# Adaptation strategies of the Icelandic horse with induced forelimb lameness at walk, trot and tölt 

Ineke H. Smit ${ }^{1} \odot \quad \mid E l i n$ Hernlund ${ }^{2} \odot \mid E m m a$ Persson-Sjodin ${ }^{2}$ © | Sigridur Björnsdóttir ${ }^{3}$ © | Helga Gunnarsdottir ${ }^{3}$ | Vikingur Gunnarsson ${ }^{4}$ Marie Rhodin ${ }^{2}$ © | Filipe M. Serra Braganca ${ }^{1}$ ©<br>${ }^{1}$ Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands<br>${ }^{2}$ Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden<br>${ }^{3}$ Agricultural University of Iceland, Borgarnes, Iceland<br>${ }^{4}$ Department of Equine Science, Hólar University College, Hólar, Iceland

## Correspondence

Ineke H. Smit, Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, 3584CM Utrecht, The Netherlands. Email: i.h.smit@uu.nl

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#### Abstract

Background and objective: Lameness assessment in the gaited Icelandic horse is complex. We aimed to describe their kinematic and temporal adaptation strategies in response to forelimb lameness at walk, trot and tölt. Study design: In vivo experiment. Methods: Ten clinically non-lame Icelandic horses were measured before and after reversible forelimb lameness induction. Upper body and limb kinematics were measured using 11 inertial measurement units mounted on the poll, withers, pelvis (tubera sacrale) and all four limbs and hoofs (Equimoves ${ }^{\circledR}, 500 \mathrm{~Hz}$ ). Horses were measured on a straight line at walk and trot in-hand and at walk, trot and tölt while ridden. Linear mixed models were used to compare baseline and lame conditions (random factor = 'horse'), and results are presented as the difference in estimated marginal means or percentage of change. Results: Lameness induction significantly ( $p<0.05$ ) increased head vertical movement asymmetry at walk (HDmin/HDmax HAND 18.8/5.7 mm, HDmin/HDmax RIDDEN : $9.8 / 0.3 \mathrm{~mm}$ ) and trot (HDmin/HDmax HAND : $18.1 / 7.8 \mathrm{~mm}$, HDmin/HDmax ${ }_{\text {RIDDEN }}$ : 24.0/9.3 mm). At the tölt, however, HDmin did not change significantly ( 1.1 mm ), but HDmax increased by $11.2 \mathrm{~mm}(p<0.05)$. Furthermore, pelvis vertical movement asymmetry (PDmax) increased by 4.9 mm , sound side dissociation decreased ( $-8.3 \%$ ), and sound diagonal dissociation increased (6.5\%). Other temporal stride variables were also affected, such as increased stance duration of both forelimbs at walk, tölt and in-hand trot.


Main limitations: Only one degree of lameness (mild) was induced with an acute lameness model.

[^0]Conclusions: Classical forelimb lameness metrics, such as vertical head and withers movement asymmetry, were less valuable at tölt compared to walk and trot, except for HDmax. Therefore, it is advised to primarily use the walk and trot to detect and quantify forelimb lameness in the Icelandic horse.

## KEYWORDS

asymmetry, equine biomechanics, gaited horse, inertial measurement units, kinematics

## 1 | INTRODUCTION

With its ability to tölt, the Icelandic horse has been rising in popularity among recreational and competitive riders. Due to their growing presence, there is also an increased need for knowledge regarding lameness adaptations in this breed. Clinicians often testify that assessing lameness in Icelandic horses is particularly challenging. This may be related to their high stride frequencies, ${ }^{1-3}$ lower vertical range of motion of head, withers and pelvis compared to warmbloods at trot ${ }^{3}$ and/or the diversity of footfall patterns the Icelandic horse can show ${ }^{1}$ as a consequence of the high prevalence of the known mutation of the DMRT3 gene. ${ }^{4}$ Especially during tölt, a four-beat running gait either shown as a pure (while ridden) or as a mixed gait (sometimes encountered during lameness exams), our understanding of how Icelandic horses adapt to lameness is minimal.

Upper body vertical movement asymmetry parameters at the trot are currently the primary resource for quantitative lameness assessment in clinical practice. ${ }^{5}$ The most commonly used asymmetry parameters to quantify lameness are the differences between the two local vertical displacement minima/maxima within each stride for the head $\left({H D_{~ m i n ~}^{m}} /\right.$ $\left.H D_{\text {max }}\right)$, withers $\left(W D_{\text {min }} / W D_{\text {max }}\right)$ and pelvis $\left(\mathrm{PD}_{\text {min }} / \mathrm{PD}_{\text {max }}\right)$. These movement asymmetries in lame horses are related to weight redistribution between limbs, ${ }^{6-9}$ where the peak vertical ground reaction force (i.e., limb loading; GRFz) of the lame limb is reduced. Head and withers asymmetry parameters have also been shown to be reliable indicators of forelimb lameness at walk. ${ }^{10}$ Therefore, these asymmetry parameters seem logical candidates for lameness evaluation at tölt. However, at the tölt, the footfall pattern, and thereby weight distribution between limbs, differs from walk and trot. ${ }^{1,11,12}$ While trot is a symmetrical two-beat diagonal gait, ${ }^{13}$ walk and tollt are symmetrical four-beat gaits with lateral sequences of footfalls. ${ }^{1,13}$ In contrast, tölt mechanics are closer to those of running gaits such as trot, ${ }^{12,14}$ whereas the walk is a clear example of a walking gait. ${ }^{14}$ Furthermore, true tollt (TT) has no suspension phase, and uni- and bipedal support phases should alternate with a limb phase of approximately $25 \%{ }^{1,13}$ Therefore, lameness metrics studied for walk and trot might not directly apply to tölt.

Controversy exists among studies regarding the gait dynamics of Icelandic horses at tölt. The sequential placement of the limbs at tölt reduces the peak vertical accelerations of the body centre of mass, ${ }^{15,16}$ such that the transition from two-beat trot to four-beat tölt may be a possible lameness adaptation strategy without a speed reduction. Conversely, it has been suggested that to decrease the loading of the lame limb at tollt may be less effective compared to the
walk and trot as there are periods of unipedal support for each limb. ${ }^{12}$ When peak GRFz are compared between trot and tölt, one study reports lower forelimb peak GRFz at tölt compared to overground trot, ${ }^{12}$ while another study reported higher peak GRFz for the forelimbs at tölt compared to trot on a treadmill. ${ }^{17}$

This study aimed to describe the movement pattern changes in response to induced forelimb lameness at walk, trot and tölt during both in-hand and ridden conditions. We hypothesised that Icelandic horses would show vertical movement asymmetry of the head and withers in response to induced forelimb lameness at all gaits. However, these changes were expected to be larger at the faster gaits and smaller with the presence of a rider compared to in-hand conditions.

## 2 | MATERIALS AND METHODS

## 2.1 | Horses

A convenience sample of 10 clinically sound Icelandic horses (3 mares and 7 geldings; age: 7.5 [5-25] years; weight: 384 [370-405] kg; median [range]) in full training were included in this study. The horses were selected from a group of 30 horses, the majority Universityowned and two of which were privately owned. Horses were included when judged as non-lame based on pre-trial visual and objective lameness measurements (Equinosis) at trot in-hand, as well as clinical examinations of the locomotor apparatus by three experienced orthopaedic veterinarians (E.H., M.R. and F.S.B.). All horses could tölt while ridden (eight were homozygous for the AA genotype of the DMRT3 gene, and two were heterozygote AC).

## 2.2 | Kinematic data collection

The horses were equipped with 13 inertial measurement unit (IMU) nodes (of which 11 nodes were used for this study; Promove Mini, EquiMoves $\left.{ }^{\circledR}\right)^{18}$ set to a sampling frequency of 500 Hz , with the low-g accelerometer set at $\pm 16 \mathrm{~g}$ and the high-g accelerometer set at $\pm 200 \mathrm{~g}$ (Figure 1). In addition, the sacrum node was enabled with a global positioning system to measure the overground speed at 5 Hz . Data were stored using the internal memory of each node to ensure no data loss, and synchronisation between nodes was guaranteed with an error of less than 100 ns. All trials were video-recorded using standard equipment for retrospective analysis of the collected data.

FIGURE 1 IMU node placement. Sensors attached at the following locations: in the median plane at the poll, wither and tubera sacrale; over each tuber coxae, on the lateral aspect of each mid cannon bone (in a protection pad) and on the lateral wall of each hoof. This image is from another study with a similar set up. ${ }^{19}$ In the current study, riders were instructed to ride with loose rein contact, while this was not the case in the study depicted.


A baseline measurement was performed on each horse before lameness induction. The baseline and induced lameness measurements included the following trials: walk and trot in-hand followed by walk, trot and tölt while ridden. Each horse's gaits were performed at a self-selected speed based on the rider's experience. For the ridden trials, the rider was instructed to keep light rein contact and perform sitting walk, trot and tölt not to affect the movement asymmetry by posting. ${ }^{20}$ All trials for each horse were conducted on the same day and on the same hard surface (compacted lava gravel).

## 2.3 | Lameness induction

A well-established and fully reversible sole-pressure model was used to induce lameness. ${ }^{21}$ Each horse was shod with a custom-made shoe with a mediolateral bar designed for applying pressure on the tip of the frog. A flat-edged bolt was inserted into the hole of the bar and tightened to apply the pressure. The lameness was randomly induced on the left or right forelimb by lottery. Once a mild lameness was deemed visible at the in-hand trot by the experienced veterinarians in the research team (E.H., M.R. and F.S.B.), the pressure was considered sufficient, and the measurement protocol was continued. Mild lameness was defined as a score of 1 to 2 out of five on an ordinal 0-5 degree lameness scale, where the two degrees of lameness were defined as follows: 1 subtle lameness: intermittently visible at the trot; 2 mild lameness: visible in every stride at the trot. ${ }^{22}$

## 2.4 | Data analysis

Data analyses were performed in Matlab (version 2022b, MathWorks). All data (both baseline and induction) from horses with
induced right forelimb lameness were mirrored by multiplying accelerometer data (both high and low; around the $y$-axis) and gyroscope (around the $x$ - and $z$-axis) of all IMUs by -1 . This way, all horses were analysed as being lame on the left forelimb to allow exploration of group-level lameness adaptation strategies. The trials were split into gait segments (walk, trot and tölt) based on a gait classification algorithm ${ }^{19}$ and cut into strides based on the left hind (LH) impact. Hoof-on and hoof-off events for each limb were calculated based on manually labelled events from hoof-mounted IMU acceleration and gyroscope data. In short, a semi-supervised approach was used to detect stance and swing from each limb, using a time-series machine learning approach similar to a previously described algorithm. ${ }^{23}$ A sequence-to-sequence regression approach using a long-term short-memory neural network was used, using the limb and hoof sensor data as input and the swing/stance phase of each limb as output. The moments of change between the swing and the stance phase were detected and classified as hoof-on and hoof-off moments.

Furthermore, for all tölt segments, individual strides were labelled as TT, tölt with lateral couplets (TLC) or mixed tölt (TMix; tölt with tripedal support), based on the side dissociation, ipsi- and diagonal support phases of the limbs. ${ }^{1}$

## 2.5 | Primary outcome measures

Upper body asymmetry metrics and temporal stride parameters were extracted from each stride-segmented signal (Table 1). For the upper body asymmetry parameters, the stride split vertical displacement trajectories of the head $(H)$, withers $(W)$ and pelvis $(P)$ were used. For all three locations, the difference between the vertical displacement minima $\left(H D_{\min }, W D_{\min }\right.$ and $\left.P D_{\min }\right)$ and vertical displacement maxima

TABLE 1 Definitions of parameters and the units they are measured in.

| Variable | Units | Description |
| :---: | :---: | :---: |
| Kinematic |  |  |
| $\mathrm{HD}_{\text {min }} / \mathrm{WD}_{\text {min }} / \mathrm{PD}_{\text {min }}$ | mm | The difference between the minimum vertical positions reached by the head/withers/pelvis during the left vs. right stride half-cycle. |
| $\mathrm{HD}_{\text {max }} / \mathrm{WD}_{\text {max }} / \mathrm{PD}_{\text {max }}$ | mm | The difference between the maximal vertical positions reached by the head/withers/pelvis during the left vs. right stride half-cycle. |
| Head/withers/pelvis vertical range of motion (ROMz) | mm | The vertical range of motion of the head/withers/pelvis during a complete stride. |
| Temporal |  |  |
| Stance duration (LF/RF/RH/LH) | s | Time between hoof-on and hoof-off. |
| Diagonal dissociation (DD) | \%StrD | Time dissociation between diagonal limb pairs at hoof impact; positive if hindlimb precedes contralateral forelimb. Sound DD includes the left hind- and right forelimb, lame DD includes the right hind- and left forelimb. |
| Side dissociation (SD) | \%StrD | Time dissociation between ipsilateral limb pairs at hoof impact; positive if hindlimb precedes ipsilateral forelimb. Sound SD includes the right hind- and right forelimb, lame SD includes the left hind- and left forelimb. |
| LF/RF/RH on | \%StrD | The average moment of hoof-on as a percentage of the stride duration (LH not included, as this is always at 0\%StrD). |
| Transition time between contralateral limb pairs | \%StrD | Time dissociation between contralateral limb between hoof-off and hoof-on; positive if there is no overlap between contralateral limb pairs. |
| Stride duration (StrD) | s | Time between two consecutive LH impacts. |
| Stride speed | $\mathrm{m} / \mathrm{s}$ | Average speed of a complete stride. |

Abbreviations: LF, left forelimb; LH, left hindlimb; RF, right forelimb; RH, right hindlimb; \%StrD, percentage of the stride duration (calculated from LH-on to LH-on moments).
$\left(\mathrm{HD}_{\max }, \mathrm{WD}_{\max }\right.$ and $\mathrm{PD}_{\max }$ ) were calculated (Figure 2 for the walk and tölt examples).

Except for stride speed and stride duration and stance durations of all four limbs, temporal stride parameters were calculated from the time-normalised stride data (LH impact to next LH impact $=0 \%-100 \%$ of stride duration [\%StrD]). Footfalls of the individual limbs (left front [LF], right front [RF], LH and right hind $[R H]$ ) are displayed as \%StrD, where LH impact is always at 0\%StrD. Time differences between the footfalls within different limb pairs (diagonal and side dissociation) and transitions between contralateral limb pairs were also calculated from the time-normalised strides.

## 2.6 | Statistical analysis

Linear mixed models were created for the two conditions (in-hand and ridden) separately, containing all gaits performed during that condition, to test the effect of lameness induction on both the kinematic and temporal stride parameters. In addition, linear mixed models were created for the ridden condition where the tölt strides were split into TT, TLC and TMix. Stride-level data for all variables were entered into the model from the baseline and induced lameness measurements. The models were built in R-studio (version 1.1.414, RStudio Inc.) using package nlme (version 3.1-152). In each model, the factor
'lameness’ (baseline or induction) was entered as a fixed effect in interaction with 'gait' (walk, trot or tölt). Furthermore, 'horse' was entered as a random intercept. To correct for speed differences within horses between baseline and induction and to improve model fit, 'stride speed' was used as a random slope. Correlations between different gaits (due to the non-random order of gaits within the horse) were estimated using an autocorrelation component in each model. To test for speed differences between baseline and induction trials within gait, a model was created where 'stride speed' was not included as a random slope. Model fit was evaluated using AIC values, $\mathrm{q}-\mathrm{q}$ plots and residual plotting. Model estimates were represented as estimated marginal means, statistical significance was set at $\alpha=0.05$, and $p$ values were adjusted for multiple comparisons using the false discovery rate procedure. ${ }^{24}$

To identify kinematic and temporal parameters that, on a group level, would be likely associated with lameness at the tölt, the sensitivity and specificity of the variables listed in Table 1 were calculated using package pROC (version 1.18). For each horse, the 15 strides closest to the median stride were selected for both the baseline and induced conditions. Then, the sensitivity and specificity were calculated for each variable based on the difference between baseline and induction conditions. Finally, the optimal sensitivity, specificity, and corresponding threshold were calculated using Youden's index.


FIGURE 2 Explanations for upper body asymmetry value calculations. Upper body asymmetry values are calculated by subtracting left $(L)$ step values from right $(R)$ step values. This is done for the head $\left(H D_{\min } / H D_{\max }\right)$, withers and pelvis $\left(P D_{\min } / P D_{\max }\right)$ separately. For the head and withers, this means that the second peak or trough is subtracted from the first. This is reversed for the $P D_{\min }$ and $P D_{\max }$ calculations (second peak/trough minus the first peak/trough). Panels $A-C$ show the example of a walk, whereas in Panels $D-F$, the tölt example is shown. Panels $A$ and $D$ show the upper body vertical movement of the head (blue), withers (green) and pelvis (orange). Panels B-F show a footfall pattern of the Icelandic horse at walk ( $B, C$ ) tölt ( $E, F$ ). In Panels $B$ and $E$, the coloured circles indicate foot contact with the ground. In Panels $C$ and $F$, the footfalls of the left front (LF; green), right front (RF; purple), left hind (LH; pink) and right hind (RH; orange) are shown over the mean stride ( $0 \%-100 \%$ ). At the walk, negative values meant that a lower minimal and maximal position of the head and pelvis were reached during the right fore- and hindlimb stance phase, respectively. At the tölt, negative values meant that the head and withers reached a lower minimal position and the pelvis reached a lower maximal position during the stance phase of the left forelimb and that the head and withers reached a lower maximal position and the pelvis reached a lower minimal position during the stance phase of the left hindlimb.

## 3 | RESULTS

A total of 10 horses were used for the analyses of the effects of lameness on temporal and kinematic gait parameters. For both in-hand and ridden conditions, speed did not differ between baseline and induction for any gait ( $p>0.05$; in hand: walk $_{\text {Baseline }} /$ walk $_{\text {Induction }}$ $1.7 / 1.7 \mathrm{~m} / \mathrm{s}$ and trot Baseline $/$ trot $_{\text {Induction }} 3.5 / 3.5 \mathrm{~m} / \mathrm{s}$; ridden: walk Baseline / walk Induction $1.5 / 1.5 \mathrm{~m} / \mathrm{s}$, trot $_{\text {Baseline }} /$ trot $_{\text {Induction }} 3.8 / 3.9 \mathrm{~m} / \mathrm{s}$ and töl $_{\text {Baseline }} /$ tölt $_{\text {Induction }} 3.3 / 3.4 \mathrm{~m} / \mathrm{s}$ ). The ridden induction trial was lost for one horse due to missing data from one limb sensor. Therefore, the ridden data of this horse were excluded from further analyses.

## 3.1 | Kinematic parameters

Estimated marginal means for the kinematic parameters and their respective confidence intervals for each gait and condition can be found in Table 2 (in-hand trials) and Table 3 (ridden trials). The results for upper body vertical asymmetry parameters below are given in absolute difference between baseline and induction.

Upper body vertical asymmetry results of the ridden trials are shown in Figure 3. The $\mathrm{HD}_{\text {min }}$ increased significantly ( $p<0.05$ ), both
in-hand (18.8 and 18.1 mm ) and ridden ( 9.7 and 23.9 mm ; Figure 4A) for walk and trot, respectively, though remained unchanged during tölt. At the tölt, however, increases in $\mathrm{HD}_{\max }(11.2 \mathrm{~mm} ; p<0.05)$ and $\mathrm{PD}_{\max }(4.8 \mathrm{~mm} ; p<0.05)$ were found. Changes in $\mathrm{HD}_{\max }$ were found for both in-hand ( $7.8 \mathrm{~mm} ; p<0.05$ ) and ridden ( $9.3 \mathrm{~mm} ; p<0.05$ ) trot, where there was only a small change in $\mathrm{PD}_{\max }$ while ridden $(2.6 \mathrm{~mm}$; $p<0.05$ ). At the walk, $\mathrm{HD}_{\max }$ only changed in-hand 15.8 mm ; $p<0.05$ ), and there was no change in $\mathrm{PD}_{\max }$. Furthermore, after lameness induction $W D_{\text {min }}$ increased for in-hand ( 5.5 mm ) and ridden ( 4.4 mm ; $p<0.05$ ) trot and ridden walk ( 3.8 mm ; $p<0.05$ ). $\mathrm{WD}_{\max }$ changed with similar magnitude for both in-hand ( $4.4 \mathrm{~mm} ; p<0.05$ ) and ridden ( $4.7 \mathrm{~mm} ; p<0.05$ ) trot, though in-hand, the horses became more asymmetrical towards the lame limb, whereas while ridden, the asymmetry remained small but changed to the other side. At tölt, $W_{\text {max }}$ increased ( 3.4 mm ; $p<0.05$ ). During walk and trot, both in hand and ridden, the head's vertical range of motion ( ROMz ) increased after lameness induction, though more in-hand than ridden conditions. Withers ROMz increased in ridden walk ( 2.6 mm ; $p<0.05$ ) but decreased at ridden trot ( $4.4 \mathrm{~mm} ; p<0.05$ ), and pelvis ROMz increased at ridden trot ( $2.9 \mathrm{~mm} ; p<0.05$ ). At tölt, there were no changes before and after lameness induction in the head, withers and pelvis ROMz.
TABLE 2 In-hand walk and trot linear mixed models results.

| Variable | Units | Walk |  |  | Trot |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Baseline | Induction | Difference | Baseline | Induction | Difference |
| Kinematic |  |  |  |  |  |  |  |
| $H D_{\text {min }}$ | mm | 1.1 [-4.8 to 7.0] | -17.7 [ -23.8 to -11.6 ] | -18.8* | -7.2 [ -14.3 to -0.1 ] | -25.3 [-32.7 to -18.0] | -18.14* |
| $\mathrm{WD}_{\text {min }}$ | mm | 0.0 [ -2.9 to 2.8] | 0.2 [ -2.8 to 3.3] | 0.27 | 0.8 [-1.4 to 3.0] | -4.7 [-7.0 to -2.4] | -5.50* |
| $P \mathrm{P}_{\text {min }}$ | mm | -4.7 [ -7.6 to -1.7 ] | 0.1 [ -3.2 to 3.4] | 4.77* | 0.4 [-2.7 to 3.4] | -3.8 [-6.9 to -0.6] | -4.13* |
| $H D_{\text {max }}$ | mm | 0.8 [-2.4 to 3.9] | 6.5 [3.1 to 10.0] | 5.75* | -8.1 [-12.7 to -3.5] | -15.9 [-20.8 to -11.0] | -7.81* |
| $W D_{\text {max }}$ | mm | 2.8 [1.1 to 4.5] | 1.4 [ -0.4 to 3.3] | $-1.41^{*}$ | $-2[-4.5$ to 0.6$]$ | -6.4 [-9.1 to -3.7] | -4.43* |
| $\mathrm{PD}_{\text {max }}$ | mm | -1.9 [-4.1 to 0.3] | -1.2 [-3.4 to 1.1] | 0.74 | -3.9 [-7.5 to -0.2] | -1.6 [-5.4 to 2.2] | 2.29* |
| Head ROMz | mm | 80.4 [72.0 to 88.8] | 85.4 [76.7 to 94.1] | 4.97* | 59.1 [ 50.6 to 67.6] | 68.3 [ 59.6 to 77.0] | 9.20* |
| Withers ROMz | mm | 42.2 [36.3 to 48.2] | 45.6 [39.4 to 51.7] | 3.33* | 53.6 [48.1 to 59.0] | 51.0 [ 45.5 to 56.5] | -2.59* |
| Pelvis ROMz | mm | 69.6 [62.7 to 76.5] | 69.2 [62.2 to 76.3] | -0.33 | 53.4 [46.7 to 60.0] | 54.9 [48.2 to 61.5] | 1.48 |
| Temporal |  |  |  |  |  |  |  |
| Stance duration LF | $s$ | 0.54 [0.53 to 0.56] | 0.56 [0.54 to 0.58] | 0.02* | 0.25 [ 0.25 to 0.26] | 0.28 [0.27 to 0.29] | 0.03* |
| Stance duration RF | s | 0.52 [0.50 to 0.54] | 0.55 [0.53 to 0.58] | 0.03* | 0.24 [0.23 to 0.25] | 0.26 [0.25 to 0.27] | 0.02* |
| Stance duration LH | s | 0.51 [0.49 to 0.53] | 0.54 [0.51 to 0.56] | 0.02* | 0.23 [0.23 to 0.24] | 0.24 [0.24 to 0.25] | 0.01* |
| Stance duration RH | 5 | 0.50 [0.47 to 0.52] | 0.53 [0.50 to 0.55] | 0.03* | 0.24 [0.23 to 0.24] | 0.25 [0.24 to 0.25] | 0.01* |
| Lame diagonal dissociation | \%StrD | 27.1 [26.0 to 28.2] | 26.9 [25.8 to 28.1] | -0.17 | 1.9 [0.9 to 2.9] | 3.4 [2.4 to 4.4] | 1.47* |
| Lame side dissociation | \%StrD | 22.9 [21.6 to 24.2] | 22.6 [21.3 to 24.0] | -0.22 | 46.7 [45.5 to 47.9] | 45.0 [ 43.8 to 46.2] | -1.69* |
| Sound diagonal dissociation | \%StrD | 27.5 [26.4 to 28.7] | 27.8 [26.6 to 29.0] | 0.27 | 4.1 [3.1 to 5.1] | 5.9 [4.8 to 6.9] | 1.77* |
| Sound side dissociation | \%StrD | 22.7 [21.8 to 23.6] | 22.9 [22.0 to 23.8] | 0.15 | 46.9 [46.2 to 47.7] | 45.3 [44.5 to 46.1] | $-1.60^{*}$ |
| LF on | \%StrD | 22.9 [21.5 to 24.3] | 22.8 [21.3 to 24.2] | -0.10 | 46.8 [ 45.5 to 48.0] | 45.0 [ 43.7 to 46.3] | $-1.76{ }^{*}$ |
| RF on | \%StrD | 72.9 [71.7 to 74.1] | 72.7 [71.4 to 73.9] | -0.24 | 95.8 [94.6 to 96.9] | 93.7 [92.6 to 94.8] | $-2.03^{*}$ |
| RH on | \%StrD | 49.6 [49.2 to 50.1] | 49.3 [48.8 to 49.8] | -0.34* | 49.1 [48.5 to 49.6] | 48.9 [48.3 to 49.4] | -0.20 |
| Transition RF to LF | \%StrD | -12.8 [-13.5 to -12.2] | -13.0 [-13.7 to -12.3] | -0.19 | 5.3 [4.2 to 6.5] | 3.8 [2.6 to 5.0] | -1.54* |
| Transition LF to RF | \%StrD | -12.5 [-13.4 to -11.7] | -13.1 [-14 to -12.2] | -0.56 | 4.5 [3.3 to 5.6] | 2.0 [0.9 to 3.2] | $-2.44^{*}$ |
| Transition RH to LH | \%StrD | -12.4 [-13.4 to -11.4] | -12.1 [-13.1 to -11.1] | 0.33 | 6.6 [5.3 to 7.8] | 6.1 [4.8 to 7.4] | -0.43* |
| Transition LH to RH | \%StrD | -12.3 [-12.7 to -11.9] | -12.7 [-13.2 to -12.3] | -0.44* | 6.3 [5.3 to 7.3] | 5.1 [4.0 to 6.1] | -1.25 * |

 $95 \%$ confidence interval [lower border to upper border].

TABLE 3 Ridden walk, trot and tölt linear mixed models results.

| Variable | Units | Walk |  |  | Trot |  |  | Tölt |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Baseline | Induction | Difference | Baseline | Induction | Difference | Baseline | Induction | Difference |
| Kinematic |  |  |  |  |  |  |  |  |  |  |
| $H D_{\text {min }}$ | mm | 2.7 [ -2.5 to 7.9 ] | -7.1 [-12.3 to -1.9] | -9.74* | -4.8[-9.4 to -0.3] | -28.8 [-33.5 to -24.0 ] | -23.92 * | -3.0 [-7.2 to 1.2] | -4.1 [-8.3 to 0.1] | -1.09 |
| $W D_{\text {min }}$ | mm | 1.3 [ -0.5 to 3.1] | -2.5 [-4.3 to -0.7 ] | -3.76* | -1.5 [-3.5 to 0.5] | -5.9 [-8.0 to -3.7 ] | -4.37* | -0.2 [-2.0 to 1.5] | -1.1 [-2.9 to 0.7] | -0.85 |
| $P \mathrm{P}_{\text {min }}$ | mm | -4.9 [-8.1 to -1.8] | -2.0 [ -5.2 to 1.2] | 2.91* | 1.6 [-1.6 to 4.8] | -1.3 [-4.6 to 1.9] | -2.94* | -0.1 [ -3.0 to 2.9] | -1.3 [-4.2 to 1.7] | -1.21 |
| $\mathrm{HD}_{\text {max }}$ | mm | 1.1 [ -2.2 to 4.5] | 0.8 [-2.5 to 4.2] | -0.31 | -3.8 [-10.0 to 2.4] | -13.1 [-19.6 to -6.6 ] | -9.28* | 1.5 [ -3.2 to 6.3] | 12.7 [7.9 to 17.6] | 11.19* |
| $W D_{\text {max }}$ | mm | 2.0 [1.0 to 3.0] | 1.8 [0.8 to 2.8] | -0.16 | -2.2 [-4.6 to 0.2] | 2.5 [0.0 to 5.0] | 4.70* | 2.8 [1.0 to 4.6] | 6.2 [4.4 to 8.1] | 3.37* |
| $\mathrm{PD}_{\text {max }}$ | mm | -2.7 [-5.0 to -0.3] | -2.4 [ -4.8 to 0.0] | 0.26 | -0.2 [-3.2 to 2.8] | 2.4 [ -0.7 to 5.4] | 2.56* | -3.0 [-5.7 to -0.2] | -7.9 [-10.7 to -5.0] | $-4.88{ }^{*}$ |
| Head ROMz | mm | 56.3 [48.7 to 63.8] | 57.8 [50.2 to 65.4] | 1.54 | 75.0 [67.1 to 82.9] | 84.9 [76.8 to 93] | 9.90* | 77.9 [70.5 to 85.2] | 75.4 [68 to 82.8] | -2.48 |
| Withers ROMz | mm | 21.4 [18.5 to 24.3] | 24 [21.1 to 27.0] | 2.63* | 42.4 [39.5 to 45.2] | 37.9 [35.0 to 40.9] | -4.43* | 41.2 [38.5 to 43.8] | 39.7 [37.1 to 42.4] | -1.43 |
| Pelvis ROMz | mm | 56.5 [51.6 to 61.3] | 56.4 [ 51.5 to 61.2] | -0.11 | 48.0 [43.1 to 52.9] | 50.9 [46.0 to 55.8] | 2.86* | 38.1 [33.3 to 42.9] | 40.5 [35.7 to 45.3] | 2.42 * |
| Temporal |  |  |  |  |  |  |  |  |  |  |
| Stance duration LF | s | 0.54 [0.52 to 0.55] | 0.56 [0.55 to 0.58] | 0.03* | 0.23 [0.22 to 0.24] | 0.23 [0.22 to 0.24] | 0.00 | 0.23 [0.22 to 0.24] | 0.25 [0.24 to 0.26] | 0.02* |
| Stance duration RF | $s$ | 0.51 [0.50 to 0.53] | 0.54 [0.53 to 0.56] | 0.03* | 0.21 [0.20 to 0.22] | 0.22 [0.20 to 0.23] | 0.01 | 0.23 [0.22 to 0.24] | 0.23 [0.22 to 0.24] | 0.008* |
| Stance duration LH | $s$ | 0.55 [0.53 to 0.57] | 0.58 [0.56 to 0.60] | 0.03* | 0.23 [0.22 to 0.24] | 0.23 [0.23 to 0.24] | 0.005 | 0.26 [0.25 to 0.27] | 0.27 [0.26 to 0.28] | 0.01 |
| Stance duration RH |  | 0.55 [0.53 to 0.57] | 0.57 [0.55 to 0.59] | 0.02* | 0.22 [0.21 to 0.22] | 0.22 [0.22 to 0.23] | 0.009* | 0.25 [0.24 to 0.26] | 0.25 [0.24 to 0.26] | 0.00 |
| Lame diagonal dissociation | \%StrD | 26.1 [24.2 to 28.0] | 26.1 [24.2 to 27.9] | -0.06 | 7.2 [3.8 to 10.6] | 5.9 [3.3 to 8.5] | -1.37 | 31.4 [29.5 to 33.4] | 31.9 [29.9 to 33.8] | 0.41 |
| Lame side dissociation | \%StrD | 23.3 [21.5 to 25.0] | 22.7 [20.9 to 24.5] | -0.54 | 43.2 [40.8 to 45.7] | 45.7 [43.3 to 48.2] | 2.49* | 17.8 [16.0 to 19.5] | 16.8 [15.0 to 18.5] | -1.00* |
| Sound diagonal dissociation | \%StrD | 26.9 [25.5 to 28.2] | 28.2 [26.8 to 29.5] | 1.30* | 7.4 [5.8 to 8.9] | 5.8 [4.4 to 7.2] | $-1.54 *$ | 33.2 [31.9 to 34.6] | 35.4 [34.0 to 36.7] | 2.17* |
| Sound side dissociation | \%StrD | 23.5 [21.9 to 25.2] | 22.7 [21.1 to 24.3] | -0.85* | 43.1 [41.3 to 44.9] | 42.0 [40.3 to 43.8] | -1.05 | 17.4 [15.8 to 18.9] | 15.9 [14.3 to 17.5] | $-1.44^{*}$ |
| LF on | \%StrD | 23.0 [21.2 to 24.7] | 22.5 [20.8 to 24.3] | -0.42 | 45.2 [43.0 to 47.4] | 46.6 [ 44.4 to 48.7] | 1.38 | 18.0 [16.2 to 19.7] | 16.9 [15.1 to 18.6] | -1.09* |
| RF on | \%StrD | 73.5 [72.2 to 74.7] | 71.8 [70.5 to 73.0] | $-1.71^{*}$ | 93.9 [92.7 to 95.2] | 94.5 [93.3 to 95.8] | 0.59 | 66.9 [65.7 to 68.2] | 64.4 [63.1 to 65.6] | $-2.58{ }^{*}$ |
| RH on | \%StrD | 50.0 [49.6 to 50.4] | 49.4 [49.0 to 49.8] | $-0.57 *$ | 50.7 [ 50.2 to 51.2] | 50.6 [ 50.0 to 51.1] | -0.14 | 49.5 [49.1 to 50] | 48.9 [48.4 to 49.4] | $-0.64^{*}$ |
| Transition RF to LF | \%StrD | -13.2 [-14.6 to -11.8] | -13.2 [-14.6 to -11.8] | -0.01 | 2.9 [1.3 to 4.5] | 2.8 [1.2 to 4.3] | -0.14 | 0.1 [-1.4 to 1.6] | 1.0 [ -0.5 to 2.5] | 0.85* |
| Transition LF to RF | \%StrD | -8.8 [-10.4 to -7.3] | -11.1 [-12.6 to -9.5 ] | $-2.24 *$ | 4.4 [2.8 to 6] | 2.5 [0.9 to 4.1] | -1.86* | 1.8 [0.3 to 3.4] | -1.5 [ -3.1 to 0.0 ] | $-3.37^{*}$ |
| $\begin{aligned} & \text { Transition RH to } \\ & \text { LH } \end{aligned}$ | \%StrD | -15.7 [-17.0 to -14.4] | -15.1 [-16.4 to -13.8] | 0.58 | 0.1 [-1.3 to 1.6] | -1.6 [-3.0 to -0.1] | -1.68 * | -6.6 [-8.0 to -5.2] | $-5.9[-7.2$ to -4.5$]$ | 0.71 |
| Transition LH to RH RH | \%StrD | -12.9 [-14.2 to -11.6] | -12.9 [-14.1 to -11.6] | 0.04 | 2.9 [1.6 to 4.2] | 1.4 [0.1 to 2.7] | -1.48 * | $-5.2[-6.5$ to -4 ] | $-6.8[-8.0$ to -5.5 ] | -1.53 * | 95\% confidence interval [lower border to upper border].

 ${ }^{*} p<0.01$.


FIGURE 3 Graphical representation of linear mixed model results for upper body asymmetry during ridden trials. Linear mixed model results (estimated marginal means and confidence intervals) of the upper body symmetry values at the ridden walk, trot and tölt, before (green triangle) and after (red circle) lameness induction. Upper body asymmetry values of the head $(H)$, withers $(W)$ and pelvis ( $P$ ) are given in mm. Both the difference between the two vertical displacement minima $\left(H D_{\min }, W D_{\min }\right.$ and $\left.P D_{\min }\right)$ and between the vertical displacement maxima $\left(H D_{\max }\right.$, $W D_{\max }$ and $P D_{\text {max }}$ ) are shown. The data represent a group ( $n=9$ ) estimated marginal means (EMmeans) with the $95 \%$ confidence interval as crosshairs.

## 3.2 | Temporal parameters

The effects of induced lameness on temporal stride parameters for each gait are summarised in Figure 4, and statistical results can be found in Table 2 for in-hand conditions and Table 3 for ridden conditions. At tölt, significant changes ( $p<0.05$ ) in temporal stride parameters include an increase in sound diagonal dissociation ( $2.2 \% \mathrm{StrD}$ ) and a decrease in sound side dissociation ( $-1.4 \% \mathrm{StrD}$ ), resulting in a lower percentage of TT strides after lameness induction ( $30.7 \%$ at baseline, 20.1\% after induction). This is a result of an earlier landing of the sound forelimb (Figure 4) ( $-2.6 \%$ StrD; $p<0.05$ ), with no relevant changes in the footfall timings of the other limbs. Also, at the ridden walk ( $-1.7 \%$ StrD; $p<0.05$ ) and at in-hand trot ( $-2.0 \%$ StrD; $p<0.05$ ), the sound forelimb lands earlier relative to the stride cycle. This results in significant changes ( $p<0.05$ ) in sound diagonal dissociation (1.8\%StrD and $1.5 \%$ StrD) and sound side dissociation ( $-1.6 \%$ StrD and $-1.5 \%$ StrD) at in-hand and ridden trot, respectively, but not at walk. This earlier landing of the sound forelimb is also represented in the significant ( $p<0.05$ ) differences in the transition times from lame forelimb to sound forelimb, which decreases with $-2.2 \%$ StrD, $-1.9 \%$ StrD and $-3.4 \%$ StrD for ridden walk, trot and tölt, respectively, and with $-0.6 \%$ StrD and $-2.4 \%$ StrD for in-hand walk and trot.

## 3.3 | Results of splitting the tölt

When comparing the effects of lameness between the different types of tölt, upper body vertical asymmetry for head, withers and pelvis changed the least for strides labelled as TT , except for $\mathrm{HD}_{\text {min }}(6.8 \mathrm{~mm}$
[ $p<0.05$ ], 0.5 mm and 0.2 mm [n.s.] for TT, TLC and TMix, respectively) and $W D D_{\text {min }}(4.3,2.1[p<0.05]$ and 3.1 mm [n.s.] for TT , TLC and TMix, respectively). Changes were larger for TLC and TMix strides in $\mathrm{HD}_{\text {max }}$ ( 14.1 mm for TLC and 16.3 mm TMix; $p<0.05$ ) and $\mathrm{PD}_{\text {max }}$ ( 8.1 mm for TLC and 8.6 mm for TMix; $p<0.05$ ) compared to TT strides ( 7.3 mm for $\mathrm{HD}_{\max }$ and 6.3 mm for $\mathrm{PD}_{\max } ; p<0.05$ ). The other effects of induced forelimb lameness during the different types of tölt (TT, TLC and TMix) are summarised in Table S1 and Figure S1.

## 3.4 | Sensitivity and specificity

The sensitivity and specificity results can be found in Table 4 and were also plotted in ROC curves (Figures S2-S4). For walk and trot, both in-hand and ridden, $\mathrm{HD}_{\text {min }}$ is the most sensitive and specific variable to discriminate between baseline and induction. $W D_{\text {min }}$ seems to be a relevant variable only in in-hand (not ridden) trot (specificity $=0.77$, sensitivity $=0.66$ ). For tölt, kinematic variables have either low specificity, low sensitivity or both. For temporal variables, the transition time from LF to RF seems to be most sensitive for tölt and in-hand walk, whereas right-side dissociation seems to be most sensitive and specific for in-hand trot.

## 4 | DISCUSSION

This is the first study that describes the effect of induced forelimb lameness on the gait kinematics of Icelandic horses, presenting both upper body asymmetry and stride temporal parameters. Our results

(B) Trot, in hand

(C) Walk, ridden

(D) Trot, ridden

(E) Tölt, ridden


FIGURE 4 Graphical representation of the temporal changes. Footfall patterns during (A) walk in-hand, (B) trot in-hand, (C) ridden walk, (D) ridden trot and (E) ridden tolt, before (filled) and after (shaded) lameness induction of the left front (LF) limb. Significant changes in the footfall of the right front (RF) limb are displayed with the grey hatched area, which relates to the significant changes in diagonal dissociation (DD) (solid lines) and side dissociation (SD) (dashed lines). Black lines represent the baseline DD and SD, whereas grey lines represent DD and SD after induction. The data represents the group ( $n=10$ for in-hand trials and $n=9$ for ridden trials) mean footfall patterns.
TABLE 4 Receiver operating characteristic (ROC) analyses results for discrimination between sound and lame.

|  | Units |  | Walk in-hand |  |  |  | Trot in-hand |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Variable |  |  | Threshold | Specificity |  | Sensitivity | Threshold | Specificity |  | Sensitivity |
| Kinematic |  |  |  |  |  |  |  |  |  |  |
| $\mathrm{HD}_{\text {min }}$ |  | mm | -7.32 | 0.74 [0.67 to 0.81] |  | 0.71 [0.63 to 0.80] | -24.49 | 0.93 | [ 89 to 0.97] | 0.64 [0.56 to 0.73] |
| $W D_{\text {min }}$ |  | mm | 0.69 | 0.59 [0.50 to 0.67] |  | 0.47 [0.38 to 0.56] | -4.15 | 0.77 | [ 70 to 0.84] | 0.66 [0.57 to 0.74] |
| $\mathrm{PD}_{\text {min }}$ |  | mm | -0.56 | 0.57 [0.49 to 0.65] |  | 0.58 [0.49 to 0.67] | -4.61 | 0.73 [0. | [ 65 to 0.80] | 0.49 [0.40 to 0.58] |
| $H D_{\text {max }}$ |  | mm | 21.01 | 0.93 [0.88 to 0.97] |  | 0.19 [0.13 to 0.27] | -11.06 | 0.72 [ | [ 64 to 0.80] | 0.58 [0.48 to 0.67] |
| $\mathrm{WD}_{\text {max }}$ |  | mm | -3.55 | 0.84 [0.77 to 0.90] |  | 0.29 [0.21 to 0.36] | -8.16 | 0.87 | [ 81 to 0.92] | 0.42 [0.33 to 0.51] |
| PD ${ }_{\text {max }}$ |  | mm | -3.48 | 0.67 [0.60 to 0.75] |  | 0.39 [0.31 to 0.48] | 10.12 | 0.93 | [ 89 to 0.97] | 0.33 [0.24 to 0.41] |
| Temporal |  |  |  |  |  |  |  |  |  |  |
| Lame diagonal dissociation |  | s | 25.78 | 0.84 [0.78 to 0.90] |  | 0.36 [0.28 to 0.46] | 2.88 | 0.65 | . 57 to 0.73] | 0.74 [0.66 to 0.82] |
| Lame side dissociation |  | s | 24.72 | 0.93 [0.87 to 0.96] |  | 0.21 [0.13 to 0.28] | 46.04 | 0.67 | [. 59 to 0.74] | 0.69 [0.60 to 0.77] |
| Sound diagonal dissociation |  | s | 24.82 | 0.91 [0.86 to 0.95] |  | 0.18 [0.11 to 0.25] | 4.51 | 0.68 | [0.60 to 0.76] | 0.75 [0.67 to 0.83] |
| Sound side dissociation |  | $s$ | 23.49 | 0.75 [0.67 to 0.82] |  | 0.41 [0.33 to 0.50] | 46.96 | 0.57 | [ 49 to 0.65] | 0.95 [0.91 to 0.98] |
| LF on |  | \%StrD | 48.65 | 0.85 [0.79 to 0.91] |  | 0.36 [0.27 to 0.44] | 50.33 | 0.87 | [ .81 to 0.93] | 0.30 [0.22 to 0.38] |
| RF on |  | \%StrD | 24.89 | 0.93 [0.89 to 0.97] |  | 0.22 [ 0.15 to 0.30] | 45.88 | 0.70 [0. | [ 63 to 0.78] | 0.65 [0.56 to 0.74] |
| RH on |  | \%StrD | -26.94 | 0.72 [0.63 to 0.79] |  | 0.37 [0.29 to 0.46] | -4.58 | 0.69 [0. | [ 60 to 0.76] | 0.75 [0.68 to 0.83] |
| Transition RF to LF |  | \%StrD | -13.99 | 0.76 [0.68 to 0.83] |  | 0.33 [0.25 to 0.42] | 8.74 | 0.48 | [ 40 to 0.57] | 0.80 [0.72 to 0.87] |
| Transition LF to RF |  | \%StrD | -11.30 | 0.31 [0.23 to 0.40] |  | 0.88 [0.82 to 0.94] | 5.56 | 0.77 | [ 69 to 0.84] | 0.65 [0.57 to 0.74] |
| Transition RH to LH |  | \%StrD | -10.76 | 0.90 [0.85 to 0.95] |  | 0.28 [0.20 to 0.36] | 10.07 | 0.53 [ | . 45 to 0.62] | 0.73 [0.65 to 0.81] |
| Transition LH to RH |  | \%StrD | -13.27 | 0.78 [0.71 to 0.85] |  | 0.41 [0.32 to 0.50] | 8.88 | 0.55 | . 47 to 0.64] | 0.58 [0.48 to 0.65] |
|  | Units | Walk ridden |  |  | Trot ridden |  |  | Tölt ridden |  |  |
| Variable |  | Threshold | Specificity | Sensitivity | Threshold | Specificity | Sensitivity | Threshold | Specificity | Sensitivity |
| Kinematic |  |  |  |  |  |  |  |  |  |  |
| $H D_{\text {min }}$ | mm | -0.24 | 0.57 [0.49 to 0.65] | 0.65 [0.58 to 0.73] | -13.88 | 0.80 [0.72 to 0.87] | 0.79 [0.71 to 0.87] | 45.74 | 0.09 [0.05 to 0.14] | 0.98 [0.96 to 1.0] |
| $\mathrm{WD}_{\text {min }}$ | mm | -2.64 | 0.84 [0.77 to 0.91] | 0.45 [0.37 to 0.54] | -8.09 | 0.85 [0.79 to 0.91] | 0.43 [0.33 to 0.52] | -3.92 | 0.74 [0.66 to 0.82] | 0.43 [0.35 to 0.52] |
| $\mathrm{PD}_{\text {min }}$ | mm | -0.21 | 0.71 [0.64 to 0.79] | 0.49 [0.41 to 0.57] | -6.45 | 0.88 [0.82 to 0.94] | 0.32 [0.24 to 0.41] | -11.03 | 0.12 [0.07 to 0.18] | 0.93 [0.89 to 0.97] |
| $H_{\text {max }}$ | mm | 0.81 | 0.57 [0.48 to 0.65] | 0.51 [0.44 to 0.59] | -24.94 | 0.86 [0.80 to 0.92] | 0.38 [0.29 to 0.48] | -5.67 | 0.47 [0.39 to 0.56] | 0.77 [0.71 to 0.85] |
| $\mathrm{WD}_{\text {max }}$ | mm | -0.32 | 0.48 [0.38 to 0.57] | 0.66 [0.58 to 0.74] | -2.99 | 0.48 [0.39 to 0.58] | 0.78 [0.69 to 0.86] | 9.28 | 0.72 [0.65 to 0.80] | 0.49 [0.40 to 0.57] |
| PD ${ }_{\text {max }}$ | mm | -0.09 | 0.75 [0.68 to 0.82] | 0.40 [0.32 to 0.48] | 2.6 | 0.76 [0.69 to 0.84] | 0.51 [0.42 to 0.60] | 3.91 | 0.26 [0.19 to 0.34] | 0.87 [0.81 to 0.92] |
| Temporal |  |  |  |  |  |  |  |  |  |  |
| Lame diagonal dissociation | s | 26.92 | 0.64 [0.56 to 0.72] | 0.49 [0.40 to 0.56] | 4.62 | 0.79 [0.71 to 0.87] | 0.36 [0.26 to 0.45] | 31.96 | 0.55 [0.46 to 0.63] | 0.74 [0.66 to 0.81] |
| Lame side dissociation | 5 | 26.23 | 0.30 [0.22 to 0.38] | 0.86 [0.80 to 0.91] | 45.04 | 0.82 [0.75 to 0.88] | 0.36 [0.28 to 0.46] | 14.64 | 0.82 [0.76 to 0.89] | 0.47 [0.39 to 0.56] |
| Sound diagonal dissociation | s | 25.42 | 0.50 [0.41 to 0.59] | 0.73 [0.66 to 0.80] | 5.82 | 0.90 [0.84 to 0.95] | 0.40 [0.31 to 0.50] | 37.1 | 0.87 [0.81 to 0.92] | 0.36 [0.28 to 0.44] |

TABLE 4 (Continued)

|  |  | Walk ridden |  |  | Trot ridden |  |  | Tölt ridden |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Variable | Units | Threshold | Specificity | Sensitivity | Threshold | Specificity | Sensitivity | Threshold | Specificity | Sensitivity |
| Sound side dissociation | s | 26.35 | 0.39 [0.30 to 0.47] | 0.81 [0.75 to 0.87] | 43.8 | 0.95 [0.91 to 0.98] | 0.31 [0.23 to 0.40] | 16.22 | 0.64 [0.56 to 0.72] | 0.67 [0.59 to 0.75] |
| LF on | \%StrD | 49.36 | 0.66 [0.58 to 0.74] | 0.52 [0.44 to 0.61] | 48.95 | 0.78 [0.70 to 0.85] | 0.39 [0.30 to 0.49] | 48.97 | 0.56 [0.48 to 0.65] | 0.49 [0.41 to 0.58] |
| RF on | \%StrD | 23.54 | 0.55 [0.46 to 0.63] | 0.61 [0.53 to 0.69] | 45.04 | 0.8 [0.72 to 0.87] | 0.34 [0.25 to 0.43] | 14.56 | 0.81 [0.74 to 0.87] | 0.47 [0.38 to 0.55] |
| RH on | \%StrD | -25.82 | 0.57 [0.48 to 0.66] | 0.68 [0.60 to 0.75] | -6.35 | 0.96 [0.91 to 0.99] | 0.32 [0.23 to 0.41] | -33.84 | 0.54 [0.45 to 0.62] | 0.68 [0.59 to 0.75] |
| Transition RF to LF | \%StrD | -13.69 | 0.67 [0.59 to 0.76] | 0.49 [0.40 to 0.57] | 7.75 | 0.45 [0.35 to 0.54] | 0.70 [0.60 to 0.80] | 3.99 | 0.60 [0.52 to 0.67] | 0.82 [0.76 to 0.88] |
| Transition LF to RF | \%StrD | -11.04 | 0.42 [0.33 to 0.50] | 0.87 [0.81 to 0.92] | 3.72 | 0.96 [0.93 to 0.99] | 0.46 [0.37 to 0.56] | 2.53 | 0.56 [0.47 to 0.65] | 0.91 [0.86 to 0.95] |
| Transition RH to LH | \%StrD | -16.28 | 0.40 [0.31 to 0.49] | 0.80 [0.73 to 0.86] | 7.98 | 0.60 [0.51 to 0.69] | 0.67 [0.57 to 0.76] | 2.3 | 0.25 [0.17 to 0.33] | 0.96 [0.93 to 0.99] |
| Transition LH to RH | \%StrD | -15.23 | 0.52 [0.43 to 0.61] | 0.60 [0.52 to 0.68] | 6.44 | 0.85 [0.77 to 0.91] | 0.57 [0.47 to 0.66] | -0.85 | 0.43 [0.34 to 0.52] | 0.94 [0.90 to 0.98] |

Note: The threshold with corresponding specificity and sensitivity are given for all variables, both kinematic and temporal for in-hand (A) and ridden (B) trials. The 'Youden index' method was used and the values represent the
 upper border].
Abbreviations: LF, left forelimb; LH, left hindlimb; RF, right forelimb; RH, right hindlimb; \%StrD, percentage of the stride duration (calculated from LH-on to LH-on moments).
support our hypothesis that Icelandic horses show compensatory mechanisms to induce forelimb lameness, but these differ between the studied gaits. Both walk and trot, as well as tölt, were included in the study to better understand lameness adaptations in both walking and running gaits. This was particularly interesting since, based on empirical evidence, orthopaedic veterinarians often state that Icelandic horses can have problems performing trot during lameness exams. Other gaits, such as pace and canter, were not included in our study for safety reasons. Previous attempts to quantify lameness in canter have failed, mainly due to the canter being an asymmetrical gait, which does not allow for the calculation of asymmetry variables. ${ }^{25}$

As expected, at walk and trot, both in hand and ridden, vertical movement asymmetry of the head increased. The absolute magnitude of the head vertical movement asymmetry was larger at trot compared to walk, which is consistent with previous forelimb lameness studies in warmblood horses. ${ }^{5}$ Furthermore, withers' vertical movement asymmetry $\left(W D_{\text {min }}\right)$ changed in the same direction as $H D_{\text {min }}$ at in-hand trot, which is consistent with findings in lame warmblood horses with forelimb lameness. ${ }^{22}$ Based on a study by Pfau, the vertical movement asymmetries after lameness induction should be large enough to be detectable by veterinarians. ${ }^{26}$ However, that study was performed using warmblood horses and needs to be repeated for smaller breeds such as the Icelandic horse. Nonetheless, Icelandic horses seem to adapt to forelimb lameness in a similar fashion as warmblood horses do at walk and trot in terms of upper body movement asymmetry.

The increase in vertical movement asymmetry of the head, indicating impact lameness $\left(\mathrm{HD}_{\text {min }}\right)$ at the trot and, to a lesser extent, at the walk, was not found at tölt. However, more systematic increases in asymmetry between the maximum positions of the head $\left(H D_{\max }\right)$ and the pelvis $\left(P D_{\max }\right)$ were found, although some horses showed similar values for $\mathrm{HD}_{\max }$ at baseline. The pattern in vertical displacement maxima differences at tölt seems similar, though opposite and with smaller changes, to the pattern in vertical displacement minima differences after forelimb lameness induction at trot. ${ }^{22}$ That is to say, the head and withers asymmetry are pointing towards the same side, whereas the pelvis asymmetry points towards the opposite side. Following lameness induction, a lower maximum head position was reached when the lame limb pushed off from the ground. The lower maximum position of the pelvis occurs when the lame forelimb is in midstance and the ipsilateral hindlimb pushes off. This could indicate that the horse is pitching its body backwards, trying to use the pelvis and back to decrease the loading of the lame forelimb. The increase in pelvis vertical movement asymmetry may confuse the observer in determining the affected limb, as asymmetries of the pelvis are often related to hindlimb lameness. ${ }^{6,8,26}$

Some apparent adaptations to lameness at tölt, and to a lesser extent at walk and trot, seem to occur in the footfall pattern. Our results show that the Icelandic horse tries to decrease the loading of the lame forelimb by advancing the placement (i.e., the earlier timing of the hoof impact) of the sound forelimb. This earlier placement ultimately decreases the transition time from the lame to the sound
forelimb, whereas the transition time from the sound to the lame forelimb remains unaffected. This might be caused by lower peak vertical forces and impulses produced by the lame forelimb. ${ }^{6,7,10,21}$ Similar adaptations have been found in warmblood horses with forelimb lameness at trot, where the suspension from the lame to the sound diagonal was $61 \%$ shorter compared to baseline and not even half as long as the suspension from the sound to the lame diagonal. ${ }^{27}$ Due to the earlier landing of the sound forelimb, the same asymmetry translates into the differences in diagonal dissociation and side dissociation between the sound and the lame side. At trot and tölt, right diagonal dissociation and right-side dissociation, which include the non-lame forelimb, change, while left diagonal dissociation and left side dissociation, which include the lame forelimb, remain unaffected.

The stance duration of both forelimbs increased during all gaits and conditions except for the ridden trot. The increase in stance duration has been described in trot as one of the primary mechanisms horses use to reduce peak GRFz of the lame limb. ${ }^{21}$ As tölt is also considered a running gait, it was not surprising to find increases in stance duration of the lame forelimb after lameness induction in this gait. However, a recent study showed a reduction in stance duration of the lame limb at walk, ${ }^{10}$ which contrasts with our results. Differences between treadmill and overground locomotion may cause this discrepancy, as it is known that in trot, the stance duration of the forelimbs is higher on the treadmill compared to overground conditions. ${ }^{28}$ However, whether this holds true for walk is unknown. Another cause may be that speed could not be perfectly matched between baseline and induction trials in our study. Even though on average speed did not change after lameness induction and stride speed was corrected for in the statistical models, the horses could have compensated by slightly changing the speed between trials. Finally, the increase in stance duration at the walk is likely not a rider effect since stance duration at the walk after lameness induction increased with similar magnitude during both in-hand and ridden conditions.

The increase in upper body vertical movement asymmetry was more considerable during the in-hand conditions compared to ridden conditions, even though the rider was asked to perform a sitting trot and only have light rein contact to reduce the rider's influence in the head movement. It is known that, by posting, riders can increase or decrease vertical movement asymmetry. ${ }^{20,29}$ However, the rider was not expected to affect the movement asymmetry in our study, as the rider stayed seated during all gaits to avoid asymmetrical loading. ${ }^{30,31}$

The horses used for this study were judged as non-lame before inclusion. This soundness was further confirmed by the absence of significant asymmetries at both in-hand and ridden walk and trot during the baseline measurements. Interestingly, substantial upper body vertical movement asymmetries were found in the baseline tölt trials. A possible explanation is that the tölt might be a more complex task for the horse than to walk or to trot. It can be found in the literature that elite dressage horses are more symmetrical at trot compared to passage or piaffe in terms of the vertical centre of mass movement. ${ }^{32}$ Furthermore, it has been shown from human studies that healthy young adults move more asymmetrically in terms of limb kinematics and vertical centre of mass movement when dual tasks need to be performed, thus when a task has increased complexity. ${ }^{33}$ The
increased complexity theory is supported by the notice that Icelandic horses, unless extremely good tölters, do not perform a pure tölt unless ridden, for instance, when they are out in the field or run inhand. These baseline asymmetries at tölt in sound Icelandic horses possibly make it more challenging to examine a horse for lameness and to distinguish between physiological and pain-related movement asymmetries.

Studies have shown that lameness is easier to quantify at a trot compared to walk in warmblood horses. ${ }^{10,34}$ It is suggested that this can be attributed to higher speed and the two-beat nature of the trot, which ultimately increases peak GRFz, resulting in higher levels of discomfort when the lame limb is on the ground. Also, the centre of mass moves more vertically in trot when compared to walk, and this higher vertical centre of mass movement contributes to the higher peak vertical forces on the limbs. ${ }^{10}$ However, with increasing speed, stride frequency increases, resulting in lower visibility of the asymmetries that might be present. ${ }^{2}$ Like trot, tölt also has higher stride speed and frequency compared to walk ${ }^{1,3,11,12,17}$; therefore, higher peak GRFz can be expected. Following this reasoning, we expected to find increased upper body movement asymmetries after lameness induction at tölt. However, this was not found in the same manner as at the walk and trot. In contrast, similar to walk, the tölt has no suspension phase, and for the largest part of the stride cycle, at least two limbs are in contact with the ground. This might make it easier to redistribute weight away from the lame limb. Moreover, Polet showed that the vertical centre of mass motion should decrease with more distributed footfalls during the gait cycle. ${ }^{16}$ This might explain why differences in $H D_{\text {min }}$ and $W D_{\text {min }}$ were not expressed at tölt compared to trot. Although it is still counterintuitive that substantial changes in these parameters are found at walk, but not at tölt after induction.

Since the horses in this study were only measured at tölt while being ridden, we could not discriminate between the effects of lameness and the possible confounding effects between the rider and lameness on the measured parameters at tölt. Substantial differences were observed between in-hand and ridden walk and trot, where movement asymmetries after lameness induction were smaller in ridden conditions. Some orthopaedic veterinarians mention that when Icelandic horses are presented for lameness exams, it is difficult to get them to or keep them in, trot. As a result, they may often show a tölt mixture or a pacy trot. We found that adaptations to lameness may differ between different 'types' of tölt, where the asymmetries were most prominent in this so-called TMix. Movement asymmetries could have been more pronounced if the horses had been tölted in hand, even though the quality of this tölt may have been low. Further research needs to be done with clinical cases to confirm this.

There were some limitations to this study. The first limitation is that we used an acute but reversible sole-pressure lameness induction model, which differs from many pathologies in lame horses. However, when the same lameness induction method on warmbloods was used, ${ }^{22}$ lameness patterns were similar to those seen later in clinical cases with different kinds of pathologies. ${ }^{35}$ Second, only one surface type was investigated, which is known to affect kinematics and stride temporal parameters. ${ }^{36}$ Finally, only subtle/mild lameness was
induced to minimise the discomfort experienced by the horses during this study. Therefore, we could not investigate if different degrees of lameness result in different adaptation strategies.

Understanding the compensation mechanisms for lameness across gaits in the Icelandic horse is essential for a proper lameness diagnosis. Compensatory mechanisms are complex and demonstrate that, in general, kinematic adaptations at tölt are different from those at walk and trot. Furthermore, adaptations seem larger in in-hand compared to ridden conditions. At tölt, as opposed to walk and trot, asymmetry in the vertical movement minima of the head appears less indicative of forelimb lameness, making visual assessment more challenging. Based on the results of this study, it is advised to primarily use the walk and trot for lameness assessments in the Icelandic horse. If a horse is unable to perform trot in-hand, we advise performing the lameness examination while ridden at trot, with the rider performing sitting trot or standing in the stirrups.

## AUTHOR CONTRIBUTIONS

Ineke H. Smit contributed to data analysis and interpretation, and drafting the article. Filipe M. Serra Braganca is responsible for the integrity of the data. Filipe M. Serra Braganca, Elin Hernlund and Marie Rhodin contributed to the conception of the work, data collection, interpretation of the results and critical revision of the article. Filipe M. Serra Braganca and Marie Rhodin contributed equally to the work. Sigridur Björnsdóttir, Helga Gunnarsdottir, Emma PerssonSjodin and Vikingur Gunnarsson contributed to the conception of the work, data collection and revisions of the article. All authors gave final approval of the version to be published.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## PEER REVIEW

The peer review history for this article is available at https:// www.webofscience.com/api/gateway/wos/peer-review/10.1111/ evj. 13998.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at https://doi.org/10.24416/UU01-19THCV.

## ETHICAL ANIMAL RESEARCH

The study was approved by the Icelandic Food and Veterinary Authority MAST (approval number: 2020-02-12).

## INFORMED CONSENT

Informed consent was provided for inclusion of client-owned horses.

## ORCID

Ineke H. Smit (1) https://orcid.org/0000-0002-9269-9634
Elin Hernlund (D) https://orcid.org/0000-0002-5769-3958
Emma Persson-Sjodin (I) https://orcid.org/0000-0002-0331-6970
Sigridur Björnsdóttir (D) https://orcid.org/0000-0001-5521-475X
Marie Rhodin (D) https://orcid.org/0000-0003-0575-2765
Filipe M. Serra Braganca (D) https://orcid.org/0000-0001-8514-7949

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## SUPPORTING INFORMATION

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