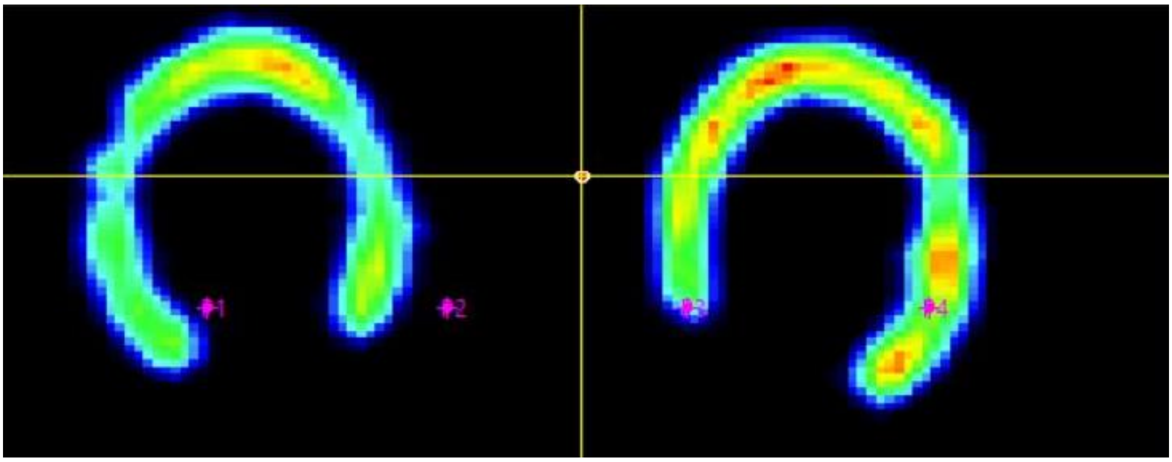
A cross sectional survey of forelimb loading bias and examination of centre of pressure and the association with workload between counter-clockwise and clockwise trained Thoroughbred racehorses in New Zealand



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Abstract

Background: Musculoskeletal injury (MSI) is one of the major reasons for wastage of horses in the racing industry. The use of a pressure plate may be useful in the early detection of lameness by measuring the Centre of Pressure (CoP). High-intensity asymmetric training and workload are associated with asymmetry in the load distribution of the forelimbs of Thoroughbred racehorses. At present, there is no published data on asymmetry in the load distribution of clockwise trained racehorses.

Objectives: To investigate the difference in load distribution of the forelimbs between clockwise and counter-clockwise trained Thoroughbred racehorses and the CoP displacement in association with workload.

Methods: CoP data were measured in 17 counter-clockwise, 30 clockwise and 10 in both directions trained Thoroughbred racehorses. A 0.5 m Footscan pressure plate was used for data collection. Horses stood squarely positioned with their forelimbs in the centre of the pressure plate for a duration of 66.7 seconds while measuring at a sampling frequency of 15 Hz. Duplicate static images were performed with once the left limb and once the right limb placed first to calculate loading distribution between the forelimbs. CoP data were filtered and amplitude, velocity and frequency of the CoP displacement were calculated.

Results: The loading of the right forelimb was 54.31% in the counter-clockwise trained group, 52.95% in the clockwise trained group and 57.07% in the both directions trained group. There were no significant differences in loading between the three groups. The mean amplitude was 0.80±0.34 mm, the mean velocity was 0.89±0.38 mm/s and the mean frequency was 0.34±0.05 Hz. There was a significant decrease in frequency with accumulated gallops (R^2=0.162, p=0.002) as well as total cumulative gallop distance (R^2=0.124, p=0.007).

Conclusion: The loading of the right forelimb was higher than the loading of the left forelimb, irrespective of training direction and workload. This implies a biologically normal level of asymmetry and laterality. The decrease in frequency with a higher workload suggest a greater postural stability within racehorses with a higher workload. However, further research is required to investigate possible changes in the frequency due to a higher workload.

Contents

Introduction

Horses are important domestic animals in New Zealand and are used for racing and sport. New Zealand has an estimated 30 horses per 1000 people and 3.7 horses per 1000 hectares. In New Zealand the majority of horses are used for Thoroughbred racing, Standardbred racing, sport, recreation and work. The production of horses has a major export focus, with approximately 40 per cent of the Thoroughbred foals born exported, to markets such as Australia, Hong Kong, Singapore and Macau. The combined sport horse and racing industries generate approximately NZ$2 billion in gross domestic product (Rogers et al., 2017).

The racing industry is one of the most prominent sectors of the equine industry in New Zealand. There are approximately 2,900 flat races and 120 jump races run, with respectively ~5,500 and 300 horses starting each year. Most horses are 3, 4 or 5 years old in flat racing and have a median of five starts per season (Bolwell et al., in press). The better-performing horses are used for breeding after finishing their racing career. The production of racehorses in New Zealand is pasture-based and the annual foal crop is approximately 4500 foals (Gee et al., in press; Rogers et al., in press). Weaning of foals is between 4 and 6 months of age. Weaned foals are reared at pasture in cohorts of foals based on commercial appeal and sex. The ideal age to sell Thoroughbreds is as yearlings and identification of these horses takes place around weaning. The yearlings selected for the yearling sales in late January and early February start with a preparation approximately 13 weeks before the sales. Controlled exercise is part of the preparation; this is usually performed as in-hand walking 3-5 days per week. The exercise is generally provided as part of the education, rather than as a physical conditioning program. 1500 yearlings are annually sold at the yearling sales (Rogers et al., in press).

The training of the majority of Thoroughbred racehorses starts when they are long yearlings, at 18-19 months of age. The population of racehorses in training is predominantly 2- and 3-year-old. However, there are relatively few race starts within the 2-year olds. Thoroughbred racehorses have a median racing career of 8-11 start or 2-3 years. The loss of horses from the racing industry occurs at various moments. Approximately 33% are lost before they start to train and race. Tanner *et al.* (2013) reported that 46% of the horses that were registered with a trainer never trialled and 55% never started in a race (Tanner et al., 2013). Most of the retirements of racehorses from training and racing were based on a trainer's decision due to poor performance or lack of talent (Bolwell et al., in press). The second important reason for loss from racing is musculoskeletal injury (MSI). Musculoskeletal injury accounts for 78% of forced interruptions to training and 25% of retirements from racing or deaths (Perkins et al., 2005a). Risk factors for MSI are horse age, gender, race distance, race type, racetrack, season, pre-existing pathology involving the musculoskeletal system,

exercise intensity, horseshoe characteristics and various activities that occur during a race such as stumbling, interaction with other horses, use of the whip and load changes (Perkins et al., 2005b). The most common MSI's in Thoroughbred racehorses are shin soreness and tendon and ligament conditions. Less frequently occurring injuries are fractures, wounds or laceration and back disorders (Perkins et al., 2005a). The majority of MSI's in racehorses occur in the forelimb (85%) and are located in the distal limb (84%) (Perkins et al., 2005b). Some (15.8% of the horses) exit the racing industry after the first MSI occurrence. The number of horses exiting with fractures and tendon and ligament injuries is high, 42.9% of the horses with fractures exit the racing industry after first MSI occurrence. Of the group of horses with tendon and ligament injuries 41.7% leave the industry. Meanwhile, only 2.6% of horses with shin soreness leave the industry (Perkins et al., 2005a). Juvenile Thoroughbred racehorses have a higher risk of developing dorsal metacarpal disease (bucked shins, shin splints, sore shins, stress fractures), while tendon and soft tissue injuries are more frequent in older staying racehorses (Rogers et al., 2012). It was reported that accumulation of high-speed gallop exercise has a protective effect on the risk of fracture in racehorses. However, an increasing facture risk was associated with increasing distances at canter and gallop training in short time periods (up to one month). Therefore, physical exercise has effects on bone adaptation and injury risk (Verheyen et al., 2006).
Ramzan & Palmer (2011) reported in the United Kingdom an occurrence of MSI of approximately 25% of the horses per calendar year (Ramzan & Palmer, 2011). For some injury types there was a predominance of right-sided injuries, 81.5% of carpal fractures, 69.2% of superficial digital flexor tendonitis injuries and 68.8% of proximal phalanx fractures were reported on the right side. The horses in this study performed most daily cantering exercise on straight tracks, the fast work was predominantly performed on straight or straight/counter-clockwise tracks (Ramzan & Palmer, 2011). It was proposed that laterality of gait may have been a factor in the predominance of right-sided injuries (Ramzan & Palmer, 2011; Williams & Norris, 2007).

Laterality is defined as the functional and structural differences between the right and left side of the brain. It is physically observed as differences between the left and right side of the body in perception of stimuli, sidedness of motor behaviour and information processing. It is displayed at individual or population level, when most of the individuals in the population have the same direction bias. There are various forms of individual and population handedness reported in different horse breeds. Furthermore, there are differences in and between populations due to sex, age, training, breeding, handling, arousal and morphological proportions (Cully et al., 2018). It is reported that motor bias becomes stronger with age (McGreevy & Thomson, 2006). Observations of racehorses showed a significant increased bias in Thoroughbreds older than two years compared to those under two years old (McGreevy & Rogers, 2005). Murphy *et al.* (2005) showed that male horses exhibit significantly more left leading leg preferences, while female horses exhibit more right leading leg preferences (Murphy et al., 2005). In a cohort of 2-year-old racehorses trained for one year it was reported that at the end of the one-year race training the proximal phalanx of the right forelimb had a significantly thicker palmer cortex than that of the left forelimb. The horses in this study were predominantly trained and raced in clockwise direction (Beccati et al., 2011). Another finding in Thoroughbred racehorses in training is flat feet. Labuschagne *et al.* (2017) reported higher prevalence of a flat left foot than a flat right foot in counter-clockwise trained racehorses in New Zealand (Labuschagne et al., 2017).

The racing in New Zealand occurs predominantly in a counter-clockwise direction (left-handed). However, at 10 of 49 racetracks racing occurs in a clockwise direction (right-handed). These clockwise racetracks are situated in the northern part of the North Island (New Zealand Thoroughbred Racing, 2019). Thoroughbred racing in New Zealand is exclusively performed on grass tracks, whilst all weather surfaces and sand are comprehensively used for training. Most racetracks are oval shaped (27 of 49), other racetracks are egg shaped (16 of 49) or have a different shape (5 of 49) (Rogers et al., 2014).

Previous studies have shown a greater pressure distribution under the right forelimb than the left forelimb in Thoroughbred racehorses (Lichtenauer et al., 2016; Plantinga et al., 2018). The same loading bias was also found in New Zealand Sport horses (Heuver, 2019; Schuurmans, 2019). Plantinga *et al.* (2018) reported a smaller centre of pressure amplitude in Thoroughbred racehorses with >10 training gallops, while horses with 0-10 training gallops had a larger amplitude (Plantinga et al., 2018). While Schuurmans (2019) reported no significant association of workload and the centre of pressure displacement in New Zealand Sport horses (Schuurmans, 2019). The studies of Lichtenauer *et al.* (2016) and Plantinga *et al.* (2018) identified an asymmetrical pattern of the centre of pressure (CoP) in Thoroughbred racehorses. These results were proposed to have a relationship with the counter-clockwise training protocol (Lichtenauer et al., 2016).

The CoP may be considered as the net output variable of the interaction of the torque and forces that occur in the limb and in its inertial properties. The CoP qualifies the dynamic load distribution under the hoof (Nauwelaerts et al., 2017). The CoP pattern reflects the postural sway movements of the horse. Postural sway is the process of continuous invisible minor movements during stable stance, due to small adjustments of muscle tension. It is essential to maintain the horse's centre of mass in a horizontal plane within narrow limits. The CoP moves horizontal in mediolateral (ML) and craniocaudal (CC) directions over a period of time. The CoP movement can be described in the variables amplitude, velocity and frequency (Clayton et al., 2003; Gomes-Costa et al., 2015). The CoP moving pattern provide an indication of the balance and stability of the horse's centre of mass (Clayton et al., 2003). Postural sway measurements in humans are often used for diagnosing balance problems and neurological diseases. Musculoskeletal injuries in humans have been associated with a negative effect on the postural sway. Changes in postural sway can indicate impaired motor control. Postural sway measurements may be a useful way to diagnose MSI in horses in an early state (King et al., 2013). A study of Lichtenauer (2018) showed a difference in frequency of the CoP pattern in Thoroughbred racehorses after eight weeks of high-intensity training, this might be an indication for painful limbs (Lichtenauer, 2018). The CoP can be measured while a horse is standing on a pressure plate. Loading patterns can be made visible by using pressure plates and are useful to diagnose musculoskeletal injury in an early stage. Horses with musculoskeletal injury have a different loading pattern than healthy horses (Nauwelaerts et al., 2017). Pressure plates can in a clinical setting be used combined to force plate for the quantification of symmetry in the forelimb (Oosterlinck et al., 2010).

The aim of this study is to investigate the effect of the way of training (i.e. clockwise, counter-clockwise or both directions) on the load distribution and the pattern of the CoP between the forelimbs in the standing horse in a cross sectional population of New Zealand Thoroughbred racehorses. It was hypothesized that there is a difference in loading of the left versus the right forelimb between clockwise, counter-clockwise and in both directions trained racehorses. Furthermore, it was hypothesized that horses with a greater workload would have a larger loading bias to the right or to the left forelimb when counter-clockwise or clockwise trained, respectively. Furthermore, it was hypothesized that there would be no difference in CoP variables (i.e. velocity, amplitude and frequency) between clockwise, counter-clockwise and in both directions trained racehorses. In addition, it was hypothesized that there would be no relationship between workload and CoP variables.

Materials and methods

Horses

Data were collected via a cross-sectional survey of 57 Thoroughbred racehorses in the North Island of New Zealand in November 2019. Seventeen horses were predominantly trained counter-clockwise (left-handed), ten horses were trained in both directions or on a straight line and thirty horses were predominantly trained clockwise (right-handed). All horses were at different stage of their race preparation program.

Data collection

During field data collection general information was collected with a questionnaire for each horse (racing name, sire, age, sex). Information on workload was collected as months in training, average gallop distance, number of accumulated gallop days and whether the horse was trained counter-clockwise, clockwise or in both directions. Information on racing career was obtained from the online database of New Zealand Thoroughbred Racing.

Pressure plate

The RsScan footscan® 0.5m high-end system (RScan International NV, Belgium) was used to collect the CoP measurements. The pressure plate surface was covered with a 3-mm-thick rubber mat to reduce the risk of horseshoe nail head penetration from shod horses. The pressure plate was surrounded by a plywood frame to reduce the possibility of damaging the plate because of a horse standing on the edge of it, which was also covered with a rubber sheet. The horses had to stand square with the forelimbs in the centre of the plate during the data collection. The measurements were performed on a horizontal surface. Data of the CoP oscillations were collected at a sampling frequency of 15Hz for a duration of 66.7 seconds. The handler stood close to the horse without physical contact. The horse had to keep his head straight ahead, obvious movements of the horse during the measurement were noted and these data were excluded from analysis. The measurement was considered successful if the horse stood still for at least 15 seconds. The data for the limb loading were collected on duplicate single collections (~1 second), of which during one measurement the right limb was placed last and during the other measurement the left limb was placed last. The Footscan 7 gait 2nd generation and Footscan Balance 7.72 software (RScan International) were used to collect the limb loading data.

Data analysis

Questionnaire data were manually transcribed into Microsoft Excel. Total gallop distance was derived from the numbers of gallops and average gallop distance. The variables were tested for normality and the median and interquartile range (IQR) were calculated using RStudio. The snapshots displaying the distribution underneath the limbs were analysed in the Footscan® 7 gait 2nd generation to calculate the percentage of loading of each limb. The results were transcribed to Microsoft Excel and the mean loading was calculated between the two measurements. RStudio was used to calculate the average loading of the limbs of all the horses, the median and the IQR.
The CoP data was recorded as x and y coordinates during time. The x-axis represents the Medio-Lateral (ML) axis, positive to the left, while the y-axis represents the Cranio-Caudal (CC) axis, positive cranially. The data was visually screened for movements in the Footscan® 7.72 Balance software. The data was exported to Microsoft Excel and sections without visible movements were used with a minimum length of 15 seconds. The data of the x coordinate were exported to RStudio and filtered using a filter and smoothing script to remove high frequency noise. The variables amplitude, velocity and frequency of the mediolateral direction were calculated in RStudio to describe the CoP movements. Amplitude was calculated as the average difference between the consecutive maximal and minimal values of the x coordinate, differences exceeding 3 mm were excluded for calculation because larger displacements are associated with gross motor movements. Velocity was calculated by dividing the accumulated amplitudes by the accumulated time differences between maximal and minimal values of the x coordinate. Frequency was calculated by dividing the total amount of maximal values of the x coordinate by the total time.

Statistical analysis

The data was analysed using RStudio 1.2.5019 with significance set at $P<0.05$. The differences in months in training between counter-clockwise, clockwise and in both directions trained horses was tested using an ANOVA test. The relationship between age and the workload (number of accumulated gallops and total gallop distance) was described by using linear regression. Each variable (velocity, amplitude, frequency) was tested for normality and the mean and IQR were calculated. The variables were tested for a significant difference between counter-clockwise, clockwise and in both directions trained horses using an ANOVA test. Linear regression was used to describe the relationship of the workload parameters (number of accumulated gallops and total gallop distance) and the CoP variables. The relationship of the CoP variables and age was described by using linear regression. Association between loading bias of the limbs and counter-clockwise, clockwise and in both directions training was tested using an ANOVA test. The difference between order of placing of the limb (i.e. right or left forelimb first) was tested for significance with a t-test. The relationship of the workload parameters and loading bias within the counter-clockwise, clockwise and both directions training groups was described by using linear regression. The significance of the bias within all groups was tested using a t-test.

Results

Horse population

Data were collected from 57 Thoroughbred racehorses (table 1). Ten horses were in training with a trainer at the Foxton-track, eleven horses were with a trainer at the Awapuni-track and thirty-six horses were in training with four different trainers at the Pukekohe-track. The horses were divided in three groups depending on the predominant direction of training. Horses trained at Foxton- (8/17) and Awapuni-track (9/17) were classified as Left (17/57). Horses trained at Pukekohe-track were classified as Right (30/57). Three horses trained and raced in both directions and therefore classified as Both. Seven horses had never galloped on the track and were therefore also classified as Both. The majority of the counter-clockwise and clockwise trained horses were geldings (64%, 30/47), while there was an equal distribution of gender in the Both-category. The horses ranged in age from two to nine years old. Two-year-old horses were overrepresented within the Both category (6/10). There were less 2-year-old horses within the Left- and Right-category (0/17 and 3/30, respectively). Most of the horses were shod with conventional fullered steel shoes (54/57). One horse had egg bar shoes and two horses had no shoes.

Table 1: Descriptive demographics of the sample population of Thoroughbred racehorses obtained via cross-sectional survey at six trainers in New Zealand, categorized according to the direction in which they were trained.

Descriptor	Left	Both	Right	Total
Number of horses	17	10	30	57
Gender				
Gelding	11	5	19	35
Mare	6	5	10	21
Stallion	0	0	1	1
Age	5 (IQR 3-6)	3.5 (IQR 2-5.5)	4 (IQR 3-5)	4 (IQR 3-5)

Workload

Description of workload data of the Thoroughbred racehorses during this preparation is shown in Table 2. Counter-clockwise trained horses had been in training during this preparation longer than clockwise trained horses and horses trained in both directions (6 months (IQR 4-8) vs. 4.3 (IQR 3.5-5) vs. 2.5 months (IQR 0.75-2), p=0.044 and p=0.001, respectively). There was no significant difference in months in training between Both and Right (p=0.110). The number of accumulated gallops was similar in the Left- (22 (IQR 4-28)) and Right-categories (21 (IQR 15-24)), whilst the Both-category had performed less gallops (10 (IQR 0-0)). Only the 6-year-old horses (n=2) within the Both-category had performed gallops during this preparation. The average gallop distance was similar in the Both- (1100m (IQR 1100-1100)) and Right-categories (1083m (IQR 1000-1200)), whilst the Left-category galloped an average distance of 885m (IQR 700-1100) (p=0.092). Counter-clockwise trained horses had performed most races and trials this season (4 (IQR 0-5)), followed by clockwise trained horses (3 (IQR 1-4)) and in both directions trained horses (2 (IQR 0-0), p=0.057). Horses of the Left-category had run most races in their career (14 (IQR 1-18)), followed by the Both- (7 (IQR 0-12)) and Right-categories (4 (IQR 0-6), p=0.082). Horses trained counter-clockwise had the highest total accumulated gallop distance this preparation (26469m (IQR 13200-23100)), followed by horses trained clockwise and both directions (23531m (IQR 15400-28800) and 20074m (IQR 1600-25600), p=0.127 respectively). Most horses (41/57) did pace work (canter work at a lower speed than a gallop) on the track 3 to 6 times each week, depending on the trainers' choices and upcoming races. Four horses did pace work on a straight line on the beach 6 times a week. Eleven horses were trained using a water treadmill 4 to 5 times a week instead of pace work. One horse was trained in the horse walker 7 days a week. Most horses (n=47) galloped each week with a maximum of twice a week, also depending on the trainers' choices and upcoming races. Almost half of the horses (27/57) were trained in the horse walker once a week.

Table 2: Workload of this preparation in a sample population of Thoroughbred racehorses.

Workload	Left	Both	Right	Total
Months in training	6 (IQR 4-8)	2.5 (IQR 0.75-2)	4.3 (IQR 3.5-5)	4.5 (IQR 3-5.5)
Horses performing gallops	13/17	2/10	29/30	44/57
Number of accumulated gallops	22 (IQR 4-28)	10 (IQR 0-0)	21 (IQR 15-24)	19 (IQR 2-24)
Average gallop distance (m)	885 (IQR 700-1100)	1100 (IQR 1100-1100)	1083 (IQR 1000-1200)	1025 (IQR 800-1100)
Races and trials this season	4 (IQR 0-5)	2 (IQR 0-0)	3 (IQR 1-4)	3 (IQR 2-4)
Total races	14 (IQR 1-18)	7 (IQR 0-12)	4 (IQR 0-6)	8 (IQR 0-10)
Total gallop distance (m)	26469 (IQR 13200-23100)	20074 (IQR 1600-25600)	23531 (IQR 15400- 28800)	26005 (IQR 15150-31000)

Loading of the forelimbs

An example of the pressure distribution scan is shown in figure 1. The average loading of the forelimbs of each horse is shown in figure 2. Horse 17, a counter-clockwise trained horse, was excluded due to poor measurements. Horse 40, a clockwise trained horse, was excluded for further calculations due to symptoms of soreness; this horse refused to shift weight to his left limb.

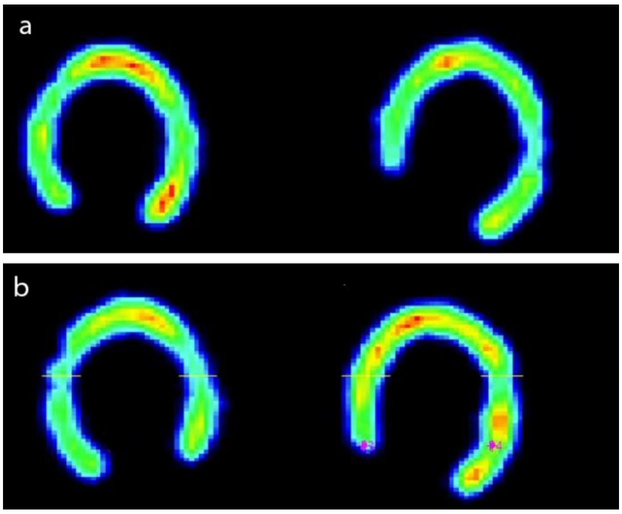

Figure 1: Example of a pressure scan. a) Left limb placed first, b) Right limb placed first.

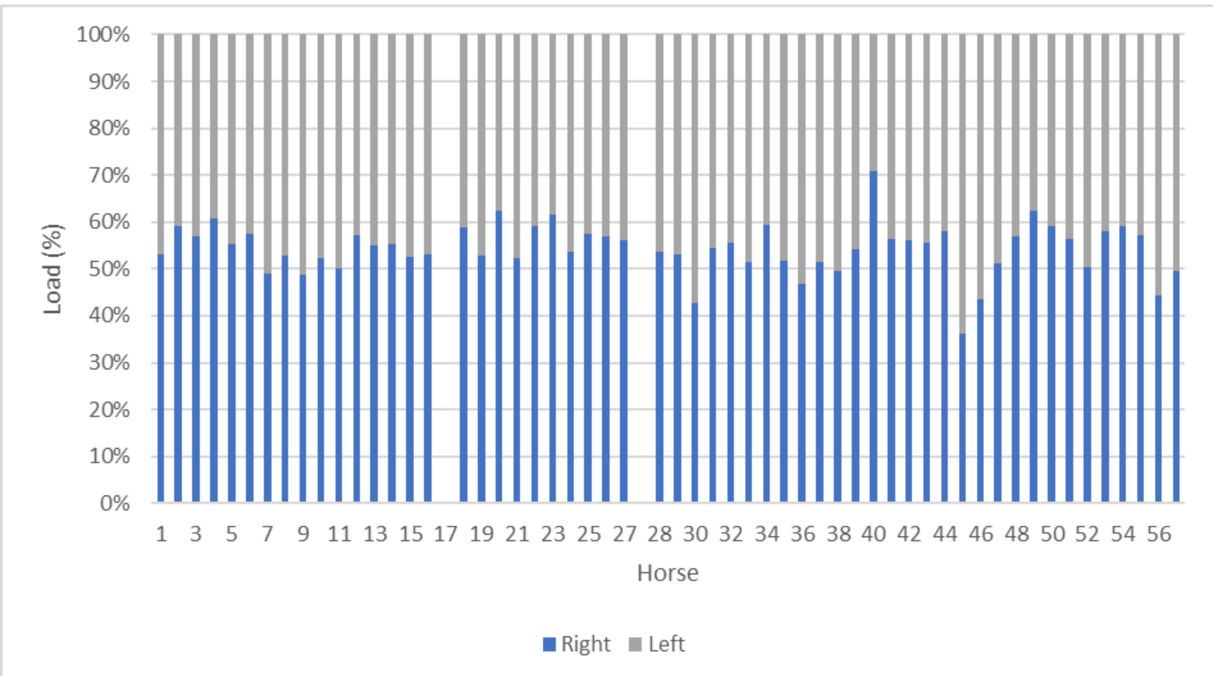

Figure 2: Load distribution between the left and right forelimb. Horses 1-17 are Left-category, horses 18-27 are Both-category and horses 28-57 are Right-category.

The horses trained in both directions had the highest average load under the right forelimb (57.07% (SE 3.50)), followed by the counter-clockwise (54.31% (SE 3.48)) and clockwise trained horses

(53.55% (SE 6.62), p=0.150). Table 3 shows the mean loading of the right forelimb of each group. The distribution of the average loading of the right forelimb within the three groups is shown in figure 3. There was a significant difference in all horses between the measurement where the left limb was placed first and the measurement where the right limb was placed first (17.68±9.14, $p<0.001$).

Table 3: Loading of the right forelimb (%) within three groups of differently trained Thoroughbred racehorses.

	Left	Both	Right	Total
Loading left limb placed first	44.74 (SE 4.76)	50.05 (SE 6.92)	43.62 (SE 7.34)	45.12 (SE 6.93)
Loading right limb placed first	63.88 (SE 4.67)	64.10 (SE 2.18)	62.28 (SE 8.33)	63.08 (SE 6.60)
Average limb loading	54.31 (SE 3.48)	57.07 (SE 3.50)	52.95 (SE 5.87)	54.10 (SE 5.06)

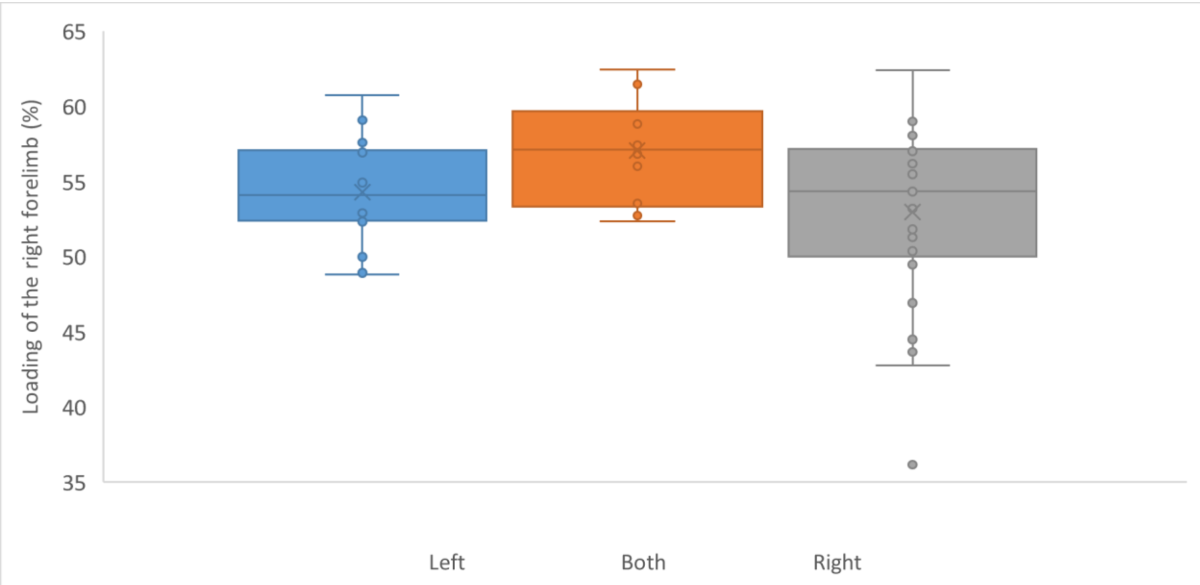

Figure 3: Boxplots of the loading of the right forelimb (%) in Thoroughbred racehorses trained counter-clockwise, clockwise and in both directions.

There was no significant difference in loading of the right forelimb between horses in any of the training direction categories (p=0.150). There was no linear relationship between age and loading of the right forelimb in all groups. Levels of asymmetry for loading are reported in table 4. Most horses (50/55) had an 80% symmetry (loading of each limb between 40-60%), less horses (24/55) had a 90% symmetry (loading of each limb between 45-55%) and a small group of horses (12/55) had a 95% symmetry (loading of each limb between 47.5-52.5%). The bias was predominantly to the right forelimb within all groups (45/55). The horses trained in both directions were all biased to the right (10/10), counter-clockwise and clockwise trained horses were predominantly biased to the right

(13/16 and 22/29, respectively). Across all horses, and across all training direction groups there was a significant bias to the right (p<0.001).

Table 4: Bias of the loading of the limbs in Thoroughbred racehorses. Three thresholds of symmetry within the groups of horses, showing the number of horses of the population with any kind of bias and the number of horses biased to a specific side.

	Left	**Both**	**Right**	**Total**
95% symmetry	4/16	1/10	7/29	12/55
90% symmetry	9/16	3/10	12/29	24/55
80% symmetry	15/16	8/10	27/29	50/55
Right-bias	13/16	10/10	22/29	45/55
Left-bias	3/16	0/10	7/29	10/55

The relationship between workload, expressed in total gallop distance, and loading is presented in figure 4. There was no linear relationship between workload and loading of the right forelimb in the Left- (p=0.419), Both- (p=0.967) and Right-categories (p=0.707).

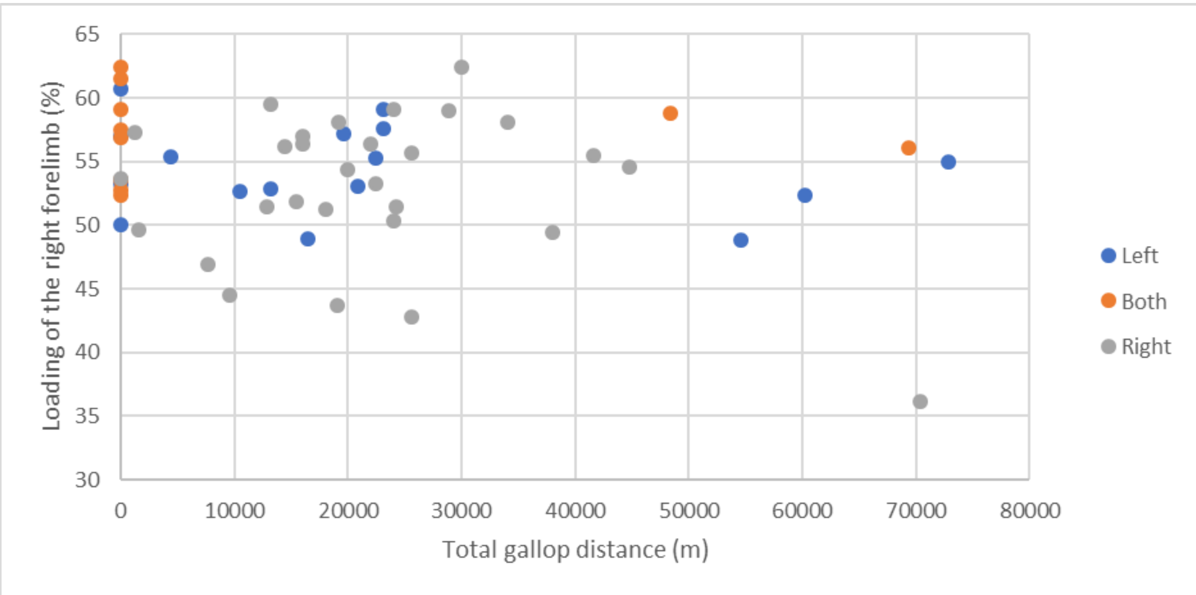

Figure 4: Relationship between workload and loading of the right forelimb (%) in three groups of Thoroughbred racehorses with different training.

Age

The correlation between age and workload in the population is shown in figure 5. There was a weak linear relationship between age and number of gallops (R^2=0.082, p=0.018) and a weak linear relationship between age and total gallop distance (R^2=0.083, p=0.017).

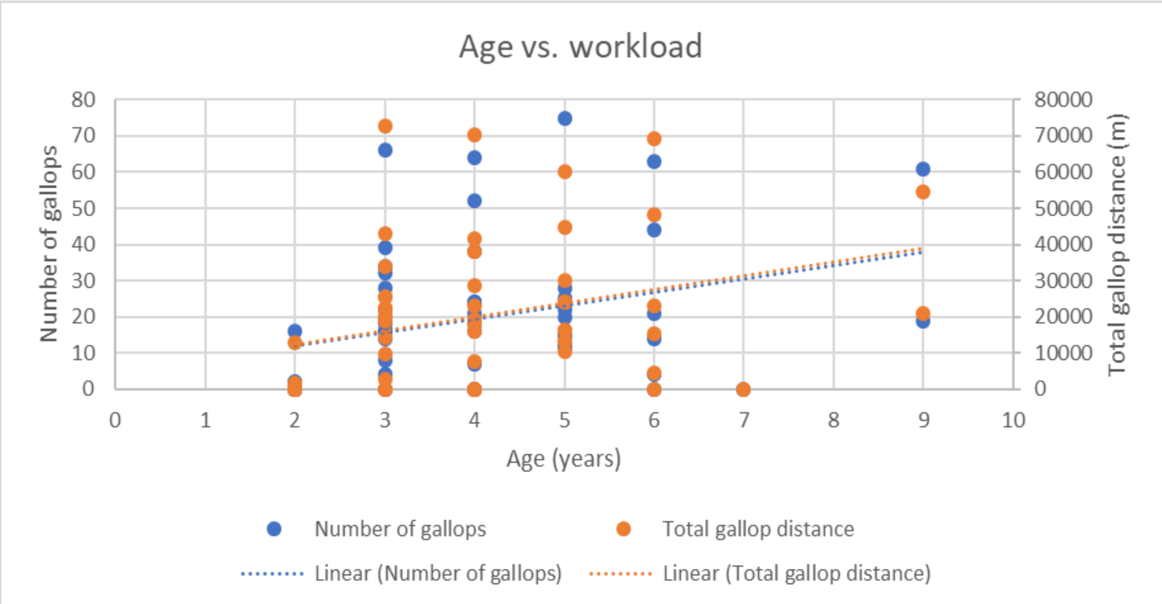

Figure 5: Relationship between age and workload in terms of number of gallops (left Y-axis) and total gallop distance (right Y-axis) in a population of Thoroughbred racehorses.

CoP displacement

Table 5: Average amplitude, velocity and frequency of the CoP displacement of three groups of Thoroughbred racehorses with different training.

	Left	**Both**	**Right**	**Total**
Amplitude (mm)	0.84 (SE 0.25)	0.74 (SE 0.38)	0.80 (SE 0.38)	0.80 (SE 0.34)
Velocity (mm/s)	0.88 (SE 0.33)	0.74 (SE 0.26)	0.94 (SE 0.43)	0.89 (SE 0.38)
Frequency (Hz)	0.40 (SE 0.05)	0.39 (SE 0.07)	0.38 (SE 0.04)	0.38 (SE 0.05)

Table 5 shows the CoP displacement of the three groups of Thoroughbred racehorses with different training. Horses 1, 16, 18, 22, 36 and 38 were excluded due to bad measurements with too much gross motor movement, preventing identification of the CoP signal. There were no significant differences in amplitude, velocity and frequency between the three groups. The average amplitude of the population was 0.80±0.34 mm, the velocity was 0.89±0.38 mm/s and the frequency was 0.38±0.05 Hz. The standard error of the average frequency was small (0.05 Hz), while there was a larger standard deviation of the average velocity (0.38 mm/s) and average amplitude (0.34 mm).

There was no association of age and the CoP variables; amplitude (p=0.833), velocity (p=0.407) and frequency (p=0.347). Figure 6 shows a weak linear relationship between total gallop distance and frequency (R^2=0.124, p=0.007). There was no association between total gallop distance and amplitude (p=0.519), or velocity (p=0.110).

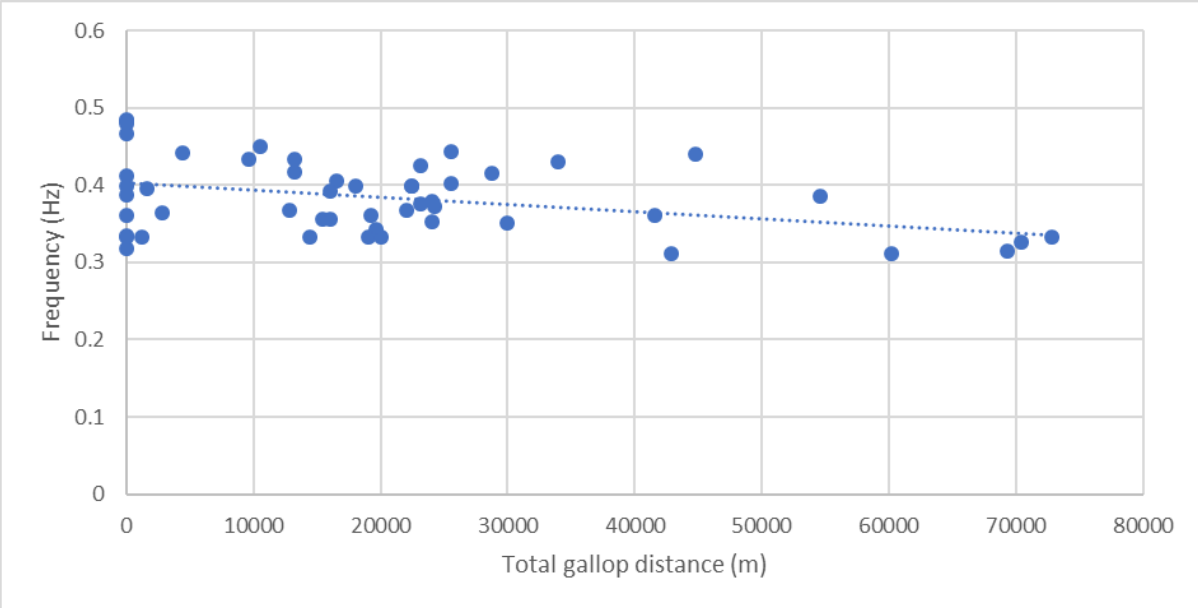

Figure 6: The relationship between total gallop distance and frequency of the CoP displacement in a population of Thoroughbred racehorses.

There was no association between the number of gallops and amplitude (p=0.422). However, there was a weak linear relationship between number of gallops and frequency (R^2=0.162, p=0.002) and velocity (R^2=0.061, p=0.044), shown in figure 7 and figure 8. However, there was no significant linear relationship between number of gallops and velocity if horse 10 (number of gallops=75, velocity=1.61mm/s) was excluded (p=0.172).

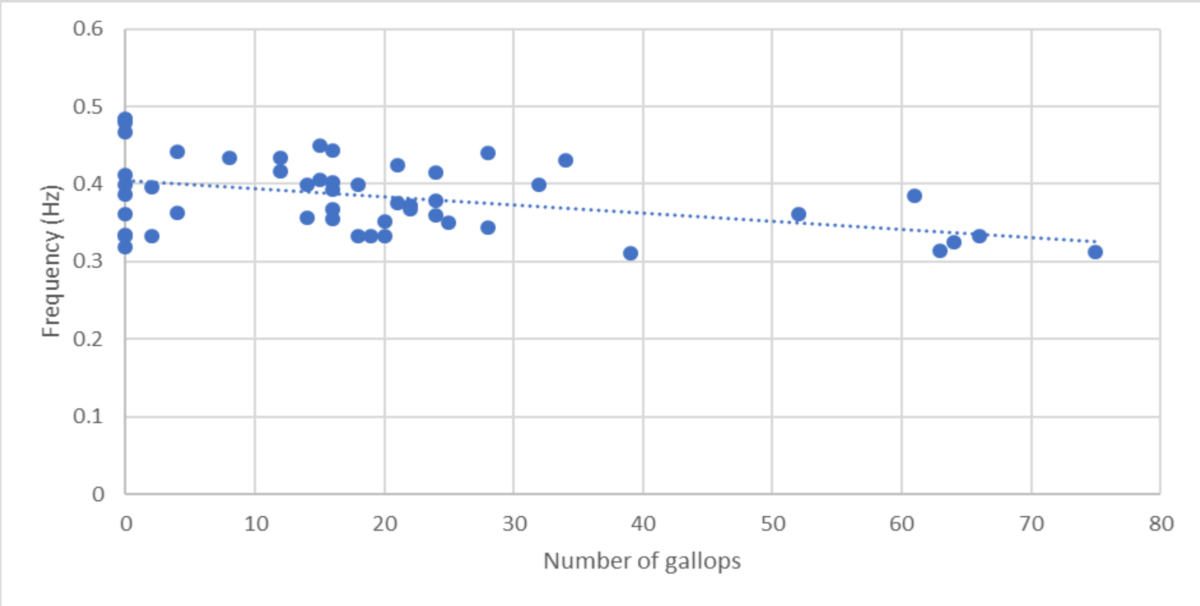

Figure 7: The relationship between number of gallops and frequency of the CoP displacement in a population of Thoroughbred racehorses.

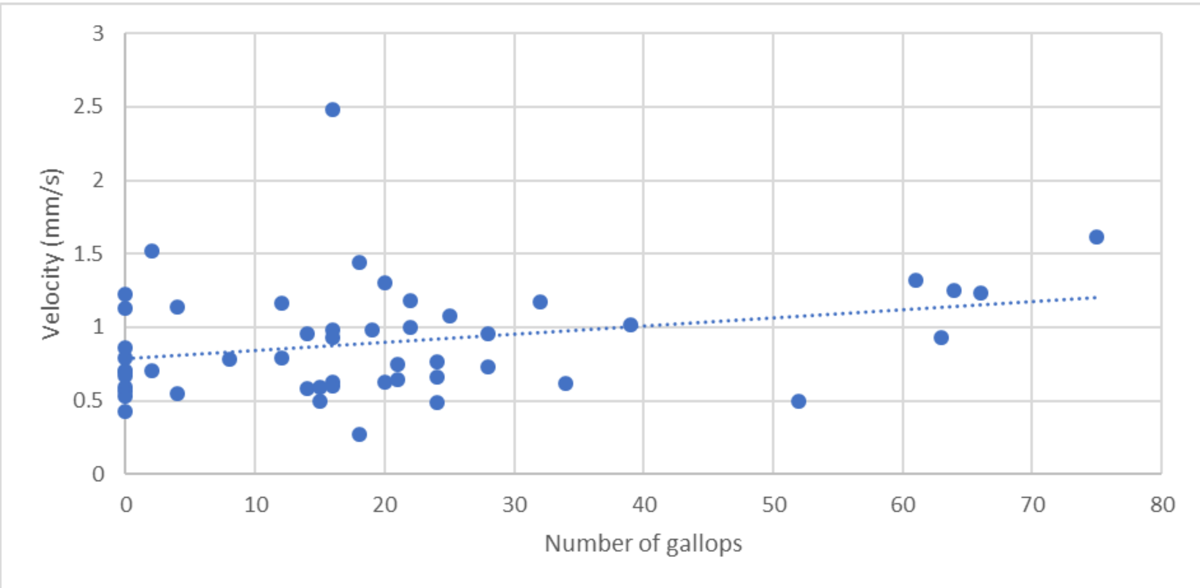

Figure 8: The relationship between number of gallops and velocity of the CoP displacement in a population of Thoroughbred racehorses.

Discussion

This study was the first to investigate the differences in loading distribution of the forelimbs and CoP displacement between counter-clockwise, clockwise and both ways trained Thoroughbred racehorses. The study population consisted a cohort of 2- to 9-year-old racehorses of different trainers across the North Island of New Zealand. Loading distribution of the forelimbs and frequency, velocity and amplitude of the CoP displacement were measured. The data used for this study were collected at trainers enrolled in the study through a convenience sample based on location. The way of training depends on the track used for training. The racetracks in the southern part of the North Island are predominantly counter-clockwise (i.e. left-handed), while racetracks in the northern part are predominantly clockwise (i.e. right-handed) (New Zealand Thoroughbred Racing, 2019). The population was a convenience sample and not homogeneous, since there were differences in age, workload, trainer and housing. All the clockwise trained horses were trained at Pukekohe-track but were situated at four different training yards. The counter-clockwise trained horses were trained at two different tracks, namely Foxton and Awapuni. There was an unequal distribution of age within the both directions trained groups, since 6/10 horses were 2-year-old. However, within the primary groups of interest (counter-clockwise and clockwise) there was a more equitable distribution of age.

The study demonstrates that there is no association of the loading bias and direction of training. This is contradictory to the hypothesized association that there would be a difference in loading of the forelimbs between counter-clockwise and clockwise trained horses. Furthermore, the results also contradict the hypothesis that a greater workload would result in a larger bias to the right or to the left forelimb when counter-clockwise or clockwise trained, respectively. Previous studies showed a loading bias to the right in counter-clockwise trained racehorses and sport horses (Heuver, 2019; Lichtenauer, 2018; Plantinga et al., 2018; Schuurmans, 2019). The average loading of the right forelimb in sport horses was 56.5% in the study of Heuver (2019) and 52.6% in the study of Schuurmans (2019). In racehorses a loading of the right forelimb of 55.1% was found in the study of Lichtenauer (2018) and Plantinga *et al*. (2018) reported a similar value with loading of 53.8%. It was suggested the bias was the result of the asymmetrical training. The aim of this study was to investigate the influence of training on the loading bias. The measurements showed a loading to the right forelimb of 54.31% in counter-clockwise trained horses and 52.95% in clockwise trained horses. This difference is not significant and therefore implies that there is a loading bias to the right irrespective of the direction of training. Furthermore, there was no association between loading and age. These results contribute to a clearer understanding of the loading bias to the right in horses. While previous studies have suggested that the loading bias could be the result from training, these results demonstrate that training had no significant influence. There was an unequal distribution of horses between groups in the current study. There were less horses (n=17) in the counter-clockwise trained category than in the clockwise trained category (n=30). However, previous studies with counter-clockwise trained racehorses have showed a loading bias to the right within this group (Lichtenauer, 2018; Plantinga et al., 2018). The goal of the current study was to investigate the loading bias within clockwise trained racehorses. The group of clockwise trained horses (n=30) can be considered a reliable sample size to conclude that there is a generalized significant loading bias to the right within horses. The study of Lichtenauer (2018) suggested the loading bias might be the result of cyclic overload. The effects of training on the musculoskeletal system have been extensively investigated. The musculoskeletal system is positively affected by early exercise in the juvenile horse (Rogers et al., 2012). Firth *et al.* (2005) reported a greater bone size, density and strength index in trained horses compared to untrained horses (Firth et al., 2005). The study of Beccati *et al.* (2011) showed a significantly thicker palmaer cortex of the proximal phalanx of the right forelimb than the left forelimb after one year of race training in predominantly a clockwise direction. It has been found that the leading forelimb is subjected to the highest vertical ground reaction force, both in a turn and on a straight line (Beccati et al., 2011). It is common for horses to use the left limb leading stride pattern in curves when galloping counter-clockwise and vice versa when galloping clockwise (Cully et al., 2018). This could explain the difference in palmaer cortex thickness found in the study of Becatti *et al.* (2011). The majority of the horses change stride between the turns and straight part of the track (Williams & Norris, 2007). To avoid excessive muscular fatigue racehorses change stride ≥ 8 times/mile when performing asymmetric work (Clayton & van Weeren, 2001). During racing most injuries occur to the leading leg, this is irrespective of the course direction (Ueda et al., 1993).

It was suggested that pain in the leading limb might cause a higher loading in the nonleading limb. This would explain the loading bias to the right that was found in the counter-clockwise trained racehorses (Lichtenauer, 2018; Plantinga et al., 2018). This might suggest that clockwise trained racehorses would have a load bias to the left and horses trained both ways might have a more symmetrical load distribution between the forelimbs. However, the current study showed that horses are predominantly biased to the right, irrespective of training direction. A possible explanation of the consistent load bias to the right is laterality of the horse. Motor and sensory laterality of horses has been reported several times (Cully et al., 2018; van Heel et al., 2010; McGreevy & Rogers, 2005; McGreevy & Thomson, 2006; Murphy et al., 2005; Williams & Norris, 2007). A study of Colborne *et al.* (2009) showed an asymmetric moment pattern between the forelimbs. This suggests a different contribution of the two forelimbs to propulsion and thus a different loading (Colborne et al., 2009; Colborne et al., 2016; Heaps et al., 2011). Skeletal asymmetry has also been described in Thoroughbred racehorses. It has been found that the right metacarpal bone is longer than the left in 76% of the Thoroughbred racehorses. This asymmetry is also found in horses and ponies of various breeds (Leśniak, 2013; Watson et al., 2003). This might be the result of asymmetric gene expression, that has been described in many classes of animals, including horses (Shiratori et al., 2001). Asymmetry and laterality are described at different levels in various species (Gibbons et al., 2012; Gough & McGuire, 2015; Lorincz & Fabre-Thorpe, 1996; Manns et al., 2018). To our knowledge a similar loading bias as found in this Thoroughbred racehorse population is not described in other species yet. The loading bias reported might be the result of a biologically normal population asymmetry. Further research is needed to determine the cause of the bias. It would be interesting to investigate the loading of each limb, with both the fore- and hindlimbs on a bigger pressure plate, to determine the loading distribution between the hindlimbs in comparison with the forelimbs.

Unfortunately, only the study of Heuver (2019) noticed the effect of the placing order of the forelimbs on the load distribution. The analysis of the two different measurements (one right limb placed first and one with the left limb placed first) showed a significant difference of the load distribution in relationship to which limb is placed first. The mean loading of the right forelimb when the right forelimb is placed first is 63.08%, while it is only 45.12% when the left forelimb is placed first. These results agree with the findings of Heuver (2019) that the first placed limb carries relatively more weight than the last placed limb. The studies of Lichtenauer (2018), Plantinga *et al.* (2018) and Schuurmans (2019) calculated the loading of the forelimbs with a single snapshot. The average load distribution could be influenced by this type of measurement. However, it is plausible that there is an equal ratio between left and right placed first measurements, eventually resulting in a reliable result.

There was a larger standard error in the mean loading of the right forelimb within the group trained in clockwise direction despite the larger number of individuals within this group. This shows more variation in the load distribution is present in the clockwise trained group compared to the counter-clockwise and both directions trained groups. However, there was no association between loading of the right forelimb and workload. Counter-clockwise training might be preferred by the majority of the racehorses due to laterality. This might lead to a small discomfort to the horses when trained clockwise. This might result in a shift in the load distribution between the forelimbs, resulting in the larger standard error found in this study. However, further research within a larger population is needed to establish whether this result is generalizable on the population or just an accidental finding.

The horses used in this study had a various workloads and were at different stages of their race preparation program. In line with the hypothesis, no difference in CoP variables (i.e. velocity, frequency and amplitude) was found between the groups with different training directions. The CoP frequency found in this study (0.38±0.05 Hz) was slightly higher than reported in previous studies. Schuurmans (2019) and Heuver (2019) found a frequency of 0.34 Hz, while other studies reported a frequency of 0.30 Hz (Clayton & Nauwelaerts, 2014; Lichtenauer, 2018; Plantinga et al., 2018). The velocity found (0.89±0.38 mm/s) was in agreement with the report of Schuurmans (2019) (0.78 mm/s), Heuver (2019) (0.84 mm/s) and Plantinga (2018) (1.07 mm/s). However, other studies reported different values of the CoP velocity. Clayton (2014) reported a velocity of 2.2 mm/s, Gomes-Costa (2015) found 4.3 mm/s and Lichtenauer (2018) reported 1.88 mm/s (Clayton & Nauwelaerts, 2014; Gomes-Costa et al., 2015; Lichtenauer, 2018). The CoP amplitude found in this study (0.80±0.34 mm) is in agreement with the report of Heuver (2019) (1.05 mm). However, previous reports described a variety of amplitudes. Clayton (2014) found an amplitude of 9.3 mm, Gomes-Costa (2015) reported 3.2 mm and Lichtenauer (2018) found 22.6 mm.

Contrary to the hypothesized association, the data suggest that there is a significant relationship between the number of gallops and CoP frequency, as well between the total gallop distance and CoP frequency. The frequency decreased significantly with an increasing workload. The results contradict with the results of Lichtenauer (2018). That study found an increased frequency in 15 Thoroughbred racehorses after eight weeks of training. The mean frequency that was found in week 1 was 0.28 Hz, in week 8 the mean frequency was 0.30 Hz. However, three horses in the study did not gallop during the trial and four horses were spelled during the trial. This might have caused a lower average workload at the second measurement, resulting in a higher frequency. It was suggested that the higher frequency after eight weeks might be the result of musculoskeletal pain due to overtraining. It is more likely that the decrease found in the current study might be the result of a better balance due to training. The study of King *et al.* (2013) showed a significant improvement of static balance control in horses with carpal joint osteoarthritis after underwater treadmill exercise (King et al., 2013). The study of Schuurmans (2019) found no association between workload and CoP frequency. However, the tested population had little variation in workload compared to the population racehorses in the current study. This might explain why no association was found. The relationship in the current study might be the result of quality of horses with a higher workload. Tanner *et al.* (2013) reported that 46% of the horses that were registered with a trainer never trialled and 55% never started in a race. The most important reason for loss from racing is poor performance or lack of talent, followed by musculoskeletal injury (Bolwell et al., in press). This implies that horses with a higher workload are performing better and may have a better physical health, than horses with a lower workload. Since the selection of better horses takes place during the training process due to trainer decisions and occurrence of injuries, this might result in a healthy horse effect with the better and stronger racehorses associated with a higher workload cohort (Bolwell et al., in press). Physically stronger horses possibly have a better balance resulting in a lower CoP frequency. This could explain the association between workload and CoP frequency, but further research is needed. It would be interesting to follow the horses over a period of time to see whether the frequency changes. If the frequency increases after a period of training, it might be the result of pain due to cyclic overload. On the other hand, a decrease of the frequency would indicate that training leads to a better balance and therefore a lower frequency.

The reported study has several limitations, such as the reliability of the CoP amplitude and velocity measurements. CoP amplitude and velocity appeared to depend on the quality of the measurement. This means that visible movement of a horse during the measurement often results in a larger amplitude and velocity. Since the studied Thoroughbred racehorses were fit to compete, it was sometimes hard to make a horse stand still for a full minute during the measurement. The program in R studio filters out most of the noise from the gross motor movements. Unfortunately, there are differences in quality of measurements, affecting the amplitude and velocity. The frequency is only affected when the quality of the measurement is too poor; this resulted in excluding 6 horses from the calculations. Further research is needed to improve the determination of the amplitude and velocity. An appropriate setup is needed to get a more homogenous measurement quality. In our study we used a different setup at every trainer due to differences in location options. We have experienced that the pressure plate should be situated in a quiet place (i.e. no horses and people walking by), where the horse feels comfortable and has the opportunity to see the things happening around him. In that setup a baseline of CoP variables could be determined to hopefully use the pressure plate in a clinical setting in the future.

Another limitation of the current study is the reliability of the workload data obtained from the trainers. The data about the workload of the racehorses was an estimation by the trainer in most cases. Some trainers in the study documented all information in a detailed electronical program, which helped providing us an exact number of training days and number of gallops. Unfortunately, the majority of the trainers gave an estimation of the workload and no exact numbers. Hence, the actual workload might deviate from the number reported in this study.

Conclusion

The present study provides reliable data on the load distribution between the forelimbs within New Zealand Thoroughbred racehorses. The loading of the right forelimb is higher than the loading of the left forelimb, irrespective of training direction and workload. This implies a biologically normal level of asymmetry and laterality. A linear relationship was found between the Centre of Pressure frequency and the workload, the frequency decreases with a higher workload. This suggest a greater postural stability within racehorses with a higher workload, but further research is required to investigate possible changes in the frequency due to a higher workload.

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