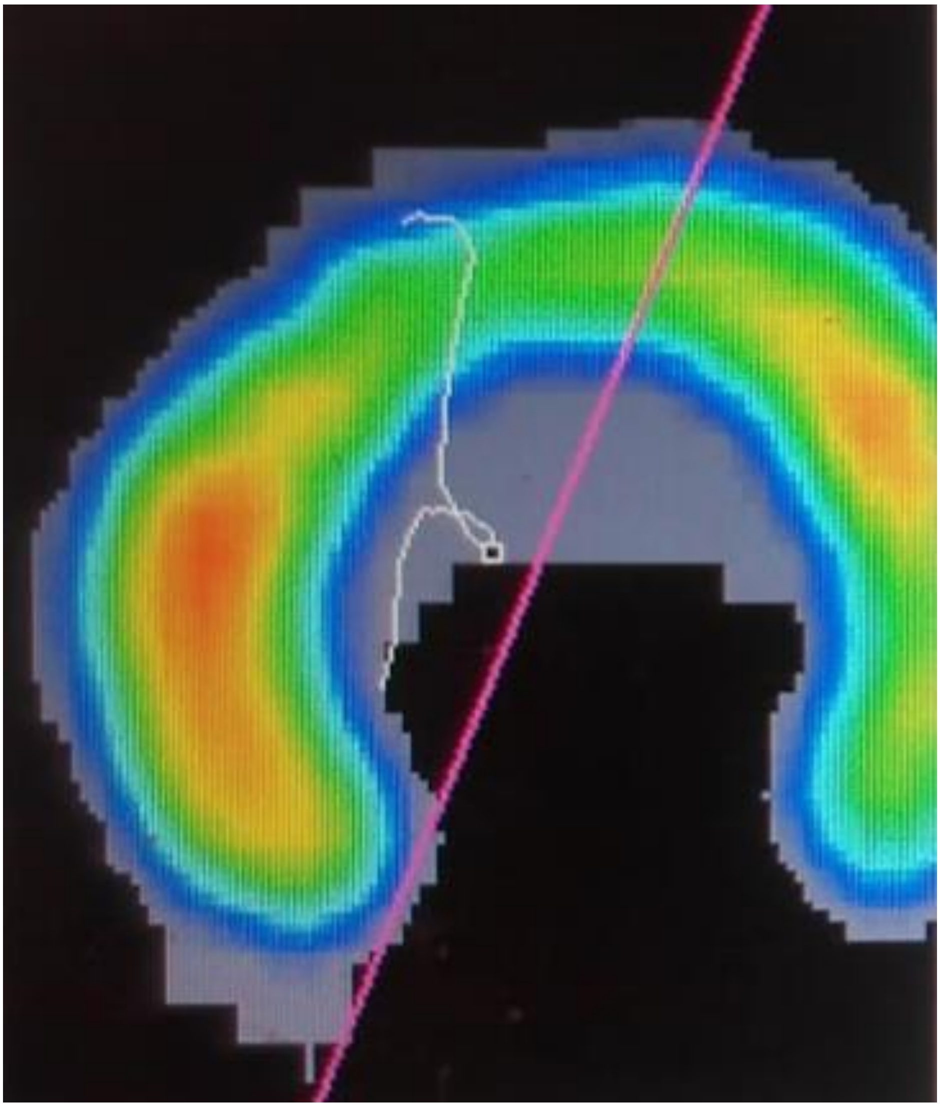
Repeatability of the Center of Pressure during a six-week shoeing interval



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Laura van Hemmen August 2019
Studentnumber: 3671542

Supervisors:
- Dr. Sandra Nauwelaerts
Department of Biology, Faculty of Science, University of Antwerp, Belgium
- Dr. Wim Back
Department of Equine Sciences, Faculty Veterinary medicine, Utrecht University, The Netherlands

Assessor:
Dr. Harold Brommer
Department of Equine Sciences, Faculty Veterinary medicine, Utrecht University, The Netherlands

Abstract

Lameness in the horse is one of the most common equine health problems in the world. Several systems, based on kinetics or kinematics, have been developed to make the observations of the lameness more objective. A new system based on measuring the center of pressure (COP) is using a combination of both. The center of pressure (COP) pathway quantifies the dynamic load distribution under the hoof. From this pattern it is concluded that the repeatability in one day is high. The objective was to investigate the repeatability of this pattern during a six-week shoeing interval. The repeatability of the COP pathway system is also compared with the repeatability of the Qhorse system.

A group of six owner- sound horses were used in the study which lasted, due to a period of quarantine of the horses in the middle of the study, 55 days. Measurements took place every other day. For the Qhorse data the horses trotted three on the straight line. Using 3D optical motion capture kinematic data were collected. Symmetry parameters minDiff and maxDiff (difference between the two minima/maxima of the movement) of the head, withers and pelvis were calculated. For the COP data the horses trotted over a pressure plate until four hits per hoof were obtained. In a hoof-bound coordinate system the COP path was determined. An analysis of variance was used for both systems to test for the effect of measurement day and horse or limb, the sum of squares of the between and within subject effects were calculated to access repeatability.

The repeatability of the COP pattern during a six- week shoeing cycle was low . The repeatability of the Qhorse system for individual horse was high and values of minDiff and maxDiff for head, withers and pelvis were consistent for an individual. The repeatability of the COP pattern and Qhorse were low ($r < 0,4$) for measuring the locomotion of a horse every other day during one trimming cycle. Main limitations of the study were the position of the markers, the extended study period and the possible influence of the handler on the measurements. Further research of the repeatability of the COP pattern is required with a system that measures more than one step at the pressure plates. A longer period in which several hoof trimming intervals can be compared is recommended.

Table of Content

Introduction

Lameness

Lameness in horses is a widespread problem. It is considered the main cause of loss of use in horses. Moreover, it is known as one of the most expensive medical problems (USDA). There are several definitions of lameness, but the most common definition is: ‘lameness is the result of a disorder in locomotion function´. Lameness could be an indication of a functional or structural problem in the locomotor system. It is not per se a disease, but a clinical sign that results in gait changes characterized by limping. Due to these changes, lameness is a clinical problem and it is one of the main reasons for equine veterinary consultation (Nielsen et al., 2014; Ross et al., 2011a; van Weeren et al., 2017). Lameness has been classified into three categories; weight bearing, non-weight bearing and a combination of both. Weight bearing or supporting leg lameness describes a lameness that is most of the time pain related, because of the weight on the lame leg in the stance phase of the stride. Most lameness conditions are of this type. Non-weight bearing or swinging limb lameness describes a lameness in which the way the horse carries the lame limb is affected; the swing phase of the lame limb reduces (Ross et al., 2011b). Forelimb lameness can be recognized by dropping of the head and neck when the non-lame foot lands, and rising of the head when the lame limb is weight bearing. Similarly, the symmetry of the pelvic movement is used to diagnose hind limb lameness. An asymmetrical movement of the pelvis or pelvic hike is an important recognition. The pelvic hike is the upwards movement of the pelvis when the lame limb hits the ground, and a downward movement when the sound limb is weight bearing. In other words, an asymmetrical movement of the pelvis occurs (Buchner et al., 1996; Ross et al., 2011b).

Lameness detection

The most used way of detecting lameness in clinical practice is a subjective visual examination of the gait of the horse under different conditions, like walking and/or trotting on a straight line and a circle. The most important and commonly used features in detecting lameness are the presence or absence of asymmetry in the locomotor system and the degree of asymmetry (van Weeren et al., 2017). When the asymmetry is above predefined threshold this is an indication for lameness (Weishaupt et al., 2006). There are several lameness scoring systems that allow the veterinarian to quantify lameness, for example the system by the American Association of Equine Practitioners (AAEP) with a scale from 0 to 5. Ideally the scoring method should be consistent worldwide, however every system has its own scale and definitions (Ross et al., 2011b). Added to this, the ability of the eye to correctly recognize asymmetries is limited and there is a difference in consistency between experienced and non-experienced veterinarians (Parkes et al., 2009). In conclusion, visual examination is not an objective and accurate way of examination. Therefore, in recent years, several systems have been developed to assist the veterinarian in lameness detection, and to make the observations more objective (Keegan et al., 2010). The measurement techniques for objective gait assessment are either based on kinetics (forces arising from the musculoskeletal system) or kinematics (motion of the musculoskeletal system). In this proposed study, we will compare the Qhorse system with a system that combines kinetics (pressure plates) with kinematics (Qualisys cameras). This system measures the Center of Pressure (COP) pathway.

Qhorse

Horses move upwards and downwards twice in the stride cycle with their head, withers and pelvis. In a non-lame horse during straight line trot, the amplitude of the movement is symmetrical and the curves of the vertical displacement of the head, withers and tuber sacral show a sinusoidal pattern. At the moment that a horse is lame, the sinusoidal pattern becomes asymmetrical and the changes in vertical displacement curves can be measured (Buchner et al., 1996; Keegan et al., 2011). Due to changes in the amplitude of the vertical displacement of the lame limb, the minimum (during stance phase) and maximum (end of the stance phase) positions of the head, withers and pelvis, differ in comparison with the non-lame limb. In a lame limb those positions reach a minimum that is higher and a maximum that is lower when compared with the contralateral non-lame limb. The difference between minimum positions between two consecutive steps is the minDiff value and the difference between maximum positions between two consecutive steps is the maxDiff value (Fig. 1). When lameness occurs, the values of the minDiff and maxDiff increase in the lame limb (Buchner et al., 1996; Ross et al., 2011b; Keegan et al., 2011).

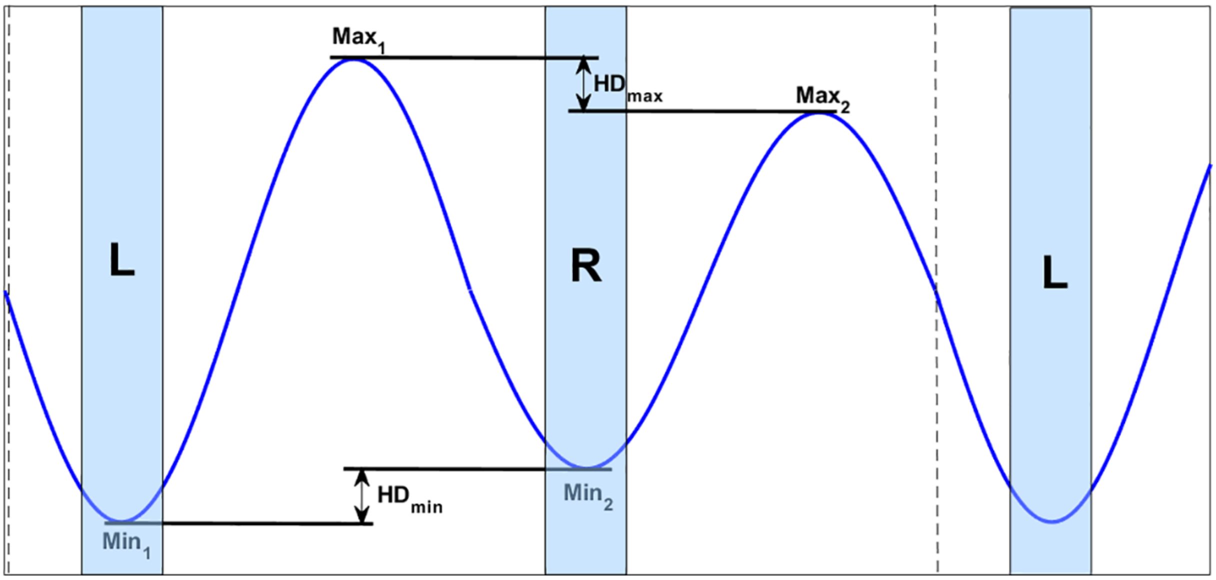

Figure 1. Example of vertical head movement in case of a right forelimb lameness, resulting in positive HDmin (minDiff) and HDmax (maxDiff) values. The light blue bars indicate which forelimb (L= left/ R= right) is in the stance phase. The vertical withers and pelvis movements are calculated in an identical way (Rhodin et al., 2017).

One of the systems today for objective kinematic gait analysis in horses is the Qhorse system (Qualisys AB, Sweden); a three-dimensional optical motion capture (OMC) system that uses several cameras positioned around a calibrated measuring volume to quantify the movement pattern of the horse by tracking the position of several reflective markers on the horses body. The system measures the asymmetry in the head, trunk and pelvis movement and lameness detection is based on minDiff and maxDiff values. The asymmetry analysis tool, Qhorse, calculates and visualizes the locomotion of the horse and enables users to capture movements that are hard or impossible to detect with the naked eye (Qhorse booklet, 2019). A 3D OMC system is highly accurate and precise and therefore it is considered the 'gold standard' for kinematic gait analysis (Serra Braganca et al., 2018).

COP

The Center of Pressure (COP) is a unique point underneath the hoof where all net pressures of the interaction between all of the forces and torques that occur in the limb comes together and it quantifies the dynamic load distribution under the hoofs (Nauwelaerts et al., 2017). Several studies have shown that the human gait and the COP can be used as a measure for foot function (De Cock et al., 2008). By tracking the position of the COP over time, the COP path, the balance and subject's mode of locomotion can be determined during the stance phase. Research shows that the COP is highly consistent in normal gait (Han et al., 1999). In horses, the repeatability of the COP path in one day is high. Different shapes in COP path were detected, but a shape is unique to the limb and the individual (Nauwelaerts et al., 2017).

Repeatability

By calculating the repeatability it is possible to show how consistent measurements are (Harper, 1994). In an ideal situation, repeated measurements of the same horse would be identical. However, due to inevitably errors of observers and change in trait size between observations it is not. Besides, the gait of a horse can be very variable, within an individual as well as across different individuals. The range of the repeatability (r) is from 0 to 1. The value expresses the proportion of variation in a trait that is due to differences among individuals, not due to differences within an individual. The average within individual variation will be low if the average individual is consistent. So the ratio of among individual variation to within individual variation (the repeatability) will be high. A repeatability of zero shows that a repeated measurement of the same horse is no more similar than those collected from different, randomly chosen, horses. A repeatability of one indicates perfectly consistent measurements. Repeatability's with negative values are theoretically possible (minimum -1), but uncommon. The repeatability will automatically increase by taking several measurements of the same trait during one trial.

Aim of the Study

In this study, a new system that measures the COP pathway in a six-week shoeing interval will be compared with an already proven system, the 'golden standard' at this moment. The Qhorse system investigates the horse in total and looks to asymmetries in head, trunk and pelvis movements. The COP pathway system focusses on one leg. Lameness is not always characterized by the typical locomotor asymmetries. Horses suffering from bilateral lameness have a locomotion pattern described as short, stiff or shuffling, characteristics that are difficult to differentiate from individual normal locomotor patterns of a horse (Buchner et al., 1995). There are also several owner-sound horses that show motion asymmetries, but it is not clear in which way these asymmetries are caused by pain and hence a potential welfare problem or related to bad performances (Rhodin et al., 2017). Because the Qhorse system is only searching for the asymmetries, not all the lameness can be detected or the interpretation of an asymmetry is possibly wrong. By using the COP pathway system, that focussed on changes of the locomotion system in one leg, in a longitudinal study the research team hopes to solve these problems. This study is part of a larger study. Long term purpose of the large study is to see if changes in the locomotor apparatus can be picked up at an early stage by the COP path and could therefore be used as an early detection tool for lameness/asymmetry (Nauwelaerts et al., 2017). Not only for veterinarians, but also for horse owners in their daily routine.

As said before the repeatability of the COP path in one day is high, however the repeatability of the COP path in a six- week shoeing interval still needs to be investigated. The first aim of the study is about the repeatability of the COP system during a six- week shoeing interval. Is the repeatability of the measurements over several days as high as in one day? The second aim of this study is to investigate whether both objective systems for gait analysis, the COP pathway system or the Qhorse system, have similar repeatability's. If the COP path is not repeatable in a six- week shoeing interval, is that due to the system or is it due to way of measuring the COP path? To test this idea the COP system is compared with the Qhorse system, of which it is know that the repeatability of measurements is high.

Hypothesis

H_0 = The COP path is as repeatable during a six-week shoeing interval as in one day.

H_1 = The COP path is not as repeatable during a six-week shoeing interval as in one day.

H_0 = The COP pathway system and the Qhorse system are as repeatable in evaluating the locomotion apparatus of the horse over the entire trimming cycle.

H_1 = The COP pathway system and the Qhorse system are not as repeatable in evaluating the locomotion apparatus of the horse over the entire trimming cycle.

Materials and Methods

Horses

A group (n=6) of visually owner-sound horses (mares) of different breeds (one riding type Royal Dutch Warmblood horse, four harness type Royal Dutch Warmblood horses and one Friesian), age range of 8 to 14 years (average 11 years), with a body mass of 517 to 653 kg (mean 580 kg) participated in this study (Table 1.). All horses were shod. The horses are owned by Utrecht University and are used for the education of veterinary science students and participate in riding lessons by study association V.S.R. de Solleysel. On day zero of the study all the horses were trimmed shod by a farrier. Each horse was measured every other weekday for six weeks (one shoeing cycle).

Table 1. Overview of the horses participated in the study.

Horse	Name	Breed
1.	Amalia	Royal Dutch Warmblood – Harness type
2.	Brianna	Royal Dutch Warmblood – Harness type
3.	Colinda	Royal Dutch Warmblood – Harness type
4.	Emillita	Royal Dutch Warmblood – Riding type
5.	Vlotte	Friesian
6.	Willarda	Royal Dutch Warmblood – Harness type

Data collection

Qhorse

The first method focused on symmetry in the horse gait. An 3D optical motion capture system (Qualisys AB Sweden, QTM software,) was used for data collection. The capture volume connected to 12 infrared cameras (Oqus) measuring at 400 fps around the straight line. Calibration before the start of the measurement was done daily by the same person according to the manufacturer's instructions. Nine hard reflective markers (12 mm), attached to the skin using double- sided adhesive tape, were place in triads on the head, withers and pelvis (Fig. 2). Guided by a handler, all the horses trotted three times on a hard surface straight line every measurement day. The Qhorse measurement were done by an orthopedic specialist. In addition to the objective measurements, the specialist also visually assessed each horse for lameness to ensure that they are still owner-sound.

Figure 2. Position of the reflective markers (red circles) in triad at the head, withers and pelvis of the horse.

COP measurements

A Qualisys motion analysis system consisting of 8 high-speed Oqus cameras was synchronized with three Footscan pressure plates (RsScan International) in series, one 2 meter plate (256x64 sensors, A) and two 1 meter (128x64 sensors, B & C) plates (Fig. 3). Both systems recorded at 125 Hz. Prior to each experiment, the pressure plates were calibrated by walking over the plates by the same person with known body weight. Cameras were calibrated using a triangular L-frame with 4 reflective markers. The markers were placed on the left upper corner of plate A. The calibration was progressed with a calibration stick with 2 markers by motion in all directions in a height between 0-30 cm above the pressure plate and was accepted when it was between 1,0 - 1,5. It takes 2 minutes till all cameras detected the 6 reflective markers and calibration could be accepted.

After calibration a rubber mat was pulled over the plates for protection. Prior to the trotting experiments, the horse was weighted and 2 soft reflective markers (19 mm) were attached on a fixed position to each hoof using double- sided adhesive tape; one marker medial and one marker lateral against the coronet (Fig. 4). The exact placement of the markers was marked on the hoof and was visible for several days; for consistency, the same person always marked the hoofs.

A handler led each horse in trot in a straight line and in a consistent velocity over the pressure plates over the entire length of the rubber mat. The COP pattern was measured by the Footscan pressure plates and the cameras identify the attached markers on the hoof. Each horse trotted over the Footscan pressure plates until 4 good hits from each hoof were detected. After each run the data were visually inspected. For further analysis, trials were selected that met the following criteria: they obtained the pressure underneath the limb during the complete stance phase and the markers were present visible in the kinematics data for the entire stance phase.

Figure 3. Research set-up of the COP system. With the pressure plates in series (A, B, C) and the camera's positioned around the pressure plates.

Figure 4. The reflective markers at the medial and lateral side of the hoof against the coronet.

Data processing

Qhorse

The 3D tracked data was visually inspected after the measurements to ensure that all the markers had been tracked adequately. Kinematic data were analyzed using the proprietary Qualisys capture software Qhorse. Based on the vertical displacement of the head, withers and pelvis the symmetry parameters minDiff and maxDiff were calculated. Normally the

Qhorse software calculates an average of these parameters for the whole trail, however to make a comparison between the repeatability of the COP data and the Qhorse data the values of the minDiff and maxDiff for one step was taken. With a custom- written code by F.M. Serra Bragança (Utrecht University) the minDiff and maxDiff values for the eighth step on the straight line were calculated in Matlab. The eighth step was on the middle of the straight line.

COP

COP data were analyzed by the Footscan gait software, Qualisys software (Qualysis Track Manager) and a custom-written code by S. Nauwelaerts (University of Antwerp) in Matlab.

In the Qualysis Track Manager kinematics of the hoofs were checked. All the markers were labeled for the side (lateral or medial) and the hoof (right front, right hind, left front, left hind). Gaps less than 10 frames during the stance phase and three following frames in the swing phase were restored. Based on the positions of the reflective markers, a local hoof-bound coordinate system was created. The X-axis was orientated from the lateral to the medial marker, the Y-axis perpendicular on the X-axis through the middle.

For further analysis the sensors in the pressure plates were transformed to coordinates. Based on the position of the hoof at the Footscan pressure plate, X- and Y- coordinates of the COP were recalculated into a hoof-bound coordinate system by the custom written Matlab code. The coordinate system of the kinematic data and the coordinate system of the Footscan pressure plates were recalculated and combined with each other. There was also correction for rotation and displacement. The COP path was calculated in this local hoofbound coordinate system and the origin of the local coordinate system was always in the middle of the hoof.

Data analysis

Qhorse

From the collected data the parameters of interest were the minDiff and maxdiff values of the head, withers and pelvis measured from the eighth step on the straight line. This gave a total of 6 parameters for each horse.

For the statistical analysis of the data, a two- way analysis of variance (ANOVA) was conducted. Day and horse were fixed factors, the dependent factors were the mindiff and maxdiff values of the head, withers and pelvis. Significance was set at $P<0{,}05$. First interaction between day and horse was checked, when P-values $> 0{,}05$ were obtained the test was recalculated without interaction effect. In the descriptive statistics a boxplot was made for every horse per dependent factor. Also the overall mean and standard deviation was calculated for every dependent factor.

COP

Parameters of interest of the COP data were the centroidsize, centroid-X, centroid-Y and centroidkine. The centroidsize is the size of the calculated COP path at the pressure plate. The coordinates within the hoof are the centroid with coordinate (0,0) as middle of the hoof. The centroid-X and the Centroid-Y are respectively the X- and Y- coordinate of the centroid.

The centroidsize within the hoofbound coordinate system of the hoof, corrected for the kinematics, is called the centroidkine.

For the statistical analysis of the COP data, also a two-way ANOVA was conducted. Each limb of the horse was considered as an individual and got its own limbcode (1 – LH, 2 – RH, 3- LF, 4 - RF). Fixed factors in this analysis were limbcode and day, the dependent factors centroidsize, centroid-X, centroid-Y and centroidkine. Significance was set at $P<0,05$. In this test the interaction was calculated as well. A boxplot, categorized by horse and limbcode, was made as well for every dependent factor. For every parameter the average of the outcomes in a day was used to make the boxplot more readable, otherwise too many outliers showed up. Also the overall mean and standard deviation of the centroidsize, centroid-X, centroid-Y and centroidkine were calculated.

Repeatability

From an Analysis of Variance (ANOVA) repeatability is calculated by using the following formula;

$r = S^2A / (S^2 + S^2A)$

- S^2A: between group variance.
    - $S^2A = (MSa – MSw)/ N$. → N = weighted average number of observation per group.
- S^2: within group variance

The levels of repeatability and the corresponding descriptions are shown in Table 2 .

Repeatability for the Qhorse data was calculated based on the between and within sum of squares of the horse and day for the values minDiff/ maxdiff Head, minDiff/ maxdiff withers and minDiff/maxDiff pelvis. N = 3 observations per group were used.

For the COP data the repeatability was calculated based on the between and within sum of squares of the limbcode and day for the values centroidsize, centroid-X, centroid-Y and centroidkine. N= 3,58 observations per group were used.

Table 2. Repeatability values

Repeatability	Meaning
r less than 0,2	Slight repeatability
r between 0,2 – 0,4	Low repeatability
r between 0,4 – 0,7	Moderate repeatability
r between 0,7 – 0,9	High repeatability
r greater than 0,9	Very high repeatability

Results

All six horses completed the study and no horses were excluded on one of the measurements days due to lameness. The study period was extended from 42 days to 52- 55 days due to a quarantine period of the horses in the middle of the study period. In total 14 (n=3) or 15 (n=3) measurement days took place for both the Qhorse system as well as the COP system. This resulted in a total of 260 measurements for the Qhorse system per variable and in a total of 1392 measurements for the COP system per variable.

Qhorse data

In total 258/ 260 measurement per variable were left for further analysis. The results of these measurements are shown in the boxplots below in figure 5 - 10. The horses (1-6) are individually displayed on the X-axis and the dependent variable (minDiff or maxDiff) is on the Y-axis. The boxes represent the interquartile range of the values of each horse, also the median is shown. The whiskers show the minimal and maximal values of each set. A couple of outliers were detected as well, these are marked by open dots or asterisk (extreme outlier).

Figure 5 and 6 represent the data of the minDiff and maxDiff values of the head of the horse.

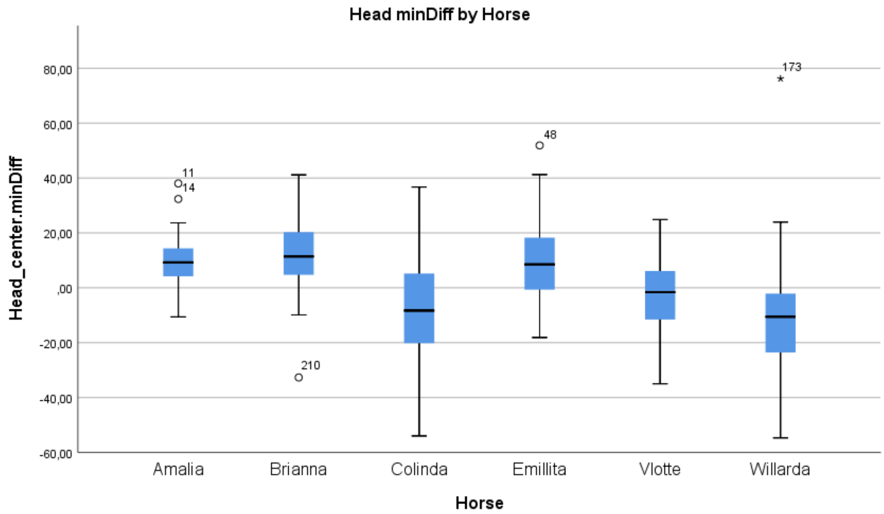

Fig. 5: Boxplot per horse for Head minDiff values

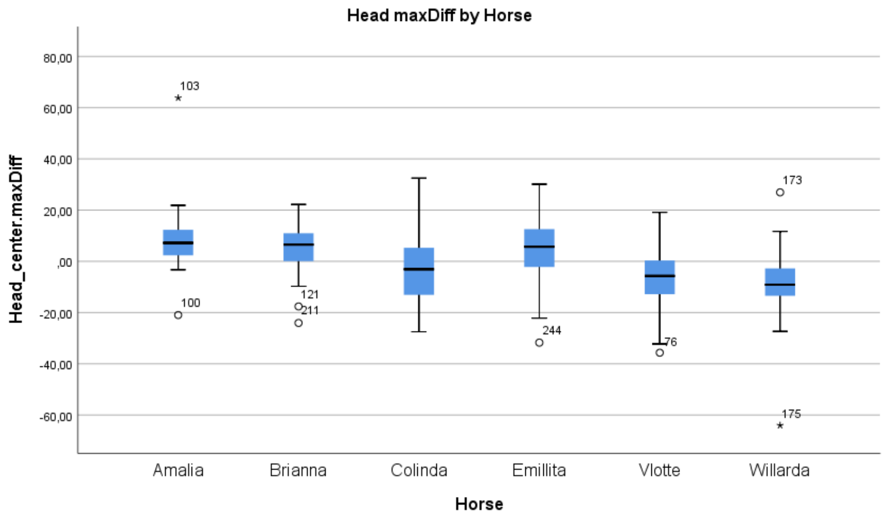

Fig. 6: Boxplot per horse for head maxDiff values

The measured values of the minDiff and maxDiff of the withers of the horses are represent in Figure 7 and 8.

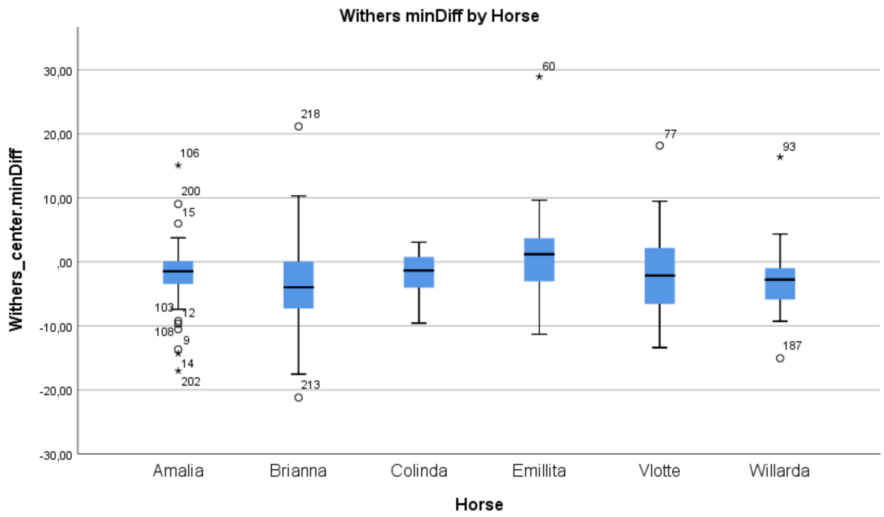

Figure. 7: Boxplot per horse for withers minDiff values

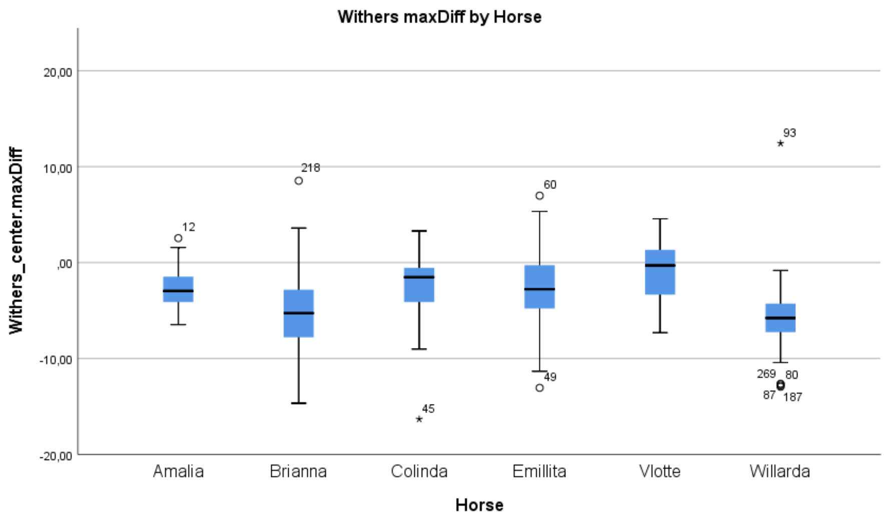

Figure. 8: Boxplot per horse for withers maxDiff values

Figure 9 and 10 represent the data of the minDiff and maxDiff values of the pelvis of the horse.

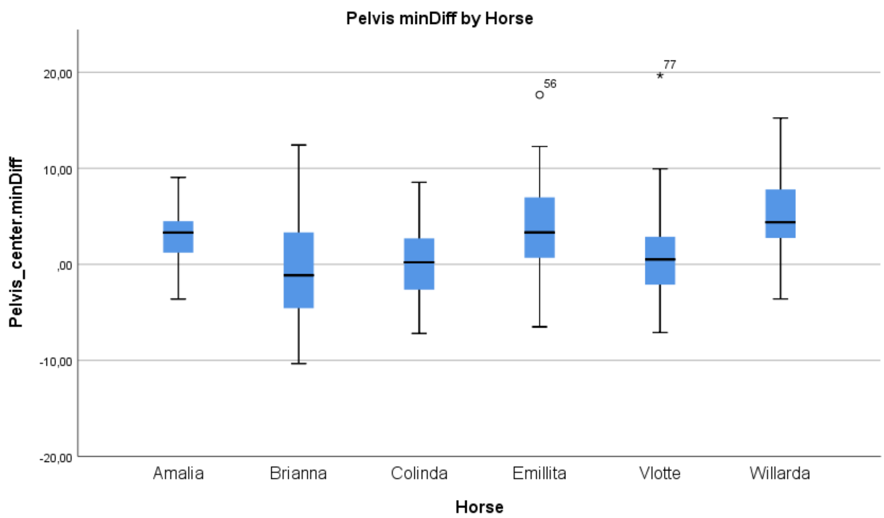

Figure. 9: Boxplot per horse for pelvis minDiff values

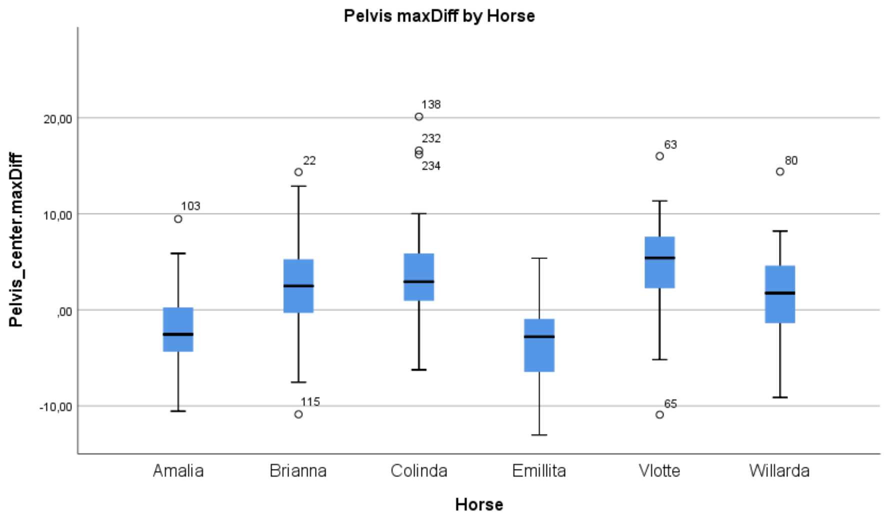

Figure. 10: Boxplot per horse for pelvis maxDiff values

The overall means and standard deviations, of the eighth step per trial, of all the horses together are also calculated for every minDiff/maxDiff value of the head, withers and pelvis. The results are shown below in Table 3.

Table 3. Descriptive statistics of the Qhorse measurements by showing the number of trials *(N) and overall* mean and standard deviation of the minDiff and maxdiff *values.*

Descriptive Statistics

	N	Mean	Std. Deviation
Head_center.minDiff	258	1,88567	17,965547
Head_center.maxDiff	258	,06149	13,589945
Pelvis_center.minDiff	258	2,06346	4,720351
Pelvis_center.maxDiff	258	1,21011	5,462878
Withers_center.minDiff	258	-1,79573	6,108092
Withers_center.maxDiff	258	-3,22642	3,897968
Valid N (listwise)	258		

SPSS output of the ANOVA for the Qhorse parameters can be found in Supplementary Item1.

Summarized there was a significant effect of horse (F(5,238)= 17,157; P=0,000) and day (F(14,238)= 2,703; P=0,001) on the dependent variable head minDiff. There was as significant effect of horse (F(5,238)= 14,090; P=0,000) and a nonsignificant effect of day (F(14,238)= 0,776; P=0,695) on the defendant variable head maxDiff. No interaction effect for both parameters was observed.
There was an interaction effect of horse and day on the withers minDiff (F(67,171) = 2,738; P=0,000). No interaction was observed for the withers maxDiff. The effect of horse on the dependent factor was significant (F(5,238)= 10,440; P=0,000), the effect of day (F(14,238)= 0,642; P=0,828) was not significant again.
An interaction effect of horse and day was observed on the pelvis minDiff (F(67,171)= 1,585; P=0,009). Also the pelvis maxDiff had an interaction effect of horse and day (F(67,171)=1,989; P=0,000).

Repeatability is calculated per Qhorse parameters, the results are visible in Table 4.

Table 4. Repeatability of the Qhorse variables per individual horse and day

Variable	Individual (horse)	Day
Head minDiff	0,84	0,36
Head maxDiff	0,81	-0,08
Withers minDiff	0,5	0,03
Withers maxDiff	0,76	-0,14
Pelvis minDiff	0,83	0,23
Pelvis maxDiff	0,89	0,04

The repeatability of the Qhorse system for the individual horse is high (r > 0,7), except for the parameter withers minDiff. More than 50% of the variation is due to differences among horses, this can only occur if individuals are consistent. An individual can be recognized by looking at the movement of the head and pelvis (r > 0,81), withers are not a good parameter for recognizing the horse by movement (r: 0,5 – 0,76). Repeatability for measurement day is low, r < 0,4. The outcome of the measurement days is not consistent, there are lots of outcome differences between days.

COP

In total 1304/1392 measurements of the centroidsize and 1300/ 1392 measurements of the variables centroid-X, centroid-Y and centroidkine were useful for further analysis. The results are shown in the boxplots below in figure 11- 14. The horses (1-6) are individually displayed on the X-axis and the dependent variable (centroidsize, centroid-X, centroid-Y and centroidkine) is on the Y-axis. Per horse the limbs (1-4) are plotted in different colors. A lot of outliers were detected in the data, these are marked by open dots or asterisk (extreme outlier) in the boxplot.

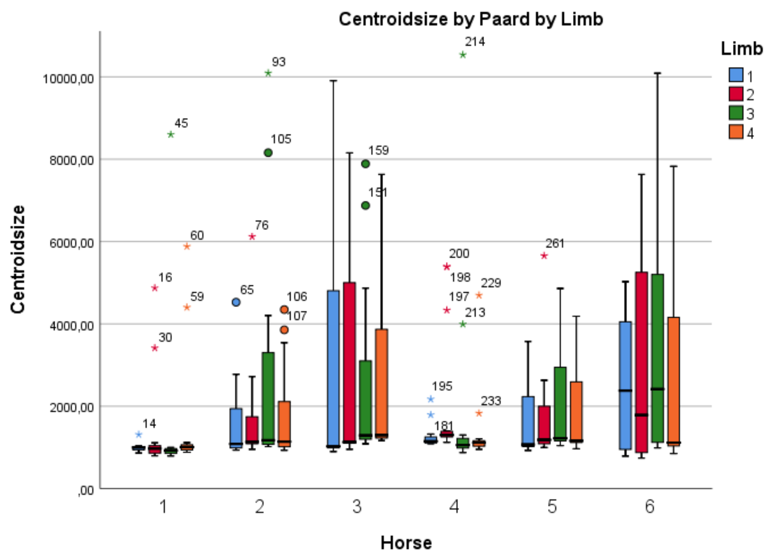

Figure 11. Boxplot of the centroidsize per horse per limb

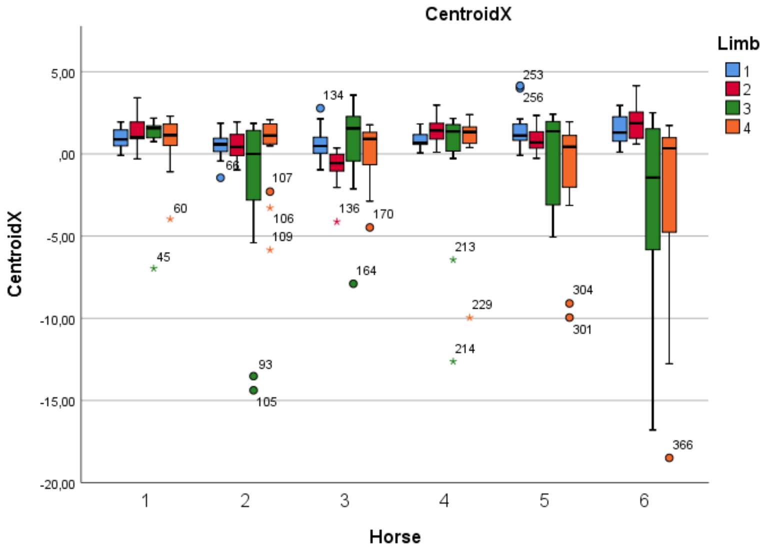

Figure 12. Boxplot of the centroid-X per horse per leg

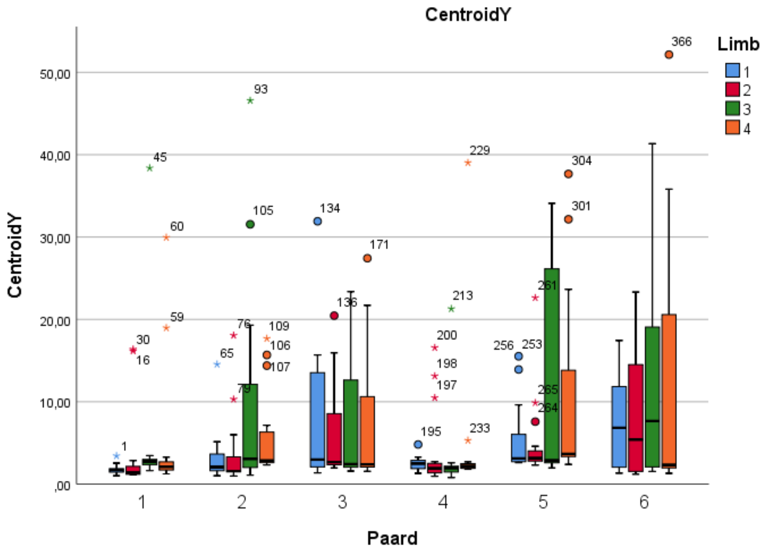

Figure 13. Boxplot of the centroid-Y per horse per leg.

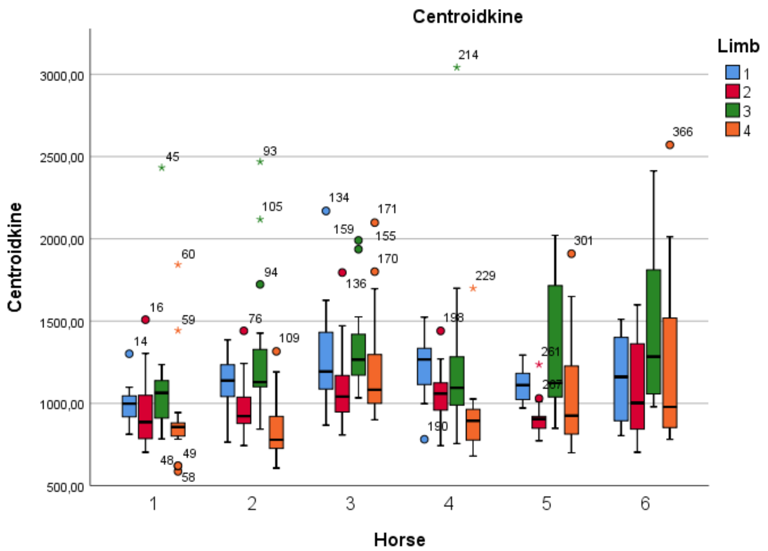

Figure 14. Boxplot Average centroidkine per horse per leg

The overall means and standard deviations are also calculated for the centroisize, centroid-X, centroid-Y and centroidkine. The results are shown below in Table 5.

Table 5. Descriptive statistics of the COP measurements by showing the number of trials *(N) and overall* mean and standard deviation of the parameters.

Descriptive Statistics

	N	Mean	Std. Deviation
centroidsize	1304	2174,7898	3628,09643
centroidkine	1300	1135,9658	601,25470
centroidx	1300	,1093	4,94348
centroidy	1300	6,9400	16,24344
Valid N (listwise)	1300		

SPSS output of the ANOVA for the COP parameters can be found in Supplementary Item 2.

Summarized there was a significant effect of limbcode (F(23,1242)= 1,591; P= 0,038) and day (F(38,1242)= 2,395; P= 0,000) on the dependent variable centroidsize. No interaction effect was observed. There was a significant effect of interaction on the centroidkine (F(310,928)= 1,168; P=0,043). A significant interaction effect (F(310,928)= 1,170; P=0,42) of limbcode and day was also observed for the centroid-X. No interaction effect of limbcode and day was observed for the centroid-Y. The effects of limbcode (F(23,1238)= 1,649; P=0,028) and day (F(38,1238)= 2,270; P= 0,000) were statistically significant.

Repeatability is calculated per COP parameters, the results are visible in Table 6.

Table 6. Repeatability of the COP values for individual limb and day

Variable	Individual (limb)	Day
CentroidSize	0,14	0,28
CentroidKine	0,47	0,23
CentroidX	0,47	0,27
CentroidY	0,15	0,26

The repeatability of the individual limb of a horse is low for the parameters centroidsize (r = 0,14) and the centroid-Y (r=0,15). The repeatability of the centroidkine (r=0,47) and the centroid-X (r=0,47) are moderate. The repeatability of the measurement day is low (r<0,3) for all the parameters. Less than 50% of variation is due to differences among horses. There are a lot of differences between the horses and between the outcome variables of the days.

Discussion

In this study a comparison is made between the new COP pathway system and the 'gold standard' for kinematic gait analysis at this moment; a 3D OMC system with the asymmetry analysis tool Qhorse (Qualisys AB, Sweden). The first aim om the study was to investigate the repeatability of the COP path during a six- week shoeing cycle. The second aim of the study was to investigate whether both systems for objective gait analysis had similar repeatability's during this six-week trimming cycle.

Based on the results of the measurements of the COP path during this study the first hypothesis "The COP path is as repeatable during a six-week shoeing interval as in one day" must be rejected. Nauwelaerts et al., (2017) concluded that the COP pattern is highly repeatable (r = 0,79) during a day by using a local coordinate system that moves with the hoof (the effect of sliding forward is removed). In this study the repeatability for the individual limb is low for the parameters centroidsize (r = 0,14) and the centroid-Y (r=0,15). The repeatability of the centroidkine (r = 0,47) and the centroid-X (r=0,47) are moderate. The repeatability of the measurement day is low (r < 0,3) for all the parameters as well. On the basis of the results of this research, we conclude that the COP pattern is not consistent over time and in one leg. A lot of outliers in the data showed up and there was a lot of variance in the values of the centroidsize, centroidkine, and centroid-X and -Y between the horses as well as within and between the legs of one horse. This can be due to the growing of the hoof during the cycle or due to other reasons like measurement faults. However, this is the first study in which the COP path is observed over time by combining pressure plates and kinematic data. The question is whether this outcome comes through the system or whether it is due to the way of measuring the COP path. To test this idea we compared the repeatability of the COP path with the Qhorse system, a system of which it is known that the repeatability of measurements is high (Serra Braganca et al., 2018).

The second hypothesis "the COP pathway system and the Qhorse system are as repeatable in evaluating the locomotion apparatus of the horse over the entire trimming cycle" can be accepted due to the results of this study. As seen in the results, the repeatability for the Qhorse system was high for the individual horse; the gait of the individual horse picked up by a kinematic gait analysis system is consistent. The repeatability of the head and pelvis is greater than 0,8 and highly consistent. The withers are less consistent, r = 0,5 for the minDiff value and r = 0,7 for the maxDiff value. In this study in time, so comparing all measurement days during one hoof trimming cycle, the repeatable is low (r < 0,4). A lot of differences between outcomes for the minDiff and maxDiff values of the head, pelvis and withers can be seen on different days and the measurements are not consistent. For the COP system the repeatability during one trimming cycle is also low (r < 0,4). For this reason we hypothesize that both systems are as low repeatable over time for measuring the used parameters. Both systems did not show consistent measurements over the days. The Qhorse system can still recognize differences between the different horses, the COP system cannot distinguish the different legs. However, we have to keep in mind that both systems are based on different ways of lameness detection (kinematics/ kinematics) and due to that it is almost impossible to say that both systems have comparable repeatability's.

The outcome of the Qhorse measurements is in contrast with a study of Hardeman et al., (2019) in which the head symmetry parameters showed significantly more variation compared to the withers and pelvis. The mean intra-class correlation coefficient (ICC) was lower for the head (0,68) than for the withers (0,76) and pelvis (0,85). Also the study of Keegan et al., (2011), using body mounted accelerometers, showed that the asymmetry parameters of the pelvis on the straight line had a better repeatability than head minDiff and maxDiff values. However, an important difference of both studies compared with our study is that more than one step per trial was used to get the asymmetry values. One step is just a snapshot and can have a lot of influence on the outcome of the values. The average of the measurements of the whole trial automatically ensures better outcomes. Hardeman et al., (2019) also observed a tendency of increased between- measurement variation. When the repetition increases during a day less variation is seen in the asymmetry parameters, less variation was also seen in the trails on consecutive days.

Nauwelaerts et al., (2017) concluded that there is a lot of difference between horses and limbs in the pattern of the COP. A lot of shapes are possible, but they are unique to the individual and the limb. It is concluded that ideal COP paths do not exist, but that the resulting path is the best possible solution for that individual limb. By measuring the COP pattern over time the results of this study did not show a very consistence pattern for individual horses in one hoof trimming cycle. However, it could still be that the COP pattern follows the same way every trimming cycle and that the whole cycle is repeatable for the horse instead of the individual measurement every day. In that case this method is still useful to provide an early detection method for lameness.

Experimental design challenges

A conclusion of the pilot study prior to this research project (unpublished data) was that the most common used period of a hoof trimming cycle was about 6 weeks. For that reason data collection would last for that period of 6 weeks. However, due to an outbreak of fever in the stables of the horses, all horses were put in quarantine for about 14- 17 days. This period started around day 34- 40 of the trimming cycle of the horses. All horses that participated in the study had fever for a couple of days. No further measurements could be done before the horses were out of quarantine, so it is decided to measure the horses one last time at the day they came out of quarantine 14- 17 days later. Therefore, measurements of the intermediate period are missing and the hoof trimming cycle was extend to 52- 55 days. This could have had influence on the results of the Qhorse and COP measurement. In the first place due to growing of the hoof in the meantime. Sickness of the horse could have had influence on the normal growth. Besides that, the horses were not trained in this period of isolation. Through this the horses were more excited than normal during the last measurement day and it was not representing the horse’s normal behavior. Normally the horses were not warmed up before starting the measurements, but at the last day warming up was done by having al the horses in trot at the lunge for about 10 minutes. Gait can improve with mild exercise so this could also have had effect on the measurements (Keegan et al., 2011).

Practical limitations

Different handlers let the horses during the Qhorse trials and the COP trails. The handler can have influence on the gait of the horses (Hardeman et al., 2019), so due to that they were instructed to minimize their influence on the horse's movement by leading with a loose rope. However, the handler will always influence the head position to a certain extent (Hardeman et al., 2019). In our study it is also tried to measure at a constant velocity, but with different horses, different breeds and more than one handler it was sometimes difficult to arrange the consistency of the gait. In further research this will be a point of attention.

Qhorse

In the study period all the horses were measured every other day except for the weekends. For the Qhorse measurements it was not possible to fix the position of the markers on the horses for several days. The markers were attached on the horse by an experienced person, but is not guaranteed that the position would be exact the same every measurement day. In further research, we would suggest to clip a small proportion of hair to ensure exact replacement of markers every measurement day. In total 258 of 260 trials of the Qhorse system were left for further analysis. Due to problems with the Qhorse measurement system a couple of measurements on two measurement days have been lost. This can be prevented by checking and saving the data directly after the measurements and do the trial again if necessary.

The mean minDiff and maxDiff values of head, withers and pelvis are calculated for the eighth step on the hard straight with the Qhorse system. In other researches, for example the Hardeman et al., (2019) and Rhodin et al., (2017), minDiff and maxdiff values were calculated by taking all the steps on the straight line together. The mean values of the parameters differ a lot with our study. What is possible to explain because one step is only a snapshot. This can also be due to the small amount of horses we used for the study or due to the type of horses. Five out of the six horses we used were not of a riding type sport horse. The gait of harness type horses and cold blood horses differs a lot from the warm blood type horses used in the other studies. On the other hand, the results did not show a clear difference between the six horses.

COP

The place of the markers on the hoof was marked with a colored marker by the same person every measurement day to ensure that the position was always the same. However, the horses were also used for riding lessons and were outside during the day, so it could not be prevented that the markings were sometimes hard to see. We aimed at placing the markers every time on the same spot, but small shifts remain possible. During the measurement trials, the horses sometimes lost the markers by hitting them with their hoofs. The markers had to then be replaced. We suggest to mark marker placement every day to ensure they will be always visible and at the same placement.

From the COP data average 3,58 of 4 observations per variable per limb were suitable for further analysis. A minimum of two correct measurements per COP value per day was required. Due to missing frames in the stance of swing phase of the kinematic data or incomplete hoof prints on the pressure plate measurements were excluded for further analysis.

Conclusion

The repeatability as observed in this study is low for the COP pattern in a six- week shoeing interval. The repeatability of the Qhorse system is high for a horse and values of minDiff and maxDiff for head, withers and pelvis are consistent for an individual. An individual horse can be recognized by the locomotion pattern, by using the COP system this is not possible. Comparison of the repeatability of the COP pathway system and the Qhorse system shows that the repeatability of both systems are comparable low ($r < 0,4$) for measuring the locomotion of a horse every other day during one trimming cycle. However, both systems are based on complete different types (kinetics/ kinematics) of lameness detection. It is know that the repeatability of the Qhorse system is high by measuring more than one step. For this reason we hypothesize that the design of measuring the COP pattern during a shoeing cycle was not correct. Further research of the repeatability of the COP pattern is required with a system that measures more than one step at the pressure plates. A longer study period in which several shoeing intervals can be compared is recommended. The influence of the place of the markers and the effect of the handler has to be taken in account.

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Supplementary Item 1 - SPSS output ANOVA Qhorse parameters

Head

Tests of Between-Subjects Effects

Dependent Variable: 1 Head_center.minDiff

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28467.801[a]	19	1498.305	6.545	.000
Intercept	991.413	1	991.413	4.331	.038
Horse	19638.034	5	3927.607	17.157	.000
Day	8661.346	14	618.668	2.703	.001
Error	54481.749	238	228.915		
Total	83866.937	258			
Corrected Total	82949.550	257			

a. R Squared = .343 (Adjusted R Squared = .291)

Tests of Between-Subjects Effects

Dependent Variable: 2 Head_center.maxDiff

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12108.405[a]	19	637.284	4.290	.000
Intercept	4.136	1	4.136	.028	.868
Horse	10465.671	5	2093.134	14.090	.000
Day	1613.792	14	115.271	.776	.695
Error	35356.052	238	148.555		
Total	47465.432	258			
Corrected Total	47464.457	257			

a. R Squared = .255 (Adjusted R Squared = .196)

Pelvis

Tests of Between-Subjects Effects

Dependent Variable: 3 Pelvis_center.minDiff

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3173.731[a]	86	36.904	2.472	.000
Intercept	1001.217	1	1001.217	67.070	.000
Horse	1189.392	5	237.878	15.935	.000
Day	399.656	14	28.547	1.912	.028
Horse * Day	1585.292	67	23.661	1.585	.009
Error	2552.669	171	14.928		
Total	6824.925	258			
Corrected Total	5726.399	257			

a. R Squared = .554 (Adjusted R Squared = .330)

Tests of Between-Subjects Effects

Dependent Variable: 4 Pelvis_center.maxDiff

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4773,981[a]	86	55,511	3,278	,000
Intercept	398,129	1	398,129	23,511	,000
Horse	2202,699	5	440,540	26,015	,000
Day	269,725	14	19,266	1,138	,329
Day * Horse	2256,137	67	33,674	1,989	,000
Error	2895,680	171	16,934		
Total	8047,467	258			
Corrected Total	7669,660	257			

a. R Squared = ,622 (Adjusted R Squared = ,433)

Withers

Tests of Between-Subjects Effects

Dependent Variable: 5 Withers_center.minDiff

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5354,090[a]	86	62,257	2,514	,000
Intercept	699,629	1	699,629	28,254	,000
Horse	465,544	5	93,109	3,760	,003
Day	378,182	14	27,013	1,091	,369
Day * Horse	4542,725	67	67,802	2,738	,000
Error	4234,269	171	24,762		
Total	10420,314	258			
Corrected Total	9588,359	257			

a. R Squared = ,558 (Adjusted R Squared = ,336)

Tests of Between-Subjects Effects

Dependent Variable: 6 Withers_center.maxDiff

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	798.167[a]	19	42.009	3.218	.000
Intercept	2526.285	1	2526.285	193.533	.000
Horse	681.394	5	136.279	10.440	.000
Day	117.376	14	8.384	.642	.828
Error	3106.731	238	13.053		
Total	6590.618	258			
Corrected Total	3904.898	257			

a. R Squared = .204 (Adjusted R Squared = .141)

Supplementary Item 2 - SPSS output ANOVA COP parameters

Centroidsize

Tests of Between-Subjects Effects

Dependent Variable: centroidsize

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1796270544,352[a]	61	29447058,104	2,382	,000
Intercept	1746877566,104	1	1746877566,104	141,295	,000
limbcode	452491483,410	23	19673542,757	1,591	,038
Day	1125185772,919	38	29610151,919	2,395	,000
Error	15355227545,521	1242	12363307,203		
Total	23319040952,397	1304			
Corrected Total	17151498089,873	1303			

a. R Squared = ,105 (Adjusted R Squared = ,061)

Centroidkine

Tests of Between-Subjects Effects

Dependent Variable: centroidkine

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	177203059,542[a]	371	477636,279	1,516	,000
Intercept	1003366786,848	1	1003366786,848	3184,476	,000
day	24528957,175	38	645498,873	2,049	,000
limbcode	30674140,617	23	1333658,288	4,233	,000
day * limbcode	114104219,157	310	368078,126	1,168	,043
Error	292394814,427	928	315080,619		
Total	2147141631,322	1300			
Corrected Total	469597873,969	1299			

a. R Squared = ,377 (Adjusted R Squared = ,128)

Centroid- X

Tests of Between-Subjects Effects

Dependent Variable: centroidx

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12126,963[a]	371	32,687	1,546	,000
Intercept	18,913	1	18,913	,895	,344
day	1866,025	38	49,106	2,323	,000
limbcode	2001,756	23	87,033	4,117	,000
day * limbcode	7667,203	310	24,733	1,170	,042
Error	19618,052	928	21,140		
Total	31760,557	1300			
Corrected Total	31745,015	1299			

a. R Squared = ,382 (Adjusted R Squared = ,135)

Centroid-Y

Tests of Between-Subjects Effects

Dependent Variable: centroidy

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	34015,087[a]	61	557,624	2,236	,000
Intercept	17411,344	1	17411,344	69,820	,000
day	21508,825	38	566,022	2,270	,000
limbcode	9457,162	23	411,181	1,649	,028
Error	308725,276	1238	249,374		
Total	405353,548	1300			
Corrected Total	342740,364	1299			

a. R Squared = ,099 (Adjusted R Squared = ,055)