	**0.008**	0.091	-26.12 ± 0.49 (-6.96)	-25.64 ± 0.21 (-7.79)	0.197	0.879
Neck inclination at hoof impact (°)	-78.88 ± 1.31 (-6.43)	-79.94 ± 0.51 (-6.58)	0.221	0.759				
Neck inclination at hoof lift off (°)	-78.42 ± 1.51 (-6.67)	-79.46 ± 0.53 (-6.4)	0.651					
Scapula inclination at hoof impact (°)	36.78 ± 0.86 (9.6)	36.24 ± 0.29 (8.85)	0.073	0.430	37.59 ± 1.01 (9.34)	35.80 ± 0.42 (10.93)	**0.002**	0.326
Scapula inclination at hoof lift off (°)	22.84 ± 0.84 (15.69)	22.87 ± 0.40 (16.54)	0.782	0.757	24.14 ± 1.36 (19.49)	23.14 ± 0.45 (17.35)	0.08	0.599
Shoulder angle at hoof impact (°)	-83.07 ± 1.39 (-6.91)	-82.73 ± 0.49 (-6.5)	0.968	0.451	-79.2 ± 1.28 (-5.61)	-79.46 ± 0.41 (-4.73)	0.181	0.829
Shoulder angle at hoof lift off (°)	-80.72 ± 1.09 (-5.74)	-81.15 ± 0.38 (-4.95)	0.236	0.716	-78.83 ± 1.30 (-5.71)	-78.86 ± 0.42 (-4.71)	0.551	0.998
Hindlimb inclination at hoof impact (°)	13.52 ± 0.76 (23.15)	12.52 ± 0.37 (32.53)	0.184	0.647	10.10 ± 1.13 (41.84)	8.79 ± 0.41 (45.01)	**0.003**	0.975
Hindlimb inclination at hoof lift off (°)	-31.26 ± 0.69 (-9.6)	-30.67 ± 0.23 (-7.71)	**0.002**	0.345	-33.92 ± 0.56 (-5.9)	-33.29 ± 0.25 (-7.19)	0.134	0.289

9.4.2 Neuromuscular activity

The normalised integrated EMG according to awareness of the abrupt changes is presented in table 9.4.4. When the horses were aware of the change from the firm to soft surface condition, the contribution of the *trapezius cervicis* (cranial site) **$F_{(1, 61)} = 6.69$, $P = 0.012$** and *extensor carpi radialis* **$F_{(1, 47)} = 11.13$, $P = 0.002$** significantly increased during stance. The *long head of the triceps* (caudal site) however, showed a significant **$F_{(1, 116)} = 6.66$, $P = 0.011$** reduction during pre-activation. When the horses were aware of the change from the soft to firm surface condition, the contribution of the *splenius* **$F_{(1, 52)} = 4.2$, $P = 0.045$**, *trapezius cervicis* (caudal site) **$F_{(1, 22)} = 20.81$, $P < 0.0001$** and *ulnaris lateralis* **$F_{(1, 45)} = 27.3$, $P < 0.0001$** significantly increased during stance (Table 9.4.4).

*Table 9.4.4: Mean ± SE (coefficient of variation) normalised integrated EMG (iEMG) values (%) during pre-activation (100 ms preceding stance) and stance according to awareness of the abrupt change. The P-value for the interaction (Int.) between stride location and awareness has also been reported. Values of P <0.05 denote a significant difference between 'not aware' and 'aware' on the corresponding surface condition. A * represents data that was transformed due to the residual values generated from the GLM not being normally distributed.*

	Firm → Soft			**Int.**	**Soft → Firm**			**Int.**
Parameter	Not aware	Aware	***P*-value**	***P*-value**	Not aware	Aware	***P*-value**	***P*-value**
Splenius								
Pre-activation	73.34 ± 6.33 (27.28)	63.48 ±1.75 (23.37)	0.066	**0.003** (Fig 9.2.8)	59.21 ± 8.14 (49.55)	67.62 ± 3.47 (38.07)	0.606	0.463
Stance	72.05 ± 4.76 (20.91)	62.87 ± 2.40 (31.7)	0.738	0.331	54.85 ± 5.34 (32.3)	64.86 ± 3.29 (36.23)	**0.045 ***	0.678 *
Trapezius Cervicis								
Cranial site pre-activation	40.03 ± 6.33 (50.03)	50.91 ± 2.99 (47.42)	0.185*	**0.035*** (Fig 9.2.9)	44.87 ± 4.82 (37.23)	61.13 ± 2.85 (35.52)	0.107 *	0.602 *
Cranial site stance	52.52 ± 3.86 (23.21)	65.99 ± 2.36 (27.95)	**0.012**	0.669	71.41 ± 6.35 (28.14)	70.71 ± 3.21 (26.44)	0.869	0.508
Caudal site pre-activation	35.28 ±7.67 (48.59)	56.64 ±3.37 (40.75)	0.095	0.967	49.48 ± 7.78 (47.14)	54.54 ± 4.15 (48.7)	0.765	0.570
Caudal site stance	54.44 ± 5.56 (22.84)	72.09 ± 2.77 (26.32)	0.087	0.359	53.52 ± 6.53 (29.88)	82.01 ± 3.10 (17.73)	**<0.0001**	0.554
Long head of the Triceps								
Cranial site pre-activation	68.73 ± 3.97 (20.81)	74.05 ± 1.51 (21.72)	0.441	0.161	61.07 ± 5.30 (33.63)	68.17 ± 2.03 (28.19)	0.331	0.842
Cranial site stance	65.95 ± 5.47 (29.9)	70.90 ± 1.55 (22.81)	0.893*	0.093*	74.55 ± 5.01 (22.27)	72.53 ± 1.98 (23.81)	0.670	0.833
Caudal site pre-activation	78.07 ± 4.49 (23.03)	70.22 ± 1.36 (20.45)	**0.011**	0.122	66.63 ± 5.69 (33.05)	69.55 ± 2.16 (27.39)	0.386	0.561
Caudal site stance	69.10 ± 5.12 (29.62)	67.23 ± 1.78 (26.46)	0.849	0.430	69.93 ± 7.17 (32.43)	70.05 ± 2.18 (26.07)	0.552 *	0.347 *
Extensor Carpi Radialis								
Pre-activation	55.8 ± 10.3 (58.13)	52.92 ± 3.25 (46.41)	0.561	0.963	62.48 ± 7.50 (43.26)	57.91 ± 4.27 (44.9)	0.563	0.363
Stance	59.48 ± 6.96 (43.77)	71.67 ± 2.67 (24.76)	**0.001***	0.574*	76.55 ± 5.04 (22.82)	76.64 ± 2.38 (20.85)	0.549	0.768
Ulnaris Lateralis								
Pre-activation	69.91 ± 7.07 (35.05)	73.09 ± 2.32 (20.36)	0.060 *	0.210 *	75.85 ± 4.82 (26.2)	72.98 ± 2.29 (20.8)	0.319 *	0.378 *
Stance	75.00 ± 5.70 (26.34)	77.88 ± 2.10 (20.32)	0.736	0.758	67.70 ± 4.26 (23.53)	82.0 ± 2.13 (16.64)	**< 0.0001**	0.333

Significant interactions were observed between stride location and awareness for the *splenius* **F (2, 72) = 6.45, *P* =0.003** and *trapezius cervicis* (cranial site) **F (2, 65) = 3.53, *P* =0.035** muscle during pre-activation (Figure 9.4.1 and 9.4.2). Both muscles showed a more consistent contribution across all strides when the horses were aware of the abrupt change. The interaction for the *splenius* muscle occurred during the stride on the firm surface and the second stride on the soft surface where the normalised values for no awareness reduced below that of when the horses were aware of the change. The interaction for the *trapezius cervicis* (cranial site) occurred during the first stride on the soft surface where the normalised values for no awareness increased above that of the values for when the horses were aware of the change. No significant (P > 0.05) interactions were observed for any of the muscles during pre-activation or stance when the horses encountered the soft to firm surface condition.

Figure 9.4.1: Interaction plot for the splenius during pre-activation

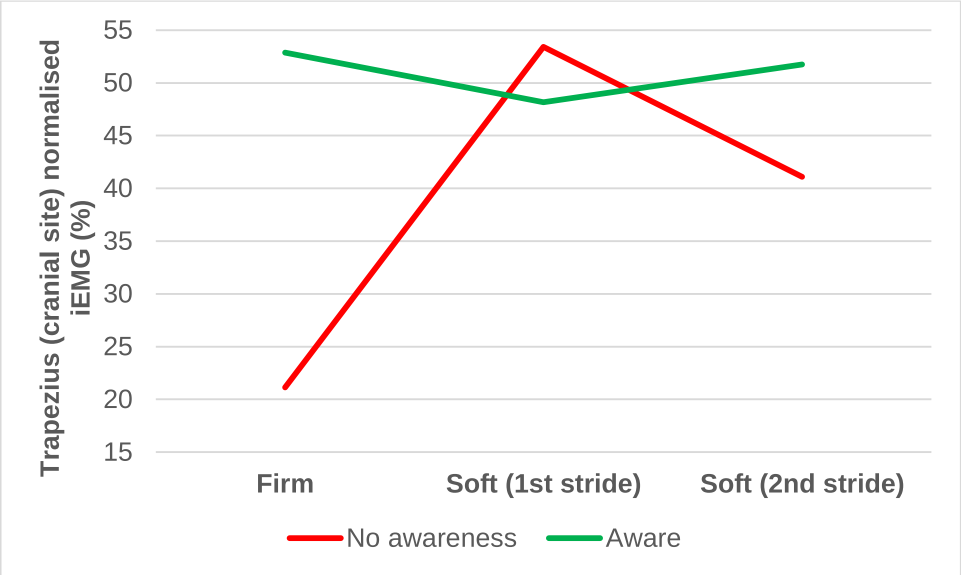


Figure 9.4.2: Interaction plot for the trapezius cranial site during pre-activation

9.5 Stride to stride changes and the effect of awareness on effective limb stiffness

The estimated peak vertical force, length change and effective limb stiffness of the fore and hind limbs on the different surface conditions was analysed. When the horses moved from the firm to soft surface condition, the parameters showed no significant (*P* >0.05) changes in the fore and hind limbs between strides (Table 9.5.1). When the horses were aware of the change, total length change in the fore limb significantly **F (1, 114) = 15.02, *P* <0.0001** reduced and effective fore limb stiffness significantly **F (1, 114) = 13.09, *P* <0.0001** increased.

Table 9.5.1: Mean ± SE (coefficient of variation) predicted peak vGRF, total length change and effective stiffness of the fore and hind limbs according to stride location and awareness when the horses travelled from the firm to soft surface condition. The P-value for the interaction (Int.) between stride location and awareness has also been reported. Values of P <0.05 denote significant differences in relation to stride location and awareness.

	Stride location				**Awareness**			**Int.**
Parameter	Firm	Soft (stride 1)	Soft (stride 2)	***P* - value**	Not aware	Aware	***P* - value**	***P* - value**
Fore vGRF (kN)	6.33 ± 0.11 (12.3)	6.34 ± 0.11 (12.78)	6.36 ± 0.16 (12.99)	0.386	6.42 ± 0.19 (12.54)	6.33 ± 0.07 (12.6)	0.218	0.175
Total Fore length change (m)	0.123 ± 0.002 (10.47)	0.123 ± 0.002 (10.51)	0.124 ±0.002 (9.88)	0.619	0.129 [a] ± 0.003 (8.07)	0.123 [b] ± 0.001 (10.5)	**<0.0001**	0.573
Fore stiffness (kN/m)	52.6 ± 1.47 (18.73)	52.17 ± 1.35 (19.02)	51.95 ± 2.08 (20.84)	0.359	50.83 [b] ± 2.06 (16.68)	52.5 [a] ± 0.98 (19.53)	**<0.0001**	0.331
Hind vGRF (kN)	5.77 ± 0.13 (15.74)	5.74 ± 0.12 (15.3)	5.84 ± 0.18 (15.35)	0.2	5.80 ± 0.22 (16.35)	5.78 ± 0.08 (15.27)	0.876	0.53
Hind length change (m)	0.036 ± 0.007 (119.95)	0.04 ± 0.005 (96.47)	0.036 ± 0.008 (115.85)	0.542	0.04 ± 0.011 (111.63)	0.037 ± 0.004 (107.54)	0.098	0.254
Hind stiffness (kN/m)	96.6 ± 28.7 (194.48)	59.4 ± 62.4 (757.97)	112.8 ± 39.9 (176.75)	0.706	93.2 ± 57.8 (255.69)	82.3 ± 33.7 (415.19)	0.966	0.811

When the horses moved from the soft to firm surface condition, estimated peak vertical force **F (1, 93) = 7.07, *P* = 0.009** and length change **F (1, 84) = 5.68, *P* = 0.019** in the forelimbs significantly reduced between stride one and two (Table 9.5.2). When the horses were aware of the change in surface condition, estimated peak vertical force in the fore limb significantly **Ant F (1, 93) = 3.99, *P* = 0.049** increased. This marginally increased effective fore limb stiffness, however this was not significant.

Table 9.5.2: Mean ± SE (coefficient of variation) predicted peak vGRF, total length change and effective stiffness of the fore and hind limbs according to stride location and awareness when the horses travelled from the soft to firm surface condition. The P-value for the interaction (Int.) between stride location and awareness has also been reported. Values of P <0.05 denote significant differences in relation to stride location and awareness.

	Stride location			**Awareness**			**Int.**
Parameter	Soft	Firm (stride 1)	***P* - value**	Not aware	Aware	***P* - value**	***P* - value**
Fore vGRF (kN)	6.24 [a] ± 0.1 (11.81)	6.23 [b] ± 0.11 (12.32)	**0.009**	6.14 [b] ± 0.2 (12.36)	6.25 [a] ± 0.08 (12.0)	**0.049**	0.299
Total Fore length change (m)	0.123 [a] ± 0.003 (16.21)	0.117 [b] ± 0.003 (15.93)	**0.019**	0.123 ± 0.006 (18.11)	0.12 ± 0.002 (15.99)	0.323	0.888
Fore stiffness (kN/m)	52.27 ± 1.63 (22.26)	53.29 ± 1.68 (20.73)	0.131	51.03 ± 3.39 (22.99)	52.99 ± 1.25 (21.35)	0.218	0.975
Hind vGRF (kN)	5.56 ± 0.13 (16.76)	5.58 ± 0.12 (15.53)	0.427	5.55 ± 0.24 (16.21)	5.58 ± 0.09 (16.13)	0.478	0.720
Hind length change (m)	0.061 ± 0.004 (40.36)	0.058 ± 0.003 (41.03)	0.802	0.065 ± 0.006 (33.86)	0.059 ± 0.003 (41.69)	0.198	0.144
Hind stiffness (kN/m)	115.8 ± 10.9 (61.56)	133.7 ± 18.9 (101.81)	0.711	102.1 ± 16.0 (54.36)	129.0 ± 12.9 (90.79)	0.504	0.660

When the horses moved on the continuous surfaces, estimated peak vertical force, length change and effective limb stiffness in the fore and hind limbs showed no significant (P >0.05) change between stride one and two (Table 9.5.3). The contribution of the distal and proximal springs to the total length change in the fore limb according to surface condition and awareness is presented in figures 9.5.1 and 9.5.2. The length change in the hind limb is also presented in figure 9.5.3.

Table 9.5.3: Mean ± SE (coefficient of variation) predicted peak vGRF, total length change and effective stiffness of the fore and hind limbs according to stride location on the continuous surfaces. Values of P <0.05 denote significant differences between stride one and two on the corresponding surface condition.

	Continuous Firm Surface				Continuous Soft Surface		
Parameter	Stride 1	Stride 2	***P*- value**		Stride 1	Stride 2	***P*- value**
Fore vGRF (kN)	6.44 ± 0.16 (14.66)	6.52 ± 0.16 (14.26)	0.171		6.20 ± 0.17 (16.15)	6.29 ± 0.16 (14.89)	0.195
Total Fore length change (m)	0.111 ± 0.004 (22.17)	0.109 ± 0.005 (27.64)	0.814		0.126 ± 0.003 (13.39)	0.126 ± 0.003 (14.13)	0.974
Fore stiffness (kN/m)	61.92 ± 3.69 (33.20)	165 ± 104 (357.11)	0.390		49.79 ± 1.59 (18.31)	50.79 ± 1.61 (18.25)	0.520
Hind vGRF (kN)	5.53 ± 0.18 (17.12)	5.63 ± 0.18 (16.60)	0.065		5.04 ± 0.14 (15.53)	5.04 ± 0.13 (13.14)	0.999
Hind length change (m)	0.051 ± 0.005 (46.4)	0.046 ± 0.005 (60.08)	0.249		0.085 ± 0.003 (19.58)	0.079 ± 0.005 (33.47)	0.558
Hind stiffness (kN/m)	190.6 ± 57.1 (149.89)	226 ± 135 (303.74)	0.810		62.21 ± 3.19 (28.11)	172.2 ± 99.3 (299.47)	0.328

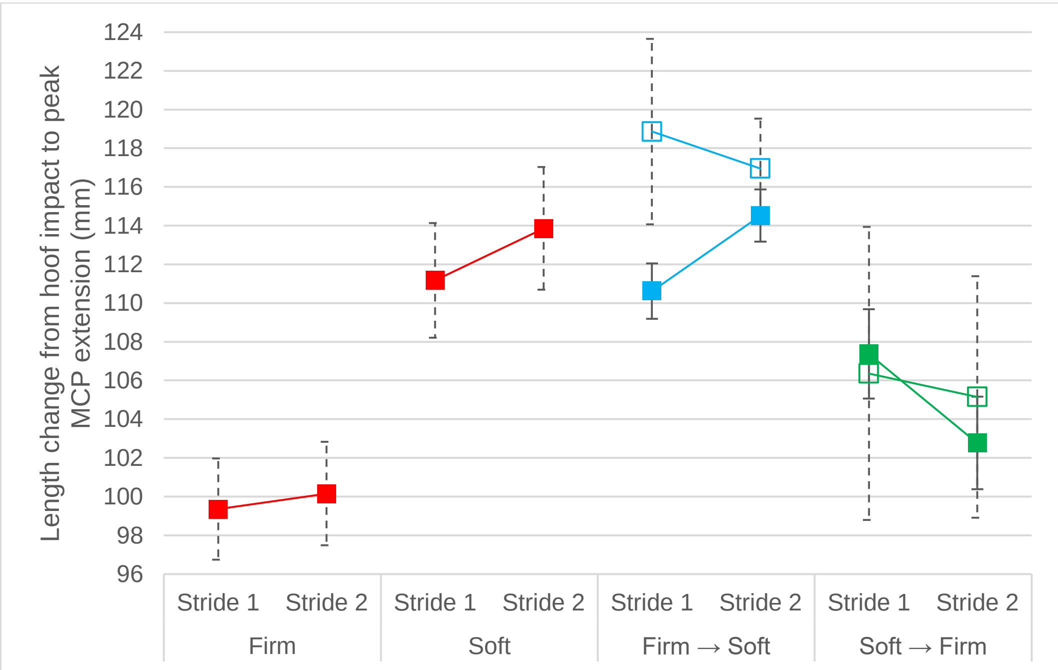


*Figure 9.5.1: Mean (±SE) length change (mm) of the **distal** spring for stride one and two on the different surface conditions. The data from the abrupt change in surface condition has also been split according to awareness (open marker: not aware; closed marker: aware). Length change was calculated from hoof impact to peak MCP extension. Data has been grouped from all horses.*

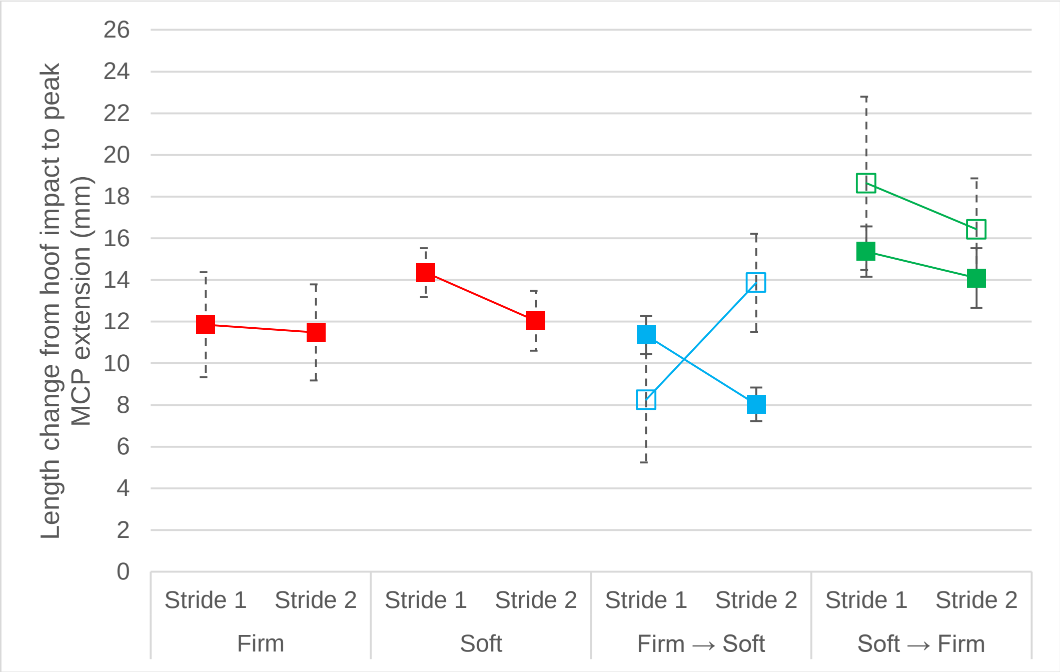


Figure 9.5.2: Mean (±SE) length change (mm) of the ***proximal*** *spring for stride one and two on the different surface conditions. The data from the abrupt change in surface condition has also been split according to awareness (open marker: not aware; closed marker: aware). Length change was calculated from hoof impact to peak MCP extension. Data has been grouped from all horses.*

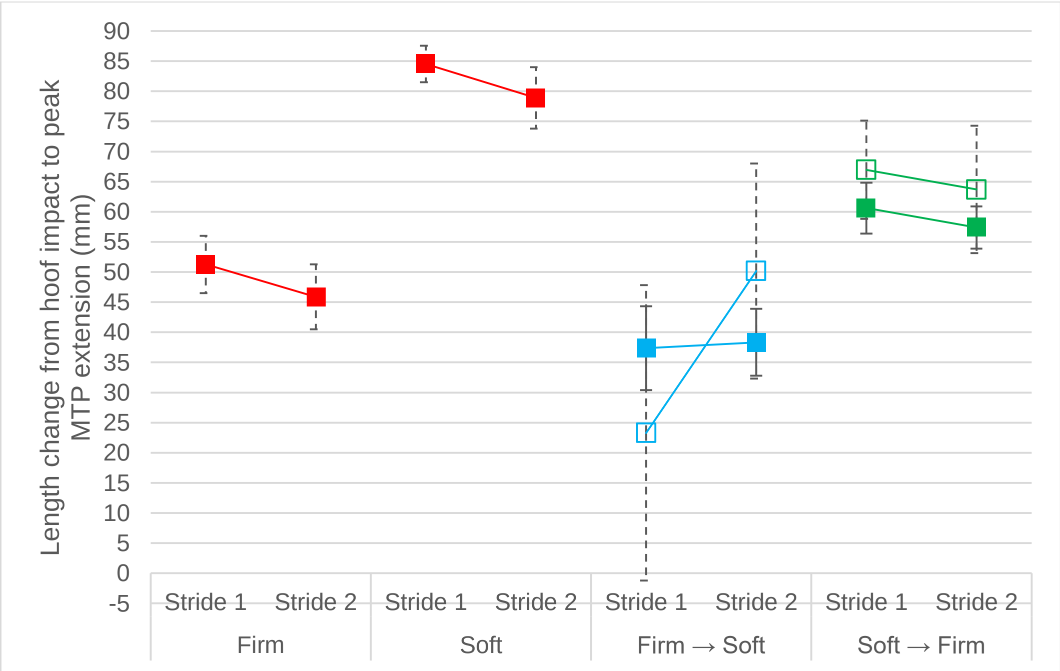


Figure 9.5.3: Mean (±SE) length change (mm) of the ***hind limb*** *for stride one and two on the different surface conditions. The data from the abrupt change in surface condition has also been split according to awareness (open marker: not aware; closed marker: aware). Length change was calculated from hoof impact to peak MTP extension. Data has been grouped from all horses.*

9.6 Kinematic and EMG Results Summary

The significant kinematic and EMG findings from the entire study were collated and presented according to stride to stride changes on the different surface conditions (Table 9.6.1) and awareness of the abrupt change in surface condition (Table 9.6.2).

Table 9.6.1: Significant kinematic and EMG stride to stride changes. The arrows represent an increase or decrease between two consecutive strides for the corresponding surface condition. The firm → soft condition includes data from three consecutive strides. The grey shaded boxes represent no significant findings.

	Firm → Soft	Soft → Firm	Firm	Soft
Temporal		Hind limb stance duration ↑ Hind limb duty factor ↑	Timing of peak MTP extension ↓	Hind limb stance duration ↑
Linear	Hind limb stride length ↓	Fore and hind limb stride length ↓	No stats available	No stats available
Angular	Fore and hind limb inc. at hoof lift off ↓ Shoulder angle at hoof lift off ↓	Peak MCP extension ↓ Scapula inclination at hoof impact ↑		
Effective limb stiffness		Estimated fore vGRF ↓ Total fore limb length change ↓		
Normalised iEMG pre-activation	*Splenius* ↓ (stride 2 &3) *Triceps* cranial site ↑ (stride 1 and 3)			*Triceps* caudal site ↓
Normalised iEMG stance	*Triceps* cranial site ↓ (stride 3)			*Trapezius* caudal site ↓ *UL* ↑

Table 9.6.2: Significant kinematic and EMG according to awareness of the abrupt change in surface condition. The arrows represent an increase or decrease with awareness. The grey shaded boxes represent no significant findings.

	Firm → Soft	**Soft → Firm**
Temporal	Fore and hind limb stance duration ↑ Fore and hind limb duty factor ↑ Ipsilateral contact time ↑	Hind limb stance duration ↑ Hind limb duty factor ↑ Ipsilateral contact time ↑
Linear	Fore and hind limb stride length ↓	Fore limb stride length ↓
Angular	Fore and hind limb inc. at hoof lift off ↓	Peak MCP extension ↑ Scapula inclination at hoof impact ↓ Hind limb inc. at hoof impact ↓
Effective limb stiffness	Total fore limb length change ↓ Effective fore limb stiffness ↑	Estimated fore vGRF ↑
Normalised iEMG pre-activation	*Triceps* caudal site ↓	
Normalised iEMG stance	*Trapezius* cranial site ↑ *ECR* ↑	*Splenius* ↑ *Trapezius* caudal site ↑ *UL* ↑

9.7 Post-hoc observational analysis

Further measurements relating to the hypotheses were produced to aid interpretation of the significant results and to enable comparisons with other literature. The data presented in this section were not analysed statistically and are explored further in the discussion (Chapter 10.0).

Fore and hind limb retraction at hoof lift off

When the horses moved from the firm to soft surface condition, fore and hind limb inclination at hoof lift off demonstrated stride to stride changes and also alterations with awareness. The data from the individual horses was explored further and presented for the fore (Figure 9.7.1) and hind (Figure 9.7.2) limbs. The figures demonstrate the stride to stride variations with no awareness and reveal a more consistent reaction with awareness, especially in the fore limbs. Fore limb retraction tended to increase (more negative) during the second stride without awareness, which subsequently decreased with each stride for every subject with awareness.

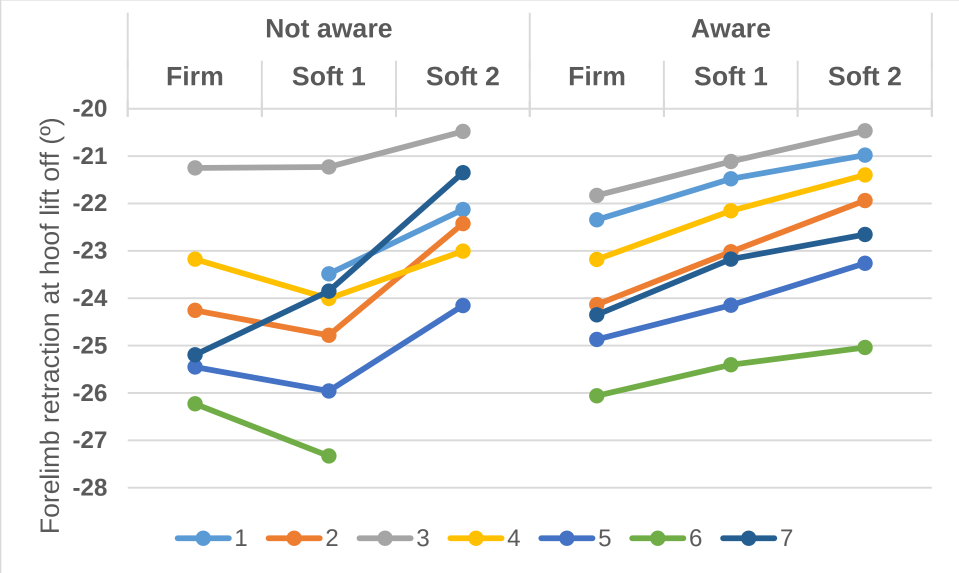


Figure 9.7.1: Forelimb retraction for the individual horses (n =7) in relation to awareness and before and after the change in surface condition. A more negative value represents greater limb retraction.

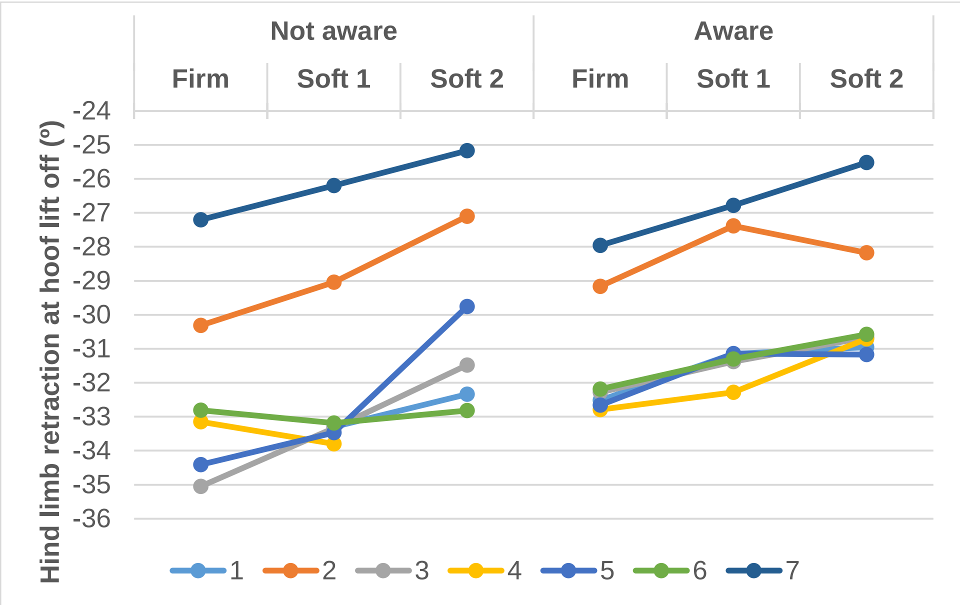


Figure 9.7.2: Hind limb retraction for the individual horses (n =7) in relation to awareness and before and after the change in surface condition. A more negative value represents greater limb retraction.

Ipsilateral contact times

The ipsilateral contact time demonstrated a significant increase with awareness on both surfaces presenting an abrupt change (Figure 9.7.3 and 9.7.4), suggesting the hind hoof impact occurred earlier in relation to fore hoof lift off. The timing was also greater on the continuous firm surface condition in comparison to the continuous soft surface (Figure 9.7.5). This alteration relates to hypothesis one and is potential evidence to suggest that the horses altered their balancing strategy. A longer hind limb stance duration appeared to be responsible for the greater contact time. The possibility of horses preserving the timing of hoof lift off in the hind limb regardless of awareness and surface condition was explored in Chapter 10.0.

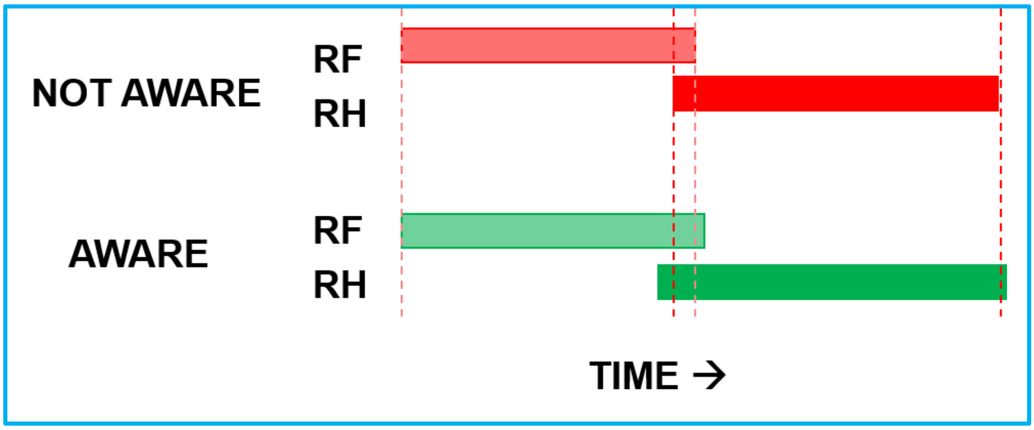


Figure 9.7.3: An illustration showing changes in the stance duration of the right fore and hind limb according to awareness of the abrupt change (Firm → Soft). The stance duration bars for the right hind limb have been positioned to represent the contact timing of the ipsilateral limb pair. The dashed vertical lines represent hoof impact and lift off for the fore and hind limbs when the horses were not aware of the change in surface condition (Not aware: 25 ms before hoof lift off vs. aware: 52 ms). The differences shown represent the mean changes on the different surface conditions.

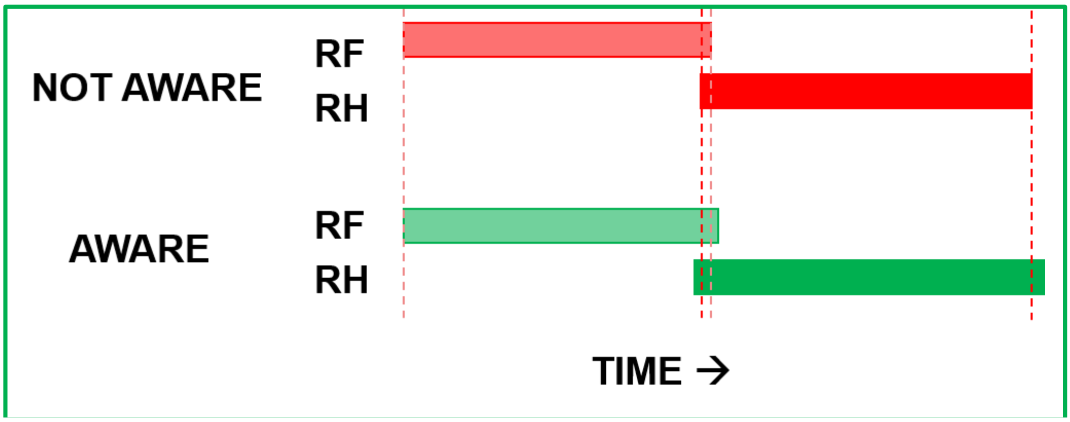


Figure 9.7.4: An illustration showing changes in the stance duration of the right fore and hind limb according to awareness of the abrupt change (Soft → Firm). The stance duration bars for the right hind limb have been positioned to represent the contact timing of the ipsilateral limb pair. The dashed vertical lines represent hoof impact and lift off for the fore and hind limbs when the horses were not aware of the change in surface condition. (Not aware: 12 ms before hoof lift off vs. aware: 26 ms) The differences shown represent the mean changes on the different surface conditions.

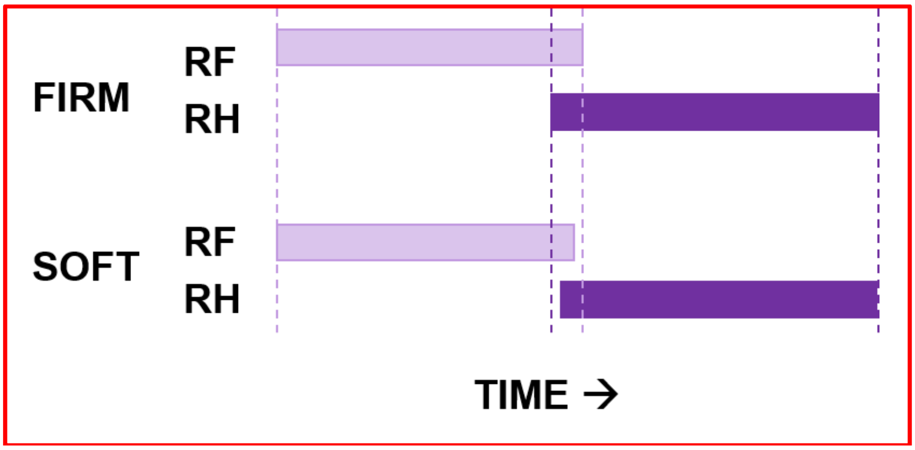


Figure 9.7.5: An illustration showing changes in the stance duration of the right fore and hind limb on the continuous firm and soft surface condition. The stance duration bars for the right hind limb have been positioned to represent the contact timing of the ipsilateral limb pair. The dashed vertical lines represent hoof impact and lift off for the fore and hind limbs on the firm surface condition. (Firm: 37 ms before hoof lift off vs. soft: 16 ms). The differences shown represent the mean changes on the different surface conditions.

Findings on a continuous surface

In order to explain some of the kinematic and electromyographic differences observed on the continuous firm and soft surfaces, extra parameters were calculated. The mean trajectory of the tarsal angle during the swing phase was assessed to explore differences in stride length on the two surface conditions (Figure 9.7.6). Peak tarsal flexion during swing was 10.07° greater on the soft surface condition.

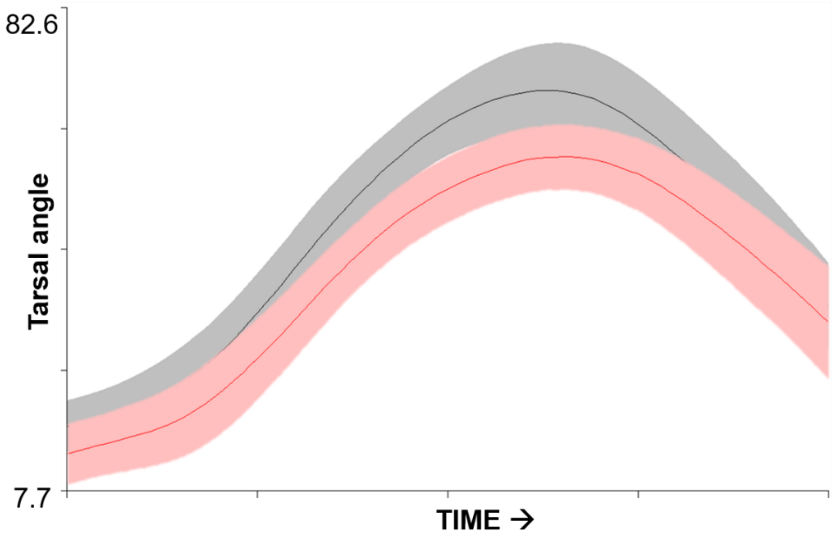


Figure 9.7.6: Mean tarsal angle trajectory during the swing phase of the right hind limb on the firm (red line) and soft (black line) surface conditions. Data has been grouped from all horses and trials on each continuous surface condition. The shaded areas represent the standard deviation. A greater angle denotes more flexion.

The way in which the data had been normalised and presented in section 9.3 and 9.4 poses a limitation when comparing data from different days. It is possible to obtain the average contribution of the muscle on a particular surface condition by looking at the normalised values, however this does not reveal how the overall magnitude differs between days. To address this issue, the static trials were used to assess how the magnitude of muscle activity differed on the two surface conditions post hoc. The mean baseline magnitude was extracted from the individual static trials using a 100 millisecond window for each muscle. Data was grouped from the different horses before calculating the average magnitude for each muscle on the different days. The percentage difference in magnitude was subsequently calculated relative to the firm surface condition (Table 9.7.1). It is important to note that the magnitude may have been influenced by slight differences in sensor placement on the different days. It was not necessary to calculate the baseline magnitude for day 2 and 3 when the horses encountered an abrupt change due to data not being compared between days.

Table 9.7.1: % difference in magnitude of sEMG activity comparing the continuous soft to the continuous firm surface. sEMG data was normalized to the muscle activity recorded in the standing trials. Values less than 100% represent a greater muscle contribution on the firm surface condition.

Muscle	**Difference in magnitude on firm vs. soft**
Splenius	71.84%
Trapezius cranial site	24.16%
Trapezius caudal site	45.96%
Triceps cranial site	16.13%
Triceps caudal site	61.44%
Extensor carpi radialis	493.60%
Ulnaris lateralis	369.05%

Metatarsophalangeal joint

Based on the significant findings, it was possible to establish that horses altered limb posture with respect to surface condition. This has been found previously in the hind limb of race horses galloping at high speed (12-17 m/s) on two different surface types (Symons *et al*., 2013). To allow for a direct comparison with the study by Symons *et al*. (2013), MTP angle at hoof impact was measured (Table 9.7.2). Stride to stride changes and differences between surface conditions were negligible suggesting horses did not alter dynamic posture in the same way at trot.

Table 9.7.2: Mean MTP angle at hoof impact. Data has been grouped from all horses and categorised according to surface condition and awareness.

	MTP angle at hoof impact (º)
Firm no change	
1	180.44
2	180.41
Soft no change	
1	181.06
2	181.11
Firm → Soft	
Not Aware	
1	181.11
2	181.31
Aware	
1	180.63
2	180.67
Soft → Firm	
Not Aware	
1	180.95
2	181.06
Aware	
1	180.69
2	180.7

9.8 Surface response

Surface moisture content did not alter significantly **$F_{(2, 27)} = 2.42$, $P = 0.108$** for the duration of data collection. The Orono Biomechanical Surface Tester was used to quantify the change in functional surface properties each day before (fresh track) and after (horse track) the horses had been through the recording zone. On day one and four, ten single drops were made on each track (fresh and horse track as described in section 5.2.4). On day two and three, five single drops were made on each track due to the change in surface condition. There were no data for the fresh track on day one so the change in functional surface properties between the fresh and the horse track cannot be quantified. The functional surface properties have been analysed in such a way so it is possible to establish differences between: days; the firm and soft surface conditions and; the fresh and horse track.

Cushioning

The peak vertical force, which represents surface cushioning (greater force = less cushioning) showed significant changes according to day **$F_{(3, 64)} = 16.41$, $P < 0.0001$**, surface condition **$F_{(1, 64)} = 10.31$, $P = 0.002$** and track **$F_{(1, 64)} = 5.06$, $P = 0.028$** (Figure 9.8.1). Cushioning on day four was greater than day two and three and the soft surface condition offered more cushioning than the firm surface condition. The lower peak force measured on the horse track rather than the fresh track suggests cushioning increased each day as data was collected, with the exception of the soft surface on day 3 where the horse track had less cushioning.

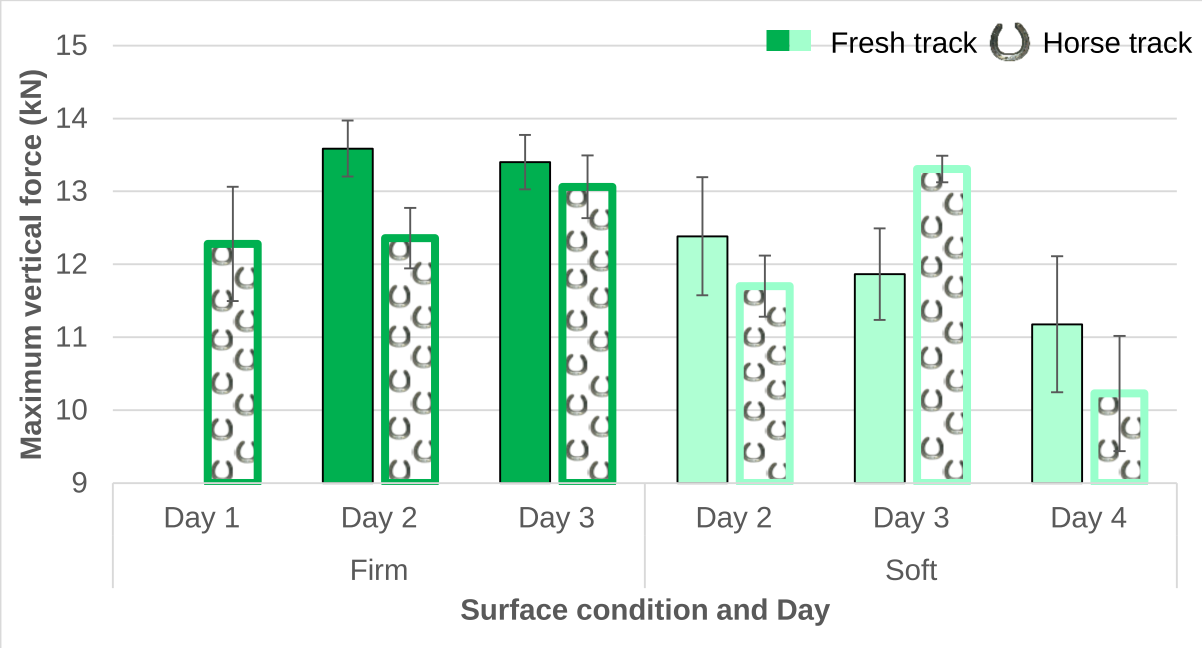


Figure 9.8.1: Mean (±SE) peak vertical force, representing cushioning of the different surface conditions throughout data collection.

Impact Firmness

The maximum vertical acceleration, which represents surface impact firmness demonstrated exactly the same pattern as peak load and significantly changed according to day **F (3, 64) = 14.25, *P* <0.0001**, surface condition **F (1, 64) = 29.93, *P* <0.0001** and track **F (1, 64) = 8.63, *P* =0.005** (Figure 9.8.2). Impact firmness on day four was lower than day two and three and the soft surface condition had less impact firmness than the firm surface condition. The lower impact firmness measured on the horse track suggests the surface became less compact as data was collected with the exception of the soft surface on day 3 where the horse track increased in firmness.

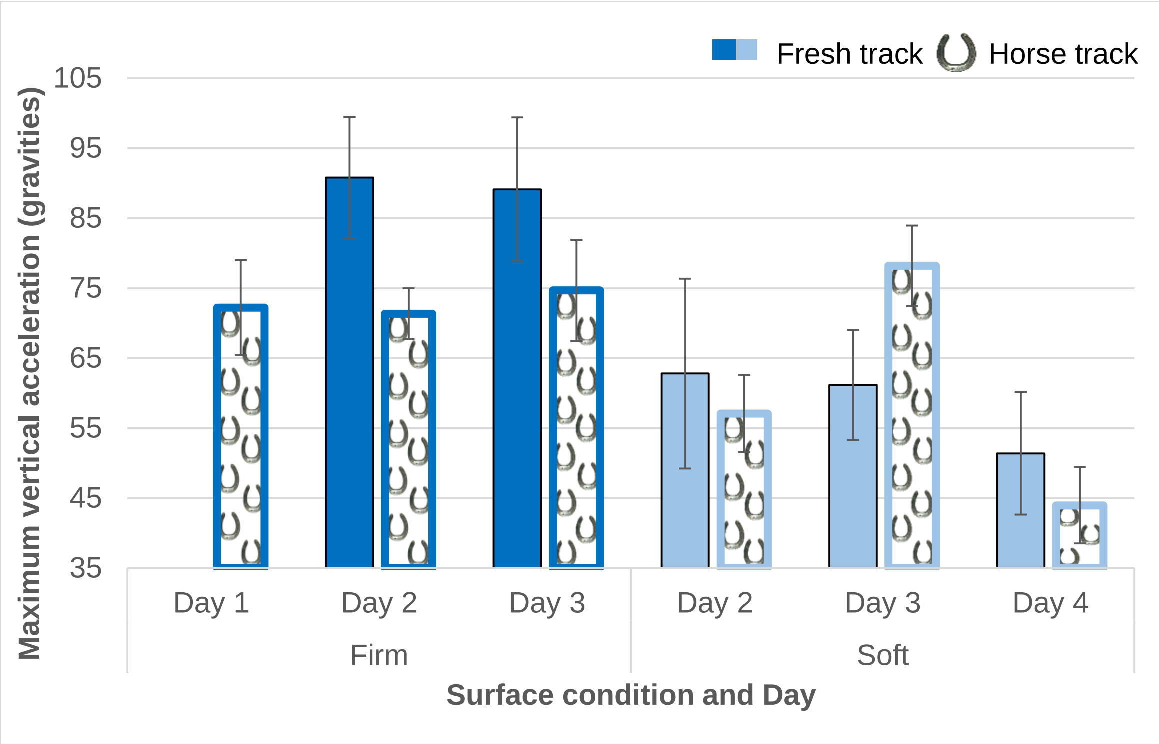


Figure 9.8.2: Mean (±SE) maximum vertical acceleration, representing impact firmness of the different surface conditions throughout data collection.

Responsiveness

The responsiveness of the surface showed significant changes according to day **F (3, 64) = 12.79, *P* <0.0001**, surface condition **F (1, 64) = 10.84, *P* =0.002** and track **F (1, 64) = 9.49, *P* =0.003** (Figure 9.8.3). Responsiveness was greater on day one and two in comparison to day three and four. A greater responsiveness was also recorded on the soft surface condition and horse track in comparison to the firm surface and fresh track respectively. A greater responsiveness represents a shorter rebound time of the surface after loading and therefore the surface is able to respond faster.

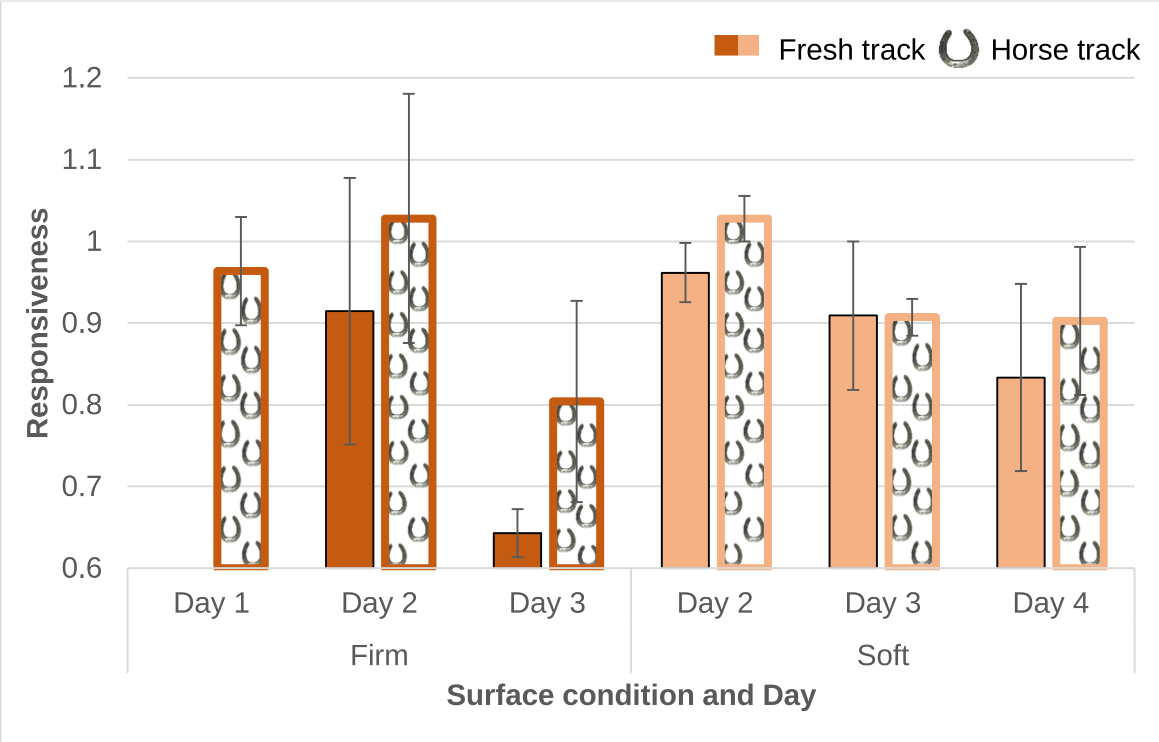


Figure 9.8.3: Mean (±SE) responsiveness of the different surface conditions throughout data collection.

Grip

Four drops were rejected (all on day 2; 1 on the firm horse track, 1 on the soft horse track and 2 on the soft fresh track) for this parameter due to the signal for the string potentiometer velocity not following the usual profile. The maximum horizontal displacement represents linear grip where a greater displacement suggests less grip. Grip significantly changed according to day **F (3, 64) = 13.86, *P* <0.0001** and surface condition **F (1, 64) = 24.0, *P* <0.0001** but did not significant alter according to track **F (1, 64) = 2.58, *P* =0.114** (Figure 9.8.4). Grip was lower on day four than day two and three and also on the soft surface condition in comparison to the firm surface condition. The grip demonstrated a similar pattern to the change in cushioning and impact firmness where grip was generally lower on surfaces that offered more cushioning and lower impact firmness.

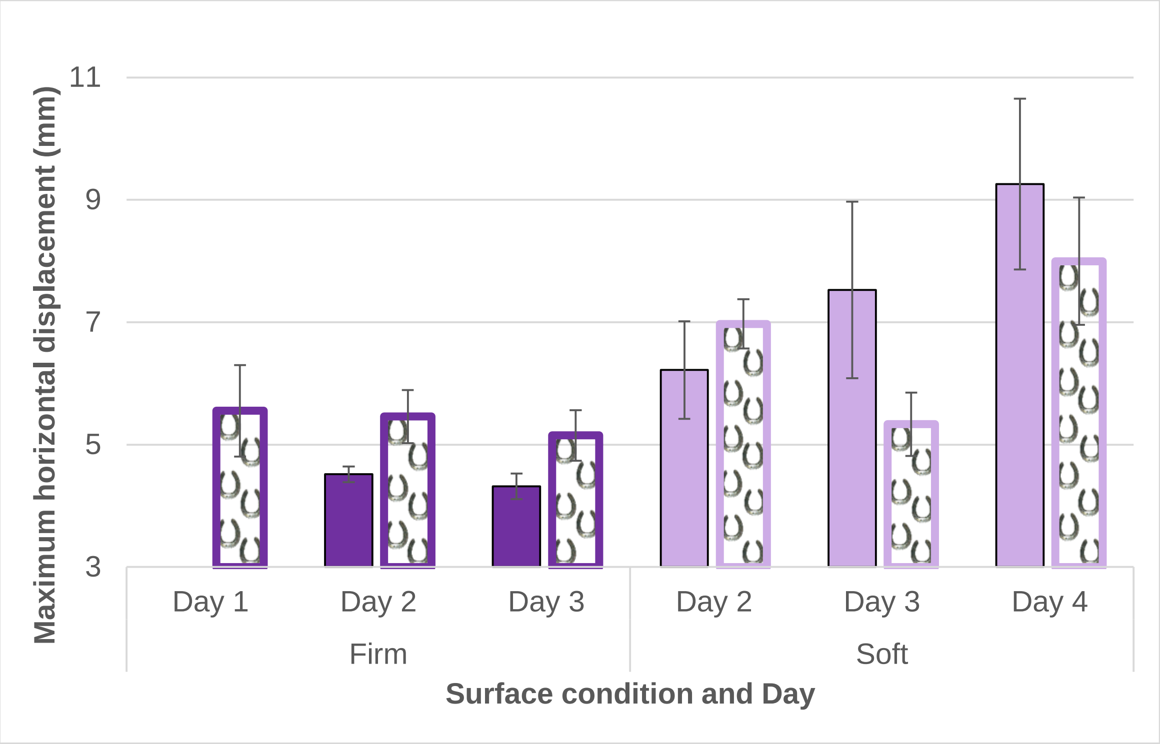


Figure 9.8.4: Mean (±SE) maximum horizontal displacement of the OBST hoof during loading, representing grip characteristics of the different surface conditions throughout data collection. A smaller amount of displacement reflects a greater amount of grip.

Uniformity

The mean values reported for each functional surface property provide a good indication of how this property differs according to surface condition. It is also important to consider the range in values recorded using the OBST because this reveals information about surface uniformity or how consistent the surface is within the test area. Contour plots were used to demonstrate the variation that existed in cushioning (Figure 9.8.5) and impact firmness (Figure 9.8.6) within the recording zone on each day.

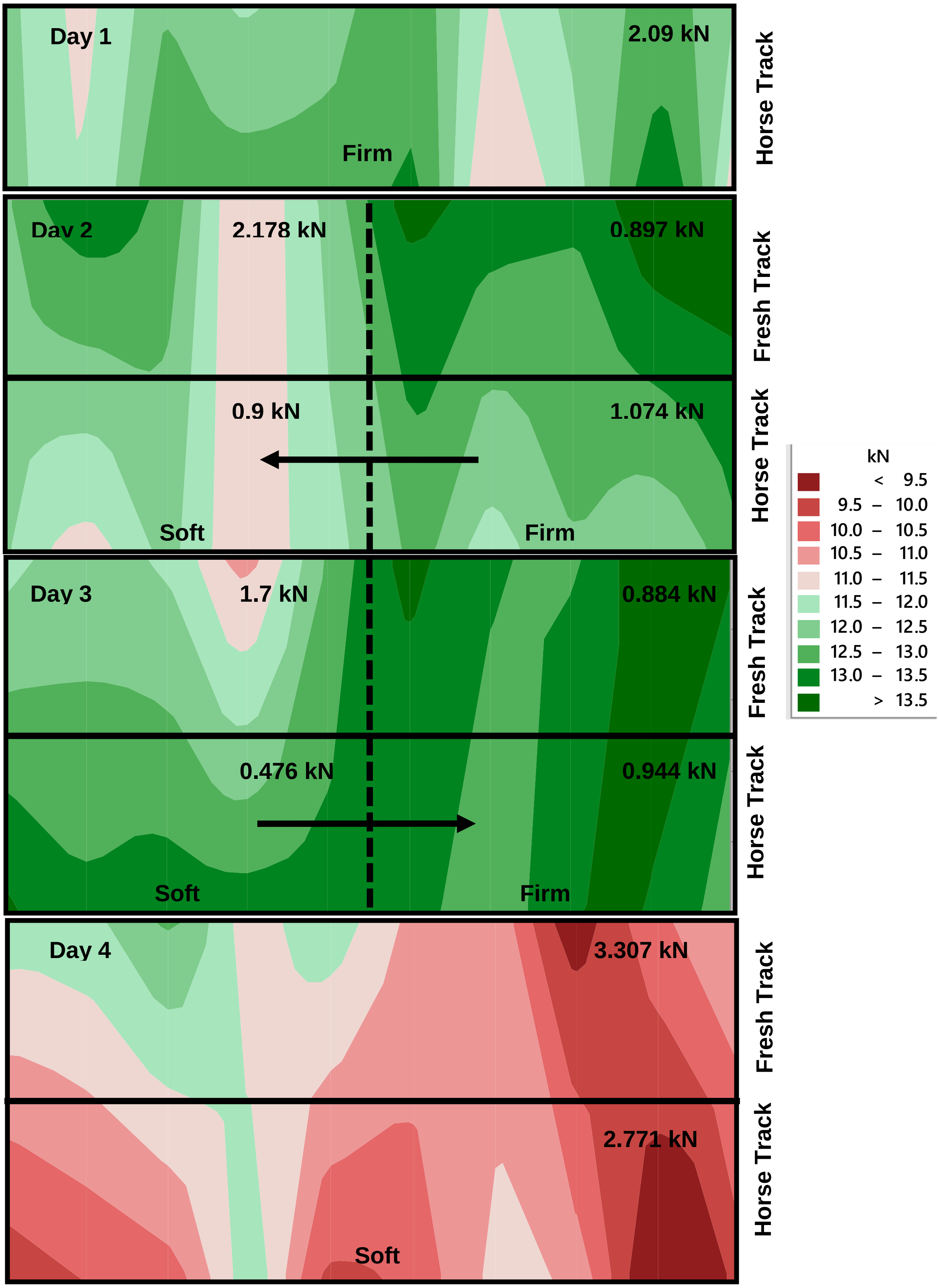


Figure 9.8.5: Contour plot demonstrating the variation in cushioning (peak vertical force) across different tracks and days. The values represent the range in cushioning where a lower range presented on each surface condition suggests better uniformity.

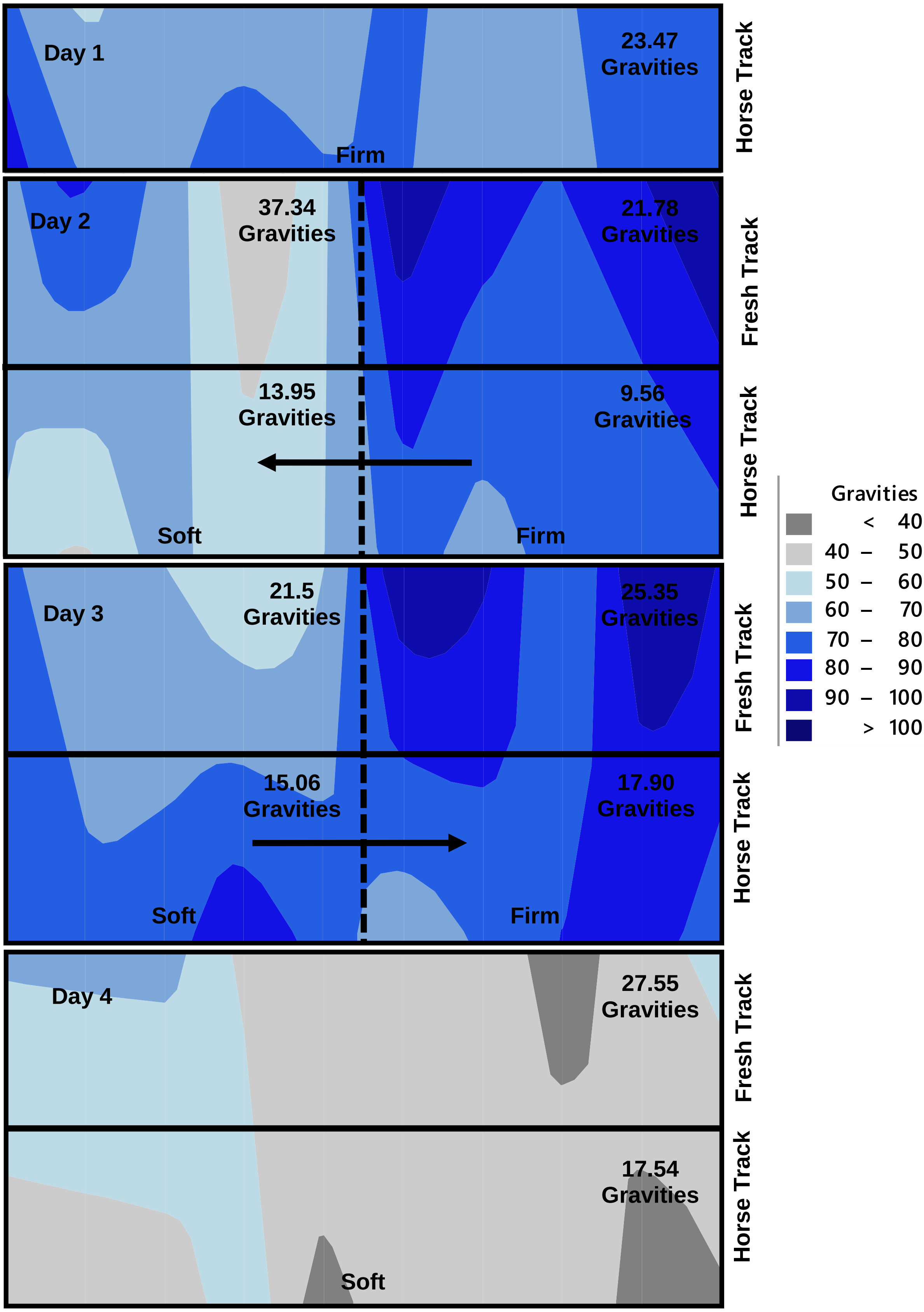


Figure 9.8.6: Contour plot demonstrating the variation in impact firmness (maximum vertical acceleration) across different tracks and days. The values represent the range in impact firmness where a lower range presented on each surface condition suggests better uniformity.

Surface stiffness

Surface stiffness was significantly **$F_{(3, 64)} = 20.64$, $P < 0.0001$** lower on day four in comparison to the other days (Table 9.8.1). The firm surface condition had a significantly **$F_{(1, 64)} = 10.27$, $P = 0.002$** greater stiffness in comparison to the soft surface condition. There was no significant ($P = 0.297$) difference between the fresh and horse track on any of the data collection days.

Table 9.8.1: Mean ± SE surface stiffness (kN/m) according to day, surface condition and whether the data was collected on the fresh or horse track.

	Firm [a]		**Soft [b]**	
Day	Fresh	Horse	Fresh	Horse
1. Firm [a]		983.8 ± 17.7		
2. Firm → Soft [a]	1080.8 ± 14.1	1006.3 ± 14.0	990.8 ± 25.3	958.0 ± 15.3
3. Soft → Firm [a]	1052.9 ± 12.7	1043.2 ± 13.5	939.3 ± 21.2	1062.0 ± 3.8
4. Soft [b]			876.3 ± 22.4	826.6 ± 19.1

10.0 Discussion

The equine athlete encounters spatial variations in the functional properties of surfaces during training and competition, which is a risk factor for injury (Murray *et al.*, 2010b; Tranquille *et al.*, 2015). The biomechanical response of the horse to spatial variations has not previously been quantified, so predicted gait adaptations and resulting level of risk were unknown. The main aim of this project was to address this gap in knowledge and to quantify the kinematic and electromyographic response of a sample of horses to a camouflaged, abrupt change in the functional surface properties. It was important to camouflage the change, as spatial variations in functional properties often occur with no visual cues (Tranquille *et al.*, 2015; Northrop *et al.*, 2016) and visual cues may induce a preparatory response. This has been observed in humans previously (Ferris *et al.*, 1999). The track used in the study simulated an arena that had been maintained so visually it looked uniform, but variations in the functional surface properties were present.

It was hypothesised that gait modifications: 1) may occur with an earlier contact timing of the ipsilateral hind limb, 2) may occur by altering dynamic posture i.e. limb position, 3) may occur by increasing the active contribution of muscles which would alter ground reaction forces. All of these modifications have been found in trotting horses to assist in managing balance (Hobbs *et al.*, 2016). In addition, gait modifications: 4) may occur by increasing the magnitude of pre-activation control of limb muscles in order to stabilise the limb prior to ground contact, 5) may represent a more cautious gait when horses are aware of an abrupt change in surface condition and 6) will not occur through changes in equine limb stiffness as a function of surface stiffness.

There were no significant stride to stride changes to the ipsilateral contact timing, suggesting hypothesis one can be rejected. Other temporal parameters within the hind limb however did show significant stride to stride changes except on the firm → soft surface condition, which implies that horses may have used a different strategy to modify their gait. The ipsilateral contact timing did show significant alterations with awareness of the abrupt change however, which provides support for hypothesis four. There was strong evidence to support hypothesis two, where some of the angular parameters representing limb position or posture demonstrated stride to stride alterations on the firm → soft and soft → firm surface condition.

There was very little evidence to support hypothesis three. The only muscle to show an increase in contribution during the second stride was the *ulnaris lateralis* on the continuous soft surface condition. Stride to stride changes were recorded in other muscles including the *long head of the triceps* on firm → soft and the *trapezius* on the continuous soft surface condition but this was a reduction in contribution. The alterations in muscle activity did not always correspond with the kinematic changes. This was attributed to the presence of eccentric muscle contractions, which were expected to play a role in stabilising the forelimb. There was also very little evidence to support hypothesis four where the magnitude of pre-activation only increased within the cranial site of the *long head of the triceps* from stride one to three on the firm → soft surface condition. The activity observed was implicated in tuning the limb properties prior to hoof impact. The other changes in pre-activation showed a reduction in magnitude with each stride including the *splenius* on firm → soft and the caudal site of the *long head of the triceps*.

There was strong evidence to support hypothesis five. Awareness of the abrupt change in surface condition elicited changes in several kinematic and electromyographic parameters, which were consistent with a more cautious gait. Hypothesis six can also be accepted. Surface stiffness was significantly higher on the firm surface condition in comparison to the soft surface condition. Effective fore and hind limb stiffness did not change as a function of surface stiffness. The only stride to stride changes were estimated peak force in the fore limb and total length change of the fore limb, which demonstrated reductions on the soft → firm surface condition. This chapter will explore the mechanisms behind the changes observed in conjunction with the possible implications of making such alterations.

The abrupt change in surface conditions initiated stride to stride modifications in some of the kinematic and electromyographic parameters. It has been claimed that the equine forelimb has limited capacity to adapt to changes in the surface by altering limb stiffness, which makes it vulnerable to changes in the external environment (Parkes and Witte, 2015). This is the first study to address if equine gait modifications are possible in response to an abrupt change in surface condition. The changes observed in this project provide evidence that horses can adapt and alter their balancing strategy but by using mechanisms other than altering limb stiffness. The modifications may be subtle during trotting at moderate speed, but at higher speeds these changes may become a risk factor for injury (Chateau *et al.*, 2010).

<u>10.1 Strategies observed for stride to stride gait modifications on firm → soft and soft → firm</u>

During steady state, unperturbed equine locomotion, the horse's body moves forward continuously with a sinusoidal pattern in both vertical and longitudinal velocity profiles of the centre of mass (COM) (Hobbs and Clayton, 2013). This is due to the GRF effects in regulating vertical body motion, maintaining forward speed and controlling trunk orientation (Hobbs and Clayton, 2013). The changes in height of the COM as the body moves forward mean that there are two distinct stages in each stride: 1) a period of low energetic cost when the COM direction changes from moving up to down and occurs as a result of the passive action of gravity; 2) a period of high energetic cost when the COM direction changes from moving down to up and work must be performed by the limbs to prevent energy being lost from the system (Bertram, 2013). These changes of COM height are natural aspects of all gaits but they are key factors determining the consequences of any gait strategy (Bertram, 2013). Balancing pitching moments will be an important stability consideration for a cursorial mammal with long legs and high COM position (Hobbs *et al.*, 2016). There are three important motor control strategies that can be altered to have a balancing effect: 1) relative fore-aft contact timing (i.e. diagonal dissociation) (Hobbs *et al.*, 2016); 2) foot contact position (Lee *et al.*, 1999); 3) fore-aft vertical force distribution (Lee *et al.*, 1999).

A steady bouncing gait will be maintained if during each stance phase: 1) the forward speed remains the same and 2) the angle swept by the leg spring during stance is the same (McMahon and Cheng, 1990). As no difference in speed was found between strides when encountering an abrupt change, forward speed was considered to be maintained. Changes in limb protraction and retraction would indicate changes in the angle swept by the limb during stance. Fore and hind limb retraction at hoof lift off demonstrated a reduction with each stride as the horses made the transition from the firm to soft surface condition (Figure 10.1.1). The changes in forelimb retraction were only significant between the stride before the change and the 2nd stride after the change whereas a significant reduction in hind limb retraction angle was observed with each stride. By altering the angle swept by the leg spring, the vertical excursion of the COM can be changed (McMahon and Cheng, 1990; Farley *et al.*, 1993; Farley and Gonzalez, 1996), which suggests when horses encountered the abrupt change they altered their gait strategy as discussed by Bertram (2013).

The reduction in retraction after the abrupt change suggests the COM position relative to the fore and hind limb foot position at hoof lift off was more caudal (Crevier Denoix *et al.*, 2010). Altering take-off conditions has been associated with self-stabilisation in runners previously, which was thought to provide excellent touch-down conditions for the next stance phase (Grimmer *et al.*, 2008). The change in retraction in this project may have been a functional strategy to aid balance and prevent a ‘falling forward posture’ (Hobbs *et al.*, 2016). A more falling forward posture has been linked to a fore-first dissociation at hoof impact of the diagonal limb pair due to a more cranial COM to COP separation (Hobbs *et al.*, 2016). With this posture, the COM and gravity would develop greater forward and downward moments prior to the transition between braking and propulsion to balance the earlier negative ground reaction force moments during braking (Hobbs *et al.*, 2016). By reducing retraction at hoof lift off with each stride in this project, this may reduce the impact of the forward and downward moments during limb loading, especially when the horses had moved onto the soft surface condition.

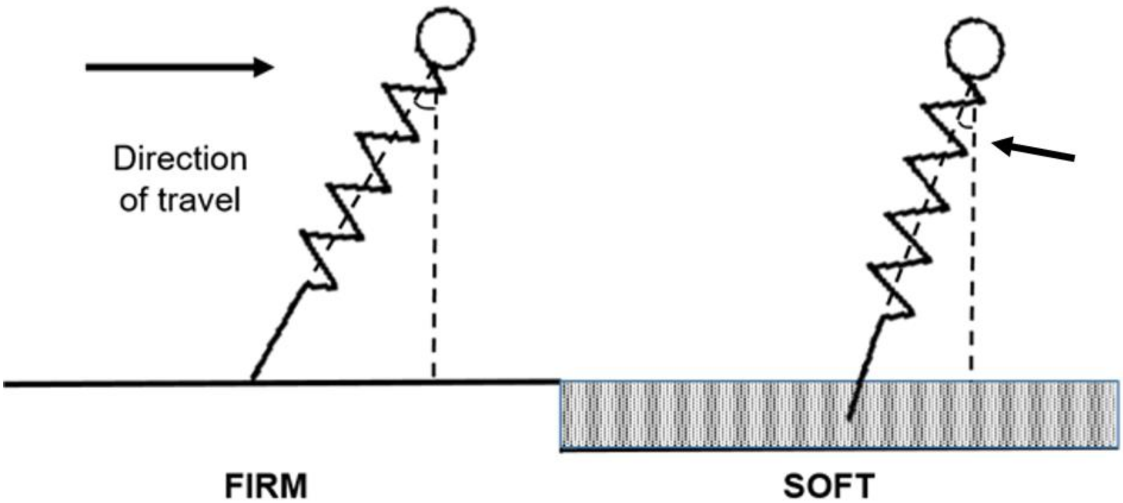


Figure 10.1.1: An illustration demonstrating the change in fore and hind limb angle at hoof lift off on the firm and soft surface condition. Limb retraction is reduced on the soft surface condition as demonstrated by a smaller angle from the vertical and represented by the arrow.

The spring system has been shown to adjust in order to operate at higher speeds by increasing the angle swept by the leg spring during stance (Farley *et al.*, 1993). This generates greater vertical forces, which results in an increased compression of the leg spring and a flatter trajectory of the COM (Farley *et al.*, 1993). A greater arc that is swept by the limb implies that limb is inclined closer to the horizontal at impact (more protraction) and at the end of stance (more retraction), which potentially means there is more scope for changes to occur within the system due to gravitational forces. Based on the findings reported here, if a change in surface condition induced similar changes in fore and hind limb retraction at faster speeds the energetic cost of maintaining the COM trajectory would ultimately increase and hasten the onset of fatigue.

The change in forelimb retraction corresponded with a reduction in shoulder flexion at hoof lift off. This represents changes to the posture of the proximal forelimb in response to the change in surface condition. During steady state locomotion, the shoulder joint has been reported to flex during the first 34% of stance before extending gradually until hoof lift off (Clayton *et al.*, 1998). The power profile measured by Clayton *et al.* (1998) demonstrates elastic energy storage and release, followed by active propulsion in the final part of the stance phase. A greater extension of the shoulder joint at hoof lift off after the abrupt change in surface condition may have assisted the propulsive phase of the next stride.

The *long head of the triceps* showed a reduction in contribution with each stride, which may also explain the reduction in shoulder flexion at hoof lift off. The reduced activity would suggest less eccentric work to stabilize the shoulder. The reduction in retraction suggests a higher COM position at hoof lift off and possibly less assistance from gravity to aid forward motion so it is envisaged that contributions from other muscles that were not measured may have increased. The *biceps brachii* for example, which is responsible for limb protraction has demonstrated activity at late stance during trot (Harrison *et al.*, 2012), suggesting a possible stabilising role. The *latissimus dorsi* retracts the limb (Payne *et al.*, 2004) and may also be implicated in co-contraction with the *biceps brachii* at the end of stance to improve postural control.

The contribution of the *triceps brachii* (cranial site) demonstrated a significant increase during the pre-activation window with each stride on the firm → soft surface condition. Pre-activation control has been identified as a key factor for altering leg posture in humans in preparation for altered ground properties (Müller *et al.*, 2010). This infers a change in limb placement at hoof impact may have occurred but no measurable kinematic changes were recorded. A study by Shinya *et al.* (2009) found muscle activity prior to touch down in humans did not change the positions or angles of body segments but may contribute to absorbing the impact of the actual touch down. Pre-activation in this project may represent more of a stabilising role. The *splenius* muscle showed a decrease in pre-activation with each stride. A lower magnitude suggests a reduced requirement to stabilise the head and neck but no corresponding changes were recorded in neck inclination.

Hind limb stride length reduced by 7 cm with a reduction in retraction as the horses made the transition from the firm to soft surface condition. Reducing the horizontal distance travelled by the hind DIPJ and impacting the ground sooner may have been a balancing strategy to help reduce the rotational effect of the falling forward posture. Upon further investigation of the data, it was evident that changes had been made to the hindlimb swing phase mechanics. The shorter stride length was achieved by a marginally higher vertical displacement of the hind DIPJ by 6 mm and a 2^{o} increase in peak tarsal flexion. This modification represents a possible feedback mechanism to maintain balance after the abrupt change in surface condition.

In a study on humans stepping down expected and unexpected level changes, stride length increased when the subjects were expecting a step down (van Dieen *et al.*, 2007). A greater step length was thought to assist in controlling forward horizontal and angular momentum. A different study by Müller *et al.* (2014) found subjects chose between a reduced step length or a more upright trunk orientation at touch down when encountering a step down to reduce the forward linear momentum of the body and prevent the upper body from falling forward. Small trunk deviations can significantly affect COM position and therefore trunk orientation at touch down is thought to play an important role in the control of balance in humans (Müller *et al.*, 2014).

Controlling trunk orientation has also been identified as an important balancing strategy in horses in order to maintain pitch stability (Hobbs and Clayton, 2013). There were no stride to stride postural changes at touch down or hoof impact when the horses moved from the firm to soft surface condition, however the reduced forelimb retraction at hoof lift off appeared to play a similar role at ensuring a more caudal positioning of the COM relative to hoof position and prevented a falling forward posture. Whist the change in surface condition in this project did not simulate a visual 'step down', the increased deformation of the soft surface certainly meant the horses stepped down off the firm surface condition. It is important to note the response of the humans differed to that of horses after the step down. Trunk inclination appeared to control posture at touch down but it was significantly more flexed at the end of contact on the step down (Müller *et al.*, 2014). This suggests the subjects had a more falling forward posture (Müller *et al.*, 2014) but the mechanisms used to oppose this posture after the second step was unknown. The different responses at the end of the contact phase suggest balancing strategies adopted by horses and humans differ after a perturbation.

Fore and hind limb retraction did not change when the horses travelled from the soft to firm surface condition unlike when the horses travelled from the firm to soft surface condition. Preventing a falling forward posture was perhaps more important as the horses moved onto the soft surface condition and so a different balancing strategy was anticipated when the horse travelled in the opposite direction. A more proximal alteration in limb posture was observed where scapula inclination at hoof impact increased by the first step on the firm surface. This indicates that the scapula was inclined further forward, closer to the horizontal. This may be expected because as the horses stepped 'up' onto the firm surface condition, the reduced deformability meant the hoof was impacting the ground higher up and therefore the scapula was inclined more forward. This could explain the stride to stride reduction in fore limb stride length by 9 cm, if the forward and downward phase of stance was physically stopped earlier by the step up.

Peak MCP extension demonstrated a small but significant reduction of 0.71° as the horses stepped onto the firm surface condition. In the absence of force data, peak MCP extension can be used to predict peak vertical forces with reasonably high accuracy (McGuigan and Wilson, 2003) and therefore this change suggests a marginally lower amount of force production. The cushioning values from the OBST data suggest the firm surface offered less cushioning (higher peak vertical force) so a greater peak MCP extension would be expected. So why did peak MCP extension reduce as the horses stepped onto the firm surface condition after the abrupt change? The change in surface condition initiated a different dynamic response in the horse and the data suggests vertical impulses within the forelimb changed. Impulses are the integral of a force acting on an object with respect to time. Stance duration within the fore limb did not change and therefore it is postulated that vertical impulses reduced due to the reduction in force.

Mean hind limb stride length also significantly reduced as the horses stepped onto the firm surface condition by 12 cm. This was accompanied by an increase in hind limb stance duration and duty factor unlike with the forelimb. Even though peak MTP extension did not change, suggesting no change in force production, the longer stance duration would enable the horse to generate greater vertical impulses within the hind limb (Hobbs *et al.*, 2014b). Impulse changes the momentum of an object and as a result a large force applied for a short period of time can produce the same momentum change as a small force applied for a long period of time. The longer stance duration in the hind limb suggests there was a redistribution of the proportion of vertical impulse between the fore and hind limbs, which may help pitch stability (Hobbs *et al.*, 2014) and enable the horses to make a smoother transition from one surface condition to another.

It is well documented that vertical impulses are greater in the forelimbs (Dutto *et al.*, 2004; Hobbs and Clayton, 2013) but this is during trot at a steady speed on a level, consistent surface. On an incline however, the vertical impulses have been shown to reduce in the fore limbs and increase in the hind limbs (Dutto *et al.*, 2004), which is supported by the findings of this project. It was proposed by Dutto *et al.* (2004) that this was due to the slight backward or downward shift of the torso of the horse on the incline and a greater force distribution to the hind limbs (Dutto *et al.*, 2004). Trunk inclination was not measured for this project but the change in distribution of the vertical impulses between the fore and hind limbs suggests the horses adopted a similar orientation as they stepped onto the firm surface condition.

10.2 Strategies observed when the horses were aware of the abrupt change

When the horses became aware of the transition from the firm to soft surface condition, fore and hind limb retraction showed a significant reduction. The changes in retraction angle for each horse according to surface condition and awareness are presented for the fore and hind limbs in Figure 9.7.1 and 9.7.2, section 9.7 respectively. There was a large degree of variation in forelimb retraction between subjects with at least four out of the seven horses adopting a 'falling forward' posture (more negative value) when there was no awareness during the first stride on the soft surface condition. This subsequently changed into a similar pattern for all horses in the presence of awareness where the COM position relative to foot position at hoof lift off was more caudal with each stride. Hind limb retraction reduced whether the horses were aware or not with the exception of horse four and six. Awareness may have had a larger influence on the forelimb in this case because this limb was the first to interact with the new surface condition.

The *long head of the triceps brachii* (cranial site), *extensor carpi radialis* and *trapezius cervicis* (cranial site) appeared to contribute to the postural changes recorded in the forelimb when the horses were aware of the change (Firm → soft). The *extensor carpi radialis* flexes the elbow and extends the carpus and generally demonstrates a low magnitude during stance (Jansen *et al.*, 1992). A greater active contribution from the *extensor carpi radialis* during stance was found with awareness, which corresponds with the reduction in forelimb retraction. A greater active contribution from the *trapezius cervicis* during stance was found with awareness. This acts to elevate the shoulder and draw the scapula forward, however no corresponding kinematic changes were observed. The increased activity may have played a stabilising role instead in order to maintain posture of the axial skeleton whilst the horses reduced the amount of limb retraction at hoof lift off. The contribution of the *long head of the triceps brachii* during the pre-activation window reduced with awareness, suggesting less eccentric work to stabilize the shoulder prior to hoof impact. This may explain the greater active contribution of the *ECR* during stance, which helped to stabilise and control limb posture from a more distal location. Contributions from other muscles that were not measured for this project should not be disregarded.

When the horses became aware of the transition from the soft to firm surface condition a significant reduction in hind limb protraction was recorded. Alterations in the angle of attack have been observed by Seyfarth *et al.* (2003) where leg retraction in humans prior to impact is thought to be a simple strategy to improve the stability of spring-mass running. The ability to select an angle of attack during running sustains a desired movement pattern and has been shown to enhance tolerance to ground disturbances (Seyfarth *et al.*, 2003). A study by Grimmer *et al.* (2008) found that running on uneven ground with a step up shortens the swing phase and decreases the angle of attack, which supports the changes observed in hind limb protraction in this project. Burn and Usmar (2005) also claimed that following equine limb protraction, the limb is accelerated in retraction, which decreases the velocity of the hoof relative to the ground prior to impact. Contributions from limb muscles such as the *ulnaris lateralis* and *triceps brachii* will have been required to actively retract and stabilise the limb prior to impact,

The reduced protraction observed in this project with awareness (soft → firm) may have been as a result of greater limb retraction prior to hoof impact. This response may have been an attempt to modulate the impact acceleration on the firmer surface and control the hoof-surface interaction to some degree. With awareness, it appears that this can be modified further in the hind limb. Fore and hind limb protraction was lower on day three overall when the horses made the transition from soft to firm in comparison to any of the other days by up to 5.7°. Based on the findings of Seyfarth *et al.* (2003), it can be postulated that more stabilisation of the system was required when the horses encountered a change in surface condition that involved a 'step up'.

When the horses were aware of the change from the firm to soft surface condition, hindlimb stride length reduced by 9 cm and this was accompanied with an increase in stance duration and duty factor. Forelimb stride length also reduced by 10 cm with awareness and was similarly accompanied with an increase in stance duration and duty factor. The temporal changes suggest the horses used all of the available balancing strategies reported by Hobbs *et al.* (2016) to remain stable. The greater duty factor implies the limb spent less time in swing during each stride. Longer flight times have been implicated in higher landing velocities and higher GRFs in humans previously (Cavanagh and Lafortune, 1980). The shorter stride length in conjunction with the shorter flight time may not only represent a more cautious gait but may also have a protective effect on gait stability.

Although speed was maintained, the longer fore and hind limb stance durations suggest that vertical impulses within the fore and hind limbs may have increased with awareness. A longer forelimb stance duration and duty factor and a shorter stride length has been reported in trotters on a deep wet sand track when compared to a firm wet sand track (Crevier Denoix *et al.*, 2010). Peak vertical ground reaction forces significantly reduced, which was attributed to the greater damping properties of the deep wet sand, so the increased stance duration was required to maintain impulse and therefore speed (Crevier

Denoix *et al.*, 2010). Peak MCP and MTP extension did not show changes with awareness, which suggests force production was maintained and therefore vertical impulses did indeed increase with awareness. An increase in vertical impulse of both the fore and hind limbs may have been required to maintain speed and move the COM forward when the horses encountered the change in surface condition if gravity could not be used as effectively to do this.

Temporal changes were only observed in the hind limb when the horses became aware of the abrupt change when moving from the soft to firm surface condition. Hindlimb stance duration and duty factor increased, suggesting greater vertical impulses with awareness. This may have been necessary to maintain pitch stability (Hobbs *et al.* (2014b) as suggested before. Peak MCP extension however, showed a marginal but significant increase with awareness, which demonstrates a slight change in the distribution of the vertical impulses between the fore and hind limbs as proposed before with the stride to stride changes. This would resemble a distribution consistent with horses moving on a level surface (Dutto *et al.*, 2004; Hobbs *et al.*, 2014b) rather than on an incline. Raising the COM during the second phase of stance is principally achieved by the forelimbs (Hobbs *et al.*, 2014b) and so the forelimbs may have played a larger role in raising the forehand with awareness of the abrupt change, whereas the hindlimbs played a larger role when horses were unaware of the change.

The ipsilateral contact time also showed a significant increase by more than two fold when the horses became aware of the change from firm to soft and soft to firm. This increase was also observed on the continuous firm surface condition when compared to the continuous soft surface and appeared to occur in conjunction with a longer stance duration and greater duty factor in the fore and hind limbs. This meant that the right hind limb hoof impact was occurring significantly earlier prior to the right fore hoof lift off. The increase in ipsilateral contact time appears to offset the longer stance duration within the fore and/or hind limbs and helps to maintain the same timing for the termination of hind limb stance under all surface conditions. The change in stance duration and ipsilateral limb contact timing in relation to awareness of the change in surface condition and on the continuous surface conditions is illustrated in figures 9.7.3, 4 and 5, section 9.7. If the surface induces temporal changes, is the ultimate aim to preserve the same timing for hoof lift off of the hind limb in order to prevent disruptions to gait? The earlier contact time of the hind limb with an increase in stance duration potentially means the timing of peak force production within the hindlimb relative to the ipsilateral forelimb occurs earlier with awareness and on the firm surface condition. This supports the third balancing strategy proposed by Hobbs *et al.* (2016) and also the claims made by Alexander (2002) where dynamic stability may be achieved by altering the timing of peak force production within the limbs.

Limb contact timing and sequence have a substantial effect on the magnitude of collisional losses (Hobbs *et al.*, 2016). In the gallop for example, collisional losses are reduced by using a limb sequence that distributes changes in the COM angular deflection between limbs (Ruina *et al.*, 2005). This reduces the net deflection angle during each contact (Ruina *et al.*, 2005). During trot, there is virtually no overlap between contacts and so there is little opportunity to reduce collisional losses by applying a pre-emptive thrust prior to the next contact (Bertram, 2013). The sequencing pattern is far more discrete during trot but there is evidence to show that collisional losses during absorption are lower with a hind-first dissociation (Hobbs *et al.*, 2016). The earlier impact time of the right hind limb prior to right fore hoof lift off observed consistently throughout this project, which occurred even earlier when the horses were aware of the change in surface condition, suggests there may be more scope to influence collisional losses experienced.

Although no temporal changes were observed in the forelimb with awareness on the soft to firm surface condition, stride length reduced by 11 cm. Greater carpal flexion was not observed with awareness so how did stride length reduce by such a large amount? Forelimb retraction was marginally lower and scapula inclination at hoof impact showed a significant reduction with awareness. This represents a more backward orientation of the scapula at impact and so more proximal alterations may have been responsible for the shortened stride length. It is interesting that the contribution of the *trapezius cervicis* (caudal site) muscle during stance increased from 53.52% to 82.01% with awareness. The *trapezius cervicis* elevates the shoulder and draws the scapula forward, which conflicts with the changes observed in scapula inclination. A stabilising role of the *trapezius* has been proposed by Payne *et al.* (2004) and so it is most likely that the increased activation was related to eccentric work to stabilise the shoulder when the horses were aware of the change.

The contribution of the *ulnaris lateralis* during stance significantly increased from 67.7% to 82% with awareness. This muscle acts down the posterior aspect of the forelimb to flex the carpal joint and extend the elbow. A greater contribution during stance may have helped to stabilise the limb like the *trapezius* muscle. The contribution of the *splenius* muscle during stance also increased by 10% with awareness. It was unfortunately not possible to measure neck inclination on this day due to having intermittent marker trajectories. A greater contribution may have been expected because the muscle elevates the head and neck and may have aided the transition as the horses stepped up on to the firm surface condition. The greater *splenius* activity is also thought to provide trunk stability and changes the spine into a rigid platform from which the limbs articulate (Rooney, 1982). This may have helped to improve pitch stability (Hobbs *et al.*, 2016) when larger vertical impulses were being applied by the hind limbs.

Feed-forward anticipatory control, intrinsic mechanical effects and reflex feedback all play important roles in the control of locomotion (Daley *et al.*, 2006). The prior experience and awareness of the horse influenced these control mechanisms, which was perhaps responsible for initiating larger changes in the biomechanical response of the horses than the actual stride to stride changes in surface condition. Recent experiences may allow the central nervous system to fine tune a general gait adjustment according to Heiden *et al.* (2006). Guinea fowl have previously shown that they were unable to compensate fully for an unexpected drop in substrate height but were successful in maintaining overall dynamic stability (Daley *et al.*, 2006). The guinea fowl adopted a different strategy when encountering a visible drop and were capable of completely adjusting limb mechanics within the perturbed step to maintain COM trajectory but only if they accurately anticipated the change based on visual information (Daley *et al.*, 2006).

Awareness has also shown to influence hopping (Moritz and Farley, 2004) and running (Müller *et al.*, 2012) mechanics in humans. When humans expect an increase in surface stiffness during hopping, hop height, knee flexion and leg muscle activation increases before contact in order to compensate for the change in surface condition (Moritz and Farley, 2004). When runners became aware of a perturbation in ground level, they can adjust their leg parameters to the visually estimated requirements during the stride before the perturbation (Müller *et al.*, 2012). The effect of a visual change in surface condition was not investigated during this project. Postural changes in fore and hind limb inclination and alterations to the vertical impulses were recorded with awareness, regardless of the change being camouflaged, which suggests that control mechanisms were in place to help maintain dynamic stability.

<u>10.3 Can horses alter limb stiffness as a function of surface stiffness like humans can?</u>

Muscle activation patterns in humans change in response to different surface conditions in order to tune the limb properties (Müller *et al.*, 2010). Greater muscle activity during human jumping has been recorded prior to touch down, which results in an increased stiffness of the lower extremities and reduced the vertical downward displacement of the

COM (Arampatzis *et al.*, 2001). This can be represented as an increase in the stiffness of the leg spring presented in figure 10.1.1. Changes in human running mechanics can also be achieved through altering the stiffness of the leg spring, which enables them to run over changing surfaces with ease and cope with irregularities in the surface condition (Müller *et al.*, 2010).

When encountering sudden changes in surface stiffness or damping (Ferris and Farley, 1997; Ferris *et al.*, 1998; Kerdok *et al.*, 2002) or uneven ground with changes in terrain height (Grimmer *et al.*, 2008) there is strong evidence to show that humans use spring-mass dynamics to help passively stabilise their locomotory trajectory (McMahon and Cheng, 1990). It is possible for humans to increase limb stiffness during running to offset reductions in surface stiffness (Ferris *et al.*, 1998; 1999). Leg stiffness of human runners has been recorded to alter by as much as 68% on surfaces with different stiffnesses (Ferris *et al.*, 1998). Another study by Ferris *et al.* (1999) investigated the effect of an abrupt change in surface stiffness on limb stiffness during running. There was evidence to show that the runners could alter their limb stiffness as they made their first step onto the new surface when they were aware of the change. Limb stiffness reduced by 29% as surface stiffness increased by more than 25-fold, and increased by 20% as the surface stiffness reduced. Maintaining the trajectory of the COM by conserving the combination of leg-surface stiffness is considered to be an important control strategy in humans (Ferris *et al.*, 1999; Moritz and Farley, 2003).

If horses have the ability to maintain a consistent sinusoidal COM trajectory when encountering an abrupt change in surface condition like humans, the energetic cost of moving the COM position forward and upward must increase to offset differences in surface compression. Current research suggests that horses cannot alter fore limb stiffness due to their large passive properties (McGuigan and Wilson, 2003). The muscle fibres relative to tendon length within the limbs are short, which means there is potentially less scope for control within the system (McGuigan and Wilson, 2003).

Based on the cushioning values from the OBST and the maximum displacement recorded with the linear potentiometer, the average surface stiffness was 1025.5 kN/m and 919.6 kN/m (10% reduction) for the firm and soft surface condition respectively. The horses moved at a steady state trot during data collection so what mechanisms were present to help offset changes in surface stiffness?

Changes in limb length may constitute changes in limb stiffness based on the calculation presented in section 6.0.3. There was more variation in the fore and hind limb length changes recorded when there was no awareness of the change in surface condition, which suggests that the individual horses used different balancing strategies. Variation subsequently reduced with awareness, which was a similar observation made with limb retraction in section 10.1 and suggests a more consistent balancing strategy had been adopted. It has been suggested by McGuigan and Wilson (2003) that the proximal spring acts in series with the predominantly passive distal spring to tune the properties of the whole limb for locomotion under varying conditions. This is in agreement with the results from this project where a greater length change of the distal spring appeared to coincide with a smaller length change of the proximal spring except when the horses travelled from the soft to firm surface condition. The ipsilateral hind limb showed a very similar pattern of length change to the proximal spring, suggesting this may have also played a role in maintaining the properties of the whole system as the horses travelled forward. Vertical length changes will have occurred as a direct result of a change in the joint angles. Peak MCP extension, which significantly reduced when the horses made the transition from the soft to firm surface condition had the potential to influence the maximal compression values of the distal spring. The total fore limb length change did indeed demonstrate a significant reduction with peak MCP extension.

The neuromuscular contributions may have also played a role in influencing the length change of the fore and hind limbs. A reduction in *triceps brachii* (cranial site only) muscle activity during stance was observed with each stride (76.41% →67.84% →63.05%) as the horses made the transition from the firm to soft surface condition. The reduction was only significant between the first and second stride after the change onto the soft surface condition. A reduction in activity would suggest more elasticity of the proximal spring and may explain the marginal increase in total fore limb length change with each stride. The contribution of the *triceps brachii* (cranial site) during pre-activation showed an increase with each stride on the same surface condition (70.5% → 74.13% → 77.19%). This muscle may have been responsible for stiffening the proximal spring prior to hoof impact and resisting a greater length change with awareness, providing further support that this spring may have a tuning effect on the limb. An increase in pre-activation of selected limb muscles has also enabled humans to increase stiffness of the lower extremities in preparation for ground contact and reduce vertical downward displacement of the COM (Arampatzis *et al.*, 2001).

The contribution of the *triceps brachii* (caudal site) during pre-activation on the continuous soft surface also significantly reduced (79.97% → 70.22%) during the second stride. This however, did not coincide with a more elastic spring and a greater length change of the proximal spring from hoof impact to peak MCP extension. No stride to stride changes were recorded on the other surface conditions even though the length changes were of a similar magnitude. It is possible that contributions from other muscles influenced the length changes observed. The *ulnaris lateralis* for example, which extends the elbow and flexes the carpal joint showed a large increase during stance (78.51% → 93.17%) between stride one and two on the continuous soft surface. This may have played a compensatory role for the reduction in pre-activation within the *triceps brachii*. It is also important to acknowledge the effects of skin displacement around the proximal markers used to calculate the length changes from (van Weeren *et al.*, 1990; Leach and Dyson, 1998). The kinematic filtering techniques employed will have reduced this issue as much as possible but some of the length change recorded may still be as a result of skin displacement.

Whilst some length changes were observed, effective fore and hind limb stiffness showed no stride to stride changes on any surface condition. Effective fore limb stiffness significantly increased with awareness of the abrupt change from firm to soft, however this was not in response to a change in surface stiffness. The results from this project support initial claims, where the mechanisms responsible for gait adaptations differ between the human and equine species.

10.4 Observations on the continuous surface conditions

Our current knowledge surrounding the biomechanical response of the horse to synthetic surfaces is based on steady-state locomotion without any induced perturbations in the surface conditions. It was important to assess the horses on a continuous surface for this project in order to obtain baseline data as a reference and to enable comparisons with other literature. The limited stride to stride changes on the continuous surfaces further supports the results found when there was an abrupt change in surface condition. Whilst limited variations were reported on the same surface condition, the two continuous surfaces induced a different biomechanical response.

The speed on the continuous soft surface condition was significantly slower than on the firm surface condition by 0.23 m/s (3.57 m/s vs. 3.8 m/s respectively). This difference is smaller than the standard error recorded during some studies within treatment groups (Gustås *et al.*, 2006; Robin *et al.*, 2009; Chateau *et al.*, 2010; Hobbs *et al.*, 2016), suggesting that speed had a minimal effect on the differences reported in this section. A study by Robert *et al.* (2002) also suggested that variations in velocity of 0.5 m/s and above result in significant changes in equine locomotion, further reinforcing this claim.

10.4.1 Kinematic response

All of the temporal kinematic parameters were lower in magnitude on the continuous soft surface condition in comparison to the continuous firm surface. Stance duration reduced by approximately 3% in both the fore (10 ms) and hind limbs (13 ms) on the soft surface. Stance duration is speed dependent where a longer contact time is generally recorded at slower speeds (Crevier Denoix *et al.*, 2010; Chateau *et al.*, 2010). Why did the results from this project differ to previous findings?

Stance duration has also been shown to be influenced by surface properties. A significantly longer stance duration has been recorded on a deep, wet sand track when compared to a firm wet sand track (Chateau *et al.*, 2010). Although not significant, the speed was also slightly slower on the deep wet sand track (6.65 m/s vs. 7.20 m/s), which may have contributed towards the longer stance duration (Chateau *et al.*, 2010). Horses have been tested at the same speed on three different surface types by Burn and Usmar *et al.* (2005). An increase in stance duration was recorded with an increase in surface deformability, which demonstrates that the surface properties alone can have an impact (Burn and Usmar, 2005). The longer stance duration could be explained by a greater longitudinal braking duration (Chateau *et al.*, 2010) because the time before the hoof completely stabilised after the primary impact is longer on the surfaces with more deformation. The findings from this current project conflict with the results from other work where a longer stance duration was recorded when the horses were travelling marginally faster and on a surface with less deformation. Duty factor also reduced in conjunction with stance duration, suggesting the fore and hind limbs spent longer in swing on the soft surface condition. It is important to acknowledge that the sub-base under the research test track and the way in which the soft surface condition had been prepared may have been responsible for some of the differences observed. The surface, which was laid to a depth of 150 mm had been tillered to a depth of 100 mm to create the soft surface condition. This meant that the horses were travelling through the top 100 mm of the surface, however the remaining 50 mm of surface was extremely compact. This in conjunction with the permavoid sub-base may have provided a base of support for the horses during limb loading.

The surface construction should not detract from the fact that the soft surface cushioning was significantly greater and impact firmness was significantly lower than for the firm surface. The responsiveness value recorded on the soft surface condition was lower than on the firm surface condition. The rebound timing of the soft surface during unloading was therefore slightly longer, which would be expected due to a reduction in stiffness (lower peak force and greater deformation). This may have represented a time closer to the point at which the heels elevated from the surface during breakover and provided an ‘active’ feel to the surface (Hobbs *et al.*, 2014a). The altered surface rebound may be closer to the natural frequency/tuning of the horse’s limbs and actually optimised performance by reducing stance duration on the soft surface. An improved performance has been recorded in humans on a running track with greater compliance previously by optimising the surface stiffness relative to the runner’s stiffness (McMahon and Greene, 1979). This was evidenced by a shorter stance duration and longer step length. These findings were also accompanied with speed enhancement, as maximal running speed increased. In this study however the goal was to maintain a consistent, rather than a maximal speed, so it is more difficult to interpret in relation to performance enhancements. That said, the greater responsiveness of the firm surface meant that the surface may have been rebounding when the limb was still in contact with the ground. This would not assist in elevating the hoof from the ground during breakover but instead would provide a ‘dead’ feel to the surface (Hobbs *et al.*, 2014) and may represent additional force that must be dissipated by the limbs (Ratzlaff *et al.*, 1997).

Peak MCP and MTP extension showed a large reduction (2^{o} and 5.69^{o} respectively) on the soft surface. This suggests that peak forces within the limbs were lower (McGuigan and Wilson, 2003) and can be further supported by the lower peak load values recorded with the OBST. Peak MCP (Northrop *et al.*, 2013) and MTP (Symons *et al.*, 2013) angles have shown to alter in response to loading previously, which strengthens claims that the change in peak MCP and MTP angle is closely linked to peak forces generated by a surface.

The ipsilateral contact time was much lower on the soft surface condition, suggesting the hind limb impacted the ground closer in time to right fore hoof lift off. The stride length in the hind limb was shorter on the soft surface condition, so why did the hoof impact the ground later? The longer swing duration of the right hind limb suggests there may have been greater vertical displacement of the marker trajectories. After exploring the swing phase kinematics of the hind limb further, peak tarsal flexion during swing was 10.07^{o} greater on the soft surface condition, which would suggest that the vertical trajectory of the hind distal interphalangeal joint (hind DIPJ) was higher (Figure 9.7.6, section 9.7). Upon observing the data, the vertical height of the hind DIPJ during swing was indeed 20 mm higher on the soft surface condition. A higher flight arc may represent a greater active contribution of the hind limb muscles (Clayton *et al.*, 2011) in response to the more cushioned soft surface.

10.4.2 Neuromuscular response

It was important to establish how the overall magnitude of muscle activity differed in order to reveal the active contribution required to sustain locomotion on the two continuous surface conditions. The way in which the data had been normalised does pose a limitation when comparing data from different days. It is possible to obtain the average contribution of the muscle on a particular surface condition by looking at the normalised values, however this does not reveal how the magnitude differs between days. To address this issue, the percentage difference in magnitude during a static trial was subsequently calculated relative to the firm surface condition (Table 9.7.1, section 9.7).

The baseline magnitude of the *splenius* muscle on the soft surface was 71.84% of the magnitude recorded on the firm surface condition, which suggests that a greater active contribution was required on the firm surface condition. The *splenius* muscle elevates the head and neck and is claimed to be a functional stabiliser of these structures against gravitational forces (Robert *et al.*, 2002; Zsoldos *et al.*, 2010; Kienapfel, 2015). Based on the muscle function and results from this project, it would be expected that the neck inclination was higher on the firm surface condition. Whilst there were no significant differences between neck inclination at hoof impact and lift off on the two continuous surfaces, the neck was indeed inclined consistently higher on the firm surface condition.

The neck inclination did not alter more than 20 degrees on each surface condition, suggesting the neck was rotationally stabilised in the pitch plane during pre-activation and stance (Dunbar *et al.*, 2008). This was during steady state locomotion without any surface perturbations, so this was expected. Dunbar *et al.* (2008) found neck posture shifted to a more vertical orientation with the transition from slower to faster gaits. The gait remained the same in this project, however a marginally faster speed was recorded on the continuous firm surface, which may contribute to the differences observed. At faster speeds, the passive forces exerted on the head and neck increase and so it has been suggested that greater muscle activity of the *splenius* counteracts these larger forces (Robert *et al.*, 2002; Zsoldos *et al.*, 2010).

The *trapezius cervicis* showed a much lower magnitude on the soft surface condition where the baseline magnitude on the soft surface was 24.46% and 45.96% of the magnitude on the firm surface at the cranial and caudal sites respectively. The *trapezius cervicis* draws the scapula forward and up, which may explain why the scapula was inclined further forwards at hoof impact and lift off on the firm surface condition. The *trapezius* is also thought to play a role in carrying the head and neck against gravity and may support the action of the *splenius* (Kienapfel, 2015). The *triceps brachii* showed a much lower magnitude on the soft surface condition where the baseline magnitude was 16.13% and 61.44% of the magnitude on the firm surface at the cranial and caudal sites respectively. The long head of the *triceps brachii* flexes the shoulder joint and extends the elbow, which would contribute to limb retraction. During the stance phase, eccentric contractions within the *triceps brachii* also help to stabilise the elbow, counteracting the effects of inertia (Robert *et al.*, 2002). This may explain the increase in shoulder flexion at hoof impact and lift off and greater forelimb retraction at hoof lift off on the firm surface condition.

There was a distinct divide in the magnitude of the distal and proximal muscles on the two surface conditions (Table 9.7.1). The proximal muscles required more active contribution on the firm surface and this appeared to coincide with the kinematic changes observed. The magnitude of muscle activity in the *extensor carpi radialis* and *ulnaris lateralis* was much greater on the soft surface condition (423.91% and 493.60 % respectively). The *extensor carpi radialis* extends the carpus and flexes the elbow joint whereas the *ulnaris lateralis* has the opposite action. Co-contraction of the *extensor carpi radialis* and *ulnaris lateralis* was recorded. Simultaneous activation of muscles with actions that oppose one another, suggests the muscles played a role in stabilising the limb. This was speculated by Jansen *et al.* in 1992 and since then, Harrison *et al.* (2012) has drawn much stronger conclusions suggesting co-contraction improves joint stability and positional control of the limb. A study by Tokuriki (1979) found that canine *extensor carpi radialis* muscle activates before foot strike to decelerate the limb and stabilise the carpus prior to ground contact. This could also explain the increase in pre-activation of the *extensor carpi radialis* on the soft surface condition.

A study by Wang *et al.* (2014) found muscle activity in humans altered according to surface stiffness. An increase in muscle activity of the biceps femoris and rectus femoris was recorded on a stiff, concrete surface when compared to grass. It was thought that a greater active contribution was required from the muscles in order to support the limb when experiencing higher forces (Wang *et al.*, 2014). This may explain the greater magnitude recorded on the firm surface condition in this project but why was the same trend not seen in the more distal muscles? A different study by Pinnington *et al.* (2005) did find an increase in EMG amplitude on a soft, dry sand surface in comparison to a firm, wooden surface. The soft surface was comparable to soft beach sand and so it is envisaged that this surface was not at all supportive during running, leading to an increase in propulsive effort and larger muscle contributions to sustain the same speed. This is an important consideration during lameness assessments since softer surfaces are usually used to identify muscular issues. This may enable assessments of the distal muscles based on the data presented here but the greater baseline activity of the proximal muscles on the firm surface suggests muscular issues may also be revealed on a hard surface.

10.5 Limitations of the project

There were some limitations of this project surrounding study design and statistical analysis, which required consideration. A Latin Square design was considered the best study design for this project but it was unfortunately not possible to randomise the order of surface conditions for each horse. This was due to the difficulty in creating a firm surface condition again once the surface had been prepared with the tiller. The length of time to collapse and set up the equipment on the other side of the test track to collect data from the horses moving from the soft to firm condition (Plate 5.2.3) was also a limiting factor.

The data from this project was analysed using univariate analyses. This inherently increases the risk of a type II error where the null hypothesis can be wrongly accepted. Statistical power calculations were performed to test the robustness of the study design. A large power was generated (0.9 – 1.0), suggesting the analyses used was sufficient to detect a practically important difference. The statistical power reduced for the 'not aware' treatment group due to this containing data from just the first trial. A greater sample size will have increased the power but this was not possible due to time constraints and horse availability. Multivariate analysis may have been a better statistical approach to understand the interaction between several kinematic and electromyographic parameters. This was not possible however, due to missing data points within the data set. The normalised iEMG data in particular had large gaps due to several trials being discarded with poor signal quality.

The signal quality of the EMG data was variable at times. Surface EMG data is renowned for noise contamination whether it originates from movement artefact, cross talk or an inconsistent skin-electrode interface. Signals with poor quality were rejected from further analysis but this did reduce the data set. The *extensor carpi radialis* and *ulnaris lateralis* signals were most affected, possibly because of the more distal location and greater sensor movement expected during locomotion. Fine wire needle EMG may have improved signal quality and reduced the number of discarded files. This technique has been associated with changes in motion due to mechanical resistance (Zsoldos *et al.*, 2010) and was therefore deemed unsuitable for this project.

Changes in muscle contributions did not always correspond with the kinematic alterations. A study by Müller *et al.* (2010) found that the presence of muscle activity correlated well with kinematic and kinetic changes in humans. Equine musculature in comparison to humans is very different and the ability to collect clean signals was challenging, which may explain why a strong link between neuromuscular and kinematic changes was not observed in this project. Factors such as skin thickness, distribution of sweat glands and subcutaneous fat can also lead to large variations in the sEMG signal (Nordander *et al.*, 2003). Despite a strong correlation being found by Müller *et al.* (2010) between muscle activity patterns and kinematic parameters, the authors still reported a high degree of variability within and between participants. When single experiments (individual trials) were investigated further by Müller *et al.* (2010) these correlations were highly significant. Individual trials were not explored further in this project in terms of muscle activity and kinematic changes but this may have provided a better understanding of the individual responses.

It is important to note that some of the changes in muscular contributions in this project were implicated in eccentric muscular control and therefore were expected to have a stabilisation effect. This assisted postural control during the stance phase of the stride and provides further support for hypothesis three. A different study by van der Krogt *et al.* (2009) found changes in human mechanics do not always result from a change in muscle activity. Leg stiffness was shown to passively adjust to unexpected surface changes. The limited neuromuscular changes to support all of the kinematic changes in this project suggests some of the gait modifications may have been a passive response, such as the change in scapula inclination at hoof impact when the horses made the transition from the soft to firm surface condition. A full electromyographic profile was not recorded during this project and it is important to acknowledge the possible contributions of other muscles to the kinematic changes reported.

The *biceps brachii* for example has been reported to be a large contributor to limb protraction (Payne *et al.*, 2004), however fine wire needle EMG is required to measure this muscle, which may create changes in motion due to mechanical resistance (Zsoldos *et al.*, 2010). The digital flexors and *supraspinatus* are also claimed to help resist gravitational and inertial forces (McGuigan and Wilson, 2003). The muscles of the back and hindlimb were not assessed during this project, which may have explained some of the postural changes. The *latissimus dorsi* is a large contributor to forelimb retraction with the potential for a high degree of power production (Payne *et al.*, 2004) whilst the long digital extensor muscle may have contributed to the greater peak tarsal flexion recorded on some of the surface conditions. Another factor to consider, which was not measured in this project, is the role of the trunk in postural control. It was suggested that trunk inclination altered as the horses made the transition from the soft to firm surface condition with changes in the distribution of the vertical impulses between the fore and hind limbs. Trunk receptors make important contributions to spatial orientation for balance and posture (Dunbar *et al.*, 2008). Rotation of the trunk in the pitch plane is minimal at trot compared to walk and canter, suggesting it is rotationally stabilised (Dunbar *et al.*, 2008).

10.6 General discussion

The abrupt change in surface condition initiated a different biomechanical response in the subjects depending on the direction of travel. Stride to stride modifications in some of the angular and linear parameters were observed in both directions. Stride to stride temporal changes were only observed in the hind limb when the horses made the transition from soft to firm. It was possible to conclude that horses do not alter limb stiffness as a function of surface stiffness in the same manner as humans. There was evidence however that horses can alter the angle swept by the limb during stance by altering the amount of fore and hind limb retraction at hoof lift off on the firm to soft surface condition, which is one of the balancing strategies used by trotting horses (Hobbs *et al.*, 2016).

Hind limb posture at hoof impact has been found to differ with respect to surface type at racing speeds up to 17 m/s (Symons *et al.*, 2013). A straighter fetlock configuration was reported on a synthetic track versus a dirt track, which was associated with reducing the strains on the elastic structures supporting the MTP joint (Symons *et al.*, 2013). These findings were consistent with human athletes who have shown to reduce hip, knee and ankle flexion at touch down and increase leg stiffness during hopping on more damped surfaces (Moritz *et al.*, 2004). To allow for comparisons, the MTP angle recorded at hoof impact during this project is presented in table 9.7.2, section 9.7. Symons *et al.* (2013) reported a heel strike angle of 178 º (mean speed: 15.8 m/s) on the synthetic surface and 192 º (mean speed: 15.7 m/s) on the dirt surface. The speed and completely different surface types used may be responsible for such a large change in comparison to the data presented here. At slower speeds on an arena surface with perhaps more subtle changes in composition, the horses do not appear to employ stride to stride changes in MTP posture at hoof impact in order to cope with the different surface conditions.

A study by Northrop *et al.* (2013) has reported a change in MCIII roll inclination (adduction) at hoof impact on a harrowed vs. rolled arena surface. The different surface preparations presented much more subtle differences in the functional surface properties and it was proposed that this may be a proprioceptive alteration to the different surface preparations (Northrop *et al.*, 2013). Horses have demonstrated the ability to alter hoof placement relative to the body, which will affect limb adduction (Hobbs *et al.*, 2011). Northrop *et al.*, (2013) also found greater MCIII adduction during the latter part of stance, especially in the canter. It was suggested that this may provide better dynamic balance since during a single support of the leading limb at canter, the sternum moves closer to the hoof in the mediolateral plane bringing the COM over the base of support (Northrop *et al.*, 2013). This was not measured during this project but demonstrates that different functional strategies exist within the horse under different experimental conditions.

The presence of awareness induced a greater change in the biomechanical response of the horses in this project, providing support for hypothesis five. The subjects appeared to adopt a more cautious gait by reducing stride length in the fore and hind (firm → soft only) limbs and increasing stance duration and duty factor in the fore (firm → soft only) and hind limbs. These changes may enable the horse to cope with the surface disruption but comes at the cost of reduced performance quality based on the subjective assessments reported by Holmstrom *et al.*, (1994). Contact timing of the ipsilateral limb pair consistently altered in response to the change in stance duration and duty factor. Timing increased with an increase in stance duration, which implies that right hind hoof impact occurred earlier prior to right fore hoof lift off. This resulted in a comparable timing for hoof lift off of the right hindlimb under all conditions. Hindlimb contact timing therefore appears to be an important balancing strategy during trotting, considering it was observed on the continuous surfaces and when the horses encountered the abrupt change in both directions. This also supports the findings of Hobbs *et al.* (2016) and hypothesis one of this project.

The current project demonstrates that there can be differences in muscle activity patterns within the same muscle. The contributions recorded at the caudal and cranial site of the *trapezius* cervicis differed in the majority of trials, especially during pre-activation. More consistency was observed at the two sites within the *triceps brachii* muscle, where approximately 70% of the trials showed a similar trend in muscle contribution. The influence of cross talk from neighbouring muscles cannot be eradicated. The *rhomboideus cervicis* underlies the *trapezius cervicis* and the cranial site on the *long head of the triceps brachii* was relatively close to the *lateral head*, which could have affected the signals recorded. It is possible that the difference in contributions at the two sites may represent a change in muscle function. Regional variations have shown to occur in the activation of the equine *longissimus dorsi* previously (Wakeling *et al.*, 2007), which demonstrates a difference in function within the same muscle. It has been postulated by Wakeling *et al.* (2008) that local activity is linked via the local action of stretch reflexes from the muscle spindles but the mechanisms that control the regional variations in activity is still unknown. Based on this, researchers should consider measuring two or more sites on muscles with a significant surface area in future work. Making comparisons between studies should also be done with caution because it is only possible to obtain a 'snapshot' of muscle function with the EMG sensor during a particular activity for larger muscles.

Once the subjects were aware of the change in surface condition (trial 2-8), it was anticipated that more changes in muscle activity may have been observed in the pre-activation window. A change in pre-activation has been recorded in human runners previously when they were visually aware of a change in step height (Müller *et al.*, 2010). Pre-activation control was responsible for altering leg posture in preparation for altered ground properties (Müller *et al.*, 2010). The muscles measured in this project showed little change during the pre-activation window and the caudal site of the *triceps brachii* was the only location to show a change with awareness. An increase in muscle contribution may have been expected based on the findings of Müller *et al.* (2010) but instead a reduction in activity was observed. This is expected to represent a reduction in the amount of eccentric muscular work required to position the limb at hoof impact and a possible improvement in efficiency of this particular muscle with awareness.

10.7 Conclusion

Contrary to current thinking, horses can make stride to stride modifications. Changes were subtle but still significant nonetheless. There is evidence to suggest that horses can maintain dynamic stability to prevent disruptions to locomotion, which supports some of the hypotheses proposed. When the horses made the transition from the firm to soft surface condition, postural changes were recorded, which were influenced by differences in contributions from the *triceps brachii* and *splenius*. Fore and hind limb retraction reduced, which demonstrated a balancing strategy and was associated with reducing a falling forward posture. During the step up from the soft to firm surface condition, the horses demonstrated a posture that was consistent with moving on an incline. Temporal changes within the hind limb suggested vertical impulse had increased, which was thought to aid the transition between the two surface conditions.

The horses adopted slightly different strategies when they were aware of the change, some of which was supported by an increase in muscular contributions, predominantly during stance. When the horses made the transition from the firm to soft surface condition, a reduction in fore and hind limb retraction was still evident and vertical impulses also increased within both limbs. When the horses made the transition from the soft to firm surface condition, a change in the distribution of vertical impulses between the fore and hind limbs was expected based on the kinematic data. The horses appeared to increase vertical impulses within the forelimbs, which more closely resembled the strategy adopted when travelling on a level surface. The longer stance duration recorded with awareness and on the continuous firm surface increased the ipsilateral contact timing. The earlier right hindlimb hoof impact meant the timing of peak force production relative to the ipsilateral fore limb was earlier and may have been implicated in improving dynamic stability.

To translate the findings of this project to an arena setting, maintaining uniformity and consistency of the functional properties in order to reduce spatial variations is essential. Based on the results presented here, a highly variable surface would require frequent stride to stride modifications, which has the potential to increase injury risk especially if the horses are unaware of the changes present. The horses were measured with one abrupt change in this project and it is still unknown how many alterations a horse can adapt to and tolerate before tripping or falling occurs. The data was collected from un-ridden horses and different results may be observed in the ridden horse as a result of aids being applied by the rider. Cues from the rider to maintain gait and posture may influence the horse's ability to adapt and therefore future work should focus on assessing the effect of several changes in surface condition on the ridden horse.

Maintaining the uniformity of surfaces used for high speed activities is also absolutely paramount based on the results from this project. At high speeds, it is envisaged that the horse will have to work harder to maintain dynamic stability. The angle swept by the limb during stance is greater, in order to flatten the COM trajectory. When suddenly encountering a softer surface at speed, a greater and possibly more rapid reduction in limb retraction to reduce the impact of a falling forward posture may be required to maintain balance. It is anticipated that an increase in the active contribution of the muscles controlling neck inclination such as the *splenius* and limb retraction such as the *latissimus dorsi* and the *extensor carpi radialis,* will be required to help control posture and limb placement. This would hasten the onset of muscular fatigue and increase the risk of injury. Since stance duration reduces with increasing speed, a longer stance duration to increase vertical impulses may be associated with reducing speed. If this occurred during racing, this would have a significant impact on performance, which highlights the importance of consistent going.

It is more likely that horses will be aware of changes in surface conditions during training due to the repeated exposure to that particular surface. The modifications observed with awareness appear to more favourable in terms of the horses being able to re-balance to the change in surface condition. The muscles that demonstrated a change in contribution during stance all increased in activity with awareness however. This suggests a greater active contribution will be required to support the postural changes. Horses that are unfit or performing intense training sessions that they are not conditioned for will particularly be at risk of fatigue induced injuries.

At competition, it is unlikely that horses are aware of changes in the surface conditions. A lack of awareness was implicated in a more falling forward posture when encountering a change to a soft surface condition and more generally lower vertical impulses within the fore and hind limbs. This may increase the risk of tripping or falling, especially at faster speeds. As horse traffic increases throughout the competition, it is envisaged that surface consistency will reduce, which potentially increases the risk further for horses competing later on in the day. This highlights the importance of maintenance throughout the competition to improve surface uniformity.

Ackland, D. C. and Pandy, M. G. (2009) Lines of action and stabilising potential of the shoulder musculature. *Journal of Anatomy*, **215**, 184-197.

Alexander, R.M. (1988) *Elastic Mechanisms in Animal Movement*. Cambridge University Press, Cambridge.

Alexander, R.M. (2002) Stability and manoeuvrability of terrestrial vertebrates. Integrated and Comparative Biology **42**,158-164 DOI 10.1093/icb/42.1.158.

Arampatzis, A., Brüggemann, G. –P. and Klapsing, G. M. (2001) Leg stiffness and mechanical energetic processes during jumping on a sprung surface. *Medicine and Science in Sports and Exercise*, 923-931.

Back, W. and Clayton, H. M. (2013) *Equine Locomotion*. 2nd ed. Philadelphia: Elsevier Saunders.

Back, W., Schamhardt, H. C., Hartman, W. and Barneveld, A. (1995) Kinematic differences between the distal portions of the forelimbs and hindlimbs of horses at the trot. *Am J Vet Res*, **56** (11), 1522-1528.

Barrey. E., Landjerit, B and Walter, R. (1991) Shock and vibration during hoof impact on different surfaces. *Equine Exercise physiology*. 3. 97-106.

Bertram, J.E.A. (2013) Gait as solution, but what is the problem? Exploring cost, economy and compromise in locomotion. *Veterinary Journal Supplement,* **198**:e3_e8 DOI 10.1016/j.tvjl.2013.09.025.

Biewener, A. A. (1998) Muscle-tendon stresses and elastic energy storage during locomotion in the horse. *Comparative Biochemistry and Physiology Part B*, **120**, 73-87.

Biewener, A. A. (2006) Patterns of mechanical energy change in tetrapod gait: pendula, springs and work. *J. Exp. Zoolog. A Comp. Exp. Biol.* **305**, 899-911.

Blickhan, R., Seyfarth, A., Geyer, H., Grimmer, S., Wagner, H. and Günther, M. (2007) Intelligence by mechanics. *Philos. Trans. A,* **365**, 199-220.

Boye, J. K., Thomsen, M. H., Pfau, T. and Olsen, E. (2014) Accuracy and precision of gait events derived from motion capture in horses during walk and trot. *Journal of Biomechanics*, **47** (5), 1220-4. doi: 10.1016/j.jbiomech.2013.12.018

Brown, N. A. T., Kawcak, C. E., McIlwraith, C. W. and Pandy, M. G. (2003) Architectural properties of distal forelimb muscles in horses, *equus caballus. Journal of Morphology*, **258**, 106-114.

Burn, J. F. and Usmar, S. J. (2005) Hoof landing velocity is related to track surface properties in trotting horses. *Equine and Comparative Exercise Physiology*, **2**(1), 37–41.

Burn, J. F., Wilson, A. and Nason, G. P. (1997) Impact during equine locomotion: techniques for measurement and analysis. *Equine Veterinary Journal Supplement*, **23**, 9-12.

Cavanagh, P. R. and Lafortune, M. A. (1980) Ground reaction forces in distant running. *Journal of Biomechanics,* **13** (5), 397-406.

Cham, R. and Redfern, M. S. (2002) Changes in gait when anticipating slippery floors. *Gait and Posture*, **15**, 159-171.

Chateau, H., Camus, M., Holden-Douilly, L., Falala, S., Ravary, B., Vergari, C., Lepley, J., Denoix, J-M., Pourcelot, P. and Crevier-Denoix, N. (2013) Kinetics of the forelimb in horses circling on different ground surfaces at the trot. *The Veterinary Journal*, **198**, 20-26.

Chateau, H., Holden, L., Robin, D., Falala, S., Pourcelot, P., Estroup. P., Denoix, J.-M. and Crevier-Denoix, N. (2010) Biomechanical analysis of hoof landing and stride parameters in harness trotter horses running on different tracks of a sand beach (from wet to dry) and on an asphalt road. *Equine Veterinary Journal*, **42** (38), 488-495.

Chateau, H., Robin, D., Falala, S., Pourcelot, P., Valette, J-P., Ravary, B., Denoix, J.-M. and Crevier-Denoix, N. (2009) Effects of a synthetic all-weather waxed track versus a crushed sand track on 3D acceleration of the front hoof in three horses trotting at high speed. *Equine Veterinary Journal,* **41** (3), 247-251.

Clayton, H. M. (1989) The effect of an acute hoof wall angulation on the stride kinematics of trotting horses. *Equine Veterinary Journal Supplement*, **9**, 86-90.

Clayton, H.M. (1994) Comparison of the smde kinematics of the collected,working, medium and extended trot in horses. ***Equine vet. J.*** **26**, 230-234.

Clayton, H. M. (2004) The Dynamic Horse: A biomechanical guide to equine movement and performance. Mason, Sports Horse Publications.

Clayton, H. M., Chateau, H. and Back, W. "Forelimb function". *Equine Locomotion*. W. Back and H. C. Clayton. 2nd ed. Philadelphia: Elsevier Saunders, 2013. 55-76. Print.

Clayton, H. M. and H. C. Schamhardt. "Measurement Techniques For Gait Analysis". *Equine Locomotion*. W. Back and H. C. Clayton. 2nd ed. Philadelphia: Elsevier Saunders, 2013. 55-76. Print.

Clayton, H. M., Lanovaz, J. L., Schamhardt, H. C., Willemen, M. A. and Colborne, G. R. (1998) Net joint moments and powers in the equine forelimb during the stance phase of the trot. *Equine Veterinary Journal*, **30**, 384-389.

Crevier-Denoix, N., Pourcelot, P., Ravary, B., Robin, D., Falala, S., Uzel, S., Grison, A., Valtte, J-P., Denoic, J. M. and Chateau, H. (2009) Influence of track surface on the equine superficial digital flexor tendon loading in two horses at high speed trot. *Equine Veterinary Journal*, **41** (3), 257-261.

Crevier-Denoix, N., Robin, D., Pourcelot, P., Falala, S., Holden, L., Estoup, P., Desquilbet, L., Denoix, J. M. and Chateau, H. (2010) Ground reaction force and kinematic analysis of limb loading on two different beach sand tracks in harness trotters. *Equine Veterinary Journal*, 42 (supplement 38), 544-551.

Dagg, L. -A., Northrop, A. J., Brigden, C. V., Martin, J. H., Owen, A. G. and Hobbs, S. J. (2012) Comparison of metacarpophalangeal joint flexion-extension: Agreement between planar and three dimensional methods of calculation from in-field measurement. International Conference on Equine Exercise Physiology Proceedings.

Daley, M. A., Usherwood, J. R., Felix, G. and Biewener, A. A. (2006) Running over rough terrain: guinea fowl maintain dynamic stability despite a large unexpected change in substrate height. *The Journal of Experimental Biology*, **809**, 171-187.

Delsys® (2016) *Trigno™ Wireless Systems and Smart Sensors*. Accessed via: http://www.delsys.com/products/wireless-emg/

De Luca, C. J. (1997) The use of surface electromyography in biomechanics. *Journal of Applied Biomechanics*, **13**, 135-163.

De Luca, C. J., Gilmore, L. D., Kuznetsov, M. and Roy, S. H. (2010) Filtering the surface EMG signal: Movement artefact and baseline noise contamination. *Journal of Biomechanics*, **43**, 1573-1579.

De Luca, C. J., Kuznetsov, M., Gilmore, L. D. and Roy, S. H. (2012) Inter-electrode spacing of surface EMG sensors: reduction of crosstalk contamination during voluntary contractions. *Journal of Biomechanics*, **45** (3), 555-561.

Dixon, S. J., Collop, A. C., Batt, M. E. (2000) Surface effects on ground reaction forces and lower extremity kinematics in running. *Medicine and Science in Sports and Exercise*, **32** (11), 1919-26.

Dunbar, D.C., Macpherson, J.M., Simmons, R.W. and Zarcades, A. (2008) Stabilization and mobility of the head, neck and trunk in horses during overground locomotion: comparisons with humans and other primates. *Journal of Experimental Biology,* **211**,3889-3907 DOI 10.1242/jeb.020578.

Dutto, D.J., Hoyt, D.F., Cogger, E.A. and Wickler, S.J. (2004) Ground reaction forces in horses trotting up an incline and on the level over a range of speeds. *Journal of Experimental Biology*, **207**, 3507-3514 DOI 10.1242/jeb.01171.

Drevemo, S., Dalin, G., Fredricson, I. and Hjertén (1980) Equine locomotion: 1. The analysis of linear and temporal stride characteristics of trotting Standardbreds. *Equine Veterinary Journal*, **12** (2), 60-65.

Drevemo, S. and Hjertén, G. (1991) Evaluation of a shock absorbing woodchip layer on a harness race track. *Equine Exercise Physiology*, **3**, 107-112.

Dyson, S. (2000) Lameness and poor performance in the sports horse: Dressage, Show Jumping and Horse Trials (Eventing). *AAEP Proceedings*, **46**, 308-315.

Faber, M., Johnston, C., van Weeren, P. R. and Barneveld, A. (2002) Repeatability of back kinematics in horses during treadmill locomotion, *Equine Veterinary Journal*, **34** (3), 235-241.

Farina, D. (2006) Interpretation of the surface electromyogram in dynamic contractions. *Exercise and Sport Science Reviews*, **34** (3), 121-7.

Farley, C. T., Glasheen, J. and McMahon, T. A. (1993) Running springs: speed and animal size. *Journal of Experimental Biology*, **185**, 71-86.

Farley, C. T. and González, O. (1996) Leg stiffness and stride frequency in human running. *Journal of Biomechanics*, **29** (2), 181-186.

Farley, C. T., Houdijk, H. H. P., van Strien, C. and Louie, M. (1998) Mechanism of leg stiffness adjustment for hopping on surfaces of different stiffnesses. *Journal of Applied Physiology*, **85**, 1044-1055.

Ferris, D. P. and Farley, C. T. (1997) Interaction of leg stiffness and surface stiffness during human hopping. *Journal of Applied Physiology*, **82**, 15-22.

Ferris, D. P., Liang, K. and Farley, C. T. (1999) Runners adjust leg stiffness for their first step on a new running surface. *Journal of Biomechanics*, **32**, 787-794.

Ferris, D. P., Louie, M. and Farley, C. T. (1998) Running in the real world: adjusting leg stiffness for different surfaces. *Proceedings of the Royal Society B: Biological Sciences*, 265, 989-994.

Firth, E. C. (2006) The response of bone, articular cartilage and tendon to exercise in the horse. *Journal of Anatomy*, **208**, 513-526.

Figueiredo, J. P., Muir, W. W., Smith, J. and Wolfrom, G. W. (2005) Sedative and analgesic effects of romifidine in horses. *Intern J Appl Res Vet Med*, **3** (3), 249-258.

Fong, S-M. and Ng, G. Y. (2006) The effects on sensorimotor performance and balance with tai chi training. *Archives of Physical Medicine and Rehabilitation*, **87** (1), 82-7.

Gibson, O. (2011) Olympic organisers looking into concerns over 'dead' equestrian surface. The Guardian. http://www.theguardian.com/sport/2011/nov/23/olympic-organisers-equestrian-surface-greenwich

Grimmer, S., Ernst, M., Günther, M. and Blickhan, R. (2008) Running on uneven ground: leg adjustment to vertical steps and self-stability. *J. Exp. Biol,* **211**, 2989-3000.

Groesel, M., Zsoldos, R. R., Kotschwar, A., Gfoehler, M. and Peham C. (2010) A preliminary model study of the equine back including activity of *longissimus dorsi* muscle. *Equine Veterinary Journal*, **42**, 401-406.

Gustås, P., Johnston, C., and Drevemo, S. (2006a) Ground reaction force and hoof deceleration patterns on two different surfaces at the trot. *Equine and comparative exercise physiology,* **3**, (4) 209 – 216.

Gustås, P., Johnston, C., Hedenstrom, U., Roepstorff, L. and Drevemo, S. (2006b) A field study on hoof deceleration at impact in Standardbred totters at various speeds. *Equine and Comparative Exercise Physiology*, **3** (3), 161-168.

Halaki, M. and Ginn, K. (2012) Normalization of EMG signals: To normalize or not to normalize and what to normalize to? *INTECH*, 175-194.

Hall, C. A. and Cassaday, H. J. (2006) An investigation into the effect of floor colour on the behaviour of the horse. *Applied Animal Behaviour Science*, **99**, 301-314.

Harrison, S. M., Whitton, R. C., Kawcak, C. E., Stover, S. M. and Pandy, M. G. (2010) Relationship between muscle forces, joint loading and utilization of elastic strain energy in equine locomotion. *J. Exp. Biol,* **213**, 3998-4009.

Harrison, S. M., Whitton, R. C., King, M., Haussler, K. K., Kawcak, C. E., Stover, S. M. and Pandy, M. G. (2012) Forelimb muscle activity during equine locomotion. *The Journal of Experimental Biology*, **215**, 2980-2991.

Hart, S. (2011) London 2012 Olympics: organisers confident new equestrian surface will win approval of angry riders. The Telegraph. http://www.telegraph.co.uk/sport/olympics/8976182/London-2012-Olympics-organisers-confident-new-equestrian-surface-will-win-approval-of-angry-riders.html

Heaps, L.A., Franklin, S.H. and Colbourne, G.R. (2011) Horizontal moment around the hoof centre of pressure during walking on right and left circles. *Equine Veterinary Journal*, **43** (2), 190-195.

Heiden, T. L., Sanderson, D. J., Inglis, J. T. and Siegmund, G. P. (2006) Adaptations to normal human gait on potentially slippery surfaces: The effects of awareness and prior slip experience. *Gait and Posture*, **24**, 237-246.

Hernlund, E. (2016) *Sports Surfaces in Show Jumping*. PhD Thesis

Hill T.W. (2003). Survey of injuries in Thoroughbred at the New York Racing Association tracks. *Clin. Tech. Equine Prac.* **2**, 323–328.

Hjertén, G. and Drevemo, S. (1994) Semi-quantitative analysis of hoof strike in the horse. *Journal of Biomechanics*, **27** (8), 997-1004.

Hobara, H., Kanosue, K. and Suzuki, S. (2007) Changes in muscle activity with increase in leg stiffness during hopping. *Neuroscience Letters*, **418**, 55-59.

Hobbs, S. J., Richards, J., Matuszewski, B. and Brigden, C. (2006) Development and evaluation of a noninvasive marker cluster technique to assess three dimensional kinematics of the distal portion of the forelimb in horses. *American Journal of Veterinary Research*, **67** (9), 1511-1518.

Hobbs, S. J., Bertram, J. E. A. and Clayton, H. M. (2016) An exploration of the influence of diagonal dissociation and moderate changes in speed on locomotor parameters in trotting horses. *Peer J*, **4:e2190**; DOI 10.7717/peerj.2190

Hobbs, S.J. and Clayton, H.M. (2013) Sagittal plane ground reaction forces, centre of pressure and centre of mass in trotting horses. *Veterinary Journal Supplement*, **198** (1):e14_e19 DOI 10.1016/j.tvjl.2013.09.027.

Hobbs, S.J., Licka, T. and Polman, R. (2011) The difference in kinematics of horses walking, trotting and cantering on a flat and banked 10 m circle. *Equine Veterinary Journal*, **43** (6): 686–694.

Hobbs, S.J., Northrop, A., Mahaffey, C., Martin, J., Clayton, H.M., Murray, R., Roepstorff, L. And Peterson, M. (2014a). *Equine Surfaces White Paper.* FEI Publication. http://www.fei.org/fei/about-fei/publications/fei-books

Hobbs, S. J., Orlande, O., Edmundson, C. J., Northrop, A. J. and Martin, J. H. (2010) Development of a method to identify foot strike on an arena surface: application to jump landing. *Comparative Exercise Physiology,* **7** (1), 19-25.

Hobbs, S.J., Richards, J. and Clayton, H.M. (2014b) The effect of centre of mass location on sagittal plane moments around the centre of mass in trotting horses. *Journal of Biomechanics*, **47**(6):1278_1286 DOI 10.1016/j.jbiomech.2014.02.024.

Hodson, E., Clayton, H. M. and Lanovaz, J. L. (2000) The forelimb in walking horses: 1. Kinematics and ground reaction forces. *Equine Veterinary Journal*, **32** (4), 287-294.

Hodson, E., Clayton, H. M. and Lanovaz, J. L. (2000) The hindlimb in walking horses: 1. Kinematics and ground reaction forces. *Equine Veterinary Journal*, **33** (1), 38-43.

Hodson-Tole, E. (2006) Effects of treadmill inclination and speed on forelimb muscle activity and kinematics in the horse. *Equine and Comparative Exercise Physiology*, **3** (2), 61-72.

Holmstrom, M., Fredricson, I. and Drevemo, S. (1994) Biokinematic differences between riding horses judged as good and poor at the trot. *Equine Veterinary Journal Supplement*, **17**, 51-56.

Holt, D., George, L. B., Clayton, H. M. and Hobbs, S. J. (2017) A simple method for equine kinematic gait event detection. *Equine Veterinary Journal*, **49** (5), 688-691. **DOI:** 10.1111/evj.12669

Holt, D., Northrop, A., Owen, A., Martin, J. and Hobbs, S. J. (2014) Use of surface testing devices to identify potential risk factors for synthetic equestrian surfaces. *Procedia Enginneering*, **72**, 949-954.

Huigen, E., Peper, A. and Grimbergen, C. A. (2002) Investigation into the origin of the noise of surface electrodes. *Med. Biol. Eng. Comput*., **40**, 332-338.

i.pinimg.com (2015) Equine superficial muscles. Accessed via: https://i.pinimg.com/736x/28/7b/2f/287b2f876bfe972b5f580758b8d8d0ec--animal-anatomy-horse-anatomy-study.jpg

Jansen, M. O., van Raaij, J. A. G. M., van den Bogert, A. J., Schamhardt, H. C. and Hartman, W. (1992) Quantitative analysis of computer-averaged electromyographic profiles of intrinsic limb muscles in ponies at the walk. *American Journal of Veterinary Research*, **53** (12), 2343-2349.

Johnston, C., and Back, W. (2006) Hoof ground interaction: when biomechanical stimuli challenge the tissues of the distal limb. *Equine Veterinary Journal,* **38**, (7) 634-641.

Kai, M., Takahashi, T., Aoki, O. and Oki, H., (1999) Influence of rough track surfaces on components of vertical forces in cantering thoroughbred horses. *Equine Veterinary Journal Supplement*, **30**, 214-217.

Ker, R. F. (1981) Dynamic tensile properties of the plantaris tendon of sheep (Ovis Aries). *Journal of Experimental Biology*, **93**, 283-302.

Kerdock, A. E., Biewener, A. A., McMahon, T. A., Weyand, P. G. and Herr, H. M. (2002) Energetics and mechanics of human running on surfaces of different stiffnesses. *Journal of Applied Physiology*, **92**, 469-478.

Kienapfel, K. (2015) The effect of three different head-neck positions on the average EMG activity of three important neck muscles in the horse. *Journal of Animal Physiology and Animal Nutrition*, 132-138.

Krüger, W. (1938) Ueber den bewegungsablauf an dem oberen teil der hintergliedmabe des pferdes im schritt, trab und galopp. *Tierärztl. Rundsh*, **44**, 549-557.

Kruse, L., Traulsen, I. and Krieter, J. (2012) Effects of different riding surfaces on the hoof and fetlock accelerations of horses, *Journal of Agricultural Sciences*, **4** (5), 17-24.

Latash, M. L. (2008) Neurophysiological Basis of Movement. 2nd ed. Human Kinetics, USA

Leach, D. H. and Dyson, S. (1998) Instant centres of rotation of equine limb joints and their relationship to standard skin marker locations. *Equine Veterinary Journal Supplement* 6, 113-119.

Lee DV, Bertram JEA, Todhunter RJ. (1999) Acceleration and balance in trotting dogs. *Journal of Experimental Biology,* **202**, 3565-3573.

Lewis, K., Northrop, A. J., Crook, G. M., Mather, J., Martin, J. H., Holt, D., Clayton, H. M., Roepstorff, L., Peterson, M. L. and Hobbs, S. J. (2015) Comparison of equipment used to measure shear properties in equine arena surfaces. *Biosystems Engineering*, **137**, 43-54.

Licka, T, Frey, A. and Peham, C. (2009) Electromyographic activity of the longissimus dorsi muscles in horses when walking on a treadmill. *The Veterinary Journal*, **180**, 71-76.

Marshall, P. and Murphy, B. (2003) The validity and reliability of surface EMG to assess the neuromuscular response of the abdominal muscles to rapid limb movement. *Journal of Electromyography and Kinesiology*, **13**, 477-489.

Maykut, J. N., Taylor-Haas, J. A., Paterno, M. V., DiCesare, C. A. and Ford, K. R. (2015) Concurrent validation and reliability of 2D kinematic analysis of frontal plane motion during running. *The International Journal of Sports Physical Therapy*, **10** (2), 136-146.

McGuigan, M. P. and Wilson, A. M. (2003) The effect of gait and digital flexor muscle activation on limb compliance in the forelimb of the horse *Equus caballus*. *The Journal of Experimental Biology*, **206**, 1325-1336.

McMahon, T. A. and Cheng, G. C. (1990) The mechanics of running: How does stiffness couple with speed? *Journal of Biomechanics*, **23**, 65-78.

McMahon, T. A. and Greene, P. R. (1979) The influence of track compliance on running. *Journal of Biomechanics*, 12, 893-904.

Medoff, H. (1995) Problems in defining and measuring friction on natural and artificial playing surfaces. IEEE, 171-174.

Merkens, H. W. and Schamhardt, H. C. (1994) Relationships between ground reaction force patterns and kinematics in the walking and trotting horse. *Equine Veterinary Journal Supplement*, **17**, 67-70.

Merletti, R. (1999) Standards for reporting EMG Data. *International Society of Electrophysiology and Kinesiology*. Accessed via: **http://faculty.educ.ubc.ca/sanderson/courses/HKIN473/pdf/ISEK_EMG-Standards.pdf**

Moritz, C. T. and Farley, C. T. (2004) Passive dynamics change leg mechanics for an unexpected surface during human hopping. *Journal of Applied Physiology*, **97**, 1313-1322.

Moritz, C. T., Greene, S. M. and Farley, C. T. (2004) Neuromuscular changes for hopping on a range of damped surfaces. *J. Appl. Physiol,* **96**, 1996-2004**.**

Müller, R. and Blickhan, R. (2010) Running on uneven ground: leg adjustments to altered ground level. *Human Movement Science,* **29**, 578-589**.**

Müller, R., Ernst, M. and Blickhan, R. (2012) Leg adjustments during running across visible and camouflaged incidental changes in ground level. *The Journal of Experimental Biology*, **215**, 3072-3079.

Müller, R., Grimmer, S. and Blickhan, R. (2010) Running on uneven ground: Leg adjustments by muscle pre-activation control. *Human Movement Science*, **29**, 299-310.

Müller, R., Tschiesche, K. and Blickhan, R. (2014) Kinetic and kinematic adjustments during perturbed walking across visible and camouflaged drops in ground level. *Journal of Biomechanics*, **47**, 2286-2291.

Murray, R. C., Walters, J., Snart, H., Dyson, S. and Parkin, T. (2010a) How do features of dressage arenas influence training surface properties which are potentially associated with lameness? *The Veterinary Journal*, **186**, 172-179.

Murray, R. C., Walters, J., Snart, H., Dyson, S. and Parkin, T. (2010b) Identification of risk factors for lameness in dressage horses. *The Veterinary Journal*, **184**, 27-36.

Muybridge, E. (1887) Animal locomotion: an electrophotographic investigation of consecutive phases of animal movements. University of Pennsylvania, Philadelphia. Reprinted as: (1979) Complete Human and Animal Locomotion – Vol III. Dover Publications Inc., Mineola, USA. Pp 1208-1246.

Murphy, J. and Arkins, S. (2007) Equine learning behaviour. *Behavioural Processes*, **76**, 1-13.

Nicol, C. J. (2002) Equine learning: progress and suggestions for future research. *Applied Animal Behaviour Science*, **78**, 193-208.

Nigg, B. M. and Yeadon, M. R. (1987) Biomechanical aspects of playing surfaces. *Journal of Sports Sciences*, **5** (2), 117-45.

Nollet, H., Deprez, P., van Ham, L., Dewulf, J., Decleir, A. and Vanderstraeten, G. (2004) Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. *Equine Veterinary Journal*, **36** (1), 51-57.

Nordander, C., Willner, J., Hansson, G.A., Larsson, B., Unge, J., Granquist, L. and Skerfving, S. (2003) Influence of the subcutaneous fat layer, as measured by ultrasound, skinfold calipers and BMI, on the EMG amplitude. *Eur. J. appl. Physiol.* **89**, 514-519.

Northrop, A J., Dagg, L-A., Martin, J H., Brigden, C, Owen, A G., Blundell, E. Peterson, M L. and Hobbs, S. J. (2013) The Effect of Two Preparation Procedures on an Equine Arena Surface in Relation to Motion of the Hoof and Metacarpophalangeal Joint. *The Veterinary Journal* DOI: 10.1016/j.tvjl.2013.09.048

Northrop, A. J., Hobbs, S. J., Holt, D., Clayton-Smith, E. and Martin, J. H. (2016) Spatial variation of the physical and biomechanical properties within an equestrian arena surface. *Procedia Engineering*, **147**, 866-871.

Olson, T. J., Chebny, C., Willson, J. D., Kernozek, T. W. and Straker, S. J. (2011) Comparison of 2D and 3D kinematic changes during a single leg step down following neuromuscular training. *Physical Therapy in Sport*, **12**, 93-99.

Oreszczyn, T., Hong, S. H., Ridley, I. and Wilkinson, P. (2006) Determinants of winter indoor temperatures in low income households in England. *Energy and Buildings*, **38**, 245-252.

Orlande, O., Hobbs, S. J., Martin, J. H., Owen, A. G. and Northrop, A. J. (2012) Measuring hoof slip of the leading limb on jump landing over two different equine arena surfaces. *Comparative Exercise Physiology*, **8**, (1) 33-39.

Pardoe, C. H., McGuigan, M. P., Rogers, K. M., Rowe, L. L. and Wilson, A. M. (2001) The effect of shoe material on the kinetics and kinematics of foot slip at impact on concrete. *Equine Veterinary Journal Supplement*, **33**, 70-73.

Parkes, R. S. V. and Witte, T. H. (2015) The foot-surface interaction and its impact on musculoskeletal adaptation and injury risk in the horse. Equine Veterinary Journal, **47** (5), 519-525. **DOI:** 10.1111/evj.12420

Parkin, T. D. H., Clegg, P. D., French, N. P., Proudman, C. J., Riggs, C. M., Singer, E. R., Webbon, P. M. and Morgan, K. L. (2004) Race- and course-level risk factors for fatal distal limb fracture in racing Thoroughbreds. *Equine Veterinary Journal*, **36**, (6) 521-526

Patla, A.E. (1997) Understanding the roles of vision in the control of human locomotion. *Gait Posture*, **5** (1), 54-69.

Payne, R. C., Veenman, P. and Wilson, A. M. (2004) The role of the extrinsic thoracic limb muscles in equine locomotion. *J. Anat*. **205**, 479-490.

Peham, C., Frey, A., Licka, T. and Scheidl, M. (2001a) Evaluation of the EMG activity of the long back muscle during induced back movements at stance. *Equine Veterinary Journal Supplement*, **33**, 165-168.

Peham, C., Licka, T. F. and Scheidl, M. (2001b) Evaluation of a signal-adapted filter for processing of periodic electromyography signals in horses walking on a treadmill. *American Journal of Veterinary Research*, **62** (11), 1687-1689.

Peterson, M. L. and McIlwraith, C. W. (2008) Effect of track maintenance on mechanical properties of a dirt racetrack: A preliminary study. *Equine Veterinary Journal*, **40** (6), 602-605.

Peterson, M. L., McIlwraith, C. W. and Reiser, R. F. (2008) Development of a system for the in-situ characterisation of thoroughbred horse racing track surfaces. *Biosystems Engineering*, **101**, 260-269.

Peterson, M. L., Roepstorff, L., Thomason, J. J., Mahaffey, C. and McIlwraith, C. W. (2012) Racing Surfaces White Paper. Accessed via: http://www.grayson-jockeyclub.org/resources/White_Paper_final.pdf

Pfau, T., Witte, T. H. and Wilson, A. M. (2006) Centre of mass movement and mechanical energy fluctuation during gallop locomotion in the thoroughbred racehorse. *The Journal of Experimental Biology*, **209**, 3742-3757.

Pinnington, H. C., Lloyd, D. G., Besier, T. F. and Dawson, B. (2005) Kinematic and electromyography analysis of submaximal difference running on a firm surface compared with soft, dry sand. *European Journal of Applied Physiology*, **94**, 242-253.

Potvin, J. R. and Brown, S. H. M. (2004) Less is more: high pass filtering, to remove up to 99% of the surface EMG signal power, improves EMG-based biceps brachii muscle force estimates. *Journal of Electromyography and Kinesiology*, **14**, 389-399.

Qualisys Track Manager (QTM) (2011) User Manual. Qualisys AB http://fy.chalmers.se/~f7xiz/TIF081C/QTM-usermanual.pdf

Radin, E. L., Parker, H. G., Pugh, J. W., Steinberg, R. S., Paul, I. L. and Rose R. M. (1973) Response of joints to impact loading – III Relationship between trabecular microfractures and cartilage degeneration. *Journal of Biomechanics*, **6**, 51-57.

Ratzlaff, M. H., Wilson, P. D., Hyde, M. L., Balch, O. K. and Grant B. D. (1993) Relationships between locomotor forces, hoof position and joint motion during the support phase of the stride of galloping horses. *Acta Anat*, **146**, 200-204.

Ratzlaff, M., Hyde, M. L., Hutton, D. V., Rathgeber, R. A. and Balch, O. K. (1997) Interrelationships between moisture content of the track, dynamic properties of the track and the locomotor forces exerted by galloping horses. *Journal of Equine Veterinary Science*, **17**, 35-42.

Reiser, R. F., Peterson, M. L., McIlwraith, C. W. and Woodward, B. (2000) Simulated effects of racetrack material properties on the vertical loading of the equine forelimb. *Sports Engineering*, **3** (1), 1-11.

Reynolds, R.F. and Day, B.L. (2005) Visual guidance of the human foot during a step. *J. Physiol.* **569,** 674-84..

Richards, J., Thewlis, D., Selfe, J., Cunningham, A. and Hayes, C. (2008) A biomechanical investigation of a single-limb squat: Implications for lower extremity rehabilitation exercise. *Journal of Athletic Training*, **43** (5), 477-482.

Richards, J. (2016) *The Complete Book of Clinical Biomechanics*. (in preparation)

Robert, C., Valette, J. P., Degueurce, C. and Denoix, J. M. (1999) Correlation between surface electromyography and kinematics of the hindlimb of horses at trot on a treadmill. Cells Tissues Organs, 165 (2), 113-122.

Robert, C., Valette, J. –P., Pourcelot, P., Audigié, F. and Denoix, J. –M. (2002) Effects of trotting speed on muscle activity and kinematics in saddlehorses. *Equine Veterinary Journal Supplement*, **34**, 295-301.

Robertson,D.G.E. and Dowling, J.J. (2003) Design and responses of Butterworth and critically damped digital filters. *J Electromyogr Kines*, **13**, 569-573.

Robin, D., Chateau, H., Pacquet, L., Falala, S., Valette, P., Poureclot, B., Denoix, J._m. and Crevier Denoix, N. (2009) Use of a 3D dynamometric horseshoe to assess the effects of an all-weather waxed track and a crushed sand track at high speed trot: Preliminary study. *Equine Veterinary Journal,* **41,** (3) 253-256.

Rooney, J. M. (1982) The horse's back: Biomechanics of lameness. *Equine Pract*., **4,** 17-27.

Ruina, A., Bertram, J. E. A. and Srinivasan, M. (2005) A collisional model of the energetic cost of support work qualitatively explains leg sequencing in walking and galloping, pseudo-elastic leg behaviour in running and the walk-to-run transition. *Journal of Theoretical Biology*, **237**, 170-192.

Schamhardt, H.C., Merkens, H.W., Vogel, V. and Willekens, C. (1993) External loads on the limbs of jumping horses at take-off and landing. *American Journal of Veterinary Research* 54: 675-680

Setterbo, J. J., Garcia, T. C., Campbell, I. P., Reese, J. L., Morgan, J. M., Kim, S. Y., Hubbard, M. and Stove, S. M. (2009) Hoof accelerations and ground reaction forces of Thoroughbred racehorses measured on dirt, synthetic, and turf track surfaces. *American Journal of Veterinary Research,* **70**, (10) 1220-1229.

Seyfarth, A., Geyer, H. and Herr, H. (2003) Swing-leg retraction: a simple control model for stable running. *The Journal of Experimental Biology*, *206*, 2547-2555.

Sinclair, J., Taylor, P. J. and Hobbs, S. J. (2013) Digital filtering of three-dimensional lower extremity kinematics: an assessment. *Journal of Human Kinetics*, **39**, 5-13.

Swan, P., Otago, L., Finch, C. and Payne, W. (2009) The policies and practises of sports governing bodies in relation to assessing the safety of sports grounds. *Journal of Science and Medicine in Sport*, **12**, 171-176.

Swanstrom, M. D., Zarucco, L., Stover, S. M., Hubbard, M., Hawkins, D. A., Driessen, B. and Steffey, E. P. (2005) Passive and active mechanical properties of the superficial and deep digital flexor muscles in the forelimbs of anesthetized thoroughbred horses. *Journal of Biomechanics*, **38**, 579-586.

Symons, J. E., Garcia, T. C. and Stover, S. M. (2013) Distal hindlimb kinematics of galloping Thoroughbred racehorses on dirt and synthetic racetrack surfaces. *Equine Veterinary Journal*, **46** (2), 227-232.

Thomason, J. J. and Peterson, M. L. (2008) Biomechanical and mechanical investigations of the hoof-track interface in racing horses. *Veterinary Clinics: Equine Practice*, **24**, 53-77.

Tokuriki, M. and Aoki, O. (1991) Neck muscle activity in horses during locomotion with and without a rider. *Equine Exercise Physiology 3*, 146-150.

Tokuriki, M. (1979) Cinematographic and electromyographic analysis of vertical standing jump in the dog. *Journal of Experimental Biology*, **83**, 271-282.

Tokuriki, M., Ohtsuki, R., Kai, M., Hiraga, A., Oki H., Miyahara, Y. and Aoki, O. (1999) EMG activity of the muscles of the neck and forelimbs during different forms of locomotion. *Equine Veterinary Journal Supplement*, **30**, 231-234.

Tranquille, C. A., Walker, V. A., Hernlund, E., Egenvall, A., Roepstorff, L., Peterson, M. L. and Murray, R. C. (2015) Effect of superficial harrowing on surface properties of sand with rubber and waxed-sand with fibre riding arena surfaces: a preliminary study. The Veterinary Journal, 203 (1), 59-64.

Van Boxtel, A. (2001) Optimal signal bandwidth for the recording of surface EMG activity of facial, jaw, oral, and neck muscles. *Psychophysiology*, **38**, 22-34.

van Dieen, J. H., Spaniaard, M., Konemann, R., Bron, L. and Pijnappels, M. (2007) Balance control in stepping down expected and unexpected level changes. *Journal of Biomechanics*, **40** (16), 3641-9.

van der Krogt, M. M., de Graaf, W. W., Farley, C. T., Moritz, C. T., Richard Casius, L. J., and Bobbert, M. F. (2009) Robust passive dynamics of the musculoskeletal system compensate for unexpected surface changes during human hopping. *Journal of Applied Physiology*, **107**, 801–808.

van Weeren, P. R. (2010) On surfaces and soreness, guest editorial. *The Veterinary Journal*, **186**, 129- 130.

van Weeren, P. R., van den Bogert, A. J. and Barneveld, A. (1990) A quantitative analysis of skin displacement in the trotting horse. *Equine Veterinary Journal Supplement*, **9**, 101-109.

Veterinary Medicine (2016) *Equine Skeleton*. Accessed via: http://vanat.cvm.umn.edu/run/lrg%20images/equine-skeleton.jpg

Wang, L., Hong, Y. and Li, J. X. (2014) Muscular activity of lower extremity muscles running on treadmill compared with different overground surfaces. *American Journal of Sports Science and Medicine*, **2** (4), 161-165.

Wang, J., Tang, L. and Bronlund, J. E. (2013) Surface EMG signal amplification and filtering. *International Journal of Computer Applications*, **82** (1), 15-22.

Watson, J. C. and Wilson, A. M. (2007) Muscle architecture of biceps brachii, triceps brachii and supraspinatus in the horse. *Journal of Anatomy*, **210**, 32-40.

Wakeling, J. M. (2008) The recruitment of different compartments within a muscle depends on the mechanics of the movement. *Biol. Lett.*, **5**, 30-34.

Wakeling, J. M., Ritruechai, P., Dalton, S. and Nankervis, K. (2007) Segmental variation in the activity and function of the equine longissimus dorsi muscle during walk and trot. *Equine and Comparative Exercise Physiology*,**4** (2), 95-103.

Wijnberg, I. D., Van der Kolk, J. H., Franssen, H. and Breukink, H. J. (2003) Needle electromyography in the horse compared with its principles in man: a review. *Equine Veterinary Journal*, **35** (1), 9-17.

Wilson, A. M., McGuigan, M. P., Su, A. and van den Bogert, A. J. (2001) Horses damp the spring in their step. *Nature*, **414**, 895-899.

Wilson, A. M., Watson, J. C. and Lichtwark, G. A. (2003) Biomechanics: A catapult action for rapid limb protraction. *Nature*, **421**, 35-36.

Winter DA, Sidwall HG, Hobson DA. (1974) Measurement and reduction of noise in kinematics of locomotion. *Journal of Biomechanics*, **7**, 157-159.

Witte, T. H., Knill, K. and Wilson, A. M. (2004) Determination of peak vertical ground reaction force from duty factor in the horse (*Equus caballus*), *The Journal of Experimental Biology*, **207**, 3639-3648.

Zsoldos, R. R., Kotschwar, A. B., Kotschwar, A., Groesel, M., Licka, T. and Peham, C. (2010) Electromyography activity of the equine splenius muscle and neck kinematics during walk and trot on the treadmill. *Equine Veterinary Journal*, **42** (38), 455-461.

12.0 APPENDICES

AP/NEW/PR PROJECT REFERENCE NO.

uclan
University of Central Lancashire

APPLICATION TO ANIMAL PROJECTS COMMITTEE FOR APPROVAL OF RESEARCH PROJECT

This form should be completed for all **NEW*** applications for University research support and submitted to the Chair of the Animal Projects Committee. Please refer to the notes for guidance on completion of the form. If the project involves behavioural studies of wild mammals, of animals in zoos or of invertebrates in the field please use form AP/NEW/PR/2. If making any reference to any other project in Section 6 or Section 7, the Project Reference No. should be given.

*A new project includes those where there are changes to Section 4, Section 5 or Section 6.

If this application relates (amendment or sub-project) to an application which has previously been reviewed, and approved, by AP Committee, please supply the corresponding Project reference number(s) from your decision letter(s). ______

Section 1

Is the project

- ☐ **Undergraduate**
- ☐ **Postgraduate Taught**
- ☐ **Research**

Is this application for funding?

☐ **YES**
☐ **NO**

If Yes, provide outcome of bid, if known?

Section 2

(a) Is the work to be carried out under an existing Home Office Project Licence?

☐ **YES**
☐ **NO**

(If answer is YES – a copy of the existing approved project licence application to be attached and this application to be completed in respect of this specific study/sub-project)

Home Office Project Licence No (if applicable)

Home Office Project Licence Title (if applicable)

Home Office Project Licence Approved No of Animals (species per year)

(b) Does the project require a new Home Office Licence?

☐ **YES**
☐ **NO**

Section 3

Is there a Personal licence holder?

☐ **YES**
☐ **NO**

Who holds it?

Is there a Personal licence No.?

Personal Licence No. **Date of issue**

Section 4

Duration of Project **3** **Years (maximum 5 years)**

Proposed Start Date of Project **07/2013** **(Month/Year)**

Proposed End Date of Project **06/2016** **(Month/Year)**

Section 5

Title of Project

Do horses alter their limb stiffness as a function of surface stiffness?

Section 6

Name of researcher and co-workers

Danielle Holt | **Dr Sarah Hobbs**

Dr Jaime Martin

Dr Andy Owen

Alison Northrop

Section 7

Aims and Objectives of Project (Layperson's terms)

The main aim of the study is to investigate whether equine limb stiffness alters according to large and subtle changes in surface stiffness and to determine what the implications are in terms of performance and risk of injury. The stiffness of a surface has been defined as the ratio of applied force to the amount of deflection of a surface (Nigg and Yeadon, 1987) and is potentially a risk factor for injury. Limb stiffness relates to the ratio of the peak vertical ground reaction force that is generated when the hoof strikes the ground to the maximum displacement of the centre of mass (Farley and González, 1996).

The study will test the following hypotheses (1) Equine limb stiffness will alter in response to a change in surface stiffness, (2) The muscles of the forelimb will play a role in altering the limb stiffness on impact.

In order to address the aims of the project, biomechanical and mechanical measurements will be used to determine the limb stiffness of the horses and surface stiffness respectively. The first part of the project would involve designing a force shoe that can be fitted to the underside of the hoof in order to calculate the vertical ground reaction forces (vGRF) generated when the hoof strikes the ground. The vGRF in conjunction with the amount of vertical movement of the vertebrae near the withers will enable the stiffness of the limb to be calculated. An Orono Biomechanical Hoof Tester (OBST), will be used to measure surface stiffness. Once the force shoe has been validated, a group of horses will be tested on a range of different arena surfaces that have been maintained for competition. This is expected to include a waxed sand and fibre, un-waxed sand and fibre, sand and rubber, woodchip and grass surface. A concrete surface will be used as a control surface. This protocol will enable any large or subtle alterations in limb stiffness according to surface stiffness to be quantified.

Please also attach more detailed information (Scientific if applicable)
See 'Scientific information for Section 7'

Section 8

In layperson's terms explain precisely what will happen to the animal(s);

Pilot Study

The pilot study will involve testing the force shoe to ensure it is fit for purpose and to establish whether it can produce reliable measurements. It is envisaged that one horse will be used for this purpose. The force shoe will be fitted to the hoof of the horse's right forelimb and compensatory weight and height will be added to the shoe of the left forelimb. The preliminary design involves securing the force shoe with the use of stud holes and screws, which are commonly used in practice for competitions and do not inflict any pain on the horse. In order to collect the data, a wire will run from the force shoe to a datalogger, which will be secured to the distal limb of the horse using a tendon boot. Wireless Surface Electromyography electrodes may also be secured to the muscle bodies of the proximal forelimb because muscle activity has been implicated in affecting human limb stiffness previously (Kerdok *et al*., 2002).

Data will be collected during standing and during motion from the horse and this will be repeated once the force shoe has been removed and refitted to ensure that measurements can be repeated. The horse will be led in a straight line in order to take measurements from six consecutive strides, which is a similar procedure followed by Kruse *et al*. (2012) and this will be repeated five times on two different surfaces (n=30 for each surface).

Cameras will also be positioned to capture high speed footage of the horse in motion. This will enable the data from the force shoe to be synchronised with the kinematic measurements. Markers will be applied to the horse on anatomical locations in order to measure the vertical movement of the vertebrae at the withers. Stride length, stride frequency, stance duration and joint angles of the forelimb will also be measured as these variables have been an indicator of human limb stiffness previously (Farley and González, 1996). Limb stiffness will be calculated using the following equation:

$$\frac{\textbf{Peak vertical ground reaction force}}{\boldsymbol{Maximum\ displacement\ of\ the\ vertebrae}}$$

An Orono Biomechanical Surface Tester (OBST), which was originally designed by Professor Mick Peterson, University of Maine (Peterson *et al*., 2008) will be used to quantify the surface stiffness. The OBST will be dropped in the immediate area surrounding the hoof prints made by the horse's right forelimb after the last trial. The horses will not be present in the arena when surface stiffness is being measured. The OBST is equipped in order to calculate the surface stiffness equation:

$$\frac{\textbf{Peak vertical ground reaction force}}{\textit{Maximum displacement}}$$

Experimental Protocol

A similar protocol and sampling procedure will be followed to the pilot study (n=30 for each combination of horse, gait and surface) where the sample of horses (n=8) will be walked and trotted through a recording zone. In order to determine the effects of subtle changes to a surface on equine limb stiffness, the protocol will be performed on one arena surface that has been prepared using two maintenance methods (harrowed and rolled). In order to investigate the effects of larger changes to a surface on equine limb stiffness, the protocol will be carried out on different arena surfaces that have been maintained for competition. This is expected to include a waxed sand and fibre, un-waxed sand and fibre, sand and rubber, woodchip and grass surface. A concrete surface will be used as a control surface. The protocol may involve transporting the same horses to a different venue in order to test such a variety of arena surfaces. The OBST will also be used after the biomechanical measurements in order to quantify the surface stiffness.

The risk assessments for handling horses and use of the equipment will be strictly adhered to at all times. The force shoe and other equipment to be used will not cause any pain or suffering to the sample of horses. All of the horses will be habituated to the equipment, however if there is a prolonged stress response, it will be removed from the study immediately.

Farley, C. T. and González, O. (1996) Leg stiffness and stride frequency in human running. *Journal of Biomechanics*, **29** (2), 181-186.

Kerdock, A. E., Biewener, A. A., McMahon, T. A., Weyand, P. G. and Herr, H. M. (2002) Energetics and mechanics of human running on surfaces of different stiffnesses. *Journal of Applied Physiology*, **92**, 469-478.

Kruse, L., Traulsen, I. and Krieter, J. (2012) Effects of different riding surfaces on the hoof and fetlock accelerations of horses, *Journal of Agricultural Sciences*, **4** (5), 17-24.

Nigg, B. M. and Yeadon, M. R. (1987) Biomechanical aspects of playing surfaces. *Journal of Sports Sciences*, **5**, 117-145.

Section 9

a) How many, and which species of animals are intended to be used each year of the project

Year	No of animals	Species
2014	**up to 2**	***Equus caballus***
2015	**up to 10**	***Equus caballus***

If animals are being killed/harmed complete Sections 9, 10, 11 and 12

Section 10

State any additional reasons that support this proposed use of animals to obtain the specific objectives. Is the number of animals you propose to use appropriate? – i.e. large enough to produce a satisfactory valid result and not greater, in accordance with the principles of Reduction, Refinement and Replacement.

<u>Assessment of the potential for implementation of the 3Rs into a research protocol</u>

For further information please see
http://www.nc3rs.org.uk/
and
http://www.homeoffice.gov.uk/comrace/animals/furtherinfo.html

Please use the following checklist to assist you in completing Sections 11, 12 and 13 of the application form.

If any answers require elaboration, please refer to the guidance notes for Sections 11, 12 and 13 as appropriate.

	<u>**YES**</u>	<u>**NO**</u>
Can the work be done without using animals or without using a project licence?		✓
Have you considered any information relating to the 3Rs which allows you to select a replacement alternative to achieve some or all your objectives?	✓	
Have you done a specific literature search to ensure that you have chosen the best methods for this research?	✓	
Can existing replacement alternatives be adapted, or can you develop new alternatives, to accommodate your needs without compromising scientific integrity?		✓
Can you modify your research strategy to allow you to use a replacement alternative for some stages of your work, without compromising your project? *Consider parallel animal/non-animal experiments for the validation of alternatives for your purposes; or* *Consider whether the results of experiments using replacement alternatives can improve any subsequent or complementary animal studies*		✓

Where animal experiments appear to be inevitable, have you considered limiting the pain and suffering to the animals by implementing, reducing and refinement alternatives?
Consider:-
• *Use of less sentient species*
• *Sharing animals*
• *Using only one sex*
• *Less invasive methodology*
  ○ *Injection of smaller volumes*
  ○ *Smaller needles*
  ○ *Less frequent sampling*
• *Improving husbandry*
• *Anaesthesia and analgesia*
• *More-humane endpoints*
	✓	

Have you consulted	<u>**YES**</u>	<u>**NO**</u>
A statistician	✓	
The Named Vet		✓
The Named Animal Care and Welfare Officer?	✓	

Section 11 **Indicate the balance between the pain, suffering and distress to the animals involved and the likely benefits to be gained by the research.**

Section 12 **Are there ways in which the procedures could be refined to reduce the pain or suffering to animals without affecting the scientific validity of the project?**

Section 13 **Indicate what scope exists for reduction in the number of animals used and refinement in technique as the project progresses** If the sample size is reduced, the statistical power of the project will reduce below 0.8, which is considered to be the minimum value in order to determine a significant treatment effect if one exists (Lenth, 2001). It is envisaged that up to 10 horses of similar age and height will be selected for the study in order to allow for two horses to be withdrawn from the study for various reasons without affecting the validity of the results.

Lenth, R. V. (2001) Some practical guidelines for effective sample size determination, *American Statistical Association*, **55**, (3), 187-193.

<table>
<tr><td colspan="3">Section 14

List hazardous chemical substances that will be used and expected quantities (e.g. those classified under the CHIP Regulations as toxic, harmful, corrosive, irritant, flammable, oxidising, dangerous for the environment).</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td colspan="3">Please attach COSHH assessments for the above mentioned substances.</td></tr>
</table>

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<tr><td colspan="2">List hazardous biological agents that will be used and expected quantities.</td></tr>
<tr><td></td><td></td></tr>
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<tr><td colspan="2">Please attach COSHH assessments for the above mentioned substances.</td></tr>
</table>

Details of equipment used.	
Force Shoe	Gait analysis equipment will include markers, cameras and possibly surface electromyography– please see RA for kinematic measurements
OBST	
Please attach a risk assessment for the operation of any equipment that exposes persons to a significant hazard (e.g. cut, trap, nip/crush, electrocution, entanglement, very hot / very cold etc).	

Details of animal handling operations. List precautions that will be taken to prevent injuries being sustained during contact with animals (e.g. kick, bite, crush, poison etc).
Standard handling practices will be followed during testing when taking the horses from the stable and leading them. The horses will be accustomed to lifting their limbs up whilst the force shoe and 'dummy shoe' are fitted to the right and left forelimbs respectively. It is standard practice to lift the limbs up on a daily basis to clean out debris from the underside of the hoof. The correct PPE will be worn at all times including sturdy boots with good grip and a hat and gloves will be worn when leading the horses.
Please attach relevant risk assessment. **Please see risk assessments for kinematic measurements and the force shoe.**

Details of specific hygiene or containment requirements. (e.g. animal allergen, infection risk).
All researchers must wash hands thoroughly after testing in order to avoid allergic reactions and the transmission of zoonotic diseases. Researchers who have a known allergy to horses, will not handle the horses.

Have any training needs specific to this project been identified that must be undertaken prior to the start of the project? If so give details as to nature of training and delivery and assessment methods.
☐ **YES** ☐ **NO**
Training will be required with the camera system that will be used to perform gait analysis. The principal investigator will attend weekly training at UCLan that will involve learning how to use the equipment.

Is Health Screening necessary for those carrying out this project? If so give details.
☐ **YES** ☐ **NO**
Handlers will be fit and healthy will not be allergic to horses. This will determined verbally and the handler will be given the opportunity to disclose any medical conditions and/or medication that they are taking. If handling horses exacerbates a particular medical condition, then the handler will not be

involved with data collection.

Section 15

Has approval been given by the Ethics Committee of any collaborating institution?

☐ **YES**
☐ **NO**

Please attach a copy of the relevant documentation relating to external approval.

Signature/Name of Principal Investigator Danielle Holt

Date 24/09/2013

The form should be emailed/sent to Louise Price, Committee Secretary, at least two weeks prior to the Committee meeting.

Applications can be emailed to lmprice1@uclan.ac.uk or posted to Graduate Research School, Room 12, Greenbank Building

AP/NEW/PR

Notes for Guidance on completion of application form

Projects that must be considered by the APC are ones which involve

- handling of and interaction with all animals including those protected under the 1986 Act[1]
- direct intervention with animals
- observational studies that may cause distress*

and would normally complete Form AP/NEW/PR

Animal studies which do not fall into the above categories e.g. analysis of ongoing or historic data and those based on questionnaires do not need to be considered by the Committee.

Project Reference No: All projects are allocated a reference number when submitted to the Committee. This reference number should be quoted on all correspondence/project forms e.g. closure report, renewal form

Section 1
Tick relevant box and, if yes, provide outcome of bid, if known.

Section 2
Tick relevant box and include Project Licence Number; Title and Approved No of Animals

Section 3
Tick relevant box and include name of licence holder, personal licence No. and date of issue

Section 4
Indicate the number of years (maximum 5) the project will be running together with the proposed Start Date and End Date of the Project (Month/Year)

Section 5
This should be the definitive title of the project

Section 6
All the names of research workers and co-workers should be included. If there are any external collaborators indicate the name of the Institution(s)

Section 7
The aims and objectives of the project should be made very clear as there are external members on the Committee.

---

[1] The Act regulates scientific procedures which may cause pain, suffering, distress or lasting harm to "protected animals"; it refers to these as "regulated procedures". "Protected animals" are defined in the Act as all living vertebrate animals, except man, as well as one invertebrate species, the common octopus. The definition includes foetal, larval and embryonic forms which have reached specified stages of development.

Where available more detailed information should be attached to the application. This section should be no more than 1000 words.

Section 8
You should make clear all procedures to which the animals will be subjected to, include any adverse effect to the animals e.g. tissues collected from abattoir and analysed for zymogen, induction of diabetes for *in vitro* study.

Section 9
Include any justification (e.g. numbers required for statistical analysis) of the numbers of animals to be used each year of the project. Where more than one species is to be used, indicate how many of each. If animals are being shared please complete Section 10-12.

Section 10
Give any additional reasons that support the proposed use of animals to obtain the specific objectives. State the statistical methods you would use to analyse your results and the statistical basis of your estimation of the number of animals required.

Section 11
Indicate the balance between the pain, suffering & distress to the animals involved and the likely benefits to be gained by the research.

Section 12
If the answer is YES information is required as to why this is not done. **It is expected that an explanation will be given in this section.**

Section 13
Give details of what scope exists for the reduction in the number of animals used and refinement in technique as the project progresses.

Section 14
Give details relating to Health and Safety aspects. Has the person received training on killing or is deemed competent?

Section 15
If there are any External Collaborators in Section 5 has approval been given by the Ethics Committee in the Institution(s). If the answer is **YES** provide relevant documentation.

Principal Investigator should sign and date the form or type in name if submitted electronically.

<u>Submission of Project Application Form</u>

The Animal Projects Committee normally meets four times a year. Project Application forms and all supporting documents should be with the Secretary no later than two weeks before the date of the meeting.

If the project needs to be approved immediately this should be forwarded to the Secretary and the Chair of the Committee.

Reference Documents

http://www.nc3rs.org.uk

http://www.homeoffice.gov.uk/comrace/animals/furtherinfo.html

www.hse.gov.uk

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RISK ASSESSMENT TITLE	LEARNING AREA	ASSESSMENT UNDERTAKEN	ASSESSMENT REVIEW
Kinematic measurements	Equine Research	**Signed:** Danielle Holt **Date:** September 2013	**Date:** September 2014

STEP ONE	STEP TWO	STEP THREE
List significant hazards here:	**List groups of people who are at risk from the significant hazards you have identified.**	**List existing controls or note where the information may be found. List risks which are not adequately controlled and the action needed:**
General handling, lifting and moving of equipment includes risk of back injury as well as arm, hand, leg and foot injury if equipment is dropped.	Researcher and co-workers	Ensure correct manual handling techniques are known and used at all times when moving equipment. If the item in question is considered too heavy it must be moved between two people to avoid injury. All researchers and co-workers will be aware of this. Qualified first aider and first aid kit will be on site during set up and testing. It will also be ensured that there is a mobile phone available in case the need to call the emergency services arises. A first aid box and the first aider will be located before the testing begins.

The testing procedures includes the risk of slipping when inside or exiting the arena due to the surface material underfoot.	Researcher and co-workers	Be aware at all times of the surface being stepped on and take time to clean any excess build up of surface material of shoes whilst in and outside the arena. The correct Personal Protective Equipment must be used including sturdy boots with sufficient grip.
The study includes risks of the researchers and co-workers bumping into or sliding on equipment used.	Researcher and co-workers	All electrical equipment attached to a mains power supply shall be placed as near as possible to the side of the arena so that wires are not running across the researchers or the vehicles path. All wires that do cross the floor shall be safely placed under matting and covered with the arena surface. Wires running out of the arena shall be securely taped to the floor to avoid trips. The site will have been risk assessed before hand to make sure that the arena surface is level and in good condition and that researchers are aware of entrances and exits, fire assembly points and first aid stations. No unauthorised persons shall be allowed into or around the testing area or near the equipment. The testing area will be kept as tidy as possible and not crowded with testing equipment or too many co-workers. Equipment that is not in use will be put away.
Electrical equipment could become faulty and cause injury such as an electric shock.	Researcher and co-workers	All equipment will have been PAT tested and checked prior to use for loose wires or possible problems. Equipment which needs to be connected to a main power supply will have a circuit breaker attached. The equipment will not be used in wet conditions and the weather forecast will be taken note of.
Habituation of the horse to the equipment and testing procedure includes risk of horse knocking	Researcher and co-workers	Initial habituation will take place in the stable and during in-hand work. The horse will be handled at all times by competent researchers who are experienced. The horse will then be led into

over, standing on or kicking someone.		the testing area and a sufficient warm up in walk and trot will be used to encourage relaxation and familiarisation with the equipment. Researchers must be aware at all times of their position in relation to the horse.
The study includes the risk of a horse getting loose during testing and causing injuries from being kicked or trodden on.	Researcher and co-workers	The equipment and wires will be fenced off to reduce unnecessary harm in case a horse does get loose. The horse will be caught as soon as possible by someone who is experienced and wearing the correct PPE (sturdy boots, gloves and a hat which meets a BSEN 1384 PAS 015 standard)
Positioning gait analysis markers on the forelimb includes risk of being knocked over, stood on, kicked or bitten.	Researcher and co-workers	All researchers and co-workers handling the horses will have undergone safety training and be experienced with handling horses. Researchers must be aware at all times of their position in relation to the horse. Personal protective clothing should be worn including sturdy boots and a riding hat to current safety standards PAS 015/BSEN 1384 or equivalent.
Risk of sprains/strains whilst leading the horse during testing	Researcher handling the horse and horses	The horse and handler will perform a suitable warm up and cool down that gently increases and decreases heart rate respectively. The horse and handler will be considered fit to perform the activities involved with the testing procedure. If the horse and/or handler appear to get tired, testing will be stopped and only started again when the handler is comfortable to do so and when the horse appears to have recovered.
If EMG is used, clipping the horse for EMG sensor application includes the risk of tripping or falling over wires. Also risk of	Researcher and co-workers	All clipping must be done in a safe designated area and clippers will be wireless. No electrical equipment will be left unattended during the clipping process. Clippers electrical will be checked that they are in good working order prior to use. Clipping is routine

electric shock.		practice and the horses used will be regularly clipped and will befamiliar with the process. Razors will be used to complete shaving process. Care will be taken to remove the hair and razors will be stored safely in between use.
Clipping the horse also involves risk to the handler from the horse becoming upset by the clippers or clipping process. Workers could be bitten, kicked or stood upon.	Researcher and co-workers	A riding hat to current British safety standard (BS EN 1384 or PAS 015) and sturdy footwear must be worn by the person handling the horse and the person clipping at all times. The horse involved in the study must have been clipped previously and have shown relaxed behaviour. The process must be stopped immediately if the horse shows signs of significant stress, or the handlers decide it unsafe to continue with the process. This is unlikely because all horses used in the study will be used to being clipped as part of their normal management routine.
Positioning EMG electrodes onto proximal muscle areas of the horse includes risk of being kicked, knocked over, bitten or stood on.	Researcher and co-workers	All researchers and co-workers handling the horses will have undergone safety training and be experienced with handling horses. Researchers must be aware at all times of their position in relation to the horse. Personal protective clothing should be worn including sturdy boots and a riding hat to current safety standards PAS 015/BSEN 1384 or equivalent.
Zoonotic disease through working near animals.	Researcher and co-workers	Hand washing and good hygiene will be expected by all personnel involved.
Allergic reactions to horses	Researcher and co-workers	Screen all researchers and co-workers working with horses.
The dust from the arena surfaces may cause irritation to the eyes, nose and mouth	Researcher and co-workers	The researcher and co-workers will be warned about the possible affects the dust may have and will be asked to report any discomfort to these areas to the first aider immediately.

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RISK ASSESSMENT TITLE	LEARNING AREA	ASSESSMENT UNDERTAKEN	ASSESSMENT REVIEW
Orono Biomechanical Surface Tester (OBST).	Equine	**Signed:** Danielle Holt **Date:** March 2012	**Date:** September 2013

STEP ONE	STEP TWO	STEP THREE
List significant hazards here:	**List groups of people who are at risk from the significant hazards you have identified.**	**List existing controls or note where the information may be found. List risks which are not adequately controlled and the action needed:**
General handling, lifting and moving of equipment includes risk of back injury as well as arm, hand, leg and foot injury if equipment is dropped.	Researcher and co-workers	Ensure correct manual handling techniques are known and used at all times when moving equipment. If the item in question is considered too heavy it must be moved between two people to avoid injury. All researchers and co-workers will be aware of this. All personnel need to check the location and contact details of a first aider prior to the start of any testing.
The testing procedures includes the risk of slipping when inside or	Researcher and co-workers	Be aware at all times of the surface being stepped on and take time to clean any excess build up of surface material of shoes

exiting the arena due to the surface material underfoot.		whilst in and outside the arena. The correct Personal Protective Equipment must be used; no loose clothing and sturdy boots with sufficient grip.
Electrical equipment could become faulty and cause injury.	Researcher and co-workers	All equipment connected to the mains power supply will have been PAT tested and checked prior to use for loose wires or possible problems. Equipment which needs to be connected to a main power supply will also have a circuit breaker attached. The rig will be checked fully for loose connections and care will be taken at all times to make sure that it is positioned on a safe suitable area before testing commences.
Using equipment outdoors in wet conditions.	Researcher and co-workers	When not in use, equipment should be secured to prevent contact with water. Use of suitable trip switches or circuit breakers to be used. Researchers must wear suitable PPE: no loose clothing, sturdy boots. PPE must be appropriate for the weather (i.e. waterproofs in wet conditions and sun screen and eye and head protection if necessary).
The study includes risks of the researcher and co-workers, bumping into or sliding on equipment used.	Researcher and co-workers	All electrical equipment attached to a mains power supply shall be placed as near as possible to the side of the arena so that wires are not running across the researchers or the vehicles path. All wires that do cross the floor shall be safely placed under matting and covered with the arena surface. Wires running out of the arena shall be securely taped to the floor to avoid trips. The site will have been risk assessed before hand to make sure that the arena surface is level and in good condition and that researchers are aware of entrances and exits, fire assembly points and first aid stations. No unauthorised persons shall be allowed into or around the testing area or near the equipment. The researcher responsible

		for wheeling the trolley during testing supervises the area behind the vehicle and then when the equipment is not in use (ie during a break), the trolley is wheeled next to the rig so there is no need for people to step over the wire.
The study involves the risk of driving a vehicle with attached machinery in a possibly confined space. The driver may crash and become injured or strike a researcher or member of the public	Researcher, co-workers and members of the public	All operators must hold the correct license for operating particular vehicles. e.g. car or tractor. Drivers must be taught the correct techniques for handling the machines at speeds, with implements, braking, and parking before commencement of testing. All other researchers must be aware of their position in relation to the vehicle at all times and exit the area when the vehicle is being driven from one location to the next. The driver must ensure the area is clear and safe before attempting to move the vehicle. Members of the public must be kept away from the testing area and informed by researchers of areas which are unsuitable to enter.
The study involves the risk of researchers hands, feet becoming trapped or injured by the machinery and rig whilst in use.	Researcher and co-workers	Administrative controls will be in place and all researchers must keep hands and feet away from the rig before it is activated. Researchers must be aware of the role of other researchers at all times to avoid accidental activation of any parts of the rig. All researchers must wear the correct PPE including sturdy boots with good grip (this was discussed with Steve Whittle prior to re-submission). All researchers involved with using the OBST have previous experience in handling the equipment. If it is necessary for researchers who are unfamiliar with using the OBST to use the equipment however, they will be taught basic safety procedures and the correct method to operate the equipment before testing commences. It takes a minimum of two researchers to operate the OBST, making supervision possible at all times. No researcher should handle any machinery or drive the vehicles if they are

		excessively tired or under the influence of alcohol or recreational drugs.
Zoonotic disease through working near animals.	Researcher and co-workers	Hand washing and good hygiene will be expected by all personnel involved.
Injury due to a fire in the surrounding area	Researcher and co-workers	All personnel should check fire assembly and exits and procedures in the event of a fire.

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RISK ASSESSMENT TITLE	LEARNING AREA	ASSESSMENT UNDERTAKEN	ASSESSMENT REVIEW
The force shoe	Equine Research	**Signed:** Danielle Holt **Date:** September 2013	**Date:** September 2014

STEP ONE	STEP TWO	STEP THREE
List significant hazards here:	**List groups of people who are at risk from the significant hazards you have identified.**	**List existing controls or note where the information may be found. List risks which are not adequately controlled and the action needed:**
Fitting/removing: Researcher being injured if the horse moves and tries to put its foot down whilst fitting/removing the force shoe and the 'dummy shoe' on the opposite forelimb	Researchers handling the horse	This will be performed by two researchers who are experienced in handling horses. The correct PPE will be worn including sturdy boots with good grip. The equipment required to fit the force shoe will be close to hand before the foot is lifted to reduce the time required to fit the shoe.
Researcher being injured if the horse stamps its foot whilst securing/removing the data logger	Researcher handling the horse	The researcher will practice the procedure for applying the force shoe and data logger several times before approaching the horse to reduce unnecessary handling. A tendon boot that is easy to fit

to the distal limb, which has a small wire running from the unit to the force shoe.		and remove and commonly used in practice will be fitted to the lower limb of the horse in order to secure the data logger. The researcher will not kneel or put their hands on the floor, which is standard practice on a stable yard.
Researchers being injured whist the horse is being led. This could include rope burn from the lead rope, being trodden on or kicked.	Researcher and co-workers	The researchers will wear the correct PPE including sturdy boots, gloves and a hat, which meets the BSEN 1384 PAS 015 standard when holding/leading the horse. The handlers will leave plenty of room near the limbs of the horse to avoid being trodden on or kicked. The horse will have 'dummy' equipment secured to its lower limb initially in order to habituate the horse. The equipment that will be used to secure the force shoe includes the use of holes and screws. Studs are commonly used in practice, which are secured using a similar method. If the horse does not become accustomed to the 'dummy' equipment then it will be withdrawn from the study. The actual force shoe and data logger will be fitted in the arena that the horse will be tested in to avoid any slipping or catching the equipment on the stable yard (such as in doorways).
The testing procedures includes the risk of slipping when inside or exiting the arena due to the surface material underfoot.	Researcher and co-workers	Be aware at all times of the surface being stepped on and take time to clean any excess build up of surface material of shoes whilst in and outside the arena. The correct Personal Protective Equipment must be used including sturdy boots with sufficient grip.
Loose wires between the force shoe and data logger, causing a trip hazard.	Researcher handling the horse and horse	All equipment will have been tested and checked prior to use for loose wires or possible problems. The horse will be under constant supervision and if any problems are encountered, the horse will be stopped immediately.
The force shoe and/or data logger	Researcher, co-workers and	The force shoe and data logger will be double-checked that they

may become loose, causing a trip hazard.	horses	are fitted securely prior to commencing the study. The stud screws used to fit the force shoe will be checked to make sure that they have not come loose. The Velcro/straps on the tendon boots will be in very good condition and free from bedding/surface material, which could possibly reduce the adhesion of the Velcro.
Using equipment in wet conditions that could cause faults with the wires and possible electrocution.	Researcher, co-workers and horses	The force shoe will **not** be used in wet conditions and any puddles will be avoided.
Zoonotic disease through working near animals.	Researcher and co-workers	Hand washing and good hygiene will be expected by all personnel involved.
Allergic reactions to horses	Researcher and co-workers	Screen all researchers and co-workers working with horses.
The habituation and trials both pose a risk of injury occurring	Researcher and co-workers	Qualified first aider and first aid kit will be on site during set up and testing. It will also be ensured that there is a mobile phone available in case the need to call the emergency services arises. A first aid box and the first aider will be located before the testing begins.

Accepted by EVJ as a technical note – revisions submitted Dec 2016

A simple method for equine kinematic gait event detection

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Keywords:

Biomechanics, Kinematics, Gait Event, Ridden Horse, Ground reaction force, 3D analysis

Summary

Background: Previous studies have validated methods for determining kinematic gait events using threshold-based methods, however a simple method is yet to be identified that can be successfully applied to walk, trot and canter.

Objectives: To develop a simple kinematic method to identify the timing of hoof-on, peak vertical force and hoof-off, which can be applied to walk, trot and canter.

Study Design: *In-vivo* method authentication study. The horses (*n*=3) were ridden in walk, trot and canter down a runway with four force plates arranged linearly. Three-dimensional forces were recorded at a sampling rate of 960 Hz and were synchronised with a ten-camera motion analysis system sampling at 120 Hz.

Methods: Events identified from the vertical ground reaction force (GRFz) data were hoof-on (GRFz>50N), peak vertical force ($GRFz_{peak}$) and hoof-off (GRFz<50N). Kinematic identification of hoof-on and hoof-off events was based on sagittal planar angles of the fore and hindlimbs. Peak metacarpophalangeal/metatarsophalangeal (MCP/MTP) joint extension was used to assess the time of $GRFz_{peak.}$ The accuracy (mean) and precision (SD) of the time difference between the kinetic and kinematic events were calculated for the fore and hindlimbs at each gait.

Results: Hoof-off was determined with better accuracy (range: -3.94 to 8.33ms) and precision (5.43 to 11.39ms) than hoof-on across all gaits. Peak MCP angle (5.83 to 19.65 ms) was a more precise representation of $GRFz_{peak}$ than peak MTP angle (11.49 to 67.75 ms).

Main Limitations: The sample size was small and, therefore, further validation is required. The proposed method was tested on one surface.

Conclusions: A simple kinematic method of detecting hoof-on, hoof-off and $GRFz_{peak}$ is here proposed for walk, trot and canter. Further work should focus on validating the methodology in a larger number of horses and extending the method for use on surfaces with varying compliance.

Introduction

Equine biomechanical studies rely heavily on determination of gait events and subsequent stride cycles for the accurate analysis of kinematic and kinetic variables [1]. However, a standardised, evidence-based method to objectively determine gait events using motion capture data is yet to be defined for over ground, ridden conditions[2,3]. Previous studies reported that limb force and timing of initial hoof impact can be difficult to identify using kinematic data, with force plates being widely accepted as the “gold standard” for identifying hoof contact (hoof-on) and lift off (hoof-off) [2,4,5]. Force plates are, however, rarely used outside laboratory conditions, so a reliable kinematic method of defining the time of hoof-on, hoof-off and peak vertical force ($GRFz_{peak}$) in field studies would be useful [2,6].

Previous validations of kinematic gait events against force data have reported high accuracy and precision [2,3,6,7,8,9]. Most of these studies use hoof markers for event detection but precise visual determination of hoof contact and lift off are difficult, especially on compliant surfaces [2, 10]. The objective was to use force data to evaluate a straightforward kinematic method to identify the time of hoof-on, hoof-off and $GRFz_{peak}$, which can be universally applied to all limbs of the ridden horse in walk, trot and canter.

Methods

Horses

Three Lusitano stallions (height at withers: 1.61–1.65m; mass: 535.5 - 585kg) trained to advanced level dressage were ridden by their usual trainer (mass: 65 kg). The horses were assessed by a veterinarian to be sound at walk and trot on a straight line.

Data Acquisition

Retro-reflective 3D markers were applied to the left and right side of the horse (Figure 1). A static trial of each horse standing square and at least 6 successful walk, trot and canter trials were recorded. The horses were ridden in walk (1.66 ± 0.22 m/s), trot (2.44 ± 0.25 m/s) and canter (2.95 ± 0.69 m/s) down a runway with a poured rubber surface. Speed was measured using the first derivative of a marker on the sacrum in the direction of motion. Kinematic data were captured at 120 Hz with a ten-camera motion analysis system (Eagle cameras, Motion Analysis Corp.; Cortex 1.1.4.368, Motion Analysis Corp.) and synchronised kinetic data with four force plates arranged linearly along a runway (Bertec Corporation, USA) at 960 Hz.

Data Processing

Kinematic and kinetic data were analysed using Visual 3D (C-Motion Inc.). Kinematic data were interpolated (maximum gap 10 frames) and then filtered with a low pass zero lag 4th order Butterworth digital filter (cut off frequency of 10 Hz). The same filter was also applied to the kinetic data with a cut off frequency of 100 Hz in accordance with [11]. The timing of hoof impact, lift off and peak vertical force was calculated using GRF and kinematic data.

Gait event detection using GRF data

Footfalls were rejected if the hoof was not entirely on the force platform or if another hoof was in contact with the same force platform simultaneously. The vertical ground reaction force

(GRFz) data were used to detect the time of hoof-on (GRFz>50N), peak vertical force ($GRFz_{peak}$) and hoof-off (GRFz<50N).

Gait event detection using kinematic data

To determine the kinematic hoof-on and hoof-off events for the forelimbs, a sagittal plane angle was computed using the following markers: 1) centre of rotation of the MCP joint; 2) centre of rotation of the distal interphalangeal (DIP) joint; 3) the lateral epicondyle of the humerus (Figure 1a). The hindlimb events for hoof-on and hoof-off were also identified by creating a sagittal plane angle, using the following markers: 1) centre of rotation of the MTP joint; 2) the talus representing the centre of rotation of the tarsal joint; 3) the hind DIP joint (Figure 1b). Planar angle-time curves were plotted for the fore and hindlimbs. A threshold of 0 degrees was used to define events when the two segments were aligned, with hoof-on coinciding with descent through 0 degrees and hoof-off on ascent through 0 degrees. The time of $GRFz_{peak}$ was identified with the kinematic data using maximum MCP and MTP joint extension, where maximal MCP extension has previously shown a strong correlation with peak vertical force [12].

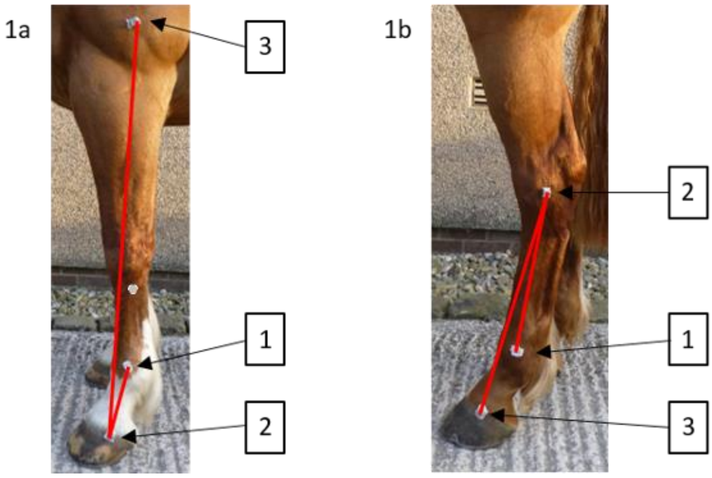

Figure 1a) The sagittal plane angle used to identify hoof-on and hoof-off events for the forelimbs; 1) MCP joint; 2) fore DIP joint; 3) lateral epicondyle of the humerus. The MCIII was created using markers on the proximal end of metacarpal IV and MCP joint. The MCP joint was created using the MCIII and fore pastern segment, which was made using the centre of rotation of the MCP joint and fore DIP joint markers. Figure 1b) The sagittal plane angle used to identify hoof-on and hoof-off events for the hindlimbs; 1) MTP joint; 2) talus; 3) hind DIP joint. The MTIII segment was created using the talus and MTP joint markers. The MTP joint was created using the MTIII and hind pastern segment, which was made using the centre of rotation of the MTP joint and hind DIP joint markers.

Data Analysis

Gait event timings were derived using the GRF and kinematic methods. The accuracy and precision of the kinematic gait events at representing the GRF events were calculated for the fore and hindlimbs at each gait in accordance with [3]. Accuracy is defined as the mean difference between kinematic and GRF events (bias) and precision as the standard deviation (SD) of the mean difference (accuracy) [3]. The smallest difference was considered the best accuracy and precision.

<u>Results</u>

A total of 227 stance phases (walk: 113; trot: 80; canter: 34) were analysed across all subjects. Accuracy and precision of the kinematic gait events for all gaits and individual limbs (Table 1) showed that hoof-off was identified more accurately than hoof-on, as shown by a much smaller deviation from the GRF event (Figure 2). Accuracy (difference in timings closer to zero) and precision (smaller standard deviation of the difference in timings) were higher for hoof-on in canter compared to walk and trot. Accuracy for hoof-off was highest at trot, but precision was highest at walk. The time of $GRFz_{peak}$ corresponded well with maximal MCP/MTP extension.

Table 1: The accuracy (mean) and precision (standard deviation) between events detected kinematically and using ground reaction force data for forelimbs and hindlimbs of all horses at each gait (ms). Canter was categorised further into leading (Le) and trailing (Tr) limbs. Positive values indicate that the kinematic event occurred before the GRF event and vice versa for negative values. Negative values for stance duration indicate that the kinematic method generates a longer timing.

		Events							
Gait	**Limb**	**Accuracy (ms)**	**Precision (ms)**	**Accuracy (ms) Peak MCP /MTP ext.**	**Precision (ms) Peak MCP /MTP ext.**	**Accuracy (ms)**	**Precision (ms)**	**Accuracy (ms)**	**Precision (ms)**
		Hoof-on (GRF-Kin)		**$GRFz_{peak}$ (GRF-Kin)**		**Hoof-off (GRF-Kin)**		**Stance Duration**	
Walk	Fore	36.91	20.83	-0.3	19.65	4.76	8.54	-31.99	27.0
	Hind	26.49	19.47	-60.61	67.75	2.62	6.64	-24.2	22.4
Trot	Fore	49.32	20.26	-25.22	11.21	8.33	5.43	-41.18	20.51
	Hind	21.99	13.53	-38.44	16.90	-3.94	7.04	-36.76	61.21
Canter	Le FL	17.86	18.28	-19.79	6.20	7.29	6.95	-10.71	22.93
	Tr FL	31.67	16.57	-21.67	5.83	7.50	8.29	-24.17	21.32
	Le HL	11.11	22.77	-62.5	11.49	2.78	11.39	-8.33	29.81
	Tr HL	12.88	8.63	-33.33	13.61	0.76	10.18	-12.12	16.82

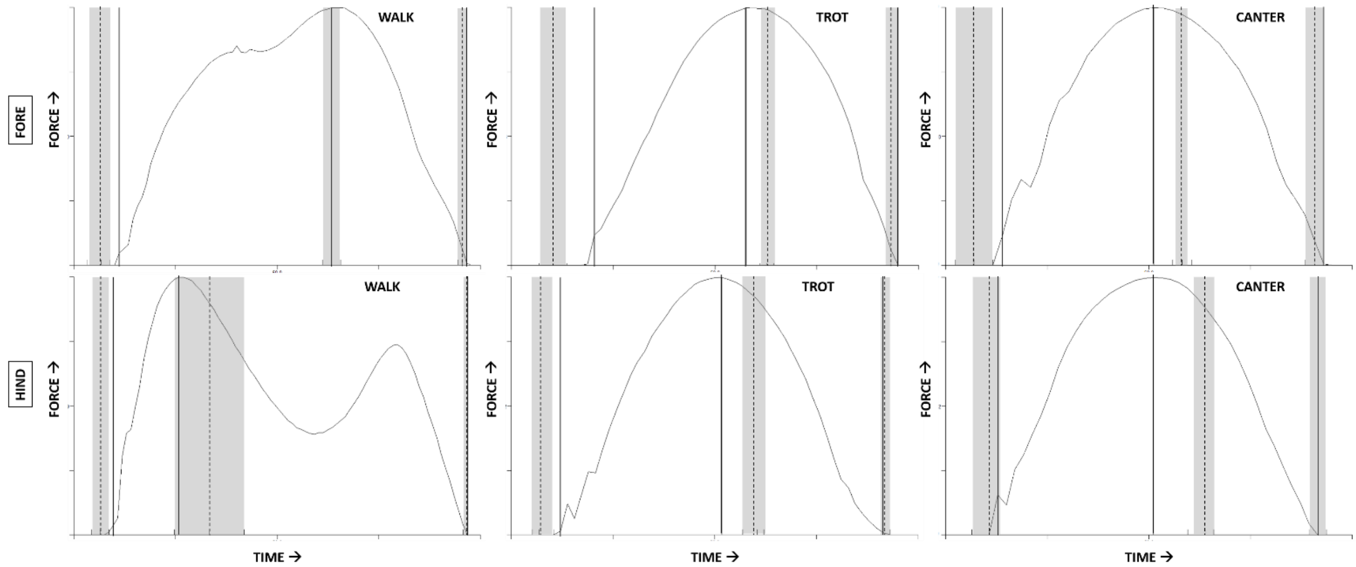

Figure 2: The accuracy and precision of the kinematic gait events for fore and hindlimbs on a GRF trace at walk, trot and canter. The solid black lines on each graph represent the GRF events at hoof-on (GRFz>50N), GRFz$_{peak}$ and hoof-off (GRFz<50N) from left to right respectively. The dotted lines represent the events identified using the kinematic methods; from left to right: hoof-on, peak MCP/MTP extension and hoof-off. The shaded areas represent the precision of each kinematic event. The canter data from the leading and trailing limbs has been grouped for the purpose of this graphical representation.

Discussion

This study evaluated a kinematic method for determining the timing of hoof-on, GRFz$_{peak}$ and hoof-off events in walk, trot and canter. The method is simple, can be applied to two dimensional or three dimensional kinematic data and can be used under most field conditions, provided the coronary band is visible. The hoof-off event was detected with better accuracy and precision than hoof-on, which was generally within one to two frames of the GRF event. The timing of GRFz$_{peak}$ also corresponded closely with maximal MCP/MTP extension.

Hoof orientation during impact was not taken into account for this study. The hoof sole has been observed to be completely flat on the ground within several milliseconds of initial impact [13], which suggests that the effect of hoof orientation on impact timing should be minimal. The distal interphalangeal joint markers are also at the centre of rotation, which therefore should make the detection method less sensitive to hoof orientation on landing. The horses in this study were tested during collected canter and further work is required to investigate the accuracy and precision of the kinematic detection methods in horses travelling at faster velocities.

Precision as low as 2 ms or less than one frame of data has been reported [9] for hoof-on at walk and trot using a velocity threshold method, which appears to be the most accurate to date. In that study a greater sample of footfalls were analysed (360-800 hoof-on events for walk and trot in a straight line), however it is important to note that differences were calculated by averaging the within-horse mean values, which will lower the overall differences between footfalls [9]. Nevertheless, the hoof-off kinematic detection method reported here demonstrated better accuracy at trot in the hind limbs than the methods used by [9]. The hoof-off event at trot was comparable to some of the methods described by [3], however the detection methods used appear to be more complex to execute in comparison to this study.

Some methods [3,7,9] are also dependent on velocity thresholds. Surface properties can influence parameters such as hoof landing velocity [10], which may affect the repeatability of these methods if used on compliant surfaces. Forelimb landing angle is affected by surface stiffness [10], which suggests that the angles used to calculate the kinematic events during this study may also be affected by the surface properties. Surface effects are not well documented [3], so pilot work is recommended before testing on compliant surfaces [9].

Peak vertical force is commonly identified in research because it is associated with the risk of musculoskeletal injuries and can be used during lameness assessments [14]. The ability to calculate the timing of this event in the absence of force data could constitute a useful tool when quantifying the entire kinematic profile of a horse during such assessments. In this study, peak MCP extension was found to be a more precise method for $GRFz_{peak}$ detection than MTP extension. A very strong positive correlation between MCP joint angle (49.4% stance) and GRF (47.7% stance) was found during *in vitro* loading [12]. In contrast, [15] suggested that maximal fetlock extension and peak force in the forelimbs during trot occur more independently. A delay in fetlock extension has been observed during trot in the forelimbs of ridden horses where it was proposed that the dynamic effect of the rider may have a greater influence after mid-stance when the horse's centre of gravity is rising [16]. This could explain why peak MCP and MTP extension occurred after $GRFz_{peak}$ in the present study.

Conclusions

A simple method of detecting force gait events using kinematic data has been identified for ridden walk, trot and canter. Further work must focus on validation using a greater sample size to establish the effect of a larger population of horses on the accuracy and precision of the detection methods under a variety of ridden and un-ridden conditions.

References

[1] Leach, D. (1993) Recommended terminology for researchers in locomotion and biomechanics of quadrupedal animals. *Cells Tissues Organs*, **146** (2-3), 130-136.

[2] Hobbs, S. J., Orlande, O., Edmundson, C. J., Northrop, A. J. and Martin, J. H. (2010) Development of a method to identify foot strike on an arena surface: application to jump landing. *Comparative Exercise Physiology,* **7** (1), 19-25. doi:10.1017/S1755254010000097

[3] Boye, J. K., Thomsen, M. H., Pfau, T. and Olsen, E. (2014) Accuracy and precision of gait events derived from motion capture in horses during walk and trot. *Journal of Biomechanics*, **47** (5), 1220-4. doi: 10.1016/j.jbiomech.2013.12.018.

[4] Schamhardt, H. C. and Merkens, H. W. (1994) Objective determination of ground contact of equine limbs at the walk and trot: comparison between ground reaction forces, accelerometer data and kinematics. *Equine vet. J. Suppl*., **17**, 75-79.

[5] Witte, T. H., Knill, K. and Wilson, A. M. (2004) Determination of peak vertical ground reaction force from duty factor in the horse (*equus caballus*). *The Journal of Experimental Biology*, **207**, 3639-3648.

[6] Olsen, E., Andersen, P. H. and Pfau, T. (2012) Accuracy and precision of equine gait event detection during walking with limb and trunk mounted inertial sensors. *Sensors*, **12**, 8145-8156. doi:10.3390/s120608145

[7] Peham, C., Scheidl, M. and Licka T. (1999) Limb locomotion – speed distribution analysis as a new method for stance phase detection. *Journal of Biomechanics*. **32**, 1119-1124.

[8] Galisteo, A. M., Garrido-Castro, J. L., Miró, F., Plaza, C. and Medina-Carnicer, R. (2010) Assessment of a method to determine the stride phases in trotting horses from video sequences under field conditions. *Vet. Med. Austria*, **97**, 65-79.

[9] Starke and Clayton (2015), A universal approach to determine footfall timings from kinematics of a single foot marker in hoofed animals. *PeerJ*, **3**,e783. DOI 10.7717/peerj.783

[10] Burn, J. F. and Usmar, S. J. (2005) Hoof landing velocity is related to track surface properties in trotting horses. *Equine and Comparative Exercise Physiology*, **2** (1), 37–41. doi.org/10.1079/ECP200542

[11] Hobbs, S. J. and Clayton, H. M. (2013) Sagittal plane ground reaction forces, centre of pressure and centre of mass in trotting horses. *The Veterinary Journal*, **198**, e14-e19. doi.org/10.1016/j.tvjl.2013.09.027

[12] McGuigan, M. P. and Wilson, A. M. (2003) The effect of gait and digital flexor muscle activation on limb compliance in the forelimb of the horse *Equus caballus*. *The Journal of Experimental Biology*, **206**, 1325-1336. doi: 10.1242/jeb.00254

[13] Merkens, H. W. and Schamhardt, H. C. (1994) Relationships between ground reaction force patterns and kinematics in the walking and trotting horse. *Equine Veterinary Journal*, **26**, (17) 67-70. DOI: 10.1111/j.2042-3306.1994.tb04877.x

[14] Merkens, H. W. and Schamhardt, H. C. (1988) Evaluation of equine locomotion during different degrees of experimentally induced lameness I: Lameness model and quantification of ground reaction force patterns of the limbs. *Equine Veterinary Journal*, **20** (S6) 99-106. DOI: 10.1111/j.2042-3306.1988.tb04655.x

[15] Abu-Ali, G. S., Mullineaux, D. R. and Clayton, H. M. (2004) Evaluation of kinetic and kinematic events in equine locomotion. *Equine and Comparative Exercise Physiology*, **1** (2), A1- A30. doi.org/10.1079/ECEP200419

[16]Clayton, H. M., Lanovaz, J. L., Schamhardt, H. C., & Wessum, R. V. (1999). The effects of a rider's mass on ground reaction forces and fetlock kinematics at the trot. *Equine Veterinary Journal*, **31** (S30), 218-221.

Ethical Considerations

All procedures were approved by the Michigan State University Institutional Animal Care and Use Committee, protocol #02/08-020-00.

Conflicts of Interest

The authors have declared no conflicts of interest.

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Registered title: Do horses alter limb stiffness as a function of surface stiffness?

Abstract

Limb stiffness (peak vGRF/vertical displacement of the limb) has been researched extensively in humans, possibly because it is thought to be related to performance and injury. Calculating limb stiffness in horses has formed the basis of the MPhil phase of the project. The aim of the project was to develop equipment and techniques capable of measuring peak vertical ground reaction force, length changes of the forelimb and associated functional characteristics in the horse during locomotion. A second aim of the project was to quantify gait variability when walking and trotting (inhand v. ridden) over a uniform surface so true variability initiated by a change in surface can be accounted for during the PhD phase.

A simple prototype force shoe was designed and is currently going through a validation process. Kinematic and surface electromyography measurements were taken to identify parameters and any evidence of muscle pre-activation (prior to ground contact) that may influence limb stiffness. The coefficient of variation was less than 15% during all of the walk trials and less than 8% during the trot trials for most of the kinematic parameters. The *ulnaris lateralis* muscle demonstrated the most evidence of pre-activation, suggesting that the end of the swing phase of the stride is possibly controlled by neuromuscular activity and there may be potential for horses to modulate limb stiffness.

Arena surfaces, which are used for training and competition predominantly for dressage and show jumping horses, have variable functional properties due to many external influences such as the weather. Horses are expected to perform consistently and therefore the ability to adapt to such variable properties may have implications in injury prevention. This has generated the unique focus for the PhD phase where limb stiffness and other associated parameters will be measured using the same technique as the MPhil phase on a surface with sudden and gradual changes in the functional properties. Adaptations to the surface, if any, will be identified and discussed in relation to equine performance and injury prevention.

Introduction

Performance and soundness are key factors to the success and longevity of a horse's career. The surface type used for training and competition is considered a risk factor for injury, which has attracted attention from several research groups worldwide. Unlike sports surfaces for human athletes, it is generally believed that a surface that poses a greater risk of injury is associated with a higher level of performance whereas a surface that has shock absorbing properties increases muscular effort and will be detrimental to performance as discussed by Chateau *et al*. (2010) and Dura *et al*. (1999). Research that contributes to improving the welfare of horses by identifying surface properties that may enhance or be of detriment to the horse in terms of injury and performance is therefore paramount.

Limb stiffness in humans, which is the relationship between the deformation of a body and a given force in its simplest sense, has generated research interest because it is thought to be related to performance and injury (Butler *et al*., 2003). Some stiffness is necessary for performance however, too much or too little may lead to injury. There is evidence that when humans encounter sudden changes in substrate stiffness or damping (Farley *et al*., 1998; Ferris *et al*., 1999; Kerdok *et al*., 2002; Moritz *et al*., 2004), or uneven ground with changes in terrain height (Grimmer *et al*., 2008; Müller and Blickhan, 2010), they adapt their limb properties (leg stiffness, orientation and length) to the altered situation. Limb adaptation appears to help maintain a consistent centre of mass trajectory and avoid disruptions to locomotion.

The human (McMahon and Cheng, 1990; Farley and Gonzalez, 1996; Müller *et al*., 2010) and equine (McGuigan and Wilson, 2003) limbs have been modelled as springs previously however the components responsible for controlling the stiffness of this spring are quite different between the two species. Alterations in human limb stiffness are mainly initiated by changes in the activity of the long muscle fibres, which is energetically expensive whereas the equine distal limb has significant passive elastic properties, which suggests that the mechanisms behind altering limb stiffness will differ (Biewener, 1998). Such findings in humans in conjunction with the limited research on the response of horses to changes in surface properties provided the motivation for investigating limb stiffness in horses for this PhD.

Equine Limb Stiffness

Equine limb compliance has been studied by McGuigan and Wilson (2003) where the forelimb of the horse was split into two components; the proximal spring (proximal spine of the scapula to the lateral epicondyle of the humerus) and the distal spring (lateral epicondyle of the humerus to the distal interphalangeal joint) (Figure 1). The absolute length changes and ratio of the proximal:distal length change between hoof impact and mid-stance were reported. The results showed that the length change in the distal spring was ten times greater than the length change in the proximal spring and demonstrates the necessity to consider the proximal and distal limb as two separate components, which contribute to whole limb stiffness. Work for this PhD calculates limb length change of the two springs separately to enable comparison with McGuigan and Wilson (2003) in order to further understand the horse's response to functional properties of a surface.

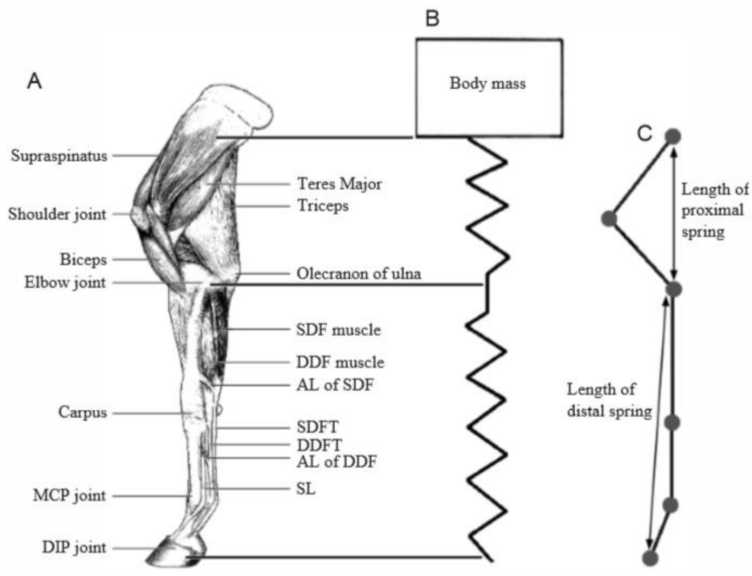


Figure 1: The distal and proximal springs; (A) The equine forelimb, showing the muscles associated with weight bearing; (B) The limb can be conceptually divided into two springs, representing the proximal part of the limb (shoulder to elbow) and the distal part of the limb (elbow to digit); (C) The length of the springs. Taken from McGuigan and Wilson (2003).

The total length change (proximal + distal segment length) will subsequently be used to calculate limb stiffness in equation 1, which has formed the basis of the MPhil work:

$$\textbf{Equation 1: Limb stiffness} = \frac{\textbf{Peak vertical ground reaction force}}{\boldsymbol{Total\ length\ change\ (proximal{+}distal\ length\ change)}}$$

MPhil Phase

Aims:

1. To develop equipment and techniques capable of measuring peak vertical ground reaction force, proximal and distal length changes and associated functional characteristics in the horse during locomotion.
2. To quantify gait variability when moving over a uniform surface.

Objectives:

1. Design a suitable prototype force shoe to enable the peak vertical ground reaction forces to be recorded during consecutive strides (Part I).
2. To develop kinematic techniques to measure proximal and distal limb length changes (Part II).
3. To investigate gait variability within horses, between days and activities (ridden v in-hand) using surface electromyography and kinematic measurements (Part II).

Part I Bespoke equipment development – Force shoe

Introduction

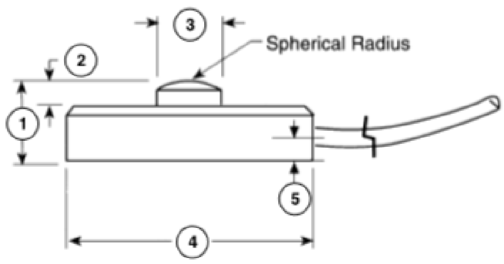
It is well established that surface properties affect the hoof-surface interaction (Barrey *et al.*, 1991; Gustås *et al.*, 2006a; Chateau et al., 2010) and therefore the forces and accelerations experienced by the horse during the stance phase. The ability to measure the ground reaction forces during equine locomotion enables the effect of different surfaces on this complex interaction to be characterised. Various methods of calculating the ground reaction forces have been developed, each of which has advantages and limitations. Force plates have been used previously for studies on humans and horses (Ferris *et al*., 1999; Clayton *et al*., 1999). Capturing an impact of the entire limb can take numerous attempts however (Merkens *et al.*, 1993). A force plate would remove the potential to record the ground reaction forces on different surfaces and would also affect the surface stiffness under which it is installed. Instrumented treadmills have been used to measure the ground reaction forces of multiple strides at different gaits and speeds (Weishaupt *et al*., 2010), but this removes the ability to test numerous surfaces that are used for training and competition.

The limitations of force plates and instrumented treadmills have been recognised previously, which has subsequently led to the development of instrumented horseshoes. This type of application enables the measurement of ground reaction forces during consecutive strides on surfaces that are commonly used for training and competition. Earlier designs were generally more fragile and not always reliable (Frederick and Henderson, 1970; Ratzlaff *et al*., 1987; Barrey *et al*., 1990; Kai *et al*., 1999) but more recent developments are considered to be robust and have demonstrated a high degree of accuracy and repeatability (Roland *et al*., 2005; Chateau *et al*., 2009). Roland *et al*. (2005) constructed the first three dimensional dynamometric horseshoe using strain gauge technology however at 0.86kg, this would significantly affect the gait of the horse (Lanovaz *et al*., 1999). A more recent dynamometric horseshoe created by Chateau *et al*. (2009) was much lighter at 0.49kg and has been instrumented with piezoelectric sensors, which were considered well suited to measure ground reaction forces and moment vectors. The total height of the dynamometric horseshoes were 21mm (Roland *et al*., 2005) and 22mm (Chateau *et al*., 2009) however, which was recognised as possibly having an effect on equine locomotion (Chateau *et al*., 2009). The most recent instrumented horseshoe design was developed by Martin *et al*. (2014) in order to measure the surface properties of race tracks and hoof impact characteristics at racing speeds. The additional height and weight was negligible because the standard shoe was used and fitted with inertial transducers and a strain gauge before being fitted by a farrier. This type of application would be most suited for this project however, they have a short life span and can only be fitted by a farrier immediately prior to data collection to one horse. It is also not possible to view data as it is being collected, which is a severe limitation if there were any problems with the system.

The aim of this work therefore, was to develop a simple force shoe within the allocated budget that was capable of measuring a proportion of the peak vertical ground reaction force of several horses during locomotion and that did not require a farrier to be present during data collection.

Development work

It was important to develop a wireless data acquisition (DAQ) system to reduce the risk of injury during data collection and to ensure that the application was simple to use. Delsys®, a company that has developed their own movement and surface electromyography sensors (sEMG) provided a solution for this project. A LEMO connector was fitted to a small, compatible load cell (figure 2) with a suitable range (5kN) in order to be connected to a Trigno™ load cell adaptor, which could send signals wirelessly to a Delsys® base station and laptop within a 30m range of the horse.



	Dimensions (mm)
1	6.4
2	0.8
3	6.1
4	19.0
5	2.5

Figure 2: Load cell (5 kN Interface model LBS miniature compression load button) dimensions.

The load cell was secured between two rigid structures within an aluminium housing in order to perform compression tests to measure the change in magnitude of the signal and determine the suitability of the material to be used as part of a force shoe. A plate with a 50mm diameter was placed on top of the button and screwed to the housing in order to prevent it from rocking and applying side loads (Plate 1). This meant that the load cell was pre-loaded, which is similar to the dynamometric horse shoe created by Chateau *et al*. (2009). Pre-loading the load cell also ensures that it remains secure between two rigid surfaces within the force shoe during dynamic loading and therefore reduces the variability of the data collected.

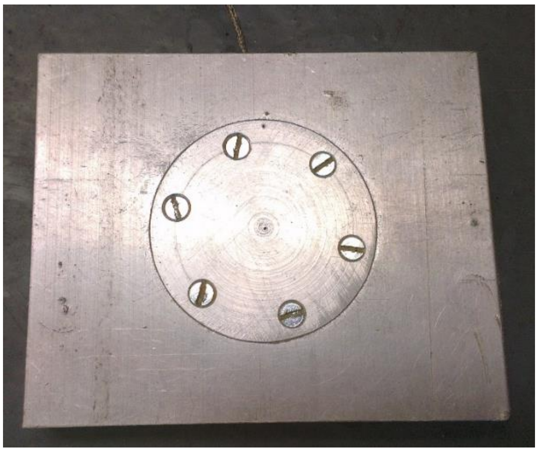


Plate 1: Aluminium housing for the load cell with the contact plate.

Force Shoe Design

The anatomy of the foot and the location of the centre of pressure (COP) has been a key consideration in the design of the force shoe. Peak vertical ground reaction forces occur at mid-stance and so positioning the load cell within the housing at the approximate COP location will provide a good representation of the vertical force being experienced by the horse. Hood *et al*. (2001) measured load distribution in horses on a deformable sand surface using a carbon fibre based transducer system. The centre of pressure was difficult to localise due to a lack of hoof wall contact but it was generally described as being located in the central third of the foot. The COP in newly shod horses (n=18) was most often located in the medio dorsal quadrant during a different study by van Heel *et al*. (2005). This relative distribution did not appear to change after an 8 week shoeing interval. A more recent study by Oosterlinck *et al*. (2014) measured contact area of the hoof using a pressure plate during walk and trot on a soft and hard surface. At mid-stance, there was more loading of the toe region on the soft surface while the medial and lateral areas were loaded equally (Oosterlinck *et al*., 2014). Based on these findings, it can be loosely concluded that the button of the load cell within the force shoe must be situated centrally to account for individual differences between horses and within the central third of the hoof (Plate 2).

Plate 2: Solar view of the right front hoof of the pilot horse with the approximate centre of pressure location at mid-stance. This is not to scale.

The aluminium housing used for the compression tests proved to be suitable material to use and was adapted so it could be secured to a standard steel horse shoe (Plate 3 and plate 4a). The standard shoe was drilled with 5mm holes at three locations before being fitted by the farrier. The height of the force shoe was 10mm and weighed 0.6 kg including the Delsys® load cell adaptor and standard steel shoe (Plate 4b). Compensatory height and weight will be added to the left fore shoe in the form of a 'dummy' force shoe in order to prevent asymmetrical movement.

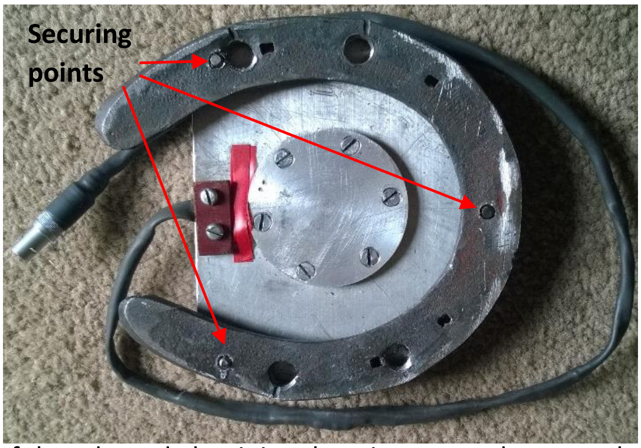


Plate 3: Dorsal view of the adapted aluminium housing secured to a used steel shoe in preparation for repeating the compression tests.

Plate 4a: Solar view of the prototype force shoe secured to the base of the right front shoe.

Plate 4b: Lateral view of the prototype force shoe.

Compression tests on the prototype force shoe

A Denison machine was used to apply up to 2 kN to the force shoe 3 at 0.2 kN increments. The compression tests were performed with the force shoe placed on a steel surface (Plate 5a) and on an arena surface (Plate 5b). The effect of three different pre-loads (200, 300 and 400 µV) on the repeatability and range of measurements was also investigated. The tests were repeated three times (Chateau *et al.*, 2009) for each increment at each pre-load to determine the accuracy of the measurements. The voltage was recorded for three seconds using EMGworks® acquisition software at a sampling rate of 1926 Hz for each repetition and values were extracted using EMGworks® analysis software.

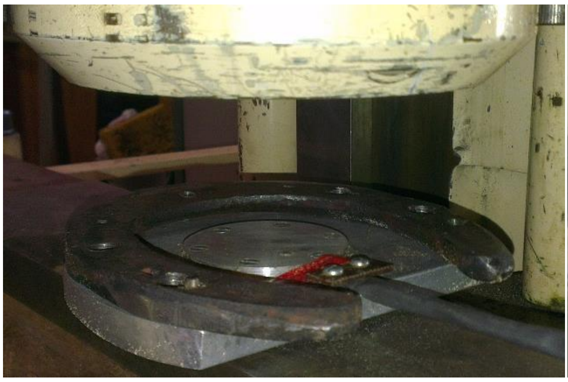


Plate 5a: Compression tests on steel

Plate 5b: Compression tests on an arena surface

The 200 µV pre-load generated the strongest correlation between the force applied and the voltage recorded and therefore will be used for future testing (Figure 3). The proportional voltage being recorded on the surface in comparison to steel was also calculated using the data in figure 3 in order to quantify the actual force being applied to the force shoe by the horse during *in-vivo* testing (Figure 4).

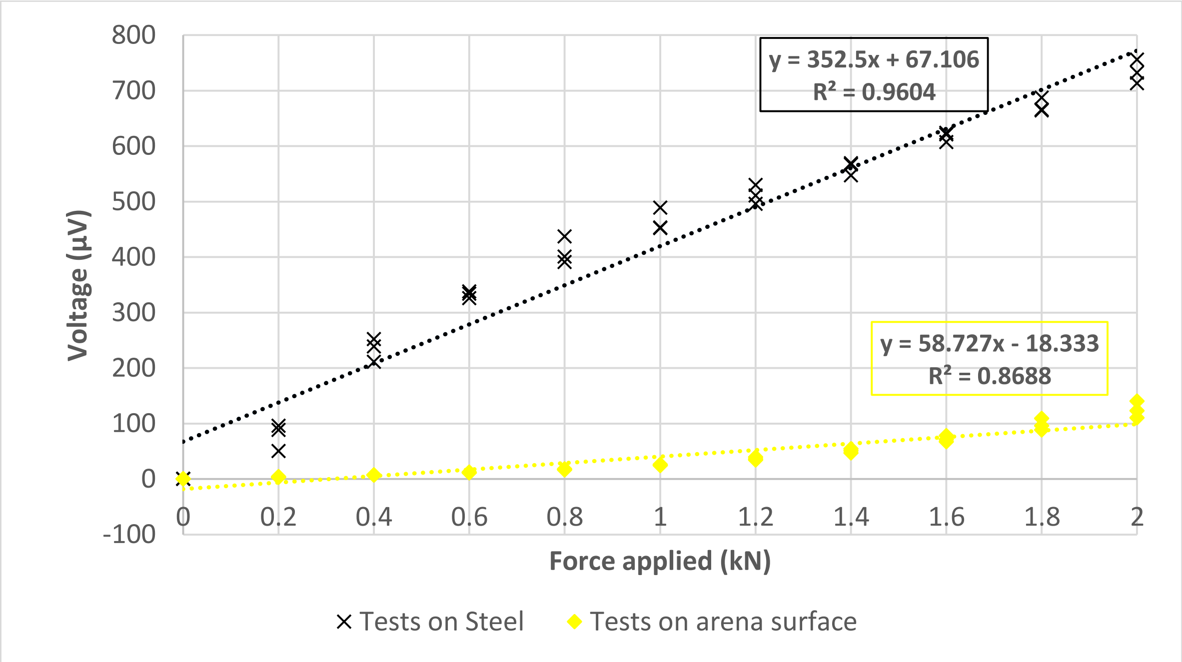


Figure 3: Voltage recorded with the force shoe pre-loaded at 200 µV on steel and on an arena surface using the LEMO connector and Delsys® system.

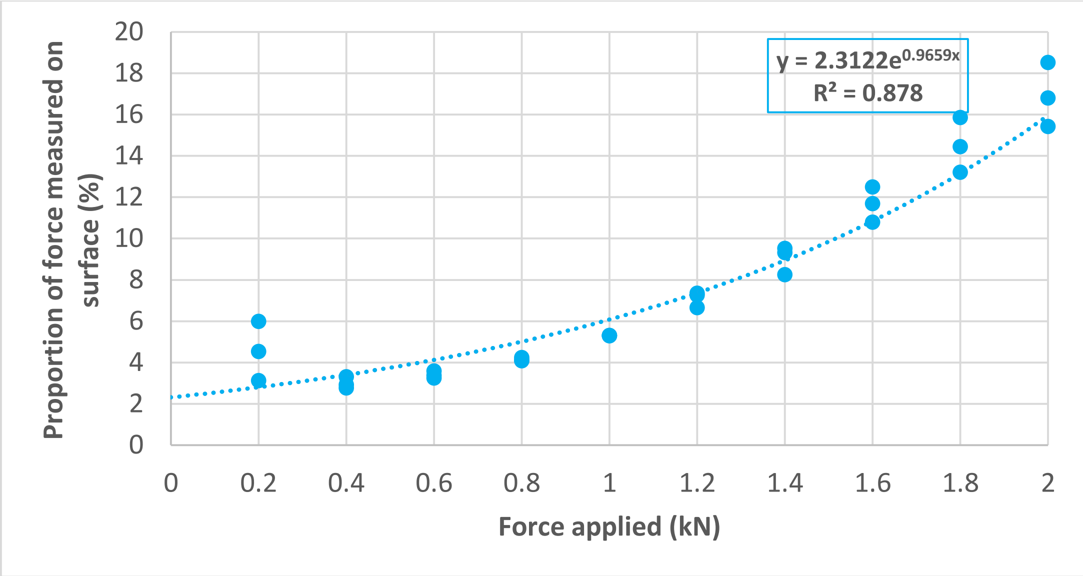


Figure 4: Proportion of the force being measured on the arena surface in comparison to steel.

Measurements are yet to be taken with the force shoe attached to the pilot horse. Work is being carried out to identify methods of securing the wire of the load cell and ensuring the safety of the horse prior to any further data collection. It is envisaged that the wire from the load cell will run out towards the bulbs of the heel (towards the bottom of plate 4a) and up into a boot, which is commonly used for training and competition and fits around the distal limb. The boot will be adapted with a lateral pocket in order to hold the Trigno™ load cell adaptor. Other studies using instrumented horseshoes have previously had a much larger data acquisition system mounted onto the horse (Roland *et al*., 2005; Chateau *et al*., 2009) and therefore the system used for this project fulfils the initial criteria of being relatively simple.

Error Analysis

The compression tests will be repeated with the Denison machine once the force shoe has been used on the pilot horse. This was also performed by Chateau *et al*. (2009) to check the repeatability of measurements after an *in vivo* test session.

Part II: Kinematic and Electromyography measurements

Introduction

Kinematic and GRF data provide useful information describing the limb movements and the forces responsible for those movements (Clayton *et al*., 2000). The effect of different surface types and preparation methods on certain kinematic parameters of the horse has been researched extensively to determine how the substrate affects performance and potentially poses a risk factor for injury. The effect of surface type on variables such as ground reaction force (Ratzlaff *et al*., 1997; Kai *et al*., 1999; Gustås *et al*., 2006; Robin *et al*., 2009; Chateau *et al*., 2010, 2013; Crevier Denoix *et al*., 2010), hoof and fetlock accelerations (Barrey *et al*., 1991; Burn and Usmar, 2005; Gustås *et al*., 2006; Chateau *et al*., 2009; Kruse *et al*., 2012) and stride parameters such as stance duration, duty factor, stride frequency (Robin *et al*., 2009; Chateau *et al*., 2013) and hoof slide during landing from a jump (Orlande *et al*., 2012) have been quantified. The effect of preparation techniques on limb kinematics at stance have also been investigated (Tranquille *et al*., 2012; Walker *et al*., 2012; Northrop *et al*., 2013).

The forces and accelerations experienced during stance on different surfaces and preparations may not be the only factors responsible for alterations seen in kinematic parameters. The muscles may play an active role in positioning the limb prior to impact and stabilising the limb during loading. Neuromuscular activity before ground contact (muscle pre-activation) has been observed in humans previously (Hobara *et al*., 2007, 2008; Müller *et al*., 2010) however, there is little research to suggest this occurs in the horse. Harrison *et al*. (2012) investigated forelimb muscle activity during equine locomotion and reported that all of the surveyed muscles (n=15), except the *Extensor carpi radialis* (ECR), were active during stance. It was noted that the muscles were activated between late swing and early stance and deactivated only in late stance (Harrison *et al*., 2012). The presence of muscle activity prior to hoof impact may be evidence of pre-activation, however this was not discussed and requires further investigation.

It is well documented that surface type influences kinematic variables, however it is important to consider the amount of variation in each parameter that would be expected under the same conditions. This will enable true differences in limb stiffness that may be initiated by subtle and large changes in the functional properties of the surface to be accounted for during the PhD phase.

Inter-horse variability and inter-stride variability of horses trotting on a circle with a 4m radius on fibre sand and asphalt has been recorded by Chateau *et al*. (2013). Inter-horse variability was greater for all of the measured kinetic and kinematic variables in comparison to inter-stride variability (Chateau *et al*., 2013), which has also been found previously (Faber *et al*., 2002; Heaps et al., 2011). It is impossible to remove this inherent variability that exists between horses and therefore horses with similar breed, age and weight will be selected for the PhD phase. The presence of a rider may also affect the variability of kinematic parameters, which has previously shown to have a dynamic effect on equine locomotion (Clayton *et al*., 1999). Changes have been observed in peak vertical ground reaction forces and fetlock kinematics of the forelimb (Clayton *et al*., 1999) and the motion patterns of the equine back (Peham *et al*., 2004). It is envisaged that the force shoe will be used on ridden horses during the PhD phase and therefore trials in the un-ridden and ridden horse are appropriate for this work.

The aim of this work therefore, was to establish the normal degree of variability of certain kinematic parameters under the same conditions on different days during ridden and unridden trials. The second aim of the study was to determine if there was any evidence of muscle pre-activation in selected superficial muscles that may play a role in limb placement and stabilisation immediately prior to and during stance.

Materials and Methods

Kinematic and electromyographic readings were taken over four consecutive days from a small sample size (n=2; Horse 1: Gelding, 15.2 hh, 15years, Anglo Arab, 494 kg; Horse 2: Mare, 14.1hh, 17 years, polo pony, 416 kg) of horses, which were kept under the same management routine and used for college students.

Kinematic measurements

Kinematic data were recorded at 250 Hz using an eight camera (Qualisys Oqus) motion analysis system with Qualisys Track Manager Software. The cameras were set up so an extended calibration could be carried out (Plate 6), which enabled as many consecutive strides to be measured as possible.

Plate 6: An 8 camera motion analysis system set up for an extended calibration

Fourteen spherical retro-reflective markers, 25 mm in diameter were placed on the following anatomical locations on the right fore and hindlimbs of two horses: Forelimbs; the proximal end of the spine of the scapula, the greater tubercle of the humerus (centre of rotation of the shoulder joint), the lateral epicondyle of the humerus (centre of rotation of the elbow joint), the lateral tuberosity of the radius, the lateral styloid process of the radius, the proximal end of metacarpal IV, centre of rotation of the metacarpophalangeal (MCIII) joint and the lateral hoof wall, approximately over the centre of rotation of the DIP joint. Hindlimbs; the most ventral part of the tuber coxae, greater trochanter, lateral epicondyle of the femur, lateral malleolous of the talus, the centre of rotation of the MCIII joint and the lateral hoof wall (Plate 7). One marker was also placed on the croup of the horse in order to track speed during the trials. The horse's hair was trimmed in the less prominent anatomical locations to ensure a repeatable marker application during the different test days.

Plate 7: Locations of the retro-reflective markers (excluding the croup)

Electromyography measurements

Surface electromyograpy (EMG) measurements were taken at a sampling rate of 2000Hz using a Delsys® base station. The EMG and kinematic data were synchronised in Qualisys Track Manager using the Delsys® Trigno Control Utility. Five muscles identified in plate 8, were selected for their associated function with limb placement and because of their suitability and reliability for taking surface EMG measurements (Jansen *et al*., 1992; Hodson-Tole, 2006; Harrison *et al*., 2012). Once the location of the selected muscle bellies had been identified, the area was shaved and cleaned with an alcohol wipe before applying the Delsys® EMG sensor with custom made adhesive labels.

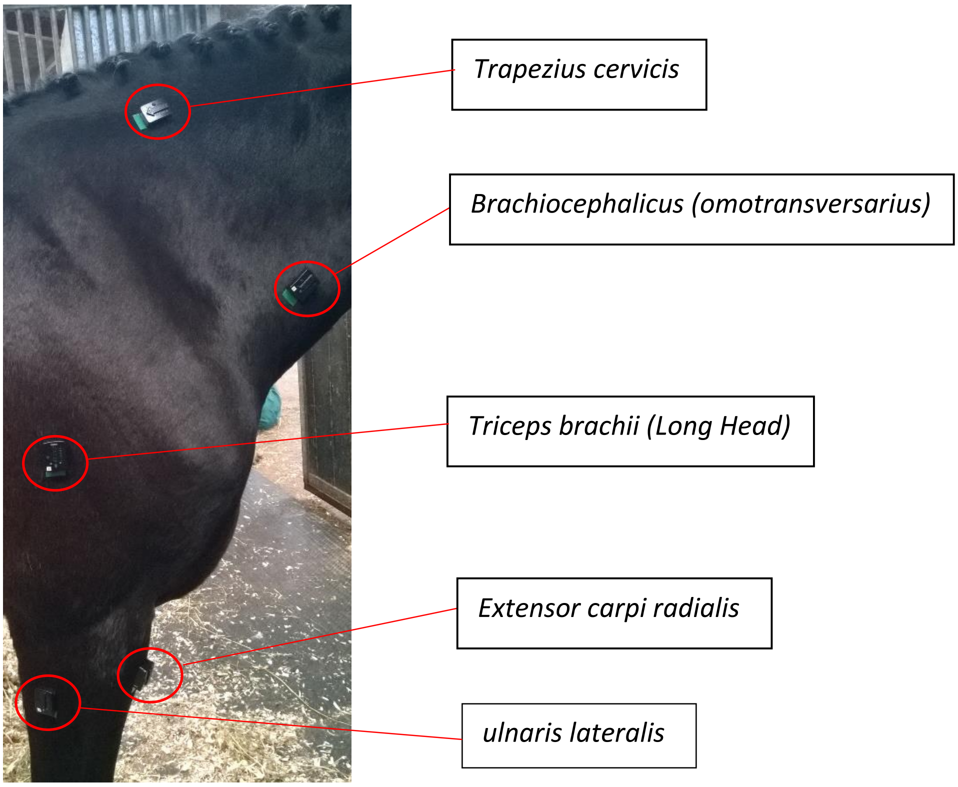


Plate 8: EMG sensor placements on horse2

Experimental Protocol

The experimental protocol was repeated over four consecutive days. An indoor arena with a well established waxed sand and fibre surface was harrowed before putting the camera system up in the same location each day. An extended calibration was performed for 180 seconds and was only accepted when the residual values were less than 1 mm for all of the cameras. The retro-reflective markers and EMG sensors were applied immediately before data collection on each day, data was collected from each horse consecutively.

Once a static file had been recorded, the horses were led in hand at walk and trot and then ridden by the same rider (BHS stage 4, female, age 25, body mass 56 kg) in walk and trot (Plate 9a and 9b). The horses were encouraged to walk and trot at a consistent speed and stride rate during the trials. The horses were led and ridden in snaffle bridles and wore the same leg wraps on all four limbs. The horses were ridden in their own general purpose saddles and were encouraged to work in an outline at a consistent medium walk and working trot.

Plate 9a: In-hand walk trial with horse 1

Plate 9b: Ridden trot trial with horse 2

Previous research investigating the effects of different surfaces on equine kinematics used from 10 (Chateau *et al*., 2013) to 30 strides (Chateau *et al*., 2010) for each condition. For this study based on the size of the calibration zone each walk and trot trial was repeated five and eight times respectively each day in order to obtain an equivalent number of strides per activity. Further trials were performed if the horse was not moving with a regular rhythm or if the rider felt the horse hollowed and a consistent contact was not maintained.

Data Processing

Data processing was carried out using Visual 3D v5 software (C-Motion Inc.). A model was created of the right scapula, humerus, radius, carpus, metacarpophalangeal joint (MCIII), pastern and hoof from each static trial. Proximal (proximal spine of scapula to the lateral epicondyle of the humerus) and distal (lateral epicondyle of the humerus to the distal interphalangeal joint) spring segments were also constructed in order to measure the length change of the springs (Figure 6). Events including hoof impact, mid-stance and toe off were identified and each stride separated accordingly, based on Hobbs *et al*. (2010). An event was also created 100 ms prior to hoof impact (Müller *et al*., 2010) in order to establish the presence of any muscle pre-activation, which may have been responsible for assisting with limb positioning on impact (RFON Prior).

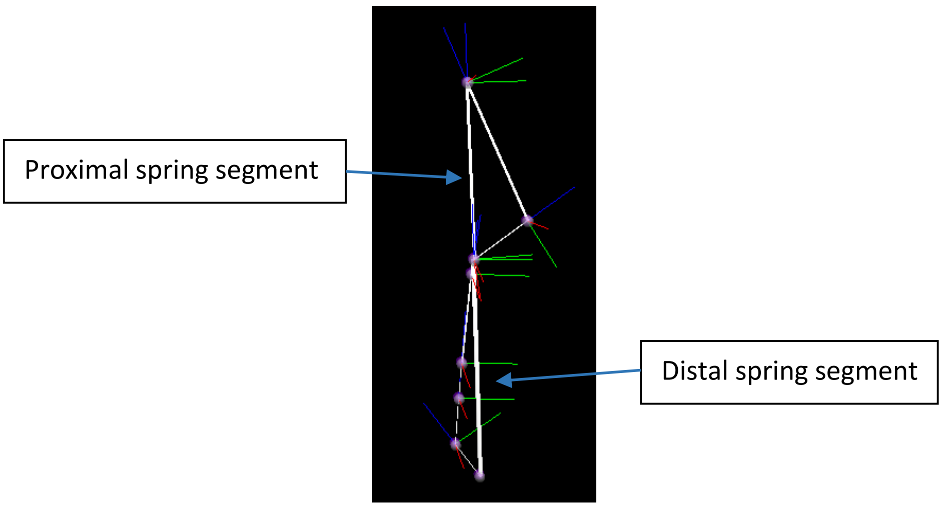


Figure 6: The forelimb model created using Visual 3D with the proximal and distal springs.

Files were rejected during data processing if markers had fallen off or if the signal was not present at identified events. Twenty strides of walk per day for each horse, 21 strides of trot per day for horse 1 and 32 strides of trot per day for horse 2 were subsequently analysed, which was still comparable to other research (Chateau *et al*., 2010; 2013). The data from day 1 were not included due to the marker trajectories not always being consistent, making comparisons difficult. The data for horse 2 on day 4 was also not included because the horse appeared asymmetrical during the in hand trials.

Kinematic Parameters

The kinematic targets were filtered using a fourth order Butterworth filter with a cut off frequency of 10 Hz. Temporal parameters including speed, stance duration and stride length were calculated to allow for comparisons with other research. Speed is a large factor influencing other parameters and files were rejected if the standard deviation (s.d.) of the mean speed increased above 0.24m/s, which was the lowest s.d. recorded by Chateau *et al*. (2013) and did not cause significant differences in speed on left and right circles.

The contribution of the distal and proximal spring to the total length change of the limb from hoof impact to mid-stance was calculated (Distal, proximal and total spring length change (hoof impact – mid-stance)). The proximal spring was not always under maximal compression at mid-stance and showed a greater length than at hoof impact, which gave a misleading representation of length change. Limb stiffness in humans has been calculated using the difference between standing leg length and the maximum displacement of the centre of mass during stance (McMahon and Cheng, 1990). There was no reference made to mid-stance, which suggests that spring length does not necessarily need to be derived from this event. The minimum length of the segments throughout the entire stance phase was therefore derived and also deducted from the length of the springs at hoof impact in order to make comparisons (Distal, proximal and total spring length change (hoof impact –min value during stance)).

MCIII inclination, which reflects the angle of distal limb placement on the surface was calculated for hoof impact and toe off relative to the global coordinate system, similar to Northrop *et al*. (2013). Peak fetlock angle and fetlock angle at mid-stance was also calculated using the pastern and MCIII as a reference segment. This has previously shown a very strong positive correlation to peak vertical ground reaction forces at mid-stance (McGuigan and Wilson, 2003) and is therefore an important parameter to consider in relation to stiffness. Angles were calculated based on an XYZ Cardan sequence.

Electromyographic Parameters

The raw EMG signals were shifted by five frames to account for electromechanical delays before being demeaned, rectified and low pass filtered at 40 Hz using the pipeline in Visual 3D to determine if there was any evidence of pre-activation.

Statistical Analysis

Mean, standard deviation and the coefficient of variance were calculated for all of the kinematic parameters. The data from each horse was grouped according to gait (walk and trot) and stacked according to horse (1 and 2), day (2-4) and activity (inhand v. ridden) and analysed using Minitab 16 Software. A General Linear Model was used to look for significant differences with horse as a random effect and day and activity as fixed effects on the different parameters. The residual values were tested for normality using a Kolmogorov-Smirnov test. Post hoc analysis was carried out using a Tukey test on normally distributed data and a Kruskal Wallis non-parametric test was used on non-normal data. Values of P<0.05 were considered statistically significant.

Results and Discussion

Activity and day effect (Inhand vs ridden) on kinematic paramaters

Activity (inhand v ridden) and day had a significant effect on most of the parameters. The mean (± standard deviation) for the different parameters are presented for both horses in table 1.

Table 1: Mean values ± (standard deviation) for both horses during inhand and ridden trials at walk and trot. Shaded boxes denote significant (P<0.001 * unless stated otherwise) differences between the mean values for each parameter for inhand and ridden at the same gait. The significant day effect on each gait is also stated.

	Activity					
Parameter	**Walk**			**Trot**		
	Inhand	**Ridden**	**Day effect**	**Inhand**	**Ridden**	**Day effect**
Speed (m/s)	1.55 ± (0.098)	1.51 ± (0.063)		3.18 ± (0.12)	3.01 ± (0.18)	P=0.04
Stride length (m)	1.81 ± (0.08)	1.68 ± (0.05)		2.27 ± (0.1)	2.17 ± (0.17)	
Stance duration (s)	0.81 ± (0.04)	0.76 ± (0.03)	P<0.001	0.36 ± (0.02)	0.38 ± (0.03)	P<0.001
Distal spring length change (hoof impact – mid-stance) (m)	0.044 ± (0.005)	0.046 ± (0.007)	P<0.001	0.097 ± (0.005)	0.095 ± (0.006) ***P=0.038**	P<0.001
Proximal spring length change (hoof impact – mid-stance) (m)	-0.0025 ± (0.003)	-0.0008 ± (0.005)	P<0.001	0.0012 ± (0.007)	0.0096 ± (0.005)	P<0.001
Total spring length change (hoof impact – mid-stance) (m)	0.041 ± (0.006)	0.046 ± (0.006)	P<0.001	0.098 ± (0.007)	0.105 ± (0.008)	P<0.001
Distal spring length change (hoof impact – min value during stance) (m)	0.063 ± (0.008)	0.053 ± (0.007)	P<0.001	0.099 ± (0.006)	0.102 ± (0.007)	P<0.001
Proximal spring length change (hoof impact – min value during stance) (m)	0.01 ± (0.005)	0.0098 ± (0.003) ***P=0.032**	P<0.001	0.0072 ± (0.007)	0.0135 ± (0.005)	P<0.001
Total spring length change (hoof impact – min value during stance) (m)	0.073 ± (0.007)	0.063 ± (0.008)	P<0.001	0.106 ± (0.008)	0.115 ± (0.008)	P<0.001
MCIII inclination impact (^{o})	24.22 ± (2.81)	23.16 ± (2.39)		23.14 ± (1.71)	24.37 ± (1.63)	
MCIII inclination toe off (^{o})	-42.06 ± (0.74)	-42.5 ± (2.47)	P<0.001	-36.31 ± (1.74)	-38.96 ± (2.09)	P<0.001
Fetlock angle mid-stance (^{o})	36.28 ± (1.07)	37.82 ± (8.18) ***P=0.022**	P<0.001	51.49 ± (3.78)	53.02 ± (7.44) ***P=0.009**	P<0.001
Peak fetlock angle (^{o})	38.21 ± (0.85)	38.66 ± (7.6)	P<0.001	51.57 ± (3.79)	53.82 ± (7.57)	P<0.001

A significant day effect was found for most parameters. The largest differences were commonly observed between the first and last day of data collection. This may have been due to the horses habituating to the same protocol and techniques used by the handler over consecutive days. It is postulated that a higher degree of variation may be expected from a horse that is not accustomed to a certain environment and this must be accounted for when looking at the response of horses to changes in surface properties during the PhD phase.

Speed was significantly different between inhand and ridden trials for each gait. The coefficient of variation was lower for the ridden trials at walk (4.14% v. 6.32%) yet greater for the ridden trials at trot (6.04% v. 3.8%). The presence of a rider has been thought to exert a stabilising effect on the horse (Peham *et al*., 2004), which would suggest a lower amount of variation may be expected during the ridden trials performed in this study. This was not always the case and parameters with higher values were usually associated with greater variation. This must be acknowledged for future work because surface properties eliciting large changes in kinematic parameters may generate a more variable data set, making trends and relationships harder to establish between the data. The coefficient of variation was less than 15% during all of the walk trials and less than 8% during the trot trials for all of the parameters except the fetlock angles during the ridden trials (14.03%-21.62%) and the proximal segment lengths (35.41%-573.63%).

Stance duration is usually dependent on speed where a shorter stance duration is observed as speed increases (Gustås *et al*., 2006b; Weishaupt *et al*., 2010), however this trend was not found during the walk trials for this study. Duty factor, which is the fraction of the stride time that the limb is in stance has been reported previously and enabled the relationship between stance and swing duration to be established for different gaits on level (Witte *et al*., 2004; Chateau *et al*., 2013) and banked surfaces (Hobbs *et al*., 2011). An inverse relationship exists between stance time and peak vertical force and Witte *et al*. (2004) has shown that it is possible to accurately predict vertical ground reaction forces using duty factor. The greater peak fetlock angle recorded during this study, which would also suggest larger vertical ground reaction forces (McGuigan and Wilson, 2003) did not always coincide with a shorter stance duration. Calculating duty factor during this study may have provided more information on the kinematic response of the horse to the surface and will be measured for the PhD phase.

MCIII inclination has been used to identify the placement of the limb in relation to the horse's body (Hobbs *et al*., 2011). This parameter has been observed to change according to the surface deformability (Burn and Usmar, 2005) and therefore is important to consider for this work. MCIII inclination during protraction and retraction did not change according to surface preparation in a different study by Northrop *et al*. (2013) and it was therefore suggested that stride length did not change either. Significant differences were found in MCIII inclination during this study however, a larger inclination at impact was not associated with a longer stride length during the trot trials. The coefficient of variation for MCIII inclination at impact (inhand =7.37%; ridden =6.7%) and stride length (inhand =5.63%; ridden =6.47%) during trot was generally low, which suggest that another parameter may be responsible for the differences observed.

Most of the total length change of the forelimb occurred in the distal spring segment for both horses. This was also hypothesised and proved by McGuigan and Wilson (2003). The proximal spring sometimes lengthened by mid-stance hence why negative values are presented in table 1 for this parameter. Absolute length changes from hoof impact to mid-stance were reported by Mcguigan and Wilson (2003) and so it was not possible to determine if the proximal spring had lengthened or shortened by mid-stance in their study. There may be a couple of reasons for the limited amount of length change in the proximal spring. This segment is much stiffer

(McGuigan and Wilson, 2003) and the determination of length change in the proximal portion of the limb is also considered to be less accurate, due to the effect of skin displacement (van Weeren *et al*., 1990). Skin displacement was expected to have some influence on the results reported and may explain why the proximal spring length had the largest coefficient of variation for all of the trials (35.41-573.63%). It is important to acknowledge this for future data collection and every effort will be made to use the same anatomical location for marker placement so the effects of skin displacement will be similar on the marker trajectories.

Total length change taken between hoof impact and mid-stance was comparable to peak fetlock extension and fetlock extension recorded at mid-stance where the ridden trials were associated with significantly greater values. An increase in fetlock extension has been associated with a greater ground reaction force previously (McGuigan and Wilson, 2003). The presence of a rider has also been shown to increase the ground reaction forces when compared to unridden trials (Clayton *et al*., 1999), due to the increased total mass, which supports the findings during this study. Based on these findings, it can be speculated that the greater total length change and greater fetlock extension observed during the ridden trials of this pilot work may have been initiated by greater peak ground reaction forces at mid-stance. It is important to note that the fetlock angles were much more variable during the ridden trials at walk and trot (2.22-7.36 – 14.03-21.62%), which necessitates unridden and ridden trials during the PhD phase if changes in the functional properties only initiate subtle changes in the kinematic parameters.

sEMG activity – Is there any evidence of pre-activation?

Muscle pre-activation before ground contact has previously been linked to modulating limb stiffness in humans (Hobara *et al*., 2007, 2008; Müller *et al*., 2010). The amount of EMG activity that is thought to be pre-activation in humans has not been stated in other literature, however an increase in magnitude prior to ground contact appears to be evidence of pre-activation (Hobara *et al*., 2007, 2008; Müller *et al*., 2010). The onset of muscular activity in another study has been defined as when the signal intensity reached 5% of its maximum and ceasing when the signal fell below 5% of its maximum (Hodson-Tole, 2006).

The muscle activity recorded during this study was variable between horses and activities. Some of the signals were interrupted by noise at times, which may have been caused by cross-talk between the muscles or an inconsistent interface between the electrode and the skin. There appeared to be no evidence of pre-activation in the *Brachiocephalicus* muscle in both horses where the muscle was generally active from the end of stance through to early swing (Figure 7). There was also no evidence of pre-activation in the *Trapezius cervicis* in both horses and the long head of the *Triceps brachii* in horse 2. This does not necessarily mean that the respective muscles do not help with supporting the limb during a stride, however it can be concluded that they do not play an active role in stabilising the limb immediately before hoof impact, which would influence limb stiffness.

The long head of the *Triceps brachii* in horse 1 was active in late swing through to early stance, which was also found by Harrison *et al*. (2012) and Hodson-Tole (2006). A strong association (r^2 =0.87) has been found between the time of the peak magnitude in the long head of the *Triceps brachii* muscle and the timing of the maximum limb protraction angle, suggesting a possible role in limb placement during impact (Hodson-Tole, 2006). It is interesting to note that the function of the long head of the *Triceps brachii* is to flex the shoulder and extend the elbow, which in theory would be associated with retracting the limb. The burst of activity may represent eccentric muscle contractions, which would suggest that the muscle may be controlling limb placement prior to impact. This can be supported by Burn and Usmar (2005) who state that the limb is accelerated in retraction after limb protraction, which is thought to decrease the velocity of the hoof relative to the ground prior to ground contact. This requires further investigation due to the pattern only being observed in one horse during this study. A burst of activity was also sometimes observed around mid-stance during the trot trials for horse 1, which may have contributed to reducing the length of the proximal spring and the more positive values obtained.

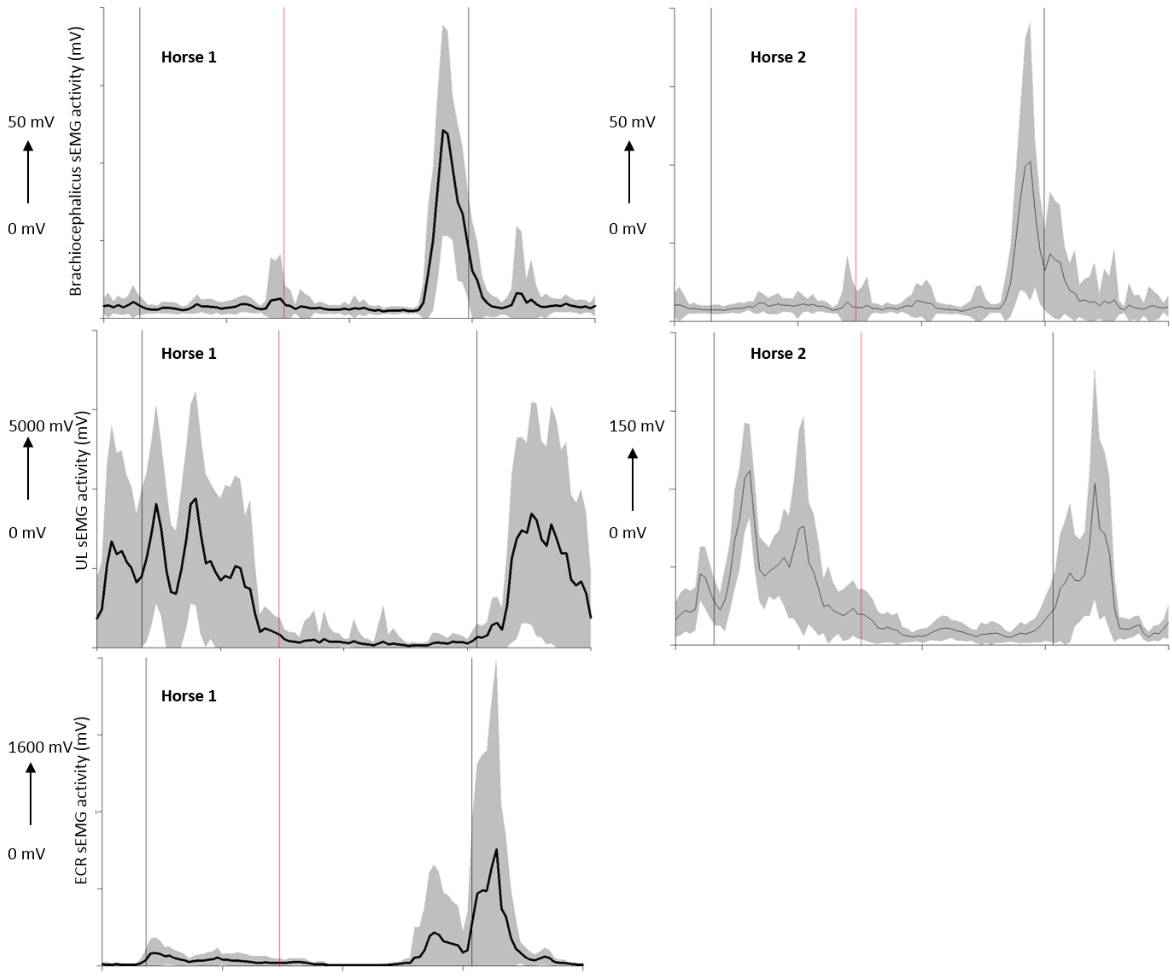


Figure 7: Mean (and standard deviation reflected by the shaded area) rectified and low pass filtered (at 40Hz) sEMG signals for the brachicephalicus muscle (inhand walk trials for horse 1 and 2), u*lnaris lateralis* (UL) muscle (ridden walk trials for horse 1 and 2) and *Extensor carpi radialis* (ECR) muscle (ridden walk trials for horse 1). Y axis -> first vertical grey line = pre-activation; first vertical grey line = hoof impact; red vertical line = mid-stance; second vertical grey line = toe off and into the swing phase.

The UL demonstrated a clear pattern of pre-activation according to the findings of other studies (Hodson-Tole, 2006; Hobara *et al*., 2008) (Figure 7). Jansen *et al*. (1992) and Harrison *et al*. (2012) also reported that the UL was active during the transition from late swing to stance when the carpus was almost extended. The UL is responsible for flexing the knee and extending the elbow, which again suggests that the limb is being slightly retracted prior to impact and aiding limb placement. Pre-activation was not evident in the ECR however co-contraction was observed between the ECR and the UL just after toe off for all of the trials in horse 1. The signals for horse 2 however, were not always reliable and so evidence of co-contraction could not be found. Double activation of the *Extensor carpi radialis* and flexor muscles primarily involved in flexion of the carpus has also been observed in a different study, which may play a role in joint stabilisation and help to control the extent of carpal flexion during early swing (Jansen *et al*., 1992; Harrison *et al*., 2012). It is envisaged that the variability of the activation patterns of the different muscles according to activity and surface stiffness will be investigated during the PhD phase. The magnitude of any pre-activation will also be recorded to determine the contribution the muscle may make to limb placement.

<u>Conclusion</u>

The prototype force shoe has proven to be a suitable design as outlined in Part I and the validation process will continue before it is used on several horses for the main study. The Delsys® system has also proved to be a reliable data acquisition system and has generated readings with a high degree of repeatability. The variation in kinematic parameters expected under the same conditions as discussed in Part II will help to inform the PhD phase of the project where differences that exceed what is considered 'normal' may be attributed to other external factors such as the functional properties of the surface. Based on the results from this study, length change of the distal and proximal springs, which will be used to calculate limb stiffness, will be derived as the difference between the length at hoof impact and the minimum length throughout the entire stance phase in order to consider maximal compression. The *ulnaris lateralis* muscle demonstrated the most evidence of pre-activation, suggesting that the end of the swing phase of the stride is possibly controlled by neuromuscular activity and there may be potential for horses to modulate limb stiffness.

<u>PhD phase</u>

Instrumented force shoes have been used in equine research to measure ground reaction forces over several decades. The length change of the limb between hoof impact and mid-stance has also been quantified by McGuigan and Wilson (2003). It has therefore been possible to calculate limb stiffness, however this has not always been the focus of work carried out previously. Limb stiffness is a concept taken from human research, which is thought to relate to both performance and injury, which may explain the extensive literature in this field.

Limb stiffness in horses has been estimated by McGuigan and Wilson (2003) based on the forces recorded during *in vitro* tests. The stiffness of the distal and proximal springs were approximated and the effect of surface stiffness (turf enabling 50mm deformation v.tarmac) was discussed. The effect of racetrack stiffness on the vertical GRFs of the forelimb during gallop has also been investigated using a dynamic model (Reiser *et al*., 2000). Alterations in surface stiffness appeared to be an important factor determining load rate, energy dissipation of the limb and stance duration. The amount of deformation during the stance phase on a range of surfaces is thought to vary from a few millimetres to several centimeters (Burn and Usmar, 2005). It has been shown that changes in track compliance of this order elicit large changes in the magnitude of hoof impact acceleration (Barrey *et al*., 1991; Kruse *et al*., 2012) and ground reaction forces (Gustås *et al*., 2006a; Chateau *et al*., 2010). The way in which limb stiffness alters according to a change in surface stiffness is yet to be quantified *in-vivo*.

This has great application to industry where horses in various disciplines are commonly exposed to changeable functional surface properties during training and competition at all levels. Event and race horses may come across a complete change in surface type where the horse must gallop across an all-weather surface to reach the next turf section. Horses used for other disciplines such as dressage and show jumping, which commonly take place on an arena surface, are not always presented with such drastic changes. These horses' still encounter spatial and temporal variations (Plate 11) in the functional properties of the same arena surface (Hobbs *et al*., 2014), which has been identified as a risk factor for injury in dressage horses previously (Murray *et al*., 2010). Horses are expected to perform consistently despite such variable properties and therefore the ability to adapt may have implications in injury prevention. This has generated the unique focus for the PhD phase where limb stiffness and other associated parameters will be measured on a surface with sudden and gradual changes in the functional properties.

Plate 11: Variation in arena surfaces; a waxed sand and fibre arena surface near to the mounting block; a build-up of a waxed sand and fibre surface against the wall due to a lack of arena maintenance; a sand and rubber surface with variable distributions of rubber.

It is important to acknowledge that awareness and prior experience of a change in surface properties may alter the kinematic response of the horse. This has been observed in humans previously where visual and camouflaged pertubations in the surface initiated different responses (Müller *et al*., 2012). It appeared that runners could cope with camouflaged drops but this was at the cost of a reduced performance and an increased risk of injury (Müller *et al*., 2012). When runners encountered visible pertubations, adaptations were prepared one step ahead by altering leg force to lift or lower the centre of mass (Müller *et al*., 2012), which is considered to smooth the COM trajectory (Blickhan *et al*., 2007). The response of the horse to the first trial during this PhD will provide extremely valuable information on how horses encounter a change when there is no prior experience and it is hypothesised that less variation in the measured parameters will be observed as trials are repeated based on the findings in humans and during Part II of the MPhil work. This is of significance because horses competing in Dressage and Show Jumping are commonly transported to different venues where there will be no prior experience of the surface.

The findings of this work potentially have great relevance to industry and may help to answer some fundamental questions on how horses encounter a change in the functional properties by possibly altering limb stiffness and other kinematic parameters. Identifying a range in properties that leads to variation in parameters that may be of detriment and exceeds what is considered 'normal' as identified during the MPhil phase, would undoubtedly provide an essential contribution to current surface research.

<u>PhD Phase</u>

Aims

To investigate whether equine limb stiffness alters according to large and subtle changes in surface stiffness and to determine what the implications are in terms of performance and risk of injury.

Objectives

1. To determine the repeatability and reliability of the vertical ground reaction forces measured with the force shoe on one pilot horse.
2. To investigate the effect of subtle and large changes in the functional surface properties on force, kinematic and surface electromyography measurements of several horses.
3. To investigate the effect of visual and camouflaged perturbations on the force, kinematic and EMG variables.
4. To calculate the resultant limb stiffness and establish if horses can adjust limb stiffness according to surface stiffness.
5. To use the Orono Biomechanical Surface Tester, which is equipped to measure surface stiffness.

A Gantt chart has been formulated to provide deadlines to work towards during the PhD (Table 3).

Table 3: Projected deadlines from September 2014 to June 2016.

Task	**Sep-14**	**Oct-14**	**Nov-14**	**Dec-14**	**Jan-15**	**Feb-15**	**Mar-15**	**Apr-15**	**May-15**	**Jun-15**	**Jul-15**	**Aug-15**	**Sep-15**	**Oct-15**	**Nov-15**	**Dec-15**	**Jan-16**	**Feb-16**	**Mar-16**	**Apr-16**	**May-16**	**Jun-16**
RDSC3 report and VIVA	■	■	■	■																		
Force shoe validation with one test horse				■	■																	
Error analysis of the force shoe after *in-vivo* tests					■																	
Consider publication of the force shoe design and validation					■	■	■															
Literature review repeated and experimental protocol designed for main study		■	■	■	■	■																
Pilot work for main study							■	■														
Force, kinematic and electromyographic measurements taken from several horses on the same surface with visual and camoflauged large								■	■	■												

and subtle changes.																						
Data analysis and write-up																						
Further data collection if required																						
Further data analysis																						
Write-up and consider publication of the data collected during the PhD																						

References

Barrey, E. (1990) Investigation of the vertical hoof force distribution in the equine forelimb with an instrumented horseboot. *Equine Veterinary Journal Supplement*, (9), 35-38.

Barrey. E., Landjerit, B and Walter, R. (1991) Shock and vibration during hoof impact on different surfaces. *Equine Exercise physiology*. 3. 97-106.

Biewener, A. A. (1998) Muscle-tendon stresses and elastic energy storage during locomotion in the horse. *Comparative Biochemistry and Physiology Part B*, **120**, 73-87.

Burn, J. F. and Usmar, S. J. (2005) Hoof landing velocity is related to track surface properties in trotting horses. *Equine and Comparative Exercise Physiology*, **2**(1), 37–41.

Butler, R. J., Crowell III, H. P. and McClay Davis, I. (2003) Lower extremity stiffness: implications for performance and injury. *Clinical Biomechanics*, **18**, 511-517.

Chateau, H., Camus, M., Holden-Douilly, L., Falala, S., Ravary, B., Vergari, C., Lepley, J., Denoix, J-M., Pourcelot, P. and Crevier-Denoix, N. (2013) Kinetics of the forelimb in horses circling on different ground surfaces at the trot. *The Veterinary Journal*, **198**, 20-26.

Chateau, H., Holden, L., Robin, D., Falala, S., Pourcelot, P., Estroup. P., Denoix, J.-M. and Crevier-Denoix, N. (2010) Biomechanical analysis of hoof landing and stride parameters in harness trotter horses running on different tracks of a sand beach (from wet to dry) and on an asphalt road. *Equine Veterinary Journal*, **42** (38), 488-495.

Chateau, H., Robin, D., Falala, S., Pourcelot, P., Valette, J-P., Ravary, B., Denoix, J.-M. and Crevier-Denoix, N. (2009) Effects of a synthetic all-weather waxed track versus a crushed sand track on 3D acceleration of the front hoof in three horses trotting at high speed. *Equine Veterinary Journal,* **41** (3), 247-251.

Clayton, H. M., Hodson, E. and Lanovaz, J. L. (2000) The forelimb in walking horses: 2. Net joint moments and joint powers. *Equine Veterinary Journal*, **32** (4), 295-299.

Clayton, H. M., Lanovaz, J. L., Schamhardt, H. C. and van Wessum, R. (1999) The effects of a rider's mass on ground reaction forces and fetlock kinematics at the trot. *Equine Veterinary Journal Supplement*, **30**, 218-221.

Crevier-Denoix, N., Robin, D., Pourcelot, P., Falala, S., Holden, L., Estoup, P., Desquilbet, L., Denoix, J. M. and Chateau, H. (2010) Ground reaction force and kinematic analysis of limb loading on two different beach sand tracks in harness trotters. *Equine Veterinary Journal*, 42 (supplement 38), 544-551.

Durá, J. V., Hoyos, J. V., Lozano, L. and Martínez, A. (1999) The effect of shock absorbing sports surfaces in jumping. Sports Engineering, 2, 103-108.

Faber, M., Johnston, C., van Weeren, P. R. and Barneveld, A. (2002) Repeatability of back kinematics in horses during treadmill locomotion, *Equine Veterinary Journal*, **34** (3), 235-241.

Farley, C. T. and González, O. (1996) Leg stiffness and stride frequency in human running. *Journal of Biomechanics*, **29** (2), 181-186.

Farley, C. T., Houdijk, H. H. P., van Strien, C. and Louie, M. (1998) Mechanism of leg stiffness adjustment for hopping on surfaces of different stiffnesses. *Journal of Applied Physiology*, **85**, 1044-1055.

Ferris, D. P., Liang, K. and Farley, C. T. (1999) Runners adjust leg stiffness for their first step on a new running surface. *Journal of Biomechanics*, **32**, 787-794.

Frederick, F. H. and Henderson, J. M. (1970) Impact force measurement using preloaded transducers. *American Journal of Veterinary Research.* **31** (12), 2279-2283.

Grimmer, S., Ernst, M., Günther, M. and Blickhan, R. (2008) Running on uneven ground: leg adjustment to vertical steps and self-stability. *J. Exp. Biol,* **211**, 2989-3000.

Gustås, P., Johnston, C., and Drevemo, S. (2006a) Ground reaction force and hoof deceleration patterns on two different surfaces at the trot. *Equine and comparative exercise physiology,* **3**, (4) 209 – 216.

Gustås, P., Johnston, C., Hedenstrom, U., Roepstorff, L. and Drevemo, S. (2006b) A field study on hoof deceleration at impact in Standardbred totters at various speeds. *Equine and Comparative Exercise Physiology*, **3** (3), 161-168.

Harrison, S. M., Whitton, R. C., King, M., Haussler, K. K., Kawcak, C. E., Stover, S. M. and Pandy, M. G. (2012) Forelimb muscle activity during equine locomotion. *The Journal of Experimental Biology*, **215**, 2980-2991.

Heaps, L.A., Franklin, S.H. and Colbourne, G.R. (2011) Horizontal moment around the hoof centre of pressure during walking on right and left circles. *Equine Veterinary Journal*, **43** (2), 190-195.

Hobara, H., Kanosue, K. and Suzuki, S. (2007) Changes in muscle activity with increase in leg stiffness during hopping. *Neuroscience Letters*, **418**, 55-59.

Hobara, H., Kimura, K., Omuro, K., Gomi, K., Muraoka, T., Iso, S. and Kanosue, K. (2008) Determinants of difference in leg stiffness between endurance- and power-trained athletes. *Journal of Biomechanics*, **41**, 506-514.

Hobbs, S.J., Licka, T. and Polman, R. (2011) The difference in kinematics of horses walking, trotting and cantering on a flat and banked 10 m circle. Equine Veterinary Journal, 43 (6): 686–694.

Hobbs, S.J., Northrop, A., Mahaffey, C., Martin, J., Clayton, H.M., Murray, R., Roepstorff, L. And Peterson, M. (2014). *Equine Surfaces White Paper.* FEI Publication%. http://www.fei.org/fei/about-fei/publications/fei-books

Hobbs, S. J., Orlande, O., Edmundson, C. J., Northrop, A. J. and Martin, J. H. (2010) Development of a method to identify foot strike on an arena surface: application to jump landing. *Comparative Exercise Physiology,* **7** (1), 19-25.

Hodson-Tole, E. (2006) Effects of treadmill inclination and speed on forelimb muscle activity and kinematics in the horse. *Equine and Comparative Exercise Physiology*, **3** (2), 61-72.

Hood, D. M., Taylor, D. and Wagner, I. P. (2001) Effects of ground surface deformability, trimming, and shoeing on quasistatic hoof loading patterns in horses. *American Journal of Veterinary Research,* **62** (6), 895-900.

Jansen, M. O., van Raaij, J. A. G. M., van den Bogert, A. J., Schamhardt, H. C. and Hartman, W. (1992) Quantitative analysis of computer-averaged electromyographic profiles of intrinsic limb muscles in ponies at the walk. *American Journal of Veterinary Research*, **53** (12), 2343-2349.

Kai, M., Takahashi, T., Aoki, O. and Oki, H., (1999) Influence of rough track surfaces on components of vertical forces in cantering thoroughbred horses. *Equine Veterinary Journal Supplement*, **30**, 214-217.

Kerdock, A. E., Biewener, A. A., McMahon, T. A., Weyand, P. G. and Herr, H. M. (2002) Energetics and mechanics of human running on surfaces of different stiffnesses. *Journal of Applied Physiology*, **92**, 469-478.

Kruse, L., Traulsen, I. and Krieter, J. (2012) Effects of different riding surfaces on the hoof and fetlock accelerations of horses, *Journal of Agricultural Sciences*, **4** (5), 17-24.

Lanovaz, J. L., Clayton, H., Colborne, G. R. and Schamhardt, H. C. (1999) Forelimb kinematics and net joint moments during the swing phase of the trot. *Equine Exercise Physiology Supplement*, **30**, 235-239.

Martin, C., Merritt, J. and Davie, H. (2014) 'Track surface identification using an instrumented horseshoe'. *In Proceedings: International Conference on Equine Exercise Physiology:* June, 2014, Chester, p.46.

McGuigan, M. P. and Wilson, A. M. (2003) The effect of gait and digital flexor muscle activation on limb compliance in the forelimb of the horse *Equus caballus*. *The Journal of Experimental Biology*, **206**, 1325-1336.

McMahon, T. A. and Cheng, G. C. (1990) The mechanics of running: How does stiffness couple with speed? *Journal of Biomechanics*, **23**, 65-78.

Merkens, H. W., Schamhardt, H. C. and van Osch, G. J. V. M. (1993) Ground reaction force patterns of Dutch Warmblood horses at normal trot. *Equine Veterinary Journal*, **25**, (2), 134-137.

Moritz, C. T., Greene, S. M. and Farley, C. T. (2004) Neuromuscular changes for hopping on a range of damped surfaces. *J. Appl. Physiol,* **96**, 1996-2004**.**

Müller, R. and Blickhan, R. (2010) Running on uneven ground: leg adjustments to altered ground level. *Human Movement Science,* **29**, 578-589**.**

Müller, R., Ernst, M. and Blickhan, R. (2012) Leg adjustments during running across visible and camouflaged incidental changes in ground level. *The Journal of Experimental Biology*, **215**, 3072-3079.

Müller, R., Grimmer, S. and Blickhan, R. (2010) Running on uneven ground: Leg adjustments by muscle pre-activation control. *Human Movement Science*, **29**, 299-310.

Murray, R. C., Walters, J., Snart, H., Dyson, S. and Parkin, T. (2010) Identification of risk factors for lameness in dressage horses. *The Veterinary Journal*, **184**, 27-36.

Northrop, A J., Dagg, L-A., Martin, J H., Brigden, C, Owen, A G., Blundell, E. Peterson, M L. and Hobbs, S. J. (2013) The Effect of Two Preparation Procedures on an Equine Arena Surface in Relation to Motion of the Hoof and Metacarpophalangeal Joint. *The Veterinary Journal* DOI: 10.1016/j.tvjl.2013.09.048

Oosterlinck, M., Royaux, E. and Pille, F. (2014) 'Pressure plate evaluation of forelimb toe-heel and medio-lateral hoof balance on a hard versus a soft surface in sound ponies at the walk and trot'. *In Proceedings: International Conference on Equine Exercise Physiology:* June, 2014, Chester, p.46.

Orlande, O., Hobbs, S. J., Martin, J. H., Owen, A. G. and Northrop, A. J. (2012) Measuring hoof slip of the leading limb on jump landing over two different equine arena surfaces. *Comparative Exercise Physiology*, **8**, (1) 33-39.

Peham, C., Licka, T., Schobesberger, H. and Meschan, E. (2004) Influence of the rider on the variability of the equine gait. *Human Movement Science*, **23** (5): 663–671.

Ratzlaff, M.H., Grant, B.D., Frame, J.M. and Hyde, M.L. (1987) Locomotor forces in galloping horses. *Equine Exercise Physiology*, **2**, 574-586.

Ratzlaff, M., Hyde, M. L., Hutton, D. V., Rathgeber, R. A. and Balch, O. K. (1997) Interrelationships between moisture content of the track, dynamic properties of the track and the locomotor forces exerted by galloping horses. *Journal of Equine Veterinary Science*, **17**, 35-42.

Reiser, R. F., Peterson, M. L., McIlwraith, C. W. and Woodward, B. (2000) Simulated effects of racetrack material properties on the vertical loading of the equine forelimb. *Sports Engineering*, **3** (1), 1-11.

Robin, D., Chateau, H., Pacquet, L., Falala, S., Valette, P., Poureclot, B., Denoix, J._m. and Crevier Denoix, N. (2009) Use of a 3D dynamometric horseshoe to assess the effects of an all-weather waxed track and a crushed sand track at high speed trot: Preliminary study. *Equine Veterinary Journal,* **41,** (3) 253-256.

Roland, E. S., Hull, M. L. and Stover, S. M. (2005) Design and demonstration of a dynamometric horseshoe for measuring ground reaction loads of horses during racing conditions. Journal of Biomechanics, 38, 2102-2112.

Tranquille, C. A., Walker, V. A., Roepstorff, L., Hernlund, E. and Murray, R. C. (2012) 'Does harrowing have an effect on the mechanical properties of waxed sand with rubber or waxed sand with fibre surfaces?'. *In Proceedings: BEVA Congress*: September, 2012, Birmingham, p158.

van Heel, M. C. V., Moleman, M., Barneveld, A., VanWeeren, P. R. and Back, W. (2005) Changes in location of centre of pressure and hoof-unrollment pattern in relation to an 8-week shoeing interval in the horse. *Equine Veterinary Journal*, **37** (6), 536-540.

van Weeren, P. R., van den Bogert, A. J. and Barneveld, A. (1990) A quantitative analysis of skin displacement in the trotting horse. *Equine Veterinary Journal Supplement*, **9**, 101-109.

Walker, V. A., Tranquille, C. A., Roepstorff, L., Landolt, C., Brandham, J. and Murray, R. C. (2012) 'The effect of harrowing and watering on arena surface characteristics and kinematics of the working trot'. *In Proceedings: BEVA Congress*: September, 2012, Birmingham, p159.

Weishaupt, M. A., Hogg, H. P., Auer, J. A. and Wiestner, T. (2010) Velocity-dependent changes of time, force and spatial parameters in Warmblood horses walking and trotting on a treadmill. *Equine Veterinary Journal*, **42** (supplement 38), 530-537.

Witte, T. H., Knill, K. and Wilson, A. M. (2004) Determination of peak vertical ground reaction force from duty factor in the horse (*Equus caballus*), *The Journal of Experimental Biology*, **207**, 3639-3648.

General Linear Model: RF SD versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	0.034390	0.034390	143.48	0.000
HORSE RF	6	0.071232	0.011872	49.53	0.000
Surface condition RF	5	0.017818	0.003564	14.87	0.000
Error	333	0.079817	0.000240		
Lack-of-Fit	270	0.072581	0.000269	2.34	0.000
Pure Error	63	0.007237	0.000115		
Total	345	0.195569			

General Linear Model: RFMid% of stance versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	308.0	308.029	59.69	0.000
HORSE RF	6	1460.0	243.328	47.15	0.000
Surface condition RF	5	151.1	30.221	5.86	0.000
Error	326	1682.3	5.160		
Lack-of-Fit	264	1529.6	5.794	2.35	0.000
Pure Error	62	152.7	2.462		

General Linear Model: RF Fetlock angle max versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	101.9	101.895	16.80	0.000
HORSE RF	6	2255.3	375.888	61.96	0.000
Surface condition RF	5	182.7	36.536	6.02	0.000
Error	329	1995.8	6.066		
Lack-of-Fit	264	1886.5	7.146	4.25	0.000
Pure Error	65	109.3	1.682		
Total	341	4682.1			

General Linear Model: RF Duty Factor versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	55.04	55.040	22.44	0.000
HORSE RF	6	521.60	86.934	35.45	0.000
Surface condition RF	5	242.41	48.482	19.77	0.000
Error	205	502.77	2.453		
Total	217	1335.30			

General Linear Model: RF stride length versus mean speed, HORSE RF, Surface conditio

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	2.9064	2.90644	747.31	0.000
HORSE RF	6	2.5986	0.43310	111.36	0.000
Surface condition RF	5	0.2188	0.04376	11.25	0.000
Error	206	0.8012	0.00389		
Total	218	8.1756			

General Linear Model: RFON forelimb inc. versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	100.61	100.615	60.66	0.000
HORSE RF	6	1168.28	194.713	117.39	0.000
Surface condition RF	5	731.29	146.258	88.18	0.000
Error	333	552.34	1.659		
Lack-of-Fit	267	497.20	1.862	2.23	0.000
Pure Error	66	55.14	0.835		
Total	345	2481.54			

General Linear Model: RFOFF forelimb inc. versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	50.47	50.466	25.81	0.000
HORSE RF	6	459.08	76.513	39.14	0.000
Surface condition RF	5	386.04	77.209	39.49	0.000
Error	334	652.97	1.955		
Lack-of-Fit	271	558.79	2.062	1.38	0.063
Pure Error	63	94.19	1.495		
Total	346	1706.69			

General Linear Model: RFON neck inc versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	4	1 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	139.22	139.222	8.25	0.005
HORSE RF	6	1648.06	274.676	16.27	0.000
Surface condition RF	3	41.25	13.749	0.81	0.487
Error	183	3089.24	16.881		
Lack-of-Fit	142	2804.53	19.750	2.84	0.000
Pure Error	41	284.72	6.944		
Total	193	5412.57			

General Linear Model: RFON shoulder inc versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	1.79	1.791	0.53	0.469
HORSE RF	6	2441.18	406.863	119.61	0.000
Surface condition RF	5	68.69	13.739	4.04	0.001
Error	316	1074.92	3.402		
Lack-of-Fit	254	1024.85	4.035	5.00	0.000
Pure Error	62	50.07	0.808		
Total	328	3650.01			

General Linear Model: RFON shoulder angle versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	66.52	66.524	5.79	0.017
HORSE RF	6	3630.24	605.041	52.63	0.000
Surface condition RF	5	685.18	137.036	11.92	0.000
Error	316	3632.55	11.495		
Lack-of-Fit	254	3565.05	14.036	12.89	0.000
Pure Error	62	67.51	1.089		
Total	328	8173.24			

General Linear Model: RFOFF neck inc versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	4	1 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	355.03	355.029	21.36	0.000
HORSE RF	6	1455.69	242.615	14.59	0.000
Surface condition RF	3	77.37	25.791	1.55	0.203
Error	184	3058.76	16.624		
Lack-of-Fit	147	2828.69	19.243	3.09	0.000
Pure Error	37	230.07	6.218		
Total	194	5514.98			

General Linear Model: RFOFF shoulder inc versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	26.49	26.489	5.72	0.017
HORSE RF	6	2446.67	407.778	88.04	0.000
Surface condition RF	5	68.78	13.757	2.97	0.012
Error	314	1454.34	4.632		
Lack-of-Fit	253	1397.22	5.523	5.90	0.000
Pure Error	61	57.12	0.936		
Total	326	4182.27			

General Linear Model: RFOFF shoulder angle versus mean speed, HORSE RF, Surface condition RF

Factor Information

Factor	Type	Levels	Values
HORSE RF	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RF	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, Firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed	1	70.76	70.763	6.34	0.012
HORSE RF	6	2234.43	372.406	33.36	0.000
Surface condition RF	5	345.32	69.064	6.19	0.000
Error	314	3505.53	11.164		
Lack-of-Fit	253	3491.11	13.799	58.38	0.000
Pure Error	61	14.42	0.236		
Total	326	6184.05			

General Linear Model: RH SD versus mean speed_1, HORSE RH, Surface condition RH

Factor Information

Factor	Type	Levels	Values
HORSE RH	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RH	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed_1	1	0.06148	0.061476	135.31	0.000
HORSE RH	6	0.07716	0.012860	28.30	0.000
Surface condition RH	5	0.06071	0.012143	26.73	0.000
Error	317	0.14403	0.000454		
Lack-of-Fit	266	0.13365	0.000502	2.47	0.000
Pure Error	51	0.01038	0.000203		
Total	329	0.32383			

General Linear Model: RHMid% of stance versus mean speed_1, HORSE RH, Surface condition RH

Factor Information

Factor	Type	Levels	Values
HORSE RH	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RH	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed_1	1	0.04	0.039	0.00	0.956
HORSE RH	6	2822.29	470.381	36.71	0.000
Surface condition RH	5	102.12	20.424	1.59	0.161
Error	305	3907.66	12.812		
Lack-of-Fit	255	3627.34	14.225	2.54	0.000
Pure Error	50	280.32	5.606		

General Linear Model: RH fetlock angle versus mean speed_1, HORSE RH, Surface conditio

Factor Information

Factor	Type	Levels	Values
HORSE RH	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RH	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed_1	1	43.15	43.145	4.58	0.033
HORSE RH	6	4819.48	803.247	85.23	0.000
Surface condition RH	5	1477.37	295.474	31.35	0.000
Error	307	2893.41	9.425		
Lack-of-Fit	256	2811.72	10.983	6.86	0.000
Pure Error	51	81.69	1.602		
Total	319	9763.55			

General Linear Model: RH Duty factor versus mean speed_1, HORSE RH, Surface condition RH

Factor Information

Factor	Type	Levels	Values
HORSE RH	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RH	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed_1	1	274.6	274.621	44.79	0.000
HORSE RH	6	2688.7	448.124	73.09	0.000
Surface condition RH	5	855.1	171.018	27.89	0.000
Error	205	1256.9	6.131		
Total	217	5053.1			

General Linear Model: RH adjusted SL versus mean speed_1, HORSE RH, Surface condition RH

Factor Information

Factor	Type	Levels	Values
HORSE RH	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RH	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed_1	1	2.4094	2.40943	401.74	0.000
HORSE RH	6	3.0127	0.50212	83.72	0.000
Surface condition RH	5	0.2914	0.05828	9.72	0.000
Error	204	1.2235	0.00600		
Total	216	8.5457			

General Linear Model: RFOFF-RHON versus mean speed_1, HORSE RH, Surface condition RH

Factor Information

Factor	Type	Levels	Values
HORSE RH	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RH	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed_1	1	0.023688	0.023688	71.94	0.000
HORSE RH	6	0.190406	0.031734	96.37	0.000
Surface condition RH	5	0.045798	0.009160	27.82	0.000
Error	311	0.102411	0.000329		
Lack-of-Fit	260	0.093493	0.000360	2.06	0.001
Pure Error	51	0.008918	0.000175		
Total	323	0.339322			

General Linear Model: RHON hindlimb inc. versus mean speed_1, HORSE RH, Surface condition RH

Factor Information

Factor	Type	Levels	Values
HORSE RH	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RH	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed_1	1	59.17	59.174	7.03	0.008
HORSE RH	6	2937.46	489.577	58.12	0.000
Surface condition RH	5	1648.05	329.610	39.13	0.000
Error	312	2627.95	8.423		
Lack-of-Fit	258	2539.29	9.842	5.99	0.000
Pure Error	54	88.67	1.642		
Total	324	7177.00			

General Linear Model: RHOFF Hindlimb inc versus mean speed_1, HORSE RH, Surface condition RH

Factor Information

Factor	Type	Levels	Values
HORSE RH	Random	7	Ben, Fly, Polly, Raison, Ruby, Terry, Theo
Surface condition RH	Fixed	6	1 firm, 1 soft, 2 firm, 2 soft, firm, soft

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
mean speed_1	1	195.84	195.836	59.90	0.000
HORSE RH	6	1024.55	170.759	52.23	0.000
Surface condition RH	5	632.36	126.471	38.68	0.000
Error	310	1013.56	3.270		
Lack-of-Fit	261	948.41	3.634	2.73	0.000
Pure Error	49	65.15	1.330		
Total	322	3010.04			