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Chemical composition of horse hooves with functional qualities for competing barefoot

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Abstract

Barefoot racing is a common practice within the harness racing industry, but not all horses have hooves of sufficient quality to race sustainably without shoes. There is currently no objective approach available to assess whether a horse's hooves are suitable for barefoot racing, raising animal welfare issues if trainers misjudge the functional qualities of hooves. This study compared chemical composition of the hoof wall and fatty acid (FA) composition of the digital cushion in a group of horses that had raced barefoot often (RB) and a group of horses that could not race barefoot and therefore raced with shoes (RS). Trimmings from the hind hoof wall were collected from the lateral quarters in one sub-cohort postmortem and in another sub-cohort of live horses and analyzed for macro- and microelements, nitrogen, dry matter (DM), and total and free amino acid content. For the postmortem horses, samples of the digital cushion were also collected and analyzed for total and free FAs. RB horses had lower concentrations of copper in the hoof wall (17.5 ± 3.9 vs. 32.8 ± 4.7 mg/kg DM, $P = 0.02$) than RS horses. RB horses also tended ($P < 0.1$) to have higher concentrations of nitrogen (164.2 ± 0.2 vs. 163.5 ± 0.3 g/kg DM) and sulfur (22.9 ± 0.2 vs. 22.3 ± 0.3 g/kg DM). RB horses had higher hoof wall concentrations of arginine (10.51 ± 0.05 vs. 10.34 ± 0.06 g/100 g DM, $P = 0.03$) and showed a trend ($P < 0.1$) for higher hoof wall concentrations of cysteine (6.14 ± 0.10 vs. 5.82 ± 0.13 g/100 g DM) and proline (4.62 ± 0.05 vs. 4.49 ± 0.06 g/100 g DM). There were no differences between the groups for any other element or amino acid analyzed. There were also no differences between the two groups in terms of FA composition of the digital cushion. These results indicate that chemical composition, especially with respect to copper, arginine, nitrogen, sulfur, cysteine, and proline, may be important for the functional qualities of the hoof capsule and the ability to race barefoot without wearing the hoof down. However, chemical analysis of hoof wall tissue and of the fat content of the digital cushion does not seem to be a definitive method for distinguishing horses that have hooves suitable for barefoot racing from those that do not.

Lay Summary

Barefoot racing is a common practice within the harness racing industry, as it may make a horse run faster. However, not all horses have hooves of sufficient quality to withstand the wear from the track surface during racing, creating a risk of hoof damage. Therefore, an objective method is needed to distinguish between horses that have hooves suitable for barefoot racing and those that do not. In this study, we compared the chemical composition of hoof walls and the fatty acid (FA) composition of the digital cushion in horses that had raced barefoot often and horses that could not race barefoot frequently. We found differences between the two groups of horses in terms of mineral- and amino acid concentrations in the hoof wall, but not in the FA composition of the digital cushion. This indicates that chemical composition may be important for the functional qualities of the hoof capsule and the ability to race barefoot without hoof wear and damage. However, chemical analysis of hooves is not a definitive method for distinguishing horses suitable for barefoot racing from horses that are not suitable.

Key words: amino acids, fatty acid composition, harness racing, race horses

Abbreviations: DM, dry matter; FA, fatty acids; MUFA, sum of mono-unsaturated fatty acids;N, nitrogen; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids; PUFA, sum of poly-unsaturated fatty acids; RB, barefoot racing horses; RS, shod racing horses; SAFAtot, sum of saturated fatty acids; UFAtot, sum of unsaturated fatty acids

Introduction

Shoeing horses is common practice in most equestrian and race disciplines. The shoe affects the physical characteristics of the hoof by modifying friction and grip on the ground (Horan et al., 2022). It also influences widening of the hoof during load and protects it from wear and damage. However, in Swedish harness racing, competing without shoes ('racing barefoot') is common, owing to a strong perception among trainers that racing barefoot makes horses run faster, which we recently confirmed (Solé et al., 2020). In the same study, we also found that racing barefoot on all hooves is associated with an increased risk of galloping or being disqualified, although interestingly this risk is eliminated in horses racing with shoes on the hind hooves (Solé et al., 2020). The reason for this is unclear, but it could indicate that the hind hooves of horses are the limiting factor with respect to wear and tear, which is supported by anecdotal information provided by experienced trainers. Also in cows, there are reports of the hind claws being more susceptible to damage than the front claws (Vermunt and Greenough, 1996).

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The practice of racing barefoot requires hooves with appropriate mechanical qualities so that they do not break or wear down. The general practice is to remove shoes when competing, and keep the horse shoed the rest of the time in order to protect the hoofs from the daily wear and tear and maximise the performance during races. As an objective measure of hoof quality is currently not available, trainers form an opinion about the abilities of a horse to race barefoot based on experience, anecdotal knowledge and, likely, trial and error. Since horses have occasionally been observed bleeding from the sole after races, this approach may compromise animal welfare, indicating a need for objective measures of hoof physical properties. It could be hypothesized that hooves suitable for barefoot racing differ in terms of chemical properties of the hoof capsule from hooves that are not suitable. For example, mineral content differs between different parts of the bovine claw, indicating an association with load-bearing properties (Assis et al., 2017), and hoof wall sulfur content seems to be correlated to hoof wall tensile strength in horses (Ley et al., 1998).

A softer horn may affect the functional properties of the hoof, e.g., significantly lower hoof hardness has been observed in lame cows compared with healthy cows (Zhao et al., 2015). Some horses have been bred for strong hooves (Han et al., 2023) and the genetic background is suggested to influence hoof strength (Ott and Johnson, 2001; Tocci and Sargentini, 2020; Han et al., 2023), hoof mineral content (Tocci et al., 2017; Tocci and Sargentini, 2020) and hoof amino acid composition (Samata and Matsuuda, 1988), suggesting that horse genetics influence the properties and function of the hoof, possibly through regulation of hoof protein synthesis. The major structural protein in hooves is keratin, composed of polypeptide chains that curl into an α-helix. The sulfur-containing amino acid cysteine is essential in keratin, but other constituents such as histidine, methionine, alanine, glycine, and serine also have important functions (Chilakamarry et al., 2021). Against this background, it is of interest to compare the mineral and amino acid composition of hooves in horses suitable for barefoot racing and horses that are not suitable.

Another region of interest in the hoof is the digital cushion, as it may affect the functions of the hoof. For example, it has been shown that thickness of the digital cushion is a strong predictor of lameness in cattle (Bicalho et al., 2009) and that a less thick digital cushion can increase the incidence of sole ulcers and white line disease in dairy cows (Machado et al., 2011). In cattle hooves, the lipid content in the digital cushion has been found to be lower in tissue situated directly under the distal phalanx than in other parts, indicating that the lipid content may affect the weight-bearing capacity of the hoof (Räber et al., 2006). The digital cushion in horse hooves has been less well studied, but Proske et al. (2016) found that digital cushion thickness is greater when horses are barefoot than when they are shod. It is also possible that fatty acid (FA) composition affects the load-absorbing properties of the digital cushion, as the physical properties of different fatty acids vary (Ellis and Isbell, 1926; Maw et al., 2003); however, to the best of our knowledge, this has not been documented in horses.

The aim of this study was to compare the chemical composition of hoof horn and the lipid composition of the digital cushion in the hind hooves of horses that had raced barefoot often (RB) and in horses that could not race well barefoot and therefore raced with shoes (RS). Our hypothesis was that the chemical composition of hind hoof walls and the FA composition of the digital cushion differ between horses that are able to race barefoot and horses that cannot race barefoot.

Methods

The study was performed in Sweden from 2019 to 2020. It did not require ethical permission under Swedish regulations, as the material collected originated from horses culled for purposes other than the study or from live horses during regular hoof trimming.

Animals and inclusion criteria

Standardbred trotters and North Swedish and Norwegian coldblooded trotters (5 to 20 years) with a minimum of eight races during their career were included in the study. The inclusion criterion for horses in group RB was that horses must have raced barefoot 3 times within a 1-mo (31 d) period at least once in their career. For inclusion in group RS, horses could occasionally have raced barefoot, but with a minimum of 45 d between races, and the trainer had to confirm that the horse could not race barefoot often due to problems with wear and tear of the hind hooves. Data on racing conditions (barefoot or not) and trainers were obtained from the Swedish Trotting Association.

Hoof material for chemical analysis was collected from two sub-cohorts (live and culled horses) and digital cushion material for lipid analysis (see below) was collected from culled horses only. The culled horses were culled for reasons other than this study and consisted of 18 horses (age 13 ± 5 yr), of which 11 were included in group RB (age 11 ± 5 yr) and seven in group RS (age 5 ± 8 yr). One horse in the culled group was a coldblooded trotter (in group RS) and the remaining 17 were Standardbred trotters. The sub-cohort of live horses consisted of 23 horses (age 10 ± 3 yr), of which 13 were included in group RB (age 11 ± 3 yr) and 10 in group RS (age 10 ± 3 yr). Two of the RB horses and one of the RS horses in the live group were coldblooded trotters and the rest were Standardbred trotters. In the live cohort, three barefoot racing horses were from a trainer without any horses racing with shoes available for the study. The remaining 20 horses were from four different trainers, all contributing with both barefoot and shod horses. In the culled cohort, six horses were from three trainers contributing with both barefoot and shod horses and two barefoot horses were from a trainer without any horses racing with shoes available for the study. The remaining nine horses were from trainers contributing with one horse each and both horses racing barefoot and horses racing with shoes were represented.

Sample collection

In the sub-cohort of culled horses, hoof wall, and digital cushion samples were collected immediately after culling and transported to the laboratory on ice. At the laboratory, trimmings (minimum 2 g) from the hind hoof wall (stratum medium) were collected from the quarters at the lateral side of each horse (Figure 1). For analysis of amino acids, trimmings from the right hind hoof and medial side were also collected. The hoof samples were divided in two in a sagittal section using a band saw. Samples (minimum 1 g) from the digital cushion were collected from the medial half of the hoof (Figure 1). In the sub-cohort of live horses, trimmings

Figure 1. Hoof sample sites. (A) Sample site for lipid analysis in the digital cushion (B) Sample site for chemical analysis of hoof trimmings.

(minimum 2 g) were collected in the stable from the lateral side of both hind hooves. Samples from both cohorts were wrapped in plastic and stored at −20 °C until sample preparation and analysis. Hoof color was assessed from images for all hooves except two (where photographs were lacking) and categorized into "dark" (n = 26) or "light/mixed" (n = 13).

Chemical analysis

Hoof trimmings from both the culled and live horses were sent to Agrilab AB (Uppsala, Sweden) for analysis of chemical composition. The samples were milled before analysis. Dry matter (DM) content was determined by drying at 105 °C for at least 24 h. Analysis of total nitrogen (N) content was performed by dry combustion on a LECO CN928 macro analyzer (LECO Corporation, MI, USA) calibrated for every 10th sample. Concentrations of macro- and micronutrients (Ca, P, Mg, K, Na, Mn, S, Cu, and Zn) were analyzed by inductively coupled plasma optical emission spectroscopy using a Spectroblue ICP-OES analyzer (SPECTRO Analytical Instruments, Kleve, Germany) after dissolving in nitric acid and distilled water on a heat block for an hour. Iron was excluded from the analyses due to expected contamination from horseshoes, nails, and tools used during the sampling procedure.

Analyses of free and total amino acid concentrations in the hoof wall were performed at a commercial laboratory (Eurofins, Linköping, Sweden) according to ISO 13903:2005; EU 152/2009.

Lipid analysis

Total lipids were extracted from tissue by homogenization in hexane/isopropanol (3:2, v/v) (Hara and Radin, 1978). The raw lipid content was quantified gravimetrically. Extracted lipids (2 mg) were used for identification of FA proximate composition, after methylation performed according to the method of Appelqvist (1968). Methylated FA were analyzed by gas chromatography (Trace Ultra FID; Thermo Scientific, Milan, Italy) using a BPX-70 50m fused silica capillary column (id. 0.22 mm, 0.25 µm film thickness, SGE, USA). Peaks were identified by comparing sample retention times to retention times of the standard mixture GLC-68-A (Nu-Chek Prep, Elysian, USA).

Statistical analyses

All statistical analyses were performed in SAS 9.4. The level of statistical significance was set at $P \leq 0.05$, with statistical trends reported for $0.05 < P < 0.1$.

Differences in chemical composition of the hoof walls between groups were analyzed using a mixed model including the fixed effects of group (RB or RS), breed (Standardbred or coldblooded), hoof wall color (dark or light/mixed), sample origin (live or culled horse), raced within the previous year (yes, n = 22 or no, n = 19), and horse age. If there was no effect of breed, color, or raced within the previous year ($P > 0.1$), these were removed from the model. Amino acid concentrations were analyzed using a mixed model including the fixed effects of group, sample origin, raced within the previous year, and horse age. If P was > 0.1 for "raced within the previous year," this was removed. The lipid concentration of the digital cushion and the composition in terms of percentage of each FA in total FA were analyzed using the same model as for the amino acids but without the effect of sample origin, since all samples were from the same cohort. Results are presented as least square means (LSmeans) ± standard error (SE).

Results

Effects of group on chemical composition of hoof walls

There was no effect of horse breed or hoof wall color on any of the elements analyzed, so these effects were excluded from further analyses. RB horses had lower concentrations ($P \leq 0.5$) of Cu than RS horses (Table 1). There was also a statistical trend for higher concentrations of N and S in RB horses than in RS horses ($P < 0.1$, Table 1). Horses that had raced within the previous year had higher concentrations of K and Mn in their hooves and showed a trend for more Mg and less S and Zn than horses not active in racing during the previous year (Supplementary Table S1). Sample origin (live or culled horse) had an effect on hoof concentrations of DM, N, Ca, Mg, K, S, and Mn, but not on hoof concentrations of Na, P, Cu, or Zn (Supplementary Table S1).

Effects of group on amino acid composition of hoof walls

Due to small sample size for five horses (too little hoof material removed at trimming), only hoof wall samples from 36 horses were analyzed for amino acid concentrations (RB $n = 22$, RS $n = 14$). The concentrations of hydroxyproline and ornithine were below the limit of quantification in all samples and were therefore not analyzed statistically. Arginine concentrations were higher ($P = 0.03$) in RB horses than in RS horses (Table 2), while proline and cysteine showed statistical trends ($P < 0.1$) for higher concentrations in RB horses than in RS horses. For the remaining amino acids analyzed, no differences were found between RB and RS horses (Table 2). Hoof trimmings from horses active in racing during the previous year showed higher concentrations of alanine, aspartic acid, glutamic acid, isoleucine, leucine and lysine, and lower concentrations of cysteine (Supplementary Table S2). Sample origin (culled or live horse) had an effect on hoof wall concentrations of arginine, histidine, phenylalanine, proline, serine, cysteine, and methionine (Supplementary Table S2).

Effects of group on FA composition of the digital cushion

There was no difference in digital cushion FA content or FA composition between RB and RS horses (Table 3). Whether the horse had been active in racing during the previous year had no effect on either total amount of FAs or FA composition of the digital cushion ($P > 0.05$).

Effects of horse age on hoof wall composition

There was no difference in horse age between RB and RS horses ($P > 0.05$). Horse age had an effect on the hoof wall concentration of K ($P = 0.01$), with K concentration increasing by 0.04 ± 0.04 g/kg DM for each year of age. Age also

Table 1. Dry matter (DM) content and concentrations of nitrogen (N) and different minerals in the hoof wall of horses that had raced barefoot at high frequency (RB) or only occasionally (RS). All values presented are LSmean ± SE

Measure	RB	RS	*P*-value
DM, %	73.0 ± 0.6	72.3 ± 0.7	n.s.
N, g/kg DM	164.2 ± 0.2	163.5 ± 0.3	0.09
Ca, g/kg DM	1.1 ± 0.1	1.1 ± 0.1	n.s.
K, g/kg DM	1.7 ± 0.2	1.8 ± 0.2	n.s.
Mg, g/kg DM	0.3 ± 0.0	0.3 ± 0.0	n.s.
Na, g/kg DM	0.5 ± 0.0	0.5 ± 0.0	n.s.
P, g/kg DM	0.3 ± 0.0	0.3 ± 0.0	n.s.
S, g/kg DM	22.9 ± 0.2	22.3 ± 0.3	0.09
Cu, mg/kg DM	17.5 ± 3.9	32.8 ± 4.7	0.02
Mn, mg/kg DM	20.9 ± 4.2	25.5 ± 5.1	n.s.
Zn, mg/kg DM	168.7 ± 4.6	167.0 ± 5.6	n.s.

Table 2. Concentrations of different amino acids (g/100 g DM) in hoof trimmings from horses that had raced barefoot at high frequency (RB, $n = 22$) or only occasionally (RS, $n = 14$)

	RB	RS	*P*-value
Alanine	4.18 ± 0.03	4.20 ± 0.03	n.s.
Arginine	10.51 ± 0.05	10.34 ± 0.06	0.03
Aspartic acid	7.90 ± 0.05	7.91 ± 0.07	n.s.
Glutamic acid	15.41 ± 0.10	15.40 ± 0.12	n.s.
Glycine	4.62 ± 0.06	4.70 ± 0.08	n.s.
Histidine	1.22 ± 0.01	1.22 ± 0.01	n.s.
Isoleucine	3.69 ± 0.02	3.68 ± 0.02	n.s.
Leucine	9.19 ± 0.05	9.22 ± 0.06	n.s.
Lysine	3.93 ± 0.05	4.01 ± 0.06	n.s.
Phenylalanine	3.17 ± 0.02	3.22 ± 0.02	n.s.
Proline	4.62 ± 0.05	4.49 ± 0.06	0.09
Serine	8.04 ± 0.04	7.98 ± 0.05	n.s.
Threonine	4.95 ± 0.03	4.90 ± 0.03	n.s.
Tyrosine	4.45 ± 0.06	4.48 ± 0.08	n.s.
Valine	5.22 ± 0.03	5.19 ± 0.04	n.s.
Cysteine	6.14 ± 0.10	5.82 ± 0.13	0.06
Methionine	0.79 ± 0.02	0.83 ± 0.02	n.s.

Table 3. Total concentration of lipids (% of DM) and fatty acid composition of total lipids (% of total FA) in the left hind digital cushion of horses that had raced barefoot at high frequency (RB, $n = 11$) or only occasionally (RS, $n = 7$)

Fatty acid	RB	RS	*P*-value
C14:0	3.0 ± 0.2	3.4 ± 0.2	n.s.
C16:0	26.8 ± 0.5	27.2 ± 0.7	n.s.
C16:1 *n-9*	1.2 ± 0.1	1.3 ± 0.1	n.s.
C16:1 *n-7*	11.4 ± 0.6	11.8 ± 0.8	n.s.
C18:0	4.8 ± 0.5	4.6 ± 0.7	n.s.
C18:1 *n-9*	27.5 ± 0.9	26.0 ± 1.2	n.s.
C18:1 *n-7*	2.9 ± 0.1	3.1 ± 0.2	n.s.
C18:2 *n-6*	11.2 ± 0.7	12.0 ± 0.9	n.s.
C18:3 *n-3*	4.8 ± 0.5	3.8 ± 0.6	n.s.
C20:1 *n-9*	0.4 ± 0.0	0.4 ± 0.1	n.s.
C20:2 *n-6*	0.3 ± 0.1	0.4 ± 0.1	n.s.
C20:3 *n-6*	0.7 ± 0.1	0.6 ± 0.1	n.s.
C20:4 *n-6*	1.7 ± 0.3	1.5 ± 0.3	n.s.
C20:3 *n-3*	0.4 ± 0.1	0.5 ± 0.1	n.s.
C22:5 *n-3*	0.6 ± 0.1	0.6 ± 0.1	n.s.
Total lipids, % of dry matter	0.7 ± 0.2	1.1 ± 0.2	n.s.
SAFAtot[1]	34.6 ± 0.8	35.2 ± 1.0	n.s.
UFAtot[2]	63.1 ± 0.8	61.9 ± 1.1	n.s.
MUFA[3]	43.4 ± 1.3	42.6 ± 1.7	n.s.
PUFA[4]	19.7 ± 1.2	19.3 ± 1.5	n.s.
UFA/SAFA[5]	1.8 ± 0.1	1.8 ± 0.1	n.s.
n-3 PUFA[4]	5.8 ± 0.7	4.8 ± 0.6	n.s.
n-6 PUFA[4]	13.9 ± 1.0	14.4 ± 1.2	n.s.
n-3/*n-6*[6]	0.4 ± 0.0	0.3 ± 0.1	n.s.

[1]Sum of saturated fatty acids,
[2]sum of unsaturated fatty acids,
[3]sum of mono-unsaturated fatty acids,
[4]sum of poly-unsaturated fatty acids,
[5]ratio of unsaturated fatty acids and saturated fatty acids,
[6]ratio of omega-3 and omega-6 fatty acids.

tended to affect hoof wall concentrations of Mn and Zn ($P < 0.1$), with Mn concentration decreasing by 1.6 ± 0.9 mg/kg DM and Zn concentration decreasing by 0.9 ± 0.9 mg/kg DM for each year of age.

Age had an effect on arginine, isoleucine, and leucine, the concentrations of which increased by 0.02 ± 0.01, 0.01 ± 0.00, and 0.02 ± 0.01 g/100 mg DM, respectively ($P < 0.05$), for each year of age. Age also tended to affect the concentration of glutamic acid ($P < 0.1$), which increased by 0.04 ± 0.02 g/100 mg DM for each year of age.

Total FA concentration, the proportion of C18:0, and the total amount of PUFA in the digital cushion increased with horse age, while the proportion of C18:1 *n*-9 and MUFA decreased (Table 4).

Table 4. Effect of horse age on fatty acid composition (% of total FA) of the digital cushion in 18 horses. Change by year as estimate ± SE

Fatty acid	Change by year, % of total FA	*P*-value
C14:0	−0.05 ± 0.03	0.06
C18:0	+0.21 ± 0.09	0.03
C18:1 *n*-9	−0.40 ± 0.16	0.02
C20:4 *n*-6	+0.02 ± 0.01	0.08
C20:3 *n*-3		n.s.
MUFA	−0.59 ± 0.23	0.02
PUFA	+0.49 ± 0.20	0.03
n-3 PUFA	+0.16 ± 0.08	0.07
n-6 PUFA	+0.34 ± 0.16	0.06
Total lipids, % of dry matter	+0.07 ± 0.03	0.04

Discussion

To the best of our knowledge, this is the first study to compare the chemical composition of hoof trimmings from horses able to race barefoot and horses with hooves not suitable for barefoot racing. As hypothesized, there were differences in the chemical composition of hoof trimmings from RB and RS horses.

Hoof trimmings from RB horses tended to have more N, implying a greater protein proportion overall. The primary constituent of hooves is α-keratin, a fibrous protein consisting of polypeptide chains that curl into an α-helix. Hoof material from RB horses showed a trend for higher concentration of cysteine and significantly higher concentration of arginine, both of which are important for the properties of keratin (Bragulla and Homberger, 2009), suggesting differences in keratin amount and/or composition between RB and RS

horses. There was also a tendency for higher S concentration in hoof material from RB horses, indicating that there could be a difference in the concentration of S-containing amino acids (for example, cysteine). The cysteine residues in keratin have strong, covalent disulfide bonds that link the polypeptide chains together (Marston, 1946; Bragulla and Homberger, 2009). These S-cross bridges contribute to the hardness of keratin, with hard keratin having more S-cross bridges and a higher protein concentration than soft keratin (Tomlinson et al., 2004; McKittrick et al., 2012). In a previous study on horses, hoof tensile strength was positively associated with S content (Ley et al., 1998). Studies in buffalo have also found higher S concentrations in the portions of the claws bearing the most weight (Assis et al., 2017). In horses, there is anecdotal information that Thoroughbreds generally have poorer hoof quality than horses of other breeds, which may be related to lower cysteine concentrations in Thoroughbred hooves compared with horses of other breeds (Samata and Matsuuda, 1988). Ott and Johnson (2001) have confirmed that Thoroughbreds had lower breaking strength in their hooves than Quarter Horses. The trend for a difference in cysteine content, and consequently in S content, between the hooves of RB and RS horses may therefore be one reason for their different capabilities to withstand the loads of barefoot racing.

The properties and functions of keratin are affected by the amino acid sequence, and the position of amino acids can influence the entire three-dimensional architecture of the molecule (Bragulla and Homberger, 2009). Keratins can go through post-translational modifications such as deimination (also called citrullination) that influence their physiochemical properties. During this process, arginine is converted to citrulline and thereby loses its positive charge, which may lead to altered properties of the keratin (Bragulla and Homberger, 2009). The lower arginine content observed in hoof walls of RS horses than RB horses might thus be a result of higher deimination activity and associated changes in keratin properties. The difference in hoof wall arginine content may also be connected to the trend for greater proline concentration in hooves of RB horses, as proline can be synthesized from arginine and vice versa (Wu et al., 2011).

Horses able to race barefoot had lower concentrations of Cu in their hoof walls than RS horses. This was unexpected, as a Cu-dependent enzymatic process is crucial for stabilization of keratin filaments (Marston, 1946; Tomlinson et al., 2004). Copper has been reported to affect the soundness of the hoof in both horses and cattle, for example, in a study of horses, the incidence of white line disease decreased when the diet was changed to a feed with higher Cu and Zn concentrations (Higami, 1999). In contrast to results of the present study, previous studies indicate a positive relationship between hoof wall Cu concentration and hoof health. One explanation could be that the function of Cu in hoof wall qualities is related to the formation of keratin, rather than to its concentration in the already-formed tissue. The formation of the disulfide bonds between cysteine residues in keratin filaments is dependent on thiol oxidase enzyme (O'Dell, 1990). This enzyme is activated by Copper and Cu is thereby essential for structural strength on cellular level to give rigidity to the keratinized cell matrix. This process occurs in living cells, taking place in the coronet where the keratinocytes are cornified. However, the samples analyzed in the present study were from the distal part of the hoof, where cells are dead and already keratinized. Therefore, available Cu may still be essential for the formation of keratin without its presence being reflected in the distal part of the hoof wall. The same line of reasoning might be relevant for the Cu/Zn SOD enzyme, acting to prevent oxidative damage (Tomlinson et al., 2004). Our results also contradict findings by Zhao et al. (2015) of higher concentrations of Cu in feet of lame animals (cows). However, their samples were obtained from the bulbar zone of the bovine claw, where the horn is softer than in the abaxial wall (Manson and Leaver, 1988) and therefore less keratinized. Sargentini et al. (2012) found no significant correlation between hoof hardness and Cu concentration in hoof walls of donkeys. Likewise, van Marle-Köster et al. (2019) found no correlation between Cu concentration and tensile strength in the hoof wall of beef cattle. In a previous study of tensile strength in horse hooves, Cu content did not explain tensile strength variation (Rueda-Carrillo et al., 2022). In our study, one horse had considerably higher hoof wall Cu concentrations than the others (114 mg/kg DM vs. 21 ± 14 mg/kg DM), but even with this outlier removed from the statistical analyses, the difference between RB and RS horses remained ($P = 0.03$). Interestingly, according to the farrier, this horse had very bad hooves displaying poor growth and fragile hoof horns. The mechanism behind this negative impact of high Cu concentrations in hoof wall tissue remains to be elucidated. Further studies should be conducted to increase understanding of the relationship between Cu and functional qualities of the hoof.

FAs in the digital cushion

There were no differences in FA composition of the digital cushion between RS and RB horses. However, older horses had a greater concentration of total FAs in the digital cushion than younger horses. This is in agreement with findings for cattle, where heifers have a lower lipid content in the digital cushion than cows (Räber et al., 2006). Older horses also appeared to have higher PUFA concentration in the digital cushion than younger horses. This may be explained by older horses (post-racing career) being fed a more forage-based diet than younger horses, reflecting different levels of work. However, as we did not control for diet, we cannot confirm this suggestion. It is known that diet has an influence on the FA composition of body fat in horses (Belaunzaran et al., 2015) and has also been suggested to affect the fat content of the digital cushion in cows (Räber et al., 2006), with increased forage:concentrate ration in the diet resulting in more PUFA. The physical-chemical properties of PUFA vs. saturated FA are well known (more double bonds, softer fat). This could influence digital cushion properties, so more investigations on lipid quality are needed.

Effects of age

In the present study, age affected several components of the hoof wall, including amino acids, K, Zn, Mn, and FA composition. Previous reports on age-related changes in the amino acid composition of hoof walls in horses are scarce. The observed increases in concentrations of arginine, isoleucine, leucine, and to some extent also glutamic acid imply a shift in keratin composition with age. The reason for the increased concentration of K is not clear, but increasing K concentration with age and a correlated decrease in tensile strength have been observed in human hair (Kim et al., 2013). With increasing horse age, the total FA concentration, the proportion of

C18:0, and the total amount of PUFA in the digital cushion increased and the proportion of C18:1 *n*-9 and MUFA decreased. However, this effect could be confounded by dietary and other factors, and it is not clear to what extent the observed correlations in the present study were an actual effect of age. The study design did not permit investigation of a possible effect of age on hoof biochemistry and the effects found were unexpected. In future studies, it might therefore be relevant to consider age as a factor when studying hoof quality in horses.

The chemical composition of hooves in horses able to race barefoot (RB horses) differed from that in horses unable to race barefoot (RS horses). Although this study was not designed to assess causality, it indicates that chemical composition with respect to Cu and arginine as well as N, S, cysteine, and proline may be important for the functional qualities of the hoof capsule in horses. However, chemical analysis of hoof wall tissue and of the fat content in the digital cushion does not seem to be a clinically feasible method for accurately identifying the suitability of hooves for racing barefoot or shod.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Disclosures

The authors declare no conflict of interest.

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