The effects of water on limb and upper-body kinematics in horses walking and trotting in a water track

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Abstract

Rehabilitation after a musculoskeletal injury has become an essential part of veterinary care. Various forms of exercise in water have been described as an effective medium to improve function while preventing further injuries. This study evaluated kinematic changes of horses walking and trotting on a water track (WTr) versus overground (OG). Eleven horses, equipped with IMU sensors (EquiMoves®; attached to the cannon bones of each leg, the head, withers, back (T15), tuber sacral and L/R tuber coxae) were measured at walk and trot overground (OG1), while going through a water track (85m long; water height 33cm), and once more overground (OG2). Linear mixed models were used to compare the different conditions ($p < 0.05$). There was no significant difference in symmetry of head, withers and sacrum at walk. At trot, symmetry index of head, withers and sacrum decreased (-45.7%, -41.7% and -41.2%) in the WTr. Speed was significantly lower under WTr conditions compared with OG conditions at walk. At trot, speeds were similar. At walk and trot in the WTr, there was a significant increased stride duration (+14.9% and +15.6%), swing duration of both FLs (+19.1% and +38.5%) and HLs (+19.2% and +19.1%), and stance duration of both FLs (+13.5% and +7.9%) and HLs (+11.6% and +10%). Stride length increased only at trot in the WTr (+11.7%). At walk, FL pro- and retraction decreased (-14.2% and -16.2%) and HL protraction increased (+48.4%). At trot, pro- and retraction of only the HLs increased (+68.4% and +20.8%). No differences between OG1 and OG2 were found. The main conclusion of this study is that there is a combined effect of gait and water on upper-body symmetry, limb sagittal angles and stride timings, where increased hindlimb pro- and retraction yielded the most considerable change compared to overground locomotion. The reduced speed in water at walk, should be considered as a confounding factor. Since speeds were not different between conditions at trot, these effects seem to have been caused by the water. This information can help clinicians to meet individual rehabilitation requirements. Nevertheless, more studies are required to determine the long-term effects of WTr exercise.

Keywords
Horse; water exercise; water track; symmetry; limb timing; limb angles; rehabilitation

Introduction

Aquatic exercise is frequently used for rehabilitation and as an additional training method for sport horses, whereby there is improving knowledge of the benefits of horses exercising in water. Nevertheless, there is still a lot unclear about equine kinematics during water exercise and therefore rehabilitation and training programs are still limited evidence based (Scott et al., 2010). There are certain studies available about the biomechanical responses of horses' back and limbs using a water treadmill. Results of the study of Mooij et al. (2013) revealed that training in a water treadmill with water levels at hoof, fetlock and carpal joint gave an increased pelvic flexion and axial rotation, and a reduced lateral bending relative to water levels at elbow and shoulder joints. They also found that water levels at the height of the fetlock and carpal joint triggered a response to lift both front limbs (FLs) and hind limbs (HLs) to step over the water, to make this movement there must be an increased axial rotation of the equine back. When the water level is too high for the horse to step over the surface, the locomotion pattern changes, which is also caused by the greater resistance of water by increasing depth (Mooij et al., 2013). Water will assist the horse in lifting the limb in the vertical plane because of the buoyancy it provides, but there is a greater resistance to movement in the sagittal plane (Scott et al., 2010). Therefore, water height is proven to be relevant for the locomotion pattern of the horse, whereas water heights at the levels of the elbow and shoulder resulted in different locomotion patterns than water heights at the levels of hoof, fetlock and carpal joint, due to the increased resistance of water by increasing water levels (Mooij et al., 2013). The largest range of joint motion (ROM) lies at different water depths for different joints and the greatest flexion and extension of a joint is not always at the same water height (Mendez-Angulo et al., 2013). The flexion-extension ROM of the thoracolumbar spine becomes greater with increasing water depth (Nankervis et al., 2016).

Studies showed that water heights at the level of the carpus results in a lower stride frequency and a larger stride length (Scott et al. 2010; McCrae et al., 2021). The change in stride timing is not accompanied by an increase in workload because there was no significant difference in heart rate detected (Scott et al., 2010). Therefore, water exercise could be a useful tool in revalidation programs to train horses without significantly increasing the workload.

However, these studies were all performed with horses using a water treadmill, whereby there are several differences between a water treadmill and the water track (WTr) used in the present study. For instance, speed and water height is easily controlled and changed with a water treadmill, which cannot be done in the WTr. This could both be an advantage and a disadvantage because the optimal speed during water exercise is not yet determined and probably differs for each individual horse. During exercise in the WTr horses move at their own preferred speed. Thereby, FL stance duration is longer on a treadmill compared to overground (OG) and the FLs and HLs are moved further to caudal during the retraction, whereby there are no differences in the angle of protraction. Workload of treadmill exercise is lower (Van Oldruitenborgh-Oosterbaan & Clayton, 1999), and the lumbar motion is less but more symmetrical compared to exercise OG (Alvarez et al., 2009). Nevertheless, treadmill research is easier to standardize and therefore has a greater repeatability (Van Oldruitenborgh-Oosterbaan & Clayton, 1999). By using a WTr the effect of the treadmill can be removed making it possible to focus simply on the effects of water on the locomotion pattern of the horse.

The aim of the current study was to objectively describe the kinematics of upper- and lower-body parameters during water exercise in a WTr (walk and trot) compared to kinematics OG and determine the effect of water on the locomotion pattern of the horse to help making water training and rehabilitation programs more evidence based. We hypothesized that (1) WTr exercise will improve the kinematics of the equine upper-body and limbs OG, and (2) the presence of water affects horses' upper-body symmetry and limb kinematics in a WTr.

Material and methods

The study protocol was approved by the IvD (Animal Welfare Body Utrecht) and informed consent forms for publication of research data have been signed by the owners of the participating horses prior to the study.

Horses

Kinematics were measured in eleven owner-sound sport horses with no recent history of lameness and with a mean age of 10.4 years (sd ±5.2) and a mean height at the withers of 170.3 centimeters (sd ±5.5), while they were walking and trotting OG and through a WTr both at their own preferred speed. Exact body weight of the horses is unknown. The horses were all experienced with WTr exercise and of various genders (mare, n=4; gelding, n=6; stallion, n=1), breeds (KWPN, n=6; Oldenburg, n=1; Hannoveranian, n=1; AES, n=2; Rheinland, n=1) and level in sport.

Data collection protocol and devices

Firstly, horses underwent a quick clinical exam to exclude external signs of lameness subjectively and afterwards were harnessed with ten wireless IMU (inertial measurement unit) sensors of the EquiMoves® system. Sensors were attached to the head, withers, back at T15 (the 15th thoracic vertebra), tuber sacrale, the left and right tuber coxae and one on each leg to objectively capture horse motion (Bosch et al., 2018). All sensors were incorporated in waterproof polythene pouches. The head sensor was attached to the headpiece of the halter, leg sensors were laterally attached to the cannon bones via soft lightweight tendon boots with Vetrap® and Leukoplast® for extra firmness, the withers sensor was secured to the highest point of the girth and the remaining sensors were attached to the skin with animal polster and double-sided tape. An overview of the sensors attached to each horse is shown in Figure 1. Before data collection, each horse was walked in hand for ten minutes on a firm surface as warming-up and to get familiar with the equipment. During the warming-up, horses were subjectively judged on soundness by experienced clinicians. After checking the sensor connection to the computer containing the EquiMoves software, all horses stood still for a few seconds until calibration of the IMU's was completed. The sensors record data at a frequency of 200 Hz, acceleration and angular velocity is measured using accelerometers and gyroscopes (Bosch et al., 2018) and speed was measured with a global navigation satellite system (GNSS). The EquiMoves software distinguishes the gait of the horse. Measurements started with a baseline measurement OG by walking and trotting in hand in a straight line on a firm surface. Second, the horses were guided between two lunging lines through the WTr in walk and trot. At last, there was another measurement OG followed by removal of the sensors. Data was collected continuously in each condition (OG1, WTr and OG2), and between every measurement calibration of the sensors was checked. Measurements took place on several days and horses were led by different handlers during OG measurements, guidance trough the WTr was done by the same two experienced handlers and all handlers exercised as little influence as possible on the locomotion pattern of the horse. The WTr has a length of 85 meters and water height was set on 33

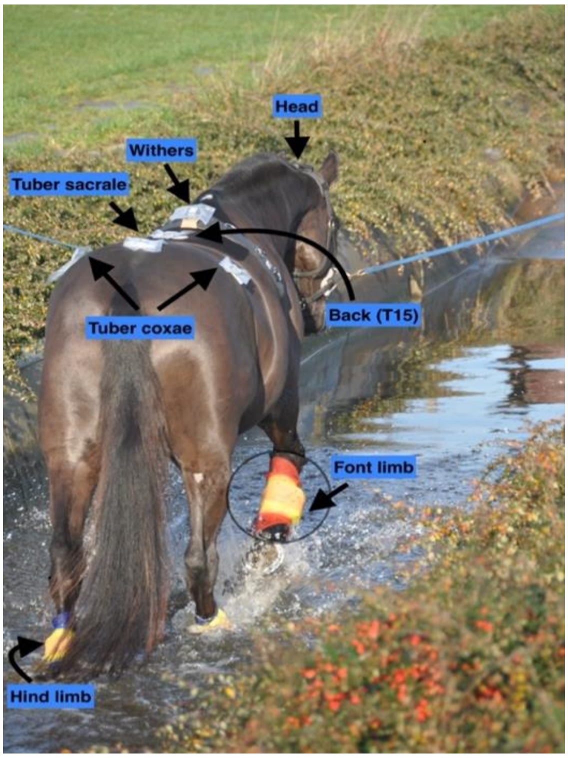

Figure 1. *Overview of all EquiMoves® sensors attached to the horse.*

centimeters (approximately at the level of the carpal joint) based on studies and personal experience; an overview of the WTr used in this study is shown in figure 2. It was predetermined that the length of both WTr and OG were long enough to collect enough numbers of steps for sufficient and valuable data for interpretation.

Figure 2. *Overview of the water track used in this study. The water height was set on 33 centimeters and the track has a length of 85 meters.*

Upper-body parameters

Symmetry or range of motion (ROM) of head, withers and sacrum was measured, whereby the symmetry index (value between 0 and 1) displays the vertical displacement of the body at each of these positions. The upper body moves up and down two times within each stride for the consecutive right and left limbs, meaning there are two peaks and two troughs (Bosch et al., 2018). Maximal difference displays the difference in millimeters between two peaks of the vertical displacement and minimal difference displays the difference in millimeters between two troughs of the vertical displacement. Thus, dorsoventral displacement of the sensors measured the vertical ROM. In perfectly symmetrical moving horses, there is no difference between the peaks and troughs of the vertical displacement (Starke et al., 2012). Data from the sensor on the back (T15) was not processed for this thesis. Sensors of tuber coxae and tuber sacrale were combined to one parameter, named sacrum.

Lower-body parameters

GNSS speed

Speed cannot be controlled in the WTr as is done in water treadmills, therefore the GNSS speed was incorporated in the EquiMoves software to measure the speed.

Limb timing parameters

Each limb separately generates a stride cycle while moving, whereby the stride cycle is delimited by two successive moments that the hoof contacts the ground. Within the stride cycle there is a swing- and a stance phase. The swing phase begins with lifting the hoof (hoof-off moment) and ends when the hoof contacts the ground again (hoof-on moment), whereby the period between these moments displays the swing phase. The period that the hoof is in contact with the ground displays the stance phase (Bosch et al., 2018). Stride duration is the amount of time between two successive hoof-on moments of a single limb in seconds and stride length is the distance covered during one stride cycle of a limb in meters. Hoof-on and hoof-off moments are captured by the IMU's, which enables a distinction between the swing- and stance phase and determination of the stride duration (Bragança et al., 2017).

Limb angle parameters

By processing the limb angle parameters, the orientation of the IMU's attached to each limb was used to determine the angle of the limb at the position of the cannon bone relative to the vertical line (Bosch et al., 2018). There are angles in the sagittal or anterior-posterior plane (pro- and retraction) and in the coronal or medial-lateral plane (ab- and adduction). The forward extension of a limb is defined as the protraction, whereby a maximum is reached prior to the hoof-on moment. Opposite, the backward extension of a limb is defined as the retraction, whereby a maximum is reached after the hoof-off moment. When pro- and retraction is mentioned in this study, the amount of pro- and retraction of the cannon bone is meant. All angles are displayed in degrees. Differences in height of the lifted limb was not measured. The maximal ROM of a limb in the sagittal plane is the total angular distance between protraction and retraction. Ab- and adduction angles display the respectively outward and inward tilting of the limb, these parameters were measured by the IMU's but not implemented in this study (Bosch et al., 2018).

Data analysis

As mentioned previously, the EquiMoves system was used to collect and process the data. Statistical analysis was performed using R-studio (version 4.1.0) with package emmeans and lme4. Starting, all useable data from the EquiMoves software was converted into an Excel file. Following, the data of both left and right FLs and HLs was put together as we did not expect large differences between sides as all horses were declared sound. All variables were assessed for homoscedasticity and normality first and afterwards box plots and spaghetti plots (figure 10 – 12, appendix) were created for the three different conditions (OG1, WTr and OG2) in walk and trot to identify potential outliers and to observe the effect of water. The aim of this study was to determine the degree of asymmetry instead of establishing right or left asymmetry, therefore positive and negative values of the upper-body parameters were converted to absolute values. Protraction angles were implemented as positive values into the dataset and retraction angles were implemented as negative values. Linear mixed models were used for all variables with additional a post-hoc test for pair-wise comparison with a false discovery rate correction for multiple testing to compare the different conditions and determine the effect of water. Horse identity was incorporated as a random effect and the three different conditions (OG1, WTr and OG2) as fixed effects. P-values <0.05 were given to be statistically significant. Not all variables met the criteria of homoscedasticity and normality, but to optimize the data used in the linear mixed model difficult adjustments were necessary. Therefore, it was decided that for this thesis the used model complies. However, this means that the findings are not completely reliable, which must be kept in mind when looking at the results.

Results

All eleven participating horses were included in data analysis. There was no significant difference between kinematics overground before and after WTr exercise (OG1 vs. OG2) in both upper-body and lower-body parameters, therefore the first hypothesis is rejected. Nevertheless, there are significant differences between OG kinematics and kinematics in the WTr, which supports the second hypothesis.

Upper-body parameters at walk

According to table 4 (appendix), there were no significant differences in the upper-body parameters between horses walking OG compared to horses walking in the WTr, meaning there was no significant difference in symmetry index, minimal difference and maximal difference of head, withers and sacrum and thereby no change in vertical ROM at walk.

Upper-body parameters at trot

In contrast to the findings at walk, there were significant differences in upper-body parameters between horses trotting OG compared to horses trotting in the WTr. Table 1a shows a significant p-value <0.05 for head symmetry and p-values <0.01 for withers and sacrum symmetry at trot. Symmetry index of head, withers and sacrum decreased (-45.7%, -41.7% and -41.2%) in the Wtr compared to OG conditions. Minimal difference and maximal difference showed no significant differences, according to table 1b (appendix). The effect of water on the symmetry index of head, withers and sacrum at walk and trot during the three different conditions are shown in the box-and-whisker plots in figure 3a, which gives an overview of the spread of the values.

Symmetry index at trot						
		Estimated mean	Lower C.I.	Upper C.I.	p-value	Difference in %
Head	OG1	0.35	0.23	0.48	-	-
	WTr	0.19	-0.06	0.46	<0.05	-45.7 %
	OG2	0.35	-0.09	0.61	0.95	0 %
Withers	OG1	0.12	0.06	0.17	-	-
	WTr	0.07	-0.03	0.15	<0.01	-41.7%
	OG2	0.10	0.01	0.19	0.32	-16.7%
Sacrum	OG1	0.17	0.11	0.23	-	-
	WTr	0.10	0	0.20	<0.01	-41.2%
	OG2	0.13	0.03	0	0.044	-23.5%

Table 1a. Effects of water on the symmetry in trot. *Estimated mean from mixed model analysis with 95% confidence intervals for the symmetry index of the upper-body parameters (head, withers and sacrum) and p-values of pairwise comparisons between trot conditions (trot in hand overground before water exercise (OG1) vs. trot through the water track (WTr) vs. trot in hand overground after water exercise (OG2)).*

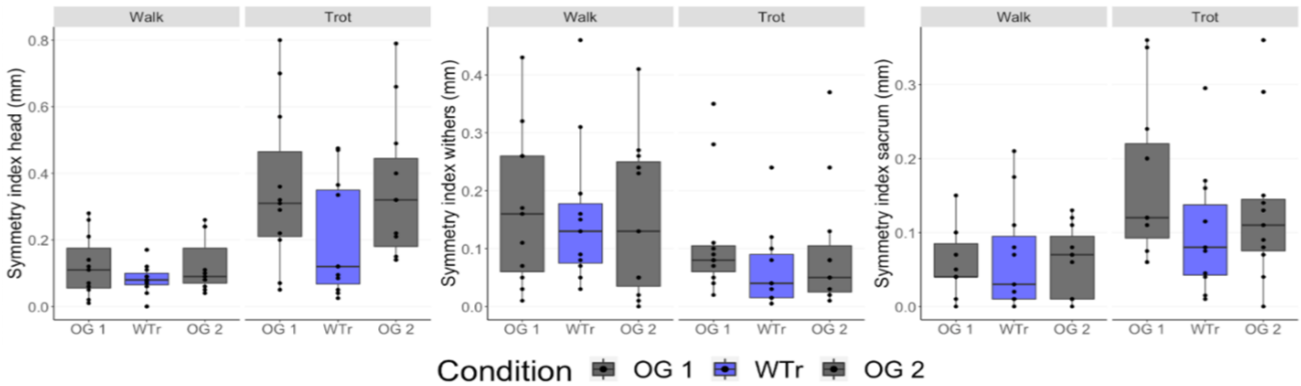

Figure 3a. *Box-and-whisker plots of the symmetry index in millimeters (mm) of head, withers and sacrum in walk and trot during the three different conditions. OG 1 = overground 1 (grey), WTr = water track (blue) and OG 2 = overground 2 (grey). The box represents the measurements between the first and third quartile, the horizontal line within the box indicates the median and the whiskers connect the minimum and maximum values, except for the outliers. All individual values are shown separately with dots.*

Lower-body parameters at walk

Outputs of the linear mixed model with the effect of water on speed, limb timing parameters and sagittal limb angle parameters at walk is partial shown in table 2a (only significant relevant values) and completely in table 2b (appendix). All significant relevant values had a p-value <0.01. Speed was significantly lower under WTr conditions then under OG conditions at walk (-10.3%). Stride duration in walking horses increased (+14.9%) in the WTr. Figure 4 shows the box-and-whisker plots of the stride duration and stride length during the three different conditions at walk and trot. There was no difference in stride length between OG conditions and WTr conditions at walk. According to table 2a, there was a significant decrease (-14.2% and -16.2%) in FL pro- and retraction and HL protraction increased (+48.4%) significantly. There was no significant difference in HL retraction in the WTr. Swing and stance duration of both FLs (+19.1% and +13.5%) and HLs (+19.2% and +11.6%) increased at walk. The effects of water on maximal protraction and retraction angles of both FLs and HLs, and swing and stance duration of both FLs and HLs at walk and trot during the three different conditions are illustrated in figure 5 and 6, respectively.

Lower-body parameters at walk

		Estimated mean	Lower C.I.	Upper C.I.	p-value	Difference
GNSS speed	OG1	1.56	1.50	1.61	-	-
	WTr	1.40	1.27	1.53	<0.01	-10.3%
	OG2	1.63	1.49	1.75	0.088	+4.5%
Stride duration	OG1	1.21	1.17	1.25	-	
	WTr	1.39	1.32	1.46	<0.01	+14.9%
	OG2	1.20	1.13	1.27	0.46	-0.8%
FL protraction	OG1	34.26	32.85	35.68	-	-
	WTr	29.39	26.43	32.36	<0.01	-14.2%
	OG2	33.89	30.93	36.86	0.64	-1.1%
FL retraction	OG1	-35.59	-38.85	-32.33	-	-
	WTr	-41.34	-46.48	-36.20	<0.01	-16.2%
	OG2	-36.32	-41.46	-31.18	0.46	+2.1%
HL protraction	OG1	28.76	27.40	30.12	-	-
	WTr	42.68	39.41	45.96	<0.01	+48.4%
	OG2	28.93	25.66	32.21	0.86	+0.6%
FL swing duration	OG1	0.47	0.45	0.48	-	-
	WTr	0.56	0.52	0.58	<0.01	+19.1%
	OG2	0.47	0.43	0.49	0.59	0%
HL swing duration	OG1	0.52	0.50	0.54	-	-
	WTr	0.62	0.58	0.65	<0.01	+19.2%
	OG2	0.51	0.48	0.55	0.32	-1.9%
FL stance duration	OG1	0.74	0.71	0.77	-	-
	WTr	0.84	0.78	0.89	<0.01	+13.5%
	OG2	0.73	0.68	0.79	0.48	-1.4%
HL stance duration	OG1	0.69	0.67	0.72	-	-
	WTr	0.77	0.73	0.82	<0.01	+11.6%
	OG2	0.69	0.64	0.74	0.68	0%

Table 2a. Effects of water on limb timing and sagittal limb angles at walk. *Estimated means from mixed model analysis with 95% confidence intervals for the most important lower- body parameters and p-values of pairwise comparisons between walk conditions (walk in hand overground before water exercise (OG1) vs. walk through the water track (WTr) vs. walk in hand overground after water exercise (OG2)).*

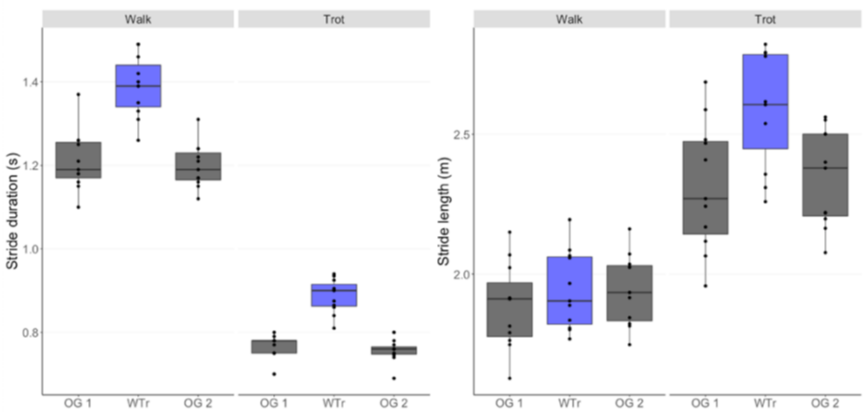

Figure 4. *Boxplots of the stride duration in seconds (s) and stride length in meters (m) in walk and trot during the three different conditions. OG 1 = overground 1 (grey), WTr = water track (blue) and OG 2 = overground 2 (grey).*

The box represents the measurements between the first and third quartile, the horizontal line within the box indicates the median and the whiskers connect the minimum and maximum values, except for the outliers. All individual values are shown separately with dots.

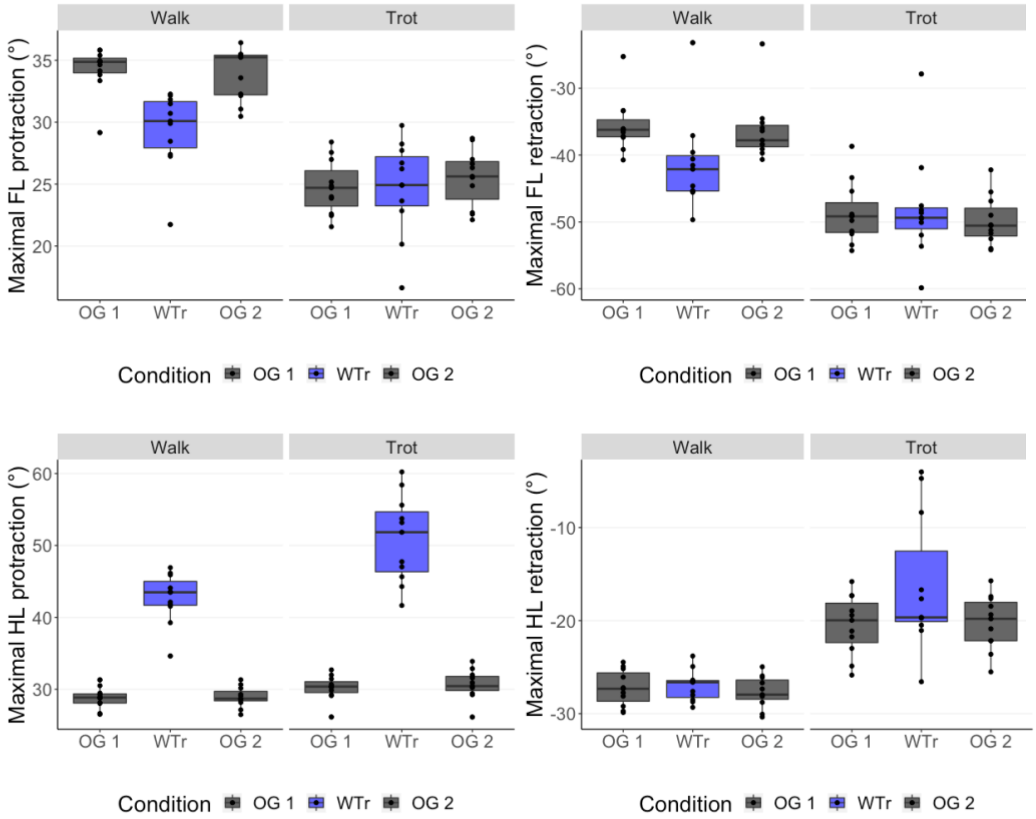

Figure 5. *Box-and-whisker plots of maximal protraction (FL and HL) and maximal retraction (FL and HL) in degrees (°) at walk and trot during the three different conditions.*

OG 1 = overground 1 (grey), WTr = water track (blue) and OG 2 = overground 2 (grey).

The box represents the measurements between the first and third quartile, the horizontal line within the box indicates the median and the whiskers connect the minimum and maximum values, except for the outliers. All individual values are shown separately with dots.

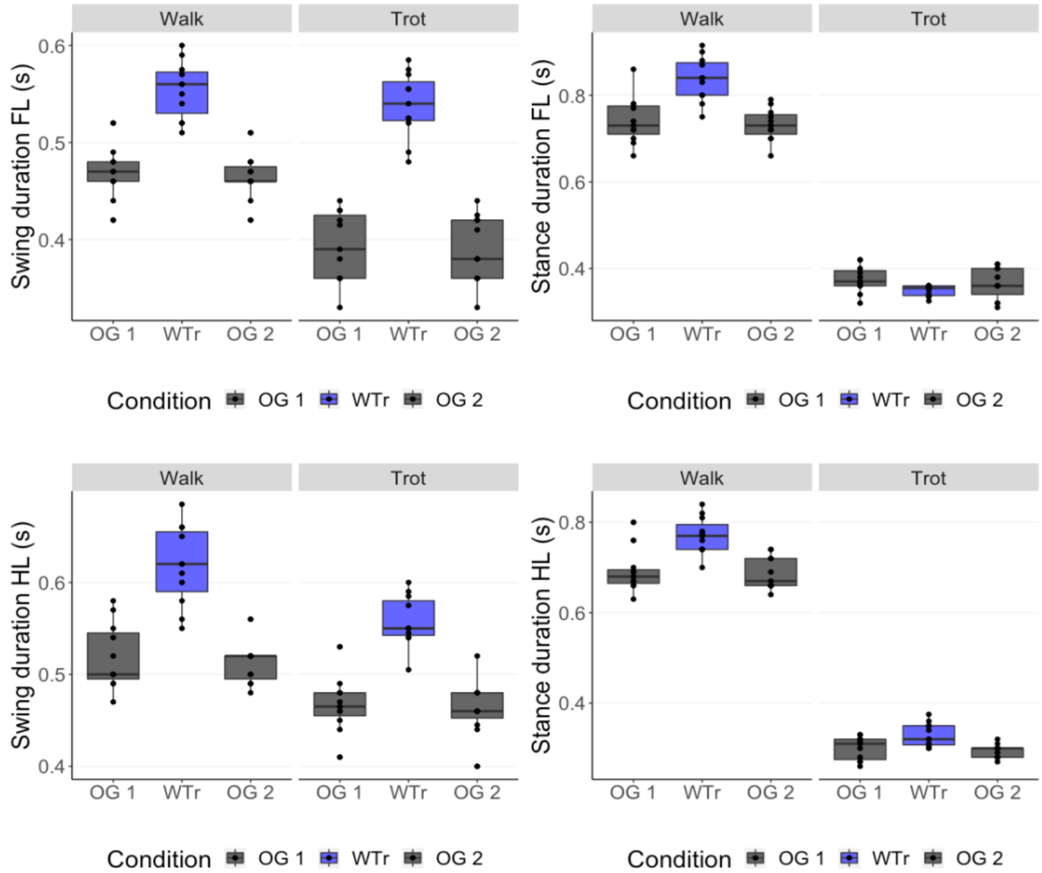

Figure 6. *Box-and-whisker plots of swing and stance duration in seconds (s) of both FLs and HLs at walk and trot during the three different conditions.*

OG 1 = overground 1 (grey), WTr = water track (blue) and OG 2 = overground 2 (grey).

The box represents the measurements between the first and third quartile, the horizontal line within the box indicates the median and the whiskers connect the minimum and maximum values, except for the outliers. All individual values are shown separately with dots.

Lower-body parameters at trot

The effects of water on limb timing and limb angle parameters at trot are illustrated in figure 4, 5 and 6. Table 3a shows the output of linear mixed model analysis between the different conditions (complete table with all lower-body parameters at trot in appendix, figure 3b). At trot, speeds in the WTr were similar to speeds OG (p=0.08). Stride duration increased in the WTr (+15.6%), as well as stride length (+11.7%), with p-value <0.01 for both. In WTr conditions FL swing and stance duration increased (+38.5% and +7.9%), just as swing and stance duration of the HLs increased (+19.1% and +10%), with p-value <0.01 for each variable. The swing and stance phase displayed by the EquiMoves software during one stride cycle of both FLs and HLs in the WTr at trot is illustrated in figure 7. At trot, HL protraction increased with 68.4% (p<0.01) and HL retraction increased with 20.75% (p<0.05) in the WTr. There was no significance difference in FL pro- and retraction in the WTr at trot. Figure 8 and 9 respectively, illustrate the pro- and retraction angles of FLs and HLs in the WTr during one stride cycle of a particular horse displayed by the EquiMoves software.

Lower-body parameters at trot

		Estimated mean	Lower C.I.	Upper C.I.	p-value	Difference
GNSS speed	OG1	3.02	2.89	3.15	-	-
	WTr	2.92	2.68	3.16	0.080	-3.3%
	OG2	3.10	2.86	3.34	0.15	+2.7%
Stride duration	OG1	0.77	0.75	0.79	-	-
	WTr	0.89	0.84	0.94	<0.01	+15.6%
	OG2	0.76	0.71	0.80	0.39	-1.3%
Stride length	OG1	2.31	2.19	2.43	-	-
	WTr	2.58	2.36	2.81	<0.01	+11.7%
	OG2	2.34	2.11	2.57	0.60	+1.3%
HL protraction	OG1	30.20	27.99	32.41	-	-
	WTr	50.86	45.81	55.90	<0.01	+68.4%
	OG2	30.64	25.59	35.69	0.77	+1.5%
HL retraction	OG1	-20.48	-23.37	-17.60	-	-
	WTr	-16.23	-22.10	-10.37	<0.05	+20.8%
	OG2	-20.24	-26.11	-14.38	0.88	+1.2%
Front Swing	OG1	0.39	0.37	0.41	-	-
	WTr	0.54	0.49	0.58	<0.01	+38.5%
	OG2	0.39	0.34	0.43	0.82	0%
Hind Swing	OG1	0.47	0.45	0.48	-	-
	WTr	0.56	0.52	0.59	<0.01	+19.2%
	OG2	0.47	0.43	0.49	0.61	0%
Front Stance	OG1	0.38	0.36	0.39	-	-
	WTr	0.35	0.32	0.38	<0.01	-7.9%
	OG2	0.38	0.34	0.40	0.35	0%

Hind Stance	OG1	0.30	0.29	0.31	-	-
	WTr	0.33	0.30	0.36	<0.01	+10.0%
	OG2	0.29	0.27	0.32	0.41	-3.4%

Table 3a. Effects of water on limb timing and sagittal limb angles at trot. *Estimated means from mixed model analysis with 95% confidence intervals for the lower- body parameters and p-values of pairwise comparisons between trot conditions (trot in hand overground before water exercise (OG1) vs. trot through the water track (WTr) vs. trot in hand overground after water exercise (OG2)).*

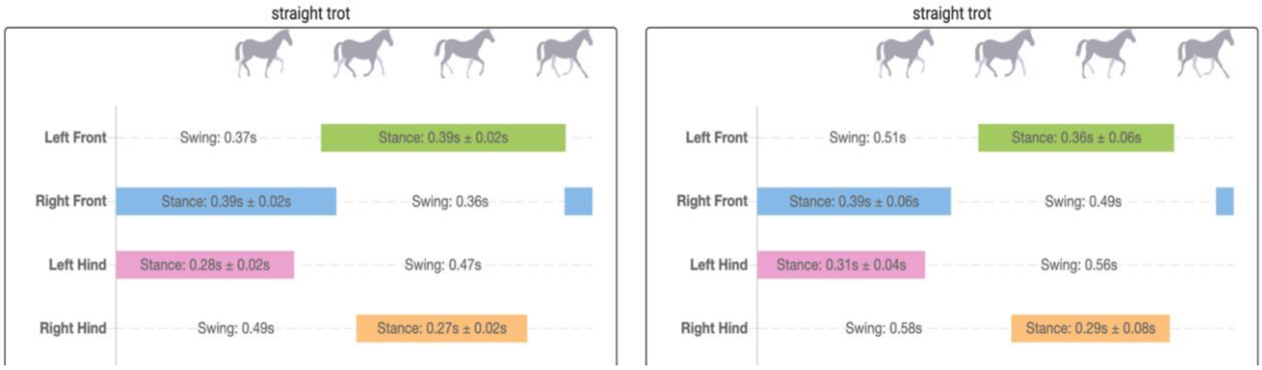

Figure 7. *Illustration of limb timing in second (s) of both FLs and HL during one stride cycle OG (left) and in the WTr (right) at trot. Stance duration for each limb is represented by the colored bars and swing duration is illustrated by the transparent dashed lines. © EquiMoves software*

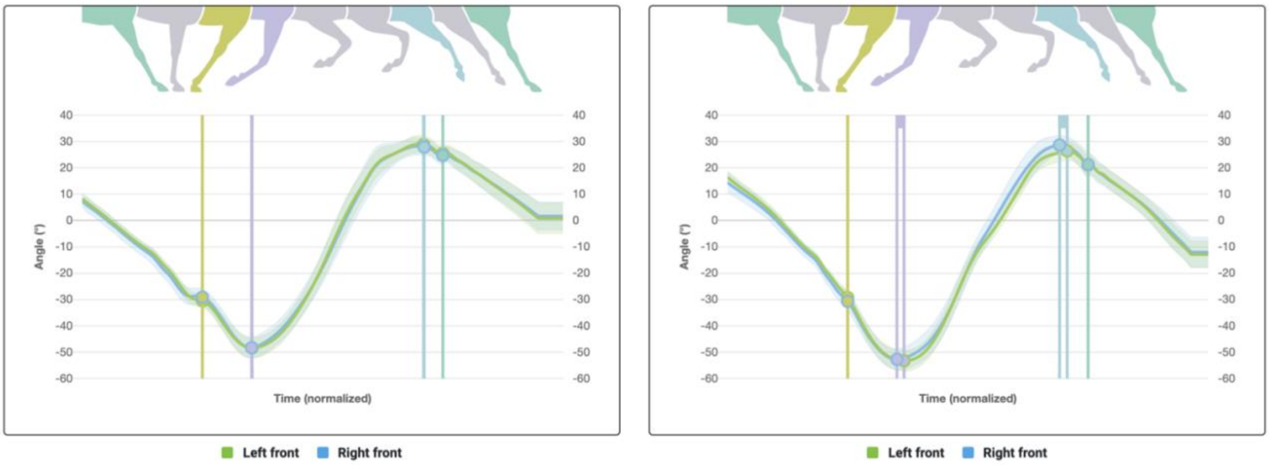

Figure 8. *Illustration of protraction and retraction angles in degrees (°) of both FLs during one stride cycle OG (left) and in the WTr (right) at trot. The mean angle is represented by the blue line (RF) and the green line (LF), and the transparent zones represent the distribution of all stride cycle values in time. © EquiMoves software*

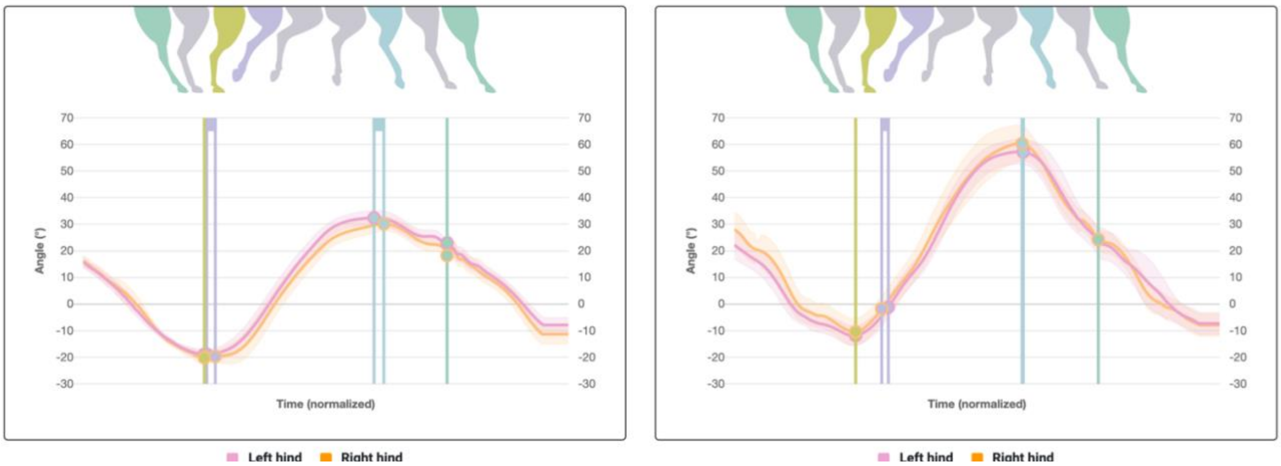

Figure 9. *Illustration of protraction and retraction angles in degrees (°) of both HLs during one stride cycle OG (left) and in the WTr (right) at trot. The mean angle is represented by the orange line (RH) and the pink line (LH), and the transparent zones represent the distribution of all stride cycle values in time. © EquiMoves software*

Discussion

The aim of this study was to objectively evaluate kinematic changes of upper-body parameters and limb timing and angle parameters of horses walking and trotting through a WTr versus kinematics OG, to determine the effect of water on the equine locomotion pattern.

Effect of water on upper-body kinematics

It was hypothesized that horses would be more symmetrical in the presence of water during WTr exercise. The results of the present study confirm this hypothesis only at trot, since horses became more symmetrical in the WTr at trot compared to OG locomotion. There is a significant increase in vertical ROM during WTr exercise at trot, suggesting that the bigger effort horses have to stabilize their body make them more symmetrical.

Effect of water on limb kinematics

It was hypothesized that the presence of water would affect limb kinematics during exercise in the WTr compared to OG locomotion.

Limb timing parameters

The main significant findings in WTr conditions confirm this hypothesis, for there was an increased stride duration at walk and trot and an increased stride length at trot only. Swing and stance duration of both FLs and HLs increased at walk and trot in the WTr. The modified limb timing parameters in the WTr are most likely the result of the buoyancy and drag force of water. Buoyancy enables the upward moving of limbs and therefore affect stride timing. Presumably, the increased duration in swing phase in water is the outcome of the exaggerated flight arc powered by the bouyancy and drag force of water (Scott et al., 2010).

Limb angle parameters

The current study confirms the hypothesis, since there were significant differences in sagittal limb angles at walk and trot in the WTr. Surprisingly, pro- and retraction of the FLs decreased at walk in WTr conditions. At trot, there was no significant difference in pro- and retraction of the FLs in the WTr compared to OG. At walk, protraction of the HLs nearly doubled in the WTr and no significant difference for retraction was found. Maximal protraction of the HLs increased even more in the WTr at trot and maximal retraction of the HLs increased as well. It is known that increased HL protraction causes lumbar flexion of the back in horses (Wolschrijn et al., 2013), which is considered a preferred movement in sport horses. However, the increased HL retraction at trot in the WTr results in thoracolumbar extension of the back (Wolschrijn et al., 2013). Nankervis & Lefrancois (2018) stated that horses with abnormal back conformation, back pathologies or stiffness are less likely to increase HL protraction during water exercise and prefer to increase HL retraction (Nankervis & Lefrancois, 2018). Nevertheless, there was an increase in HL protraction and no increase in HL retraction at walk in the WTr in the present study, implying that horses need to increase HL retraction to compensate for the great increase in HL protraction.

Effect of water height

A water height of 33 centimeters was used in the current study, based on personal experience and supported by the study of Mooij et al. (2013) that described that water levels at the height of the carpal joint triggered a response to lift both FLs and HLs to step over the water and thereby influence the kinematics compared to OG locomotion (Mooij et al., 2013). The effect of buoyancy and drag force become greater with increasing water levels and there is evidence that there is a decreased stance duration with increasing water depths (Nankervis et al., 2016; McCrae et al., 2021). Possibly, the water height used in the present study was too low for the decrease in stance duration to occur. Therefore, variation in water heights will greatly influence the results.

Effect of speed

At walk, there was a lower speed in the WTr compared to OG. There was no significant difference in speed at trot during the different conditions. Several options were considered to process the effect of lower speed in the WTr at walk, to determine whether changes in kinematics were achieved by the presence of water or whether speed was of influence. The effect of speed could be implemented in the statistical model, which would complicate statistical analysis. Therefore, for this thesis the assumption was made that a reduced speed in the WTr at walk should be considered as a confounding factor since speeds were not significantly different between WTr and OG conditions at trot. Therefore, these effects seem to have been caused by the presence of water. The decreased FL pro- and retraction at walk in the WTr, may be related to the reduced speed in the WTr at walk. Furthermore, more information about the preferred speed of horses' during WTr exercise is necessary, whereby the results can be translated to speeds on water treadmills.

Effect of one-time WTr exercise on OG locomotion

It was hypothesized that water exercise improves the kinematics of the equine upper-body and limbs overground. The current study denies this hypothesis since there was no significant difference between OG1 and OG2 measurements after a short session in the WTr, meaning there was no change in both OG upper-body kinematics and limb kinematics before and after performing WTr exercise. The horses in this study had no time to adapt to the WTr prior to the measurements and only had a short one-time session in the WTr (walk and trot back and forth). Previous studies have shown that musculoskeletal adaptations take more time than the amount of time that the horses spent in the WTr (Prochno et al., 2020). Therefore, no long-term effects of WTr exercise on OG kinematics could be assessed in the current study, and longitudinal research is required. There is no hard evidence yet that regularly performing water training influences OG kinematics. However, Murray et al. (2020) found an increased development of gluteal and HL musculature after 20 weeks of regular water treadmill training, utilizing subjective assessment of muscle development (Murray et al., 2020).

Long-term effect of WTr exercise

There was no significant difference between kinematics overground before and after performing WTr exercise (OG1 vs. OG2). Nevertheless, performing aquatic exercise on a regular basis may result in changed kinematics OG. The current study is based on measurements immediately after doing WTr exercise and with horses whereby WTr exercise is not part of their training schedule. To figure out whether there is a correlation between WTr exercise and adapted OG kinematics, more long-term research is necessary. In both human and veterinary medicine, aquatic therapy has long been recognized as having beneficial effects on the musculoskeletal system (Geytenbeek, 2002; Drum et al., 2015). Additionally, aquatic exercise is proven to be effective for more body modifications then only affecting kinematics, such as increased aerobic capacity (Greco-Otto et al., 2020) and improvement in horses diagnosed with osteoarthritis was seen compared to the control group using water levels at the point of the shoulder (King et al., 2013). As mentioned before, the effects are greatly influenced by the water height, therefore more research is necessary to determine which water height is beneficial in various conditions and injuries. Skin problems can be a contra-indication for water exercise (Tranquille et al., 2018). Nevertheless, there is no research available where a similar WTr is used because water treadmills are frequently used for studying kinematics of horses and dogs. For this reason, comparing the result of water treadmill studies to the findings of the present study is difficult.

The use of a WTr in rehabilitation programs

WTr exercise provides the opportunity to train horses on a firm surface and in a straight line without additional weight of the rider, making it beneficial in rehabilitation programs for horses with musculoskeletal pathologies to give equine athletes controlled exercise without losing important locomotory muscle strength (Tokuriki et al., 1999) and cardiovascular capacity (Greco-Otto et al., 2020; Lindner et al., 2012) during injuries. This is in accordance with human (Geytenbeek, 2002; Mooventhan & Nivethitha, 2014) and canine (Drum et al., 2015) studies. In veterinary medicine, aquatic therapy is already frequently used in (post-operative) rehabilitation programs and musculoskeletal pathologies in dogs (Monk et al., 2006; Preston & Wills, 2018). Water exercise needs to be customized to the individual horse for achieving the best results, since multiple factors play a role to successful use (Nankervis et al., 2021). Incorrect use of water tracks and water treadmills can lead to opposite results than the intended effect. The current study shows that horses move more symmetrical during water exercise in a WTr, which can be beneficial for asymmetrical horses. In addition, the rider can induce or exacerbate asymmetry and therefore WTr exercise can be advantageous (MacKechnie-Guire et al., 2020). Nevertheless, it is important to diagnose the underlying cause of the asymmetry before starting water exercise to determine if water exercise could indeed be beneficial. The greater ROM underlying the increasing symmetry is not advantageous in certain injuries. A great benefit of the WTr compared to a water treadmill is that it is possible to train a horse under the saddle, which may be beneficial in rehabilitation programs to build up training intensity and workload. Furthermore, horse walkers are frequently used to give horses controlled movement during rehabilitation. However, walking in a circle has disadvantages of introducing asymmetrical workload of the distal limbs (Davies & Merritt, 2004) and an asymmetry in limb inclination (Hobbs et al., 2011). Therefore, moving in a straight line is more beneficial and can be accomplished using a WTr or water treadmill. Moreover, exercise without additional weight of the rider has the advantage that there is no increased workload on the back and limbs which is desirable for injured horses (Clayton et al., 1999). The thoracolumbar extension of the back caused by the increased HL retraction during water exercise is undesirable in the rehabilitation of horses with musculoskeletal injuries to the back and certain HL pathologies (Muñoz et al., 2019).

Comparison between WTr and water treadmill

Similar studies using IMU's were done with a water treadmill to investigate the effects of water and belt speed on the upper-body and limb kinematics in horses at walk using 5 different speeds. The vertical ROM of head, withers, T15 and sacrum significantly increased in the presence of water on a water treadmill with a water height of 30 cm at walk (Pasman, 2020), whereby in the current study there was only a bigger ROM of head, withers and sacrum at trot. Incremental belt speeds increased the vertical ROM of head and sacrum (Pasman, 2020), which corresponds to the fact that there was a greater ROM of the upper-body parameters at trot in the WTr in this study and not at walk. Regarding the limb timing parameters in a similar study on a water treadmill with a water height of 30 cm, stride duration and swing and stance duration of both FLs and HLs increased (de Geer, 2021), these findings are similar to the results in this study. Moreover, incremental belt speeds reduced all limb timing parameters (de Geer, 2021), which is controversial to the findings in the WTr whereby increased stride timing results were more convincing in trotting horses. The presence of water caused an increase in HL protraction and an increased retraction of both FLs and HLs (de Geer, 2021). This is partially in accordance with the results of this study during WTr exercise, whereby HL protraction increased at walk with similar values and both HL protraction and retraction increased at trot, with a greater value in the current study. However, there was a decrease in FL protraction and retraction at walk in the present study and there was only increased HL retraction at trot and no effect on FL sagittal angles at trot. The different results could be explained by the effect of using a treadmill, causing a prolonged relative stance further caudal compared to OG locomotion (Buchner et al., 1994).

Limitations of the study

The current study had several limitations that should be considered when evaluating the results. First, the study population included horses from different breeds, ages, genders, and levels in sport, which could be of influence at the locomotion pattern of the individual horse. Flaws in study population and study design attenuated the strength of the research evidence. Second, the experience with WTr exercise varied between horses in the present study. All study horses were familiar with exercise in the WTr, but some horses performed WTr exercise on a more regular and recent basis than other horses. This may have resulted in better adapted horses to WTr exercise with development of important locomotory musculature for water exercise and therefore may also affect OG locomotion. In addition, it might be possible that the locomotion pattern of the study horses was not fully habituated to the WTr yet at the time of the measurements what may have resulted in wider standard deviations in comparison with a study population including horses fully habituated to WTr exercise. Furthermore, horses were declared sound by the owners, but the IMU sensors detected mild to moderate lameness in a few horses during the warming-up. This could have resulted in potential unreliable data for these specific horses. Finally, this was the first study using the EquiMoves sensors in a WTr. The sensors were incorporated in waterproof pouches and attached as close to the surface of interest as possible. Due to the moving water hitting the sensors there may have been changes in position of the sensors, especially at the site of the limbs.

Conclusion

The main conclusion of the current study was that the presence of water with a height of 33 cm significantly affects upper-body symmetry and limb kinematics at walk and trot during WTr exercise. There is a combined effect of gait and water on upper-body symmetry, limb sagittal angles and stride timings, where increased HL pro- and retraction in the WTr at trot yielded the largest change compared to OG locomotion. Furthermore, speed was lower in the WTr at walk which was considered as a confounding factor. There were no significant differences in kinematics OG before and after conducting water exercise in the WTr.

In recent years, there has been an increasing interest in the use of water exercise in training and rehabilitation programs of equine athletes. This study provides new insides into the effects of WTr exercise on equine kinematics and can help clinicians establishing training or rehabilitation programs. Moreover, the present study fills a gap in the literature by focusing only on the effects of water and exclude the effects of a treadmill. Nevertheless, results are greatly influenced by the used water height and therefore the results of this study can contribute to determine water height and possibly appropriate belt speed on a water treadmill.

The present study focused on the vertical ROM of the upper-body. The effect of water on lateral bending and rotation and flexion-extension of the upper-body is still unclear and should be further investigated. Only sagittal limb angle parameters were processed, the effect of WTr exercise on coronal limb angles is yet to be determined. In addition, research is required to define not only the pro- and retraction but also the height of the lifted limb. Furthermore, longitudinal research is required to determine long-term effects of WTr exercise on OG kinematics and musculoskeletal pathologies.

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Conflict of interest

The author declares no conflict of interest.

Ethical considerations

The study protocol was approved by the Animal Welfare Body (IvD) of Utrecht before initiation of the study.

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Appendix

Upper-body parameters at trot

			Estimated mean	Lower C.I.	Upper C.I.	p-value
Head	Symmetry index	OG1	0.35	0.23	0.48	-
		WTr	0.19	-0.06	0.46	0.031
		OG2	0.35	-0.09	0.61	0.95
	Minimal difference	OG1	13.40	4.62	22.17	-
		WTr	17.77	0.98	34.54	0.30
		OG2	12.85	-3.94	29.63	0.89
	Maximal difference	OG1	14.29	8.62	19.95	-
		WTr	17.35	4.19	30.49	0.44
		OG2	12.54	-0.61	25.68	0.65
Withers	Symmetry index	OG1	0.12	0.06	0.17	-
		WTr	0.07	-0.03	0.15	<0.01
		OG2	0.10	0.01	0.19	0.32
	Minimal difference	OG1	5.33	2.82	7.83	-
		WTr	5.98	0.39	11.55	0.69
		OG2	4.24	-1.35	9.82	0.50
	Maximal difference	OG1	5.54	1.57	9.50	-
		WTr	7.19	1.17	13.38	0.16
		OG2	5.95	-0.25	12.15	0.72
Sacrum	Symmetry index	OG1	0.17	0.11	0.23	-
		WTr	0.10	0	0.20	<0.01
		OG2	0.13	0.03	0.23	0.044
	Minimal difference	OG1	6.70	3.70	9.71	-
		WTr	7.65	2.24	13.07	0.45
		OG2	6.19	0.78	11.61	0.68
	Maximal difference	OG1	10.9	5.69	16.1	-
		WTr	13.4	4.83	21.95	0.16
		OG2	10.0	1.43	18.55	0.60

Table 1b. Effects of water on upper-body parameters at trot. *Complete estimated means from mixed model analysis with 95% confidence intervals for the upper- body parameters and p-values of pairwise comparisons between trot conditions (trot in hand overground before water exercise (OG1) vs. trot through the water track (WTr) vs. trot in hand overground after water exercise (OG2)).*

Lower-body parameters at walk

		Estimated mean	Lower C.I.	Upper C.I.	p-value
Number of strides	OG1	58.55	50.03	67.06	-
	WTr	67.14	46.64	87.63	0.18
	OG2	56.73	36.23	77.22	0.77
GNSS speed	OG1	1.56	1.50	1.61	-
	WTr	1.40	1.27	1.53	<0.01
	OG2	1.63	1.49	1.75	0.088
Stride duration	OG1	1.21	1.17	1.25	-
	WTr	1.39	1.32	1.46	<0.01
	OG2	1.20	1.13	1.27	0.46
Stride length	OG1	1.88	1.80	1.97	-
	WTr	1.94	1.77	2.12	0.211
	OG2	1.94	1.77	2.12	0.20
FL protraction	OG1	34.26	32.85	35.68	-
	WTr	29.39	26.43	32.36	<0.01
	OG2	33.89	30.93	36.86	0.64
FL retraction	OG1	-35.59	-38.85	-32.33	-
	WTr	-41.34	-46.48	-36.20	<0.01
	OG2	-36.32	-41.46	-31.18	0.46
HL protraction	OG1	28.76	27.40	30.12	-
	WTr	42.68	39.41	45.96	<0.01
	OG2	28.93	25.66	32.21	0.86
HL retraction	OG1	-27.25	-28.29	-26.21	-
	WTr	-26.99	-29.07	-24.90	0.63
	OG2	-27.66	-29.75	-25.57	0.45
FL swing duration	OG1	0.47	0.45	0.48	-
	WTr	0.56	0.52	0.58	<0.01
	OG2	0.47	0.43	0.49	0.59
HL swing duration	OG1	0.52	0.50	0.54	-
	WTr	0.62	0.58	0.65	<0.01
	OG2	0.51	0.48	0.55	0.32
FL stance duration	OG1	0.74	0.71	0.77	-
	WTr	0.84	0.78	0.89	<0.01
	OG2	0.73	0.68	0.79	0.48
HL stance duration	OG1	0.69	0.67	0.72	-
	WTr	0.77	0.73	0.82	<0.01
	OG2	0.69	0.64	0.74	0.68

Table 2b. Effects of water on limb timing and sagittal limb angles at walk. *Complete estimated means from mixed model analysis with 95% confidence intervals for the lower- body parameters and p-values of pairwise comparisons between walk conditions (walk in hand overground before water exercise (OG1) vs. walk through the water track (WTr) vs. walk in hand overground after water exercise (OG2)).*

Lower-body parameters at trot

		Estimated mean	Lower C.I.	Upper C.I.	p-value
GNSS speed	OG1	3.02	2.89	3.15	-
	WTr	2.92	2.68	3.16	0.08
	OG2	3.10	2.86	3.34	0.15
Stride duration	OG1	0.77	0.75	0.79	-
	WTr	0.89	0.84	0.94	<0.01
	OG2	0.76	0.71	0.80	0.39
Stride length	OG1	2.31	2.19	2.43	-
	WTr	2.58	2.36	2.81	<0.01
	OG2	2.34	2.11	2.57	0.60
FL protraction	OG1	24.74	23.07	26.41	-
	WTr	24.71	21.14	28.27	0.972
	OG2	25.52	21.95	29.08	0.43
FL retraction	OG1	-48.65	-51.99	-45.31	-
	WTr	-48.05	-55.64	-40.47	0.785
	OG2	-49.83	-57.42	-42.25	0.59
HL protraction	OG1	30.20	27.99	32.41	-
	WTr	50.86	45.81	55.90	<0.01
	OG2	30.64	25.59	35.69	0.77
HL retraction	OG1	-20.48	-23.37	-17.60	-
	WTr	-16.23	-22.10	-10.37	0.0114
	OG2	-20.24	-26.11	-14.38	0.88
FL Swing	OG1	0.39	0.37	0.41	-
	WTr	0.54	0.49	0.58	<0.01
	OG2	0.39	0.34	0.43	0.82
HL Swing	OG1	0.47	0.45	0.48	-
	WTr	0.56	0.52	0.59	<0.01
	OG2	0.47	0.43	0.49	0.61
FL Stance	OG1	0.38	0.36	0.39	-
	WTr	0.35	0.32	0.38	<0.01
	OG2	0.38	0.34	0.40	0.35
HL Stance	OG1	0.30	0.29	0.31	-
	WTr	0.33	0.30	0.36	<0.01
	OG2	0.29	0.27	0.32	0.41

Table 3b. Effects of water on limb timing and sagittal limb angles at trot. *Complete estimated means from mixed model analysis with 95% confidence intervals for the lower- body parameters and p-values of pairwise comparisons between trot conditions (trot in hand overground before water exercise (OG1) vs. trot through the water track (WTr) vs. trot in hand overground after water exercise (OG2)).*

Upper-body parameters at walk

			Estimated mean	Lower C.I.	Upper C.I.	p-value
Head	Symmetry index	OG1	0.12	-0.08	0.17	-
		WTr	0.08	-0.17	0.18	0.132
		OG2	0.12	-0.13	0.22	0.942
	Minimal difference	OG1	9.86	5.64	14.07	-
		WTr	12.27	2.13	22.39	0.437
		OG2	8.29	-1.48	18.41	0.609
	Maximal difference	OG1	6.46	3.05	9.86	-
		WTr	5.86	-1.75	13.46	0.783
		OG2	7.70	0.09	15.29	0.571
Withers	Symmetry index	OG1	0.17	0.09	0.25	-
		WTr	0.16	0	0.31	0.730
		OG2	0.15	0	0.31	0.635
	Minimal difference	OG1	3.70	1.44	5.96	-
		WTr	5.32	1.20	9.44	0.104
		OG2	4.06	-0.06	8.18	0.707
	Maximal difference	OG1	3.16	1.08	5.24	-
		WTr	2.90	-0.91	6.71	0.769
		OG2	2.77	-1.04	6.58	0.664
Sacrum	Symmetry index	OG1	0.06	0.03	0.09	-
		WTr	0.07	0	0.14	0.721
		OG2	0.06	-0.01	0.13	0.899
	Minimal difference	OG1	5.57	2.46	8.69	-
		WTr	6.46	-0.72	13.64	0.675
		OG2	6.12	-1.06	13.30	0.796
	Maximal difference	OG1	3.46	2.24	4.69	-
		WTr	1.89	-0.85	4.63	0.056
		OG2	4.45	1.72	7.19	0.215

Table 4. Effects of water on upper-body parameters at walk. *Complete estimated means from mixed model analysis with 95% confidence intervals for the upper- body parameters and p-values of pairwise comparisons between walk conditions (walk in hand overground before water exercise (OG1) vs. walk through the water track (WTr) vs. walk in hand overground after water exercise (OG2)).*

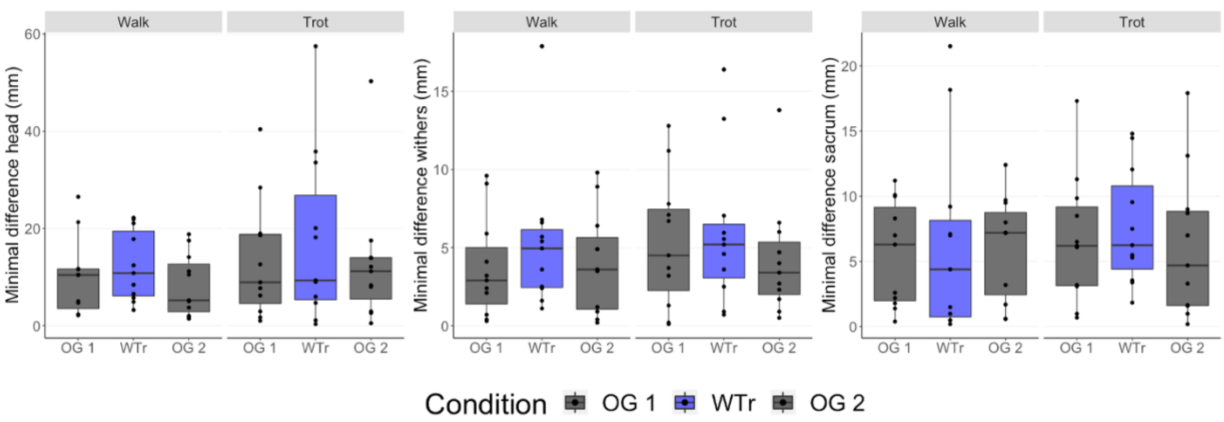

Figure 3b. *Box-and-whisker plots of the minimal difference in millimeters (mm) of head, withers and sacrum in walk and trot during the three different conditions. OG 1 = overground 1 (grey), WTr = water track (blue) and OG 2 = overground 2 (grey). The box represents the measurements between the first and third quartile, the horizontal line within the box indicates the median and the whiskers connect the minimum and maximum values, except for the outliers. All individual values are shown separately with dots.*

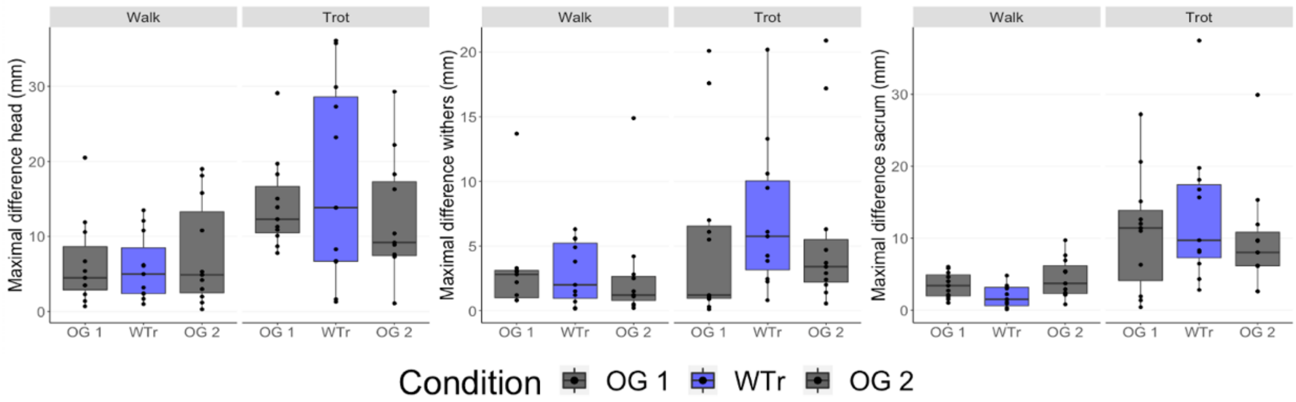

Figure 3c. *Box-and-whisker plots of the maximal difference in millimeters (mm) of head, withers and sacrum in walk and trot during the three different conditions. OG 1 = overground 1 (grey), WTr = water track (blue) and OG 2 = overground 2 (grey). The box represents the measurements between the first and third quartile, the horizontal line within the box indicates the median and the whiskers connect the minimum and maximum values, except for the outliers. All individual values are shown separately with dots.*

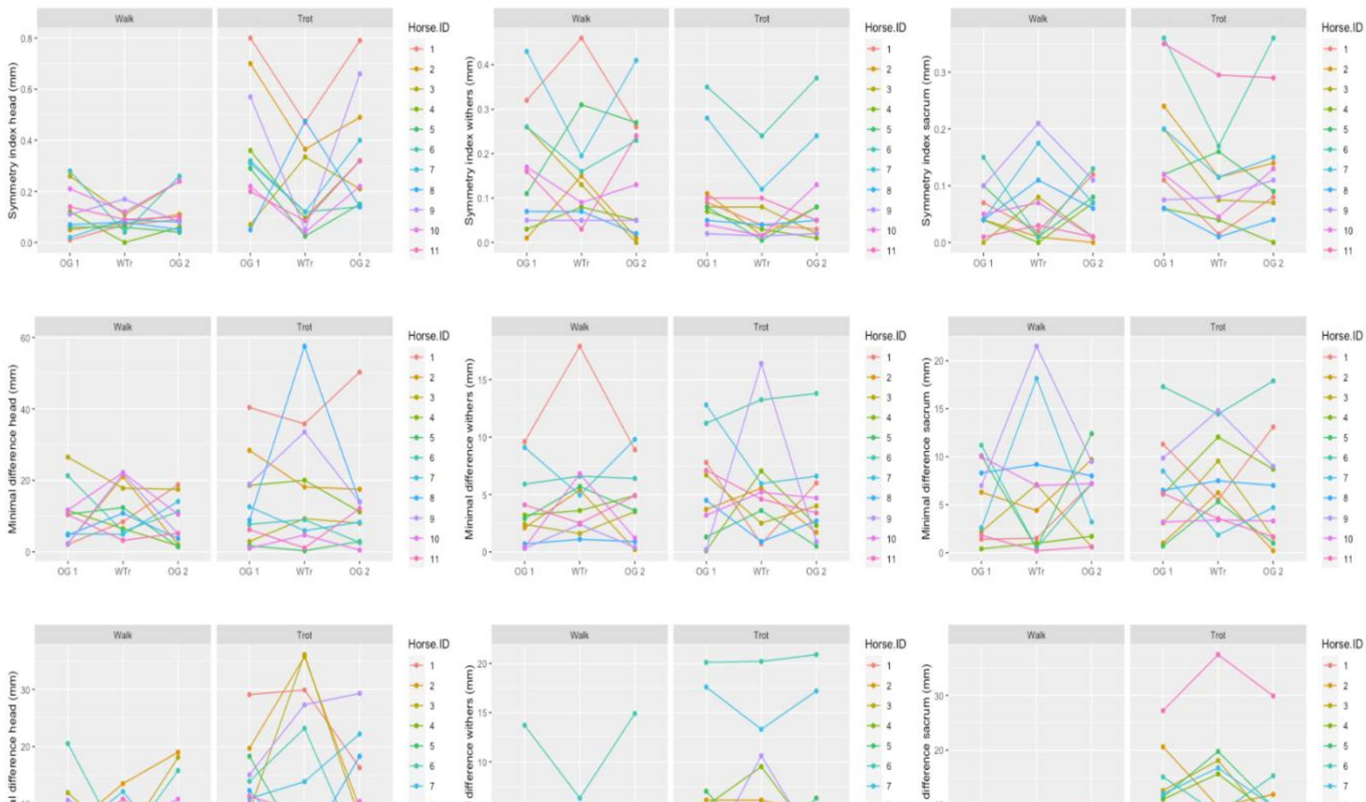

Figure 10. *Spaghetti plots of the upper-body parameters (symmetry index, minimal difference and maximal difference) in millimeters (mm) at walk and trot during the three different conditions (OG1, WTr and OG2). Each color represents an individual horse (horse ID 1-11) and the dots represent the mean value of the variable measured for a horse at each condition (OG1, WTr and OG2).*

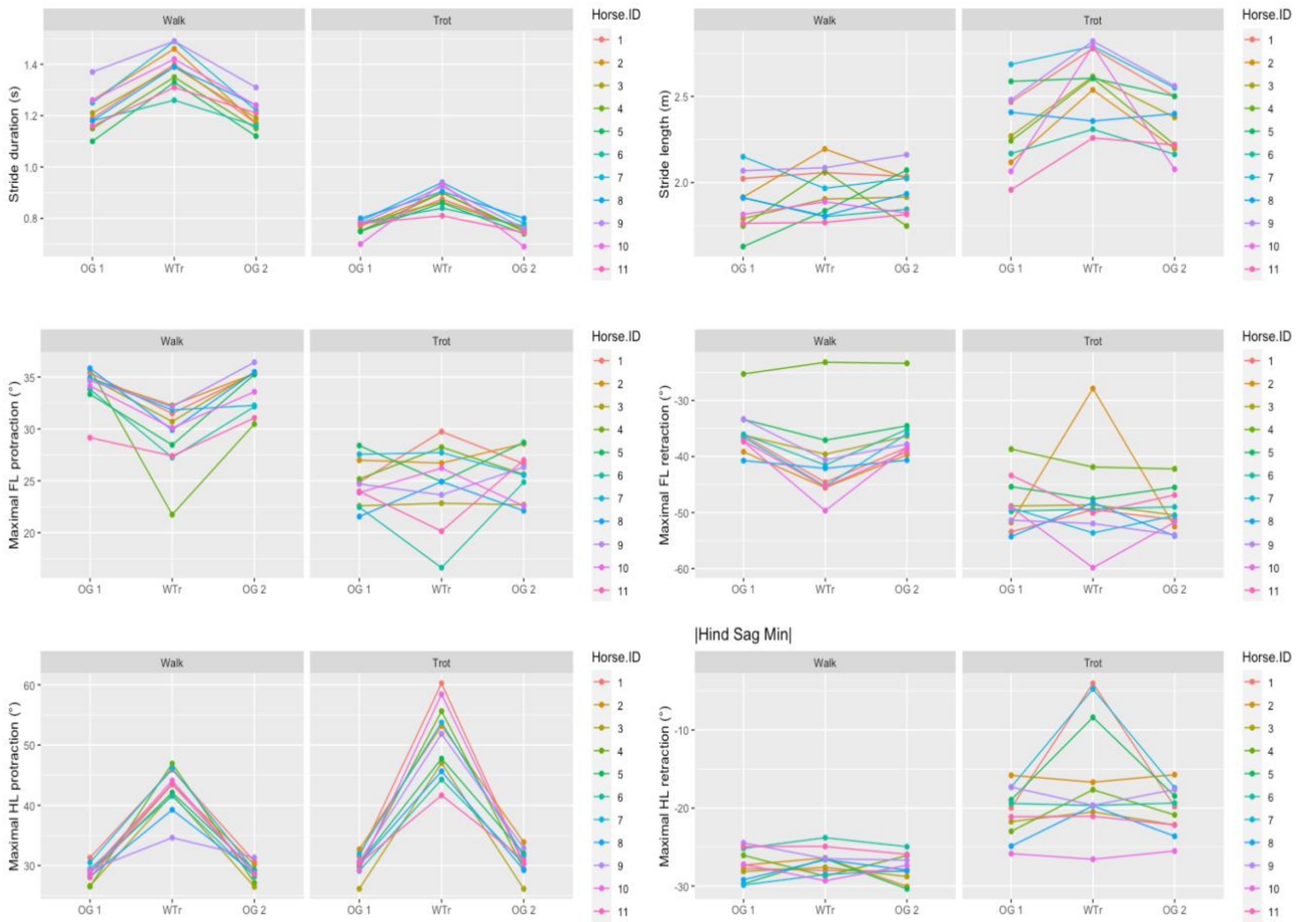

Figure 11. *Spaghetti plots of the limb timing parameters, stride duration in seconds (s) and stride length in meters(m), and limb angle parameters (maximal pro- and retraction of both FLs and HLs) in degrees (°) at walk and trot during the three different conditions (OG1, WTr and OG2). Each color represents an individual horse (horse ID 1-11) and the dots represent the mean value of the variable measured for a horse at each condition (OG1, WTr and OG2).*

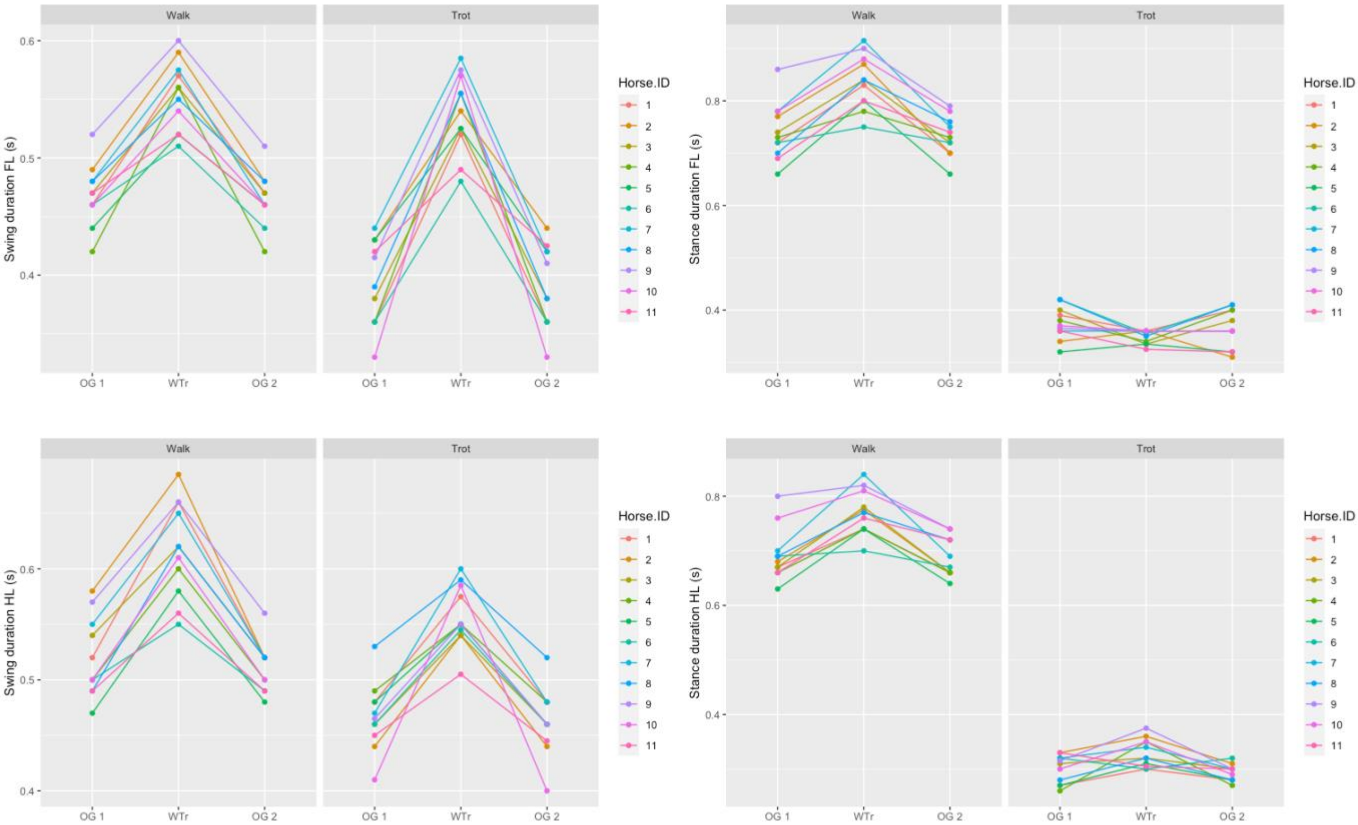

Figure 12. *Spaghetti plots of limb angle parameters (swing and stance duration) in seconds (s) at walk and trot during the three different conditions (OG1, WTr and OG2). Each color represents an individual horse (horse ID 1-11) and the dots represent the mean value of the variable measured for a horse at each condition (OG1, WTr and OG2).*