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Hoof Characteristics of Anglo Arabian, Haflinger, Monterufoli, and Maremmano Horse

Research Article

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ABSTRACT

In this work the hoof quality of 9 Anglo Arabian, 10 Haflinger, 7 Maremmano, and 15 Monterufoli equines was evaluated. Anglo Arabian is a cosmopolitan breed, others are Italian horses: Haflinger derives from Trentino Alto Adige, Maremmano and Monterufoli from Tuscany. Hoof samples, from the front left foot, were collected during the trimming in order to assess the physical-chemical characteristics and mineral content in nail. Collected data were submitted to ANOVA, principal component and discriminant analyses and a heathmap of the square distance among mineral content in hoof of different breeds was carried out. The softest hoof was found in Monterufoli Pony, having also highest dry matter content. Highest Fe, Ni, Pb, Se content, characterized the Anglo Arabian hoof, while Monterufoli Pony hoof showed the highest Na content. The multivariate analyses have shown K, Li, Mn, Na, and P were the most identifying minerals in the hoof nail. In Anglo Arabian, Haflinger, Maremmano hoof there was a high bioaccumulation of minerals, while the Monterufoli Pony hoof quickly remove the minerals, probably through the nail consumption and the osmoregulation activity. All statistical analysis showed that Monterufoli Pony hoof was different from the hoof of other considered breeds. All animals have shown foot of good quality suitable for the barefoot practice.

KEY WORDS Anglo Arabian horse, Haflinger horse, hoof characteristics, Maremmano horse, Monterufoli Pony.

INTRODUCTION

This study has dealt with the quality of the hoof of different breed reared in Tuscany. Monterufoli pony (MP) is an endangered breed which derives from the Pisa province, and it is now used as saddle and driving equine (Tocci *et al.* 2007). Anglo-Arabian (AA) is a widespread breed used for endurance rides (Lynghaug, 2009). Haflinger (HA) horse, which was used in agriculture, is a breed native to South Tirol that was improved with Arabian stallions, and it is one of the most common Italian breeds (Tocci *et al.* 2017). This breed is now used as riding and driving horse. Maremmano (MA) horse is a Tuscan Latial breed. At the origin of the breed contributed oriental horses and large north European equines. Within the end of XIX and the beginning of XX century the selection and the improvement of the breed began, through the introduction of Arabian, Thoroughbred, Hackney horses (Felicetti *et al.* 2010).

The environment and selection determine the hoof characteristics, which are an index of environmental adaptability (Tocci *et al.* 2017). The nail of animals tends to cumulate heavy metals (Skibniewska *et al.* 2015), which are excrete through the consumption (Tocci *et al.* 2017). The soundness of a foot also depends on its arteriovenous activity (Sargentini *et al.* 2012). Because their role in the keratin structuring (Noormohammady *et al.* 2018) macroelements, microelements and oligominerals are very important for the quality of nail and for the foot health (Tocci *et al.* 2017). Extraneous minerals and heavy metals in small amounts can play an important role in the bone mineralization and the reproduction (Stachurska *et al.* 2011). Feet condition the horse movement and a healthy and strong foot promotes the barefoot practice and the animal welfare. The common shoeing practice is not always a benefit because it can involve some potential deleterious effects on soundness. The horse shoeing can hind the waste removal in nail and reduce the arteriovenous activity, because the shoe avoids the natural changes of foot in its external dimension and avoids the sole, bars and frog natural expansion (Clayton *et al.* 2011). The aim of this study was to compare the morphological, physical, chemical, and mineral characteristics of hooves of AA, HA, MA horses and MP reared in Tuscan farms.

MATERIALS AND METHODS

In this study the morphological, physical-chemical and mineralogical characteristics were evaluated in the front left hoof of 41 adult unshod equines. The study considered 9 AA horses (4 males and 5 females), 10 HA horses (6 males and 4 females), 7 MA horses (7 males) and 15 MP (8 males and 7 females) reared in the farms of the "Carabinieri Forestali - ex Corpo Forestale dello Stato" in Arezzo and Siena provinces and in a private farm of Livorno province. The diet was similar for all animals and was based on local fodder (1.2 kg/100 kg live weight) and concentrate meal (0.2 kg/100 kg live weight). The trial was performed during the fall-winter season 2015-2016.

After trimming, the morphological and qualitative characteristics were considered: foot conicity was evaluated through the crown circumferences and foot plantar circumference relationship. The hoof hardness was evaluated through a digital durometer Sama tools (Shore D). To obtain the mean values on every hoof portion, a double measurement on wall, white line (WL) and sole were performed. The thickness of wall and white line was measured through a digital caliper (SAMA Tools IP67 L200).

During the trimming, nail samples from the left front foot of each equine were taken. The hoof samples were washed with water and ethyl alcohol (Sargentini *et al.* 2012), then were submitted to pre-drying (60 $^{\circ}$C /24 h.), followed by the recovery humidity room (24 h). The samples were dried and crushed with an electric mill, then with an analytic mill "A 11 basic", grinding through a discontinuous shock rotating knife (Sargentini *et al.* 2012). The chemical composition of the samples were performed in order determine the water content, obtained with a preliminary sample hoof prehydratation (60 $^{\circ}$C /24 h.) and following drying up (105 $^{\circ}$C/4 h.); the crude protein was evaluated and crude ashes were evaluated as reported by Tocci *et al.* (2017).

The mineral content in nail was carried out in CeRA laboratories of the "Dipartimento di Scienze e Tecnologie Agrarie, Alimentari e Forestali (DAGRI)", through the Inductive Coupled plasma-optical emission spectroscopy (ICP-AES - IRIS INTREPID II XSP). Were considered the main animal body minerals; the macroelements calcium, potassium, magnesium, sodium, phosphorous, the microelements copper, iron, manganese, selenium, and zinc, the oligoelement nickel. Some extraneous elements in nail were also considered: aluminium, lithium, lead, strontium. The mineral data were submitted to two way ANOVA, using JMP statistical software JMP 10 (SAS, 2001), considering as fixed factor the breed. The differences among means were compared with the Tukey test, considering as limit P-value $\leq$ 0.001.

On mineral content a principal component analysis (PCA) was also performed. PCA is a technique for reducing the dimensionality of such datasets, increasing interpretability but at the same time minimizing information loss. It does so by creating new uncorrelated variables that successively maximize variance (Jolliffe and Cadima, 2016). The number of factors to rotate was chosen following the eigenvalues-greater-than-one rule proposed by Kaiser (Sargentini *et al.* 2018), applying the Varimax rotation, which allows the transformation of the solution so that the Rotated Component Matrix can be relatively easy to understand (Abdi and Williams, 2010).

Discriminant Canonical Analysis was also applied on mineral content and the distance among breeds was used to construct the graphic representation of centroid distances. Furthermore, squared distances among all arrays were used to draw a heatmap (Haarman *et al.* 2015), which is a graphical representation of data that uses a system of color-coding to represent different values. All statistical analyses were performed through JMP 10 (SAS, 2001).

RESULTS AND DISCUSSION

The morphological characteristics of the study indicated that the hoof size of the animals was larger in AA and MA horse breeds, intermediate size in HA horse breed and smaller in MP pony breed (Table 1). In all breeds the front foot ideal conicity (5/6) (Sargentini *et al.* 2012) was shown in this study. The results concerning the sole, white line and wall hardness (Table 1) have shown significant variations among breeds and the MP hoof has shown the lowest values. The wall and white line thickness were the largest in the MA hoof horse and the lowest in MP hoof.

The percentage of chemical composition in hoof nail was shown in Table 2.

Table 1 Measures and physical characteristics of hoof of studied horses

Item		AA	HA	MA	MP	RSD
Crown circumference	cm	36.00±2.28^{ab}	38.22±1.08^{ab}	42.00±1.32^{a}	35.81±0.81^{b}	3.24
Sole circumference	cm	42.00±1.40^{b}	43.55±0.93^{b}	48.16±1.14^{a}	41.69±0.70^{b}	2.80
Conicity index		0.84±0.04	0.88±0.02	0.87±0.02	0.86±0.01	0.006
Sole hardness	H	107.24±5.36^{a}	88.22±5.08^{ab}	81.27±5.69^{b}	73.28±4.02^{b}	16.08
White line hardness	H	76.06±3.45^{a}	81.43±4.32^{a}	77.52±4.67^{a}	60.80±2.86^{b}	11.43
Wall hardness	H	117.82±4.88^{a}	104.81±5.12^{ab}	98.08±4.88^{bc}	81.34±4.04^{c}	16.18
Wall thickness	mm	10.63±0.60^{b}	11.99±0.76^{ab}	13.82±0.90^{a}	6.78±0.82^{c}	2.01
White line thickness	mm	4.76±0.27^{ab}	4.05±0.33^{bc}	5.60±0.40^{a}	2.77±0.36^{c}	0.89

AA: Anglo-Arabian; HA: Haflinger; MA: MA: Maremmano and MP: Monterufoli pony.
The means within the same row with at least one common letter, do not have significant difference (P>0.01).
RSD: residual standard deviation.

Table 2 Chemical composition of hoof of studied horses

Item		AA	HA	MA	MP	RSD
Water content	%	16.24±1.33^{b}	16.76±1.18^{b}	16.09±1.43^{b}	23.03±0.84^{a}	5.15
Dry matter	%	83.75±1.33^{a}	83.24±1.18^{a}	83.91±1.43^{a}	76.96±1.21^{b}	5.15
Crude protein	% on DM	98.53±0.18	98.86±0.18	98.43±0.18	98.24±0.14	0.615
Ashes content	% on DM	1.34±0.15	1.24±0.17	1.32±0.18	1.67±0.13	0.58

AA: Anglo-Arabian; HA: Haflinger; MA: Maremmano and MP: Monterufoli pony.
The means within the same row with at least one common letter, do not have significant difference (P>0.01).
RSD: residual standard deviation.

The water content was the highest in MP hoof (Table 2). The protein and ash content were similar among breeds. The mineral composition in order of breed was shown in Table 3. Among the macroelements, K and P have shown highest content in HA hoof, while Na content was highest in MP. Microelements and trace minerals were less present in the MP hoof, whereas in the other breeds, especially in the AA hoof, Fe, Se and Ni were present in high content. Extraneous elements have shown less content in the MP hoof. The PCA have shown 4 significant values to the Kaiser test that explained enough 65% of total variation (Table 4). The Varimax rotation in the first factor (Table 5), explaining 22.4% of the variability, was identified by 8 of 15 minerals, mainly macroelements (Ca, K, Mg, Na, P). The second factor explained mainly for microelements (Cu, Fe, Se, Zn). In PC1 two groups were individuated: on the left side was the MP hoof, on the right side were AA and HA hooves. Macroelements seemed to identify HA hoof, while microelements characterized AA hoof horse (Figure 1). In PC2 AA and HA were distinct in two groups, in the upper and in lower side respectively. The Raw canonical coefficient of canonical discriminant functions (Table 6) showed in Canonical 1 almost 64 % of the total variance explained: the most discriminant minerals were K, Li, Mn, Ni, P, Pb having positive correlation and Na having negative correlation. Canonical 2 have shown enough 25% of the total variance explained and the most discriminant minerals were K having negative correlation and Fe, Ni, Pb, Zn having positive correlation.

In Canonical 3, showing almost 11% of the total variance, the most discriminant minerals were Al, Ca, Fe, Na, Pb.

The canonical discriminant analysis showing the first against the second canonical variant of the areas indicated how MP hoof was isolated from other breeds because less influenced by the main minerals of can 1 (Figures 2 and 3), but more discriminated by Na. MP hoof was less discriminated in the comparison between the first and the third canonical (Figure 4), while it was completely integrated in the comparison between the second and the third canonical (Figure 5).

Heatmap distances (Figure 6) confirmed that part of MP hooves, indicated the medium-low distances, were distant from the hooves of other breeds. Squared distances among breeds, represented by a heatmap (Figure 6), distinguished two main groups: one very large group on the upper side of the figure and one small red group in the bottom of graphic, including hoof samples of HA (50%), AA (30%), and MP (20%); this group has shown the highest distances in the heatmap.

In the largest group there were three main subgroups with different distances indicated by different colours: blue colour indicates short distances, while red colour indicates large distances. MP hooves clustered together in the first and the second group. This group have shown in the second half the lowest distances in the heatmap. The third cluster, subdivided in two subgroups, included mainly HA and MA hooves.

Table 3 Mineral composition (ppm) of hoof of studied horses (Means±SEM)

Item	AA	HA	MA	MR	RSD
Al	449.64±105.11	356.36±94.01	152.40±116.61	335.62±76.76	420.46
Ca	1198.86±148.57	1189.46±132.87	894.97±164.82	1266.95±108.50	594.29
Cu	5.33±0.74	3.95±0.66	3.74±0.82	3.98±0.54	2.955
Fe	1722.41±310.79[a]	1108.42±277.98[ab]	458.95±344.80[b]	452.80±226.97[b]	1232.20
K	1396.84±228.47[b]	2609.00±204.35[a]	1675.28±253.46[b]	1605.18±166.85[b]	913.89
Li	0.45±0.05[a]	0.62±0.05[a]	0.54±0.06[a]	0.23±0.04[b]	0.22
Mg	304.81±30.50	300.10±27.28	245.64±33.84	260.52±22.28	122.02
Mn	98.84±36.06[ab]	220.87±32.25[a]	138.06±40.00[ab]	14.70±26.33[b]	144.23
Na	382.90±0.8[ab]	321.7± 60.90[b]	162.06±75.53[b]	567.07±49.72[a]	272.35
Ni	3.75±0.55[a]	2.59±0.49[ab]	1.99±0.61[ab]	1.03±0.40[b]	2.21
P	193.78±22.70[b]	317.31±20.31[a]	191.55±25.19[b]	130.05±16.58[b]	90.83
Pb	2.41±0.29[a]	2.07±0.26[a]	2.11±0.32[a]	0.68±0.21[b]	1.15
Se	0.75±0.16[a]	0.18±0.14[ab]	0.39±0.18[ab]	0.13±0.12[b]	0.65
Sr	3.38±0.52	4.75±0.47	3.23±0.58	3.42±0.38	2.09
Zn	128.92±8.75	105.64±7.83	127.30±9.72	106.53±6.40	35.04

AA: Anglo-Arabian; HA: Haflinger; MA: Maremmano and MP: Monterufoli pony.
Macroelements: Ca, K, Mg, Na and P; Microelements: Cu, Fe, Mn, Se and Zn; Trace elements: Ni and Extraneous elements: Al, Li, Pb and Sr.
The means within the same row with at least one common letter, do not have significant difference (P>0.01).
RSD: residual standard deviation.
SEM: standard error of the means.

Table 4 Eigenvalues and variability percentage of minerals in hoof nail

Number	Eigenvalue	Percentage	Cumulative percentage	Kaiser test
1	4.59	30.62	30.62	
2	2.07	13.78	44.40	*
3	1.65	10.99	55.39	*
4	1.46	9.74	65.13	*
5	0.96	6.42	71.54	*
6	0.92	6.14	77.68	NS
7	0.69	4.60	82.28	NS
8	0.61	4.07	86.35	NS
9	0.54	3.57	89.92	NS
10	0.42	2.82	92.74	NS
11	0.36	2.40	95.14	NS
12	0.27	1.82	96.96	NS
13	0.20	1.35	98.31	NS
14	0.16	1.05	99.36	NS
15	0.10	0.64	100.00	NS

* (P<0.01).
NS: non significant.

Table 5 Varimax rotation factor scores for the four-factor model for minerals in hoof nail

Item	Factor 1	Factor 2	Factor 3	Factor 4
Al	0.07	-0.07	0.06	**0.81**
Ca	**0.82**	0.25	-0.06	0.27
Cu	0.12	**0.67**	-0.06	0.20
Fe	0.05	**0.81**	0.11	-0.11
K	**0.49**	-0.16	-0.02	**-0.52**
Li	**0.33**	0.20	**0.74**	0.24
Mg	**0.90**	0.15	-0.01	-0.10
Mn	**0.62**	0.19	**0.48**	**-0.39**
Na	**0.30**	-0.06	**-0.69**	0.16
Ni	0.25	**0.75**	**0.31**	-0.06
P	**0.52**	-0.24	**0.65**	-0.12
Pb	0.05	**0.50**	**0.49**	0.21
Se	-0.08	**0.39**	0.17	**-0.37**
Sr	**0.81**	**0.33**	0.20	0.07
Zn	0.20	**0.58**	-0.09	-0.13
% of variability	22.4	18.5	14.1	10.1

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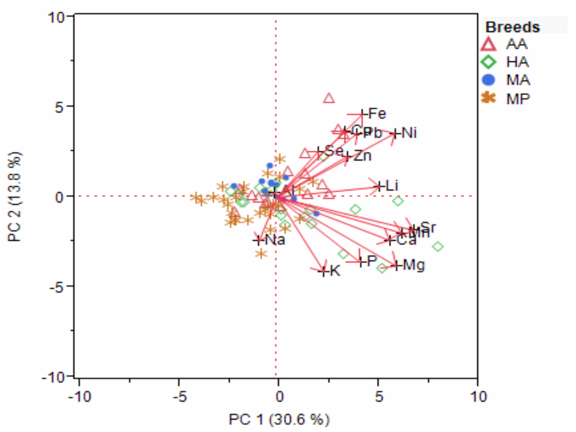

Figure 1 Biplot of mineral content in hoof of different breeds: PC1 *vs.* PC2

Table 6 Raw canonical coefficient of canonical discriminant functions[1]

Item	Canon [1]	Canon [2]	Canon [3]
Al	-0.03	0.11	**0.33**
Ca	-0.13	-0.03	**0.30**
Cu	0.00	0.23	0.14
Fe	0.21	**0.33**	**0.37**
K	**0.35**	**-0.40**	0.26
Li	**0.68**	-0.01	-0.01
Mg	0.11	0.08	0.26
Mn	**0.56**	-0.11	0.11
Na	**-0.48**	-0.09	**0.37**
Ni	**0.32**	**0.38**	0.23
P	**0.66**	-0.20	**0.37**
Pb	**0.55**	**0.36**	0.01
Se	0.11	**0.44**	0.00
Sr	0.20	-0.20	0.26
Zn	0.09	**0.33**	-0.22
Eigenvalue	0.15	**0.07**	0.04
% of total variance explained	63.80	**1.08**	0.45
Prob>F	< 0.0001	< 0.0001	0.14

[1] Lambda di Wilk 0.0607689; F approx 5.0156; DF number 55; Den DF173.63 and Prob >F < 0.0001.

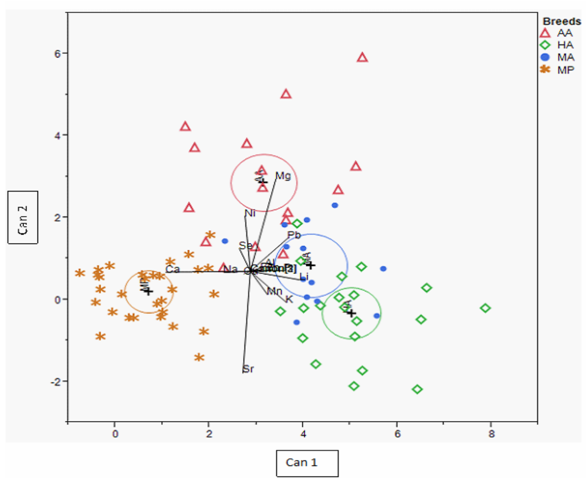

Figure 2 Biplot of canonical (CAN) discriminant analysis showing the first against the second canonical variant of the mineral content in hoof of different breeds

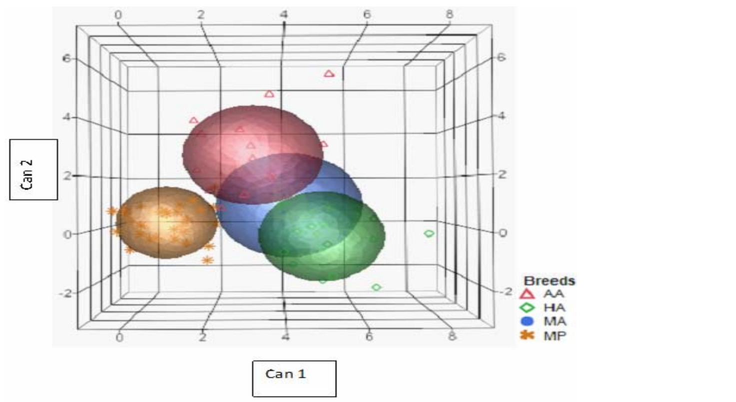

Figure 3 Three-dimensional plot of canonical (CAN) discriminant analysis showing the first against the second canonical variant of the mineral content in hoof of different breeds

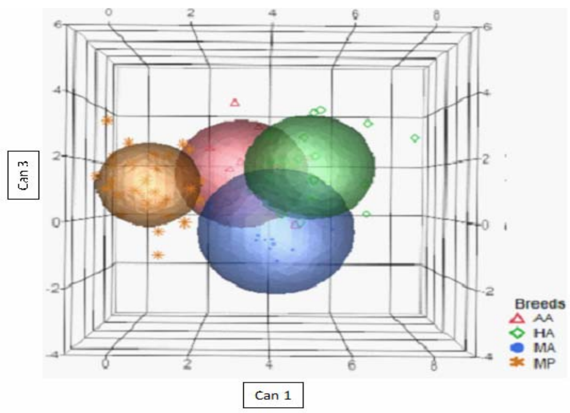

Figure 4 Three-dimensional plot of canonical (CAN) discriminant analysis showing the first against the third canonical variant of the mineral content in hoof of different breeds

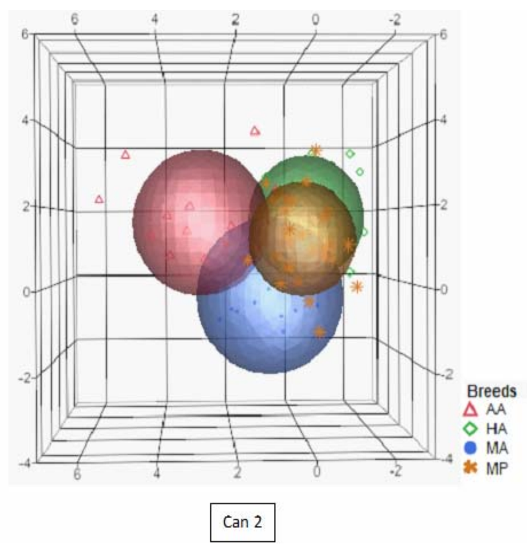

Figure 5 Three-dimensional plot of canonical (CAN) discriminant analysis showing the second against the third canonical variant of the mineral content in hoof of different breeds

The hoof morphology agreed with the morphological characteristics of the examined breeds of the trial: MA, having an average height of 163 cm and an average weight of 530 kg (Tocci *et al.* 2009) and AA, having an average height at withers of 162 cm and an average weight of 543 weight (Tocci *et al.* 2017) are the largest horses, while MP, having an average height at withers of 135.4 in males and 129.2 in females an average weight of 282 kg is the smallest, and HA, having an average height at withers of 146 and an average weight of 359 kg (Falaschini *et al.* 2003) is intermediate.

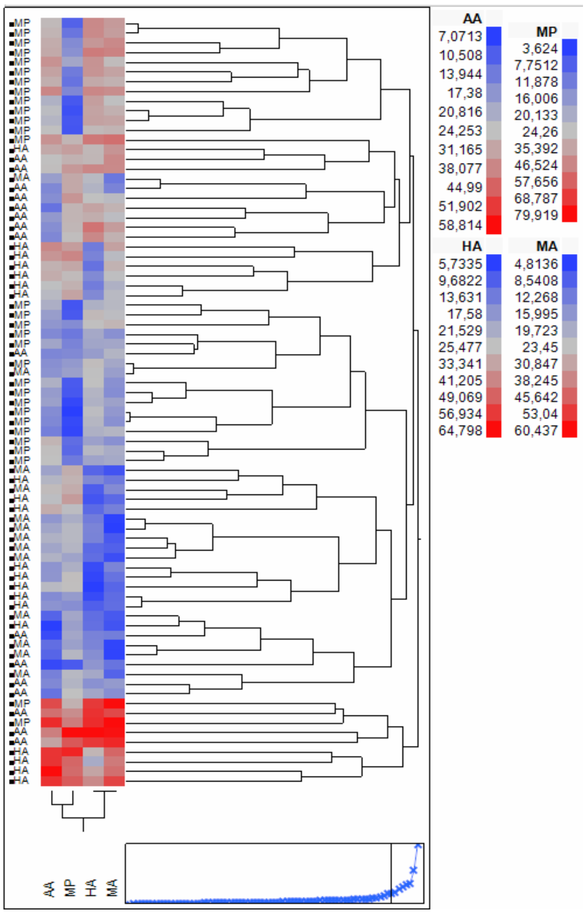

Figure 6 Heatmap of the square distance among mineral content in hoof of different breeds

The hoof hardness is conditioned by different factors: breed, dry or wet season, husbandry conditions (Tocci *et. al.* 2017). According to some authors (Sargentini *et al.* 2012) a hard hoof not necessarily allows to a resistant hoof. The water content in nail is strongly conditioned by the season (dry or wet), the soil characteristics and the rearing system (Sargentini *et al.* 2012). The different water content in nail affects the hoof elasticity; a low content of water in hoof coincides with a high hydrogen bonds quantity, with consequent nail cells hardening (Tocci *et al.* 2017). The water percentages in AA, HA and MA hooves met the literature results (Goodman and Haggis, 2009) concerning horses and ponies reared in wet regions of the Continental Europe. Nevertheless, other authors (Landers, 2006) found higher water content than that found in MP hoof. If compared with Mangalarga Marchador and Pataneiro hooves (Faria *et al.* 2005), the crude protein content was slightly higher in this study, while the ash content was slightly lower. Among the minerals, calcium content agreed with the same parameter found on Thoroughbred horse hoof (Ley *et al.* 1998). If compared with this trial, the Ca content of Mangalarga (Faria *et al.* 2005) and Arabian horse (Abdin-Bey, 2007) hooves was very low. Also, Pantaneiro horse hoof has shown lower values in Ca (Faria *et al.* 2005). P content met the literature results (Faria *et al.* 2005; Abdin-Bey, 2007). Among the microelements, Cu content agreed with literature values for Mangalarga and Arabian horse hoof, while if compared with Pantaneiro horse hoof the Cu content was lower (Faria *et al.* 2005; Abdin-Bey, 2007). Zn content agreed with Arabian and Pantaneiro horse hooves, while Mangalarga horse hoof has shown lower values. PCA analysis confirmed the results of previous studies (Tocci *et al.* 2017), where Na, which seemed to act as osmoregulator removing dangerous minerals, identified MP hoof. HA hoof was characterized by Ca, K, P, Mn, Mg, Sr and the AA hoof was indicated by Fe, Cu, Ni, Pb, Se and Zn; MA hoof was not clearly identified by the minerals the discriminant analysis and the heatmap of the square distance among mineral content in hoof have shown how MP hoof was different from that of other breeds. Both discriminant analysis and heatmap distances have shown how AA hoof was present in all clusters, indicating the past crosses of Thoroughbred and Arabian equines with the breeds considered in this study.

CONCLUSION

This study allowed individuate the quality of the horse hoof and the main minerals in hoof nail were individuated. Considering both multivariate analyses, the most important minerals in hoof were: K, Li, Mn, Na and P. K, Mn, and P characterized the HA hoof. MP hoof was far from other breeds and showed a high Na content; this result confirmed the osmoregulation activity in the MP found in previous studies. AA hoof was mainly characterized by Li. MA horse hoof was not identified by minerals. The hoof quality found in this study can suggest the barefoot practice for these breeds during the usual and not hard equestrian activities.

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