Validation and standardisation of a two dimensional motion analysis technique to measure normal conformation and gait in Arabian horses.

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Abstract

The development of standardised two dimensional motion analysis techniques to obtain baseline measures would provide the equine industry with a consistent method for analysing equine conformation and gait. The use of these methods to define breed specific conformation and gait could be utilised by the industry for conformation and gait assessment. This study focussed on validating and standardising such methods to define normal conformation and gait for the Arabian horse. Validation involved comparing the accuracy of two 2D motion analysis software programmes; Quintic© and HU-M-AN™. Static and dynamic linear and angular validation was performed by comparing known values to values calculated by the software programmes. Higher variation was established for Quintic© measurements; the margin of error was up to 20mm for static measurements and 3.36° for angular measurements. When using Quintic©, angles of different size were measured with varying amounts of accuracy; these differences were significant ($P<0.001$). The pattern of these differences was similar to a sine wave. It was concluded that Quintic© was not compatible with a normal video camera recording at a 4:3 aspect ratio which may have related to calibration or angle measurement algorithms. HU-M-AN™ was used for all further analysis due to the smaller margin of error established during validation. Intra-horse variation in conformation and stride characteristics (stride length and ROM) were measured in a group of three horses over five consecutive days. Stride length was consistently longer on day one than subsequent days for all horses ($P<0.001$), and positively correlated to velocity. Variation in stride length between days varied for each horse; some horses had more stable gait characteristics than others. Little variation was established for ROM data between days; few joints demonstrated ROM that was significantly different between days for individual horses. Providing stance of the horse, marker placement and velocity are closely regulated, baseline data can be obtained on one occasion. The standardised method previously validated was used to define normal gait for Arabian horses. Conformation and stride characteristics were assessed for a group of six purebred Arabian horses (mean age 27±10.56 months). There was no significant difference in ROM between any of the horses measured ($P>0.05$); the horses had breed-specific gait patterns which allowed normal gait to be defined for a distinct breed. A database of normal gait for the Arabian horse was created for use by Arab horse owners or breeders in the UK.

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1.0 Introduction

The development of standardised two dimensional motion analysis techniques would provide the equine industry with a consistent method for analysing equine conformation and gait, which according to Colborne (2004) would enable equine practitioners to quickly and simply obtain valuable biomechanical information. Currently there is a paucity of information about the validity of such systems; research into equine locomotion is often performed in laboratory conditions using three-dimensional methods (Barrey, 1999). These systems have previously been established as accurate and are recognised as the "gold standard" for gait analysis (Nankervis *et al.*, 2009). Accurate two dimensional methods of analysing equine gait "in-field" would be more beneficial to the equine industry, as they would be accessible to equine practitioners, including breed societies. Normal conformation and gait would have to be defined and this information used in conjunction with standardised methods in order for these systems to be utilised practically by the industry. This study focussed on validating and standardising the methods in order to define normal conformation and gait for the Arabian horse.

The official breed society for the Arabian horse in the United Kingdom is the Arab Horse Society. One of the aims of the Arab Horse Society is to ensure that good quality animals remain within the breeding population, by promoting the breeding of these animals through a Premium Scheme. Horses registered with the scheme must be graded; this involves an evaluation of conformation and gait quality, and is currently performed by judges. This grading is subjective, as the horses are scored in relation to a perceived ideal. There is no quantitative data available on what constitutes normal conformation or gait characteristics, for horses to be graded against. Defining normal conformation and gait for Arabian horses (using a quantitative method such as two dimensional motion analysis) would facilitate the creation of a database to aid the Arab Horse Society in its grading and selection process.

Assessment of conformation is also an important factor to consider when defining normal gait. Conformational studies frequently focus on Thoroughbreds (Anderson, 2004; Anderson *et al.*, 2004; Mawdsley *et al.*, 1996; Weller *et al.*, 2006a; Weller *et al.*, 2006b) and Warmbloods (Back *et al.*, 1996; Holmstrom *et al.*, 1990; Magnusson, 1985) as these sports horse breeds are more commonly used in competition. These studies

attempt to link conformation to performance or soundness rather than defining what normal conformational traits are specific to that breed. The studies that have focused on Arab conformation (Gharahveysi *et al.*, 2008; Sadek *et al.*, 2006) attempt to define normal Arab conformation, and use body measurements as an objective method to measure conformation. These studies are a start in the implementation of databases of normal conformation for Arabian horses; however gait has yet to be included.

1.1 Evaluating conformation

Equine conformation refers to the shape of the horse; the lengths of bone segments, angles of joints and deviations of segments from the vertical or horizontal (Weller *et al.*, 2006b). Quantitative measurements of these bone segments and joint angles will not change with training. Conformation therefore provides the foundation of how the horse moves, and ultimately limits performance and the probability of the horse remaining sound throughout its competitive career. Evaluation of conformation is an essential factor in any selection process.

The evaluation of conformation is assessed from the external appearance of the horse *(Weller et al.*, 2006b). Traditionally in the equine industry conformation is subjectively assessed, evaluating the conformation of a horse in relation to a perceived ideal. This subjective evaluation is dependent on the opinion and experience of the individual performing the evaluation, and has been described as an "individual feeling" (Mawdsley *et al.*, 1996) for the overall appearance of the horse that seems to be more of an art than a science (Rossdale and Butterfield, 2006). It is possible the lack of objectivity in this method of assessment will lead to poor consistency between conformation scores from different assessors. It is still the most commonly used method of evaluating conformation in the equine industry in the UK and is the method currently utilised by the Arab Horse Society.

Evaluation techniques need to be standardised in order to provide breeders with reliable and useable information. This can be done by using an objective and repeatable method for assessing conformation. Attempts have been made to quantify the evaluation of conformation using linear scoring systems. A linear scoring system was introduced in the bovine industry by the American Holstein Cattle Association in the 1970s, called the Linear Assessment Trait Evaluation Programme (Mawdsley *et al.*, 1996). This method

aimed to score specific conformational traits on a scale between two biological extremes, rather than defining good or poor conformation. This method has been modified for use in the equine industry (Mawdsley *et al.*, 1996). Foot slope for example, would be scored in relation to "upright" or "sloping" (Mawdsley *et al.*, 1996). This system quantified conformation by providing the animal with an overall conformation score, supplying more accurate information to the breed society.

Linear scoring systems have been compared with traditional conformation scoring in Irish Draught horses (Breen, 2009). A group of Irish Draught horses were assessed by a panel of experienced Irish Draught or Irish Sports horse judges. The judges scored each horse twice using the linear and traditional method. The strength of intra-judge agreement for each horse was calculated for the traditional and linear methods using intra-class correlation coefficients (ICCs). ICC values describe how closely correlated conformation scores are for each individual judge. Consistency was poor for both scoring methods. The ICC values for the traditional scoring method were low, ranging from 0.020 to 0.243, with eight of the nine traits scoring below the 0.20 cut off point. The traits with the highest ICC values were "type" and "barrel and back". The traits with the lowest ICC values were "foreleg" and hind leg". The linear scoring method showed similar low consistency of scores between judges, with ICC values ranging from 0.037 to 0.320. The traits with the highest ICC values were again, those relating to the body of the horse; "barrel and back", "scapula" and "type", with traits of the distal limb being significantly lower.

Traits that concern scoring lengths and angles had the lowest ICC values. It could be argued that it is difficult to accurately estimate lengths and angles by eye. This would explain the low ICC values for traits that involved these types of assessments. Traits that do not involve estimating lengths or angles, such as muscularity of the hindquarters and muscularity of the neck showed higher ICC values, suggesting these traits are easier to score accurately. Research has previously suggested that it is difficult to judge by eye lengths and angles, leading to inaccuracies and inconsistencies in conformation scores (Magnusson, 1985).

The low inter-judge consistency scores imply that the linear scoring method of evaluating conformation is still affected by a degree of subjectivity. The linear method does quantify conformation scores, however it is not a completely objective method of assessing conformation as the horses still have to be scored by an individual. This

means that the scores are subject to individual interpretation, decreasing the reproducibility of the method.

Lack of experience of using the linear scoring method could contribute to the low consistency between judges. The judges in study by Breen (2009) were experienced in assessing conformation using the traditional method, rather than the linear scoring method. Judges with more experience in using the linear scoring method might have produced more consistent results. It has been suggested in previous studies that inter-judge consistency would increase with experience and more detailed definitions of the traits being scored (Magnusson, 1985). Standardisation of conformation assessment, leading to a completely objective method would be expected to increase reproducibility of conformation evaluation, making the information collected more beneficial to the breed society. Information about how the conformational traits measured affect the movement and consequently the performance of the horse will also provide beneficial information to the Arab Horse Society.

1.1.1 Conformation and performance

Predicting performance using conformation is a long standing tradition in the equine industry. Elite horses may not necessarily have "ideal" conformation (van Weeren and Crevier-Denoix, 2006), therefore the traditional method of evaluating conformation will not be entirely useful. Quantification of conformation, as well as investigating direct links between particular traits and movement will provide essential information to breeders. A reliable and repeatable method of evaluating conformation by measuring traits will ultimately lead to a more in-depth understanding of how those traits affect movement and subsequently performance.

The majority of evidence supporting the relationship between conformation and performance has been anecdotal until recently. Research has been conducted to determine the effect different conformational traits have on movement, and how conformation can be related to performance. Performance is not a quantifiable measure, due to other parameters affecting overall performance (Weller *et al.*, 2006b), making comparisons between conformation and performance difficult. Research has overcome this by comparing conformation in elite and non-elite horses. Preliminary studies showed that certain static measurements of conformation were either larger or smaller in

better performing horses (van Weeren and Crevier-Denoix, 2006). This type of research makes the assumption that all elite horses perform to a higher standard than non-elite horses, whereas the non-elite horses may have the conformational potential to perform but lack the opportunity (in terms of training or resources). These studies do however show some interesting correlations between conformation and performance.

A long sloping femur; sloping scapula; long humerus and proximal phalanx length have been linked to good performance in Swedish Warmblood horses (Holmstrom *et al.*, 1990). The mean inclination of the scapula in elite horses was 65.3° and 64.4° (for dressage and show jumping respectively) compared to 66.3° for the non-elite horses. A larger scapula inclination leads to a more sloping scapula, which is preferable as it leads to a smoother more comfortable ride (Back *et al.*, 1996) and has also been linked to a longer stride length (Weller *et al.*, 2006b) which is desirable in most disciplines. It has also been noted that a long radius, short third metacarpal and flat ilium are all desirable traits for good movement (Holmstrom, 2000).

1.1.2 Conformation and soundness

Conformation evaluation is used to select horses with a low risk of developing lameness (Back *et al.*, 1996) as conformation can be a predisposing factor in the development of musculoskeletal injuries. Studies have been conducted into correlations between conformational measurements and soundness. An upright scapula (a larger inclination of the scapula) has been linked to increased concussion of the distal limb in show jumpers (Back *et al.*, 1996) and therefore an increased risk of developing lameness. Upright proximal phalanxs have been correlated with a greater ROM of the metacarpophalangeal joint (de Souza *et al.*, 2004). Mean ROM in trot was 54.61° for horses with normal forelimb conformation, compared with 60.72° for "camped under" (upright proximal phalanxs) horses. Larger ROM of the metacarpophalangeal can put more strain on the superficial flexor tendon or increased pressure on the navicular area of the foot, leading to an increased chance of lameness (de Souza *et al.*, 2004). A small tarsal joint angle has been correlated to increased flexion in this area, which in turn minimises concussion on the joint. It could be argued therefore that a large tarsal angle could lead to increased concussion on the hind limb and therefore predisposes for lameness. A straight tarsus however has also been linked to a longer stride, increased swing duration and range of motion within the joint, all of which are desirable in most

disciplines (Back *et al.*, 1996). These studies confirm there is no "ideal" conformation for horses to be rated against in traditional evaluation methods. Traits have both negative and positive effects on performance and soundness. It is essential therefore to quantify how conformation is measured and also to quantify how these measures affect equine locomotion. One method of doing this would be to use two dimensional motion analysis techniques, such as using videography combined with a software package that will measure static conformational traits and dynamic movement.

1.2 Evaluating gait

Evaluation of equine locomotion has two main purposes; to assess gait quality and to identify gait irregularities. Gait quality is traditionally subjectively assessed, in much the same way as conformation with gait being scored in relation to an "ideal". This subjective evaluation relies on the experience or opinion of the judge doing the scoring. It is also limited by the innate restrictions of the human eye in its ability to detect subtle differences between individual horses or to register fast movement (Holmstrom *et al.*, 1990). Currently, identification of irregularities of equine gait uses linear scoring to attempt to standardise the method as well as making it more objective (Back *et al.*, 2007). The linear scale used to determine the degree of lameness is a scale from 0-10 (Fuller *et al.*, 2006) and is generic throughout the equine industry in the UK. Clinical experience has been shown to have a significant effect on ability to identify gait abnormalities accurately and the reliability of subjective assessments is poor when the lameness is mild (Keegan, 2007). Research has also shown that while intra-assessor consistency for detecting lameness is good (repeatability); inter-assessor consistency is poor (reproducibility) for horses ranging from 0 (sound) to 10 (non-weight bearing) by three veterinary surgeons (Fuller *et al.*, 2006). Gait analysis systems could be used to objectively evaluate gait, and also provide quantitative data that could be used for direct comparisons.

1.2.1 Gait analysis techniques

One method of quantifying conformation and gait is to use motion analysis techniques. These techniques have been used in research to evaluate lameness and the subsequent effect of treatment (Back *et al.*, 1993b); analyse performance (Leleu *et al.*, 2005, Deuel

and Park, 1990) and determine the predictive qualities of gait (Cano *et al.*, 1999, Cano *et al.*, 2001b, Back *et al.*, 1995a).

Some gait analysis systems consist of expensive and complicated equipment designed specifically for laboratory environments. Data recorded from these systems may be accurate however it is hard to extrapolate data recorded in a laboratory environment to "real life" situations. There is a need to develop usable tools for objectively analysing gait, and to prove they can provide reliable and accurate information to practitioners quickly and inexpensively (Colborne, 2004).

Currently, the most popular technique for "in field" motion analysis is videography in conjunction with two-dimensional (2D) motion analysis software. The method involves attaching markers to anatomical landmarks on the horse, and filming the horse in motion. The videos are downloaded and analysed using motion analysis software such as Equinalysis©, Quintic© or HU-M-AN™. The video cameras used are portable and can be taken to the site of the horse. The markers used are circular or spherical and can be attached to specific points on the body of the horse depending what is to be measured. The markers can be tracked manually or semi-automatically to determine the marker co-ordinates in space and time (Barrey, 1999). The video clips are analysed frame by frame, so (depending on the speed of the video camera used) the stride can be analysed in more detail than the capabilities of the human eye. Information such as stride length; duration; frequency of the minimum and maximum flexion and extension of the joints being measured, can be extrapolated from the video clips, as well as linear and angular measurements of static conformation.

1.3. Two-dimensional motion analysis

1.3.1 Marker placement

The minimum number of markers needed to measure a specific joint is three (Schamhardt *et al.*, 1993) although more can be used (two markers on each limb segment). Most two dimensional motion analysis techniques require markers to be placed over the approximate centre of rotation of the joints being measured (Clayton and Schamhardt, 2001) however some studies have placed markers at the proximal and distal ends of limb segments (Galisteo *et al.*, 1996). It is hard to estimate the approximate centre of rotation of joints using palpation, which leads to potential errors

in accurately calculating joint range of motion. Proximal and distal ends of limb segments are easier to palpate, therefore placement of markers is more accurate. This does not necessarily lead to more accurate data being recorded. Using two markers on each limb segment allows for the calculation of the joint angle as it changes, however if the markers are not aligned with the bone axes the joint angles calculated will be offset (Schamhardt *et al.*, 1993). Providing the marker position is known in respect to the joint segments or angles being measured; and the horse is standing square, the exact marker set being used has little influence on the accuracy of the data obtained (Schamhardt *et al.*, 1993). It could be argued that a simple marker set will produce more reliable data when repeated measures are being taken due to ease of application. It should also be noted that a simple and easy to apply marker set is essential in the development of a standardised method of gait analysis if it is to be utilised by the equine industry.

1.3.2 Soft tissue artefact

The purpose of using anatomical markers is to identify specific points of the skeleton on the surface of the skin, by palpating the muscle and underlying tissue to feel the relevant bony segments underneath. Soft tissue artefact (STA) relates to the movement of the anatomical markers placed on the skin, in relation to the underlying bone. It is one of the main sources of error when it comes to motion analysis techniques that use anatomical skin markers (Leardini *et al.*, 2005). The amount of STA is dependent on the position of the markers; some joints will display more STA than others due to the amount of underlying tissue or the way the joint moves. STA is a contributing factor to distortion or "noise" that is visible when it comes to analysing the movement that has been recorded. The nature of the STA is often similar to the actual movement of the horse, therefore it is difficult to distinguish between the two (Schamhardt *et al.*, 1993).

Soft tissue artefact on the human carpus has been reported as up to 21mm distally and 23mm posteriorally (Leardini *et al.*, 2005); up to 10mm on the human tibia (Leardini *et al.*, 2005). Equine studies have shown similar results, with the distal limb having smaller deviations than more proximal locations. Measurements of 8mm were found for the metacarpus compared to 142mm for the greater trochanter (van Weeren *et al.*, 1990a). These results are supported by further research (van Weeren *et al.*, 1990b) where deviations of 10 to 40mm were found for the scapula and 130mm to 170mm for the greater trochanter. This difference could be accounted for by the difference in the

amount of skin and underlying tissue in these areas. The distal limb has no underlying muscle or adipose tissues (the distal limb contains only tendons and ligaments under the skin) whereas more proximal locations, such as the femorotibial joint, have greater amounts of tissue, including muscle and adipose tissue. STA can be corrected for using different techniques. Van Weeren *at al.* (1990ab) attempted to quantify the amount of skin displacement caused by STA for different joints. The quantification of skin displacement means that algorithms can be utilised to correct for errors caused by STA. Schamhardt *et al.* (1993) suggested another method to overcome STA would be use to choose anatomical sites to place markers where skin movement is negligible, therefore not an issue. This is not an accurate or practical method of correcting for skin displacement; it may be practical for distal limb markers where there is little STA, but not for proximal markers. Some distortions from STA can be minimised using data smoothing and filtering techniques applied after data is downloaded and digitised. This is a quick and simple method therefore appropriate to be used in this study.

1.3.3 Repeated measures

Baseline measurements of stride characteristics are obtained using repeated measurements for each horse. Number of repeats recorded for each horse varies from three (Drevemo *et al.*, 1980a) to twelve (Clayton *et al.*, 2002). Degueurce *et al.* (1997) used five repeats to measure stride characteristics to ensure precision when investigating variability of limb joint patterns. A study by Cano *et al.* (1999) recorded a high number of repeats for each horse, and then randomly selected five to analyse further. Research by Drevemo *et al.* (1980a) recorded eight successive strides per horse and found very little variance in individual horses. Based on these results it was estimated that three to five strides would be a sufficient number to record in order to determine baseline gait characteristics for individual horses.

Further research (Drevemo *et al.*, 1980b) recorded the same group of horses immediately after the first recording, to test the reproducibility of equine gait on more than one occasion. The overall means and standard deviations were very similar on both occasions, however there were some small variations within individual horses. Additional research (Degueurce *et al.*, 1997, Sloet van Oldruitenborgh-Oosterbaan *et al.*, 1996, van Weeren *et al.*, 1993, Back *et al.*, 1994b, Leleu *et al.*, 2004) has shown similar results. Minor variations could be due to instrumental errors or soft tissue

artefact, such as skin displacement (Chiari *et al.*, 2005; Leardini *et al.*, 2005), but results suggest that horses have stable locomotion patterns, which would allow normal gait to be quantified.

1.4 Variation in equine gait

Small intra-horse variation in gait is desirable as it means that baseline measures can be obtained on a single occasion. Intra-horse variation in range of motion of specific joints changes depending on what joint is being measured, as different joints move in different ways. In French Saddle horses, variability of joint angles was shown to differ depending which joint was being measured (Degueurce *et al.*, 1997). The coxofemoral joint ROM had the lowest variability of 1°, the joints with the largest variability were the fore and hind metacarpophalangeal and distal interphalangeal joints. The variability of these joints ranged from 3.2° to 3.5°. The low intra-horse variability confirms other studies that have concluded individual horses have stable locomotion patterns that are repeated for each stride (Degueurce *et al.*, 1997, van Weeren *et al.*, 1993, Back *et al.*, 1994a). The intra-horse variability was greater for joints of the distal limb, rather than the proximal. Inter-horse variability (IEV) was greater than intra-horse variability. The inter-horse variability ranged from 0.9° (coxofemoral) to 6.3° (fore distal interphalangeal). There was greater variability in the distal joints compared to the proximal joints. Galisteo *et al.* (1996) investigated the variability of angular joint parameters in Andalusian horses. Intra and inter-individual variability were recorded. The results indicate low variability in most of the joints measured (less than 10%), however some joints showed a significantly higher variance. The intra-individual variation in the scapulohumeral joint was 24.9%, and the inter-individual variation was 33.1%. The high variation between repeats of the same horse, as well as between different horses could be due to the way the scapulohumeral joint moves, or limitations of the method for example soft tissue artefact. The scapula has a higher degree of muscle or fat mass than bone segments in the distal limb (Holmstrom *et al.*, 1990), leading to increased soft tissue artefact (more movement of the skin and underlying tissues over the bone). This could lead to an increased intra and inter-horse variability in the range of motion for the scapulohumeral joint.

A study by Drevemo *et al.* (1980a) analysed linear and temporal stride characteristics in 30 Standardbred trotters. Intra-individual variation within horses was considerably smaller than inter-individual variation between horses. Intra-horse variation for stride

length ranged from 8.5cm to 10.8cm compared to 31.4cm to 32.1cm for the inter-horse variation. Similar results were found for stride duration, with intra-horse variation ranging from 7.2 milliseconds to 9.3 milliseconds, and inter-horse variation ranging from 23.3 milliseconds to 23.8 milliseconds. The inter-horse variation was larger than the intra-horse variation, which is to be expected as no two horses will display the same stride characteristics. The differences were surprisingly large however, considering the horses used were all the same breed (Standardbred trotters). This suggests that conformation could be a contributing factor to the inter-horse differences. It could be possible that horses of the same breed vary considerably in conformation, therefore an assessment of conformation should be included in any study into equine gait.

Drevemo *et al.* (1980b) published further work into the short and long term repeatability of equine stride characteristics. The same group of horses from the first study (Drevemo *et al.*, 1980a) were recorded again immediately after the first recording, then again four years later. The most constant stride parameters were stride length with a mean standard deviation of 11.4cm on the first recording and 9.7cm on the second; swing (7.0msecs compared to 7.3msecs); step (5.4msecs compared to 6.2msecs) and suspension duration (7.3msecs compared to 6.8msecs). These results show that the short term reproducibility of equine gait is good due to the stability of stride parameters. The long term study indicated that the horses changed locomotion patterns between the two recordings (three years later). The largest change was seen in stride duration, increasing from a mean of 460.6 milliseconds to 487.3 milliseconds. The swing phase also increased significantly (from a mean of 352.3 milliseconds to 375.2 milliseconds), which could be the cause of the increased stride duration. The decrease in long term reproducibility in equine gait could be accounted for by age or training effects.

1.5 Breed

Equine breeds exhibit distinct characteristics, developed through selective breeding performed over a number of generations (Galisteo *et al.*, 1997). There is a huge amount of variation between equine breeds, with horses being selected for different attributes such as strength, speed or beauty (Cano *et al.*, 2001b). Conformation has a direct effect on the locomotion of the horse (Holmstrom *et al.*, 1990) and is inherently different between breeds. It could be argued therefore that horses will exhibit breed-specific patterns of locomotion. Few studies have focussed on exactly what these specific patterns are related to different breeds, as most studies into locomotion and breed are comparative, and sometimes contradictory (Cano *et al.*, 2001b, Galisteo *et al.*, 1997, Galisteo *et al.*, 2001b). One study established Arabian horses had significantly larger scapulohumeral range of motion (ROM) in walk compared to Andalusian horses; but smaller than Anglo-Arab horses (Galisteo *et al.*, 2001b). The Arabian horses had a mean ROM of 17.6±3.4° compared to 15.7±2.5° and 19.0±4.3° for the Anglo-Arabians and Andalusians respectively. A study by Cano *et al.* (2001b) contradicts these results (table 1.0); Arabians had the largest mean scapulohumeral ROM (25.3°) compared to Anglo –Arab (16.8°) and Andalusian (22.3°). The horses in this study were recorded in trot, compared to the horses in the previous study (Galisteo *et al.*, 2001b) that were recorded in walk. The difference in gait could account for the difference in ROM. Both studies established significant differences between breeds for all joints measures; highlighting the possibility of defining breed-specific gait patterns. The Arabian horses measured by Cano *et al.* (2001b) had a significantly shorter stride length in trot; demonstrated in table 1.0 (page 13). The standard deviation of the mean Arabian stride length was the largest out of the three, suggesting Arab horses have more variable gait. Variability in gait of Arab horses could present some difficulties for the present study when attempting to define normal gait for the breed.

Table 1.0: Stride length (in trot) and range of motion reported for various breeds (adapted from Galisteo *et al.*, 1997; Cano *et al.*, 2001b).

Trait/Breed	Arab	Anglo-Arab	Andalusian	Dutch Warmblood
Stride length (m)	2.6	2.6	2.7	3.1
Velocity (m/s)	4.8	3.7	3.9	4.0
Scapulohumeral ROM (°)	25.3	16.8	22.3	19.3
Humeroradial ROM(°)	58.4	60.1	67.1	57.8
Carpus ROM(°)	83.2	95.2	108.0	98.5
Metacarpophalangeal ROM(°)	85.3	88.2	103.0	82.6
Coxofemoral ROM(°)	29.5	24.8	29.6	N/A
Tarsus ROM(°)	58.8	61.3	69.0	N/A
Metatarsophalangeal ROM(°)	99.1	102.6	122.5	N/A

1.6 Age

Selection of horses for breeding and performance is often performed at a young age, therefore it is important to ascertain how locomotion develops as the horse grows. Consequently there have been a range of studies investigating how locomotion changes with age (Cano *et al.*, 2001a, Back *et al.*, 1993a, Back *et al.*, 1994a, Cano *et al.*, 1999, Back *et al.*, 1995a), providing some contradictory results. The studies by Cano *et al.* (2001a) and Cano *et al.* (1999) indicate that horses do not have inherent locomotion patterns from birth, they change and evolve with age. These studies measured stride characteristics of young and mature Andalusian horses using two dimensional motion analysis techniques. The greatest amount of modification to locomotion was found to be between 12 and 24 months, with some changes still occurring up to 36 months (Cano *et al.*, 2001a). Other studies, such as those by Back *et al.* (1994a; 1993a) indicate that horses' locomotion stabilises at a much younger age (four months) as no differences were found in temporal and angular characteristics in Dutch Warmblood horses aged four to 26 months. The studies by Cano *et al.* (1999; 2001a) recorded the horses being led in-hand on a track, whereas the studies by Back *et al.* (1993a; 1994a) recorded the horses on a treadmill. It has been reported that treadmill stride characteristics differ from those over ground (Barrey *et al.*, 1993; Buchner *et al.*, 1994). Higher stride frequency and longer stride length, (Barrey *et al.*, 1993) as well as an increase in stance duration (Buchner *et al.*, 1994) were established in horses trotting on a treadmill compared to over ground. The differences between gait using these two methods of data

collection makes it difficult to formulate direct comparisons between gait analysed in the Cano *et al.* and Back *et al.* studies.

1.7 Velocity

The quantification of equine gait requires stable locomotion patterns, with little intra-horse variation. Low intra-horse variation means that baseline data can easily be obtained, therefore when collecting baseline locomotion data consideration of factors affecting variability of gait must be considered. Velocity is one factor that can affect locomotion patterns in horses. At faster velocities longer distances are covered in a shorter space of time; stride length increases and stride duration decreases (Clayton *et al.*, 2002). A change in velocity has been shown to have a significant effect on stride characteristics in foals (Back *et al.*, 1993b). As velocity increased, stance phase duration decreased and flexion of the humeroradial, metacarpophalangeal and carpal joints increased. The data in this study was collected using a treadmill, which has been shown to have significant effects on equine locomotion (Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999). Over ground, horses will select a speed at which they are most comfortable (Peham *et al.*, 1998). This optimum speed has also been shown to be the speed at which there is the least variation between cycles of successive strides (Peham *et al.*, 1998). In this study, the smallest standard deviation between successive strides was found when horses were travelling at their optimum speed on a treadmill, and as the speed of the treadmill altered so did intra-horse variation between strides (with standard deviations ranging from 0.6cm to 5.8cm for one horse studied). The study also showed that each horse had different optimum speeds, ranging from 3.7 to 6.9m/s. Evidently velocity does have a significant effect on the variability of equine gait, efforts should be made when measuring variability to ensure all horses travel at similar speeds.

1.8 Validation

When using videography combined with motion analysis software as a method of equine gait analysis, the size of the horse can present some difficulty due to the amplitude of movement (Degueurce *et al.*, 1996). A large subject, such as a horse requires a large field of view in order to capture the full range of movement. Equine stride length can range from 2.63m to 3.07m at trot (see table 1.0, page 12). Human stride length has been reported as on average 1.4m (White and Lage, 1993; Dubost *et*

al., 2008). The video camera must be situated further from the subject than human motion analysis studies. It is essential that the motion analysis techniques used are still accurate and reliable at this distance to ensure they are a valuable tool for the equine industry to use. In order to validate the accuracy of the software, standard reference lengths or angles can be used (true values); accuracy is determined by the amount of conformity between the true and measured values. Defining the limits of the system's accuracy will enable effective and accurate interpretations of the results (Deluzio *et al.*, 1993; Wilson *et al.*, 1999).

Results of validation studies reported on one system will not generalise to other systems, therefore the accuracy of each system requires definition (Klein, 1995). Results of validation on some commercially available systems can be seen in table 1.1 (page 19). The majority of validation studies so far determine the accuracy of three-dimensional motion analysis techniques, there appears to be very little validation research using two-dimensional equivalents or the specific software programmes used in this research (Quintic© and HU-M-AN™). There is currently no published research into the validation of Quintic© or HU-M-AN™ in the use of measuring static and dynamic values or the tracking of movement in horses. There has been research into the reliability of Quintic© to measure tibial rotation in humans (Lovett, 2006). This study used Quintic© in conjunction with a tibial pointer device to measure the amount of rotation. The intra-class correlation coefficients (ICC) were calculated from two separate digitisations of the same ten subjects. The ICC values were >0.7 in 70% of the subjects, indicating a good reliability. This study used two measurement techniques (Quintic© and the tibial pointer device) therefore it is hard to distinguish the reliability of Quintic© alone from this study. HU-M-AN™ has been used in previous research to validate the temporal accuracy of digital video-based motion capture systems (Teeple *et al.*, 2009). This study did not test the accuracy of the software alone, but the accuracy of different camera systems (HU-M-AN™ was used to digitise the videos). The study revealed that differences (up to 5% for angular position and up to 15% for angular velocity) for different camera systems were caused by the method of compression used (to download the video clips onto the computer), rather than the software.

1.8.1 Static linear validation

Static linear accuracy is applicable when analysing equine conformation; the assessment of linear conformation traits involves the measurement of varying static lengths (measuring the distance between two points on limb segments).Linear validation is normally calculated using inter-marker distance measurements (Chiari *et al.*, 2005). This is done by placing makers a set distance apart and comparing this true value to the value measured by the software. It is a basic requirement of all motion analysis systems yet despite this, very few studies have validated static linear measurements when calculating the accuracy of motion analysis software. The majority of studies into linear validation are regarding dynamic rather than static measurements. Two studies that measured static linear accuracy were Klein and De Haven (1995) and Thornton *et al.*, (1998). The two motion analysis software packages (Ariel and Kinemetrix 3-D) were considered accurate with a mean error of 500±1.3mm (Klein and De Haven, 1995), and 80±0.1mm (Thornton *et al.* 1998). The studies only tested one length respectively (500mm and 80mm), rather than a range of lengths. It is possible that different lengths could be measured to varying degrees of accuracy, therefore a range of different lengths should be tested.

1.8.2 Static angular validation

Angular validation involves comparing true values to measured values (as with linear validation). True angles are frequently taken from recording a goniometer; an established tool to objectively measure individual joint movement in human patients (Edgar *et al.*, 2009) and in assessments of static conformation in horses, prior to the use of photography or videography (Magnusson, 1985). Scholtz (1989) validated a motion analysis software programme called WATSMART (Waterloo Spatial Motion Analysis Recording Technique) using a goniometer. The goniometer had infrared light emitting diodes attached to the axis and arms. Scholtz recorded the goniometer at 12 angles, varying by 5°, from 45° to 100°, using a video camera, 10 times for each angle. The variation for each angle was less than 0.5° ($P<0.05$), however it was noted that if the goniometer rotated out of plane the reliability and accuracy decreased. The software will recognise the pixels comprising the marker and automatically calculate the centre point. This automatic identification will be limited if distortions occur (if the camera or marker is rotated out of plane). These inaccuracies due to out of plane rotations were

also noted by Wilson *et al.* (1999). Further research by Klein and De Haven (1995) used a similar method to calculate angular accuracy of the Ariel Performance Analysis System. The goniometer was manually positioned at each angle (from 10° to 180° in 10° increments) and recorded using a video camera. This process was repeated ten times. The average deviation was <0.03° (more accurate than WATSMART), and considered to be within the normal error range and the software was deemed reliable at accurately measuring static angles. Angular consistency was measured using the same process as above but moving the goniometer through the field of view. Accuracy was consistent for angles <120° however angles >120° there was increased variability between the goniometer reading and the value shown using the software. The decrease in accuracy for angles >120° is supported by further research by Linden (1992). This study used a similar method (a goniometer with markers attached) and motion analysis software (Motion Analysis™). The mean differences were smaller for angles <90° (0.5°) compared to the mean differences for angles >90° (1°). The system demonstrated the greatest error in calculating the 180° angle (between 1.5° and 2.4°). The system was less accurate at calculating angles greater than 180°. The author suggests this could be due to the algorithm the software uses to calculate the angles. The algorithm uses the cosine rule to calculate the angle, and as the cosine approaches one, the software appears to be limited when the opposite side becomes very small (Linden *et al.*, 1992). None of these studies validated angles >180°. When assessing equine conformation, some joints can have angular values of over 180° for tarsal, proximal phalanx and carpus angles (Anderson and McIlwraith, 2004), therefore it is important that validation of the software reflects these types of measurements.

1.8.3 Dynamic validation

Calculating the accuracy of dynamic measurements commonly uses a device of known value (length) moving throughout a calibrated field of view. Linden *et al.* (1992) used two spherical markers attached to each end of a rigid wooden bar (178.5mm apart). The wooden bar was moved randomly within the field of view, and recorded using a video camera. The system calculated the distance between the markers from 174.1mm to 177.6 mm, giving an error value of between 0.9 mm and 4.4mm. This method was repeated by Degueurce *et al.* (1996) to evaluate the use of a three dimensional opto-electronic system for capturing movement. The absolute and calculated distances were

tested for variance, and showed no significant differences ($P<0.05$), with a mean error of less than 5mm for a reference length of 60.9cm.

Dynamic validation has also been performed using a pendulum test (Wilson *et al.*, 1997; Chiari *et al.*, 2005), although this is not as widely used. Markers were attached to either end of a wooden bar, which was then oscillated. The linear distance was calculated for the distance between the markers as the pendulum was in motion. The swing phase of the equine distal limb is likened to an inverted pendulum (Back *et al.*, 1993) as has the human athlete (Chiari *et al.*, 2005), therefore it would seem appropriate to use a pendulum to test the accuracy of motion analysis software that will be used for equine locomotion analysis. This simple test could be developed to enable the accuracy of linear and angular measurements, as well as velocity to be calculated.

A common method of validating dynamic accuracy of motion analysis systems is to compare the system being tested to a "gold standard" system-one that has already been validated as accurate and is widely used in research. One such system is the Qualisys Oqus motion capture system. This is an optoelectronic system that uses both active and passive markers to track moving objects. This system is accurate to within 1mm, and a similar system (ProReflex) has been used in previous studies as the standard with which to compare other systems to (Nankervis *et al.*, 2009).

The study by Chiari *et al.* (2005) also reported the results of validation tests of various motion analysis systems that are commercially available. Accuracy was reported in a typical gait analysis setting for marker distance estimates. The standard error and standard deviation ranged from 0.1mm (standard deviation 0.53mm) to 5.3mm (standard deviation 4.2mm). For most of the systems tested, the standard error was greater than 1mm. There was variation in errors reported for each system, which shows the importance of testing the accuracy of each motion analysis system prior to use.

Table 1.1: Results of validation tests performed on various commercially available systems (adapted from Wilson *et al.*, 1999; Chiari *et al.*, 2005).

Author (s)	Software	Type of validation	True value (s)	Margin of error
Klein and De Haven (1995)	Ariel Performance Analysis System	Static linear and angular	Inter-marker distance value of 50cm Goniometer values from 10° to 180° in 10° increments	1.3mm <0.03°
Thornton *et al.* (1998)	Kinemetrix 3-D	Static linear	Inter-marker distance value of 80mm	0.1mm
Scholz (1989)	WATSMART (Waterloo Spatial Motion Analysis Recording Technique)	Static angular	Goniometer values from 45° to 100° in 5° increments	0.5°
Linden *et al.* (1992)	Motion Analysis System	Static angular	Goniometer values from 20° to 180° in 10° increments	<0.4°
Wilson *et al.* (1997)	Ariel Performance Analysis System	Dynamic (angular)	12 angles at four initial angular positions	0.183°
Degueurce *et al.* (1996)	3D Vision	Dynamic (linear)	Inter-marker distance value of 60.9cm	<5mm
Linden *et al.* (1992)	Motion Analysis	Dynamic (linear)	Inter-marker distance value of 178.5cm	4.4mm

The provision of accurate quantitative data on normal conformation and stride characteristics of Arabian horses, creating a unique baseline dataset would provide the Arab Horse Society as well as Arab horse owners and breeders with interesting and useful information. Accurate data cannot be obtained without a valid and standardised method of data collecting. This research will form three separate but inter-linked studies. Firstly, a comparison of two commercially available two-dimensional software programmes will be undertaken to establish the accuracy and suitability of the

programmes for use with equine gait analysis. Secondly, variability in equine gait will be measured to standardise how "normal" (baseline) gait characteristics are measured using two-dimensional motion analysis techniques. These two studies will form the basis of the final section of this research; to establish normal conformation and gait in a group of purebred Arabian horses.

PART I

Validation and comparison of Quintic© and HU-M-AN™ for use in two-dimensional motion analysis.

2.0 Aim

The aim of this part of the study was to validate and compare the accuracy of Quintic© and HU-M-AN™ as two-dimensional motion analysis software packages, for static linear and angular measurements and dynamic linear and angular measurements and velocity.

2.1 Objectives

i) Record a metre ruler and measure different distances with both software packages. Compare the measured values to the known values (and to each other).

ii) Record a goniometer and measure different angles with both software packages. Compare the measured values to the known values (and to each other).

iii) Record dynamic length, angle (and velocity) using a pendulum to measure length of arc, change in angle and velocity with both software packages. Compare these values to measurements taken with a previously validated "gold standard" system (Oqus).

2.2 Hypothesis

i) There will be a significant difference between the known linear distances and the measured linear distance with both software packages.

ii) There will be a significant difference between the known angle and the measured angle with both software packages.

iii) There will be a significant difference between the standard values for dynamic distance, angle or velocity with both software packages.

3.0 Instrumentation

Manual digitising of the video clips used Quintic© Biomechanics 9.03 v14 and HU-M-AN™ (2D) v6.0. In addition to this, hardware used included a Sony DV-tape digital video camera (HDR-FX1000) recording at 50 hertz (25 frames per second, two fields per frame), and a Fujitsu Siemens laptop for downloading the video clips. A purpose built wooden cube (50cmx50cm) was used to provide a reference length and ratio for calibrating the video clips. The majority of validation tests use a standardised measurement technique or a known value to compare the software against (Klein and De Haven, 1995). The standards to be used in this study are a metre ruler (for static linear measurements), goniometer (for static angular measurements) and a pendulum (for dynamic measurements).

3.1 Data smoothing

The majority of data obtained from motion analysis is low frequency (Chiari *et al.*, 2005). This data is often smoothed to remove the high frequency noise associated with data collected using two dimensional motion analysis techniques (Howarth and Callaghan, 2009). HU-M-AN™ and Quintic© use a low pass, second order Butterworth filter for data smoothing. This is an appropriate filter for analysis of low frequency data. Cut off frequencies for the majority of human movement data is between 4 and 8 Hertz (Bartlett, 2007). There were only small amounts of distortion for the data for this investigation (no noise from impact or STA) therefore a cut off frequency of 10 Hertz was used. Data smoothing using this technique will remove any data above the cut off frequency (removing high frequency data), therefore leaving the low frequency data that represents the movement being measured, rather than the distortions.

3.2 Linear accuracy

Static linear estimations tested the accuracy of the software to calculate linear distances from a standard length. The method was developed from a similar method used by Klein and De Haven (1995). The standard length used was a metre rule, clamped in a retort stand. Circular markers (5mm diameter) were attached to the meter rule at 10cm intervals (giving ten known lengths from 10 to 100cm to calculate). The centre of the marker was used to digitise. The linear estimations tested three positions of the metre rule (figure 3.0) which was placed at different angles (against the vertical) i) 90 degrees (horizontal) ii) 0 degrees (vertical) iii) 45 degrees (diagonal). A camera on a tripod was positioned 4m away from the metre rule, and levelled using a spirit level. A calibration cube (50x50cm) was used to ensure the camera was perpendicular to the equipment and to calibrate the video clips. The calibration cube was recorded for three seconds, and then removed from the field of view. The meter rule was recorded for three seconds, three times for each position (in total nine clips were recorded).

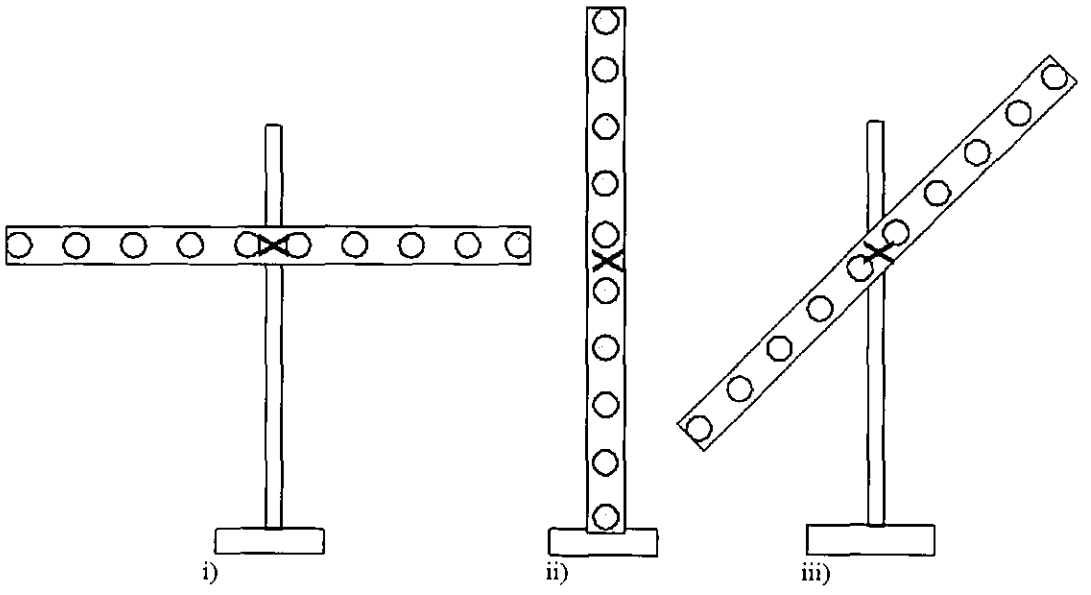


Figure 3.0: Test rig for estimation of linear accuracy: showing i) horizontal ii) vertical and iii) diagonal orientations of the meter rule. Ten, 5mm markers were placed at 10cm intervals on the metre rule (giving ten known linear distances), which was clamped into a retort stand (the clamp is marked with an X).

3.3 Angular accuracy

Static angular estimations tested the accuracy of the software to calculate static angles. The standard used was a clinical goniometer clamped in a retort stand (figure 3.1). Goniometers have previously been used as standards for angular validation studies (Klein and De Haven, 1995; Linden, 1992). The test rig was set up and calibrated as above. Three circular markers (5mm diameter) were attached to the centre pivot point, and the end of each axis of the goniometer (250mm from the centre). The reference angles used were between 10 and 360 degrees at 10 degree increments (based on the same procedure as Linden *et al.*, 1992)). The goniometer was manually positioned into each of the 36 reference points, in the vertical and horizontal plane, and recorded for three seconds three times for each angle. A total of 216 references angles were recorded.

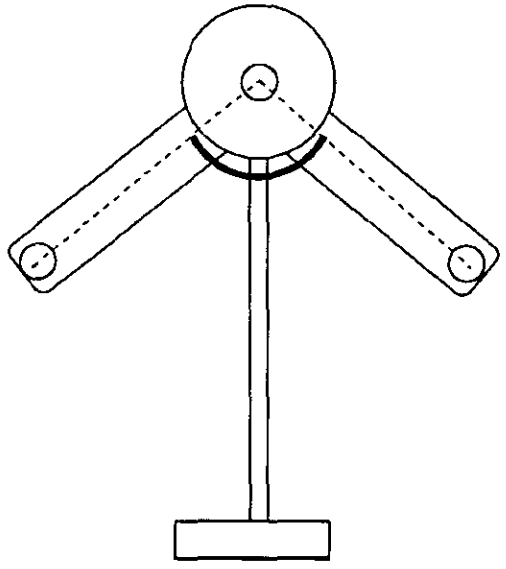


Figure 3.1: Estimation of static angles using a goniometer clamped to a retort stand. Circular markers (5mm) were attached to the centre of the pivot and each axis (shown in grey). The angle was measured between the three markers (angle shown in red). The goniometer was positioned into 36 different angles (between 10 and 360^{o}).

3.4 Dynamic accuracy

To measure the accuracy of the software in measuring dynamic distances and angles, a pendulum was used. This is not a widely used method but has been reported previously as a technique to validating dynamic measurements (Chiari *et al.*, 2005). The pendulum was constructed by hanging a mass from a retort stand using a wire cable (112cm). Circular markers (20mm, in addition to this a 5mm circular marker to identify the centre) were attached to the pendulum at the centre of the mass, centre of the pivot and base of the retort stand (figure 3.2). The pendulum mass was pulled back to starting angles of 20°, 30° and 60° (measured from the retort stand) and set in motion, five times for each angle. The pendulum was recorded simultaneously with two systems; the two dimensional system used for the previous static validation (standard digital video camera) and a three dimensional optoelectronic system (Oqus) that acted as the standard. The Oqus system digitises automatically and has previously been validated to be accurate to 1mm for dynamic measurements, and has been used as the "gold standard" for other research validating new motion analysis techniques therefore an appropriate system to compare Quintic© and HU-M-AN™ to. Once the clips were downloaded and digitised, range of motion, length of arc (linear distance travelled by the mass) and velocity (of the mass) were calculated using all three systems. The results from Quintic© and HU-M-AN™ were compared to the standard results obtained from Oqus.

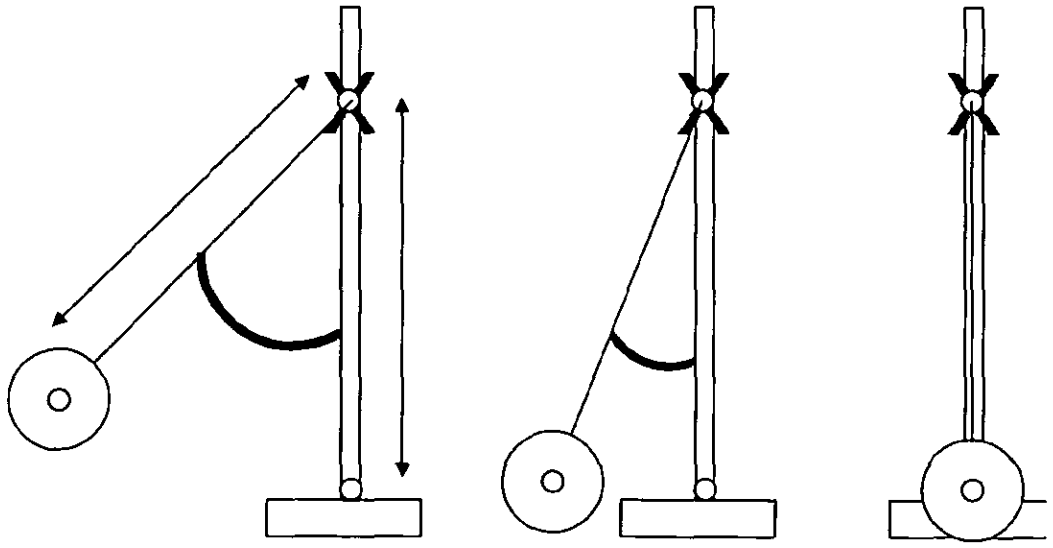


Figure 3.2: Test rig to simulate a pendulum. A mass was suspended from a retort stand from a pivot (X). Circular markers (5mm) shown in grey were attached to the centre of the mass, centre of the pivot and base of the stand (shown in grey). The angle measured is shown in red.

3.5 Statistical analysis

Descriptive statistics were calculated for the data for each true value (distance and angle) and orientation. Descriptive statistics included mean difference (the difference between the true and measured value), standard error, variance (the distance of the data from the mean value) and the coefficient of variation (the standard deviation as a percentage of the mean, which allows data sets of different values to be compared).

Data for each true value and orientation were tested for normality using an Anderson-Darling normality test. Data for the measured value, and the difference between true and measured values were tested. Data that were established as parametric were analysed further using a General Linear Model (GLM).

4.0 Linear validation

4.0.1 Comparison of Quintic© and HU-M-AN™

Tables 4.0 to 4.2 and figures 4.0-4.2 illustrate the differences between the true linear measurements taken from the software programmes in the horizontal, vertical and diagonal planes. The amount of variation between repeated measurements using the same programme has also been established. All data were parametric.

Table 4.0 Comparisons between Quintic© and HU-M-AN™ for static linear measurements in the horizontal plane. The highest and lowest mean difference values for each software have been highlighted in grey.

True value (cm)	Mean difference		SE		Variance		%COV	
	Quintic©	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*
10	-0.33	0.03	0.33	0.22	5.97	0.15	1.00	3.80
20	0.00	0.07	0.00	0.08	0.00	0.02	0.00	0.72
30	0.67	0.18	0.33	0.08	1.88	0.02	1.00	0.46
40	0.67	0.30	0.33	0.38	1.42	0.44	1.00	1.64
50	0.67	0.24	0.33	0.38	1.14	0.44	1.00	1.32
60	1.00	0.53	0.58	0.22	1.64	0.15	2.00	0.64
70	1.00	0.23	0.58	0.09	1.41	0.02	2.00	0.21
80	2.00	0.71	0.00	0.15	0.00	0.07	0.00	0.33
90	1.67	0.22	0.33	0.16	0.63	0.08	1.00	0.31
100	0.33	0.25	0.33	0.29	0.58	0.25	1.00	0.50

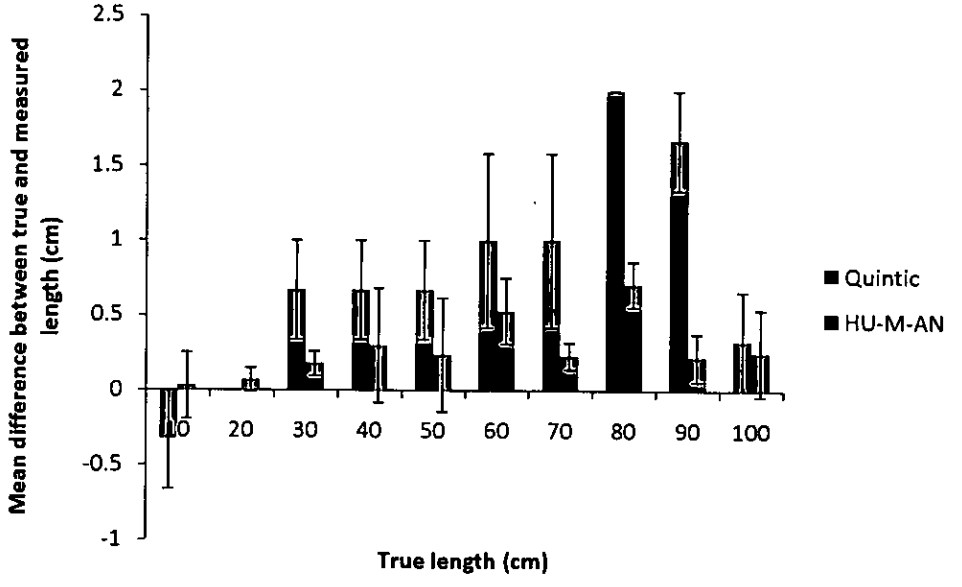


Figure 4.0: Mean difference (±SE) between true and measured distances with Quintic© and HU-M-AN™ in the horizontal plane.

Table 4.1: Comparisons between Quintic© and HU-M-AN™ for static linear measurements in the vertical plane. The highest and lowest mean difference values for each software are highlighted in grey.

True value (cm)	Mean difference		SE		Variance		%COV	
	Quintic©	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*
10	0.00	0.00	0.00	0.15	5.97	0.07	1.00	2.65
20	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.01
30	0.00	0.28	0.00	0.08	0.00	0.02	0.00	0.44
40	0.00	0.27	0.33	0.13	1.43	0.05	1.00	0.57
50	0.33	0.47	0.33	0.23	1.14	0.16	1.00	0.78
60	1.00	0.56	0.00	0.15	0.00	0.07	0.00	0.44
70	1.00	0.71	0.00	0.23	0.00	0.16	0.00	0.56
80	1.00	0.45	0.00	0.23	0.00	0.16	0.00	0.49
90	1.00	0.60	0.00	0.26	0.00	0.21	0.00	0.50
100	0.00	-0.46	0.00	0.13	0.00	0.05	0.00	0.23

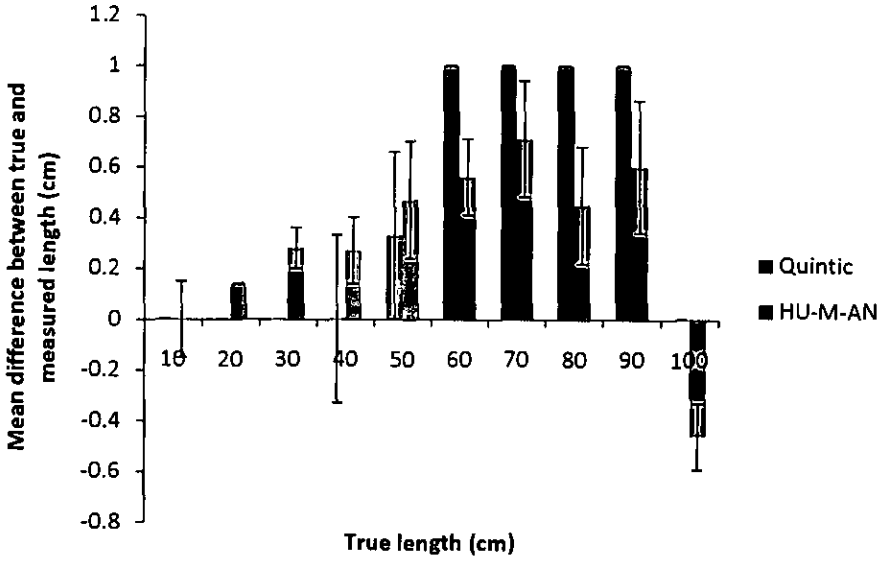


Figure 4.1: Mean difference (±SE) between true and measured distances with Quintic© and HU-M-AN™ in the vertical plane.

Table 4.2: Comparisons between variation in Quintic© and HU-M-AN™ for static linear measurements in the diagonal plane. The highest and lowest mean difference values for each software are highlighted in grey.

True value (cm)	Mean difference		SE		Variance		%COV	
	Quintic©	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*
10	0.00	-0.06	0.00	0.12	0.00	0.04	0.00	2.05
20	0.00	-0.04	0.00	0.29	0.00	0.25	0.00	2.51
30	0.00	0.33	0.00	0.26	0.00	0.21	0.00	1.50
40	0.00	0.45	0.00	0.14	0.00	0.06	0.00	0.61
50	0.33	0.50	0.33	0.25	1.15	0.19	1.00	0.86
60	1.00	0.46	0.00	0.19	0.00	0.11	0.00	0.56
70	1.00	0.46	0.00	0.06	0.00	0.01	0.00	0.16
80	1.00	0.58	0.00	0.05	0.00	0.01	0.00	0.11
90	1.00	0.58	0.00	0.05	0.00	0.01	0.00	0.09
100	0.00	0.04	0.00	0.06	0.00	0.01	0.00	0.10

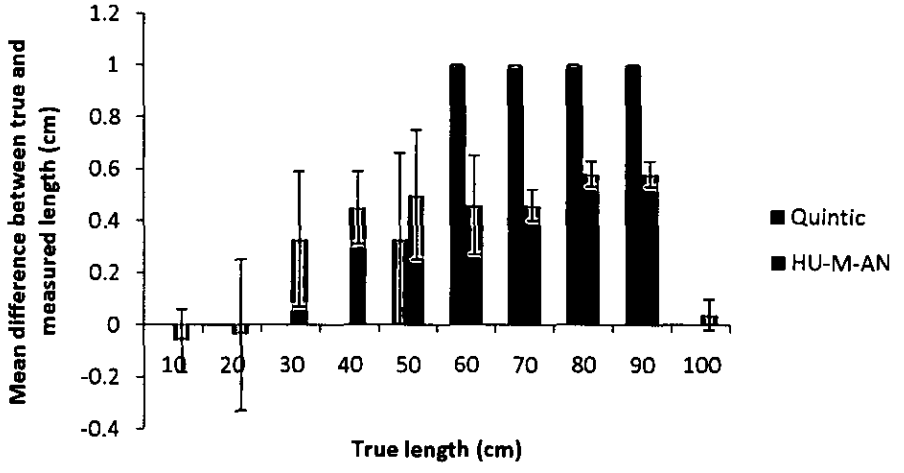


Figure 4.2: Mean difference (±SE) between true and measured distances with Quintic© and HU-M-AN™ in the diagonal plane.

Overall there appears to be higher variance with the HU-M-AN™ data. It must be noted however, that Quintic© measures linear distances to one decimal place, compared to HU-M-AN™ that measures to three. A difference of below 1cm with Quintic© will therefore be measured as zero, whereas with HU-M-AN™ this difference could be between 0.000 and 0.999cm. This needs to be taken into account when analysing the data further.

There does appear to be a trend in the mean difference values for the HU-M-AN™ data. Values in the middle range (between 30cm and 90cm) tend to display higher mean differences (figures 4.0-4.2). Values in the lower and upper ranges (10-20cm and 100cm) tend to display lower mean differences.

4.0.2 Effect of true distance, orientation and software

Table 4.3: Linear validation of Quintic© and HU-M-AN™ for the true distances of 10-100cm in three orientations (horizontal, vertical and diagonal) for the measured values and difference between true and measured values. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Measured value		Absolute difference	
	Quintic©	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*
True Distance	***	***	***	***
Orientation	*	NS	*	NS
True distance and orientation interaction	NS	NS	NS	NS
Repeat	NS	NS	NS	NS
Software	***		***	
True distance and software interaction	***		***	

Table 4.3 demonstrates Quintic© and HU-M-AN™ measure varying lengths consistently; repeat was not a significant factor for either software package (P>0.05). Depending on which software was being used, the true values were measured differently. Software was a significant influence on the measured value and the absolute difference (P<0.001). Each software package will measure varying lengths to different degrees of accuracy (true distance was a significant influence on absolute difference). Quintic© is affected by the orientation of the lengths being measured (P<0.05), whereas HU-M-AN™ is not (P>0.05), although there was no interaction between true length and orientation for either software packages (P>0.05).

4.1 Angular validation

4.1.1 Comparison between Quintic© and HU-M-AN™

Tables 4.4 and 4.5 illustrate the variation between the true angular value and measurements taken from the software programmes in the horizontal, vertical planes. All data were parametric.

Table 4.4: Comparison of Quintic© and HU-M-AN™ for angular validation in the horizontal plane. The highest and lowest mean differences have been highlighted in grey. The four lowest COV values are highlighted in yellow. * = quadrant angle.

True angle (°)	Mean difference		SE		Variance		COV(%)	
	Quintic©	HUMAN™	Quintic©	HUMAN™	Quintic©	HUMAN™	Quintic©	HUMAN™
10	1.45	0.58	0.04	0.18	0.00	0.09	0.57	2.86
20	1.93	0.42	0.30	0.09	0.28	0.02	2.40	0.74
30	2.21	0.13	0.17	0.18	0.08	0.10	0.90	1.02
40	2.67	0.28	0.20	0.17	0.12	0.09	0.82	0.74
50	3.03	0.43	0.28	0.12	0.23	0.04	0.90	0.41
60	3.36	0.63	0.31	0.17	0.29	0.09	0.85	0.48
70	2.50	0.52	0.30	0.23	0.27	0.16	0.71	0.56
80	1.94	0.56	0.10	0.17	0.03	0.09	0.21	0.36
***90**	0.53	0.60	0.42	0.20	0.52	0.12	0.80	0.37
100	0.64	0.52	0.06	0.16	0.01	0.08	0.10	0.28
110	-0.19	0.63	0.30	0.26	0.27	0.20	0.47	0.40
120	-0.20	0.36	0.22	0.22	0.14	0.14	0.32	0.31
130	-0.73	0.64	0.29	0.28	0.26	0.24	0.39	0.38
140	-0.68	0.65	0.24	0.19	0.17	0.11	0.30	0.24
150	-1.20	0.35	0.26	0.13	0.20	0.05	0.30	0.15
160	-0.35	0.20	0.34	0.09	0.35	0.02	0.37	0.10
170	0.67	0.40	0.19	0.17	0.11	0.08	0.20	0.17
***180**	-0.34	0.26	0.16	0.03	0.08	0.00	0.15	0.03
190	0.78	0.23	0.11	0.12	0.04	0.04	0.10	0.11
200	1.24	-0.07	0.32	0.37	0.30	0.41	0.27	0.32
210	2.19	0.01	0.36	0.41	0.38	0.51	0.29	0.34
220	2.70	-0.23	0.06	0.20	0.01	0.12	0.05	0.16
230	1.68	-0.50	0.28	0.33	0.24	0.32	0.21	0.25
240	1.78	-0.25	0.39	0.35	0.45	0.36	0.28	0.25
250	2.06	0.30	0.26	0.09	0.21	0.03	0.18	0.06
260	1.41	0.18	0.23	0.35	0.15	0.37	0.15	0.23
***270**	1.07	0.15	0.20	0.22	0.11	0.15	0.12	0.14
280	0.07	0.61	0.37	0.14	0.42	0.06	0.23	0.09
290	-0.29	0.23	0.22	0.08	0.14	0.02	0.13	0.05
300	-0.46	0.41	0.49	0.28	0.70	0.24	0.28	0.16
310	-0.96	0.51	0.28	0.05	0.23	0.01	0.15	0.03
320	-1.30	0.54	0.16	0.26	0.08	0.21	0.09	0.14
330	-1.01	0.32	0.15	0.18	0.07	0.09	0.08	0.09
340	-1.07	0.46	0.30	0.13	0.27	0.05	0.15	0.07
350	-0.48	-0.02	0.36	0.21	0.38	0.14	0.18	0.11
***360**	-0.22	-0.10	0.11	0.25	0.04	0.19	0.05	0.12

Table 4.5: Comparison of Quintic© and HU-M-AN™ for angular validation in the vertical plane. The highest and lowest mean differences have been highlighted in grey. The four lowest %COV values are highlighted in yellow. * = quadrant angle.

True angle (°)	Mean difference		SE		Variance		COV(%)	
	Quintic©	HUMAN™	Quintic©	HUMAN™	Quintic©	HUMAN™	Quintic©	HUMAN™
10	-0.23	0.54	0.14	0.16	0.06	0.07	2.41	2.55
20	-1.11	0.35	0.15	0.12	0.06	0.04	1.34	1.01
30	-1.24	0.60	0.05	0.12	0.01	0.04	0.32	0.67
40	-1.50	0.29	0.28	0.30	0.24	0.28	1.26	1.30
50	-1.33	0.54	0.16	0.20	0.07	0.13	0.55	0.70
60	-1.07	0.32	0.29	0.28	0.25	0.24	0.85	0.80
70	-0.86	0.24	0.15	0.16	0.07	0.07	0.37	0.38
80	-0.74	0.45	0.42	0.11	0.52	0.04	0.91	0.24
*90	0.01	0.12	0.50	0.57	0.75	0.97	0.96	1.10
100	0.87	0.25	0.17	0.13	0.09	0.05	0.29	0.22
110	2.91	0.88	0.12	0.36	0.05	0.40	0.19	0.57
120	2.28	0.47	0.41	0.10	0.52	0.03	0.59	0.14
130	1.81	0.29	0.62	0.52	1.14	0.80	0.81	0.69
140	2.38	0.37	0.21	0.17	0.14	0.08	0.26	0.21
150	1.96	0.45	0.49	0.15	0.71	0.06	0.55	0.17
160	1.47	0.54	0.21	0.08	0.14	0.02	0.23	0.09
170	0.46	-0.02	0.21	0.34	0.13	0.35	0.21	0.35
*180	-0.35	-0.17	0.32	0.11	0.31	0.04	0.31	0.11
190	-0.53	-0.13	0.09	0.10	0.02	0.03	0.08	0.09
200	-1.74	-0.21	0.03	0.34	0.00	0.34	0.03	0.29
210	-2.38	-0.32	0.38	0.33	0.43	0.33	0.32	0.27
220	-2.26	-0.16	0.26	0.11	0.21	0.04	0.21	0.09
230	-2.35	0.06	0.32	0.20	0.30	0.12	0.24	0.15
240	-1.84	0.22	0.21	0.04	0.13	0.00	0.15	0.03
250	-1.62	-0.17	0.20	0.25	0.12	0.19	0.14	0.18
260	-0.91	-0.53	0.13	0.05	0.05	0.01	0.08	0.04
*270	-0.21	-0.19	0.38	0.22	0.43	0.15	0.24	0.14
280	0.67	0.15	0.15	0.10	0.07	0.03	0.09	0.06
290	1.76	0.01	0.25	0.26	0.19	0.21	0.15	0.16
300	1.56	-0.22	0.17	0.10	0.09	0.03	0.10	0.06
310	1.63	-0.03	0.27	0.09	0.21	0.02	0.15	0.05
320	1.53	0.11	0.21	0.18	0.13	0.10	0.11	0.10
330	1.55	0.07	0.06	0.04	0.01	0.01	0.03	0.02
340	0.92	-0.01	0.13	0.24	0.05	0.18	0.07	0.12
350	0.71	-0.41	0.19	0.22	0.10	0.15	0.09	0.11
*360	-0.30	0.10	0.17	0.10	0.08	0.03	0.08	0.05

A comparison of angular data in the horizontal and vertical plane for both software packages established angles measured with Quintic© had the highest mean differences. The Quintic© data appears to follow a trend for angles measured in both horizontal and vertical planes. The lowest COV values are for the measurement of true angles that correspond closely to the four quadrant angles (90°, 180°, 270°, 360°). It seems as the

true angle approaches the quadrant angles the software becomes more accurate at measuring them. Angles measured with HU-M-AN™ illustrated a different trend; larger angles had the lower COV values compared to smaller angles. Larger angles were more accurately measured compared to smaller angles.

4.1.2 Effect of true angle, orientation and software

Table 4.6: Angular validation of Quintic© and HU-M-AN™ for all data. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Measured value		Absolute difference	
	Quintic©	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*
True angle	***	***	***	NS
Orientation	**	**	NS	NS
True angle and orientation interaction	***	NS	***	NS
Repeat	NS	NS	NS	NS
Software	NS		***	
True angle and software interaction	NS		***	

Analysis of the measured value established true angle and orientation were significant covariates (P<0.001) for both software packages. Software and repeat number were not significant factors when analysing data for measured value (P>0.05). Analysis of the absolute difference (the difference between the true and measured values) established difference between true and angles measured using Quintic© varied significantly, depending on the size of the angle being measured (P<0.001). Orientation was also established as not significant (P>0.05) for either software, however there was an interaction between true angle and orientation using Quintic© (P<0.001). Absolute difference varied significantly for angles measured in the horizontal and vertical planes, depending on the size of the angle being measured.

Software was also established as significant when analysing the absolute difference (P<0.001), the difference between true and measured angles were significantly different when the same angle was measured with Quintic© and HU-M-AN™. Repeat number was not significant (P>0.05), the true angles were consistently measured when repeated measures were taken. Figures 4.3 and 4.4 illustrate the large variation between the two

software packages being tested. Much less variation was displayed in the HU-M-AN™ results, and these results do not appear to follow a trend. Both figures appear to follow the same trend for Quintic© but with a phase shift.

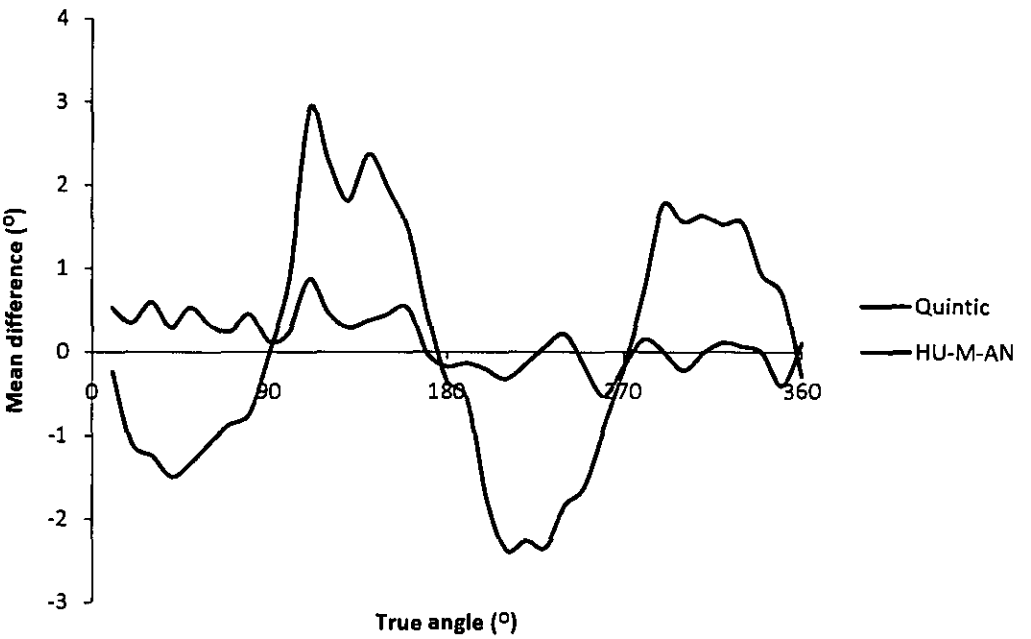


Figure 4.3: A comparison of the mean difference between true and measured angles for Quintic© and HU-M-AN™ in the horizontal plane.

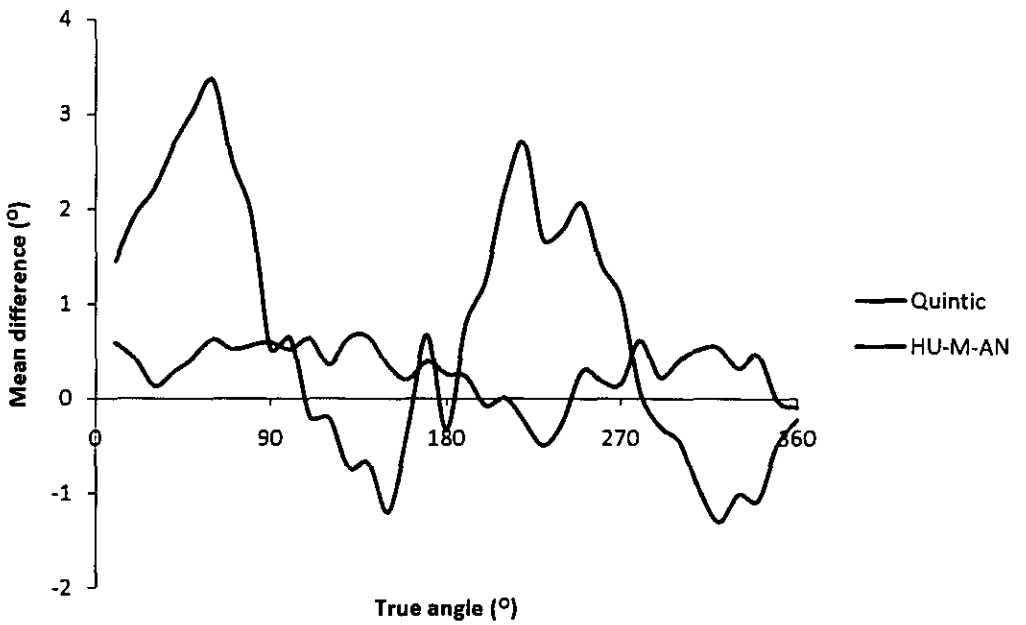


Figure 4.4: A comparison of the mean difference between true and measured angles for Quintic© and HU-M-AN™ in the vertical plane.

4.1.3 Further analysis of Quintic©

Further analysis was performed on the angular results for Quintic©. The residuals, when plotted against the true angular values, appear to follow the pattern of a sine wave. Figure 4.5 (horizontal) is similar to a sine wave whereas figure 4.6 is similar to a sine wave with a positive phase shift (along the *x* axis).

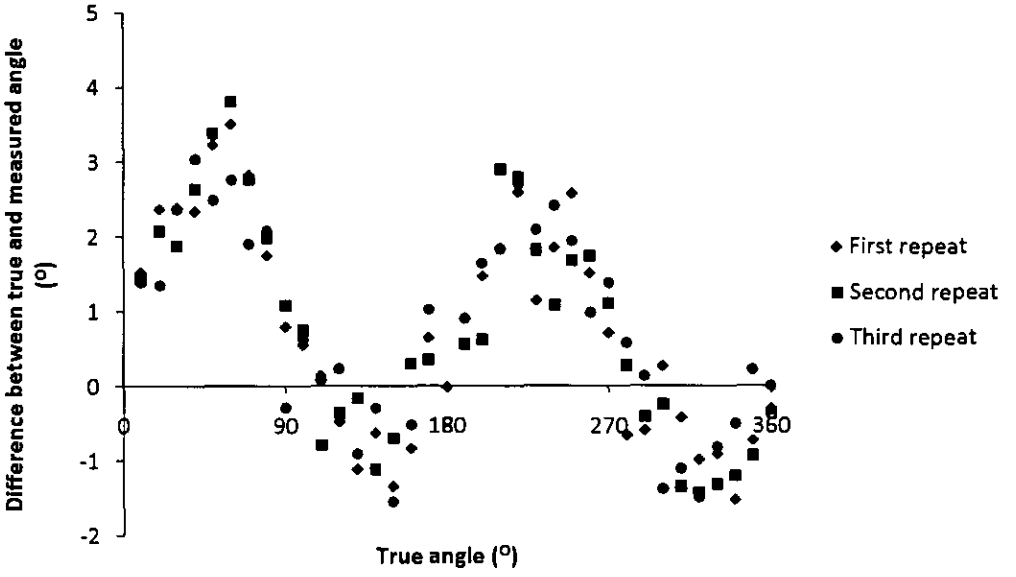


Figure 4.5: Difference between true and calculated angles measured in the horizontal plane using Quintic©

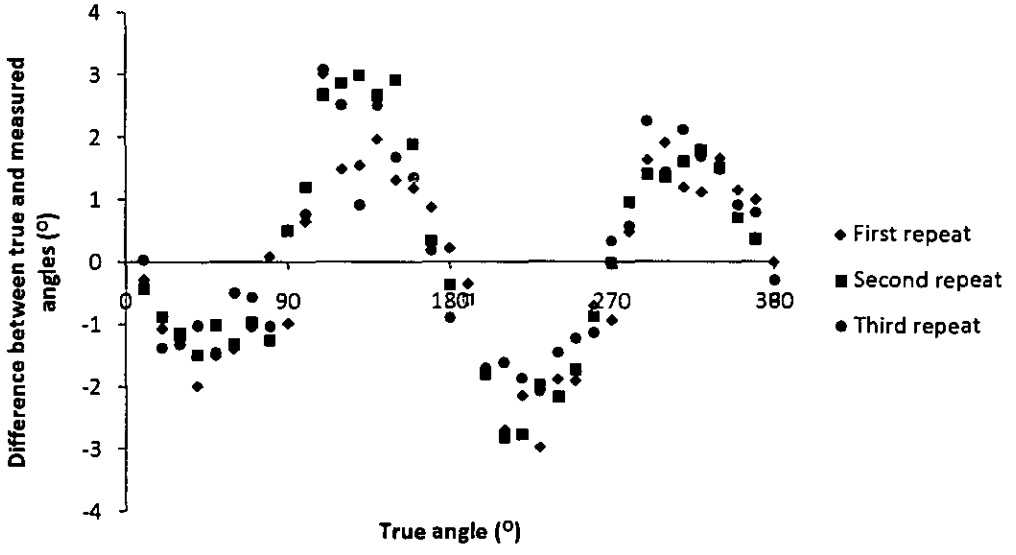


Figure 4.6: Difference between true and calculated angles measured in the vertical plane using Quintic©

4.2 Dynamic validation

4.2.1 Dynamic angular validation (Pendulum study)

Tables and figures 4.7-4.9 illustrate the amount of variation between the "gold standard" Oqus system and Quintic© and HU-M-AN™. All data were established as parametric.

There was no difference between any of the software programmes for the measurement of range of motion of a pendulum when released from position A (released from an angle of 60° from the vertical). For release positions B and C (released from angles of 30° and 20° respectively) Quintic© measurements were significantly smaller than the standard (Oqus). There was no difference between the ROM measured using HU-M-AN™ and the standard.

Table 4.7: Comparison between variation in Quintic© and HU-M-AN™ for dynamic angular measurement (ROM) of a pendulum released from three different positions. Values are in relation to the "gold standard" Oqus system.

Release Position	Mean difference		SE		Variance		%COV	
	Quintic©	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*
A	5.65	1.56	0.57	0.33	1.62	0.55	2.38	1.29
B	4.71	1.21	0.76	0.58	2.91	1.69	6.37	4.3
C	4.19	0.66	0.35	0.41	0.61	0.82	4.17	4.08

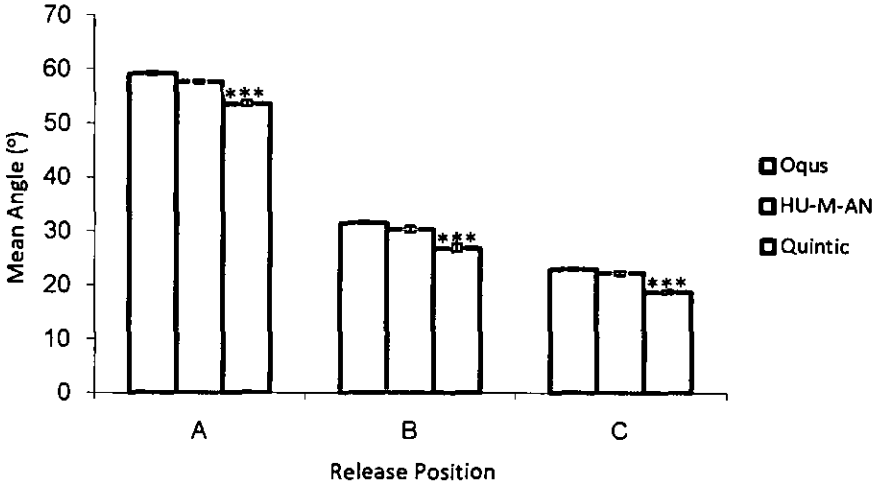


Figure 4.7: Mean ROM of one pendulum swing from three different release positions (A, B, C), comparing Quintic© and HU-M-AN™ to the "gold standard" Oqus system. (*P<0.05; **P<0.005; ***P<0.001 denotes a significant difference from the standard and level of significance).

4.2.2 Dynamic linear validation

No significant differences were established between HU-M-AN™ and the standard ($P>0.05$). In all three release positions, Quintic© measurements were significantly shorter than the standard distance, with higher levels of significance for the longer distances from the release positions A and B ($P<0.001$), compared to the shorter distance from release position C ($P<0.05$).

Table 4.8: Comparison between variation in Quintic© and HU-M-AN™ for dynamic linear measurement (cm) of a pendulum released from three different positions. Values are in relation to the "gold standard" Oqus system.

Release Position	Mean difference		SE		Variance		%COV	
	Quintic©	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*
A	13.06	0.46	1.14	0.59	6.50	1.75	2.41	1.12
B	5.98	3.45	0.98	1.05	4.80	5.55	3.88	3.58
C	5.62	3.30	0.93	0.46	4.30	1.04	5.26	2.11

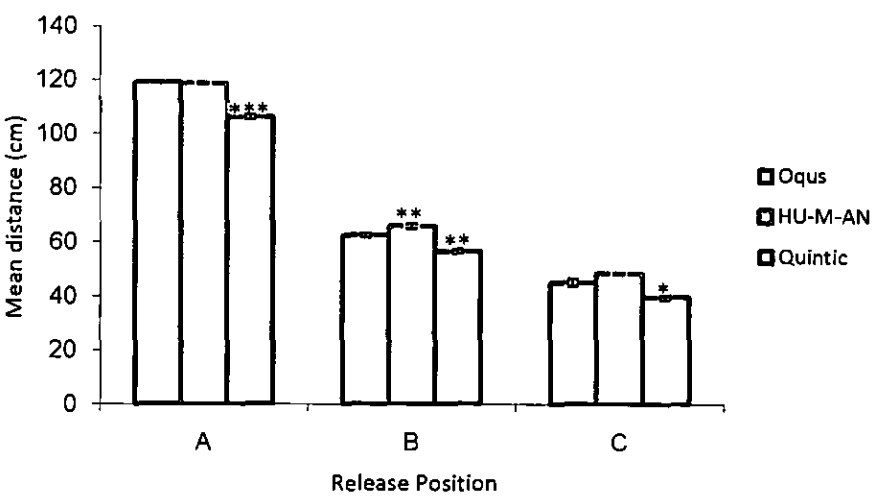


Figure 4.8: Mean distance travelled by the mass from one pendulum swing from three different release positions (A, B, C), comparing Quintic© and HU-M-AN™ to the "gold standard" Oqus system. (*P<0.05; **P<0.005; ***P<0.001 denotes a significant difference from the standard and level of significance).

4.2.3 Velocity

No significant differences were established between HU-M-AN™ and the standard for measurement of velocity. Small variations were demonstrated between Quintic© measurements and the standard (table 4.9, figure 4.9), however these variations were only significant when the pendulum was released from position B, When the pendulum was released from position B, it was measured by Quintic© as being significantly faster than the standard and HU-M-AN™ ($P<0.05$).

Table 4.9: Comparison between variation in Quintic© and HU-M-AN™ for velocity (cm/s) of a pendulum released from three different positions. Values are in relation to the "gold standard" Oqus system.

Release Position	Mean difference		SE		Variance		%COV	
	Quintic©	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*	*Quintic©*	*HU-M-AN™*
A	22.84	0.81	6.94	7.72	240.76	298.24	8.38	8.34
B	6.11	3.68	4.19	4.27	87.68	91.21	15.78	13.81
C	7.75	4.96	2.24	2.28	25.10	26.00	8.92	7.40

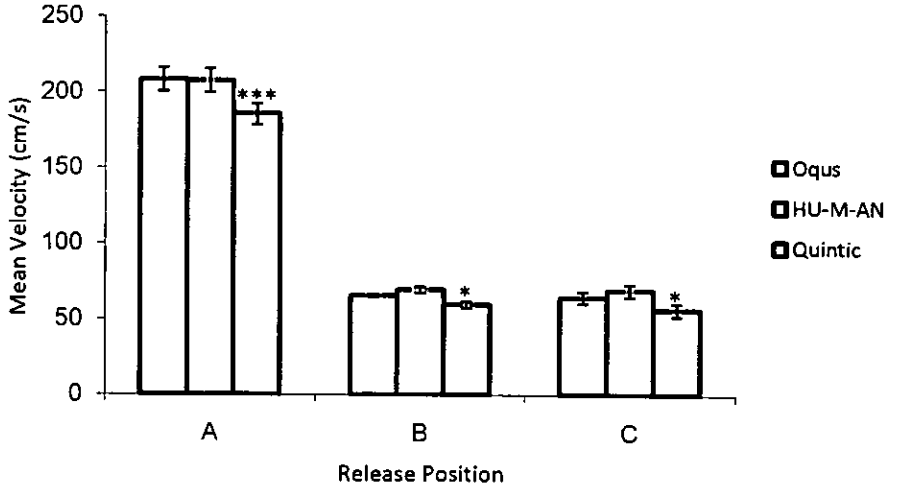


Figure 4.9: Mean velocity of the mass from one pendulum swing from three different release positions (A, B, C), comparing Quintic© and HU-M-AN™ to the "gold standard" Oqus system. (*$P<0.05$; **$P<0.005$; ***$P<0.001$ denotes a significant difference from the standard and level of significance).

4.3 Summary

The main findings for this chapter are summarised in the table below. Table 4.10 illustrates the range in margin of error for static and dynamic validation of the two software programmes.

Table 4.10: Range in margin of error values for static and dynamic validation of Quintic© and HU-M-AN™.

Type of validation	Standard measurement	Error range	
		Quintic©	*HU-M-AN™*
Static Linear	Inter-marker distance values ranging from 10-100cm (three orientations)	0.00mm-20.00mm	0.00mm-7.00mm
Static Angular	Goniometer values ranging from 10-360° (two orientations)	0.07°-3.36°	0.006°-0.88°
Dynamic Linear	Pendulum swing (distance travelled by mass)	5.62-13.06cm	0.46-3.45cm
Dynamic Angular	Pendulum swing (change in angle between mass and vertical)	4.19°-5.56°	0.66°-1.56°
Velocity	Pendulum swing (speed travelled by mass)	6.11-22.84cm/s	0.81-4.96cm/s

5.0 Discussion

Static linear and angular accuracy is fundamental when analysing equine conformation; the assessment of linear conformation traits involves the measurement of static lengths (measuring the distance between two points on limb segments), and angles of joints (measuring the angle between three points on the horse). Dynamic accuracy is vital when measuring gait characteristics; dynamic linear accuracy is applicable when measuring stride length. Dynamic angular accuracy is important when measuring range of motion of the equine joints throughout a stride cycle. It is also important to validate how accurately velocity is measured due to the importance of regulating velocity when measuring stride characteristics. The reproducibility of measurements obtained using Quintic© and HU-M-AN™ of linear and angular values (dynamic and static) and velocity established small variations between repeats, however repeat had no effect on the measurement or difference between the true and measured values ($P>0.05$). This means both software packages were consistent at performing repeated measures of the same value. Consistency is an important part of determining how accurate and appropriate the software is for equine biomechanics research; repeated measures have to be taken to obtain baseline measurements due to the intrinsic variability in equine gait (Clayton and Schamhardt, 2001). Static linear validation established higher variation between repeats using HU-M-AN™, %COV values ranged from between 0.01-3.80%, whereas the majority of %COV values for Quintic© were 0%. The same video clips were analysed with both software packages, therefore experimental set-up was discounted as a source of error in the discrepancy between the two software packages. Digitiser error may be a causal factor, due to the manual input required for digitising each video clip. Digitiser error affects the accuracy and reliability (repeatability) of the data obtained (Wilson *et al.*, 1999). Digitiser error often occurs when manually aligning the crosshairs over the marker in question; the cross hairs must be accurately positioned to gain accurate results. Surprisingly, when comparing manual and automatic digitisation, Wilson *et al.* (1999) demonstrated manually digitised clips were more accurate than automatically digitised clips, with a mean error of 0.153° for the manually digitised clips, compared to 0.223° for the automatically digitised clips, when comparing dynamic angular values (Wilson *et al.*, 1999). The authors suggest this could be due to distortions in the spherical markers. The software will recognise the pixels comprising the marker and automatically calculate the centre point. This automatic identification will be limited if distortions occur (if the camera or marker is rotated out of plane), or due to varying light conditions making identification of the pixels

inaccurate. Manual digitisers can identify the centre of the marker regardless of uneven lighting (although distortions may still be a cause of error). This source of error was also noted in research by Scholtz (1989). Conversely, automatically digitised clips produced more reliable results; errors that did occur were more consistent compared to manually digitised clips. The authors suggest this could be due to a systematic fault with the software (although this was not tested), however it could also be due to inherent inconsistencies when manual input is required for a repetitive task. This could also explain the differences in the coefficient of variation between repeats when analysing dynamic accuracy and static angular accuracy in the present study; differences were much greater for the dynamic results compared to the static ones. It is possible the effect of digitiser error was amplified due to the movement of the markers; as the camera recorded at a relatively low frame rate (50 Hz) the markers were not as clear as they would have been using a high speed camera. Quintic© measurements were significantly smaller than the standard for all types of dynamic validation ($P<0.001$). HU-M-AN™ results were smaller but not significantly, (apart from dynamic length from release position B) where HU-M-AN™ measurements were significantly longer than the standard ($P<0.05$). The manual digitisation of clips requires the researcher to pinpoint the start of movement of the pendulum (compared to Oqus where the start of motion is automatically detected). It is feasible that the researcher could not detect the initial start of movement as it was too subtle, therefore less frames were digitised leading to smaller measurements.

Analysis of the %COV for Quintic© when measuring static angles, revealed the lowest values correspond closely to quadrant angles (90°, 180°, 270°, 360°). When the true angle approached values equivalent to quadrant angles, the angles were measured more accurately; as angles deviated away from quadrant angles the angles were measured less accurately; up to 3.36° for the true angle of 60° . It is possible that due to the manual input required for digitisation, angles that are easily identifiable (quadrant angles) are more accurately measured. This is only the case for Quintic© however, as %COV values for angular validation in HU-M-AN™ do not follow the same trend. Manual input required for motion analysis is a limitation of the method. This is one possible cause of the difference in variation in dynamic accuracy between the standard (Oqus) and tested softwares. Oqus automatically digitises, which as previously discussed produces more consistent results compared to manual digitisation. Variation was less for linear, angular and velocity measurements using Oqus compared to the other two software

programmes. Manual input error was reduced for the present study as just one researcher digitised all video clips.

Using mean difference as an indicator of static linear accuracy, it was established that both software packages were within an acceptable margin of error for use in equine biomechanics research (<10mm) for linear measurements, and orientation had no effect on accuracy (P>0.05). In clinical research an error of <3.5mm is considered acceptable (Klein and De Haven, 1995). Dynamic linear validation established much larger differences for both programmes, although there was only a significant difference between Quintic© and Oqus (P<0.001). It can be stated that HU-M-AN™ is significantly more accurate than Quintic© in static and dynamic linear measurements; when analysing the difference between true and measured distances there was a significant difference between the two software packages (P<0.001). Static linear validation of Quintic© established a mean margin of error of between 0mm and 20mm compared to HU-M-AN™, where mean margin of error ranged from 0mm to 7mm. Errors that occurred were both positive and negative (the systems over estimated and under estimated lengths on different occasions). The overall mean errors for each software were 6mm for Quintic©, and 3mm for HU-M-AN™. These errors are comparable to other software programmes, where reported errors range from 0.53mm for the Elite Plus system, to 11.61mm for the Ariel Performance Analysis System (Chiari *et al.*, 2005). The majority of systems had a reported mean margin of error of between 2 and 8mm (within the range of error for HU-M-AN™ but not Quintic©). The maximum errors reported on these systems were +28.23mm (Video Locus); -26.3mm (Ariel) and +24.07mm (Ariel Performance Analysis System). The maximum errors reported for these systems are greater than the maximum errors reported for Quintic© and HU-M-AN™. True length had a significant influence on accuracy for both software packages (P<0.001); margin of error was dependent on the length being measured as some length measurements were more accurate than others. Lengths that demonstrated the most error were between 50cm and 90cm for both software packages. Both packages were more accurate at measuring lengths of 10cm and 100cm, rather than mid-range lengths. One difficulty is the accuracy to which Quintic© measures linear distances. Quintic© only allows linear distances to be measured to one decimal place (compared to HU-M-AN™ where distances can be measured to three decimal places). This means that a difference of less than 10mm with Quintic© would be measured as zero, whereas with HU-M-AN™, this difference could be between 0.000

and 9.999mm. This has led to more true lengths having an error of zero when measured with Quintic©, compared to HU-M-AN™, making direct comparisons between the two software programmes difficult. The range in margin of error for dynamic validation was larger than previously reported values. Degueurce *et al.* (1996) reported a mean margin of error of <5mm for the dynamic validation of a 3D motion analysis programme 3D Vision); Linden *et al.* (1992) reported a mean margin of error of 4.4mm for the Motion Analysis programme. 3D motion analysis is fundamentally more accurate than 2D, therefore it is hard to make direct comparisons between the two. Similarly for dynamic angular validation, a greater margin of error was established for Quintic© compared to HU-M-AN™; HU-M-AN™ error values were comparable to previously validated dynamic angular accuracy of Ariel Performance Analysis System (Wilson *et al.*, 1997) that had a mean error of 0.18°. Quintic© margin of error was much higher.

Static angular validation established more variation in the absolute difference values in Quintic©, compared to HU-M-AN™, there was a significant difference between the two software packages when analysing the absolute differences ($P<0.001$). The mean difference with Quintic© varied depending on the size of the angle being measured, true angle was a significant factor when analysing absolute differences with Quintic© ($P<0.001$), but not HU-M-AN™ ($P>0.05$), there was also an interaction between true angle and orientation using Quintic©, but not HU-M-AN™. Quintic© was more accurate at measuring some angles compared to others; and this variation depended on the orientation of the goniometer. The error values calculated for HU-M-AN™ were consistent with previous angular validation of motion analysis systems. Error values of between 0.03° (Klein and De Haven, 1995) and 0.5° (Scholtz, 1989; Linden *et al.*, 1992) were established, although these systems are 3-D rather than 2-D. The error values calculated for Quintic© were much greater than the majority of previous validation studies, except Linden *et al.* (1992) when measuring 180°. Angles <180° were measured with a error of 0.5°, however the error for 180° angles was much greater, and similar to the Quintic© error value (between 1.5° and 2.4°). Linden *et al.* (1992) suggested this was due to the cosine algorithm used to calculate angles, and the software is more inaccurate when calculating 180° angles as the cosine approaches one and the opposite side becomes very small. There is however a problem with this theory as the cosine of 180° is actually minus one, and the opposite side of an 180° angle is very large (not small). This would make more sense as a theory of why Quintic© has varying accuracy; as Quintic© is actually more accurate when measuring quadrant angles (including 180°),

not less accurate (as Motion Analysis™ was). There is however another problem with the error values for Quintic©-they follow a specific pattern.

In addition, the residuals, when plotted against the true angular values follow the pattern of a sine wave. This could be due to two factors; cosine algorithms and aspect ratio. Motion analysis software uses mathematical algorithms to calculate angles, the cosine rule is often used as an algorithm (Linden *et al.*, 1992). The cosine rule is used as it allows angles within a triangle to be calculated providing the lengths of the sides are known. There are no details available specifying what algorithms Quintic© software uses, therefore it can only be suggested that the cosine rule is being used (due to the pattern of the residuals). Angular measurement using motion analysis software requires three markers (one at each corner of the triangle), the software will measure the lengths of each side of the triangle and use the cosine algorithm to calculate the required angle. It is possible that the software has a fault in the algorithm that it uses to calculate angles, leading to a variation in accuracy when angles of different sizes are measured. Previous research that has validated static angular accuracy has suggested the cosine algorithm to be a cause of inaccuracies (Linden *et al.*, 1992). This research however noted angles became less accurately measured at 180° (whereas the current study noted better accuracy for this angle).

Aspect ratio of the camera used to record the clips could also be contributing to the angular inaccuracies. Aspect ratio is the image width divided by the height, most normal digital video cameras record at an aspect ratio of 4:3. The video camera in this study recorded at an aspect ratio of 4:3, therefore the image was 1.3 times wider than it was high (in other words the image was stretched by 1.3). When calibrating the video clips with HU-M-AN™, aspect ratio is taken into account, however there is no option using Quintic© to calibrate for the aspect ratio of the camera being used. It appeared to be less of an issue when measuring linear lengths (lengths are measured only along one axis; x or y). When calculating angles, lengths are measured along three sides, therefore any distortions that may occur due to aspect ratio will be enhanced. This could be the reason why there was a significant interaction between true angle and orientation when analysing Quintic© results. An image recorded horizontally would be more "stretched" than an image recorded vertically, due to the aspect ratio. Higher variation was established in the horizontal compared to the vertical plane when analysing Quintic© results. The aspect ratio can be accounted for by calibrating the video clips in the x and y plane. If aspect ratio cannot be accounted for, or there is a fault with the method of

calibration, the number of pixels comprising the length in either the x or y orientation will be incorrect with respect to the 4:3 image size. The inaccuracies of angular measurements in Quintic© can be reduced by minimising the sine effect of the residuals. A correction formula has been developed (using the sine of the measured angle) and a correction factor (calculated from the mean differences). The correction formula is;

$$corrected\ angle = \theta + ((\sin\theta 2) 2.21))$$

This correction formula was applied to all the angular data obtained from Quintic© and the results re-analysed (figure 5.0 and 5.1).

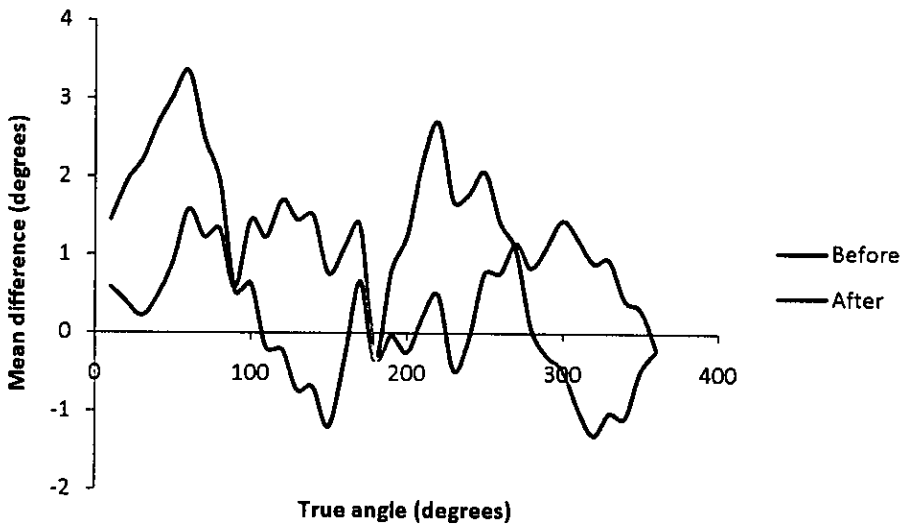


Figure 5.0: Quintic© angular measurements before and after sine correction was applied (horizontal).

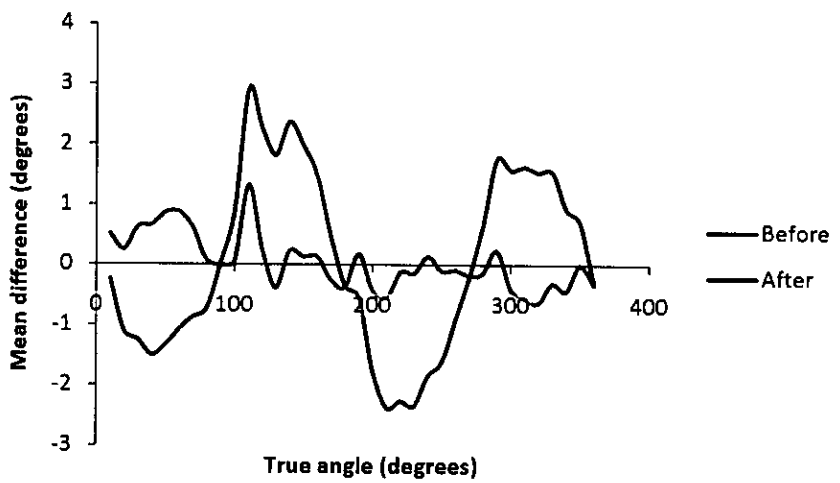


Figure 5.1: Quintic© angular measurements before and after sine correction was applied (vertical).

Table 5.0: Validation of sine corrected data for angular validation of Quintic©. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Measured value	Absolute difference
True angle	***	***
Orientation	***	***
True angle and orientation interaction	***	***
Repeat	NS	NS
Software	NS	**

Figures 5.0 and 5.1 demonstrate the sine correction formula is more effective for angles measured in the vertical plane. When analysing the corrected results (table 5.0), true angle size was still a significant factor (Quintic© measures angles of difference sizes more accurately than others), however orientation became a significant factor for the absolute difference whereas previously it was not (table 4.6 in chapter four). This is probably because due to the aspect ratio, there is less distortion vertically than horizontally. Software was still a significant factor when analysing the absolute difference, therefore HU-M-AN™ was still significantly more accurate than Quintic©.

It was decided from the results of this validation study that HU-M-AN™ is a more accurate and reliable software programme to be used for two-dimensional motion analysis research. Bearing in mind the equipment available (normal digital video camera recording at an aspect ratio of 4:3), HU-M-AN™ was used for analysing video clips for the further two parts of this study.

PART II

Standardisation of two dimensional motion analysis techniques to establish baseline data.

6.0 Aim

The aim of part II of this study was to standardise a method to obtain baseline conformation and gait parameters using two-dimensional motion analysis techniques, by establishing intra-horse variation (variation within individual horses over five consecutive days).

6.1 Objectives

i) Obtain measurements for static conformation for the left and right side of three horses for five consecutive days.

ii) Obtain measurements for stride length and range of motion for the left and right side of three horses for five consecutive days.

iii) Use statistical methods to determine intra-horse variation for the trial.

6.2 Hypothesis

i) There will be no intra-horse variation in conformation between sides

ii) There will be no intra-horse variation in conformation between days

iii) There will be no intra-horse variation in stride characteristics (stride length or range of motion) between sides.

iv) There will be no intra-horse variation in stride characteristics (stride length or range of motion) between days.

7.0 Subjects

The subjects consisted of three horses; two geldings and one mare of various breeds (Thoroughbred, Anglo-Arab, Cob). Mean age 10±3.46 years; mean height 154.43±1.02cm. The horses had a similar workload (medium work) and kept in the same management routine throughout the trial.

7.1 Instrumentation

Video clips were downloaded using Quintic© Biomechanics 9.03 v14 (splitting each frame into two fields) and digitised using HU-M-AN™ (2D) v6.0 (validated and determined accurate in part I). In addition to this, hardware used included a Sony DV-tape digital video camera (HDR-FX1000) recording at 50 hertz (25 frames per second), and a Fujitsu Siemens laptop for downloading the video clips. A wooden cube (50cmx50cm) was used to provide a reference length and aspect ratio for calibrating the video clips.

7.2 Anatomical markers

7.2.1 Dynamic and static marker sets

The majority of two dimensional motion analysis techniques use anatomical markers to measure joint ROM, usually placed at the centre of rotation of the joint to be measured (Clayton and Schamhardt, 2001). Anatomical markers used to assess conformation are often placed on the distal and proximal ends of limb segments (Holmstrom *et al.*, 1990) as lengths of limb segments as well as angles of joints are being measured. Providing the marker position is known with respect to joint angles, and in a well known position on the horse, the exact marker site has no influence on the accuracy and value of final kinematic data (Schamhardt *et al.*, 1993). One marker set was adapted from Holmstrom *et al.*, (1990) and Degueurce *et al.*, (1997) to allow static conformation and dynamic ROM to be measured. The location of the anatomical markers can be seen in figure 4.0 and table 4.0. A marker was also located at the approximate centre of mass, to determine velocity for each horse (adapted from Buchner *et al.*, 2000). Intra-horse ROM varies depending on which joints are being measured (Degueurce *et al.*, 1997); therefore both proximal and distal limb joints were investigated. In total, eight joints were analysed for ROM; four proximal joints of the fore and hind limb (scapulohumeral, humeroradial, coxofemoral, femorotibial) and four distal joints of the fore and hind limb (carpal, metacarpophalangeal , tarsal, metatarsophalangeal). The marker set identified

limb segments, rather than lengths of bones. This enabled one marker set to be used for both static and dynamic measurements. This is an essential part of ensuring the standardised method will be quick and easy to use by breed societies choosing to adopt this method of assessment.

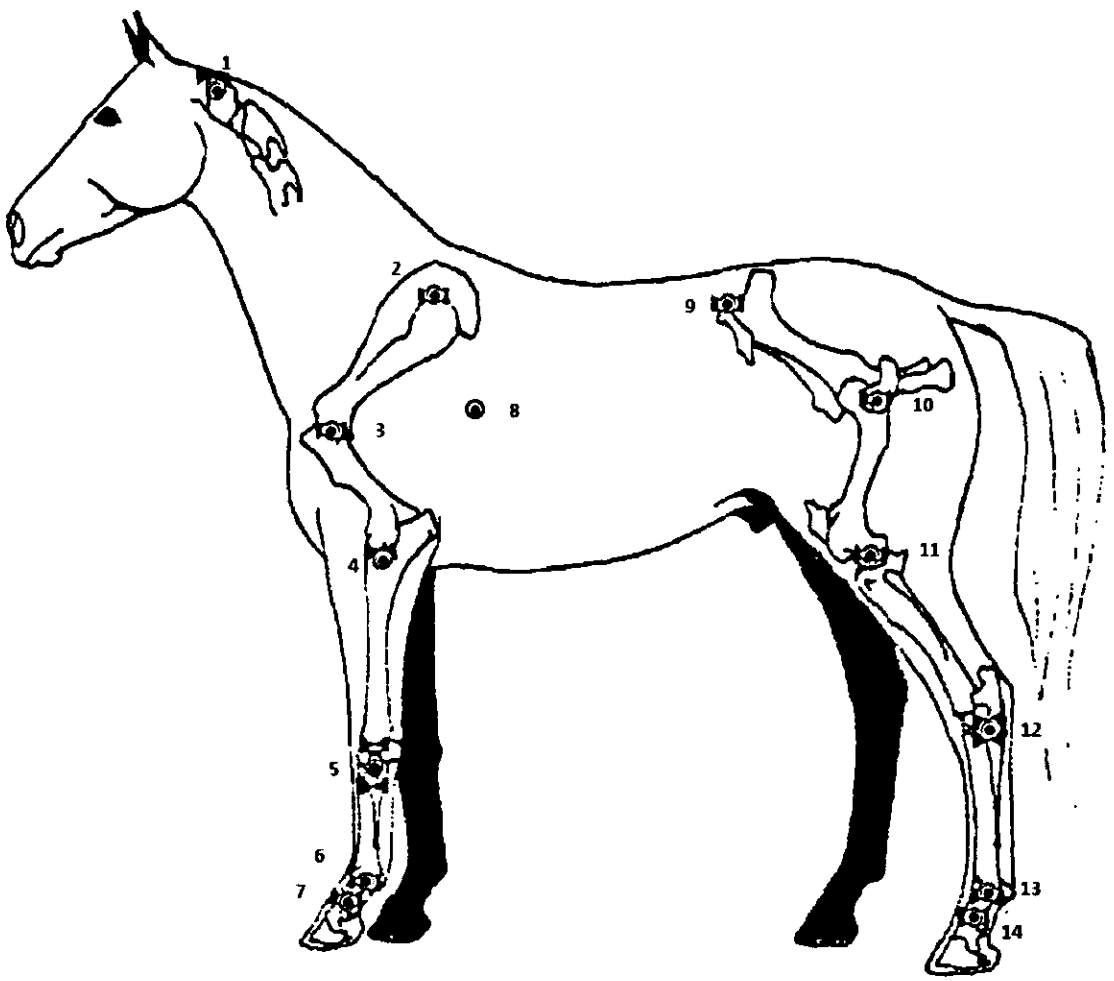


Figure 7.0: Location of anatomical markers (adapted from Holmstrom *et al.*, 1990, Degueurce *et al.*, 1997) to measure static conformation and ROM of the scapulohumeral (3), humeroradial (4), carpal (5), metacarpophalangeal (6), coxofemoral (10), femorotibial (11), tarsal (12) and metatarsophalangeal (13) joints. A marker is also located at the approximate centre of mass (8) to calculate velocity (Buchner *et al.*, 2000).

The anatomical markers used in this study were 50mm orange circular markers, with a 20mm black circular marker in the centre. The 50mm marker was solely used to provide a contrast for the 20mm black markers (it was the black markers that were digitised). The circular markers were self-adhesive and skin patch tests were performed on all subjects prior to data collection.

Table 7.0: Location of the anatomical markers in conjunction with figure 4.0, adapted from Holmstrom *et al.* (1990), Degueurce *et al.* (1997).

Forelimb	Hind limb
Cranial end of wing of atlas (1)	Approximate centre of mass (8)
Proximal end of the spine of the scapula (2)	Anterior part of the tuber coxae (9)
Caudal part of humeral head (3)	Greater trochanter (10)
Proximal part of shaft of radius (4)	Lateral epicondyle of the femur (11)
Centre of third and fourth metacarpal bones (5)	Lateral side of the talus (12)
Distal end of third metacarpal bone (6)	Distal end of the third metatarsal bone (13)
Distal end of the proximal phalanx (7)	Distal end of the proximal phalanx (14)

7.3 Filming procedure

Filming took place in an outdoor arena (the surface consisted of sand and rubber fibre). The equipment was set up prior to horses entering the arena (see figure 7.2 for experimental set-up). Prior to any horses being filmed, a calibration video was filmed in order to calibrate the videos after downloading. This involved filming a calibration cube (50x50cm) that was positioned in the field of view (same position the horses would be filmed), and in the centre of the video camera's optical axis. The calibration cube served three purposes; to ensure the equipment set up was perpendicular to the video camera; as a measurement of scale for subsequent video analysis; as a measurement of aspect ratio for subsequent video analysis. The calibration cube was recorded for three seconds, then removed from the field of view before filming of the horses began. Horses were led passed the equipment for ten minutes before recording took place. This habituated horses to the video analysis equipment as well as warming the horses up before recording. Filming took place at the same time each day (7-9am) and horses were filmed in the same order each day.

7.3.1 Conformation assessment

Conformation was measured using a quantitative method adapted from Holmstrom *et al.* (1990), based on the methods developed by Magnusson (1985). These authors used measurements obtained from static photographs to measure traits (with direct measurements for reference lengths). This method was developed to use a digital video camera to record the horses, and two-dimensional motion analysis software to measure the traits. A calibration cube was used for reference lengths. Horses were stood square (equally weight bearing on all four limbs) in the calibrated field and held by a handler. A rectangle (2x6m) was marked out in the arena surface (see figure 7.2) to ensure the

horse was perpendicular to the video camera, and stood square. Each horse was recorded for three seconds on the left and right side. This process was repeated on each day of the trial, giving a total of five repeats for each side. The linear and angular traits measured can be seen in figure 6.1.

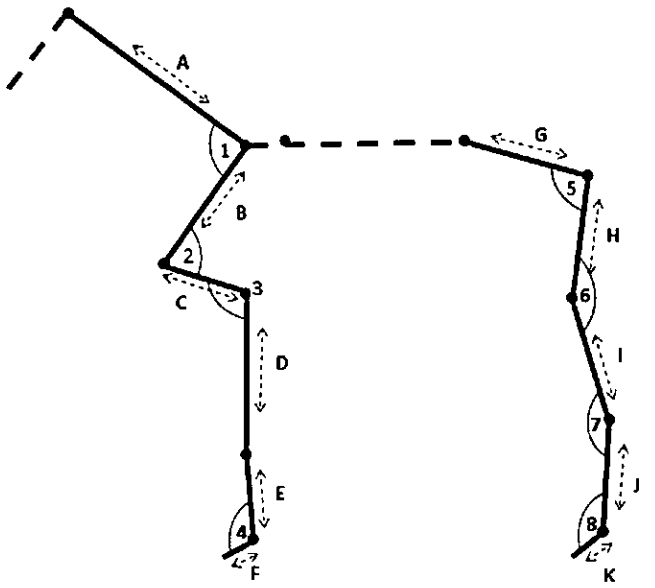


Figure 7.1: How static conformation traits were measured (horse stood square and weight bearing) and position of anatomical markers (adapted from Holmstrom *et al.*, 1990 and Degueurce *et al.* 1997). Linear traits measured; (A) neck; (B) scapula; (C) humerus; (D) radius; (E) metacarpal; (F) fore proximal phalanx; (G) ilium; (H) femur; (I) tibia; (J) metatarsal; (K) hind proximal phalanx. Angular traits measured; (1) neck; (2) scapulohumeral; (3) humeroradial; (4) metacarpophalangeal; (5) coxofemoral; (6) femorotibial; (7) tarsal; (8) metatarsophalangeal joints.

7.3.2 Stride length and range of motion assessment

Stride length and ROM were assessed in trot only. Horses were led in hand (by the same handler to maintain consistency) passed the video camera through the calibrated field of view, at a distance of 7m from the video camera (see figure 7.2) in order to capture one full stride (one full stride cycle was determined as mid-stance to mid-stance of the near fore limb). A ground pole was placed at the back of the calibrated field of view to assist the handler in leading the horse in a straight line.

Repeated measures were taken for each horse; ten repeats for each side (left and right) over five consecutive days giving 20 repeats for each horse for each day (a total of 100 repeats were recorded for each horse for the whole five day trial). Taking repeated measures to obtain baseline measures is a standard procedure in equine biomechanics

research, however number of repeats suggested as suitable varies. Figures of between three repeats (Drevemo *et al.*, 1980a) and 12 repeats (Clayton *et al.*, 2002) have been reported. Subjects in this investigation were recorded over five consecutive days, therefore it was decided ten repeats (on each side) was a sufficient number.

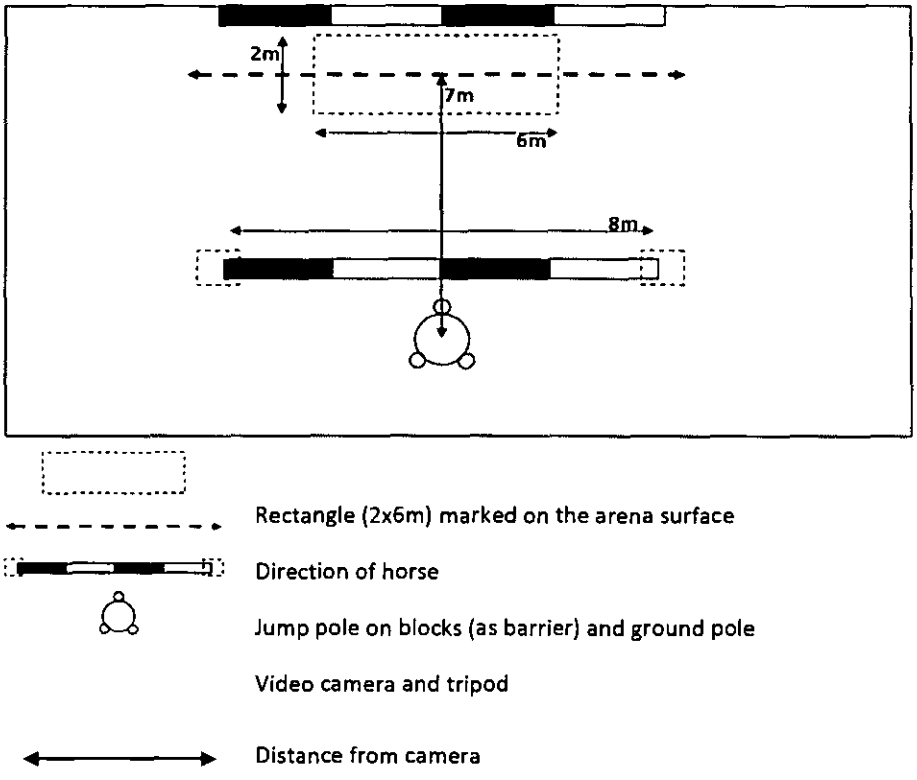


Figure 7.2: Set up of filming equipment in the arena.

Horses were recorded in the same order each day (horse 1-3), however left and right side could not be recorded simultaneously. This is because only one camera was available that recorded at the required specification (50 hertz). Order in which the left and right sides were recorded was alternated for each day. This was to minimise the effect of repeat number on stride length or ROM.

7.4 Analysis of videos

Video clips were downloaded using Quintic© Biomechanics 9.03 v14. The videos were downloaded (using a Belkin firewire 2.0 Expresscard™) and compressed using a Microsoft MPEG-4 video codec (version 2). The video file compression splits each frame into two fields (vertical and horizontal) and converts the file into an avi format. Files were then imported into HU-M-AN™ (2D) v6.0 for analysis.

7.4.1 Scale and aspect ratio

Prior to digitisation video clips were calibrated for scale and aspect ratio. To calibrate the scale, vertical and horizontal lines were drawn on the calibration cube and the true length entered (50cm). The software calculates the scaling factor and this figure was recorded. To calibrate aspect ratio, top left, top right, bottom left and bottom right corners of the cube were digitised (in order), and the aspect ratio recorded. The figures for scale and aspect ratio were entered for each video clip analysed in order to calibrate.

7.4.2 Digitisation

A trial subsequence for each clip was created (this included only the frames that were being analysed). One full stride was determined from mid-stance to mid-stance (when the limb was vertical and in contact with the ground), therefore frames before or after the required frames were discarded. A trial was created to connect anatomical markers, and define the angles being measured. This involved linking the required points and defining from which points the ROM would be calculated. Manual digitisation of the clips involved linking the markers for each frame (one full stride length consisted of 30-40 frames). Fore and hind limb were digitised separately. Before SL and ROM could be calculated, data smoothing was applied. To calculate linear velocity the centre of mass marker was digitised separately for one full stride length.

7.4.3 Data smoothing

The majority of data obtained from motion analysis is low frequency (Chiari *et al.*, 2005). This data is often smoothed to remove the high frequency noise associated with data collected using two dimensional motion analysis techniques (Howarth and Callaghan, 2009). HU-M-AN™ uses a low pass, second order Butterworth filter for data smoothing. This is an appropriate filter for analysis of low frequency data. The high frequency noise can be caused by impact (hoof strike) or distortions from soft tissue artefact. The amount of noise will vary depending on which joint is being analysed, therefore data for each joint was smoothed individually. Cut off frequencies for the majority of human movement data is between 4 and 8 Hertz (Bartlett, 2007). The cut-off frequency for this investigation was 10 Hertz due to the larger amplitude of movement in equine compared to human subjects. Data smoothing using this technique will remove any data above the cut off frequency (removing high frequency data) therefore leaving the low frequency data that represents the movement of the horse, rather than the distortions.

7.5 Statistical analysis

Descriptive statistics were calculated for the data for intra-horse variation and inter-horse variation. Descriptive statistics included mean difference (the difference between the true and measured value), standard error, variance (the distance of the data from the mean value) and the coefficient of variation (the standard deviation as a percentage of the mean, which allows data sets of different values to be compared). All data were tested for normality with an Anderson-Darling normality test. If data were established as non-parametric, skew was determined with a histogram. If data were skewed, data were transformed. For positively skewed data, the transformations $\sqrt{x}$, log x, $^1/_x$ were used. For negatively skewed data the transformations x^2, x^3 and antilog x were used. Data were then re-tested for normality.

Intra-horse and inter-horse variation and intra-group variation were established for each subject and all subjects for conformation, stride length and ROM. Parametric data was tested using a GLM with day, repeat and side as covariates. Non parametric data was tested with a Kruskal-Wallis. To determine where differences occurred, a post-hoc Tukey comparison was used.

Correlations were performed between stride length and ROM data with velocity to determine if a significant relationship existed. Parametric data were tested with a Pearson correlation, non parametric data were tested with a Spearman correlation (on ranked data). Regression analysis was also performed to determine the strength of the relationship.

8.0 Intra-horse variation

8.1 Variation in conformation

All data were established as parametric, tables 8.0-8.2 show results for subjects one-three. For subject one (table 8.0), traits that measured linear distances (lengths of bones segments) had lower SE values compared to traits that measured angular values (joint angles). There was a large variation between SE values for all conformational traits for subject two (table 8.1), with no apparent pattern between linear or angular traits (unlike subject one). Similarly to subject two, there was no obvious trend to the results for linear and angular conformation traits for subject three (table 8.2).

Table 8.0: Intra-horse variation for conformation for subject one for the five day trial. Traits with the highest and lowest %COV values are highlighted in grey.

Trait	N	Mean	SE	SD	Variance	%COV
Neck (cm)	10	79.44	2.10	6.64	44.07	8.36
Scapula (cm)	10	49.95	1.08	3.42	11.69	6.84
Humerus (cm)	10	28.82	1.02	3.24	10.47	11.23
Radius (cm)	10	51.13	1.32	4.18	17.49	8.18
Metacarpal (cm)	10	28.99	0.82	2.59	6.70	8.93
Proximal phalanx (cm)	10	11.04	0.51	1.62	2.61	14.64
Ilium (cm)	10	32.60	0.41	1.28	1.64	3.93
Femur (cm)	10	40.17	0.74	2.35	5.50	5.84
Tibia (cm)	10	44.41	1.71	5.42	29.34	12.20
Metatarsal (cm)	10	38.21	0.41	1.31	1.71	3.42
Poximal phalanx (cm)	10	11.90	0.19	0.61	0.37	5.13
Neck (°)	10	67.56	1.02	3.23	10.42	4.78
Scapulohumeral (°)	10	91.71	0.50	1.59	2.53	1.74
Humeroradial (°)	10	124.60	0.91	2.88	8.28	2.31
Metacarpophalangeal (°)	10	139.93	0.94	2.98	8.90	2.13
Coxofemoral (°)	10	108.81	1.90	6.01	36.06	5.52
Femorotibial (°)	10	169.44	2.20	6.95	48.35	4.10
Tarsus (°)	10	168.96	1.45	4.59	21.05	2.72
Metatarsophalangeal (°)	10	145.66	1.37	4.32	18.70	2.97

Table 8.1: Intra-horse variation for conformation for subject two for the five day trial. Traits with the highest and lowest %COV values are highlighted in grey.

Trait	N	Mean	SE	SD	Variance	%COV
Neck (cm)	10	76.39	1.67	5.29	27.98	6.93
Scapula (cm)	10	47.84	0.56	1.76	3.11	3.69
Humerus (cm)	10	25.74	0.52	1.65	2.72	6.41
Radius (cm)	10	52.88	0.78	2.45	6.01	4.64
Metacarpal (cm)	10	28.84	0.42	1.32	1.74	4.58
Proximal phalanx (cm)	10	10.74	0.30	0.95	0.90	8.83
Ilium (cm)	10	38.34	0.61	1.93	3.71	5.03
Femur (cm)	10	37.58	0.53	1.66	2.77	4.43
Tibia (cm)	10	44.63	0.94	2.98	8.88	6.68
Metatarsal (cm)	10	41.11	0.36	1.14	1.29	2.77
Proximal phalanx (cm)	10	10.41	0.18	0.57	0.32	5.47
Neck (°)	10	63.15	1.15	3.62	13.13	5.74
Scapulohumeral (°)	10	86.13	1.77	5.58	31.18	6.48
Humeroradial (°)	10	119.31	1.01	3.20	10.24	2.68
Metacarpophalangeal (°)	10	154.23	1.07	3.39	11.50	2.20
Coxofemoral (°)	10	105.62	1.87	5.92	35.03	5.60
Femorotibial (°)	10	158.49	1.54	4.88	23.85	3.08
Tarsus (°)	10	164.76	0.93	2.94	8.61	1.78
Metatarsophalangeal (°)	10	150.09	0.84	2.64	6.97	1.76

Table 8.2: Intra-horse variation for conformation for subject three for the five day trial. Traits with the highest and lowest %COV values are highlighted in grey.

Trait	N	Mean	SE	SD	Variance	%COV
Neck (cm)	10	62.97	1.53	4.83	23.32	7.67
Scapula (cm)	10	48.51	0.75	2.37	5.60	4.88
Humerus (cm)	10	26.24	0.46	1.44	2.07	5.48
Radius (cm)	10	50.39	1.21	3.83	14.64	7.59
Metacarpal (cm)	8	27.46	0.72	2.04	4.17	7.44
Proximal phalanx (cm)	8	10.38	0.40	1.14	1.29	10.96
Ilium (cm)	10	31.00	1.08	3.41	11.64	11.01
Femur (cm)	10	42.49	0.61	1.92	3.68	4.52
Tibia (cm)	10	38.80	0.67	2.12	4.49	5.46
Metatarsal (cm)	8	37.35	0.53	1.49	2.23	4.00
Proximal phalanx (cm)	8	10.71	0.36	1.03	1.06	9.61
Neck (°)	10	76.97	1.92	6.07	36.82	7.88
Scapulohumeral (°)	10	81.85	1.28	4.06	16.49	4.96
Humeroradial (°)	10	121.85	2.27	7.18	51.55	5.89
Metacarpophalangeal (°)	8	141.80	0.90	2.54	6.47	1.79
Coxofemoral (°)	10	119.66	0.78	2.46	6.05	2.06
Femorotibial (°)	10	156.97	1.51	4.77	22.70	3.04
Tarsus (°)	8	164.56	0.46	1.30	1.69	0.79
Metatarsophalangeal (°)	8	142.50	0.74	2.09	4.37	1.47

8.2 Investigating the effect of day and side on conformation

8.2.1 Subject One

Two traits were established as significantly different between days. Figure 8.0 demonstrates length of neck decreased from day one to three, then remained at a constant length for the rest of the trial. Scapulohumeral angle increased from day one to two and decreased on day three. The angle increased again on day four, before decreasing on the final day. Length of neck varied significantly between left and right side, whereas scapulohumeral angle did not (table 8.3).

Table 8.3: Effect of day and side on conformation for subject one. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Trait	Day	Side
	P-Value	*P-Value*
Neck (cm)	***	**
Scapulohumeral (°)	*	NS

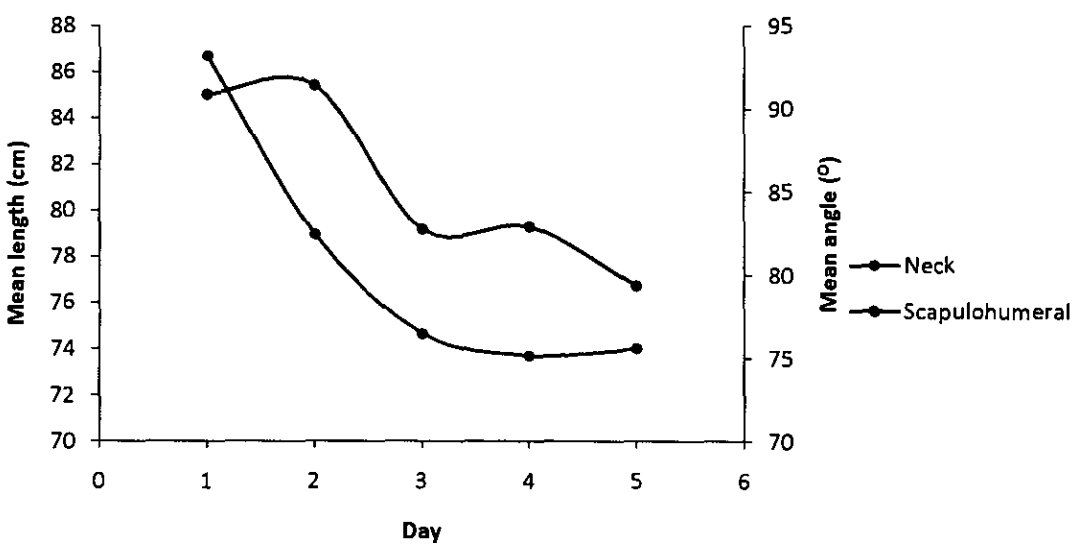


Figure 8.0: Variation in static conformation (neck length (cm) and scapulohumeral angle (°)) between days for the left side for subject one.

8.2.2 Subject Two

Six traits were established as significantly different between days for subject two all of which were linear traits (table 8.4). Figure 8.1 demonstrates that length of neck and scapula followed a similar pattern. Length did not vary between days one, two or three but decreased significantly on day four and five. Length of humerus and metacarpal followed the same pattern; with almost identical measurements. The length of these traits did not vary between days except for a decrease on day four. Figure 8.1 demonstrates small variation in length of radius between all days, however the only significant variation was the decrease in length on day four. Length of tibia did not vary between day one and two, but decreased significantly on day three. There was no variation between day three and five for this trait.

Table 8.4: Effect of day and side on conformation for subject two. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Trait	Day	Side
	P-Value	*P-Value*
Neck (cm)	*	NS
Scapula (cm)	*	NS
Humerus (cm)	*	**
Radius (cm)	*	*
Metacarpal (cm)	*	*
Tibia (cm)	*	NS
Tarsus (°)	NS	*

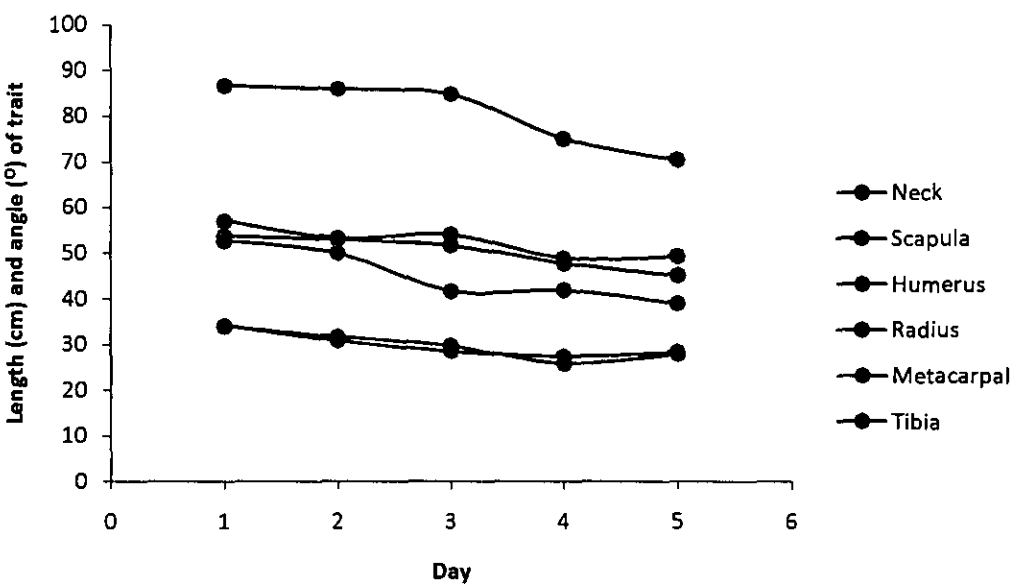


Figure 8.1: Variation in static conformation (length of neck, scapula, humerus, radius, metacarpal and tibia (cm)) between days for left side for subject two.

8.2.3 Subject Three

Angular traits were established as significantly different between days for subject three (table 8.5). Figure 8.2 demonstrates that tarsal angle decreased significantly on day three, but tarsus angle on day one and two were the same. Humeroradial angle did not change between day one and two, but increased significantly between day two and four. Humeroradial angle then decreased significantly on day five (although this angle was the same as humeroradial angle on day three). Scapulohumeral angle did not vary between day one to five consecutively, however days three and four scapulohumeral angle were significantly larger than day one.

Table 8.5: Effect of day and side on conformation for subject three. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Trait	Day	Side
	P-Value	*P-Value*
Femur (cm)	NS	*
Scapululohumeral (°)	*	NS
Humeroradial (°)	**	NS
Femorotibial (°)	NS	**
Tarsus (°)	*	NS

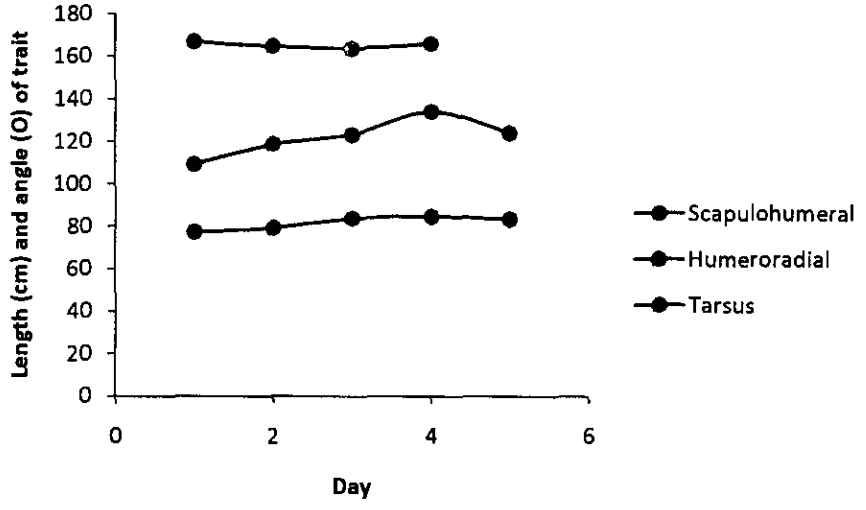


Figure 8.2: Variation in static conformation (scapulohumeral, humeroradial and tarsus angle (°)) between days for left side for subject three.

8.3 Variation in stride length and velocity over five consecutive days

Tables 8.6-8.8 illustrate intra-horse variation in stride length and velocity over five consecutive days. Data for stride length and velocity showed considerably higher variance and %COV than conformation data for all subjects. Similarly for all subjects, right side velocity had the highest SE value, and left forelimb stride length had the lowest.

Table 8.6: Intra-horse variation for stride length in trot and velocity for subject one for all days. Variables with the highest and lowest %COV are highlighted in grey.

Variable	N	Mean	SE	SD	Variance	%COV	Parametric
Left FSL	50	232.04	14.93	2.11	222.84	6.43	×
Right FSL	50	223.65	21.88	3.09	478.79	9.78	×
Log right FSL	50						✓
Left HSL	50	233.66	20.08	2.84	403.28	8.59	✓
Right HSL	50	223.01	28.34	4.01	803.14	12.71	×
Log right HSL	50						✓
Left velocity	50	318.60	29.71	4.20	882.42	9.32	×
Log left velocity	50						✓
Right velocity	50	314.88	51.21	7.24	2622.58	16.26	×
Log right velocity	50						✓

Table 8.7: Intra-horse variation for stride length in trot and velocity for subject two for all days. Variables with the highest and lowest %COV are highlighted in grey.

Variable	N	Mean	SE	SD	Variance	%COV	Parametric
Left FSL	50	236.99	21.95	3.10	481.99	9.26	×
Right FSL	48	220.44	23.97	3.46	574.72	10.88	×
Left HSL	50	229.05	24.64	3.48	606.91	10.76	✓
Right HSL	48	220.32	33.69	4.86	1135.18	15.29	×
Left velocity	50	324.08	38.34	5.42	1469.94	11.83	×
Right velocity	48	305.33	50.81	7.33	2581.15	16.64	×

Table 8.8: Intra-horse variation for stride length in trot and velocity for subject three for all days. Variables with the highest and lowest %COV are highlighted in grey.

Variable	N	Mean	SE	SD	Variance	%COV	Parametric
Left FSL	50	239.38	19.61	2.77	384.52	8.19	×
Log left FSL	50						✓
Right FSL	50	220.78	22.87	3.23	523.15	10.36	×
Log right FSL	50						✓
Left HSL	50	235.54	24.88	3.52	619.21	10.56	×
Right HSL	50	218.33	22.80	3.22	519.80	10.44	×
Left velocity	50	312.47	33.10	4.68	1095.42	10.59	×
Log left velocity	50						✓
Right velocity	50	288.60	39.04	5.52	1523.98	13.53	×

8.3.1 Subject One

Stride length and velocity were consistent between repeats on each day (table 8.9). Figure 8.3A demonstrates stride length decreased from day one to two ($P<0.001$); increased on day three ($P<0.001$), then decreased again on day four ($P<0.001$). There was no difference in stride length between days four and five, and stride length on these days was also similar to day two ($P>0.05$). When analysing the difference between left and right side, overall, left side stride length was significantly longer than right side stride length ($P<0.001$).

Table 8.9: Effect of day, repeat and side on SL and velocity for subject one. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Stride length	Velocity
Day	***	***
Repeat	NS	NS
Side	***	**
Day and repeat interaction	NS	NS
Day and side interaction	***	***

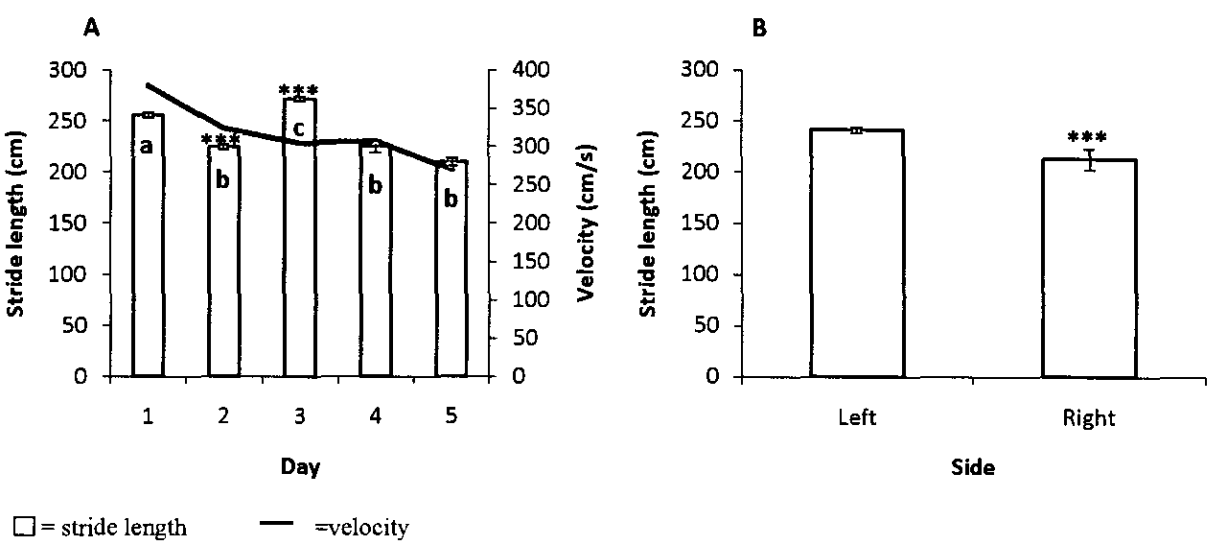


Figure 8.3: A) Mean stride length (±SE) and velocity over five consecutive days for subject one. Bars with the same letter illustrate no significant difference between days. Bars with different letters illustrate significant differences between days ($P<0.001$). B) Difference between left and right side stride length. Significant difference between sides indicated by *P<0.05; **P<0.005; ***P<0.001.

8.3.2 Subject Two

Similarly to subject one, stride length and velocity were consistent between repeats (table 8.10). Figure 8.4A demonstrates that stride length decreased significantly from day one to two ($P<0.001$) then remained at a similar length for the rest of the trial. There were small variations between days, but these were not significant. Side was a significant influence when analysing data for each day individually (table 8.10) and there was a small difference in left and right side overall, however this difference was not significant (figure 8.4B). This is probably due to large standard error (illustrated in figure 8.4B)

Table 8.10: Effect of day, repeat and side on SL and velocity for subject two. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Stride length	Velocity
Day	***	***
Repeat	NS	NS
Side	***	***
Day and repeat interaction	NS	**
Day and side interaction	***	***

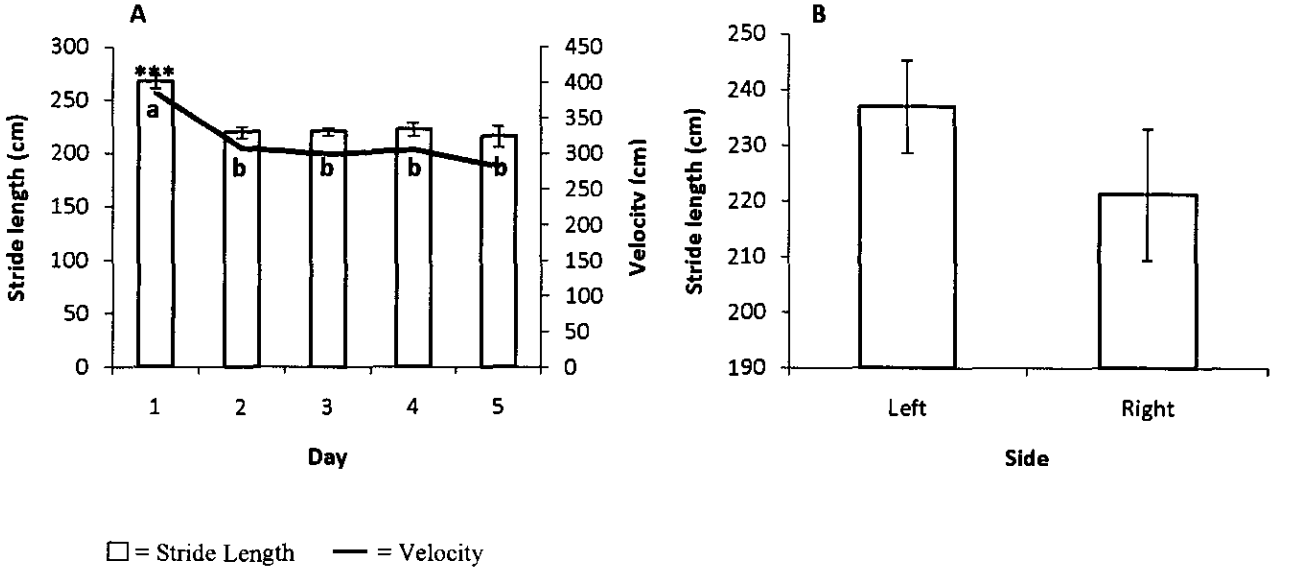


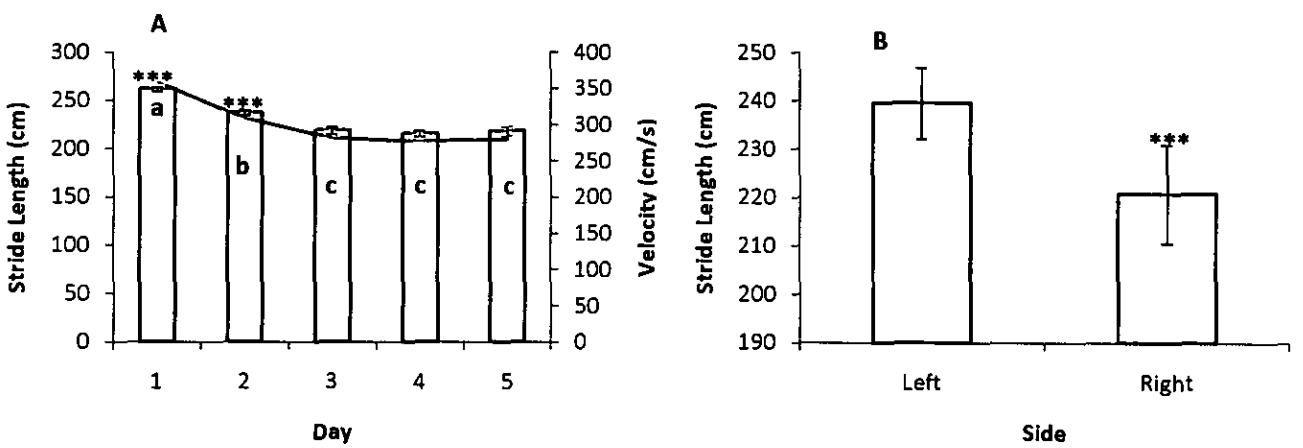
Figure 8.4: A) Mean stride length (±SE) and velocity over five consecutive days for subject two. Bars with the same letter illustrate no significant difference between days. Bars with different letters illustrate significant differences between days ($P<0.001$). B) Difference between left and right side stride length. Significant difference between sides indicated by *P<0.05; **P<0.005; ***P<0.001.

8.3.3. Subject Three

Stride length and velocity were consistent between repeats, as with the previous two subjects (table 8.11). Figure 8.5A demonstrates that stride length decreased from day one to two, and two to three ($P<0.001$), then remained at the same length for the rest of the trial ($P>0.05$). Overall, left side stride length was significantly longer than right side stride length, as illustrated in figure 8.5B.

Table 8.11: Effect of day, repeat and side on SL and velocity for subject three. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Stride length	Velocity
Day	***	***
Repeat	NS	NS
Side	***	***
Day and repeat interaction	NS	NS
Day and side interaction	NS	NS



□ = Stride Length — = Velocity

Figure 8.5: A) Mean stride length (±SE) and velocity over five consecutive days for subject three. Bars with the same letter illustrate no significant difference between days. Bars with different letters illustrate significant differences between days ($P<0.001$). B) Difference between left and right side stride length. Significant difference between sides indicated by *P<0.05; **P<0.005; ***P<0.001.

8.4 Correlation between stride length and velocity

Significant correlations were established for all subjects between stride length and velocity. Right side had the strongest correlations compared to left, and subject one had the greatest difference between left and right side correlations. This horse also had the highest and lowest R^2 values (52.7% for the left and 92.7% for the right).

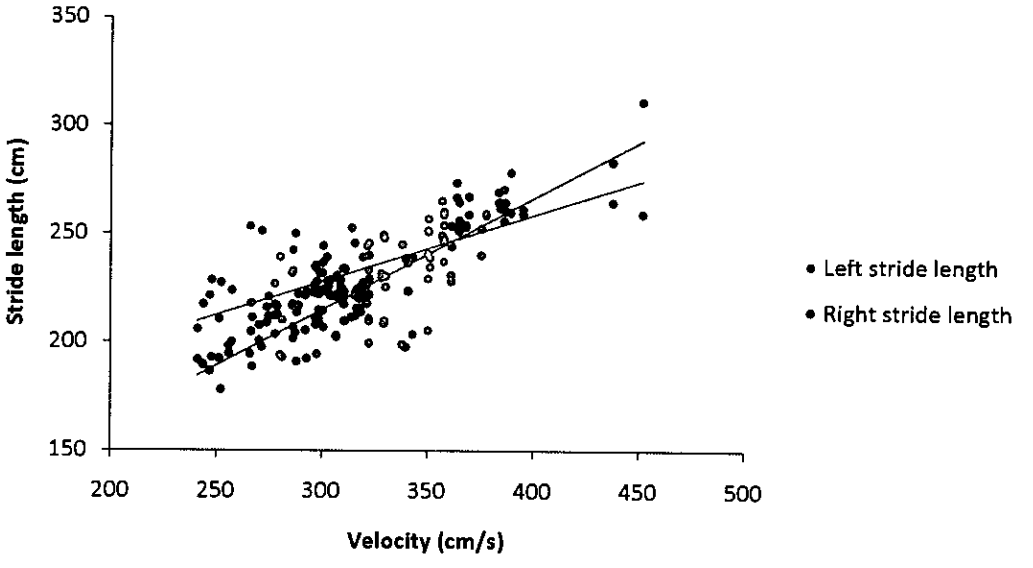


Figure 8.6: Regression analysis for stride length and velocity for left and right side for subject one (R^2=52.7%, 92.7%) showing a significant positive correlation (P<0.001).

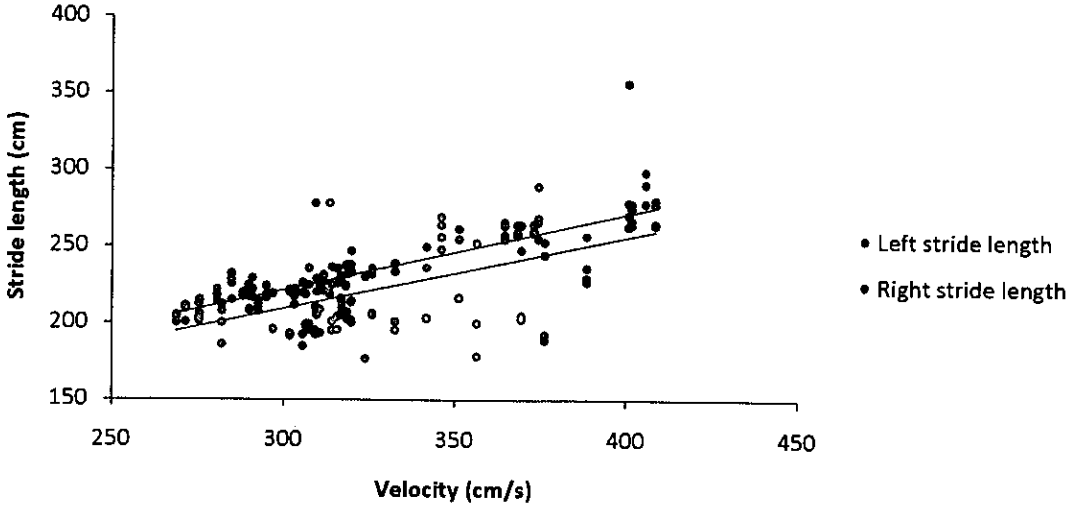


Figure 8.7: Regression analysis for stride length and velocity for left and right side for subject two (R^2=71.0%, 86.7%) showing a significant positive correlation (P<0.001).

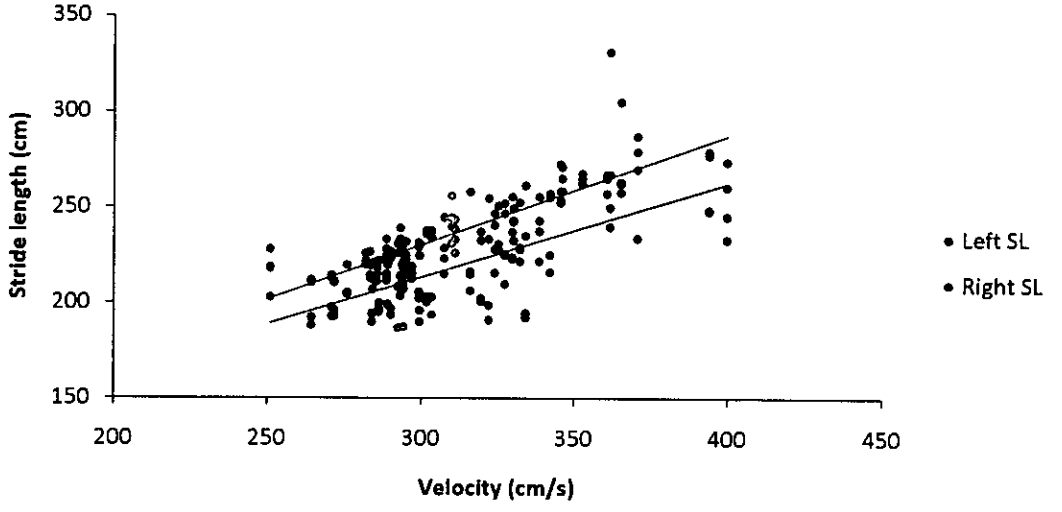


Figure 8.8: Regression analysis for stride length and velocity for left and right side for subject three (R^2=78.7%, 88.9%) showing a significant positive correlation (P<0.001).

8.5 Variation in range of motion over five consecutive days

Scapulohumeral ROM had the highest variation between days for all subjects. Metacarpophalangeal and carpus ROM had the lowest variation between days. Overall, more proximal joints (such as the scapulohumeral) had higher variation in ROM between days than distal joints (such as the metacarpophalangeal and carpus).

Table 8.12: Variation in ROM data for subject one for the five day trial. Joints with the highest and lowest %COV are highlighted in grey.

Joint	N	Mean	SE	SD	Variance	%COV	Parametric
Scapulohumeral	100	11.14	0.18	1.82	3.30	16.30	✓
Humeroradial	100	48.50	0.32	3.23	10.41	6.65	✓
Carpus	100	71.69	0.36	3.63	13.19	5.07	✓
Metacarpophalangeal	100	91.92	0.44	4.35	18.91	4.73	✓
Coxofemoral	100	24.94	0.29	2.87	8.23	11.50	✓
Femorotibial	100	46.48	0.28	2.81	7.89	6.04	✓
Tarsus	100	57.07	0.51	5.05	25.52	8.85	✓
Metatarsophalangeal	100	91.47	0.49	4.92	24.25	5.38	✓

Table 8.13: Variation in ROM data for subject two for the five day trial. Joints with the highest and lowest %COV are highlighted in grey.

Joint	N	Mean	SE	SD	Variance	%COV	Parametric
Scapulohumeral	98	15.36	0.32	3.21	10.28	20.87	✓
Humeroradial	98	52.86	0.42	4.12	16.96	7.79	✓
Carpus	98	79.22	0.57	5.68	32.29	7.17	✓
Metacarpophalangeal	98	105.12	0.79	7.84	61.42	7.46	✓
Coxofemoral	98	30.47	0.64	6.32	39.91	20.74	×
$^1/_x$	98						✓
Femorotibial	98	50.40	0.53	5.24	27.47	10.40	✓
Tarsus	98	54.21	0.36	3.60	12.96	6.64	✓
Metatarsophalangeal	98	93.09	0.50	4.97	24.68	5.34	×

Table 8.14: Variation in ROM data for subject three for the five day trial. Joints with the highest and lowest %COV are highlighted in grey.

Joint	N	Mean	SE	SD	Variance	%COV	Parametric
Scapulohumeral	100	18.04	0.47	4.75	22.55	26.33	×
Log x	100						✓
Humeroradial	100	57.58	0.46	4.56	20.76	7.91	✓
Carpus	80	86.57	0.59	5.24	27.50	6.06	✓
Metacarpophalangeal	80	109.23	0.85	7.64	58.40	7.00	✓
Coxofemoral	100	24.17	0.32	3.21	10.33	13.29	✓
Femorotibial	100	51.20	0.55	5.46	29.81	10.66	✓
Tarsus	80	57.93	0.59	5.24	27.41	9.04	✓
Metatarsophalangeal	80	97.59	1.65	14.80	219.23	15.17	✓

8.6 Effect of day, repeat and side on range of motion

8.6.1 Subject One

Table 8.15: Effect of day, repeat and side on joint ROM for subject one. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Scapulohumeral	Humeroradial	Carpus	Metacarpophalangeal	Coxofemoral	Femorotibial	Tarsus	Metatarsophalangeal
Day	NS	***	***	NS	NS	NS	**	NS
Repeat	NS	NS	NS	NS	NS	NS	NS	NS
Side	NS	NS	***	NS	NS	**	*	NS
Day and repeat interaction	NS	NS	NS	NS	NS	NS	NS	NS
Day and side interaction	*	*	**	NS	*	***	***	NS

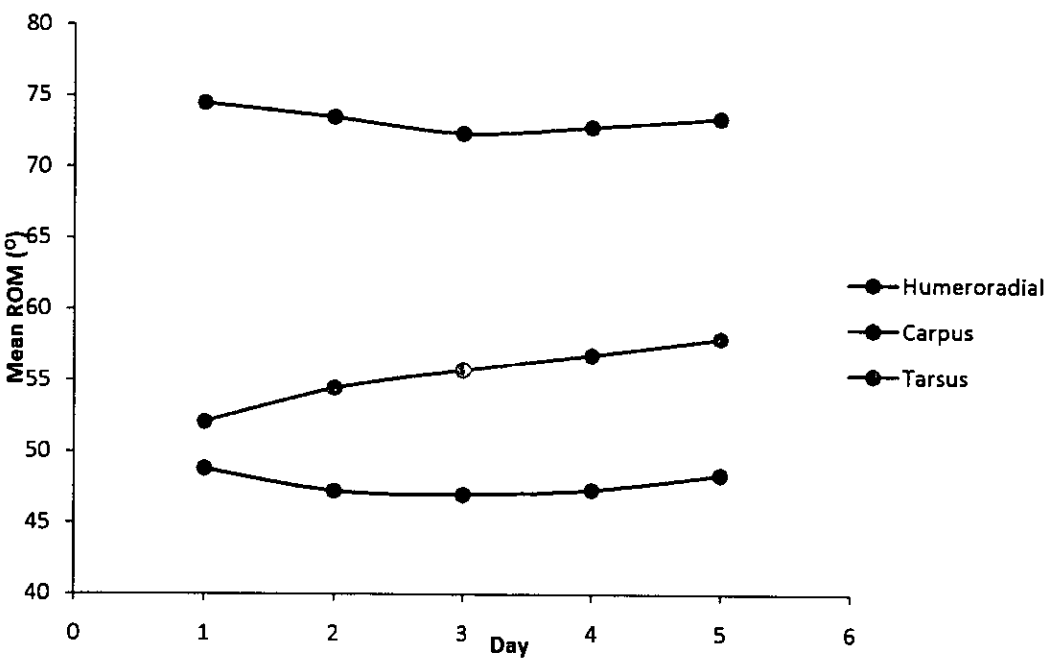


Figure 8.9: Mean ROM humeroradial, carpus and tarsus joints (left side) over five days for subject one.

ROM for each joint was consistent between repeats on each day (table 8.15); this pattern is consistent with data for SL and velocity (table 8.9). Figure 8.9 demonstrates that ROM for the scapulohumeral, metacarpophalangeal , coxofemoral, femorotibial and metatarsophalangeal did not vary significantly day to day ($P>0.05$). ROM for the humeroradial decreased from day one to two ($P<0.001$), did not vary between days two to four, then increased on day five ($P<0.001$). Carpal ROM did not vary between days one, two, four and five, however ROM decreased between day one and three, and increased between day three and four and again between four and five ($P<0.01$). Tarsal ROM did not vary between consecutive days, however tarsal angle on day five was significantly larger than tarsal angle on day one.

8.6.2 Subject Two

Table 8.16: Effect of day, repeat and side on joint ROM for subject two. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Scapulohumeral	Humeroradial	Carpus	Metacarpophalangeal	Coxofemoral	Femorotibial	Tarsus	Metatarsophalangeal
Day	***	NS	**	**	NS	***	NS	NS
Repeat	NS	NS	NS	NS	NS	NS	NS	NS
Side	**	NS	***	**	NS	NS	**	NS
Day and repeat interaction	NS	NS	NS	NS	NS	NS	NS	NS
Day and side interaction	NS	*	NS	***	NS	NS	NS	NS

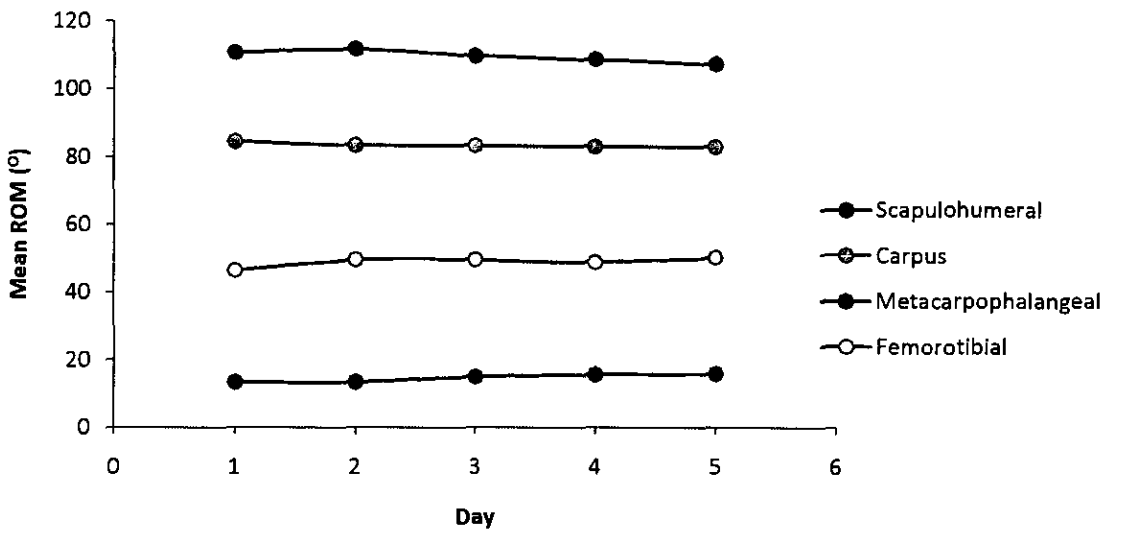


Figure 8.10: Mean ROM for scapulohumeral, carpus, metacarpophalangeal and femorotibial joints (left side) over five days for subject two.

All joint ROM was consistent between repeats (table 8.16). Figure 8.10 demonstrates ROM that changed significantly over the five day trial were for the scapulohumeral, carpus, metacarpophalangeal and femorotibial. All joints followed the same pattern of variation between days. There was no variation between day one and two, or three to five (all significant differences were established between day two and three). Metacarpophalangeal and carpal ROM decreased between day two and three ($P<0.001$), whereas femorotibial and scapulohumeral increased between day two and three ($P<0.001$).

8.6.3 Subject Three

Table 8.17: Effect of day, repeat and side on joint ROM for subject three. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Scapulohumeral	Humeroradial	Carpus	Metacarpophalangeal	Coxofemoral	Femorotibial	Tarsus	Metatarsophalangeal
Day	NS	**	NS	NS	NS	*	***	***
Repeat	NS	NS	Ns	NS	NS	NS	NS	NS
Side	NS	NS	***	**	NS	NS	**	***
Day and repeat interaction	NS	NS	NS	NS	NS	NS	NS	NS
Day and side interaction	NS	**	***	NS	NS	NS	**	***

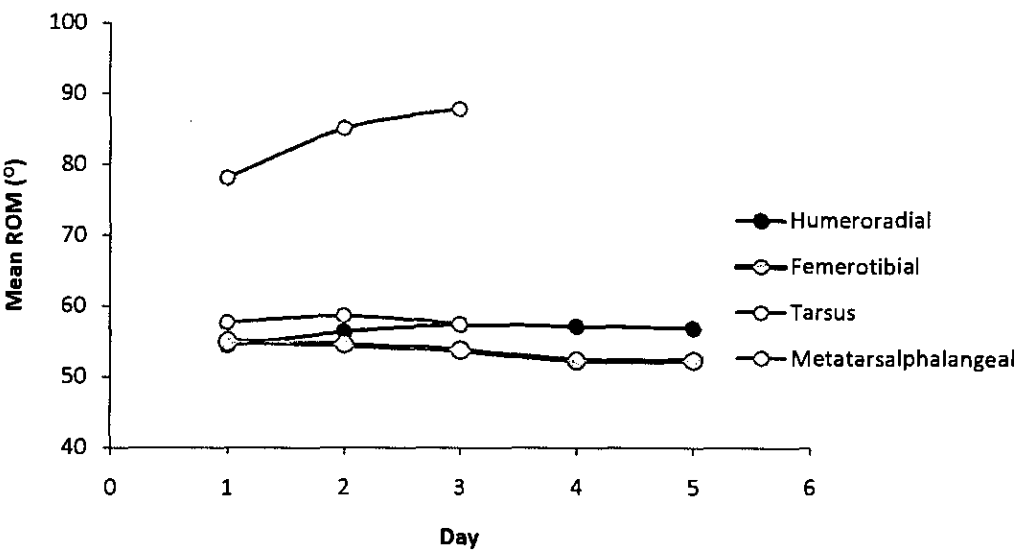


Figure 8.11: Mean ROM for the humeroradial, femorotibial, tarsus and metatarsophalangeal joints (left side) over five days for subject three.

Similarly to subjects two and three, ROM was consistent between repeats (table 8.17). Figure 8.11 demonstrates that humeroradial ROM did not change between day one and

two (P>0.05), or from day two for the remainder of the trial. ROM on day one however, was significantly smaller than days three, four and five (P<0.001). Femorotibial ROM remained constant for most of the trial, decreasing significantly on day four (P<0.001). ROM of the metatarsophalangeal increased significnatly beween day one and two (P<0.001), but did not change between day two and three (P>0.05). Day three ROM was significantly larger than day one ROM (P<0.001). There was missing data for the hind distal limb joints (tarsus and metatarsophalangeal) for days four and five.

8.7 Correlation between range of motion and velocity

Correlations were established between two joints' ROM for subjects one and two (scapulohumeral and humeroradial for subject one, carpus and tarsus for subject two). Subject three demonstrated the most significant correlations between joint ROM and velocity (five joints were correlated with velocity). More joints were established as significantly different between days for this subject (compared to subject one). Significant correlations had both positive and negative relationships, however the regression values were not as strong as the regression between SL and velocity.

Table 8.18: Correlation between ROM data for each joint (left and right side) and velocity over the five day trial for subject one. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Joint	P value	CC value	R^2 value (%)
Scapulohumeral	NS	-0.195	3.8
Humeroradial	NS	0.182	3.3
Carpus	***	0.508	25.8
Metacarpophalangeal	NS	0.191	3.6
Coxofemoral	NS	0.093	0.9
Femorotibial	NS	0.157	2.5
Tarsus	*	0.212	4.5
Metatarsophalangeal	NS	0.175	3.1

Table 8.19: Correlation between ROM data for each joint (left and right side) and velocity over the five day trial for subject two. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Joint	P value	CC value	R^2 value (%)
Scapulohumeral	*	0.250	6.3
Humeroradial	NS	0.006	0.0
Carpus	***	0.432	18.7
Metacarpophalangeal	**	-0.311	9.7
Coxofemoral	NS	-0.134	1.8
Femorotibial	*	0.228	5.2
Tarsus	NS	0.012	0.0
Metacarpophalangeal	***	-0.439	19.3

Table 8.20: Correlation between ROM data for each joint (left and right side) and velocity over the five day trial for subject three. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Joint	Significance	CC value	R^2 value (%)
Scapulohumeral	*	-0.223	5.0
Humeroradial	NS	-0.092	0.8
Carpus	*	0.248	6.1
Metacarpophalangeal	NS	-0.044	0.2
Coxofemoral	NS	0.107	1.1
Femorotibial	NS	-0.062	0.4
Tarsus	NS	-0.075	0.6
Metatarsoophalangeal	NS	0.117	1.4

8.8 Summary

Intra-horse variation established significant variation in stride length between days for all horses ($P<0.001$), and stride length was positively correlated to velocity (velocity was also established as significantly different between days). Less variation was demonstrated for intra-horse range of motion for the trial, a maximum of four joints (subjects two and three) were established as significantly different between days ($P<0.001$). The majority of joints measured did not vary significantly between days. A summary of the main findings are presented in the table below.

Table 8.21: Summary of main results for conformation (how many traits were different between sides or day); stride length (which days and sides were significantly different); ROM (how many traits were different between side and day) for each subject.

Subject	**Conformation** (number of traits)		**Stride Length** (day number)		**Range of Motion** (number of joints)	
	Side	*Day*	*Side*	*Day*	*Side*	*Day*
One	1	2	Right side >left side	One and three > two, four, five	3	3
Two	4	6	No difference	One>two-five	4	4
Three	2	3	Left side > right side	One and two > three-five	4	4

9.0 Discussion

The aim of part II was to obtain measurements for static conformation and stride characteristics for a group of horses over five consecutive days, to determine if baseline measures can be obtained on a single occasion. It has previously been established that traditional methods of assessing conformation can lead to inconsistencies between assessors (Breen, 2009). Using a quantitative method like the one used in the present study (based on Magnusson (1985) and Holmstrom *et al.*, (1990)) should lead to more consistent evaluations, providing the method is standardised. Unlike stride parameters, when assessing conformation repeated measures are rarely taken; in theory there is less variation when measuring static lengths and angles compared to dynamic ones. This was evident in part I of this research, where static validation produced smaller margin of error values than dynamic validation. The majority of conformation studies to date investigate the effect of conformation on future performance (Holmstrom *et al.*,1990; Back *et al.*, 1996; Weller *et al.*, 2006b; Holmstrom, 2000) and soundness (Back *et al.*, 1996; de Souza *et al.*, 2004). In the present study, the purpose of establishing intra-horse variation in conformation was to determine the reproducibility of the method in terms of consistency, as well as determining conformational differences between left and right side. It was important that the method was standardised and highly repeatable to make it accessible to the equine industry to use on a practical level. Limb segments were defined and measured (rather than lengths of bone segments of the limb), for ease of application. This was a preliminary investigation to determine the methodology for part III that aimed to established normal conformation for a distinct breed of horse.

Variations in intra-horse conformational measurements can be an amalgamation of inherent asymmetry between left and right side, changes in the stance, and errors in marker placement due to soft tissue artefact. These limitations will vary horse-to-horse; it is hard to distinguish between them to ascertain which limitations are causing significant variations between sides or days. The horses in this study demonstrated varying degrees of intra-horse variation in conformation, due to methodological and intrinsic limitations in the evaluation of conformation using two dimensional motion analysis techniques. One of these limitations, and a main source of error according to Weller *et al.* (2006a) is marker placement, however the actual marker set used could also be a limitation.

Markers used for dynamic analysis are usually placed at the centre of rotation of the joint to be measured (Clayton and Schamhardt, 2001), whereas for evaluating static conformation markers are often placed on the distal and proximal ends of limb segments (Holmstrom *et al.*, 1990). The current study developed a marker set that allowed conformation and gait to be assessed from one marker set; limb segments rather than actual lengths were measured. This is an essential part of ensuring the standardised method will be adopted by the equine industry as it needs to be simple and quick to obtain the data, as well as easy to process once downloaded (ease of digitising). Providing the marker position is known with respect to joint angles, and in a well known position on the horse, the exact marker site has no influence on the accuracy and value of final kinematic data (Schamhardt *et al.*, 1993). The purpose of using anatomical markers is to identify specific points of the skeleton on the surface of the skin, by palpating the muscle and underlying tissue to feel the relevant bony segments underneath. The accuracy of marker placement is dependent on the experience of the person applying the markers and the amount of soft tissue artefact (STA); the amount of tissue between the skin and bone. Soft tissue artefact is one of the main sources of error in the use of anatomical skin markers (Leardini *et al.*, 2005), and this error will vary depending on which joints are being measured. Joints with higher amounts of STA (proximal joints) will be harder to palpate to locate the correct bony segment under the skin compared to joints with smaller amounts of STA (distal joints). This has been demonstrated in the present study where scapulohumeral ROM had the highest amount of variation in all subjects, ranging from 16.30% to 26.33% (tables 8.12-8.14, page 68). This theory has been tested by Weller *et al.* (2006a), using hypodermic needles to locate the end of segment lengths and centre of rotation for joints on a whole cadaver. Radiographs were then used to determine the accuracy of the external location of the anatomical point, in relation to the actual point on the skeleton. The results confirmed that proximal locations were harder to palpate compared to distal locations; errors of less than 0.5cm were reported for points distal to the humeroradial and femorotibial joints. It was also suggested that size of the landmark may also be a factor in decreased accuracy for marker location. The proximal locations such as the greater trochanter tend to be larger areas, compared to the metacarpophalangeal for example. Taking repeated measurements over a number of days means repeated marker placement; if bony segments are hard to locate, inaccuracies could occur. This could be why it appears some conformational traits change day to day. Accuracy of marker placement is also very much dependent on the experience of the researcher doing the palpation. In other

types of assessment, level of experience has been established as a significant factor in consistency between assessors (Breen, 2009). In the present study, the same experienced researcher applied all the anatomical markers following a specified palpation protocol. This minimised chances of inconsistency in marker placement, however there was still a risk that accuracy was decreased when applying markers in the same positions over consecutive days. To reduce this risk further, small areas of hair could be clipped at the site of the anatomical landmark, to provide a reference point for marker placement. This method has been used in previous research where repeated measures have been taken from the same horses (Rose *et al.*, 2009), however could not be used in the present study due to lack of consent from the owners of the horses used. The amount of STA varies depending on individual horses, and this is strongly correlated to body condition. Horses with higher condition scores (higher fat mass) will display more STA. Subject one (Thoroughbred) had a lower body condition score compared to subjects two and three. This could be a reason why there was less variation in conformation data for subject one, compared to three, as bony segments were more easily palpated on the Thoroughbred horse. Subject one demonstrated two traits that varied significantly between days (neck length and scapulohumeral angle), compared to subjects two and three that demonstrated six and four traits respectively. Subjects two and three (Anglo-Arab and Cob) had higher amounts of STA than subject one, a possible limitation in the accuracy of marker placement. The specific marker model used could also account for some of the variation; the model aimed to enable the researcher to measure static conformation as well as ROM from the same model. Previously, research has used different marker sets depending on whether conformation or ROM is being measured. Holmstrom *et al.* (1990) used a different marker set for measuring conformation to Degueurce *et al.* (1997) for measuring ROM variability. The present study aimed to combine both these marker models, however it may have been more accurate to use a different one for measuring conformation and ROM.

In the present study, the difference between variability in linear or angular traits differed depending on the horse. Subject two demonstrated the highest variability for linear traits; six out of seven traits that were significantly different between days were linear. Subject three demonstrated higher variability for angular traits; four out of five traits that were significantly different between days were angular traits, this subject also demonstrated a significant difference between left and right side stride length ($P<0.005$), which could be accounted for by the differences in conformation. It could be

possible that intra-horse variation for subject two was mostly due to stance related variability, compared to subject three. Conformational asymmetry has previously been reported in National Hunt horses (Watson *et al.*, 2003; Weller *et al.*, 2006c). The study by Watson *et al.* (2003), measured skeletal asymmetry of the third metacarpal bone using radiographs. The right metacarpal bone was longer in 76% of horses measured. Lengths of bone segments is one factor attributing to overall conformation (Weller *et al.*, 2006b) therefore it can be assumed that asymmetry in bone lengths will lead to asymmetry in the conformation for the corresponding limb segment. Weller *et al.* (2006c) used 3D motion analysis techniques to assess conformation for 106 National Hunt horses. Results were similar to the present study where some but not all traits demonstrated left and right side asymmetry; both linear and angular traits were significantly different between sides. The authors suggested angular traits (joint angles) were more dependent on the stance of the horse, and that variations may reflect the asymmetrical stance of the horse rather than true asymmetry (Weller *et al.*, 2006a). Changes in stance could also affect measurement of conformation between days. Stance related dependency of conformation assessment has previously been tested using 3D motion analysis techniques by taking repeated measurements of the same horse, three times for each side (Weller *et al.*, 2006b). Horses were led out of the calibrated field of view, in again and repositioned between each data collection. Conformation traits varied between each repeated data collection for each horse; deviations of limb segments proved to be the most stance dependent traits measured.

True asymmetry in conformation is difficult to assess without the use of radiographs or three-dimensional analysis systems that can measure traits from both sides simultaneously. The purpose of this study was to standardise a method of assessing conformation that is not only accurate and reliable, but accessible to the equine industry. In order for the equine industry to start using a quantitative method such as the one described in this study, it needs to be practical and easily reproducible for equine practitioners to use on a daily basis for "in field" evaluations. The method is not without its limitations; which are difficult to overcome, particularly if this method is to be utilised by equine practitioners. Accuracy of marker placement can be improved by one experienced researcher applying the markers following a specific palpation method, however for this method to be adopted by the industry, the method needs to be accessible to everyone not just experienced researchers. Taking the time to ensure the horse is stood square (evenly weight bearing) and perpendicular to the camera will limit

stance related variations and distortions due to out of plane rotation, this was done in the present study by using an area marked on the surface of the school to ensure the horse was perpendicular to the camera. Differences were established between sides and days for some traits, and different traits were different on each day. This indicates the variations established in this study were due to methodological discrepancies rather than true asymmetry. Variation in conformation has been previously established for a group of Thoroughbred horses (Weller *et al.*, 2006c); therefore providing conformation is reported as a range within which normal conformation should lie, small variations should not be an issue.

Baseline measures are integral when analysing equine stride characteristics. Baseline data is used in place of an independent variable, for evaluating treatment (Stergiou and Scott, 2005) or in the present case, to define normal gait. Variability is inherent in animals (Danion *et al.*, 2003; Forner-Cordero *et al.*, 2006); it appears impossible for biological systems to repeat identical locomotion patterns on successive occasions. Equine gait characteristics may not be identical between repeats but conclusions drawn from previous studies on the variability of equine gait established small intra-horse variation (Drevemo *et al.*, 1980a; Drevemo *et al.*, 1980b; Degueurce *et al.*, 1997; Galisteo *et al.* 1996). This is desirable for gait analysis studies as it means that baseline measures can be obtained on a single occasion. It also means that "normal" gait for individual horses can be quantified. In the present study the amount of intra-horse variation in stride length varied depending on which subject was being analysed. Ten repeats were taken for each side, and no significant differences between repeats were established for stride length or velocity ($P>0.05$). Repeated measures were taken to obtain a mean value, the number of repeats can vary from three (Drevemo *et al.*, 1980b) to twelve (Clayton *et al.*, 2002), but ten were used in the present study. The results of the current study demonstrate that ten repeats are sufficient for obtaining baseline measures; stride length data obtained on one occasion is consistent. This confirms previous studies, where little variation was established between repeats of the same horse recorded on one occasion (Drevemo *et al.*, 1980a; Degueurce *et al.*, 1997). Stride length was significantly different between sides for some horses and stride length was significantly different between some but not all days ($P<0.001$), so although stride length was consistent between repeats on one day, there was less consistency between days. Intra-horse variation in stride length established differences on days one and three for subject one; stride length was significantly longer on these days than other days in

the trial (P<0.001). Subject two demonstrated significantly longer stride length on day one (P<0.001) than all subsequent days; stride length did not vary between days from day two to five. Similar results were illustrated for subject three; stride length was significantly longer on day one and two than subsequent days (P<0.001). It is evident from these results that stride length had good short term repeatability (between successive repeats on the same day), but repeatability on different days was less consistent. The level of consistency depended on the horse; stride stability is unique to individual horses. This confirms previous studies that have established horses have inherent locomotion patterns (Drevemo *et al.*, 1980a; Drevemo *et al.*, 1980b; Degueurce *et al.*, 1997; Galisteo *et al.* 1996); some locomotion patterns may be more variable than others. This variability may be due to a number of factors, all of which are limitations of the present study some of which could be accounted for to make stride characteristics less variable for future studies.

Variation in stride characteristics between sides could be partly due to asymmetrical conformation, asymmetrical conformation is a limitation that cannot be altered. Subject two demonstrated no significant difference between left stride length and right stride length overall (P>0.05); this horse also had the least asymmetry in conformation between sides. Two traits were different between sides (femur length and femorotibial angle), the small amount of conformational asymmetry is probably why there is less asymmetry when analysing stride length; conformation has a direct effect on movement (Holmstrom *et al.*, 1990; Back *et al.*, 1996; Weller *et al.*, 2006b). A horse with symmetrical conformation will probably lead to more symmetrical gait. Evaluation of conformation is an important part of any investigation into stride characteristics, due to the relationship between conformation and locomotion. It could be suggested from the present study that an assessment of conformation prior to gait analysis should provide useful information on symmetry of the individual horse, allowing decisions to be made on whether to analyse gait from one or two sides. Stride characteristics for the left and right side were not recorded simultaneously for the present study; only one camera was used therefore a comparison of left and right side was not a true comparison. Horses were also recorded for ten repeats on each side, rather than capturing each side sequentially. It could be possible that whichever side was recorded first demonstrated significantly different stride length to the subsequent side. If two cameras were used, and sides recorded simultaneously, it is possible that less variation would have been established between sides.

Correlations on the data established significant correlations between stride length and velocity, and this has been well documented in other biomechanics research (Leach and Drevemo, 1991; Peham *et al.*, 1998; Clayton *et al.*, 2001). The consistency of stride length between days was also closely correlated to consistency of velocity. Where differences between days in stride length was established, a significant different in velocity was also established. This is more than likely due to the strong correlation between stride length and velocity, and demonstrates how velocity-dependent stride length is. The dependency of stride length on velocity demonstrates how important it is to regulate velocity when aiming to obtain baseline measures in gait analysis. Velocity in the present study was not consciously regulated; consistency was improved by using the same handler to trot each horse. The variation in velocity demonstrates this is not the most reliable method of regulating velocity of trotting horses. In future work, timing gates could be utilised to ensure the horse is travelling within the same range of speed (±5% of the mean velocity) for each repeat. The analysis of inter-horse variation (results of inter-horse variation can be seen in Appendix C) established horse one had consistently shorter stride length than horses two and three. Horse one had higher variation in stride length than horse three. It is also possible that faster horses (longer stride length) were more consistent; this has been established for human athletes (Danion *et al.*, 2003), and due to the correlation of velocity and stride length in equine gait it is a plausible explanation for the increased variability seen in shorter strides.

The amount of variation in range of motion for the specific joints measured was less than variation in stride length over the five day trial. ROM for eight joints were measured, a maximum of four joints displayed variation that was established as significant between days for all subjects. Subject one demonstrated a significant reduction in carpus ROM between day one and three, but then an increase on days four and five. Carpus ROM was significantly negatively correlated to velocity, and although the correlation was not very strong (5%), ROM does follow the same pattern as change in velocity over the five days. Humeroradial and tarsus ROM were also significantly different between days; humeroradial ROM decreased between day one and two then stayed consistent until day five when it increased. Tarsus ROM increased from day one to five. Neither of these joints were significantly correlated to velocity therefore there must be another factor involved in the variation of joint ROM.

Galisteo *et al.* (1996) demonstrated similar results to the present study. Variability in the majority of joints measured was less than 10%, however the scapulohumeral joint

demonstrated the highest amount of variation (mean of 24.9%) which is comparable to the mean scapulohumeral COV value for the present study (21.2%). This study recorded the horses at walk, leading to a much lower velocity (1.68±0.15m/s). The authors did not take into account velocity due to the low SE value; they assumed velocity to be a constant parameter therefore lacking significance on stride parameters. Velocity however had the highest COV value compared to other stride parameters. It could be argued that COV is a more reliable measure of the variability of the parameter measured as it lets two sets of data to be compared that have different values as it shows the variability as a percent of the original value. Standard error is not a proportionate measure; a smaller value will have a smaller SE value, even though COV might be higher. This could be why velocity had a small SE, but larger COV value.

Intra-horse variation in ROM differed for each joint; this variation followed a pattern that was similar for all subjects. ROM for proximal joints had higher COV values compared to ROM for distal joints. Scapulohumeral ROM was the joint with the highest COV value for all subjects. The joints with the lowest COV values were metacarpophalangeal, metatarsophalangeal and carpus. It has previously been established that different joints undergo a different amount of variability (Galisteo *et al.*, 1996; Degueurce *et al.*, 1997), with contradictory results. Intra-horse variation studied by Degueurce *et al.* (1997) indicated distal joints such as the distal interphalangeal, carpus and tarsus joints displayed the lowest amount of individual variation between repeats. These joints therefore were considered to be more representative of the individual horse, whereas in proximal joints that had higher amounts of intra-horse variation, the range of motion was less characteristic of the individual horse. The study by Galisteo *et al.* (1996) revealed results more similar to the current study; proximal joints demonstrated higher amounts of variation compared to distal joints. The results indicate distal joints are more representative of the individual, compared to proximal joints (that undergo higher amounts of variation between repeats). It is possible that marker placement could be causing variation in proximal joint ROM, such as the scapulohumeral. The analysis of scapulohumeral joint conformation indicated scapulohumeral joint angle was significantly different between days for subject one and three, and scapula length for subject two. Actual scapulohumeral conformation will not have changed over the trial, one possible cause of this variation could be marker placement (as discussed earlier). Soft tissue artefact is another possible cause of the higher variation in the proximal joints. The proximal joints (scapulohumeral,

coxofemoral, femorotibial) displayed the highest COV values compared to distal joints in the current study, this is likely to be due to the varying amount of STA in these joints. Proximal joints have higher amounts of STA compared to distal joints (van Weeren *et al.*, 1990a). Variations of up to 8mm were established for distal joints, compared to values of as much as 142mm for more proximal locations. Deviations of up to 40mm have been found for the scapula (van Weeren *et al.*, 1990b), which when taking repeated measures of the same horse could be an influence on the amount of variation. A simple and accessible method of accounting for some of the distortions caused by STA is to use data smoothing and filtering techniques (Howarth and Callaghan, 2009). The present study used a Butterworth filter, at a cut-off frequency of 10 hertz to smooth all data. This technique will not account for large amounts of skin displacement, but is an acceptable and widely used protocol making it ideal to use with a standardised method aimed at equine practitioners.

The definition of gait is the "cyclic pattern of limb movements that occur during each stride" (Nicodemus and Clayton, 2002), therefore by its' very nature a stride cycle is an accumulation of the range of motion of each joint making up that stride. This is one reason why stride length data is more variable than ROM data, as it is in effect the overall movement of the horse; the sum of minor variations displayed for each joint will lead to larger variations in stride length. The aim of this chapter was to establish if gait characteristics for individual horses were stable enough to allow for baseline data to be collected on one occasion, rather than separate occasions over a number of days. It could be argued that when measuring stride length alone, due to the higher intra-horse variation baseline data should be obtained over more than one day. The amount of variation in stride length did vary depending on the horse, indicating some horses have more stable stride characteristics than others. It is possible this different amount of variation is linked to age or training effects; the mean age was 10±3.5 years and each horse has competed to different levels in different disciplines. Age and training have both previously been established as factors contributing to changes in gait characteristics (Barrey *et al.*, 1993; Buchner *et al.*, 1994), and are often linked. To minimise age and training effects, the sample of horses used should be of similar age and have undergone the same level of training. One method to ensure this is to use un-backed young horses. The horses in the present study all demonstrated significant differences between day one and two, but not all for subsequent days. This suggests that if baseline stride length data is needed, the first data collection should be discounted

(possibly due to the novelty factor) but subsequent baseline data should be stable enough to be collected on one occasion. Range of motion of specific joints demonstrated different amounts of variability, this could cause difficulties when suggesting how variable gait is overall. The standardised method developed from this research needs to be simple and accessible if it is to be utilised on a practical level by the equine industry, as well as providing equine practitioners with accurate and useful information. It can be concluded from this chapter, that although stride length is more variable than ROM, providing measures are taken to control the methodology (standardised marker placement, regulating velocity) then baseline measures to define the range of normal locomotion of the horse can be collected on one occasion (as long as stride length alone is not used as a measure of the horses gait). The results of this study indicate horses do have unique locomotion patterns and that these patterns vary between horses; it has yet to be seen if these patterns are not just unique to individual horses but within distinct breeds as well.

PART III

Defining normal gait in the Arabian Horse.

10.0 Aim

The aim of part III of this study were to apply the methods and techniques from parts I and II in order to measure normal conformation and gait of Arabian horses; and from these measurements develop a unique baseline dataset for the Arab horse.

10.1 Objectives

i) Use the standardised technique developed in part I and II to obtain measurements of static conformation of a group of Arabian horses.

ii) Use the standardised technique to obtain measurements of stride length and range of motion for a group of Arabian horses.

iii) To provide a unique data set of baseline stride and conformation data to the Arab Horse Society and Arab stud/horse owners.

10.2 Hypothesis

i) Normal conformation (determined by the mean values obtained) will be normally distributed.

ii) There will be no difference in stride length or range of motion between horses.

iii) There will be significant correlations between conformation and gait.

11.0 Subjects

The subjects consisted of six purebred Arabian horses; four mares, one gelding and one colt. Mean age 27±10.56 months. None of the horses were backed, therefore had not received any training, but were capable of trotting in hand. Five of the horses shared identical routines (lived out full time), one horse (the colt) was stabled at night. Horses were selected based on horses available at the time of the study that fulfilled the required criteria of being purebred unbroken Arabian horses.

11.1 Instrumentation

Please see section 7.1 in chapter six, part II (page 49) for details of the equipment used.

11.2 Anatomical markers

Please see section 7.2 in chapter six, part II (page 49) for details on the marker sets used to measure conformation and gait.

11.3 Filming procedure

Filming procedure is explained in detail in section 7.3 in chapter six, part II (page 51). Filming took place on location at the participating stud, on the yard which consisted of a large, flat area most suitable for trotting horses in hand. Prior to filming taking place, a calibration video was filmed using a 50 by 50cm calibration cube. Horses were led passed the equipment in walk for ten minutes before recording took place, to habituate the horses to the equipment and to warm the horses up prior to filming. Filming took place over a period of two weeks, on three separate days from 10am to 2pm.

11.3.1 Conformation assessment

Procedure for assessing conformation is explained in detail in section 7.3, chapter six (page 51). Horses were stood square (equally weight bearing on all four limbs) in the calibrated field and held by a handler, ensuring the horse was perpendicular to the camera. Horses were recorded for three seconds from the left side (traditionally the side conformation is assessed from), on one occasion. The linear and angular traits measured can be seen in figure 6.1 (chapter six).

11.3.2 Stride length and range of motion assessment

Procedure for assessing stride length and range of motion is explained in detail in chapter six. Stride length and ROM were assessed in trot only. Horses were led in hand (by the same handler to maintain consistency) passed the video camera through the calibrated field of view, at a distance of 7m from the video camera (see figure 7.2, page 52) in order to capture one full stride (one full stride cycle was determined as mid-stance to mid-stance of the near fore and hind limb). Horses were recorded from the left side only, with five repeats being taken from each horse.

11.4 Analysis of videos

Video analysis and data smoothing techniques were carried out according to the methodology described in section 7.4, chapter six (page 54).

11.5 Statistical analysis

Descriptive statistics were calculated for the data for intra-group variation and inter-horse variation. Descriptive statistics included mean, standard error, variance (the distance of the data from the mean value) and the coefficient of variation (the standard deviation as a percentage of the mean, which allows data sets of different values to be compared). All data were tested for normality with an Anderson-Darling normality test. If data were established as non-parametric, skew was determined with a histogram. If data were skewed, data were transformed. For positively skewed data, the transformations vx, log x, $^1/_x$ were used. For negatively skewed data the transformations x^2, x^3 and antilog x were used. Data were then re-tested for normality.

Inter-horse variation and intra-group variation were established for each subject and all subjects for conformation, SL and ROM. Parametric data was tested using a GLM. Non parametric data was tested with a Kruskal-Wallis. To determine where differences occurred, a post-hoc Tukey comparison was used.

Correlations were performed between SL and ROM data with velocity to determine if a significant relationship existed. Parametric data were tested with a Pearson correlation, non parametric data were tested with a Spearman correlation (on ranked data). Regression analysis was also performed to determine the strength of the relationship.

12.0 Conformation

12.0.1 Variation in conformation

Traits that measured linear distances (lengths of limb segments) overall had lower SE values than traits that measured angular values (joint angles). When comparing COV values, higher variance was established for linear traits, compared to angular traits. These results followed similar patterns to the intra-horse variation in conformation seen in part II. All data were established as parametric, therefore normally distributed within the population.

Table 12.0: Variation in conformation of a group of purebred Arabian horses. Traits with the highest and lowest %COV are highlighted in grey.

Trait	N	Mean	SE	SD	Variance	%COV
Neck (cm)	6	54.14	6.16	2.52	37.98	11.38
Scapula (cm)	6	33.17	4.04	1.65	16.31	12.17
Humerus (cm)	6	28.74	3.97	1.62	15.73	13.80
Radius (cm)	6	48.87	2.89	1.18	8.38	5.92
Metacarpal (cm)	6	30.75	3.64	1.48	13.22	11.83
Proximal phalanx (cm)	6	11.13	0.94	0.38	0.88	8.43
Pelvis (cm)	6	28.63	3.32	1.35	11.00	11.58
Femur (cm)	6	37.86	4.56	1.86	20.80	12.05
Tibia (cm)	6	42.61	1.83	0.75	3.36	4.30
Metacarpal (cm)	6	38.78	3.39	1.38	11.48	8.74
Proximal phalanx (cm)	6	11.28	2.00	0.82	4.00	17.73
Neck (°)	6	97.82	5.56	2.27	30.93	5.69
Scapulohumeral (°)	6	114.40	2.92	1.19	8.53	2.55
Humeroradial (°)	6	152.03	8.85	3.61	78.34	5.82
Metacarpophalangeal (°)	6	141.88	4.58	1.87	21.01	3.23
Coxofemoral (°)	6	116.13	2.10	0.86	4.41	1.81
Femorotibial (°)	6	162.78	10.99	4.49	120.75	6.75
Tarsus (°)	6	164.30	3.49	1.42	12.17	2.12
Metatarsophalangeal (°)	6	148.62	8.89	3.63	78.96	5.98

12.1 Stride length and velocity

12.1.1 Normal stride length in Arabian horses

Data for stride length and velocity demonstrated variance within a similar range to conformation data. Velocity had the highest %COV value forelimb and hind limb SL had similar %COV values. Data for hind limb SL and velocity were parametric (once transformed), however forelimb SL was not; data were for this trait were not normally distributed within the population.

12.1: Variation in stride length in trot and velocity for a group of purebred Arabian horses.

Trait	N	Mean	SD	SE	Variance	%COV	Parametric
Forelimb SL	30	206.38	13.24	2.42	175.25	6.41	×
Hind limb SL	30	204.80	12.50	2.28	156.28	6.10	×
Log hind limb							✓
Velocity	30	318.49	32.98	6.02	1087.60	10.35	×
Log velocity							✓

12.1.2 Inter-horse variation

There was no significant difference between fore and hind limb SL ($P>0.05$) therefore for further analysis, forelimb SL and hind limb SL were analysed together as SL (these data were established as parametric).

Table 12.2: Inter-horse variation of stride length in trot and velocity for a group of purebred Arabian horses. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variable	Stride length	Velocity
	Significance	*Significance*
Horse	***	***
Repeat	NS	NS

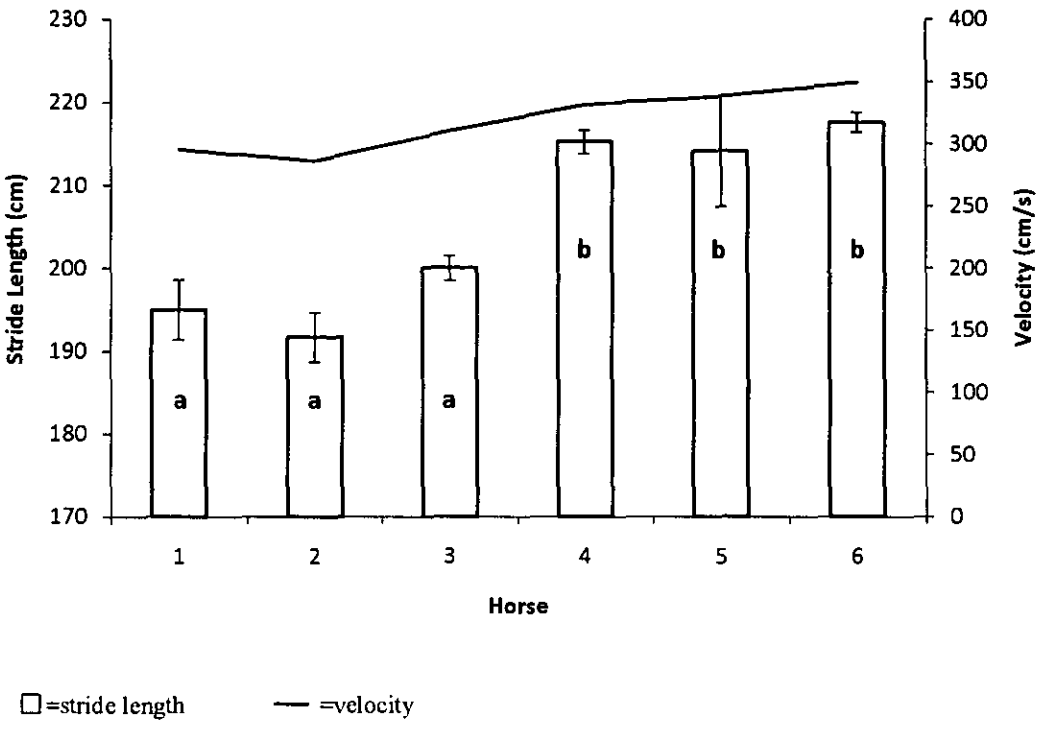


Figure 12.0: Mean stride length (±SE) and velocity for all horses. Different letters denote a significant difference between horses P<0.001). Same letter denotes no significant difference between days.

Stride length and velocity were consistent between repeats (P>0.05), this was true of all horses. There was no significant difference in SL between horses one, two or three (P>0.05), or between horses four, five and six (P>0.05). Horses one to three had significantly shorter stride length compared to horses four to six (P<0.001). A similar pattern was established for velocity. Horses one to three demonstrated no significant differences (P>0.05), horses three to six demonstrated no significant differences (P>0.05). Horses four to six were significantly faster than horses one to three (P<0.001). Horse three was faster than horses two and three, and slower than horses four to six, however this small variation was not significant. This horse also had the largest SE value (331.15±14.44cm/s).

12.1.3 Correlation between stride length and velocity

A significant positive correlation was established between mean stride length and velocity (R^2=76.2). This value is comparable to the intra-horse correlations demonstrated in part II.

Table 12.3: Correlation between fore and hind limb stride length and velocity showing, correlation coefficient (CC value) and regression value (R^2 value). Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001

Variable	Significance	CC Value	R^2 value (%)
Stride length	***	0.873	76.2

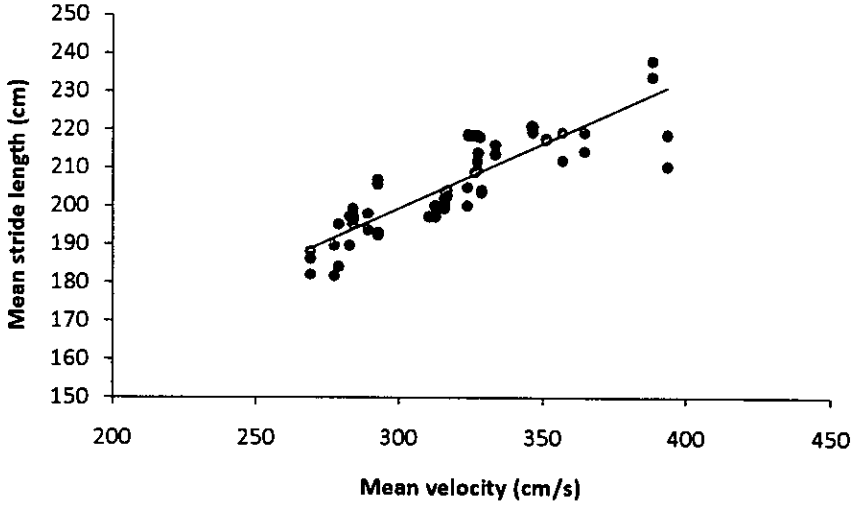


Figure 12.1: Regression analysis for stride length and velocity (76.2%) showing a significant positive correlation (P<0.001).

12.2 Range of motion

12.2.1 Normal range of motion in Arabian horses

Scapulohumeral joint ROM demonstrated the highest %COV (table 12.4), similarly to intra-group variation for ROM in part II. Variation for this joint was less for the Arab horses, compared to intra-group variation for the same joint. Tarsus joint had the lowest %COV similarly to intra-group variation. Variation was less for the Arabian horses when comparing the data to intra-group variation for the same joint. Unlike intra-horse and intra-group variation, there was no trend to the data when comparing distal and proximal limb joints.

Table 12.4: Variation in ROM for all subjects. Joints with the highest and lowest %COV are highlighted in grey.

Joint	N	Mean	SD	SE	Variance	%COV
Scapulohumeral	30	19.54	4.71	0.86	22.20	24.12
Humeroradial	30	53.67	4.37	0.80	19.09	8.14
Carpus	30	72.29	7.11	1.30	50.59	9.84
Metacarpophalangeal	30	95.81	11.17	2.04	124.70	11.66
Coxofemoral	30	29.04	3.90	0.71	15.21	13.43
Femorotibial	30	46.77	4.89	0.89	23.92	10.46
Tarsus	30	60.44	4.64	0.85	21.56	7.68
Metatarsophalangeal	30	104.29	12.92	2.36	166.96	12.39

12.2.2 Inter-horse variation in range of motion

Table 12.5 demonstrates that neither horse nor repeat had a significant effect on ROM for any of the joints measured (P>0.05). There was no significant difference between horses for each joint ROM measured (figure 12.2). Normal joint ROM for this group of Arabian horses can therefore be established from the mean values for each joint ROM.

Table 12.5: Effect of horse and repeat on joint ROM for all subjects. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variables	Scapulohumeral	Humeroradial	Carpus	Metacarpophalangeal	Coxofemoral	Femorotibial	Tarsus	Metatarsophalangeal
Horse	NS	NS	NS	NS	NS	NS	NS	NS
Repeat	NS	NS	NS	NS	NS	NS	NS	NS

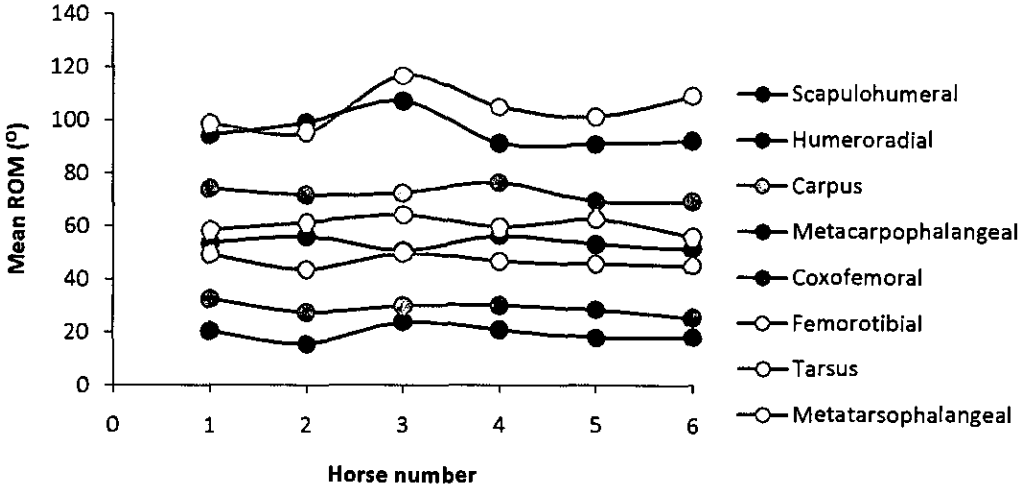


Figure 12.2: Mean ROM for all joints and subjects showing no significant differences between horses (P>0.05).

12.3 Correlations between conformation and gait

Correlation between conformation and gait are illustrated in tables 12.6 and 12.7. The majority of significant correlations between conformation traits and gait were established for hind limb traits. The correlations that were significant were all positive correlations; as the conformation trait increased in size so did the length of stride or range of motion of the joint. The regression analysis for one of these correlations (tibia length) is shown in figure 12.3. When analysing all traits together, hind limb traits could be used to predict hind limb stride length more accurately than forelimb traits (table 12.8).

Table 12.6: Correlations between forelimb stride length in trot and ROM and conformation traits showing correlation coefficient value. Significance is indicated with (*), positive and negative correlations indicated with (+) or (-).

Trait	Forelimb Stride length	Scapulohumeral ROM	Humeroradial ROM	Carpus ROM	Metacarpophalangeal ROM
Length of neck	0.298	0.055	0.462	0.167	0.107
Length of scapula	0.133	0.365	0.476	0.631	0.028
Length of humerus	0.371	0.316	0.116	0.171	0.146
Length of radius	0.187	0.494	0.004	0.365	0.465
Length of metacarpal	0.615	0.555	0.726	0.129	0.077
Length of proximal phalanx	0.649	0.316	0.527	0.222	0.465
Neck angle	0.069	0.678	0.070	0.521	0.637
Scapula angle	0.515	0.675	0.154	0.328	0.072
Humeroradial angle	0.455	0.397	0.335	0.802 *(+)	0.317
Metacarpophalangeal angle	0.289	0.560	0.135	0.791	0.221

(*) P<0.05

(**) P<0.005

(***) P<0.001

Table 12.7: Correlations between hind limb stride length in trot and hind limb conformation traits showing correlation coefficient. Significance is indicated with (*), positive and negative correlations indicated with (+) or (-).

Trait	Hind limb Stride length	Coxofemoral ROM	Femorotibial ROM	Tarsus (ROM)	Metatarsopophalangeal (ROM)
Length of pelvis	0.020	0.365	0.026	0.080	0.116
Length of femur	0.347	0.368	0.053	0.630	0.115
Length of tibia	0.933 **(+)	0.516	0.321	0.478	0.365
Length of metatarsal	0.167	0.174	0.373	0.404	0.003
Length of proximal phalanx	0.252	0.361	0.417	0.217	0.318
Coxofemoral angle	0.786	0.537	0.124	0.390	0.433
Femorotibial angle	0.075	0.823 *(+)	0.645	0.190	0.136
Tarsal angle	0.246	0.456	0.535	0.252	0.093
Metatarsophalangeal angle	0.904 *(+)	0.099	0.136	0.018	0.530

(*) $P<0.05$

(**) $P<0.005$

(***) $P<0.001$

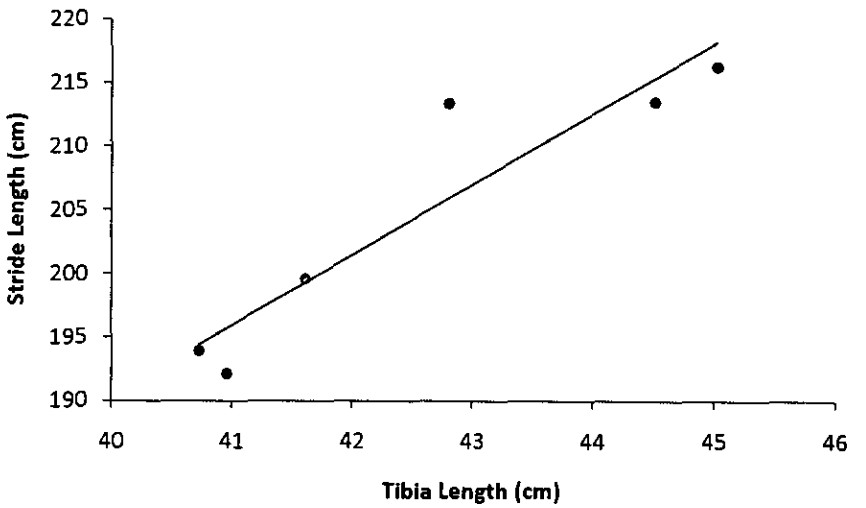


Figure 12.3: Regression for tibia length and hind limb stride length showing a significant positive correlation (P<0.005) with a regression value of 83.99%.

Table 12.8: Stepwise regression analysis of all hind limb conformation traits with hind limb stride length. Levels of significance indicated by; * P<0.05; ** P<0.005; *** P<0.001; NS not significant.

Trait	1	2	3	4
Length of tibia	**	NS	**	**
Metatarsophalangeal angle		NS	**	**
Length of proximal phalanx			**	**
Femorotibial angle				NS
Regression value (%)	83.88	93.14	99.84	100.00

13.0 Discussion

The aim of this chapter was to define the normal range of conformation and stride characteristics for a distinct breed of horse (the Arabian) to determine if a specific breed has unique conformation and gait patterns. It has previously been established in part II that individual horses have inherent locomotion patterns; more so for range of motion than stride length (small intra-horse variation), and this has also been documented previously in research using horses of different breeds (Drevemo *et al.*, 1980a; Drevemo *et al.*, 1980b; Degueurce *et al.*, 1997). To date, no attempt has been made to define normal conformation and gait in the Arabian horse, using a method that will be easily reproducible for use by the equine industry.

Table 13.0: Normal conformation and stride characteristics (in trot) for purebred Arabian horses (mean age 27±10.56 months).

Conformation traits	**Mean value (±1SE)**
Neck (cm)	54.14 ±6.16
Scapula (cm)	33.17±4.04
Humerus (cm)	28.74±3.97
Radius (cm)	48.87±2.89
Metatarsal (cm)	30.75±3.64
Proximal phalanx (cm)	11.13±0.94
Ilium (cm)	28.63±3.32
Femur (cm)	37.86±4.56
Tibia (cm)	42.61±1.83
Metatarsal (cm)	38.78±3.39
Proximal phalanx (cm)	11.28±2.00
Neck (°)	97.82±5.56
Scapulohumeral (°)	114.40±2.92
Humeroradial (°)	152.03±8.85
Metacarpophalangeal (°)	141.88±4.58
Coxofemoral (°)	116.13±2.10
Femorotibial (°)	162.78±10.99
Tarsus (°)	164.30±3.49
Metatarsophalangeal (°)	148.62±8.86
Stride Parameters (in trot)	**Mean value (±1SE)**
Forelimb stride length (cm)	206.38±2.42
Hind limb stride length (cm)	204.80±2.28
Velocity (cm/s)	318.49±6.02
Scapulohumeral ROM (°)	19.54±0.86
Humeroradial ROM (°)	53.67±0.80
Carpus ROM (°)	72.29±1.3
Metacarpophalangeal ROM (°)	95.81±2.04
Coxofemoral ROM (°)	29.04±0.71
Femorotibial ROM (°)	46.77±0.89
Tarsus ROM (°)	60.44±0.85
Metatarsophalangeal ROM (°)	104.29±2.36

The method used in this chapter was validated and standardised in the previous parts of this research, and it was established that the two dimensional motion analysis software used is accurate and reliable, and the method simple but accurate enough to be used on a day-to-day basis by equine practitioners. Inter-horse variation was used to determine if there were any significant differences between horses; intra-group variation used to develop the baseline dataset (table 13.0).

Conformation data obtained from analysing the Arab horses used in this study were parametric; the data were normally distributed within the population. In normally distributed data, 68.26% of the data is within one standard deviation of the mean, and nearly all the data (99.74%) within three standard deviations from the mean; the mean value therefore is a reliable figure to represent the population. This is important in a smaller sample size (six horses). It is almost impossible to measure all the individual members of a population (Wheater and Cook, 2000), therefore samples were used as a representation of the population being measured-in this case conformation of Arabian horses. The null hypothesis was rejected as all data were parametric. Previous studies into breed-specific conformation have also established quantitatively measured conformation traits to be normally distributed when analysing variation in conformation of a distinct breed (Weller *et al.*, 2006c).

Cano *et al.* (2001b) compared Arab conformation to other breeds, using a similar method to the present study. Angular conformation traits of seven Arab horses were measured, all measurements for joint angles were smaller than the present study except hind metacarpophalangeal angle that was larger. The standard deviations in the Cano *et al.* (2001b) study were much higher than the ones in the present study, showing the data was more spread out; there was higher variation between horses in the study by Cano *et al.* (2001b) than the present study. This could be due to age effects. Horses in the Cano *et al.* (2001b) study were older, mean age was 6.1±4.0 years, and this led to a large range in heights (which may be why there was a larger range in conformational measurements compared to the present study where horses were of a similar age). The large standard deviation shows there was also a greater range of ages compared to the horses in the present study. Age has previously been linked to training; it is possible a horse trained to a higher standard will have a different stance to a young horse that has received no training (such as the horses in the present study). It has been discussed previously how stance of the horse (when stood square for conformation assessment) has a significant effect on the reliability of conformational measurements (Weller *et al.*,

2006), and this could be why measurements were different between the two studies. The effect of stance on conformational measurements is probably accentuated in Arab show horses due to the specific training Arab horses are subjected to (particularly with in-hand showing), to achieve a particular stance in the show ring. There was no information on the type of Arabs used in the Cano *et al.* (2001b) study, or what discipline (if any) they competed in.

The coefficient of variation values for each trait follow the same pattern as with the conformation data in part II. Linear traits were more variable between horses than angular traits. It was suggested in part II that intra-horse variation was partly due to limitations of the method such as stance of the horse, marker placement and soft tissue artefact, rather than inherent variation in conformation. This is probably true with the current data, as it has previously been established that horses of a similar breed have similar conformation (Holmstrom *et al.*, 1990; Cano *et al.*, 2001b). In contemplation of this, the null hypothesis was there would be no significant differences in the quantitative measurement of conformation between Arab horses in the study. The study aimed at analysing the amount of variation between horses therefore measures of variance (COV and SE values) are of more value than analysing differences. This method of reporting variability of conformation within a population was used by Weller *et al.* (2006c) when analysing the variation in conformation of Thoroughbred horses. Weller *et al.* (2006c) used standard deviation as an indicator of the spread of conformation data within the population. Comparing the standard deviation from that study to the present study, the range of values were very similar for angular and linear traits. The Arab conformation data in the present study ranged from 0.38cm to 2.52cm for linear measurements (length of fore proximal phalanx and neck), and 2.92° to 10.99° (for scapula and femorotibial angle respectively). The Thoroughbred conformation in the study by Weller *et al.* (2006c) ranged from 0.65cm to 8.04cm (carpal and horse length), and 3.08° to 10.02° (for carpus and coxofemoral angle respectively). Similarly to part II, linear traits were less variable than angular traits. The low variation within the sample reinforces the idea that horses of similar breed have similar conformation. This suggests that due to the strong link between conformation and locomotion, horses of the same breed will have similar gait characteristics.

Stride length (fore and hind limb) and velocity were not normally distributed; data were non parametric, although hind limb stride length and velocity could be transformed. The null hypothesis therefore can only partially be rejected. The coefficient of variation for

stride length for the Arab horses was less than the intra-group variation in stride length established for the subjects in part II. These results support the hypothesis that there is less variation in stride parameters in horses of the same, or similar breeds, and has previously been established in various breeds such as Dutch Warmblood, Andalusian (Galisteo *et al.*, 1996); Standardbreds (Drevemo *et al.*, 1980a; Drevemo *et al.*, 1980b). There appears to be two separate groups within the data. Horses one, two and three were not significantly different from each other for stride length or velocity, and the same with horses four, five and six; although the second group had significantly longer stride length and velocity to the first three horses. Horses were recorded over three days, but these days do not correspond to the different groups. It was established in part II of this study that day had a significant influence on stride length ($P<0.001$), however can be discounted as a factor in this study. Age of horse may be a factor influencing the variations in stride length. Horses one, two and three were all the same age (24 months), which could explain why there was no significant difference between stride length and velocity for this group. Horses four, five and six however were different ages. Horse four was the eldest horse (48 months), horse five was the same age as horses one-three (24 months), and horse six the youngest (18 months). There must be another factor influencing velocity and stride length that would explain why the two groups are different. Gender effects might be one explanation of this. Horses one-three were all fillies; horses four to six included one mare (48 month old), one gelding (24 months) and one colt (18 months). It has previously been reported that fillies have shorter stride lengths than colts in racing Thoroughbreds (Seder *et al.*, 2003) therefore it is possible that gender is having the same effect in the present study.

A comparison of stride characteristics of Arab horse from the present study, with horses of various breeds from previous studies reveals some interesting differences. The analysis of stride length data established the Arab horses in the present study had the shortest stride length compared to Anglo-Arabs, Andalusian and Dutch Warmblood horses (Galisteo *et al.*, 1997; Cano *et al.*, 2001b). Analysis of range of motion data also revealved some interesting comparisons. The majority of joints measured, the Arab horses displayed the smallest ROM values, compared to other breeds. This would explain the shorter stride length to a certain extent. Some of the joints measured were similar to other breeds, namely ROM of the tarsus joint, where values only ranged from 24.8° (Anglo-Arab) to 29.6° (Andalusian). Two joints measured had greater ROM in the Arabs than the other breeds (except Andalusian); the metacarpophalangeal and

metatarsophalangeal. This could be accounted for by differences in age. Previous research has established age affects the kinematics of the metacarpophalangeal joint (Butcher and Ashley-Ross, 2002). Two year old Thoroughbreds had more metacarpophalangeal joint flexion compared to five year old Thoroughbreds. Increased flexion could lead to an increase in ROM, which would explain why the younger Arab horses had larger ROM for the metacarpophalangeal joint, but generally smaller ROM for other joints (and shorter stride length) compared to other breeds. These differences between breeds could be due to not just age, but differences in velocity and conformation between the different breeds as well.

Comparing the current data to previous studies that have measured Arab stride length at trot (Cano *et al.*, 2001b), revealed the horses in the present study had slightly shorter stride length (2.06±2.4m) compared to the horses in the Cano *et al.* (2001b) study (2.6m). The horses in the present study were also travelling slower, with a mean velocity of 3.2±6.0m/s compared to 4.8±0.4m/s in the Cano *et al.* (2001b) study. Velocity was regulated in the present study using a stop clock, however this was not a very reliable method of regulating velocity as there were still high levels of variation between horses (leading to increased variation in stride length). Velocity is positively correlated to stride length (Back *et al.*, 1993b; Clayton *et al.*, 2002), as velocity increases so does stride length. In the present study there was a significant positive correlation between velocity and stride length ($P<0.001$). The differences in velocity between the two studies could explain the variations in stride length. The discrepancy could also be accounted for by the variation in age and size of the horses, as previously mentioned when discussing differences in conformation. The direct link between conformation and locomotion means that a difference in conformation will probably lead to a difference in locomotion between the two groups. Regression between conformational traits measured and stride characteristics determined exactly how conformation affected gait characteristics for the Arab horses.

Correlations between conformation and gait characteristics revealed more significant correlations for the hind limb compared to forelimb. The forelimb established only the static humeroradial angle to be positively correlated to carpal ROM ($P<0.05$). Analysis of the hind limb revealed length of tibia and metatarsophalangeal angle to be positively correlated to stride length ($P<0.005$; $P<0.05$), and femorotibial angle to be positively correlated to coxofemoral ROM ($P<0.05$). The stepwise regression established when using conformation traits (length of tibia, metatarsophalangeal angle, length of hind

proximal phalanx and femorotibial angle) hind limb stride length could be very accurately predicted (regression value 100%). Hind limb stride length, compared to forelimb stride length was less variable, it is possible that stronger correlations were established because the stride was more stable. Correlations between conformation and gait have previously been established, particularly for scapula conformation and stride length (Holmstrom *et al.*, 1990; Back *et al.*, 1996; Weller *et al.*, 2006b). It is surprising therefore that no significant correlations were established between scapula conformation and stride length in the current study. Differences in conformation may also account for differences in stride length for Arab horses. The three horses with the longest stride length also had the longest scapula length, however (possibly due to the small sample size) this relationship was not significant. When analysing the traits that were significantly correlated to stride length, inter-horse conformation was fairly similar. This is reflected by the low standard deviation values for conformation data.

Intra-group variation (variation within the sample as a group) for range of motion was low, there were small variations between horses but these variations were not significant. The analysis of inter-horse variation demonstrated repeat had no significant effect on ROM ($P>0.05$) for each horse, which had been a consistent result throughout the study. This reinforces the theory that horses have good short term repeatability in stride characteristics, and five repeats is sufficient to obtain baseline measures of these characteristics. Range of motion was not significantly different between horses ($P>0.05$). This is an interesting result as it means that from the sample used (six horses), all of them displayed similar gait characteristics in terms of range of motion. This indicates normal gait can be defined for a distinct breed of horse. The ROM data in table 13.0 for the horses in the present study are smaller than the ROM data presented in table 1.0 (page 13), taken from Cano *et al.* (2001b). One joint produced similar ROM, that was the coxofemoral, with ROM values of 29.04° (present study) and 29.50° (Cano *et al.*, 2001b). This is interesting, as when comparing the conformation data between the two studies, larger angles were reported by Cano *et al.* study for the majority of joints, compared to the present study. It has previously been established there is a relationship between conformation and gait, for example larger scapulohumeral angles lead to longer stride length (Weller *et al.*, 2006b), although no correlations were established between scapula conformation and stride length or ROM in the present study. Larger joint angles measured for static conformation should lead to great ROM in that joint; a larger angle gives the joint more potential for a larger ROM providing the full potential is used. It

appears that perhaps the horses in the present study were not using the full ROM potential available to them hence why although joint angles were larger when assessing static conformation but ROM was smaller. Velocity is one possible explanation for this. The horses in the present study were over one meter per second slower (3.2m/s compared to 4.8m/s). It was established in part II that some joint ROM were correlated with velocity (table 8.24, page 78), and this accounted for the variability of horses gait between days. It has been established previously that velocity does affect stride parameters; Sloet van Oldruitenborgh-Oosterbaan and Clayton, (1999) found an increase in joint ROM for the humeroradial, metacarpophalangeal and carpal joints with an increase in velocity. In the present study the correlations were not particularly strong, with regression values ranging from 1.8% to 33.4%; this implies other factors influence range of motion. Cano *et al.* used a similar sample size to the present study (seven horses), and a similar method; horses were trotted in hand along a track although recorded their horses were recorded from the right side, instead of the left. The biggest difference between the two studies is the age of the horses used (as previously discussed). It does not state in the study by Cano *et al.* whether the horses were backed or un-backed, but it does state the horses were not involved in any specific training programme. The study also mentions that although the horses conformed to the breed standard in terms of conformation, the Arabian horses were relatively higher at the withers than normal. Age, training and height at the withers could all be possible factors to explain the discrepancies between the two data sets.

Age, height at the withers and training are all linked. Taller horses have longer stride length, and larger ROM. It is questionable as to when horses' gait patterns stop developing. Some authors suggest it can be as late as 36 months, with the largest amount of change being between 12 and 24 months (Cano *et al.*, 2001a). Other authors suggest it can be as young as four months (Back *et al.*, 1993; 1994), although these studies were performed in a treadmill therefore not comparable with the present study. Increasing age may not necessarily lead to larger ROM; a study by Butcher and Ross (2002) found that young racehorses (two year old Thoroughbreds) had more flexion in their joints compared to five year old Thoroughbreds; possibly due to immaturity of the suspensory ligaments in the distal limb allowing for more flexion. A study by Cano *et al.* (1999) contradicts these results as greater ROM was established for adult (12.3±2.9 years old) compared to young (3.7±0.2 years old) Andalusian horses. ROM increased from 17.2±5.5° to 22.8±4.9° for scapulohumeral ROM for example. A further study by

Cano *et al.* (2001a) produced similar results with humeroradial ROM increasing from54.8±3.6° to 60.2±6.6° in horses aged 12 to 36 months. Bearing this in mind, the horses in the present study may have produced smaller ROM values for the joints measured compared to the previous study Cano *et al.* (2001b) due to their age. It is also possible that ROM may have increased as these horses got older. One of the reasons for choosing young horses was to eliminate the possible effect training has on stride parameters; amount or level of training could be another explanation why the results of the two studies were different. There is plenty of evidence that training does influence stride parameters (Drevemo *et al.*, 1980b; Back *et al.*, 1995; Cano *et al.*, 2000; Ferrari *et al.*, 2009), probably due to an increase in coordination (Back *et al.*, 1999). It is plausible therefore that horses in the present study displayed smaller ROM (despite large joint angles when analysing static conformation) compared to horses in the study by Cano *et al.*, 2001a) due to their age and lack of training. It would be interesting to reassess stride parameters in the same group of horses after they had been backed to establish if there was a significant difference in stride length or ROM.

The small inter-horse variation established for stride characteristics for a sample of Arab horses used in this study illustrates that distinct breeds have specific gait patterns. Conformation data were all established as parametric, therefore the null hypothesis (conformation data would not be normally distributed within the population) can be rejected. The null hypothesis that there will be no difference between horses gait can only be partially rejected; stride length was different between some (but not all horses) but there was no significant differences in ROM between any horses. Likewise, the hypothesis there would be no correlation between conformation and gait can only be partially rejected; few traits were significantly correlated to gait (mostly hind limb traits). Gait patterns are correlated to conformation, which is inherently different between breeds (Galisteo *et al.* 1997), but may be affected by limitations such as age, gender and velocity. This reinforces the idea that if normal gait for distinct breeds is to be quantified by breed societies, the methods used to obtain these baseline measures must be standardised across all breed societies.

14.0 Conclusion

14.1 Overview

This research involved three separate but interlinked studies with the overall aim of developing an accurate method of defining normal gait in a distinct breed of horse. The study has highlighted that it is essential to validate software prior to use and to know the margin of error for the software before analysing results. The aim of the standardisation study was to determine a method to obtain baseline conformation and gait parameters using two-dimensional motion analysis techniques. This highlighted the importance of accuracy in the methodology of using 2D motion analysis, specifically in marker placement, which was noted as one of the biggest causes of error. These validated methods were then used to define normal gait in a distinct breed of horse.

14.2 Limitations

Two dimensional motion analysis techniques are not without limitations, namely distortions due to out of plane movements, when capturing three dimensional motion in two dimensions. This is an inevitable limitation when using a 2D method to record 3D movement. The use of 3D methods of gait analysis such as optoelectronic systems are more accurate, but currently are not practical to be easily used "in field". Other gait analysis systems such as accelerometers using GPS based technology are suitable for "in field" analysis, however to do not currently provide the same detailed information that 2D analysis. Despite its limitations, because 2D gait analysis is designed to be portable and simple to use, it still remains the most practical solution for this type of gait analysis.

The purpose of standardising a method of 2D motion analysis was to provide the equine industry with a practical and reproducible method to assess gait and conformation. The method therefore needed to be simple and quick to execute. It was evident however from the intra-horse variation in conformation that there were still discrepancies in marker placement, despite one researcher following the same specific protocol. In research, the experience of the person applying the markers may outweigh the limitations of the method, however if this method was to be adopted by the industry, inconsistency between equine practitioners applying the markers would be a huge limitation of the method. This could be addressed by developing a specific palpation protocol to allow the anatomical landmarks to be identified. This protocol would need

rigorous testing and validating to enable it to be deemed appropriate, a possible avenue for future development of the current study.

A further limitation of the current study is regulating the stance of the horse when assessing conformation. It is difficult to control the stance of the horse, therefore it is a limitation of using this method to measure conformation. Stance does not have a large effect on the reliability of the results, compared to marker placement, particularly if repeated measures are taken. Stance is more repeatable if conformation is measured when the horse is stood on a hard level surface and evenly weight bearing. Taking the time to ensure the horse is stood square and perpendicular to the camera will limit stance related variations and distortions due to out of plane rotation. One possible method of ensuring the horse is evenly weight bearing would be to use pressure mats. This would be another opportunity for future research, to develop the current study to attempt to improve the repeatability of measuring conformation using 2D motion analysis techniques.

It is also very hard to regulate velocity when trotting horses in hand, even when using the same handler and timing the repeated trials. Other research has accounted for intrinsic variations in velocity by using treadmills, but these have been shown to alter gait characteristics therefore it is difficult to extrapolate treadmill data to "real life" situations. The use of timing gates would be a more accurate method of timing the horses compared to a stop clock, a method which was tested in the present study then disregarded due to the time taken to set up the equipment. To overcome this problem in future research, an accelerometer could be used to measure the horses velocity for each repeated trial. The accelerometer would have to transmit the data instantly to a computer, to avoid delays between repeated measures but overall would be a more accurate method of measuring velocity.

The manual input required for processing the data once downloaded is an additional limitation; digitiser error can cause errors in accuracy and repeatability of measurements. Errors can be reduced by using the same researcher to digitise all video clips, as in the present study, however this method is not infallible. The improvement of the automatic tracking features of the software would be a worthwhile addition to the overall efficacy of the software, for both reliability whilst digitising and practicality for use in the industry. Once developed, the accuracy of automatic tracking would need to be validated. This would be a very valuable addition to the current research that

validated the overall accuracy of the software and definitely a consideration for future research.

14.3 Future research considerations

Additional considerations for future research would be to re-test the Quintic© software using a high speed camera. This would establish to what extent the aspect ratio of the camera was causing the inaccuracies (compared to the cosine algorithm), specifically in angular measurements, and therefore if the software was suitable to use with a high speed camera.

A further development would be to use the same protocol to define normal gait in more than one breed, to allow the direct comparison between gait characteristics in different breeds. If gait was measured over consecutive days (as with the standardisation study) this would also provide interesting information about the day-to-day variability of stride characteristics within different breeds.

14.4 Practical application

The purpose of this research was to inform the equine industry of the accuracy and reliability of software and protocols used for "in field" gait analysis, as well as providing information on normal gait of Arab horses. This research has direct implications for the equine industry, as it is providing vital practical information that can be used directly by equine practitioners using gait analysis. The practical implications of defining normal gait will potentially provide the Arab Horse Society (or further breed societies willing to adopt the protocol) with not only a standardised method of measuring conformation and gait, but a baseline with which to compare other horses to. This will be particularly beneficial for grading and judging performance, by providing a quantitative measure with which to score horses against, ultimately producing completely objective grading and judging systems.

14.5 *Conclusion*

The normal conformation and gait for Arab horses that were quantified in part III of this study illustrate distinct breeds have specific gait patterns. Range of motion was similar for all joints and horses, therefore a reliable indicator of normal gait in Arab horses. Gait patterns were correlated with conformation, which is inherently different between breeds. This reinforces the proposal that if normal gait for distinct breeds is to be quantified by breed societies, the methods used to obtain these baseline measures must be standardised across all societies. If details of age and training are included as part of a database of normal conformation and gait a better overall picture of breed characteristics will develop. In effect, the database would have information on the typical conformation and gait of a specific breed with the effects of age and training included. The data from this study provides useful information for future equine gait analysis research, in terms of accuracy of the software and protocols. It could also be used to provide the Arab Horse Society with helpful information about normal gait characteristics for the breed, allowing Arab horses to be classified using gait analysis.

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Appendices

REFERENCE NO.

Charlotte Robin

FACULTY OF SCIENCE

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.

Does project require a Home Office Licence? NO Project Licence no:_____

Date:25/02/2009

Title of Project:

Title: Standardisation and validation of a two dimensional motion analysis technique to develop a unique baseline data set for Arab horses.

Name of researcher and co-workers:

Charlotte Robin	**Sarah Hobbs**
Charlotte Brigden	**Jaime Martin**
Jane Allot	**Mary Bloye**

1. Aims and objectives of project:

Aims: The aims of the study are to develop and validate a standardised two-dimensional motion capture technique for equine gait analysis; to use the technique to record and analyse the static conformation of a group of Arab horses; to use the technique to record and analyse the normal gait of a group of Arab horses; to develop a unique baseline data set for Arab horses.

Objectives:

1. Standardisation and validation

-to validate a two-dimensional motion analysis capture technique using known static and dynamic measurements

-to use statistical methods to test for significant differences between the values

-to obtain measurements for static conformation every day for five days and test for variance

-to obtain measurements for stride length and range of motion every day for five days and test for variance

2. Obtaining the unique baseline data set

-to use the technique to obtain static conformation measurements for a group of Arab horses

-to use the technique to obtain measurements for stride length and range of motion for a group of Arab horses

-to provide a unique data set of baseline stride and conformation data to the Arab Horse Society and Arab stud/horse owners

2. Method

Pilot studies:

All pilot studies will take place on the yard at Myerscough College. A hard, flat surface suitable for trotting horses in hand will be used. The same horse will be used for each pilot study and will take place over two days.

1. Field of view

The field of view will be tested to determine the distance of the camera from the subject. A test area will be set up using chalk to mark one meter intervals on the ground. The horse will be trotted passed the camera three times at the set distances, to determine the field of view capable to record one full stride.

2. Camera settings

Camera settings need to be tested to determine the optimum settings according to light conditions. The settings will be tested on two days; day one (bright, clear conditions); day two (dull, overcast). The horse will be trotted passed the camera three times (at the pre-determined distance). The camera settings (shutter speed and exposure) will be altered by the researcher for each repeat. The data will be downloaded onto a laptop and analysed using a two dimensional motion analysis software package (Quintic™), and the optimum camera settings that allow the markers to be best identified (using the semi-automatic tracking feature) will be utilised.

3. Calibration

No horses will be needed for the calibration test. Three different calibration methods will be tested; a meter stick; half meter square and half meter right angle. A researcher will hold each calibration device and the device will be recorded by a video camera. The video clips will be downloaded onto a laptop and analysed using Quintic™. The calibration device that can be best aligned with the optical axis of the camera, giving the closet values to the physical measurements of the device will be used.

4. Self-adhesive tape

Prior to any markers being attached to the horse, the self-adhesive tape will be tested in a patch test. Three types of tape will be tested. A small piece of tape (10mm x 10mm) will be used to attach three markers to the horses' coat to outline the scapulohumeral joint (the same tape will be used for each marker). The horse will then be trotted in hand three times to determine if the markers remain attached securely. The horse will be inspected at the end of the test for skin irritation or changes in the condition of the coat. This will be repeated for each tape. The tape will only be used in the main study if no irritation occurs.

5. Marker size

Four varying marker sizes will be tested (50mm; 30mm; 25mm; 19mm). One horse will have three markers (same size) attached to outline the metacarpophalangeal joint. The horse will be trotted passed a video camera (at the pre-determined distance). This will be repeated for each marker size. The video clips will be downloaded onto a laptop and analysed using Quintic™, using the semi-automatic tracking feature. The smallest marker size that allows the markers to be best identified (using the semi-automatic tracking feature) will be utilised.

6. Marker material

Two marker materials will be tested (white markers on black background; markers covered in retroflective tape on black background). Three markers (of the pre-determined size) will be attached to one horse to outline the metacarpophalangeal joint. The horse will be trotted passed a video camera with 1) the white markers on black background and 2) the retroflective markers on black background. The video clips will be downloaded onto a laptop and analysed using Quintic™, using the automatic tracking feature. The markers that are most easily identifiable by Quintic™ will be utilised.

Procedure:

1. Validation

No horses will be used in the validation study. The validation study will take place in a laboratory vacuum cupboard. The validation study will verify how accurate Quintic© is.

A test rig (see figure 1.0) will be constructed to mimic a pendulum. A bearing with a low coefficient of friction will be attached to a known weight using a nylon cord (fishing line). Spherical markers (shown in black on figure 1.0) will be attached using self-adhesive tape to the centre of the pivot and on the pendulum mass. The pendulum will be set in motion, and recorded using a video camera. Ten individual pendulum swings will be recorded. The data will be downloaded onto a computer and analysed using Quintic ©. The distance travelled by the mass (length of arc); angle between the mass and the vertical; and velocity will be calculated using Quintic™ (using semi-automatic tracking) and verified using pendulum equations.

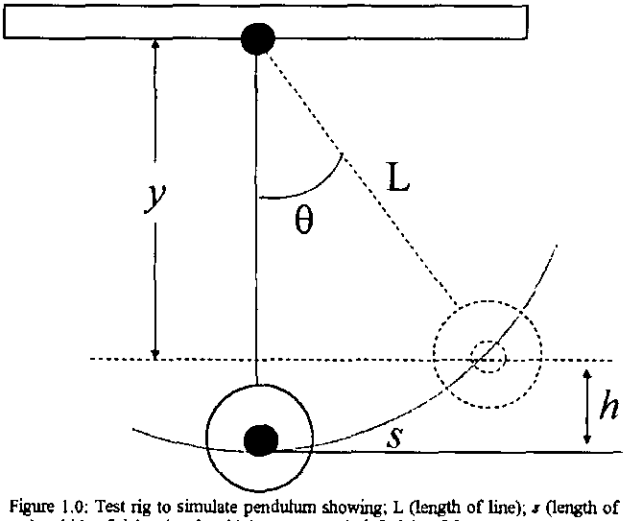

Figure 1.0: Test rig to simulate pendulum showing; L (length of line); *s* (length of arc); *y* (side of right triangle with hypotenuse L); *h* (height of the mass).

2. Standardisation of the method

This study will take place on the yard at Myerscough College using a large, flat non-slip area suitable for trotting horses in hand. The study will take place over five consecutive days.

Three horses will be used in the standardisation study to take into account the effects of inter-horse variation. Spherical markers will be attached to the horses using self-adhesive tape. The markers will be attached to specific anatomical landmarks, by the same researcher using a specific palpation method to locate the anatomical site. The horses will be stood square on a level, hard non-slip surface and recorded from the left and right (near and fore) side using two digital video cameras. The horses will be led passed the video camera to habituate the horse to the equipment. This process will also warm the horse up prior to data collection. The horses will then be trotted in hand (by the same handler to attempt to regulate velocity) in front of the cameras (at the pre-determined distance to capture one full stride) ten times for each horse. This procedure will be repeated once a day for five days (capturing 50 repeats for each horse). When the data has been downloaded, the following will be tested; variation for marker placement between days; variation for static conformation between the near and fore side; variation between stride characteristics between the near and for side; variation in stride characteristics between repeats; variation in stride characteristics between days; variation in velocity between repeats; variation in velocity between days.

3. Obtaining unique baseline data set (main study)

Data will be collected at the participating Arab studs, over a set period of time (determined by the pilot study). The data will be collected on site at the participating studs, which are all situated in the north west of the UK.

The exact method will depend on the results of the standardisation tests, however the same basic protocol (as above) will be followed. All horses used will be familiarised to the equipment (makers and video camera) prior to data collecting. The horses will be familiarised to the markers by attaching the markers on low risk areas (neck or scapula) before placing them on the distal limb. A patch test with the tape will also be performed on each horse prior to data collection. Any horse that reacts to the tape will be removed from the study. The horses will also be walked (with markers attached) passed the video camera to familiarise them to the presence of the camera (and to warm the horse up prior to data collection). The horse will be given ten minutes for the familiarisation process, if the horse appears to be stressed and is exhibiting abnormal behaviour after this time it will not be used in the study.

The results of the pilot study will be used to work out the sample size needed for the main study. It is estimated that a sample size of twenty will be adequate based on previous studies. Studies by Drevemo *et al.*, 1980a; Drevemo *et al.*, 1980b; Cano *et al.*, 1999; Degueurce *et al.*, 1997; Cano *et al.*, 2001; Galisteo *et al.*, 1996 used sample sizes ranging between 30 and 9 horses, giving a mean of 16.

Spherical markers will be attached to the horses using self-adhesive tape. The markers will be attached to specific anatomical landmarks, by the same researcher using a specific palpation method to locate the anatomical site. The horses will be stood square on a level, hard non-slip surface and recorded (from the near or fore side depending on the outcome of the pilot study) with a digital video camera. The horses will then be trotted in hand in front of the camera by the same handler (at the pre-determined settings to record one successive stride). The number of repeats will be determined by the standardisation study.

The data will be downloaded onto a laptop and analysed for stride length and range of motion using Quintic©. Inter and intra horse variation will be analysed using statistical methods. The data will be used to develop a baseline data set for each individual horse and to define normal gait characteristics for the group.

Handler / owner consent:

The owner of the stud and/or horses will be required to complete and sign an informed consent form prior to any data being collected. Handlers that will be trotting horses in front of the video camera will also be required to complete and sign an informed consent form and a physical readiness activity questionnaire (PAR-Q) form.

Confidentiality:

The horses used in the study will remain anonymous and will not be identified on the video clips or in the written report. The data will be analysed as part of a group, with no individual horses being identified. The data will be stored on a password protected computer owned by Myerscough College. Any horses or handlers of whom images are included in the thesis will give written consent for this. It may be possible that the results of this study will be published by the University or Myerscough College to peer reviewed journals and/or conferences. The results may also be released to the Arab Horse Society (written consent from the horse owner will be sought before this happens).

The information from the informed consent and PAR-Q forms will be stored in a locked filing cabinet at UCLan by the director of studies.

3a. How many, and which species of animals are intended to be used in the first year?

Pilot studies

It is intended that three horses will be used in the pilot study, and the standardisation and validation of the method. All horses will be owned and stabled at Myerscough College.

Main study

It is intended that twenty horses will be used for obtaining the unique baseline data set. All horses used in the main study will be purebred Arabs and will be owned and stabled at the various participating Arab studs.

4. What is the balance between the cost to the animals involved and the likely benefits to be gained by the research?

The risk to the horses that may occur due to the adhesive in the tape will be minimised by performing the patch test prior to data collection. Stress to the horse will be minimised prior to data being collected by familiarising the horse to the markers. This will be done by attaching the markers to low risk areas (neck and scapula) before attaching them to the distal limb. Horses will be habituated to the equipment (video camera and barrier) by walking the horse passed before data is collected.

Equine motion analysis techniques have been used in both equine locomotion research and the equine industry (Barrey, 1999; Clayton and Schamhardt, 2001). The horses used will be familiar with the process of trotting in-hand, as it is widely used in the equine industry for routine veterinary examinations and in-hand showing (Keegan, 2007). Horses will be visually assessed by the owner/ yard staff before data collection. Any horse that appears lame or exhibits behaviour that deviates from the norm will be removed from the study.

The study will provide the Arab Horse Society and Arab stud owners unique and original baseline data set of conformation and stride characteristics that can be stored and utilised at a later date.

5. Are there ways in which the procedures could be refined to reduce the cost to animals without affecting the scientific validity of the project?

The initial study to standardise the methodology will refine the procedure to reduce the cost to the animals involved. The number of repeats (how many times the horse trots passed the camera) for the main study, will be determined using two horses from the pilot study, prior to the main study taking place. This means the minimum number of repeats will be utilised in the main study, while still ensuring scientific validity.

6. Indicate what scope exists for reduction in the number of animals used and refinement in technique as the project progresses.

The method will be refined prior to data collection in the standardisation pilot studies. The sample size will be calculated statistically using the results of the pilot study and previous sample sizes from studies of a similar nature. It may be possible to reduce the sample size of the main study if inter-horse variation is minimal, without reducing the scientific validity of the study. The sample size will be calculated using the statistical power from the results of the pilot study.

7. 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.

An additional reason to justify the use of horses in this study is the benefit of the results to the Arab Horse Society, its members and the welfare of the Arab horse. The results will provide a unique baseline data set for conformation and stride characteristics of Arab horses. This has never been done before and will be very useful for future work. This data can then be utilised to monitor the effects of training, treatment or to justify selection for breeding, ultimately improving the welfare of the Arab horse and the quality of horses being bred and registered with the Arab horse society.

The costs to the horses are minimal, and the sample size will be calculated using the statistical power from the pilot study.

8. References

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Appendix 2

MYERSCOUGH COLLEGE

RISK ASSESSMENT TITLE	PROGRAMME AREA	ASSESSMENT UNDERTAKEN	ASSESSMENT REVIEW
Pilot studies (Netherby Lodge, Aughton)	Equine	**Signed: Charlotte Robin** **Date: 18th May 2009**	**Date:**

HAZARD	WHO IS AT RISK	EXISTING CONTROLS
Working alone: travelling to and working at the participating studs.	Researcher Research assistant	No one involved in the study will be working alone. The researcher will be accompanied by other members of staff. All members of the research team should carry a mobile phone on them while travelling between studs. Researchers are required to inform other members of the team of their destination and return time when travelling to and from the yard.
Working off site: risk of injury whilst not on Myerscough campus.	Researcher Research assistant	A first aid kit should be stored in the vehicle used to travel to the participating studs and should be available to all participants. A first aider should be present at the yard when data collection takes place.

Insurance: risk of accidents.	Researcher Research assistants	The yard must have public liability insurance.
Fire hazards	Researcher Research Assistant Handler	Check fire instructions in case of fire.
Loading, unloading and moving of equipment: muscle strain or injury.	Researcher Research assistant	Follow the guidelines from the Myerscough College yard induction; load to be close to the body; feet apart; do not jerk, twist or shove; straight back; use thigh muscles to push; do not lift away from body; do not lift weight you are not capable of.
Safety of electrical equipment: risk of harm due to faulty electrical equipment.	Researcher Research assistant	Portable appliances are required to be PAT tested according to the Provision and Use of Work Equipment Regulations (1998) and the Electricity at Work Regulations (1989). If equipment needs to be connected to the mains electricity supply a circuit breaker should be used.
Wires and cables on the yard: risk of injury from tripping over cables.	Researcher Research assistant Handler	Wires or cables on the yard should be covered with rubber mats to stop yard staff, students and horses walking over them. Safety notices should be displayed for personnel on the yard.
Camera positioning: camera could cause an obstruction to participants or horse. Camera (and operator) could be knocked over.	Researcher Research assistant Handler	Camera must be positioned behind a suitable barrier such as a jump wings/poles. All participants to be aware of the camera positioning. Remove all calibration equipment from the area before recording commences.

Inappropriate clothing: for working outdoors.	Researcher Research assistant Handler	Follow the guidelines from the Myerscough College yard induction. Appropriate clothing must be worn at all times, waterproof/warm clothing (dependent on weather conditions) and suitable footwear (sturdy, with a small heel). No jewellery and hair must be fastened back.
Palpation of horse, positioning and removal of skin markers: horse may be startled by procedure; horse may bite, kick, stand on, or knock into handler.	Researcher Handler	Positioning of the markers will take place in a stable. The horse must be controlled by a handler during the palpation procedure and during positioning and removal of markers. A hat must be worn when placing markers on horses to standard **BS PAS 015 1998; BS EN 1384; ASTMF 1163 95** (recommended by the BHS). Researcher and handler to stand on the same side of the horse. Stable door to be closed at all times. Horses will be familiarised to the markers by placing them on low risk areas first (neck or scapula) before placing them on the distal limb.
Leading the horse in front of the camera: horse may be startled by equipment; horse may bite, kick, stand on or knock into handler.	Researcher Research assistant Handler	Gloves and a hat must be worn when leading horses to standard **BS PAS 015 1998; BS EN 1384; ASTMF 1163 95** (recommended by the BHS). Horses will be allowed to become familiarised to the equipment (video camera and tripod) prior to data being collected. This will be done by leading the horse passed the camera to allow the horse to become accustomed to it. Researcher and handler to wear standard hat at all times during data collection.
Controlling the horse: horse may become strong when led.	Researcher Research assistant Handler	Horses can be led in bridles if appropriate. Lunge lines can be used for extra control when required (agreed by yard manager/owner).

Risk to the horse on the day: health, stress or injury.	Horse	Horses will be visually assessed by the owner and researcher prior to data collection. If the horse is lame or appears to be displaying behaviour that deviates from the norm, the horse will not be used in the study.
Allergies: risk of participants becoming ill due to allergies associated with horses.	Researcher Research assistant Handler	All participants will be required to disclose any known allergies associated with horses prior to the study taking place, and if so will not be allowed to continue with the study.

Appendix 2

MYERSCOUGH COLLEGE

RISK ASSESSMENT TITLE	PROGRAMME AREA	ASSESSMENT UNDERTAKEN	ASSESSMENT REVIEW
Main study Birkrigg Park, Kendal LA8 0DY 015395 60621 **(Liz Titterington)**	Equine	**Signed: Charlotte Robin** **Date: 19th June 2009**	**Date:**

HAZARD	WHO IS AT RISK	EXISTING CONTROLS
Working alone: travelling to and working at the participating studs.	Researcher Research assistant	No one involved in the study will be working alone. The researcher will be accompanied by other members of staff. All members of the research team should carry a mobile phone on them while travelling between studs.
Working off site: risk of injury whilst not on Myerscough campus.	Researcher Research assistant	A first aid kit should be stored in the vehicle used to travel to the participating studs and should be available to all participants. A first aider should be present at the yard when data collection takes place.

Insurance: risk of accidents.	Researcher Research assistants	The yard must have public liability insurance.
Fire hazards	Researcher Research Assistant Handler	Check fire instructions in case of fire.
Loading, unloading and moving of equipment: muscle strain or injury.	Researcher Research assistant	Follow the guidelines from the Myerscough College yard induction; load to be close to the body; feet apart; do not jerk, twist or shove; straight back; use thigh muscles to push; do not lift away from body; do not lift weight you are not capable of.
Safety of electrical equipment: risk of harm due to faulty electrical equipment.	Researcher Research assistant	Portable appliances are required to be PAT tested according to the Provision and Use of Work Equipment Regulations (1998) and the Electricity at Work Regulations (1989). If equipment needs to be connected to the mains electricity supply a circuit breaker should be used.
Wires and cables on the yard: risk of injury from tripping over cables.	Researcher Research assistant Handler	Wires or cables on the yard should be covered with rubber mats to stop yard staff, students and horses walking over them. Safety notices should be displayed for personnel on the yard.

Camera positioning: camera could cause an obstruction to participants or horse. Camera (and operator) could be knocked over.	Researcher Research assistant Handler	Camera must be positioned behind a suitable barrier such as a jump wings/poles. All participants to be aware of the camera positioning. Remove all calibration equipment from the area before recording commences.
Inappropriate clothing: for working outdoors.	Researcher Research assistant Handler	Follow the guidelines from the Myerscough College yard induction. Appropriate clothing must be worn at all times, waterproof/warm clothing (dependent on weather conditions) and suitable footwear (sturdy, with a small heel). No jewellery and hair must be fastened back.
Palpation of horse, positioning and removal of skin markers: horse may be startled by procedure; horse may bite, kick, stand on, or knock into handler.	Researcher Handler	Positioning of the markers will take place in a stable. The horse must be controlled by a handler during the palpation procedure and during positioning and removal of markers. A hat must be worn when placing markers on horses to standard **BS PAS 015 1998; BS EN 1384; ASTMF 1163 95** (recommended by the BHS). Researcher and handler to stand on the same side of the horse. Stable door to be closed at all times. Horses will be familiarised to the markers by placing them on low risk areas first (neck or scapula) before placing them on the distal limb.

Leading the horse in front of the camera: horse may be startled by equipment; horse may bite, kick, stand on or knock into handler.	Researcher Research assistant Handler	Gloves and a hat must be worn when leading horses to standard **BS PAS 015 1998; BS EN 1384; ASTMF 1163 95** (recommended by the BHS). Horses will be allowed to become familiarised to the equipment (video camera and tripod) prior to data being collected. This will be done by leading the horse passed the camera to allow the horse to become accustomed to it. Researcher and handler to wear standard hat at all times during data collection.
Controlling the horse: horse may become strong when led.	Researcher Research assistant Handler	Horses should be led in bridles if possible. In hand bridles can be used for horses that cannot wear bridles. Lunge lines should be used to provide extra control.
Risk to the horse on the day: health, stress or injury.	Horse	Horses will be visually assessed by the owner and researcher prior to data collection. If the horse is lame or appears to be displaying behaviour that deviates from the norm, the horse will not be used in the study.
Allergies: risk of participants becoming ill due to allergies associated with horses.	Researcher Research assistant Handler	All participants will be required to disclose any known allergies associated with horses prior to the study taking place, and if so will not be allowed to continue with the study. This information is on the informed consent form that is required to be read and signed by all participants.

Appendix 3

Participant Information Sheet

Horse Owner

Dear ,

You have been invited to participate in a piece of research which will aim to standardise equine motion analysis techniques. This form provides basic information regarding the testing and also asks for you to agree for your horses to be used in the testing. Such information and agreement is referred to as informed consent. The following information is designed to provide you with answers to questions you may have. Please feel free to ask any other questions to enable you to feel happy to provide consent to take part.

What will I have to do?
As the horse owner you will not be required to take part in the study, unless you request to do so. If you do want to participate in the study (to hold or lead horses) you will be required to complete a PAR-Q form (Physical Activity and You Questionnaire) which will ensure your physical ability to take part.

All the horses involved in the study will undergo the same protocol. Firstly, horses will have circular markers attached to specific anatomical landmarks using self-adhesive tape. This will be done by an experienced researcher through a set palpation method. This process will be carried out in an enclosed environment (preferably a stable) with the horse being held by a handler (either a member of your staff or the Myerscough College research team).

A video camera (on a tripod) will be set up on the yard behind a suitable barrier (such as a jump pole on a block). The horses will be led (in a bridle or head collar) passed the camera a few times to accustom the horse to the cameras presence. This process will also warm the horse up prior to data collection. The horse will be stood square and recorded for a conformation shot. The horses will then be trotted passed the camera ten times by the handler. The handler will be either a member of the Myerscough College research team, or yourself depending on personal preference. Once the data has been recorded, the horse will be returned to the stable and the markers will be carefully removed.

N.B. All Myerscough College research staff have undergone a yard induction and will be capable of trotting horses in hand.

What are the risks of taking part?
The risks to the horses taking part in the study are minimal. The main risks would be the attachment of the markers with adhesive tape which may cause irritation to the horses skin (a patch test will be performed to minimise this risk). The horses may become stressed during the procedure (either attaching the markers or trotting the horse passed the camera), causing a risk to the horse or handler, in which case it will be removed from the study. A full risk assessment has been carried out by Myerscough College to minimise potential risks to the horses and handlers, which is available on request.

Appendix 3

Do you have to take part?
Participation is entirely voluntary. You are free to withdraw at any time from the study during the testing phase. Once the testing phase is completed (for each horse) it is not possible to withdraw results as they will be anonymous and will therefore not be distinguishable from the group.

NB. Please inform the researcher if any you have allergies associated with horses prior to the study taking place, as you will be asked to withdraw from the study.

What will happen to my data?
All data that is collected from your participation will be anonymous and it will be stored numerically so it cannot be traced back to you. The results from each horse from your stud will go together with the rest of the group and they will be analysed and written up as part of my thesis. The data will be stored on a password protected computer at Myerscough College. It is also possible that the results of this study may be published by the University or College to peer reviewed journals and/or conferences. The information you provide before the study takes place (and the information from the PAR-Q) is to ensure the safety and eligibility of you and your staff. The information will be stored in a locked filing cabinet by my director of studies at the University of Central Lancashire. It will not be shared or given to any third parties.

Ethical Consent
Ethical consent for the study *has been applied for to the School of Psychology Ethics Committee, University of Central Lancashire and Myerscough College.

*N.B. This will be modified to 'has been approved by' when approval is granted

Please note that if you prefer your own yard staff to handle the horses (lead the horses in front of the camera) they will be required to sign the form as they will be recorded by the camera. The data will be stored on a password protected computer.

If you agree to undertake this testing please sign the section overleaf. It is a requirement you provide a signature to reflect agreement to perform the research.

Appendix 3

Agreement to testing

I understand the risks associated with this study and that all the data produced will be treated with confidentiality and individually. However the anonymous results may be used in possible future publications. If I wish, the results produced will be available to me.

I willingly agree to participate in the current study. I have read the above information and understand that withdrawal from the study is possible until all data has been collected.

Name of owner;

Print Name: .....

Signature: .....

Date: ...../...../.....

Name of witness;

Print name:.....

Signature:.....

Date: ...../...../.....

All communications should be made to;

Dr Sarah Jane Hobbs,

Senior Lecturer in Sport and Exercise,

Centre for Applied Sport and Exercise Sciences,

University of Central Lancashire,

Preston,

PR1 2HE

Tel: 01772 893328

Email:SJHobbs1@uclan.ac.uk

Appendix 3

Participant Information Sheet

Yard Owner

Dear

You have been invited to participate in a piece of research which will aim to define normal conformation and gait characteristics in Arab horses. This form provides basic information regarding the testing and also asks for you to agree for your horses to be used in the testing. Such information and agreement is referred to as informed consent. The following information is designed to provide you with answers to questions you may have. Please feel free to ask any other questions to enable you to feel happy to provide consent to take part.

What will I have to do?
As the stud/horse owner you will not be required to take part in the study, unless you request to do so. If you do want to participate in the study (to hold or lead horses) you will be required to complete a PAR-Q form (Physical Activity and You Questionnaire) which will ensure your physical ability to take part.

All the horses involved in the study will undergo the same protocol. Firstly, horses will have circular markers attached to specific anatomical landmarks. This will be done by an experienced researcher through a set palpation method. This process will be carried out in an enclosed environment (preferably a stable) with the horse being held by a handler (either a member of your staff or the Myerscough College research team).

A video camera (on a tripod) will be set up on the yard behind a suitable barrier (such as a jump pole on a block). A large, flat non-slip area will be needed suitable for trotting horses in hand. The horses will be led (in a bridle or head collar) onto the yard by the handler and passed the camera a few times to accustom the horse to the cameras presence. This process will also warm the horse up prior to data collection. The horse will be stood square on the yard and recorded for a conformation shot. The horses will then be trotted passed the camera five times (each side) by the handler. The handler will be either a member of the Myerscough College research team, or your own yard staff depending on personal preference. Once the data has been recorded, the horse will be returned to the stable and the markers will be carefully removed. Rugs will be put back on if appropriate.

N.B. All Myerscough College research staff have undergone a yard induction and will be capable of trotting horses in hand.

What are the risks of taking part?
The risks to the horses taking part in the study are minimal. The main risks would be the attachment of the markers which may cause irritation to the horses skin (a patch test will be performed to minimise this risk). The horses may become stressed during the procedure (either attaching the markers or trotting the horse passed the camera), causing a risk to the horse or handler, in which case it will be removed from the study. A full risk assessment has been carried out by Myerscough College to minimise potential risks to the horses and handlers, which is available on request.

Do you have to take part?
Participation is entirely voluntary. You are free to withdraw at any time from the study during the testing phase. Once the testing phase is completed (for each horse) it is not possible to withdraw results as they will be anonymous and will therefore not be distinguishable from the group.

NB. Please inform the researcher if any you have allergies associated with horses prior to the study taking place, as you will be asked to withdraw from the study.

What will happen to my data?
All data that is collected from your participation will be anonymous and it will be stored numerically so it cannot be traced back to you. The results from each horse from your stud will go together with the rest of the group and they will be analysed and written up as part of my thesis. The data will be stored on a password protected computer at Myerscough College. It is also possible that the results of this study may be published by the University or College to peer reviewed journals and/or conferences. The information you provide before the study takes place (and the information from the PAR-Q) is to ensure the safety and eligibility of you and your staff. The information will be stored in a locked filing cabinet by my director of studies at the University of Central Lancashire. It will not be shared or given to any third parties.

Ethical Consent
Ethical consent for the study *has been applied for to the School of Psychology Ethics Committee, University of Central Lancashire and Myerscough College.

*N.B. This will be modified to 'has been approved by' when approval is granted

Please note that if you prefer your own yard staff to handle the horses (lead the horses in front of the camera) they will be required to sign the form as they will be recorded by the camera. The data will be stored on a password protected computer.

If you agree to undertake this testing please sign the section overleaf. It is a requirement you provide a signature to reflect agreement to perform the research.

Appendix 3

Agreement to testing

I understand the risks associated with this study and that all the data produced will be treated with confidentiality and individually. However the anonymous results may be used in possible future publications. If I wish, the results produced will be available to me.

I willingly agree to participate in the current study. I have read the above information and understand that withdrawal from the study is possible until all data has been collected.

Name of stud owner;

Print Name: .....

Signature: .....

Date: ...../...../.....

Name of witness;

Print name:.....

Signature:.....

Date: ...../...../.....

All communications should be made to;

Dr Sarah Jane Hobbs,

Senior Lecturer in Sport and Exercise,

Centre for Applied Sport and Exercise Sciences,

University of Central Lancashire,

Preston,

PR1 2HE

Tel: 01772 893328

Email:SJHobbs1@uclan.ac.uk

Appendix 3

Participant Information Sheet

Yard Staff

Dear Sir/Madam,

You have been invited to participate in a piece of research which will aim to define normal conformation and gait characteristics in Arab horses. This form provides basic information regarding the testing and also asks for you to agree for you to take part. Such information and agreement is referred to as informed consent. The following information is designed to provide you with answers to questions you may have. Please feel free to ask any other questions to enable you to feel happy to provide consent to take part.

What will I have to do?
Yard staff may be required to participate in the study, to hold or lead horses. If you do take part in the study, you will have to complete a Physical Activity and You Questionnaire (PAR-Q) to ensure physical ability to take part.

All the horses involved in the study will undergo the same protocol. Firstly, horses will have circular markers attached to specific anatomical landmarks. This will be done by an experienced researcher through a set palpation method. This process will be carried out in an enclosed environment (preferably a stable) with the horse being held by a handler (either a member of your staff or the Myerscough College research team).

A video camera (on a tripod) will be set up on the yard behind a suitable barrier (such as a jump pole on a block). A large, flat non-slip area will be needed suitable for trotting horses in hand. The horses will be led (in a bridle or head collar) onto the yard by the handler and passed the camera a few times to accustom the horse to the cameras presence. This process will also warm the horse up prior to data collection. The horse will be stood square on the yard and recorded for a conformation shot. The horses will then be trotted passed the camera five times (each side) by the handler. The handler will be either a member of the Myerscough College research team, or your own yard staff depending on personal preference. Once the data has been recorded, the horse will be returned to the stable and the markers will be carefully removed. Rugs will be put back on if appropriate.

N.B. All Myerscough College research staff have undergone a yard induction and will be capable of trotting horses in hand.

What are the risks of taking part?
The risks to the horses taking part in the study are minimal. The main risks would be the attachment of the markers which may cause irritation to the horses skin (a patch test will be performed to minimise this risk). The horses may become stressed during the procedure (either attaching the markers or trotting the horse passed the camera), causing a risk to the horse or handler, in which case it will be removed from the study. A full risk assessment has been carried out by Myerscough College to minimise potential risks to the horses and handlers, which is available on request.

Do you have to take part?
Participation is entirely voluntary. You are free to withdraw at any time from the study during the testing phase. Once the testing phase is completed (for each horse) it is not possible to withdraw results as they will be anonymous and will therefore not be distinguishable from the group.

NB. Please inform the researcher if any you have allergies associated with horses prior to the study taking place, as you will be asked to withdraw from the study.

What will happen to my data?
All data that is collected from your participation will be anonymous and it will be stored numerically so it cannot be traced back to you. The results from each horse from your stud will go together with the rest of the group and they will be analysed and written up as part of my thesis. The data will be stored on a password protected computer at Myerscough College. It is also possible that the results of this study may be published by the University or College to peer reviewed journals and/or conferences. The information you provide before the study takes place (and the information from the PAR-Q) is to ensure the safety and eligibility of you and your staff. The information will be stored in a locked filing cabinet by my director of studies at the University of Central Lancashire. It will not be shared or given to any third parties.

Ethical Consent
Ethical consent for the study *has been applied for to the School of Psychology Ethics Committee, University of Central Lancashire and Myerscough College.

*N.B. This will be modified to 'has been approved by' when approval is granted

Please note that if you prefer your own yard staff to handle the horses (lead the horses in front of the camera) they will be required to sign the form as they will be recorded by the camera. The data will be stored on a password protected computer.

If you agree to undertake this testing please sign the section overleaf. It is a requirement you provide a signature to reflect agreement to perform the research.

Appendix 3

Agreement to testing

I understand the risks associated with this study and that all the data produced will be treated with confidentiality and individually. However the anonymous results may be used in possible future publications. If I wish, the results produced will be available to me.

I willingly agree to participate in the current study. I have read the above information and understand that withdrawal from the study is possible until all data has been collected.

Name of yard staff;

Print name:.....

Signature:.....

Date...../...../.....

Name of witness;

Print name:.....

Signature:.....

Date: ...../...../.....

All communications should be made to;

Dr Sarah Jane Hobbs,

Senior Lecturer in Sport and Exercise,

Centre for Applied Sport and Exercise Sciences,

University of Central Lancashire,

Preston,

PR1 2HE

Tel: 01772 893328

Email:SJHobbs1@uclan.ac.uk

Appendix 4

1.0 Inter -horse variation

Inter-horse variation measured the amount of variation between individual horses in the study.

1.1 Conformation

The majority of traits measured were established as significantly different between horses (16 out of 19). The traits that did not demonstrate significant differences between horses were length of radius and fore proximal phalanx and tarsal angle.

Table A4.0: Inter-horse variation in conformation traits. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Trait	Horse
	Significance
Neck (cm)	***
Scapula (cm)	*
Humerus (cm)	**
Radius (cm)	NS
Fore metacarpal (cm)	**
Fore proximal phalanx (cm)	NS
Pelvis (cm)	***
Femur (cm)	***
Tibia (cm)	***
Hind metacarpal (cm)	***
Hind proximal phalanx (cm)	***
Neck (^{O})	***
Humeroradial (^{O})	*
Coxofemoral (^{O})	***
Femorotibial (^{O})	***
Hind metacarpophalangeal (^{O})	***
Scapulohumeral angle	***
Fore metacarpophalangeal angle	***
Tarsal angle	NS

1.2 Stride length and velocity

Forelimb stride length was not significantly different between horses for the left or right side (table A4.1). Hind limb stride length did vary significantly between horses, over the trial. Horse one SL was consistently shorter than horses two and three ($P<0.001$). Small variations were established between horse two and three, however these variations were not significant ($P>0.05$) except on day four where horse two had significantly longer SL than horse three.

A4.1: Inter-horse variation in stride length in trot and velocity for forelimb, hind limb (for left and right side). Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Variable	Horse
Left forelimb stride length	NS
Right forelimb stride length	NS
Left hind limb stride length	***
Right hind limb stride length	***
Velocity	***

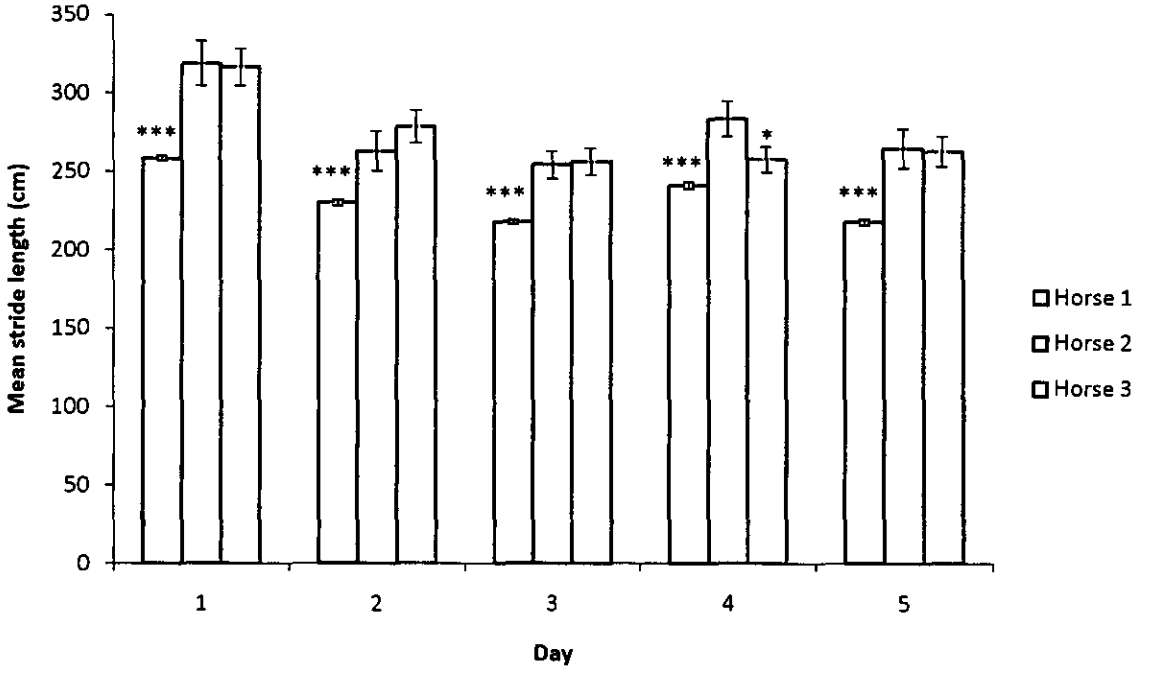

Figure A4.0: Inter-horse variation in hind limb stride length (±SE) for the left side over five consecutive days. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001.

1.3 Range of motion

All joint ROM was established as significantly different between horses (table A4.2). Figure A4.1 demonstrates scapulohumeral ROM as an example of this. Horse one scapulohumeral ROM was significantly smaller than horse two and three for all days ($P<0.001$). Initially horse three had larger scapulohumeral ROM than horse two (on days one and two), however on day three horse two had a larger scapulohumeral ROM than horse three. On day four and five, the same pattern resumed, horse three had a larger scapulohumeral ROM than horse two.

Table A4.2: Inter-horse variation in ROM for each joint measured. Levels of significance are indicated by *P<0.05; **P<0.005; ***P<0.001; NS not significant.

Joint (°)	Horse
	Significance
Scapulohumeral	***
Humeroradial	***
Carpal	***
Fore metacarpophalangeal	***
Coxofemoral	***
Femorotibial	***
Tarsal	***
Hind metacarpophalangeal	***

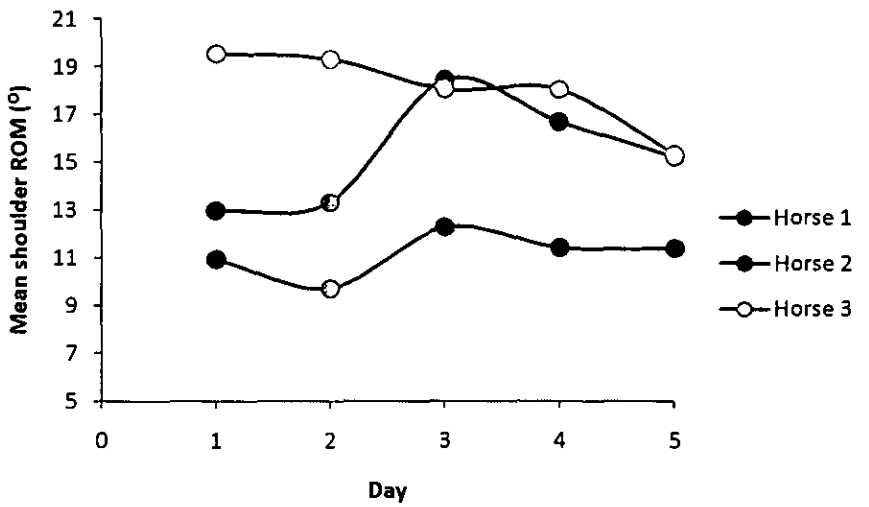

Figure A4.1: Mean scapulohumeral ROM for subjects 1-3 for five consecutive days showing a significant difference between horses ($P<0.001$).

1.4 Summary

Analysis of inter-horse variation illustrated the large amounts of variation between individual horses. Most of the conformation traits measured were established as different between horses, except for radius length, fore proximal phalanx length and tarsal angle. Stride length for horse one was significantly shorter than horses two and three on all days. Horse one also demonstrated the highest amounts of intra-horse

variation during the trial. Horses two and three had similar stride length that did not vary except on day four. ROM for all joints measured were different between horses.