# A PILOT STUDY CONCERNING FORELIMB HOOF THREE-DIMENSIONAL RECONSTRUCTIONS AND THE MAPPING OF HOOF GROWTH

Author: Désirée Geukens; Supervisor: Sandra Nauwelaerts

*Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

**ARTICLE INFO**

**Curriculum–** Researsch Traineeship Master

**Keywords–**
Horse
Hooves
3D reconstructions
Hoof growth

**Abbreviations–**
DDFT: deep digital flexor tendon
ICL: inferior check ligament
SDFT: superficial digital flexor tendon
MRI: magnetic resonance imaging
3D: three-dimensional
CT: computed tomography
VCM: virtual computer models

**ABSTRACT**

**Objective–**To investigate the results of a pilot study in making three dimensional reconstructions and the mapping of hoof growth in these models.

**Methods–** From each of the 6 horses (n=6) of various body types videos of the hooves were made during a 6-week trimming/shoeing interval. Reconstructions were made and used for mapping and measuring hoof growth.

**Results–**The software used in this study resulted in usable hoof reconstructions. Hoof growth measured in 55 points in the hoof were reduced to 8 components, consisting of points growing in a similar pattern. The contribution of each component to the total variance differs.

**Conclusions–**The software can be used to create reconstructions of the hooves and these reconstructions can be used for mapping hoof growth. This method was not yet accurate enough to measure exact hoof growth, therefore some improvements must be made in future research.

**Clinical Relevance–**The technique used in this study can be an alternative for current shoeing methods in the future, by 3D printing of individual portable hoof shoes which can be put on and off.

## Introduction

**Anatomy of the hooves–** The equine hoof is a remarkable structure which consists of several layers, each with their own function. The equine hoof wall – consisting of the following three layers: stratum externum, stratum medium, and stratum internum (lamellatum) – and the corium, form the different layers of the equine hoof from the outside to the inside [1] (**Figure 1, 2**). The thickness of the hoof wall varies per region, where the thickness gradually decreases from the toe, where the wall is thickest, to the heel [1].

The outermost external layer – the coverage of the hoof wall – is the stratum externum. The stratum externum comprises a proximally located layer and a distally located layer. The most proximal portion of this layer constitutes the transition from skin to hoof, and is also called 'the periople' (**Figure 1, 4**). The periople covers the coronary band and consists of soft and light-colored non-pigmented tissue which produces the stratum granulosum, a layer of granular cells. The stratum granulosum is resilient – due to keratohyaline, a component stored in the cytoplasmic granules – tough and impermeable. The distal portion of the layer however, contains a lot of lipids which give the equine hoof its smooth and glossy appearance. This layer is called the stratum tectorium, and because of its high-lipid content water loss from the equine hoof is decreased [1].

The majority of the hoof wall is formed from tubular and intertubular horn of the stratum medium, the middle layer [1], [2]. The proximally excavated part of the stratum medium encompasses the coronary groove which is filled with the convex coronary corium. These coronary corium composes elongated dermal papillae, which cross the coronary groove (**Figure 2, 3**), and are surrounded by horned epidermal tubules which arise from the stratum germinativum (germinal epidermis). These corneous epidermal tubules have a hollow medulla – in which the above mentioned dermal

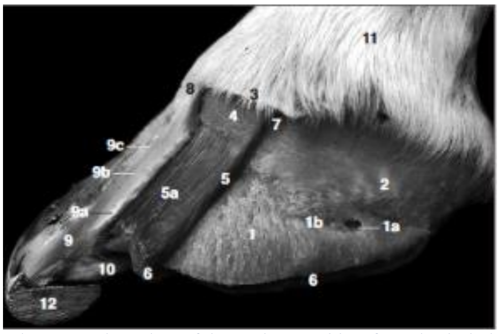


*Figure 1. Dissection of the corium and hoof. 1 Distal phalanx, 1a Foramen of the palmar process, 1b Parietal sulcus, 2 Ungular cartilage, 3 Corium limbi, 4 Corium coronae, 5 Corium parietis, 5a Dermal lamellae, 6 Corium soleae, 7 Pulvinus coronae, 8 Periople, 9 Hoof wall, 9a Stratum internum, 9b Stratum medium, 9c Stratum externum, 10 Sole, 11 Skin, 12 Shoe [3].*

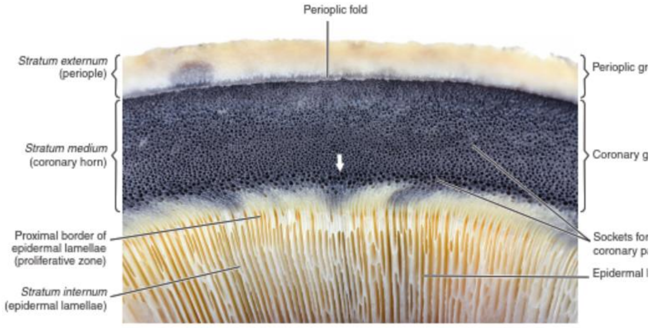


*Figure 2. "The proximal dorsal hoof wall exungulated from a normal, adult horse foot, viewed from the inner, lamellar surface" [4].*


*Figure 3. "Surface anatomy of the dorsal aspect of a normal distal forelimb" [4].*


*Figure 4. "Surface anatomy of the lateral aspect of a normal distal forelimb" [4].*

papillae fit – and a cortex of keratinized cells. The papillae and tubules are parallelly orientated across the long axis, slanting downwards, from the hoof wall. The rest of the stratum medium consist of intertubular horn and lies between the tubules containing a papillae [1]. The main component of this intertubular horn is hard keratin in which the disulphide bonds give the wall its firmness and strength [5], [6]. Keratinocytes lie in the tubules between the papillae, and while maturation of these cells in a right angle to the hoof wall proceeds they become intertubular horn [5]. Cells of the intertubular horn may contain melanin and therefore give a dark pigmented color to the hoof [1].

Reilly (1996) et al. described four distinct zones in the stratum medium from which the subdivision is based on the tubule density. Zone 1 containing the highest tubule density - with a decrease in tubular density when the percentage of hoof wall depth increases - ending with the lowest tubular density in zone 4. Reilly suggested that this pattern of tubule density in the separate zones is responsible for the gradual transmission of load-bearing stresses from the stratum medium to the stratum internum. With zone 1 as a stress-concentrating part of the hoof wall [2]. In addition, this zone is also accountable for preventing cracks from intruding into the more sensitive inward structures of the hoof wall, since the cracks propagate upwards, parallelly with the tubules [2], [6].

The stratum internum (lamellatum) is the innermost layer of the hoof wall. This layer consists of keratinized primary epidermal

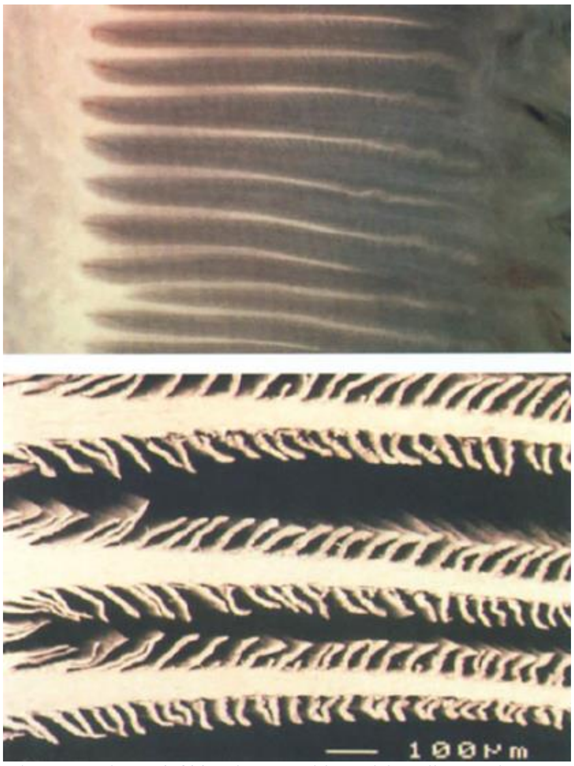


*Figure 5. The 550-600 primary epidermal lamellae, each covered with 100-150 secondary epidermal lamellae. This to increase the surface area [6].*

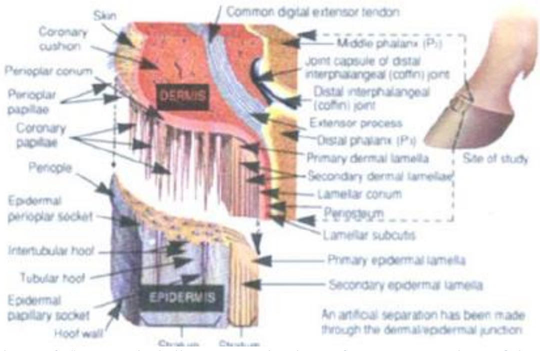


*Figure 6. "Expanded diagrammatic view of coronary region of the equine foot" [1].*

lamellae and non-keratinized secondary epidermal lamellae directed towards the distal phalanx [1], [6]. In total there are about 600 primary lamellae of which one single lamellae contains 100-150 secondary lamellae **(Figure 5)**. These epidermal lamellae connect the more external structures of the hoof with the more internal ones, since they interlock with the several lamellae of the corium. The primary lamellae move downwards from the coronary groove to distally, in 6-8 months. The primary lamellae moves past the stationary cells of the secondary epidermal lamellae – which are fixed to their basement membrane from which the basal cells are important in repairing damage by proliferation - by breaking desmosomes. The desmosomes break open and then reshape their binding whereby the primary lamellae can shift to distal. During this process the hoof is still able to support the load to which the hoof is exposed [1].

The corium (dermis) can be subdivided in a coronary region and a lamellar region. The coronary corium together with the stratum germinativum forms the so-called coronary band. The coronary corium also contains papillae, and these papillae fit into small cavities – the horned epidermal tubules - of the hoof wall. The lamellar corium, however, contains 600 primary dermal lamellae each divided into 100-200 secondary dermal lamellae. These dermal lamellae ensure the connection between the hoof wall and the underlying, more internal, structures of the hoof. The tissue of the corium consists of arteries, veins, capillaries and nerves lying in connective tissue. Therefore the main function of the corium is to provide nourishment to underlying structures and attachment of the distal phalanx to the hoof wall [1].

For a general summarizing picture of all the above mentioned structures, see figure 6 **(Figure 6)**.

**Mechanisms of hoof growth–** Germinal cells, also referred to as the epidermal basal cells, are responsible for hoof growth through the production of daughter cells by mitosis. These cells are located at the coronary band. In this region high rates of mitosis are seen, in accordance with the research of Daradka (2004) et al., which showed that the proximal regions of the hoof, the coronet and most proximal lamellae, revealed the most proliferative activity. Proliferative activity decreased significantly towards the more distal regions of the hoof wall and was absent in the most distal lamellae [7].

The germinal cells find their origin in the stratum germinitivum, a part of the stratum medium, as mentioned above. The produced daughter cells, keratinocytes, cornify during maturation adding new hoof horn to the proximal hoof wall consisting of intertubular and tubular horn. The completely keratinized cells are then called corneocytes. This newly formed hoof horn then continues to move downwards to the ground surface along the axis of the hoof wall in about 8 months, by being pushed away by the production of new cohorts of daughter cells at the basal cell layer. Because of longitudinal ridges of the secondary dermal lamellae, maturing keratinocytes are forced to move down in proximo-distal direction. Mitosis of the germinal cells is a lifelong process since the hooves need replacement due to wear and tear [6].

Hoof growth and the quality of equine hoof horn can be influenced by several external factors [8] such as heredity [9], environment [10]–[12], diet [13]–[16], and farriery [17]–[19].

Another example of a factor that may affect hoof growth are the different seasons. Lewis et al. (2014), has found significant seasonal influences on hoof growth. In the winter the least hoof horn growth was seen. No difference between front and hind hooves was found with regard to hoof growth rates, and also the gender of the horse did not affect this [20].

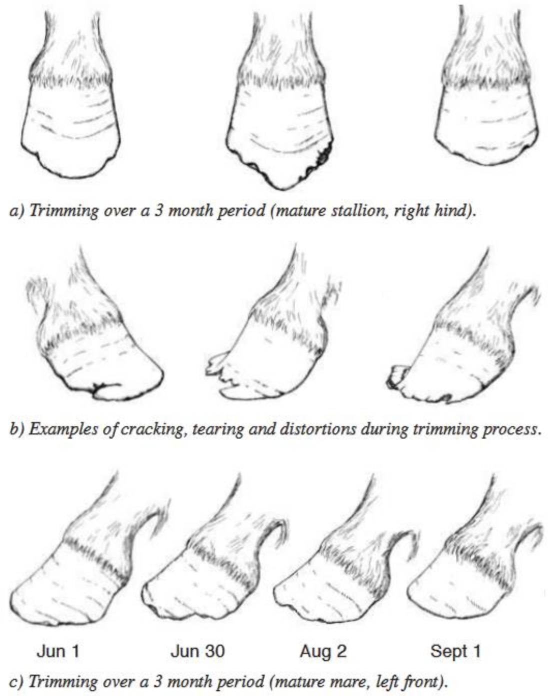


*Figure 7 Illustration of the 'self-trimming' process drawn from photographs [21].*

**Trimming and shoeing–** Trimming and shoeing of domesticated horses is a common phenomenon with regard to hoof care. However, semi-feral ponies seem to maintain proper hoof health care through a mechanism called ‘self-trimming’. Self-trimming provides a natural way of self-maintenance of the equine hoof. This process starts with tearing and cracking of the most distal hoof wall, leaving the edges of the hooves irregular **(Figure 7b)**. Depending on the type of ground on which the animals are walking, it takes a variable amount of time for the hooves to become more smooth and rounded out at the edge **(Figure 7a)**. In summer, when the horses walked on a hard and dry ground surface, it is a three month process **(Figure 7c)** [21].

Despite the fact that horses are in principle self-sustaining with regard to hoof care, domesticated horses however, are often trimmed and shod to improve hoof care. Factors involving hoof care are hoof balance, hoof length and hoof angulation [22]. There are several manners to describe proper hoof balance in horses of which the ‘natural balance’[23] takes into account the natural conformation of the hooves as is seen in (semi)feral ponies (mentioned above). However, this method seems to be unsuitable for the athletic activity we expect from the horse nowadays. Other ways to describe hoof balance are through geometric and dynamic balance. Geometric balance [24] takes into account the symmetry of the hooves in rest and therefore the hooves must be trimmed so that the long axis of the limbs will be perpendicular to the ground surface. However, dynamic balance which focusses on the horse in motion, also implies symmetry but then in the way of landing. The hooves should land symmetrically, which is often not possible [25]–[27]. However, there are several ways to describe and apply hoof balance but, there seems to be no clear evidence for one best method.

Regardless of the fact that trimming and shoeing of domesticated horses are such a common phenomenon these days, both trimming and shoeing can influence hoof growth and health. Regarding new growth of hoof horn, influences are minimal and not significant. However, a slight trend was seen in which barefoot (only trimmed hooves) tended to show the greatest amount of new hoof horn growth in comparison to shod horses. Nevertheless, a trend towards increased toe length in both the front hooves was seen in shod horses. Also the inside heel height turned out to be higher when horses are shod in comparison to unshod (barefoot) hooves [5].

Shoeing of horses can also affect other structures in the limb besides the hooves. Except of the fact that horses are shod to prevent excessive wear and protect the hooves, shoes can also be used as an therapeutic aid [28]. Egg-bar shoes, for example, can be used when there are problems with the deep digital flexor tendon (DDFT) and the inferior check ligament (ICL) [29]. Also changes in the position of joints in the distal leg of a horse can be achieved by elevating the heel or toe. In addition, this also has an effect on the more proximally located structures in the limb and the overall orientation of it. Crevier-denoix (2001) *et al.* demonstrated that elevating the heel leads to flexion of the elbow. This in turn leads to increased tension on the superficial digital flexor tendon (SDFT). However, a slight extension of the elbow is reached by elevating the toe. This might decrease the tension on the SDFT [30]. The use of heel and toe elevation can be advantageous in, for example, podotrochlear syndrome. In clinical settings it can therefore be used by means of orthopaedic shoeing [31].

Besides, general effects of shoeing must also be taken in account. Shoeing horses affects the forelimb swing phase in comparison to unshod horses. It required significant changes in all of the joints of the forelimb in both energy, to generate movement of the forelimb in the swing phase and overcome the extra weight of the shoes, and net joint moments [32].

**Photogrammetry–** To assess, among other things, hoof conformation and the effects of trimming and shoeing on the hooves of a horse several techniques have been used like radiography, magnetic resonance imaging (MRI), digital photography and so on [17], [18], [33]–[35]. However, in this study photogrammetry will be used as a technique for measurements. In photogrammetry, a series of pictures are combined to create a three-dimensional (3D) model.

Labens et al (2013) validated the use of photogrammetry as a technique for calculating the volume of an equine hoof. The aim of their study was to "develop a method for computation of virtual foot models from digital foot images allowing precise and accurate volumetric measurements" [35]. Results were prosperous and the virtual reconstructions could be used for accurate and precise volumetric measurements of the hooves. The accuracy of this method was determined by comparing the virtual computer models (VCM) to computed tomographic (CT) images of the hooves. However, the VCMs from post-trim photogrammetric measurement were deteriorated and les accurate compared to the pre-trim models. The post-trim alterations in foot volume were probably too small to detect using these models, but were detected using CT imaging. They also excluded a possible 'human error' with regard to the photographer by comparing the models made from the pictures from three different photographers. No significant differences were found between models on the basis of the person whom acquired the pictures [35].

We expand upon this technique, photogrammetry, to not only measure volume, but also hoof shape with the use of geometric morphometrics.

**Geometrics morphometrics–** We made use of geometric morphometric to study hoof shape changes over time. "Morphometrics is the study of biological shapes. [36]. Geometric morphometrics is a morphometric method in which landmark coordinates, rather than linear distances, ratios and angles, are studied. The benefit of this technique is that it captures the entire spatial arrangement of anatomical positions.

In geometric morphometrics the so called 'Cartesian landmark coordinates' need to be obtained through Procrustes superimposition (**Figure 8**), the dissociation of shape from other landmark configurations like overall size, position and orientation, generating the Procrustes shape coordinates. Then statistical analysis can be performed using these Procrustes shape coordinates which ultimately depicts the former shape of an object [37], or in our study, the hooves.

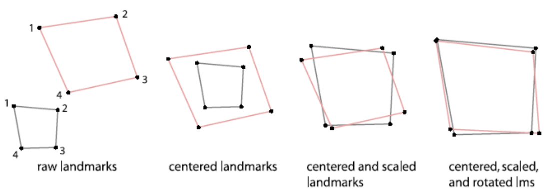


*Figure 8. "The three steps of Procrustes superimposition: translation to a common origin, scaling to unit centroid size, and rotation to minimize the sum of squared Euclidean distances among the homologous landmarks. The resulting landmark coordinates are called Procrustes shape coordinates" [37].*

Morphometric methods have been used to investigate the anatomy of different species of vertebrae regarding the skull, teeth, scapula, pelvis, femur and humerus [36]. We will apply a modification of this technique to study hoof shape changes over time.

**Aims of this study–** The versatility and high speed of a horse is mainly due through their unique physical construction which is represented by comparatively lightweight extremities and single digits surrounded by a tough cornified/keratinized layer, the hooves. Through which horses, as it were, walk on the nail of its toe. The design of these hooves are a result of the digitigrade evolution of the locomoter system of the mammalian family of Equidae resulting in monodactyl unguligrade morphology of the feet [38], [39].

One of the factors involved in the soundness of an equine is the influence of the hooves on the locomotor apparatus [20]. Since the hooves play such an important role in soundness and therefore can affect the usefulness of an equine, hoof growth rates and the quality of the hoof wall are of vital concern [14].

Aims of this study are to evaluate the results of this pilot study in using photogrammetry to make 3D reconstructions of the hooves. We also want to determine whether this technique can be used in measuring hoof growth and establish the exact amount of hoof growth. The last aim is to display and map the precise hoof growth of 55 separate points/landmarks in the hooves of the front limbs over a 6-week trimming/shoeing interval and investigate whether hoof growth is uniformly distributed, so or groups of points grow dependent of independent of each other.

## Material and methods

**Horses–** For this pilot study 6 (n=6) horses of different breeds (4 KWPN Dutch Harness horse, 1 KWPN Dutch Warmblood and 1 Friesian horse), ages (between 8 and 13 years) and weights (ranged from 514 to 622 kg) were used. All horses were clinically sound horses which were used for education at the faculty (Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University) and ridden daily in lessons. At day 0, the horses were trimmed and shod by an on–site farrier. To ensure owner-soundness of the horses during the measurement cycle of 6 weeks, an experienced clinician visually assessed the gait in trot on a hard surface and Q-horse (a lameness analysis system) data were obtained every week.

**Preparation of the hooves–** Prior to videotaping the hooves were prepped. The hooves were brushed to ensure a clean and smooth surface without debris and straws sticking out. To ensure consistency between different measurements, the hooves were marked with permanent ink markers. These markings were maintained throughout the experiment. On medial and lateral sides of the hoof a vertical perpendicular (to the coronary band) line was drawn from the coronary band to the edge of the hoof. As well as a vertical line on the dorsal wall in the ‘middle’ of the hoof was drawn using the center of the fetlock joint as an indication. The midpoint of each of these vertical lines were also marked. On both, medial and lateral, sides of the hoof a marking was drawn just in front of the periople at the heel. A final marking was drawn at the midpoint of the distance between the marking in front of the periople of the heel and the vertical line on the dorsal wall (**Figure 9**). In front of the vertical lines on medial and lateral side of the hoof, a black round marker with a screw as a center was pasted on the hoof with double sided tape. One center of these markers being red which indicated the lateral side of the hoof, the other one being uncolored indicated the medial side of the hoof. Before videotaping the hooves the distance between these centers was measured with calipers, this was needed for scaling later in the process. For the final preparatory step Helling 3-D laserscanning anti-glare spray was used on the hooves.

**Making the video–** The videos were made using a smartphone (Samsung) and a selfie stick. Two people were needed to make the video. One person for lifting up the limbs of the horse and put it in the right position, for the second person who was recording the hoof. Videos were made using a flash which was covered with a thin white paper towel in order to dim the intensity of the light. A 360 ° rotated video of the hooves was made starting and ending at the bulbs of the heel. After completing the 360 ° circle around the hoof, the camera was slightly moved in the direction of the sole to also capture this region on video. A complete video of the hoof took about 25 – 30 seconds. The hoof had to be completely in the picture

*Figure 9. Photographs from different angles to display all applied markings. (A) Markings medial side of the hoof. (B) Markings dorsal wall of the hoof. (C) Markings lateral side of the hoof.*

throughout the entire video since a large degree of overlap - overlap should be at least 80% - was important for the final 3D reconstructions. Care was taken in order to avoid movements of the limbs or the effects of backlight or overexposure of light.

**Data collection–** Once a week digital videos of the hooves of each of the six (n=6) horses were taken. Three videos per limb per horse were made to ensure having good video material for the reconstruction of a 3D model. From one horse (n=1) videos were only taken from the hooves of the forelimb because of horseshoes secured with glue on the hindfeet. This led to the impossibility of applying the correct markings on the hooves. In total 22 videos were taken every week, leading to a total data collection of 154 videos . Date, day of the horse in the cycle, indications for the hoof (left front (LF), left hind (LH), right front (RF) and right hind (RH)) and distance between the lateral and medial markers were noted on a form and used as first frames in the video. In this paper only the data from the videos of the front limbs was used.

***PHOTOGRAMMETRY,* converting videos to frames–** The first step for the photogrammetry consisted of converting the videos into individual JPG files. For the conversion, free Video to JPG Converter software was used from DVDVideoSoft. In general, videos were converted into approximately 100 frames. All frames were initially used for the next steps.

**Generating 3D reconstructions of the hoof–** The 3D models of the hoof were made in Agisoft Photoscan software. For every model a folder was added into the program containing the frames of one video.

**Generating Thin Point Cloud–** At first the photos were aligned at low accuracy - to prevent accidental artifacts which might be brought in into the photos when using high accuracy - and pair preselection disabled. After this step a thin point-cloud was generated and gave a first impression and rough outline of the model. This thin point-cloud was inspected before continuing. Attention was paid to the alignment of the photos (the blue sheets), which would ideally create a full circle around the hoof (**Figure 10**). Also the number of non-aligned photos was checked. Certainly $^2/_3$ had to be aligned properly. For the best and most complete result nearly 100 out of 100 photos were aligned.

**Generating Dense Point Cloud–** Once the photos were properly aligned and the thin point-cloud was inspected a dense point cloud was generated. The dense point cloud was created while using medium quality. In this step, the quality of the model was also assessed. The model was mainly checked for missing information. This could be seen, for example, from holes in the dense point cloud. Thereafter excessive information (the background) was deleted to create a more detailed model of the preferred object, in this case the hoof.

**Build Mesh (generating ultimate 3D model)–** Once the dense cloud points of interest were selected a highly detailed mesh was created using an arbitrary surface type, the dense cloud as source data and a face count of 500.000. To finish, the surface of the model was evaluated.

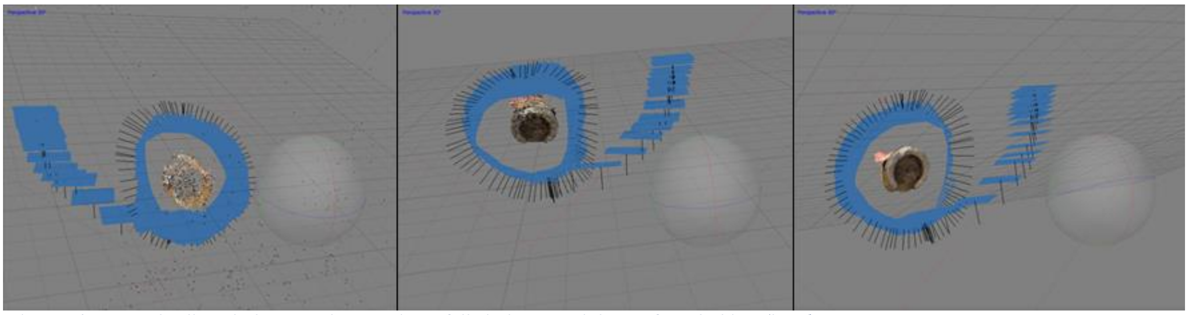

*Figure 10. Properly aligned photographs covering a full circle around the preferred object (hoof).*

When the model was positively evaluated the model was saved and exported as a .ply file. To preserve the colors of the model and enable further analysis, vertex colors, vertex normals and binary encoding were used as export parameters.

***LANDMARK EDITOR*–** Curves consisting of 11 data points were placed in the models using Landmark Editor (IDAV, Institute for Data Analysis and Visualization). PLY files were opened in the program, two single points (s1 and s2) and six curves (c0, c1, c2, c3, c4 and c5) were placed in the model (**Figure 11**). The two single points were placed in the center of the markers on both, medial and lateral, sides of the hoof. The horizontal curves were always drawn from medial to lateral side of the hoof and the vertical curves from dorsal to ventral (from the top down). Once the curves were drawn and the settings and directions were checked the file was saved and exported as .pts file to the corrsponding folder. For each model 68 points (6 x 11 for the curves and 2 single points) were loaded into the .pts file. The single points and curves were positioned for the hooves of both forelimbs (LF and RF), for every horse (n=6), every week (0, 1, 2, 3, 4, 5 and 6).

***GEOMETRIC MORPHOMETRICS,* analysis 3D hoof reconstructions–** Custom written code in MATLAB R2017b from MathWorks was used to analyze the single points and curves in the 3-D hoof reconstructions using a handwritten code. Cordinate data were scaled using the distance between the two screws. A Procrustes superimposition of the landmarks on the coronary band was performed in order to properly align the data. At first, two .pts files from two models of the same horse of week 0 were compared to establish the deviation between the curves placed (**Figure 12**). This to determine their accuracy. Subsequently the models of the other weeks (1, 2, 3, 4, 5, and 6) were compared with the first measurement, week 0, of that front limb. The outcome consisted of a scatterplot representing the 68 points per model (week 0 always shown in blue and the other weeks in red) and a variable ('difference') whereof the 3D distance between the corresponding points was calculated. From each of the horses (n=6) the values of the variable 'difference' from both front limbs (left and right) were saved in a table using Excel. This was done after every measurement for several weeks.

*Figure 11. Added curves in hoof models in Landmark Editor.*

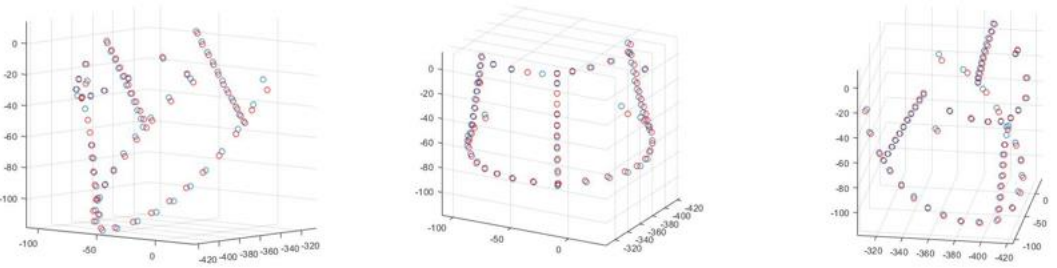

*Figure 12. Comparison and determination of accuracy from measurements of two models of week 0 (left frontlimb). (A) Lateral side of the hoof. (B) Dorsal wall of the hoof. (C) Medial side of the hoof.*

***STATISTICS–*** The data was first analyzed using SPSS v 25. A Principal Component Analysis (PCA) was performed to minimize the amount of variables of the complete dataset by aggregating correlating variables. The scale was set at continue variables, horse and limb as nominal and week as ordinal. A correlation matrix and varimax rotation were also used for the data in the PCA.

After reducing the 55 points per hoof to eight components, so every limb in every measurement had eight variables, a MANCOVA was performed. Limb and horse were set as fixed effects while week was established as a co-variable. Regressions were made per limb and per test. Several factors were tested with these regressions like the general effect of the limbs, if there was a difference between the both front limbs (left or right) and if a difference existed between individual horses. Besides, also the effect of co-variable week in these regressions was established.

## Results

The Agisoft software used for the photogrammetry was capable for construction of the 3D models of the equine hooves in this pilot study (**Figure 13**).

The raw data - 55 points per hoof – were reduced to 8 components (factors) using a Principal Component Analysis, representing separate areas in the hoof that seemed to be growing in the same pattern. Therefore every leg in every measurements has 8 variables. Some points in the hooves pertain two factors which are located at transitions between areas.

The bottom edge of the medial side of the hoof (MED), from the medial periople to the toe, represents three separate areas. At the periople the points are characterized by area 8 which quickly turns into a different growth pattern characterized by area 5. Area 5 gradually turns into area 1 towards the tip of the toe (Figure 10). The bottom edge of the lateral side of the hoof (LAT), from the toe to the lateral periople, begins with area 1 which then gradually changes into area 4. This part of the hoof is therefore only represented by two separate areas **(Table 1)(Figure 14)**.

The vertical line on the medial side of the hoof (med) is represented by two separate areas, 6 and 2. The vertical line on the dorsal hoof wall (dor) also starts with area 6 which turns quickly into area 1 in the direction of the toe. Area 1 expands and joins together with points of the MED and LAT. At last, the vertical line on the lateral side of the hoof (lat) consists of three separate areas, starting with area 7 at the height of the coronary band, and then gradually turning into area 3 **(Table 1)(Figure 14)**.

*Table 1. explanation of the abbreviations of different areas and lines in the hoof.*

| Abbreviations of areas in the hoof | |
|---|---|
| **MED** | Horizontal line along the bottom edge of the hoof on the medial side of the hoof |
| **LAT** | Horizontal line along the bottom edge of the hoof on the lateral side of the hoof |
| **Dor** | Vertical line on the dorsal hoof wall |
| **Med** | Vertical line on the medial side of the hoof |
| **lat** | Vertical line on the lateral side of the hoof |

*Figure 13. 3D models created in Agisoft.*

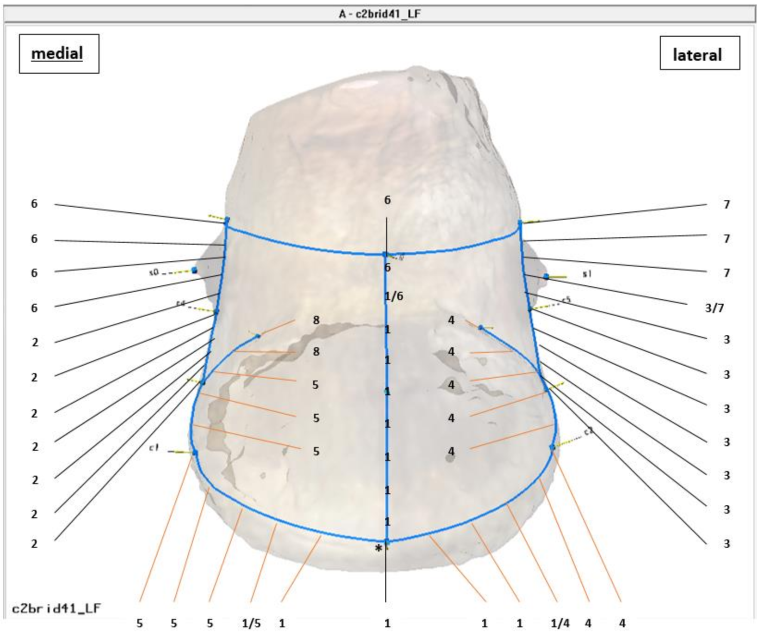


*Figure 14. Distribution of eight separate areas (eight components) concerning hoof growth in the hoof based on a Principal Component Analysis. * Three points are situated in this precise location, namely: the end-point of the medial bottom edge of the hoof, the start-point of the lateral bottom edge of the hoof and end-point of the vertical dorsal wall line.*

The contribution of each of the components differs with regard to a total amount of 100%. Components 1-8 explains 23.2%, 14.0%, 13.1%, 11.6%, 10.7%, 9.6%, 6.2% and 3.3% of the total variance respectively (**Table 2**).

MANCOVA analysis showed significant ($p<0.05$) outcomes regarding the effects of both the general effects of the horses limb (Horse*Limb) and the discrepancy per individual (horse) on hoof growth. Also the effect of the separate frontlimbs (right and left) alone was significant. As well, the effect of co-variance 'week' on hoof growth turned out to be significant. The exact p-values of week, horse, limb and horse*limb are $p=0.013$, $p=0.000$, $p=0.006$ and $p=0.000$, respectively (**Table 3**).

However, this significance does not apply to all eight areas (components) in the hooves, there were more precise differences. Co-variance week does only significantly ($p<0.05$) affect area 2 ($p=0.050$)), 3 ($p=0.037$), 6 ($p=0.003$) and 7 ($p=0.015$). The discrepancy between individuals (horse) significantly affects area 1 ($p=0.000$), 2 ($p=0.003$), 4 ($p=0.001$) and 6 ($p=0.002$). Areas 3 ($p=0.000$) and 5 ($p=0.017$) are significantly affected by the effect of the separate frontlimbs (right or left frontlimb). Last, a significant general effect of the limbs (Horse*Limb) has only been seen in areas 2 ($p=0.000$), 3 ($p=0.001$), 4 ($p=0.000$) and 6 ($p=0.000$) (**Table 4**).

*Table 2. Total Variance of each Component Explained.*

| | Rotation Sums of Squared Loadings | | |
|---|---|---|---|
| Component | Total | % of Variance | Cumulative % |
| 1 | 13.801 | 23.274 | 23.274 |
| 2 | 7.686 | 13.975 | 37.249 |
| 3 | 7.216 | 13.121 | 50.369 |
| 4 | 6.368 | 11.578 | 61.947 |
| 5 | 5.876 | 10.683 | 72.630 |
| 6 | 5.256 | 9.557 | 82.187 |
| 7 | 3.428 | 6.232 | 88.419 |
| 8 | 1.800 | 3.273 | 91.692 |

*Table 3. Significance of fixed effects horse and limb and co-variance week. b. exact statistic.*

| Multivariate test | | | | | | |
|---|---|---|---|---|---|---|
| Effect | | Value | F | Hypothesis df | Error df | Significance |
| Week | | 0.348 | $3.735^{b}$ | 9.000 | 63.000 | 0.013 |
| Horse | | 1.532 | 3.290 | 45.000 | 335.000 | 0.000 |
| Limb | | 0.292 | $2.885^{b}$ | 9.000 | 63.000 | 0.006 |
| Horse*Limb | | 1.665 | 3.715 | 45.000 | 335.000 | 0.000 |

*Table 4. Significance between effect (week, horse, limb, horse*limb) and factor (the eight components).*

| Test of Between-Subjects Effects | | |
|---|---|---|
| Effect | Factor (Component) | Significance |
| Week | 1 | 0.088 |
| | **2** | **0.050** |
| | **3** | **0.037** |
| | 4 | 0.113 |
| | 5 | 0.105 |
| | **6** | **0.003** |
| | **7** | **0.015** |
| | 8 | 0.166 |
| Horse | **1** | **0.000** |
| | **2** | **0.003** |
| | 3 | 0.457 |
| | **4** | **0.001** |
| | 5 | 0.825 |
| | **6** | **0.002** |
| | 7 | 0.863 |
| | 8 | 0.065 |
| Limb | 1 | 0.439 |
| | 2 | 0.896 |
| | **3** | **0.000** |
| | 4 | 0.660 |
| | **5** | **0.017** |
| | 6 | 0.388 |
| | 7 | 0.301 |
| | 8 | 0.687 |

| | | |
|---|---|---|
| Horse*Limb | 1 | 0.057 |
| | **2** | **0.000** |
| | **3** | **0.001** |
| | **4** | **0.000** |
| | 5 | 0.239 |
| | **6** | **0.000** |
| | 7 | 0.094 |
| | 8 | 0.177 |

## Discussion

These data describe the use of 3D-reconstructions of the hoof using several techniques and software, and the possibility of mapping hoof growth using this method and determining whether this hoof growth is uniformly distributed in the hooves. Over a 6-week trimming/shoeing interval during winter, videos of the hooves were made and successfully converted into 3D-reconstructions in which measurements were performed. This demonstrate that the techniques used in this study are suitable for creating usable 3D-reconstructions.

In this research we used a 360 ° rotated video of the hooves, starting and ending at the bulbs of the heel. After completing the 360 ° circle around the hoof, the camera was slightly moved in the direction of the sole to also capture this region on video. Jordan et all. (2000), also described a similar way of filming circular around the hoof. However, in his research to photogrammetrically measure deformations of hoof horn capsule, the hoof was filmed in standing position of the horse [40]. An advantage of our method is that the sole is also visualized.

However, before starting to make videotapes of the hooves, attention must be paid to the proper cleaning of the hoof wall. If the surface of the hoof is not flat and smooth, for instance when a small piece of straw is not removed, this will be reflected in the 3D-reconstruction by a bump. This causes, for example, the wall of the hoof to become deformed in the reconstruction. Since lines are drawn in this reconstruction to measure hoof growth, a deformed reconstruction (because of a bump) will lead to a false increase in the measured millimeters and therefore lead to an inaccuracy in your research.

Hoof growth was established in 55 points in the hoof, which were reduced to 8 factors using a Principal Component Analysis. These 8 factors arose by merging points that seem to grow in a similar pattern. Not every factor contributes in the same amount to the total variance measured. This strongly suggest that hoof growth is not uniformly distributed. The average hoof growth could not be calculated using this method because the points in the scatterplot (**Figure 12**) were not always on the same line. This can be due to 'human error' discussed later. Therefore, the 'difference' was not only measured in the direction of hoof growth, but also horizontal differences in millimeters between the points were measured. For measuring hoof growth one only should be interested in the difference in millimeters of growth on the z-axis. Daradka (2004) et all calculated that the hoof wall grows from the coronary band at an average rate of 8-10 mm per month. This rate can be influenced by several different factors, including breed, age, nutrition, and environment [7], [41]. It can also be affected by season, Lewis (2014) et al., has found significant seasonal influences on hoof growth. In the winter the least hoof horn growth was seen [20]. Our research was performed during winter. Minimal hoof growth during winter could also be a reason for not being able to measure hoof growth. In future research it could be advantageous to measure hoof growth throughout a 6-week shoeing/trimming interval during summer.

Since this research was implemented simultaneously to another ongoing study about the centre of pressure (COP) in hooves, wherefore the horses trotted a minor distance on a hard surface (concrete), it was possible that this could affect hoof growth. However, Faramarzi (2009) et al., showed that in standardbreds there was no significant change in growth of the hoof wall in response to periods of trotting exercise. Only subtle changes are expected because of the effect of loading on equine hooves according to mild exercise, as the trotting was in this research for the COP [42].

A significant effect from 'limb' (interaction between horse and limb), the difference between right- and left front leg, was seen on areas 3 and 5 in the hooves. A side note, however, is that the differences between the left front and right front legs apply based on averages, but is strongly depended of the individual horse. In brief, this calculated effect does not apply for every one of the individual horses per se.

This difference between the both front legs can be caused by a preference for right or left motor laterality, quite similar to the right- or left handedness of humans. Foals, for example, can develop a preference for one of the front legs to systematically protract while grazing. This in turn leads to differences in load bearing patterns of the both front legs and uneven feet [43]. Conformational traits of the horse can predispose in the development of lateralization [43] and can also differ between breeds [44] and sex [45].

Both the medial and lateral sides (vertically) show a transition in areas (the 8 components/factors) (**Figure 14**). On the medial side from area 6 (proximal) to 2 (distal) and on the lateral side from 7 (proximal) to 3 (distal). This can be in accordance with the coronary band, the most proximal part of the hoof, where continuous cell proliferation of the stratum germinativum leads to hoof wall growth. Cell proliferation, however, decreases in the more distal regions of the hooves and are therefore almost non-proliferative [6], [7].

Implications of this study so far, include the fact that the used software and techniques can be used in making 3D-reconstructions of the hooves of horses. Also hoof growth in a lot of points in the hoof can be established using these reconstructions. By also videotaping the sole, a 3D-reconstruction of the entire hoof was possible. So not only the hoof wall was reconstructed properly, but also the sole was projected quite accurate (**Figure 15**). Some adjustments will still have to be made and additional research has to be performed on this method to improve the accuracy and quality of the reconstructions. Nevertheless, this could be the foundation of an alternative for current shoeing methods by 3D printing of individually properly fitting hoof shoes in the future.

**Points of improvement for future research–** For the measurements the hooves were marked with a permanent marker. However, these markings faded or were worn off during the week. Resulting in having to draw these markings again every week. Although this redrawing has been carried out as accurately as possible, the markings will not always have been placed on the exact same place (human error). This resulted in some inaccuracy in determining hoof growth in millimeters. Therefore, in future research, one must think of a more permanent and accurate way to place the markings which will have to be visible for a longer period of time. One can think of branding or carving some permanent markings in the hoof wall, without causing such damage to the hoof that the animal is affected in its well-being.

The hoof growth was measured using geometrics morphometrics, the analysis of the landmark data which was added to the hoof reconstructions. For future research, however, it will be a good addition to check whether the measured distance obtained via this technique corresponds to the actual distance in the hoof to assess the accuracy of this method.

For future research it is also recommended to use a group of horses without shoes to measure hoof growth. This can then be compared with a group of horses, like in this study, with shoes. In this way the effect of shoes on hoof growth can also be determined and contribute to the concept of hoof growth.

In conclusion, this pilot study provides a good basis for future research if some improvements are made.

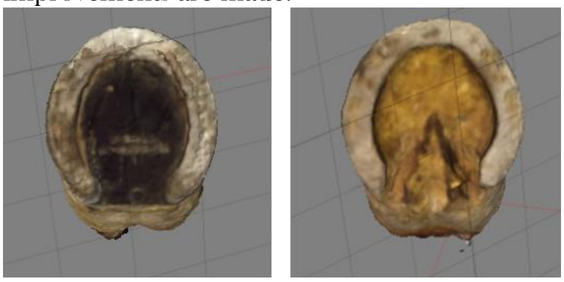

*Figure 15. The sole is also captured in the reconstructions.*

## References


[1] C. C. Pollitt, 'Clinical anatomy and physiology of the normal equine foot', *Equine Vet. Educ.*, vol. 4, no. 5, pp. 219–224, Oct. 1992.

[2] J. D. Reilly, D. F. Cottrell, R. Martin, and D. Cuddeford, 'Tubule density in equine hoof horn', *Biomimetics*, vol. 4, pp. 23–36, Jan. 1996.

[3] J.-M. Denoix, *The Equine Distal Limb : An Atlas of Clinical Anatomy and Comparative Imaging*. CRC Press, 2000.

[4] C. C. Pollitt, *The Illustrated Horse's Foot: A Comprehensive Guide*. London, United Kindom: Elsevier - Health Sciences Division, 2015.

[5] A. Morris, 'The Effects of Three Shoeing Methods on Hoof Growth and Health in Horses', *Masters Theses*, May 2015.

[6] C. C. Pollitt, 'The anatomy and physiology of the hoof wall', *Equine Vet. Educ.*, vol. 10, no. 6, pp. 318–325, Dec. 1998.

[7] M. Daradka and C. C. Pollitt, 'Epidermal cell proliferation in the equine hoof wall', *Equine Vet. J.*, vol. 36, no. 3, pp. 236–241, Apr. 2004.

[8] R. Eustace, 'Factors affecting equine hoof horn growth rate and quality', *In Pract.*, vol. 16, no. 3, pp. 129–131, May 1994.

[9] W. Zenker, H. Josseck, and H. Geyer, 'Histological and physical assessment of poor hoof horn quality in Lipizzaner horses and a therapeutic trial with biotin and a placebo', *Equine Vet. J.*, vol. 27, no. 3, pp. 183–191, 1995.

[10] J. E. A. Bertram and J. M. Gosline, 'Functional Design of Horse hoof Keratin: the Modulation of Mechanical Properties Through Hydration Effects', p. 16.

[11] B. A. Hampson, M. de Laat, P. C. Mills, and C. C. Pollitt, 'The feral horse foot. Part A: observational study of the effect of environment on the morphometrics of the feet of 100 Australian feral horses', *Aust. Vet. J.*, vol. 91, no. 1–2, pp. 14–22, 2013.

[12] M. K. Tanner *et al.*, 'Respiratory and environmental effects of recycled phone book paper versus sawdust as bedding for horses', *J. Equine Vet. Sci.*, vol. 18, no. 7, pp. 468–476, Jul. 1998.

[13] H. Josseck, W. Zenker, and H. Geyer, 'Hoof horn abnormalities in Lipizzaner horses and the effect of dietary biotin on macroscopic aspects of hoof horn quality', *Equine Vet. J.*, vol. 27, no. 3, pp. 175–182, 1995.

[14] K. D. Butler and H. F. Hintz, 'Effect of Level of Feed Intake and Gelatin Supplementation on Growth and Quality of Hoofs of Ponies', *J. Anim. Sci.*, vol. 44, no. 2, pp. 257–261, Feb. 1977.

[15] E. A. Buffa, S. S. Van Den Berg, F. J. M. Verstraete, and N. G. N. Swart, 'Effect of dietary biotin supplement on equine hoof horn growth rate and hardness', *Equine Vet. J.*, vol. 24, no. 6, pp. 472–474, Nov. 1992.

[16] J. D. Reilly, D. F. Cottrell, R. J. Martin, and D. J. Cuddeford, 'Effect of supplementary dietary biotin on hoof growth and hoof growth rate in ponies: a controlled trial', *Equine Vet. J.*, vol. 30, no. S26, pp. 51–57, Sep. 1998.

[17] M. Kummer, D. Gygax, C. Lischer, and J. Auer, 'Comparison of the trimming procedure of six different farriers by quantitative evaluation of hoof radiographs', *Vet. J.*, vol. 179, no. 3, pp. 401–406, Mar. 2009.

[18] M. Kummer, H. Geyer, I. Imboden, J. Auer, and C. Lischer, 'The effect of hoof trimming on radiographic measurements of the front feet of normal Warmblood horses', *Vet. J.*, vol. 172, no. 1, pp. 58–66, Jul. 2006.

[19] S. E. O'Grady, 'Basic Farriery for the Performance Horse', *Vet. Clin. North Am. Equine Pract.*, vol. 24, no. 1, pp. 203–218, Apr. 2008.

[20] C. Lewis, J. Nadeau, T. Hoagland, and M. Darre, 'Effect of Season on Travel Patterns and Hoof Growth of Domestic Horses', *J. Equine Vet. Sci.*, vol. 34, no. 7, pp. 918–922, Jul. 2014.

[21] L. Florence and S. M. McDonnell, 'Hoof growth and wear of semi-feral ponies during an annual summer "self-trimming" period', *Equine Vet. J.*, vol. 38, no. 7, pp. 642–645, Nov. 2006.

[22] O. Balch, K. White, D. Butler, and S. Metcalf, 'Hoof balance and lameness: improper toe length, hoof angle, and mediolateral balance', *Compend. Contin. Educ. Pract. Vet.*, vol. 17, no. 10, pp. 1275–1283, 1995.

[23] G. D. Ovnicek, B. T. Page, and G. W. Trotter, 'Natural balance trimming and shoeing: its theory and application', *Vet. Clin. North Am. Equine Pract.*, vol. 19, no. 2, pp. 353–377, Aug. 2003.

[24] M. N. Caldwell, L. A. Allan, G. L. Pinchbeck, P. D. Clegg, K. E. Kissick, and P. I. Milner, 'A test of the universal applicability of a commonly used principle of hoof balance', *Vet. J.*, vol. 207, pp. 169–176, Jan. 2016.

[25] S. E. O'Grady and D. A. Poupard, 'Physiological horseshoeing: an overview', *Equine Vet. Educ.*, vol. 13, no. 6, pp. 330–334, Dec. 2001.

[26] M. C. V. V. Heel, A. Barneveld, P. R. V. Weeren, and W. Back, 'Dynamic pressure measurements for the detailed study of hoof balance: the effect of trimming', *Equine Vet. J.*, vol. 36, no. 8, pp. 778–782, 2004.

[27] D. M. Hood, D. Taylor, and I. P. Wagner, 'Effects of ground surface deformability, trimming, and shoeing on quasistatic hoof loading patterns in horses', *Am. J. Vet. Res.*, vol. 62, no. 6, pp. 895–900, Jun. 2001.

[28] E. Eliashar, 'The Biomechanics of the Equine Foot as it Pertains to Farriery', *Vet. Clin. North Am. Equine Pract.*, vol. 28, no. 2, pp. 283–291, Aug. 2012.

[29] D. J. Riemersma, A. J. van den Bogert, M. O. Jansen, and H. C. Schamhardt, 'Influence of shoeing on ground reaction forces and tendon strains in the forelimbs of ponies', *Equine Vet. J.*, vol. 28, no. 2, pp. 126–132, Mar. 1996.

[30] N. Crevier-Denoix *et al.*, 'Effects of heel and toe elevation upon the digital joint angles in the standing horse', *Equine Vet. J.*, vol. 33, no. S33, pp. 74–78, Apr. 2001.

[31] M. A. Willemen, H. H. C. M. Savelberg, and A. Barneveld, 'The effect of orthopaedic shoeing on the force exerted by the deep digital flexor tendon on the navicular bone in horses', *Equine Vet. J.*, vol. 31, no. 1, pp. 25–30, Jan. 1999.

[32] W. H. Singleton, H. M. Clayton, J. L. Lanovaz, and M. Prades, 'Effects of shoeing on forelimb swing phase kinetics of trotting horses', *Vet. Comp. Orthop. Traumatol.*, vol. 16, no. 01, pp. 16–20, 2003.

[33] S. J. Dyson, C. A. Tranquille, S. N. Collins, T. D. H. Parkin, and R. C. Murray, 'External characteristics of the lateral aspect of the hoof differ between non-lame and lame horses', *Vet. J.*, vol. 190, no. 3, pp. 364–371, Dec. 2011.

[34] S. J. Dyson, C. A. Tranquille, S. N. Collins, T. D. H. Parkin, and R. C. Murray, 'An investigation of the relationships between angles and shapes of the hoof capsule and the distal phalanx', *Equine Vet. J.*, vol. 43, no. 3, pp. 295–301, May 2011.

[35] R. Labens, W. R. Redding, K. K. Desai, K. Vom Orde, R. A. Mansmann, and A. T. Blikslager, 'Validation of a photogrammetric technique for computing equine hoof volume', *Vet. J.*, vol. 197, no. 3, pp. 625–630, Sep. 2013.

[36] D. E. Slice, 'Geometric Morphometrics', *Annu. Rev. Anthropol.*, vol. 36, no. 1, pp. 261–281, 2007.

[37] P. Mitteroecker and P. Gunz, 'Advances in Geometric Morphometrics', *Evol. Biol.*, vol. 36, no. 2, pp. 235–247, Jun. 2009.

[38] A. Spaan A., P. Y. Sondaar, and W. Hartman, 'The structure of the evolutionary process', *Geobios*, vol. 27, no. 3, pp. 385–390, Jan. 1994.

[39] A. B. Clifford, 'The evolution of the unguligrade manus in artiodactyls', *J. Vertebr. Paleontol.*, vol. 30, no. 6, pp. 1827–1839, Dec. 2010.

[40] P. Jordan, J. Willneff, N. D'Apuzzo, M. Weishaupt, T. Wistner, and J. Auer, 'Photogrammetric measurement of deformations of horse hoof horn capsules', in *Videometrics and Optical Methods for 3D Shape Measurement*, 2000, vol. 4309, pp. 204–212.

[41] R. A. Kainer, 'Clinical Anatomy of the Equine Foot', *Vet. Clin. North Am. Equine Pract.*, vol. 5, no. 1, pp. 1–27, Apr. 1989.

[42] B. Faramarzi, J. J. Thomason, and W. C. Sears, 'Changes in growth of the hoof wall and hoof morphology in response to regular periods of trotting exercise in Standardbreds', *Am. J. Vet. Res.*, vol. 70, no. 11, pp. 1354–1364, Nov. 2009.

[43] M. C. V. V. Heel, A. M. Kroekenstoel, M. C. V. Dierendonck, P. R. V. Weeren, and W. Back, 'Uneven feet in a foal may develop as a consequence of lateral grazing behaviour induced by conformational traits', *Equine Vet. J.*, vol. 38, no. 7, pp. 646–651, 2006.

[44] P. D. McGreevy and P. C. Thomson, 'Differences in motor laterality between breeds of performance horse', *Appl. Anim. Behav. Sci.*, vol. 99, no. 1, pp. 183–190, Aug. 2006.

[45] J. Murphy, A. Sutherland, and S. Arkins, 'Idiosyncratic motor laterality in the horse', *Appl. Anim. Behav. Sci.*, vol. 91, no. 3, pp. 297–310, Jun. 2005.