Effect of Hoof Growth on the Centre of Pressure Path

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Abstract
The aim of this study was to achieve insight of the effect of hoof growth on the centre of pressure (COP) path. If the COP pattern of the individual horse is constant over a longer period of time, the COP pattern can potentially be used for the early detection of lameness. Data of ten horses was collected during two trimming intervals of six weeks and one measurement day of the third cycle. The horses were repeatedly trotted in a straight line over the three pressure mats (Footscan 3-D 1 m-system, measuring at 125 Hz) to measure the COP pattern and kinematics (8 pro-flex cameras, recording at 125 Hz) of the limbs simultaneously. Analysis of the data was performed with the dependent variables ‘‘Centroidsize’’, ‘‘Centroidsizekine’’, ‘‘Centroidsizekine X’’, ‘‘Centroidsizekine Y’’, ‘‘Centroidsizekinenorm’’ and ‘‘Stance duration’’. The variables applied in this study turned out to be too sensitive for noise. Centroidsizekinenorm was the most stable dependent variable in this study. The dependent variable stance duration showed the highest sensitivity for noisy data, this makes the variable unsuitable for the early detection of lameness in horses. The effect of day was inconsistent, significant effect was found for some of the individual legs, but not within or between all of the three trimming cycles. The shapes of the COP path, of a randomly picked legperhorsenr selected for two random days within a cycle, have been compared. The shapes of the COP path of both days show remarkable similarities. Each limb of each horse shows its own and unique COP pattern. Geometric Morphometrics Analysis, based on landmark coordinates, might be a good parameter for future research of the COP pattern with the pressure plates. The experiment should also be conducted with lame horses to see the changes of the COP pattern between the ‘owner-sound’ legs and the affected legs of the horse.

Introduction

One of the major medical concerns of sport horses is lameness which make the horses unable to perform (1). Lameness has a variety of definitions, but all of the definitions mention a disorder or loss of function which affects the gait of horses (2). Lame horses are presented to veterinarians daily. Lameness is usually evaluated visually and the interpretation of the gait of horses differs between skilled veterinarians. Due to this difference, lameness in horses can be misinterpreted (3). The high validity of computers might be a solution. The golden standard of today, to quantify lameness in an objective way, are force plates that measure the kinetics of the gait and are systems to analyse the kinematics of the gait such as the QHorse system (4). Disadvantages of these systems are their high price and the lack of mobility to transport the systems to the horse. A more affordable and mobile system is the pressure mat which can determine the COP (Centre of Pressure) under the hoof of horses. The Centre of Pressure is a single point underneath the foot of a human or animal where all pressures comes together. This point is the outcome of a calculation of the total number of the active sensors measured by the pressure plate (5). Following the position over time results in a COP pattern and gives more insight in the behaviour of feet. Studies among humans already provided more insight of the foot behaviour in motion, described and interpreted in relation to the COP pattern (6). Especially displacement and velocity of the COP turned out as good indicators to analyse the function of the foot.

However, before an alteration of the COP pattern can be assigned to lameness, it should be considered that the dynamic structure of the hoof might also influence the COP pattern of the limbs. Moreover, study of hoof growth showed an increase in length of the dorsal wall of the hoof and a decrease of the hoof angle (7). When the shape of the hoof changes due to a reaction on its environment or to natural growth and the trimming afterwards, the COP position of the hoof might change (8). Earlier research has shown a shift of the COP after trimming (9). The conformation of the hoof also influences the landing during trot, after trimming a decrease in the landing duration of especially the front feet was observed (9). The balance of the hoof can be improved by trimming. Good balance of the hoof is important during the phase of impact and provides a faster total support of the hoof during the phase of impact. This makes it enable to have a more equal load distribution over the internal structures within the hoof the horse (9). The locomotion system of the horse has a limited capacity to cope with the impact on the hoof during the landing. The horse is also unable to compensate for acute disruption of the balance of the hoof, in this scenario redistribution of the load under the hoof is limited (10).

Differences between the front and hind feet are a result of the relative increase in loading of the front feet during a shoeing interval. The natural reaction of horses in motion is to slightly distribute the body centre of mass to the forelimb (11). Previous study showed similarity between both of the front feet and both of the hind feet (7). However, the hoof-unrollment pattern of the hind limbs showed a lateral shift of the COP, where the COP pattern of the front limbs remained almost the same (7). Warmblood horses showed asymmetry in the way of landing of especially the hindfeet, a lateral landing of the hindfeet is considered as normal (9).

Nauwelearts *et al.* (2017) measured the COP pattern under the feet of horses and showed that individual sound horses have a highly repeatable and unique COP pattern for each limb within a day (12). In this cross-sectional study they suggested that the COP pattern could be a feature in early detection of lameness in horses (12). The results of this study were interpreted for just one moment of measuring. Differences in the COP pattern of the front feet and hind feet might be expected. Therefore, it would be interesting to know how the COP pattern develops during a hoof trimming cycle in a longitudinal study.

The hypothesis of this study is that the COP pattern changes in a predictable way during a hoof trimming cycle. This longitudinal study has been set up to examine the dependent variables of the COP: the centroid size corrected with kinematics, this centroid size normalised to 100 frames and the stance duration. It is questionable which of the dependent variables will have the accuracy and capacity to predict the COP pattern of the horse during a trimming cycle. Despite the dynamic character of the hoof, the repeatability within and between the cycles of the COP pattern might change in a predictable way. The independent variables day and cycle will be the analysed factors to test the predictability of the COP pattern. Therefore, the outcomes of the limbs, especially the normalised centroid size corrected with the kinematics, should be compared within and between the three trimming cycles. The usefulness of the COP pattern for the detection of early lameness in horses can be confirmed with performing an equal experiment with induced lame horses.

H0: The COP pattern does not change in a predictable way during a hoof trimming cycle.
H1: The COP pattern does change in a predictable way during a hoof trimming cycle.

Materials and methods

Horses

In this study participated ten horses of different breeds (9 KWPN, 1 Friesian), ages (mean: 9,9 years ± 2,8) and body weight (mean: 563 kg ± 40) (Table 1). The horses were all owned by the veterinary faculty and used for riding lessons or breeding. At the start of the experiment all of the horses have been examined by an experienced veterinarian and declared as 'owner-sound'. Eight horses have been shod by two experienced farriers on the first day2 of the experiment and in between the following trimming cycles. The farriers trimmed the hooves of all of the horses in a position that fits the feet in their most natural way. The study was conducted in accordance with the Ethics Commission of the University of Antwerp and the University of Utrecht.

	Breed	**Age (years)**	**Body weight (kg)**	**Shoeing**
Horse 1	KWPN	12	533	Shod
Horse 2	KWPN	11	522	Shod
Horse 3	KWPN	11	596	Shod
Horse 4	KWPN	8	538	Front shod
Horse 5	KWPN	8	631	Front shod
Horse 6	KWPN	6	582	Unshod
Horse 7	KWPN	5	506	Unshod
Horse 8	Friesian	11	599	Shod
Horse 9	KWPN	14	574	Front shod
Horse 10	KWPN	13	546	Unshod

Table 1. Overview particpating horses.

Data collection

Data of ten horses were collected during two trimming intervals of six weeks, starting from the day of trimming. In addition, one measurement during a third cycle was included in the dataset. This measurement was done after a minimum of six days, so the feet of the horse could adapt to their new conformation. Measurements were scheduled every other day during the two full cycles. Afterwards a third cycle, consisting of one measurement day, was added to increase the validity of the experiment.

Setup

Three pressure mats were placed in a straight line and covered with protective rubber mats. Two RsScan footscan pressure mats were sized one metre long and contained 128x64 sensors each. Another RsScan footscan pressure mat was sized two-metre-long and contained 256x64 sensors. The COP pattern was measured using a Footscan 3-D 1 m-system (measuring at 125 Hz) and Footscan Gait Software was used on the computers to capture the data from the Footscan 3-D 1 m-system. Matlab (v. 9.2.0556344, r2017A) was used for further analysis of the collected data.

The rubber mats were surrounded by eight pro-flex cameras (recording at 125 Hz), four cameras recording on each side of the trial (Figure 1). Two reflective markers were attached to all hooves of each horse, at the level of the coronary band. One marker at the midpoint of the lateral side of the hoof and one marker at the midpoint of the medial side of the hoof (Figure 2). The kinematics were captured with a Qualisys motion analysis system, using reflective markers on the hoof and the results were presented in the software of Qualisys Track Manager. The kinematic trails were considered valid with a maximum of three missing following frames in the swing phase and a gap of ten missing frames in the stance phase. This was the maximum amount of missing frames that could be filled in manually afterwards.

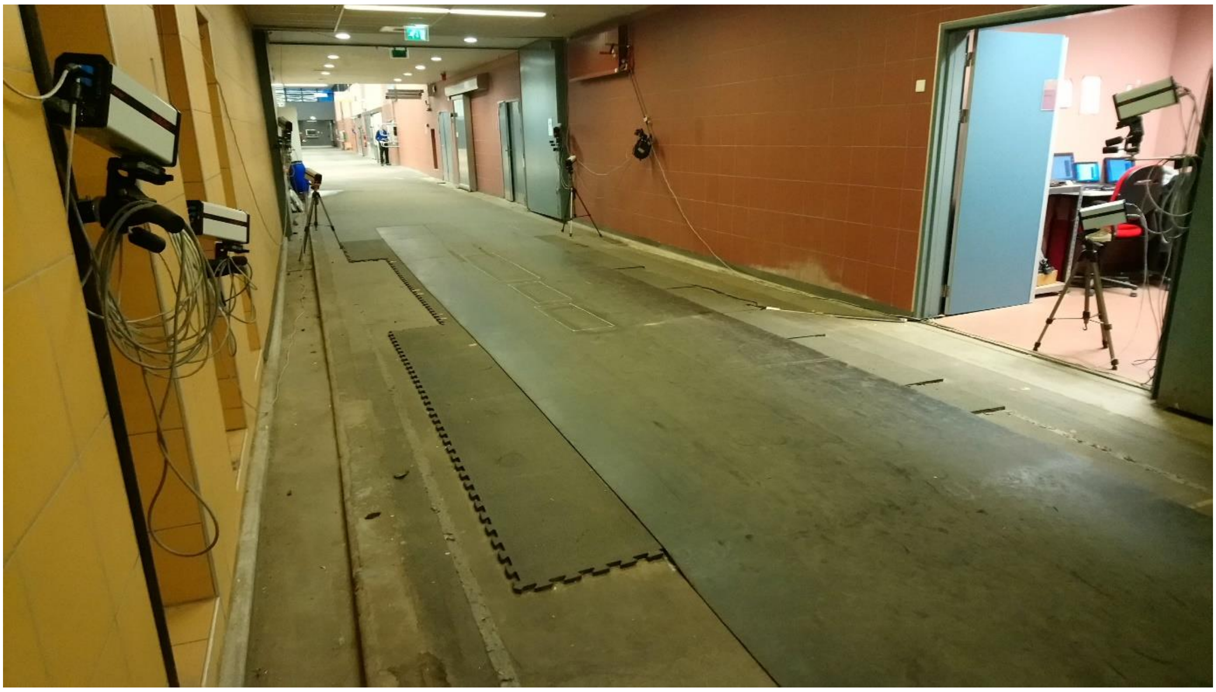


Figure 1. Setup of the experimental trial. The three pressure plates are covered with a rubber mat and the pressure plates are outlined by the chalk lines. The eight pro-flex cameras are positioned on both sides of the runway

Figure 2. Setup of the reflective markers on all four hooves.

Experiment

The horses were repeatedly trotted in a straight line over the three pressure mats to measure the COP pattern and kinematics of the limbs simultaneously. Care was taken by de handler to ensure that the horses trotted over the pressure plates in a similar and constant speed as possible. The horses entered the rubber mat in trot and were not allowed to slow down before they have passed the three mats. For each day of measurements, four pressure measurements with corresponding kinematics of each limb have been collected. Altogether, sixteen valid trials per session for each horse. The amount of pressures collected during the run over the pressure plates were varying, depending on how many hooves completely landed on the mat. The measurement was selected for further analysis if the pressure of the hoof was completely landed on the mat and the kinematics of both markers, corresponding to the pressure, were visible during the entire stance phase.

The horses were also monitored with the QHorse system to ensure that they were still owner-sound every measurement day. An experienced clinician evaluated the gait of each horse trotting a straight line. Horses judged with a clinical lameness were excluded in the measurements of that day. The QHorse system, administered by Qualisys, captures the motion of horses on a high precision (13). By evaluating the asymmetry of the horse with the QHorse system, lameness in horses can be detected at an early stage.

<u>Data analysis</u>

Data processing

The hoof pressure files have been converted to a MATLAB file for further analysis after completing the measurements. Each hoof should be completely landed on the mat for a valid hoof pressure measurement and the corresponding force plot should be recorded similarity. Each valid hoof pressure was selected by hand after the run. The corresponding force plot of the hoof pressure was selected too and was required to determine the beginning and ending of the stance phase.

The sensors of the mat needed to be transformed to coordinates for further analysis. Both markers attached on the surface of the hoof have been tracked in the X-axis and the Y-axis. Because of the instability of the trotting hoof in the relation to the X- and Y-axis, the coordinates of both systems were standardized by rotation and translation. The lateral and medial marker should have the same Y-coordinates in every frame. The transformation enables to track the movements of the kinematic markers in relation to the COP and with respect to the limbs. The movements of the kinematic markers in relation to the room are excluded.

Analysis of the kinematics, of the limb corresponding to the pressure, was performed by tracking the path of the makers. Missing frames of markers in the pathway have been filled in manually. Finally, the selected pressures and kinematics were linked and translated to a COP pattern.

The COP pattern was quantified using several variables. ‘‘Centroidsize’’ is the size of the COP path measured by the pressure plate, it is the sum of the distance of all measured locations to the centroid. The midpoint of the hoof is coordinated as [0,0]. The outcomes of the COP path are corrected for the x-, and y-axis both towards the midpoint of the hoof, the ‘‘Centroidsizekine’’. This correction was also performed for the x-, and y-axis separately, respectively the ‘‘Centroidsizekine X’’ and ‘‘Centroidsizekine Y’’. The shift of the COP to a secured point makes it enable to compare the COP outcomes with each other. Normalisation of the ‘‘Centroidsizekine’’ excludes the effect of time and standardized the measurements to 100 frames which dismisses the duration of midstance, resulting in the ‘‘Centroidsizekinenorm’’. The ‘‘Stance Duration’’ is the total of measured frames divided by 125Hz.

<u>Statistics</u>

The statistics were all performed in IBM SPSS Statistics 24. To analyse the measurements of cycle 1 and cycle 2, a multivariate analysis of covariance (MANCOVA) was used. This model was performed using ‘cycle’ (cycle 1 and cycle 2) as fixed factor and with ‘day’ as a covariate. The analysed dependent variables were ‘‘Centroidsize’’, ‘‘Centroidsizekine’’, ‘‘Centroidsizekine X’’, ‘‘Centroidsizekine Y’’, ‘‘Centroidsizekinenorm’’ and ‘‘Stance duration’’. The front limbs and hind limbs were split into two groups for further analysis of the results. Univariate analysis was used for each limb individually, the ‘‘legperhorsenr’’.

ANOVA repeated measures model with three number of levels was used to compare data between cycle 1, 2 and 3. The comparison between cycles was analyzed with a repeated measurements test, because the same measurements were performed for three periods. For this comparison the measurements of a single randomly picked day of cycle 1 and 2 have been used and compared with cycle 3. The third cycle consists of only one measurement day. The within-subject variables included ‘‘Centroidsize’’, ‘‘Centroidsizekine’’, ‘‘Centroidsizekine X’’, ‘‘Centroidsizekine Y’’, ‘‘Centroidsizekinenorm’’ and ‘‘Stance Duration’’ for all three cycles. The between-subject factor was the ‘‘legperhorsenr’’of each horse, the legs of the participated horses were numbered individually (1 to 40) to simplify the processing of the results. Furthermore, the measurements were performed in a Tukey’s b post-hoc test to compare the three different cycles with each other and to identify which of the three cycles showed significancy.

Results

During this study, 4857 (left front: 1225, left hind: 1209, right front: 1227, right hind: 1196) valid hoof pressures from 40 limbs have been collected over a period of three trimming cycles. For each valid hoof pressure were determined the dependent variables: ‘‘Centroidsize’’, ‘‘Centroidsizekine’’, ‘‘Centroidsizekine X’’, ‘‘Centroidsizekine Y’’, ‘‘Centroidsizekinenorm’’ and ‘‘Stance duration’’.

<u>Overall test</u>

Significant effect (p<0,05) was found for all of the dependent variables: ‘‘Centroidsize’’, ‘‘Centroidsizekine’’, ‘‘Centroidsizekine X’’, ‘‘Centroidsizekine Y’’, ‘‘Centroidsizekinenorm’’ and ‘‘Stance duration’’. The three-way interaction effect between day, cycle and legperhorsenr was significant (p<0,05), this means that the interaction among day and cycle is different across the levels of legperhorsenr. All of the limbs show significant differences (p<0,05) and each limb has their individual effect crossing time. Further analysis is necessary to clarify for which of the dependent variables the individual legperhorsenr show significant difference.

	day*cycle	
	Front	**Hind**
Centroidsize	7/20 (0,35)	10/20 (0,50)
Centroidsizekine	6/20 (0,30)	4/20 (0,20)
Centroidsizekine X	8/20 (0,40)	8/20 (0,40)
Centroidsizekine Y	7/20 (0,35)	6/20 (0,30)
Centroidsizekinenorm	7/20 (0,35)	1/20 (0,050)
Stance duration	7/20 (0,35)	12/20 (0,60)

*Table 2. Effect of day*cycle on the dependent variables with significant effect (p<0,05). The significant limbs per variable were summed and divided by the total number front- and hind limbs and is expressed as a percentage (X/20).*

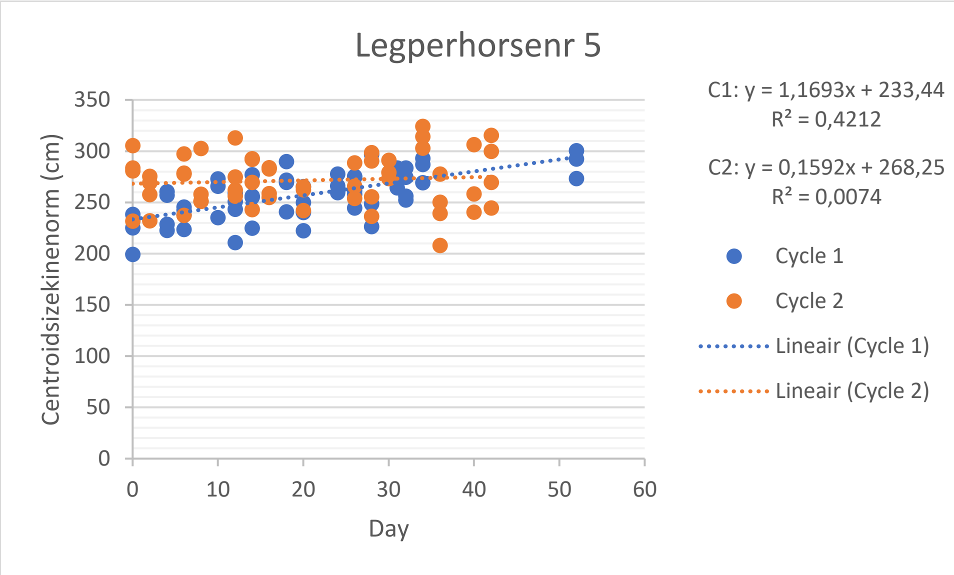
<u>Test per limb</u>

The data of cycle 1 and cycle 2 was performed per legperhorsenr to identify which dependent variables had an interaction effect (p<0,05) between day and cycle (day*cycle). Interaction effect occurs if there is an interaction between day*cycle that affects one of the dependent variables. The legperhorsenr with significant effect of day*cycle were summed and split up in a front feet and hind feet group to clarify which of the variables had the lowest and highest effect of day*cycle (Table 2). Splitting up in a front and hind group was likely, because the hoof-unrollment pattern of the hind limbs showed a lateral shift of the COP, where the position of the COP of the front limbs almost stayed the same (8). The dependent variable Centroidsizekinenorm had the lowest effect of day*cycle (8/40) and is the steadiest factor. Stance duration was the most affected dependent variable, 19 out of 40 legs had significant effect of day*cycle.

Further analysis was performed with the 8 significant affected legs, to evaluate how big the effect of day*cycle is for the steadiest dependent variable Centroidsizekinenorm. Those significant affected legs were assumed as the worst-case scenarios for the best variable Centroidsizekinenorm. The output of each limb with significant effect of day*cycle is shown in a scatterplot graph (Graph 1). For the data of each cycle is made a linear regression and their accessory R squared (R^2) was calculated. The R^2 is the percentage of the variance of the dependent variable Centroidsizekinenorm predicted by day. Furthermore, the day 0 and day 42 values of Centroidsizekinenorm were calculated with the linear formula of each cycle. The calculated values of day 0 and day 42 were still variable within both cycles of the limb. Legperhorsenr 5 had the highest calculated differences between day 0 and day 42 (49,1 cm), which is about twice the standard deviation (23,1 cm) (Table 3).

Legperhorsenr	Cycle	D0-D42 (cm)	Standard deviation (cm)	Mean difference (cm)
3	1	35,0	28,1	21,6
	2	4,50	25,9	
5	1	49,1	23,1	14,5
	2	6,69	25,3	
13	1	18,3	28,8	24,9
	2	15,3	21,7	
18	1	6,10	25,0	1,74
	2	36,9	32,6	
21	1	35,2	25,8	32,6
	2	13,4	23,9	
23	1	23,9	24,1	21,3
	2	21,6	27,8	
25	1	3,62	14,5	11,2
	2	31,8	19,3	
35	1	10,9	17,6	6,46
	2	9,48	14,4	

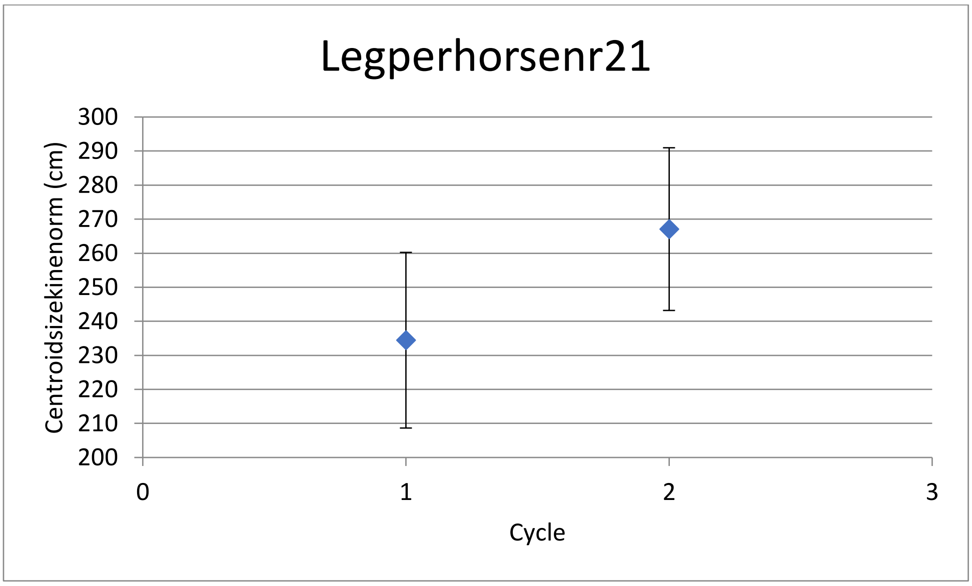
*Table 3. Overview of the legperhorsenr with significant effect of day*cycle. The difference between day 0(D0) and day 42(D42) per cycle is calculated with the linear formula. The standard deviation of each cycle and the mean difference between cycle 1 and cycle 2 are also shown in this table.*



*Graph 1. This graph shows a scatter plot of legperhorsenr 5 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R^2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*

<u>Effect per cycle</u>

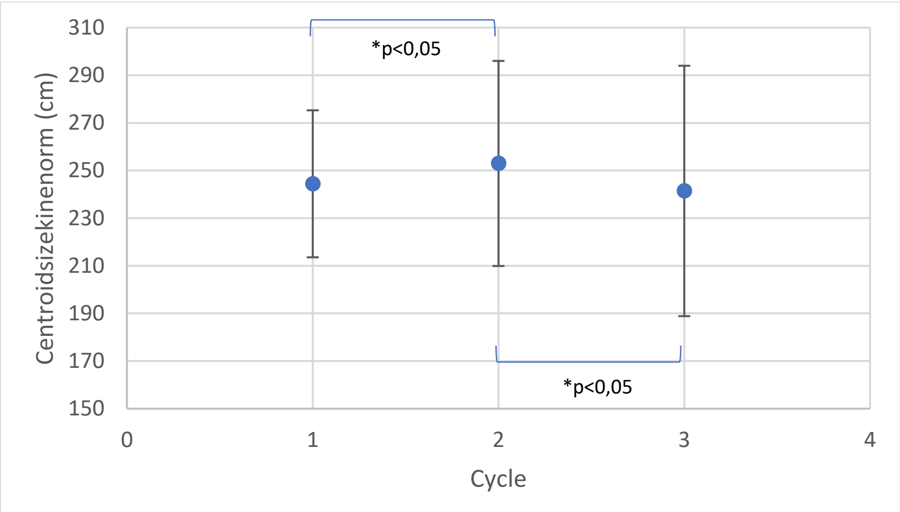
The limbs of the dependent variable Centroidsizekinenorm with effect for day*cycle were also performed in a univariate model to see how much the effect of day*cycle is. Legperhorsenr 21 showed the most deviation. For this limb, the mean and the accessory standard deviation of each cycle are shown in Graph 2. The mean difference between cycle 1 and cycle 2 of legperhorsenr 21 (32,6 cm) is higher than the standard deviation of both cycles individually (respectively 25,8 cm and 23,9 cm). The other remaining legperhorsenr do all have a mean difference which is less than the standard deviation of both cycles individually. For the graphs of the other legperhorsnr, see Appendix B.



Graph 2. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 21. The mean of cycle 1 and cycle 2 (blue spots) is extended by their standard deviation error bars.

Comparison between cycles

The mean, mean difference and standard deviation of cycle 1, cycle 2 and cycle 3 were calculated for all of the dependent variables. The mean and the accessory standard deviation of each cycle for the dependent variable Centroidsizekinenorm are shown in Graph 3. For the graphs of the other dependent variables, see appendix C. Significant effect (p<0,05) between the three cycles, determined by the mean difference, was noticed for some of the dependent variables. Cycle 2 had the most significant effect of Centroidsizekinenorm, with a mean difference between cycle 1 and cycle 2 of -8,5 cm and a mean difference between cycle 2 and cycle 3 of 11,6 cm (Graph 3). The standard deviation of cycle 1 was 30,9 cm, of cycle 2 was 43,0 cm and of cycle 3 was 52,6 cm. The mean differences of both cycles falls within the standard deviation of the three cycles. This confirms the significancy between the cycles. No significant effect found between the cycles for the variables Centroidsizekinenorm and Centroidsizekine X.



*Graph 3. The graph shows the comparison between three cycles for the variable Centroidsizekinenorm. Significant effect between the cycles is marked with *p<0,05. Each cycle presents the measurements of all legperhorsenr of one randaomly picked day. The mean of each cycle (blue spots) is extended by their standard deviation error bars.*

Shape of the COP path

The shapes of the COP path have been compared. The x- and y-coordinates of the COP path of a randomly picked legperhorsenr were selected for two random days, file 553 and file 919 (Figure 3). The coordinates are drawn with respect to the hoof-bound coordinate system and show the relative position of the of the hoof landing on the pressure plates. Comparing the COP path of this randomly picked legperhorsenr, the shapes of both days show remarkable similarities.

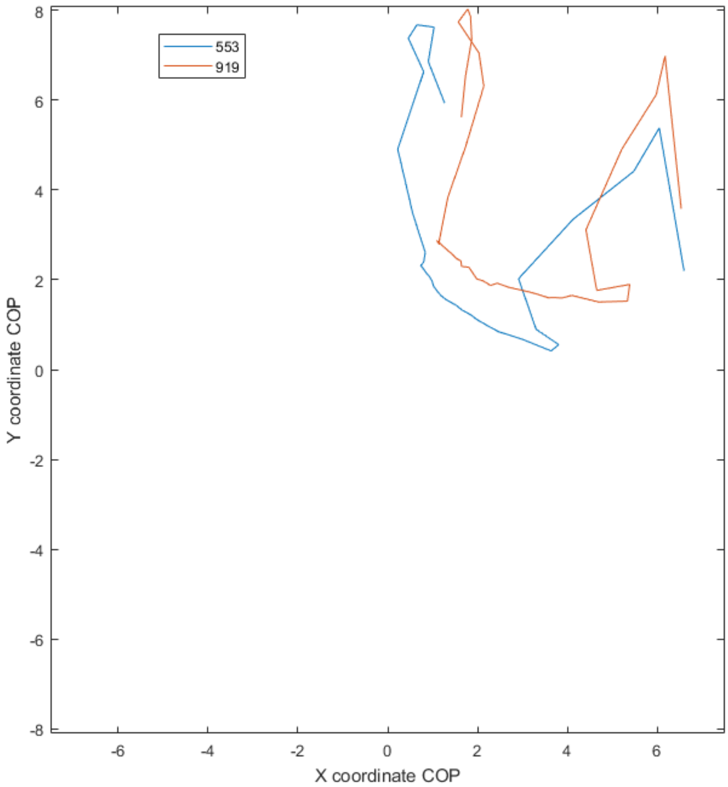


Figure 3. Comparison of the COP path of a randomly picked legperhorsenr for two random days. The x-axis presents the X coordinate of the COP path (cm) and the y-axis presents the Y coordinate of the COP path (cm). The blue line is the shape of file 553 and the blue line is the shape of file 919.

Discussion

In this study, the consistency of the COP pattern and the effect of hoof growth on the COP path over a period of three trimming cycles were evaluated. Research has shown that the COP pattern of the horse is a highly sensitive parameter which results in a repeatable COP path of the horse within a day (12). In our study the COP pattern was measured frequently to test the repeatability of the COP pattern for a long period of time. Significant effect was found for the examined dependent and independent variables of the COP pattern. The measurements of the COP patterns have been analysed by using different dependent variables. The stability, usability and predictability of the dependent variables have been examined to find the best parameter for the early detection of lameness based on the COP pattern.

The outcome per limb per dependent variable shows a lot of limbs with significant effect for day*cycle. Interaction effect between day*cycle showed the most significant limbs for the dependent variable stance duration. This implicates that the stance duration of horses varies within a day and within a cycle and therefore an unpredictable parameter. The result was contrary to what might be expected, because lame horses will have an increased stance duration (14). Otherwise, the stance duration might have been a great variable for the early detection of lameness. The stance duration in this experiment might have been influenced by an intermittent trot of the horses or by the speed of the handlers, this could explain the variance in our data. Nevertheless, the data between different days and both cycles show overlap. If the data of the stance duration was influenced by an intermitting speed of the horses or handlers, we would have found larger errors in the data. Furthermore, the horses could be not that owner sound which also affects the stance duration. The horses have been judged by an experienced clinician and with the Qhorse system to minimize the effect of lameness in this study.

Centroidsizekinenorm turns out to be the most stable dependent variable, 20 percent of the legs had effect of cycle. Though, the effect of cycle on Centroidsizekinenorm was significant for some of the individual legs, the mean difference falls within the spread of the data. To make a clear distinction between the COP pattern of owner sound legs and the lame legs, the mean difference should be bigger than the standard deviation. For the dependent variable Centroidsizekinenorm the COP pattern of the lame leg should deviate more than 50 cm. If this deviation of more than 50 cm is presented in the lame leg, the effect of cycle can be excluded. To confirm this suggestion, it is important to obtain more insight in how much the COP pattern of the lame leg differs from the owner sound leg,

To evaluate the effect of time in relation to the COP pattern the independent variables day and cycle have been examined. Effect of time on the COP path is demonstrated, but is overshadowed by the big variance of the results within a day. This is in contrast with the results of the study of the COP pattern measured for one day (12). We have examined other dependent variables in this study, which turned out to be high sensitive for noisy data. To reduce the noisy data of the measurements per limb and the outcomes within a day, should be considered to use other variables in future research.

Comparison of the three cycles, with the Tukey's-b Post-Hoc test, was performed to determine the effect of the dependent variables over a longer period of time. Significant effect between the three cycles was found for some of the dependent variables. However, the mean difference between the significant affected cycles falls within the standard deviation.

The data of the COP pattern within a day and of the individual limbs are too sensitive for noise. Nevertheless, there is an overlap of some of the COP patterns and there were no remarkable outliers in the data are barely. In this study, a relatively large data set was analysed, this makes it easier to detect noise in the data. However, the analysed dependent variables used in this study were too sensitive for noise. We should consider the statistical significance and biological relevance of the outcome of this study (15). Because of the size of this study it is questionable whether the significant affected legs differs that much to define the legs as biologically relevant. The effect of those significant affected legs might not be visible in daily practice.

The used dependent variables did not characterize the COP pattern in a good position. However, by comparing the COP path of a randomly picked legperhorsenr for two random days, the shapes of both days show remarkable similarities. This confirms our conclusion of the experiment that the COP pattern is predictable over a period of time. Given the fact that the shape of the COP pattern is similar, we have the opportunity to select variables for future research. Geometric morphometric analysis is a quantitative method based on landmark coordinates of a specific shape. Comparing the coordinates between different shapes of the COP pattern provides better insight into whether and how the shape will change. Lame horses will show a different shape of the COP pattern and the landmark coordinates of this shape will differ from 'owner-sound' horses. The shape of the COP pattern underneath the hoof appears to be a stable parameter and we should take a closer look at geometric morphometric analysis in future research.

Conclusion

The use of pressure plates is an effective method to determine the COP pattern under the hoof of the horse. We can conclude that each individual limb of each horse has its own and unique COP pattern. The results of the shape of the COP pattern shows stability of the shape within a cycle and between the cycles. However, the dependent variables which were applied in this research are too sensitive for noise. The dependent variable Centroidsizekinenorm turns out as the best and most stable variable in this study. The data of the dependent variable stance duration were the most sensitive for noise, this makes the variable unsuitable for the early detection of lameness in horses.

Nevertheless, the COP pattern of an individual leg of a horse can be picked for a random day, and the shape of the COP pattern remains the same. To provide more insight into the shape of the COP pattern based on these landmark coordinates, should be considered to select other parameters for analysis. Geometric Morphometrics Analysis might be a good recommendation for future research of the COP pattern with the pressure plates. The experiment should also be conducted with lame horses to see the changes of the COP pattern between the ‘owner-sound’ legs and the affected legs of the horse.

Acknowledgement
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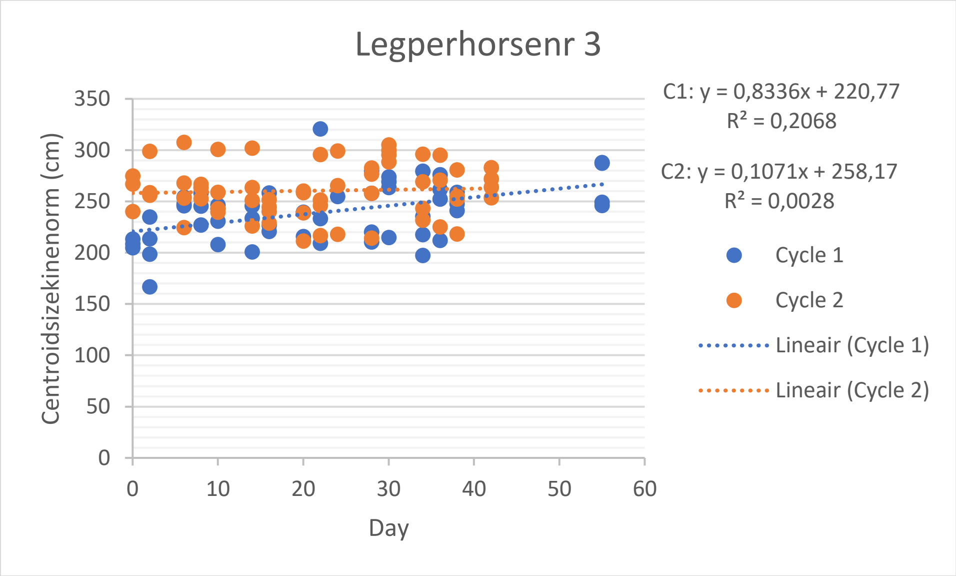
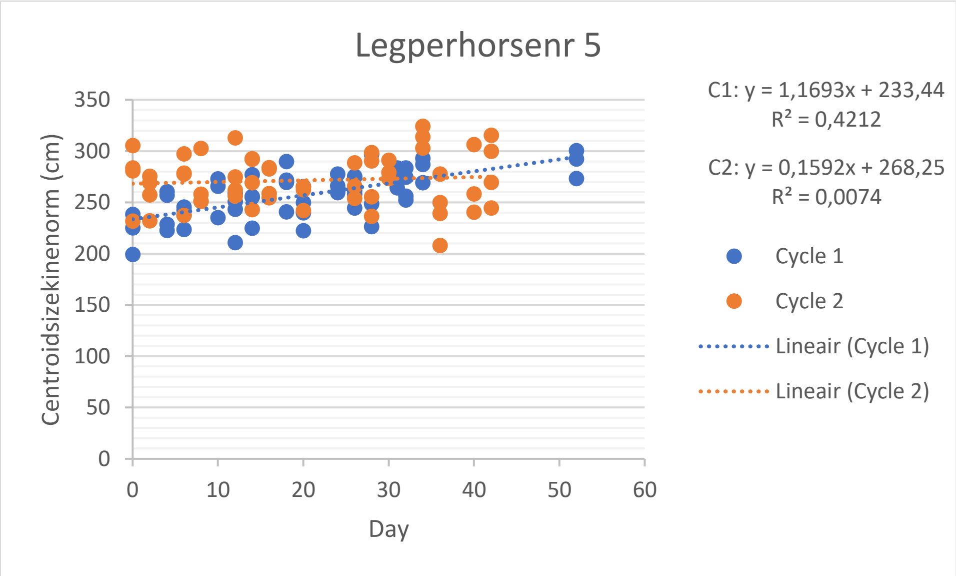
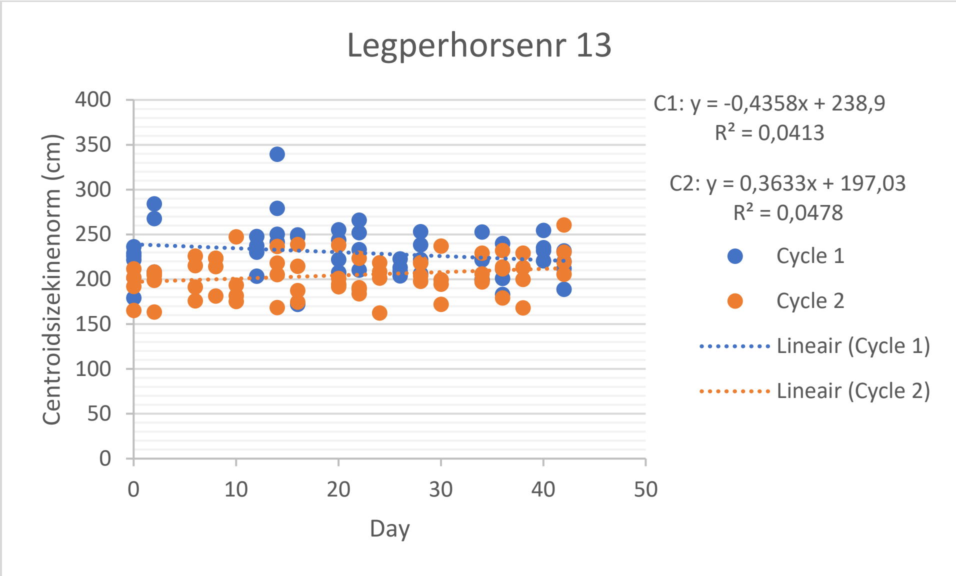
Appendix A. Test per limb



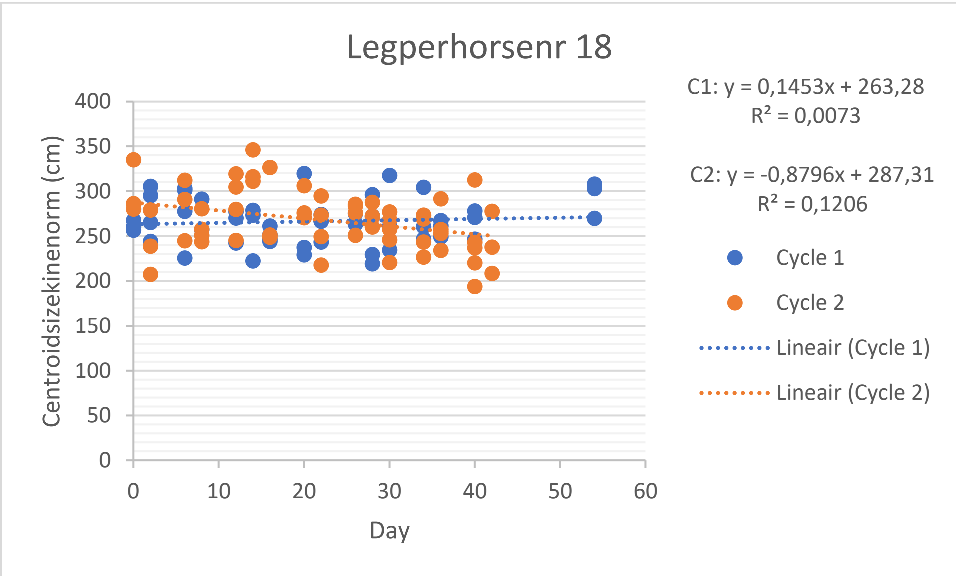
*Graph A1. This graph shows a scatter plot of legperhorsenr 3 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*



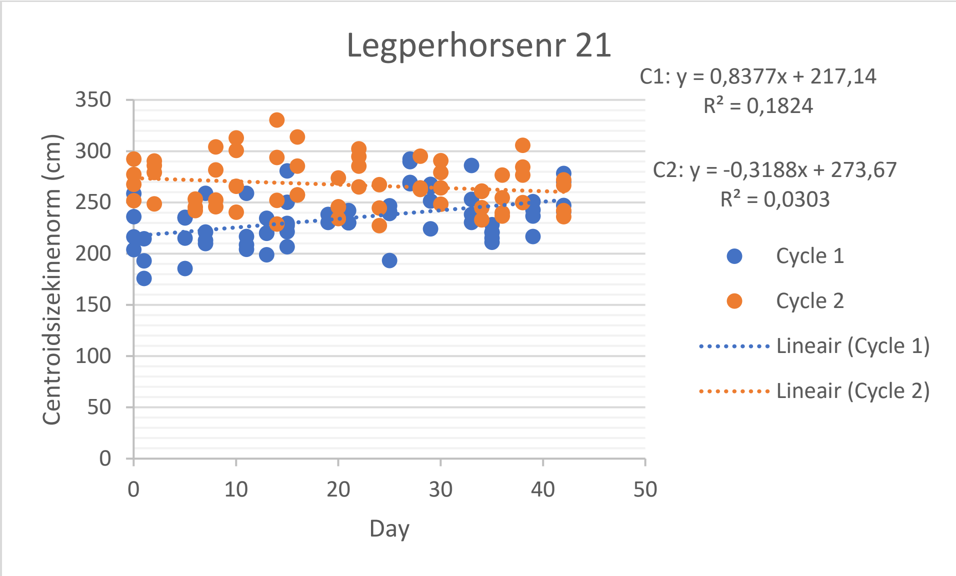
*Graph A2. This graph shows a scatter plot of legperhorsenr 5 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*



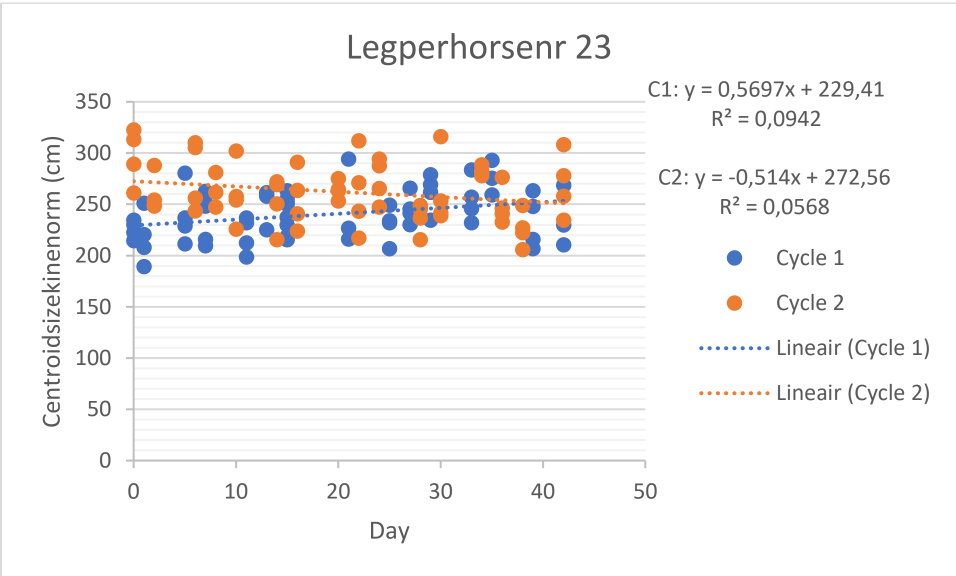
*Graph A3. This graph shows a scatter plot of legperhorsenr 13 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*



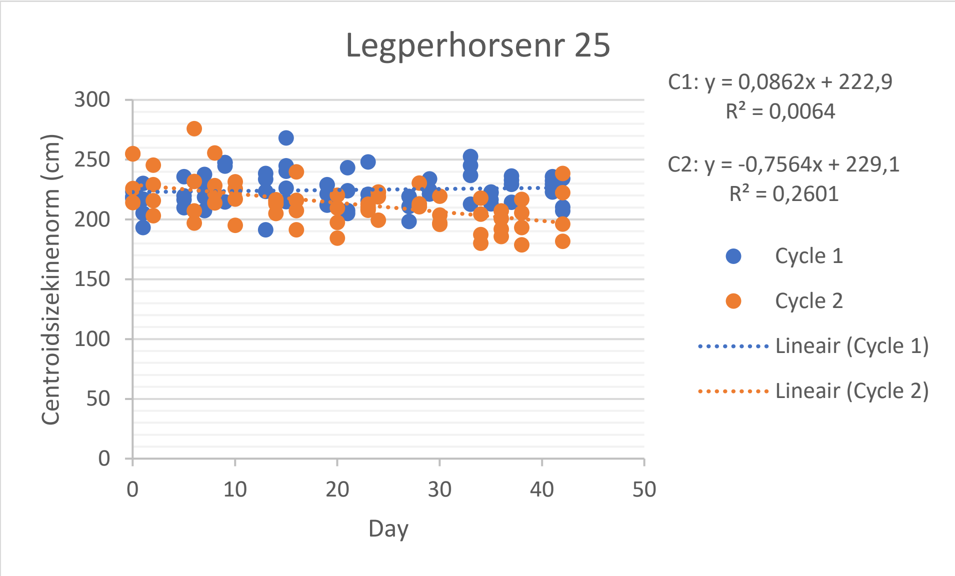
*Graph A4. This graph shows a scatter plot of legperhorsenr 18 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*



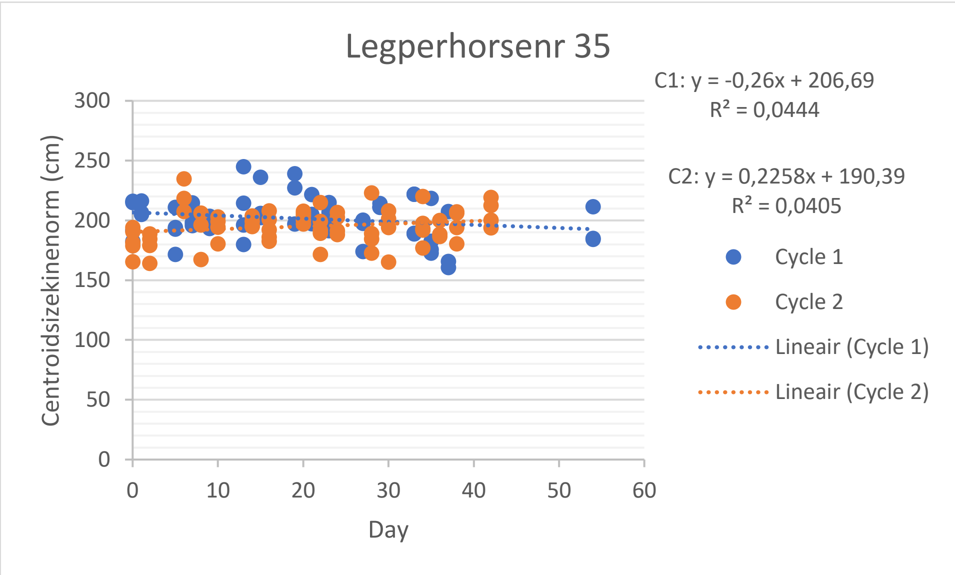
*Graph A5. This graph shows a scatter plot of legperhorsenr 21 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*



*Graph A6. This graph shows a scatter plot of legperhorsenr 23 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*

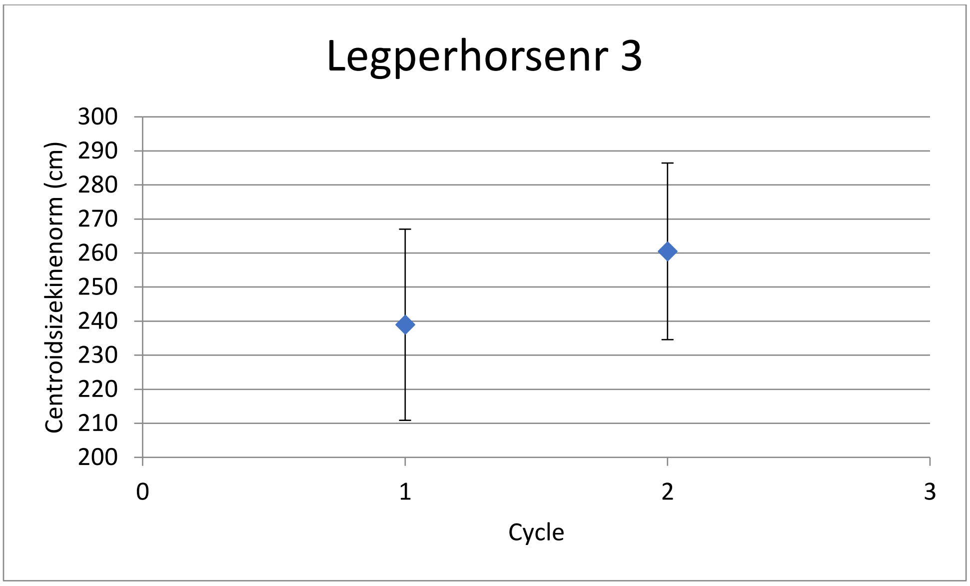


*Graph A7. This graph shows a scatter plot of legperhorsenr 25 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*

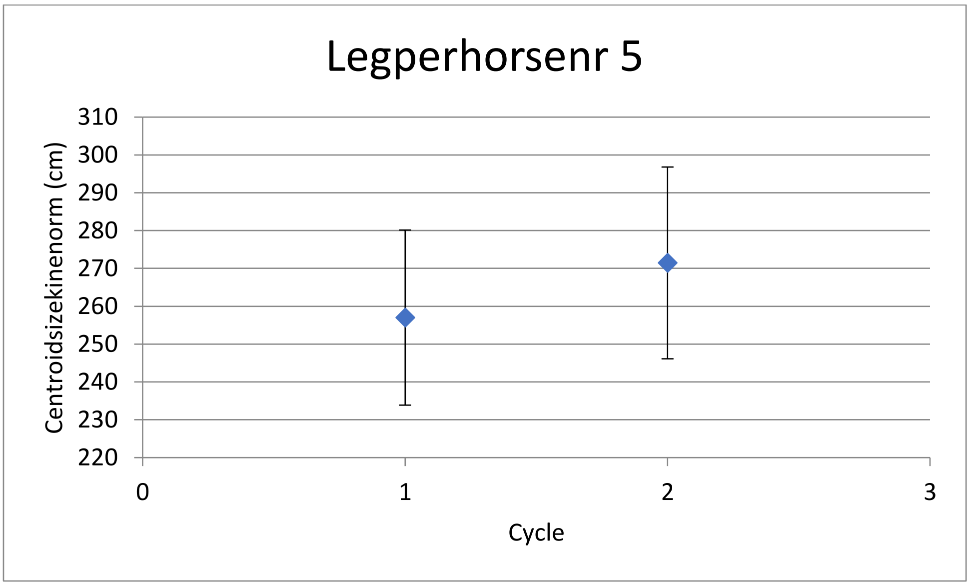


*Graph A8. This graph shows a scatter plot of legperhorsenr 35 with significant effect of day*cycle for the dependent variable Centroidsizekinenorm. For the data of cycle 1 (C1) and cycle 2 (C2) is made a linear regression. The R squared (R2) shows the coefficient of the determination of C1 and C2. The data within each day is expressed with the blue (cycle 1) and orange (cycle 2) dots.*

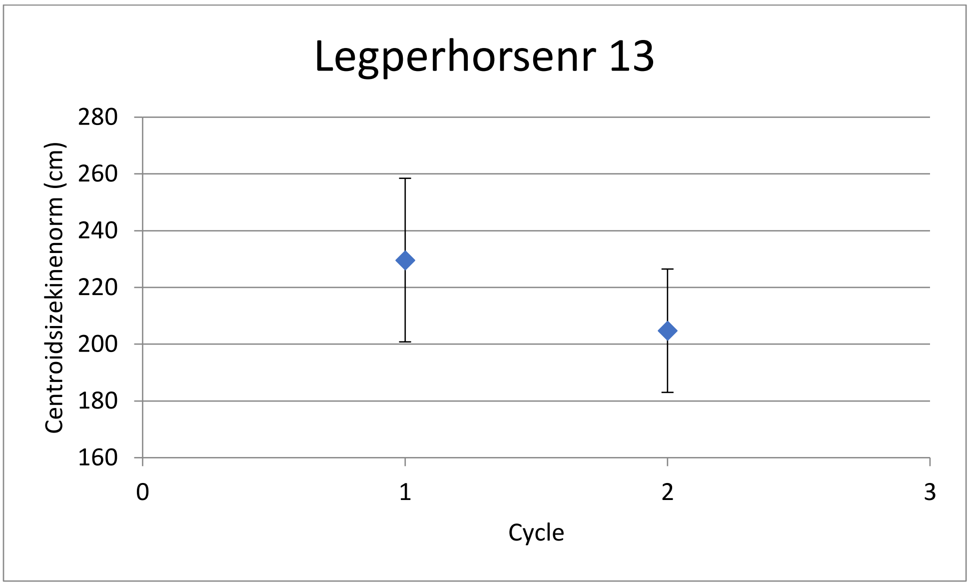
Appendix B. Effect per cycle



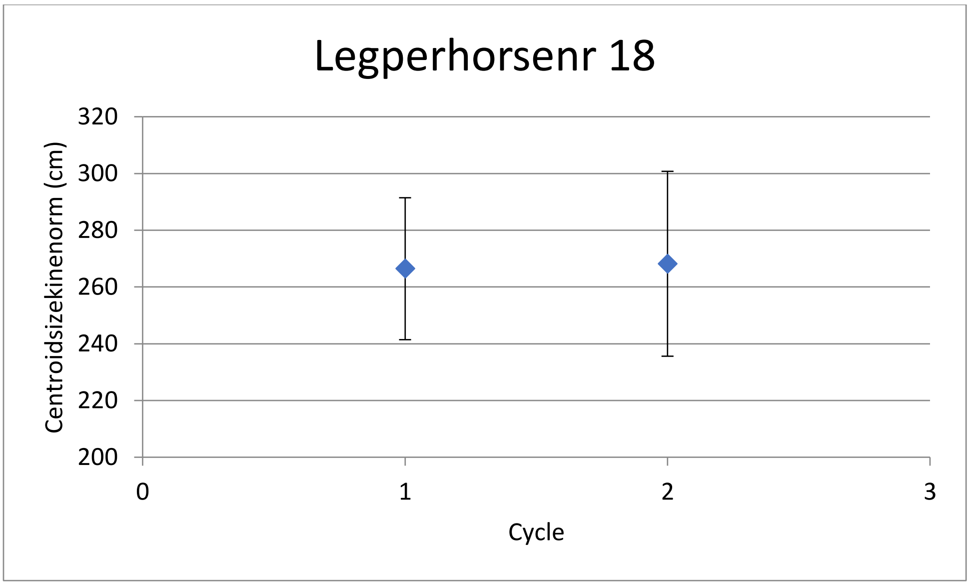
Graph B1. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 3. The means of cycle 1 and cycle 2 (blue spots) are extended by their standard deviation error bars.



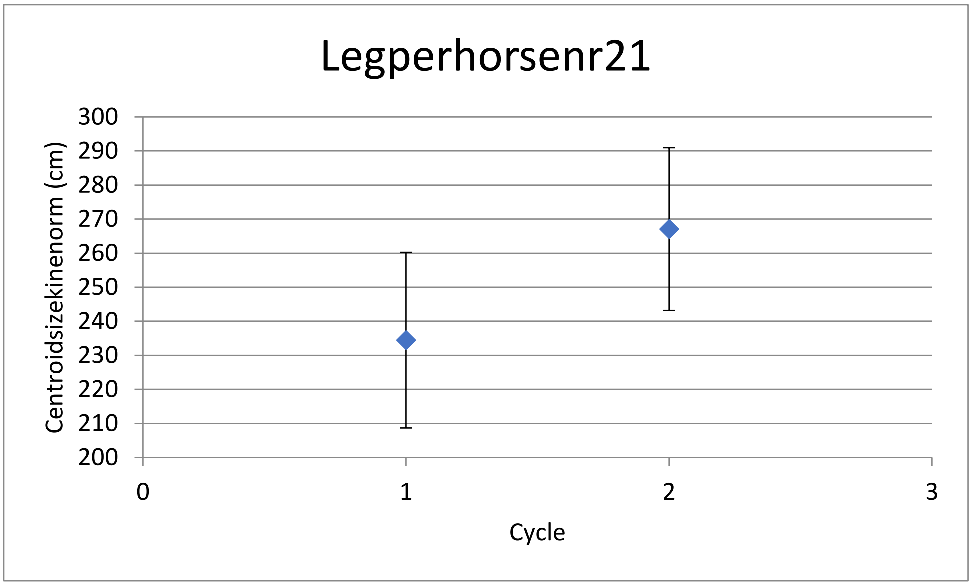
Graph B2. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 5. The means of cycle 1 and cycle 2 (blue spots) are extended by their standard deviation error bars.



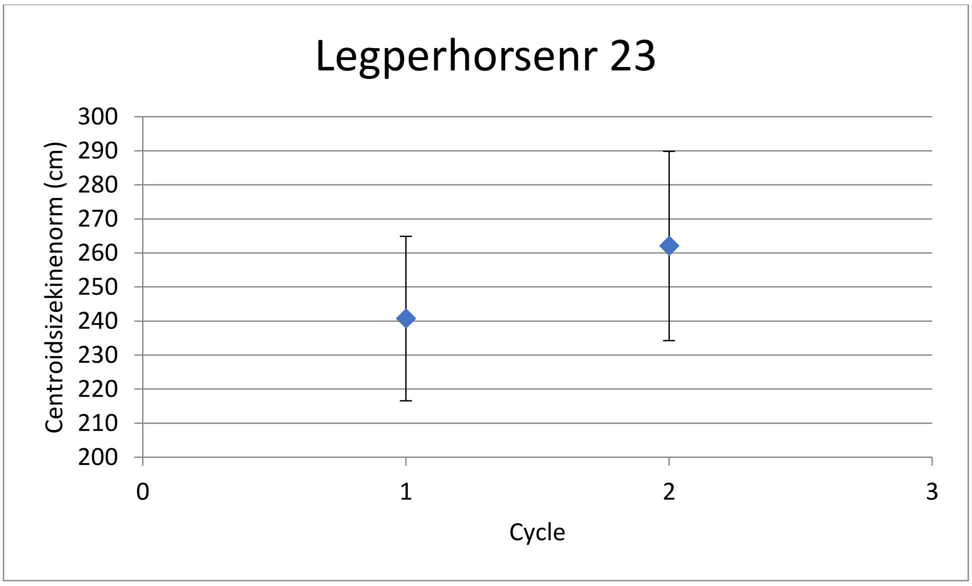
Graph B3. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 13. The means of cycle 1 and cycle 2 (blue spots) are extended by their standard deviation error bars.



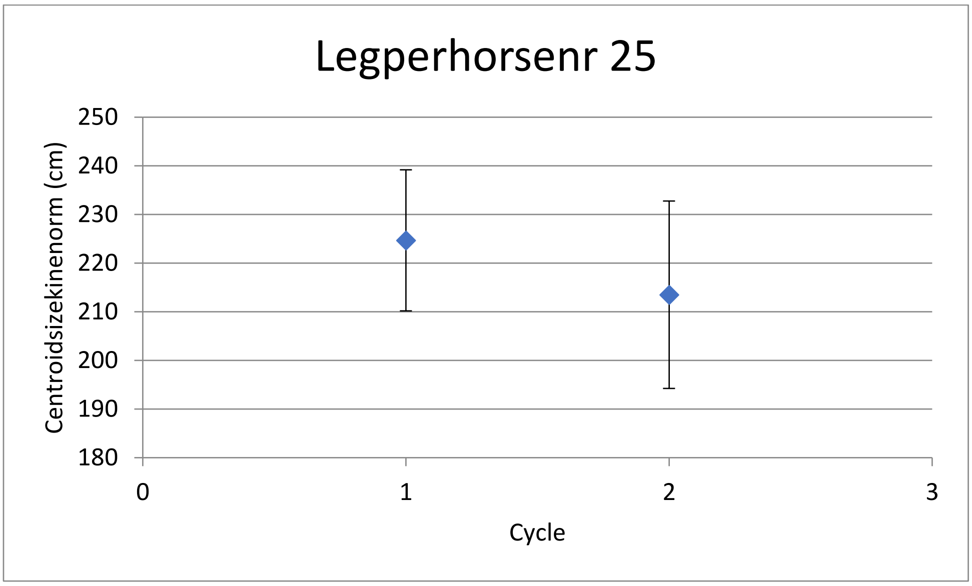
Graph B4. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 18. The means of cycle 1 and cycle 2 (blue spots) are extended by their standard deviation error bars.



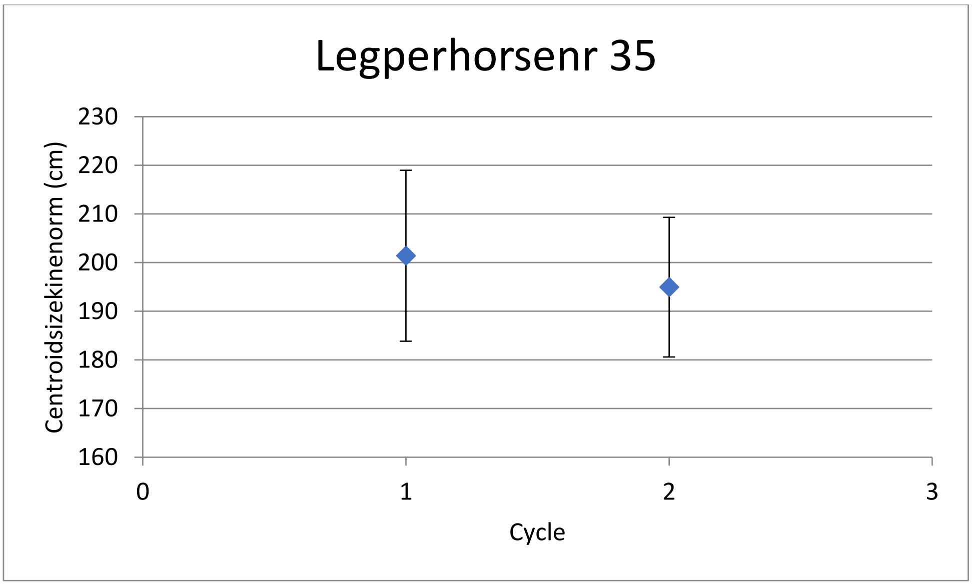
Graph B5. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 21. The means of cycle 1 and cycle 2 (blue spots) are extended by their standard deviation error bars.



Graph B6. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 23. The means of cycle 1 and cycle 2 (blue spots) are extended by their standard deviation error bars.

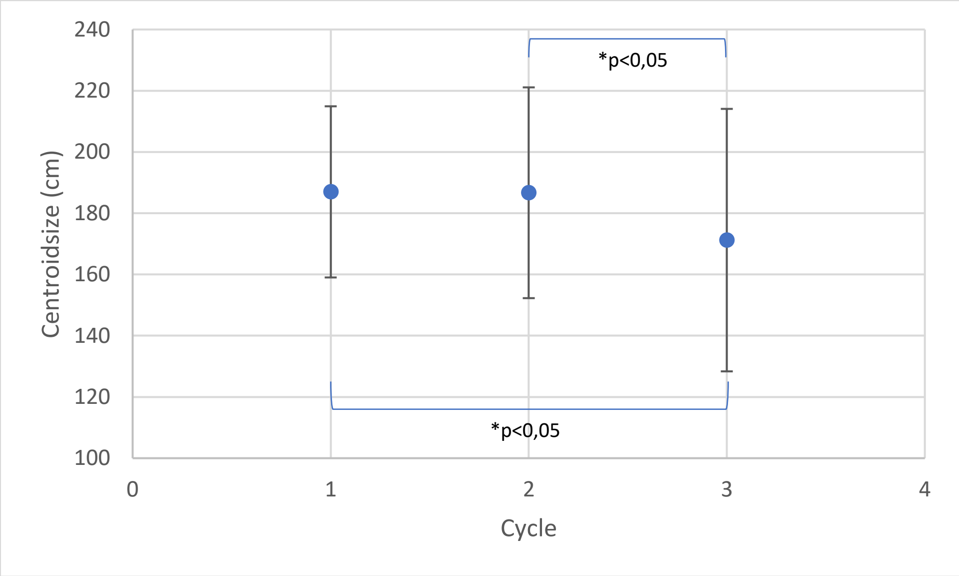


Graph B7. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 25. The means of cycle 1 and cycle 2 (blue spots) are extended by their standard deviation error bars.

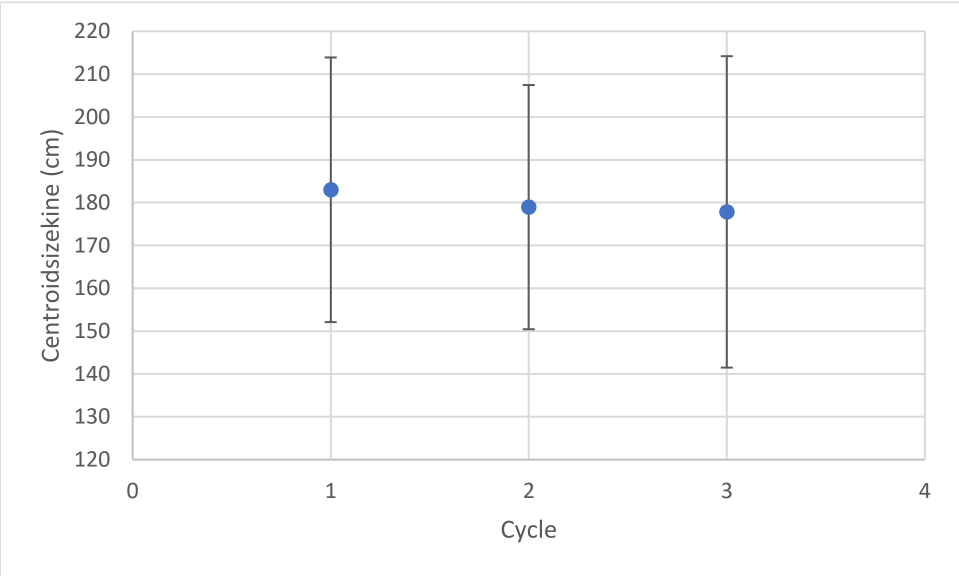


Graph B8. The graph shows the mean per cycle for the dependent variable Centroidsizekinenorm of legperhorsenr 35. The means of cycle 1 and cycle 2 (blue spots) are extended by their standard deviation error bars.

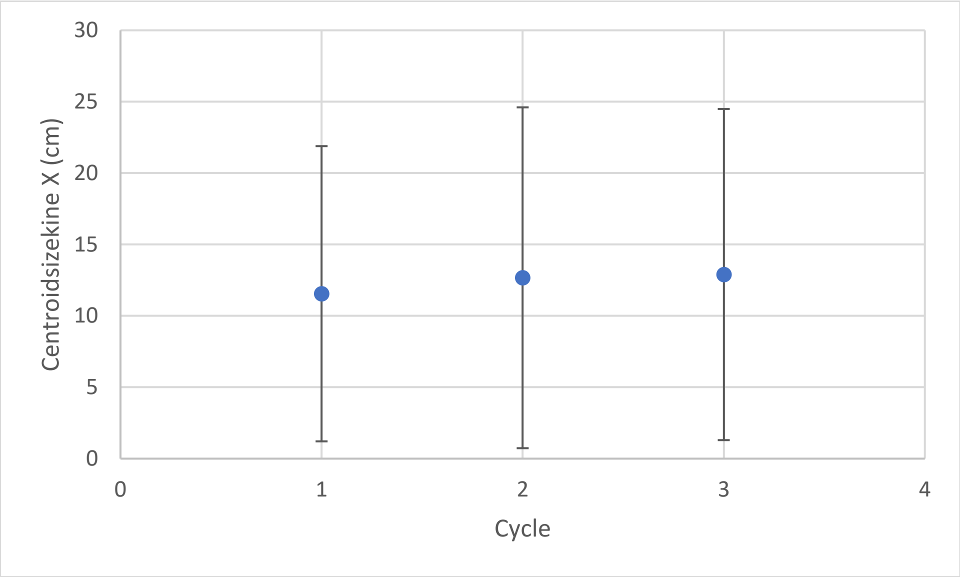
Appendix C. Comparison between cycles



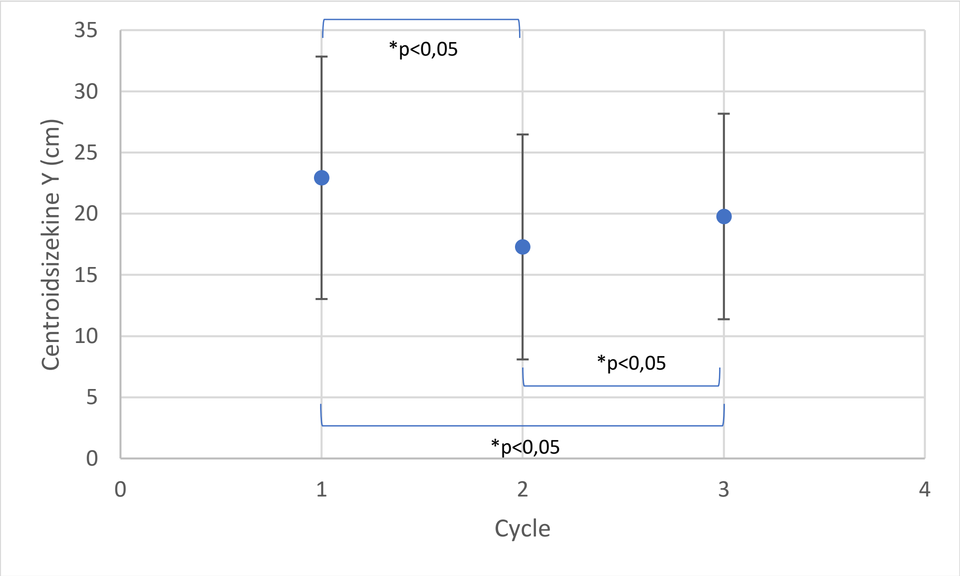
*Graph C9. The graph shows the comparison between three cycles for the variable Centroidsize. Significant effect between the cycles is marked with *p<0,05. Each cycle presents the measurements of all legperhorsenr of one randaomly picked day. The mean of each cycle (blue spots) is extended by their standard deviation error bars.*



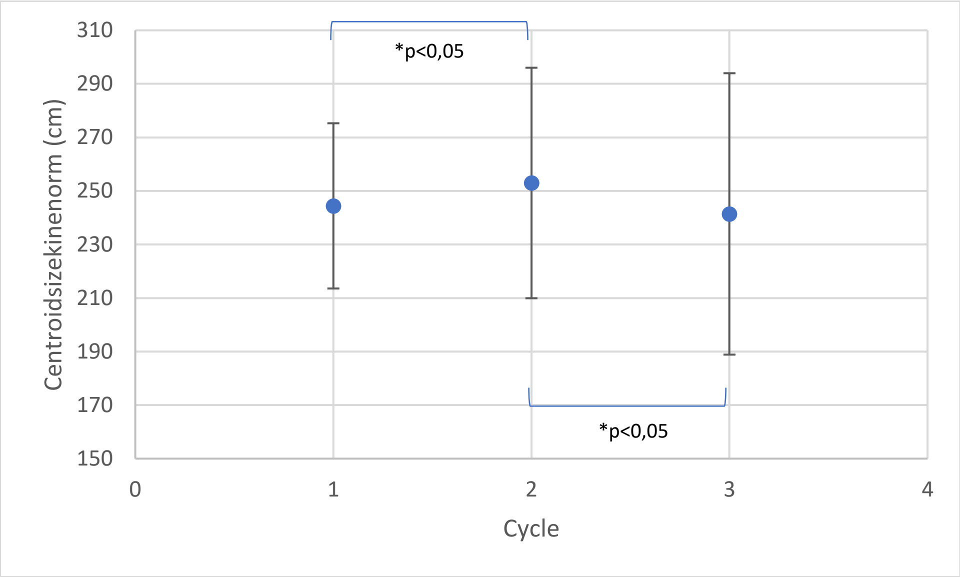
*Graph C10. The graph shows the comparison between three cycles for the variable Centroidsizekine. Significant effect between the cycles is marked with *p<0,05. Each cycle presents the measurements of all legperhorsenr of one randaomly picked day. The mean of each cycle (blue spots) is extended by their standard deviation error bars.*



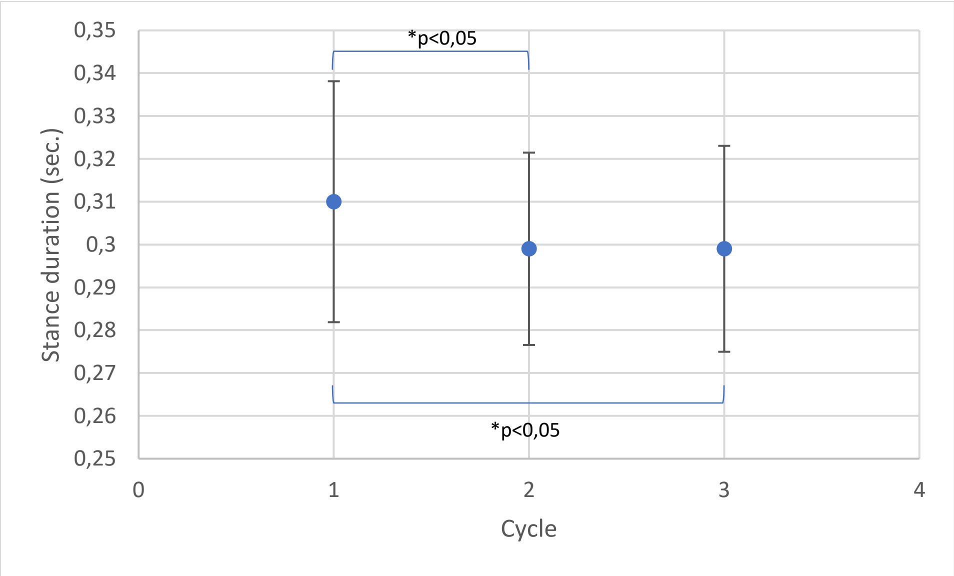
*Graph C11. The graph shows the comparison between three cycles for the variable Centroidsizekine X. Significant effect between the cycles is marked with *p<0,05. Each cycle presents the measurements of all legperhorsenr of one randaomly picked day. The mean of each cycle (blue spots) is extended by their standard deviation error bars.*



*Graph C12. The graph shows the comparison between three cycles for the variable Centroidsizekine Y. Significant effect between the cycles is marked with *p<0,05. Each cycle presents the measurements of all legperhorsenr of one randaomly picked day. The mean of each cycle (blue spots) is extended by their standard deviation error bars.*



*Graph C13. The graph shows the comparison between three cycles for the variable Centroidsizekinenorm. Significant effect between the cycles is marked with *p<0,05. Each cycle presents the measurements of all legperhorsenr of one randaomly picked day. The mean of each cycle (blue spots) is extended by their standard deviation error bars.*



*Graph C14. The graph shows the comparison between three cycles for the variable Stance duration. Significant effect between the cycles is marked with *p<0,05. Each cycle presents the measurements of all legperhorsenr of one randaomly picked day. The mean of each cycle (blue spots) is extended by their standard deviation error bars.*