 DOI: 10.56617/att.7637

Effect of the pigmentation on hardness, elasticity and chemical composition of equine hoof horn

A pigmentáció hatása a ló patájának szilárdságára, rugalmasságára és kémiai összetételére

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SUMMARY

The primary aim of our research was the analysis of the composition and quality of differently pigmented equine hooves. 7 horses were chosen to the trial so, that all of them had a wax yellow hind leg and a grey one, and one of their front legs was also grey. The tensile strength (hardness), relative elasticity of the hoof samples, as well as their moisture and lipid content, more so the quantity of four specific minerals, such as calcium, phosphor, zinc and copper were determined. Statistical analysis was carried out with Paired-sample T Test. According to our results, it seems, that the tensile strength, relative elasticity and composition of the equine hoof is not significantly influenced neither by pigmentation, nor by its anatomical position (whether it is a hind- or front leg). The quality of the hoof wall is more influenced by the individual differences, such as the grooming, foddering and weather conditions. It seems, that only the appearance of the hoof is influenced by the pigmentation.

Keywords: equine hoof horn, elasticity, hardness, chemical composition

ÖSSZEFOGLALÁS

A ló patájának minősége meghatározó jelentőségű az állat használata szempontjából. A pataszaru pigmentáltságának köszönhetően a ló patája különböző színű (palaszürke, viaszsárga, vagy sávolt) lehet. Lovasok között elterjedt nézet, hogy a viaszsárga (pigmentálatlan) paták gyengébb minőségűek a pigmentált (palaszürke) patáknál. Ezzel kapcsolatban viszonylag kevés konkrét kutatási eredmény áll rendelkezésre. Vizsgálataink elsődleges célja a különböző pigmentáltságú lópata szaru összetételének és minőségi jellemzőinek vizsgálata volt. A kísérletbe hét lovat vontunk be oly módon, hogy mindegyik egyed rendelkezett egy viaszsárga és egy szürke színű hátulsó patával, valamint az egyik elülső lábuk szintén pigmentált (palaszürke) volt. A pata minták esetében meghatároztuk a szakítószilárdságot (keménységet), a relatív rugalmasságot, továbbá a nedvesség- és nyerszsír tartalmat, valamint négy a paták szempontjából lényeges ásvány – a kalcium, a foszfor, a cink és a réz – mennyiségét. A statisztikai kiértékelést párosított t-próbával végeztük. Eredményeink alapján megállapítható, hogy a patafal szakítószilárdságát, relatív rugalmasságát és kémiai összetételét sem a pigmentáltság, sem az anatómiai elhelyezkedés (elülső vagy hátulsó láb) nem befolyásolja szignifikáns mértékben. A patafal minőségére nagyobb hatást gyakorolnak az egyedi különbségek, mint például az ápolás, a takarmányozás és az időjárási körülmények. A pigmentáltság elsősorban a pata megjelenését befolyásolja.

Kulcsszavak: pataszaru, rugalmasság, keménység, kémiai összetétel

1 Introduction

Recently, several studies have been conducted on the chemical composition of horse hooves *(Ley et al., 1998.; Spörndly-Nees et al., 2023)*. The presence and quantity of minerals greatly influence the formation of the horn and its quality. The following minerals are found in the horn: among the macroelements, Ca, P, Na, K, Mg, S, and among the microelements, Fe, Zn, Cu, Se. The sum of these is the crude ash content of the hoof *(Andrási, 2014)*. The largest part of the ash content is calcium and phosphorus, so these two minerals play an important role in the structure of the horn. Copper is an important enzyme activator. 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)*. 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. Thus, copper plays an important role in the formation of disulfide bonds in the protein molecule, which provide a significant part of the keratin matrix. Such bonds partly determine the physical properties of keratin *(Underwood, 1981)*. Zinc is component of over 200 enzyme systems *(Mulling et al., 1999)*. While the thiol oxidase enzyme is catalyzed by copper, zinc is an important component of it *(Cousins, 1996)*. Therefore, is a key element in the structure of hoof horn. Lack of it results in brittle, cracked hooves. Because of these properties, zinc is an important ingredient in most hoof care products. According to *Lukas* (2009), the zinc content of the feed had a positive effect primarily on softened hooves. *Tocci et al.* (2015) compared hoof quality of Anglo-Arabian horses and Monterufoli Ponies and reported that Anglo-Arabian horses that had stronger hooves tended to accumulate more zinc in their horn. Interestingly, however, *Noormohammady et al.* (2018) found no correlation between zinc content and horn quality in their experiment with KWPN show jumping horses. This is surprising, especially since zinc-containing products are often recommended by certain companies to increase the elasticity and hardness of the horn.

The quality of the horn is mostly influenced by external factors such as feeding, husbandry technology and weather. Genetics are also an important factor, but these encoded basic characteristics have not changed over the centuries of domestication and breeding. So the horse's hoof has the same anatomical characteristics and biological needs as before *(Mráz, 2016)*. There is an old saying, that there is no good horse without good hooves. This statement is absolutely true, as the hooves, and within them the hooves, have many tasks. The hooves carry the entire weight of the horse. The horn provides protection, protects against external mechanical influences, ensures thermal insulation, and is also responsible for the stability of movement. Therefore, good quality horn must be thick, hard, elastic and load-bearing.

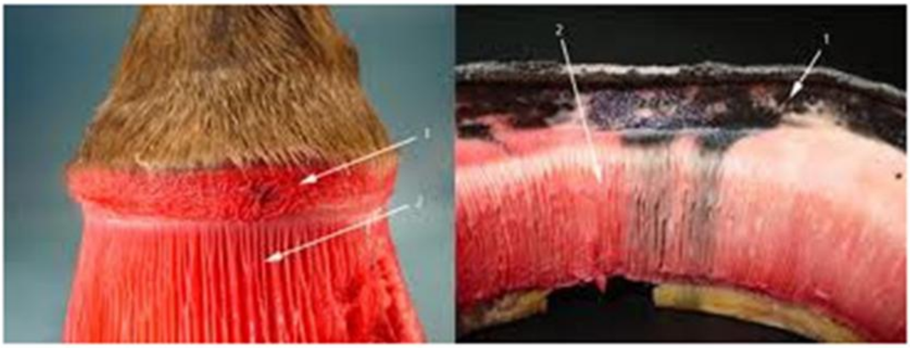
Keratin's mechanical properties are very hydration dependent, a phenomenon that has been reported in hooves *(Douglas et al., 1996; Huang et al., 2019)*. The moisture of the horns is basically ensured by circulation, but the environment and the housing technology strongly influence the moisture content of the hooves. It is important to have good quality soil, with sufficient hardness and elasticity, both in the pasture and on the track where the work takes place, to ensure adequate blood supply, thus supporting horn growth. In muddy, snowy weather, if the horse spends a lot of time outdoors, the hoof can soften, and then quickly dry out in a dry stable, in clean litter. The main reason for this may be that the sebum plugs of the cells are washed out, so when they reach a dry place, the horn easily loses moisture. Box keeping is the most common nowadays, but here an ammonia-free, dry living space and regular removal of manure are required. Due to stable housing, adequate daily exercise is necessary. Insufficient exercise will result in the hoof's self-regulation becoming less effective *(Magyar, 2016)*. Our own experience and literature data *(Smith, 1921; Lambert, 1966)* also demonstrate that the moisture content of the hoof has a significant effect on horn health. It is interesting to note, however, that *Ley et al.* (1998) in their experiment with Thoroughbreds found no significant correlations between the moisture content and the hardness and mineral composition of the hoof.

Biotin, better known as water-soluble vitamin B7 or vitamin H, contributes to faster horn growth. Its deficiency can result in softening of the white line and cracking and fragmentation of the hoof. In a previous experiment, 24 horses were studied, 16 of which had their feed supplemented with biotin for 10 months, and the other 8 horses did not receive any supplementation The results clearly showed that the horses fed biotin had a higher hooves growth rate and the horns were harder. Overall, it can be said that the hooves became healthier *(Buffa et al., 1992)*.

Polyunsaturated fatty acids (PUFA) also contribute to the development of hooves. For example, pasture grass, flaxseed or sunflower seeds are rich in these fatty acids. Horses do not have a gallbladder, but their fat digestion is excellent, which is due to the fact that bile production adapts well to the fat content of the feed *(Szabó, 2011)*.

The hoof wall consists of three layers; the stratum externum, stratum medium and the stratum internum *(Stump, 1967; Reilly et al., 1996)*. The stratum externum, or periople, is the thin, outermost layer of the hoof wall, containing completely keratinized, dead cells. The stratum medium, or tubular horn, is the middle and thickest layer of the hoof wall, where successive stages of keratinization occur. The innermost layer of the hoof wall is the stratum internum, which is in contact with the dermis and consists of longitudinal horny plates. This is the inner, germinal layer, where various undifferentiated cells are found *(Andrási, 2014; Al-Agele et al., 2019)*.

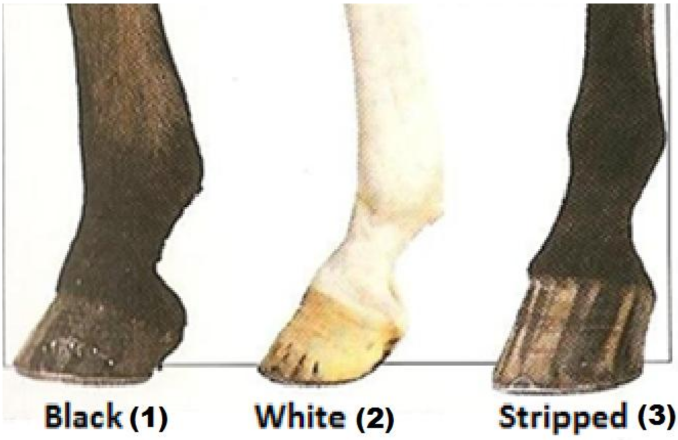
Below the layers of the stratum corneum are the layers of the dermis, which is shown in *Figure 1*. The hoof's dermis is a connective tissue containing collagen and elastic fibers, which is richly interwoven with blood vessels and nerves. The dermis contains no hair follicles at all and only some glands. On its surface there are dermal plates and dermal warts *(Fehér, 2000)*.



1. ábra. A pata irharétegének érzékeny lemezei: kapcsolat a patacsont és a szarufal között. A ló testsúlyának nagy része a szarufalra tevődik át.

Figure 1 Sensitive plates of the hoof dermal layer: connection between the hoof bone and the horn wall. Most of the horse's weight is transferred to the horn wall

The color of the hooves is due to the outermost layer of the horn wall, the periople, which is produced by the dermis of the coronary band. This top layer is mostly protective, but it is thin and less resistant, so it can easily break, crack, and even peel off in layers. Its color may be related to the hair above the hoof, but even more so to the color of the skin of the coronet. There are three types of hooves according to color *(Figure 2)*. In case of black colored lower legs usually is black hoof, in case of leg markings the hoof is usually white, by ermine marked feet the hoof can be striped, which is the transition between the previous two. The color of the hooves cannot be predicted and varies even within a horse, as all four can be the same, or they can be completely different.



2. ábra. A ló patájának színváltozatai
palaszürke (1); viaszsárga (2); sávolt (csíkos) (3)

Figure 2 Hoof color variations

It is a common belief in equestrian circles that the lighter the hoof, the poorer the quality of the horn, but this or the opposite has not been supported by any relevant research results so far. In our study, we aimed to determine whether hoof pigmentation is associated with the hardness, elasticity, moisture, fat content, and the levels of calcium, phosphorus, copper, and zinc in the hoof horn.

2 Materials and Methods

Seven horses were included in the study, six of which were from the same location *(Table 1)* and of the same age. The animals were selected so that each had white colored hind leg, the other black, and one black foreleg. The samples were taken from the hooves of the horses during their usual hoof trimming in 1st April. The excised, horseshoe-shaped hoof horn samples were stored in airtight bags at –20 °C until the commencement of the analyses.

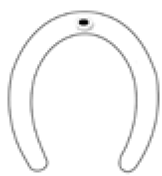
Table 1 Data on the horses included in the study

Name of Horse (1)	**Lejla (Sehi)**	**Déva Gidran I-19 (Nimbusz)**	**Gidran XXXIV-3 (Nesztor)**	**Gidran XXXIV-7 (Spektrum)**	**Behdi Gidran-18 (Irma)**	**Déva Gidran I-16 (Sordély)**	**Gidran XXXIV-5 (Micsoda)**
Breed (2)	Shagya Arabian	Gidran	Gidran	Gidran	Gidran	Gidran	Gidran
Coat color (3)	gray (6)	chestnut (7)	chestnut (7)	chestnut (7)	chestnut (7)	chestnut (7)	chestnut (7)
Age (year) (4)	17	1	1	1	2	1	1
Gender (5)	mare (8)	stallion (9)	stallion (9)	stallion (9)	mare (8)	stallion (9)	mare (8)

1. táblázat. A kutatásban szereplő lovak adatai

a ló neve (1); fajtája (2); színe (3); életkora (4); ivara (5); szürke (6); sárga (7); kanca (8); mén (9)

The hardness and elasticity tests were performed using a Zwick Roell/Z005 structural testing machine at the Kaposvar Campus of Hungarian University of Agriculture and Life Sciences. To determine the hardness and elasticity of the hooves, compression values were recorded on the toe area *(Figure 3)* using a 10 mm diameter compression steel head. The sample was placed on a 35 mm steel button *(Figure 4)*, given that the compression head approached at a speed of 50 mm/s to a distance of 1 mm, with a compression force of 5 kN.



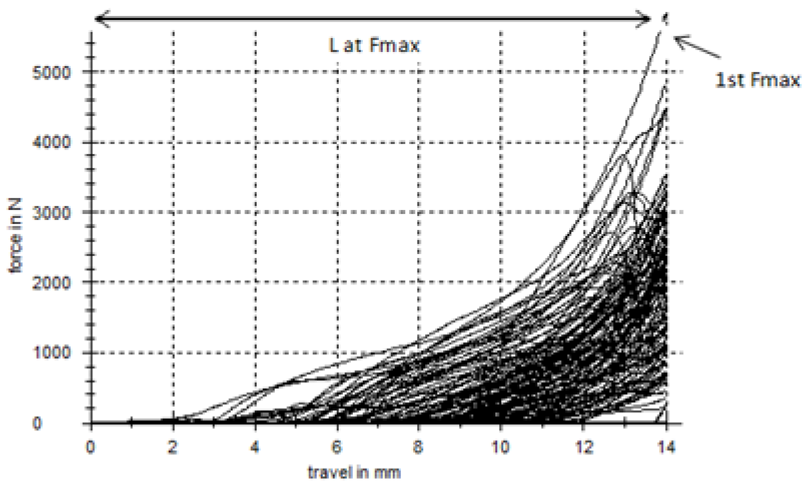
3. ábra. Mérési pont

Figure 3 Measurement point

4. ábra. Pata minta mérés közben

Figure 4 Hoof sample during measurement

At first, we measured the so-called hardness, the force necessary to cause a given deformation, the measure of which is the maximum force measured on the compression curve (1st Fmax, N) at the first compression. After that the elasticity of the hoof was measured. The elasticity was determined by compressing the hoof twice in succession, which was derived from the distance traveled by the measuring head until the maximum force was exerted, the so-called L at Fmax (mm) values. The results of the measurements were recorded using the testXpert V11.0 software supplied with the instrument *(Figure 5)*.



5. ábra. Kompressziós görbe

Figure 5 The compression curve

The measurements related to the composition of the hoof were carried out in Keszthely, in the laboratory of the "Festetics Imre" Bioinnovation Center of the Georgikon Campus of Hungarian University of Agriculture and Life Sciences. We determined the dry matter content of the hoof samples, from which we calculated the moisture content, and we also measured the crude fat and four characteristic mineral contents (phosphorus, calcium, copper and zinc) of the samples. The measurements were carried out according to Hungarian standards (*Moisture*: 152/2009/EK III. annex A; *P*: 152/2009/EK III. annex P; *Ca*: MSZ EN ISO 6869:2001 section 8.7.; *Cu, Zn*: MSZ EN ISO 6869:2001 section 8.6.).

During the study, we compared hind hooves with different pigmentation and fore and hind hooves with the same pigmentation. Our results were evaluated with a paired t-test for all examined parameters, after checking for normal distribution (Shapiro-Wilk test). The study was performed with the R statistical program *(R Core Team, 2017)*.

3. Results and discussion

Potential confounding effects of age, sex, or breed differences among the horses *(Table 1)* were partly controlled, as our study focused on comparing hooves with different pigmentation and anatomical location (forelimb vs. hindlimb) within the same individual. Furthermore, according to *Rueda-Carrillo et al.* (2022), age, breed, and sex do not significantly affect the parameters examined in this study. The results of our study were shown in Table 2. In the case of hoof hardness slightly higher values obtained by the white hoof (HS) compared to the black hind hoof (HP), while the highest value was observed by the black front (EP) hoof. The differences can not be statistically proven. The average hoof hardness of the horses we studied was similar to the average values measured in April in *Ley et al.'s* (1998) 1st trial.

The elasticity of the hoof samples was not affected by pigmentation. There were relatively large individual differences in the hooves of individual horses.

In the case of moisture content, it wasn't detected significant differences between the three groups. According to the research of *Ley et al.* (1998), this value is mostly influenced by weather and feed. The moisture content of the hooves of the horses included in the experiment, regardless of pigmentation, was similar to the results of other hoof-related research *(Butler 1974, 1976; Ley et al. 1998)*. The value of around 27% observed in all three cases is closest to the results of *Butler's* study in 1976.

The fat content of white hooves was nearly 1.5 times higher that of black, but we did not observe statistically significant differences. We detected lower fat content in our hoof samples, than in the experiment carried out by *Spörndly-Nees et al.* (2023) with harness racing horses.

When examining the mineral content of the hooves, we found no significant differences in calcium, phosphorus and copper between the groups examined, so it seems that the amount of these minerals is not affected by pigmentation. However, the difference in zinc content between white and black hind hooves is noteworthy, as this parameter was very close (P = 0.06) to being statistically significant (P < 0.05). It is therefore likely that a significant difference could be observed if the study were conducted with a higher number of elements. According to *Ley et al.* (1998), the mineral content of hooves also depends mostly on feeding and seasonal effects. The values obtained for calcium and copper are similar to the results obtained in the study of *Spörndly-Nees et al.* (2023), but lower values were observed for phosphorus and zinc. According to the their study, the mineral content of the hoof wall can also be affected by the horse's age.

Table 2 Results obtained during hoof examinations

	Average and standard deviation (1)			**p-values (2)**	
Examined parameter (3)	HS*	HP*	EP*	EP-HP	HS-HP
Hardness (1st Fmax, N) (4)	2 138 ± 436	2 019 ± 650	2 629 ± 1 427	0.58	0.71
Elasticity (L at Fmax, mm) (5)	13.59 ± 0.62	13.70 ± 0.54	13.67 ± 0.58	0.63	0.25
Moisture (%) (6)	27.96 ± 3.89	27.31 ± 2.86	27.27 ± 2.65	0.75	0.55
Crude fat (%) (7)	0.04 ± 0.012	0.024 ± 0.016	0.026 ± 0.017	0.62	0.10
Ca (%)	0.13 ± 0.015	0.12 ± 0.017	0.13 ± 0.015	0.58	0.14
P (%)	0.01 ± 0.05	0.01 ± 0.08	0.01 ± 0.08	0.77	1.00
Cu (mg/kg)	3.22 ± 0.61	2.97 ± 0.47	3.29 ± 0.60	0.53	0.22
Zn (mg/kg)	49.48 ± 4.14	44.89 ± 6.38	44.38 ± 6.64	0.42	0.06

*In the table, the sign HS was used for hind white (unpigmented) hooves, HP for hind black (pigmented), and EP for front black hooves. The differences were interpreted between HS-HP and HP-EP for all parameters examined.

2. táblázat. A patavizsgálatok eredményei

átlagok és SD értékek (1); P értékek (2); vizsgált paraméter (3); keménység (4); rugalmasság (5); nyers zsír(6)
*A táblázatban a HS jelölés a hátulsó, fehér (nem pigmentált) patákat, a HP a hátulsó, fekete (pigmentált) patákat, míg az EP az elülső, fekete patákat jelöli. Az egyes vizsgált paraméterek esetében a különbségeket a HS–HP, illetve a HP–EP csoportok között értékeltük.

Comparative data on hoof composition are available for horses and some closely related equids, such as donkeys (Equus asinus). Studies on donkey hooves have primarily reported moisture content. In their investigation, *Hopegood et al.* (2011) found that the moisture content of donkey hooves was significantly higher (P < 0.05) than that of horse hooves. However, data for other equids, such as zebras (Equus zebra and related species), remain extremely limited, and no comprehensive studies on their hoof mineral or moisture content have been published to date.

4 Conclusions

Overall, the results of this study indicate that hoof hardness, elasticity, and chemical composition are not significantly influenced by pigmentation or anatomical location (forelimb vs. hindlimb). These findings are consistent with practical observations reported by farriers *(Eldredge, 2018)*.

The results suggest that individual variation, as well as environmental factors such as nutrition, housing conditions, and weather, play a more substantial role in determining hoof quality than other parameters such as pigmentation *(Ley et al., 1998; Spörndly-Nees et al., 2023; Rueda-Carrillo et al., 2022)*. Therefore, differences in hoof care or management strategies based solely on hoof color do not appear to be justified.

Although no statistically significant differences were detected, the near-significant variation observed in zinc content between pigmented and non-pigmented hooves suggests that further investigation with a larger sample size may be warranted.

It should be noted that the relatively small number of animals included in this study represents a limitation, and future research involving a larger population and different breeds would be valuable to confirm these findings.

Despite these limitations, this study contributes to the limited body of scientific literature addressing the relationship between hoof pigmentation and hoof quality, providing a basis for further investigations in this area.

5 Aknowledgements

The publication was supported by the project EFOP-3.6.3-VEKOP-16-2017-00008. The project was implemented with the support of the European Union and co-financed by the European Social Fund.

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Érkezett/Recived: 2026. február 16.
Elfogadva/Accepted: 2026. április 28.