G.J.A.S.R

Global Journal of Animal Scientific Research

Journal homepage: www.gjasr.com

Print ISSN: 2345-4377 Online ISSN: 2345-4385

Original Article

Qualitative Hoof Characteristics in Anglo-Arabian Horses and Monterufoli Ponies Reared in the Same Farm

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ARTICLE INFO

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How to cite this article:
Tocci, R., C. Sargentini, A. Martini, and A. Giorgetti. 2015. Qualitative Hoof Characteristics in Anglo-Arabian Horses and Monterufoli Ponies Reared in the Same Farm. *Global Journal of Animal Scientific Research.* 3(1):20-29.

Article History:
Received: 2 September 2014
Revised: 15 September 2014
Accepted: 17 September 2014

ABSTRACT

This work aims the hoof morphological, physical, chemical, and mineralogical characteristics in Anglo Arabian horses, and Monterufoli ponies reared in Tuscany. 28 nail samples from wall and sole of hoof were analysed. All feet were healthy and well conformed. The hoof of Monterufoli Pony was more cylindrical, and the Anglo Arabian hoof was harder (H 112.8±4.9, and H 119.4±2.7 in sole and in wall respectively). The percentage of dry matter (83.03±0.67) was greater in Anglo-Arabian hoof, while the percentage in crude protein and in ashes in hoof was similar between breeds. The minerals were in greatest concentration in the Anglo Arabian hoof wall. Monterufoli Pony hoof seemed to excrete the minerals through the probable osmoregulation activity of Na, which has shown negative correlation with many minerals, and through the nail consumption, that is softer than that of Anglo Arabian. In principal component analyses of test results for both breeds, 10 significant factors (ratio of variance = 96.88) and 5 factors that explain 79.65% of cumulative variance were found. PCA analysis has shown a quite separation between breeds, confirming for the Anglo Arabian hoof the greater trend to accumulate minerals on nails, and for Monterufoli Pony hoof the antagonist activity of Sodium (Na) against other minerals.

Keywords: hoof quality, hoof composition, Anglo-Arabian Horse, Monterufoli Pony, hoof shaving.

INTRODUCTION

Among the studies on integumentary system on animals in livestock breeding (Cecchi *et al.*, 2011; Sargentini *et al.*, 2012; Ebrahiem *et al.*, 2014) interesting are those concerning the horse hoof characteristics. The hoof is one of the most "burdened" body-parts of the horse. On one hand its outer part is largely responsible for protecting the hoof from external influence such as abrasive, noxious substances or infectious agents (Abdin-Bey, 2007). The hoof quality is very important ("no foot no horse") in sport equines, but also in local equine breeds used in marginal areas and in rough terrain. The barefoot horse, when it's possible, is a cheaper practice for the horse breeder, because the foot care is limited to cleaning and levelling of the hoof. This work aimed the qualitative characterization of Anglo-Arabian horse and Monterufoli Pony hooves. Anglo-Arabian horse is a widespread breed, having Thoroughbred and the Arabian horse ancestors, and now used as endurance riding horse. The Monterufoli Pony is an endangered breed (Regione Toscana, 1997), which derives from the Pisa province, and now used as horseback riding, pet therapy, and sport equine (Tocci, 2010). Arabian horses are famous for their excellent hooves (Abdin-Bey, 2007), and the Monterufoli Pony is historically valued for the quality of its hoof (Braccini, 1947; Benedettini, 1999). The aim of this work was the morphological, physical, chemical, and mineralogical evaluation of healthy and well-conformed hooves of Anglo Arabian and Monterufoli Pony, reared under the same conditions in the same farm.

MATERIALS AND METHODS

In this study the morphological, physical-chemical and mineralogical characteristics were evaluated in the front left hoof of 8 Anglo-Arabian horses (age: 7.4±3.5 years; live weight: 453.4±17.9 kg) and 6 Monterufoli Ponies (age: 7.3±2.3 years; live weight: 348.0±47.9), reared in Cornocchia (SI) farm. The diet was similar in both breeds, and based on local fodder and concentrate meal. The trial was performed during the fall-winter.

To check the nail integrity, during the hoof trimming, a visual analysis was performed. The crown and the plantar surface circumferences were measured and their ratio (conicity index) was calculated (Catalano, 1984). Was also evaluated the wall, the white line and the sole hardness, measured in H (kg/mm^2), through a portable durometer shore A (Sama tools, Digital Hardness Tester HT 65 10 A).

During the trimming, 28 hoof samples were taken from the left front foot of each equine: 16 samples from Anglo-Arabian hoof (8 from wall, and 8 from sole), and 12 from Monterufoli Pony hoof (6 from wall, and 6 from sole). The hoof samples were washed with water and ethyl alcohol (Faria *et al.*, 2005), than were submitted to pre-drying (60°/24 h.), followed by the recovery humidity room (24 h). The samples were previously crushed with an electric mill, then with an analytic mill "A 11 basic", grinding through a discontinuous shock rotating knife (Sargentini *et al.*, 2012).

To determine the moisture in wall and sole, the samples were dried in stove (105°/4 h.). Ashes were also determined, through the UE official methodologies (First Commission Directive 71/250 EEC of 15 June 1971 OJ L 155/20, 12.7.1971), while the total crude protein was determined through the Kjeldahl CEE-ASPA method (Martillotti *et al.*, 1987). Crude protein and ash content had expressed as a percentage of the dry matter (DM) of a nail sample.

The hoof minerals quantitative analyses were performed in CeRA (Centro Interdipartimentale di Ricerca per la valorizzazione degli Alimenti) laboratory of Florence University, through Inductive Coupled plasma-optical emission spectroscopy (ICP-AES - IRIS INTREPID II XSP). Were determined important macroelements: Calcium (Ca), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorous (P), Ca/ P ratio; microelements: Copper (Cu), Iron (Fe), Manganese (Mn), Selenium (Se), and Zinc (Zn); some oligoelements as Boron (B), Crhome (Cr), and Nickel (Ni), and some extraneous elements: Aluminium (Al), Barium (Ba), Cadmium (Cd), Lithium (Li), Lead (Pb), Strontium (Sr).

The hoof morphological and physical data were analysed through the one way ANOVA, considering as fixed effect the breeds. The chemical and the mineralogical composition data were submitted to two way ANOVA, through JMP statistical software JMP 10 (SAS Institute, 2013), considering as fixed effects the breed (B), the hoof region, and their interaction. When the interaction was not significant, it was deleted from the model. The differences among means were compared with t Student test. The Pearson correlation coefficients were calculated between the mean hoof hardness and the mean moisture content. The reciprocal correlation among minerals was also calculated with JMP 10 (SAS Institute, 2013) considering both breeds together.

To discern the controlling process in attribute space principal component analysis (PCA), also called principal factor analysis, was used which is based on the major axes in a data cloud. These axes are derived from the correlation matrix by extracting the eigenvectors and subsequently deriving a matrix with factor loadings with an a priori chosen number of factors. Subsequently, to maximise the variance of the loadings on the factors, varimax rotation was applied (Davis, 2002). A principal component, or factor, is assumed to represent a controlling process or feature that captures a specific relevant source of variance. PCA can reduce the number of variables of the multivariate dataset to a few factors that may be indicative for mineral content in nail hooves.

RESULTS

The hoof of both breeds was well-conformed, and showed similar crown and plantar face circumferences; the crown and plantar face ratio (conicity index) was higher in Monterufoli Pony hoof (table 1). The Anglo-Arabian hoof was harder, in wall and in sole, than that of Monterufoli Pony. The white line was softer than wall and sole, and similar between breeds. The wall thickness was higher in Anglo-Arabian horse. The white line thickness in Anglo-Arabian was greater than that of MonterufoliPony hoof.

Table 1: Morphological and physical characteristics on hoof

parameters	Anglo-Arabian	Monterufoli Pony
Crown Circumference (CC) (cm)	36.0±0.7	35.1±0.9
Plantar Face Circumference (PFC) (cm)	42.9±0.8	40.3±1.0
CC/PFC ratio	0.83^{b}±0.007	0.87^{a}±0.008
Sole Hardness (H)	112.8^{a}±4.9	86.7^{b}±5.2
White line Hardness (H)	75.5±2.5	71.7±3.1
Wall Hardness (H)	119.4^{a}±2.7	113.7^{b}±3.3
Wall thickness (mm)	10.5^{a}±0.5	6.7^{b}±0.6
White line thickness (mm)	4.8^{a}±0.2	2.7^{b}±0.3

a, b – values in rows with different letters differ significantly (P≤0.05)

The dry matter content was higher in the hoof of Anglo-Arabian. The crude protein and the ash content were similar between breeds. The dry matter, crude protein, and the ash content was higher in wall respect the sole, that is wetter and elastic (table 2). A negative Pearson correlation between hardness and moisture content in wall and in sole was found: Pearson coefficient: -0.56; R2 -0.33; F ratio 0.004 in wall; Pearson coefficient: -0.50; R2 0.25; F ratio 0.0088 in sole.

Table 2: Chemical composition on Anglo-Arabian Horse and Monterufoli Pony Hoof

	Breed		Hoof Region		Significance level		
Parameter	Anglo-Arabian Horse	Monterufoli Pony	Wall	Sole	Breed	Hoof Region	Breed × Hoof Region
Dry matter (% on WB[1])	83.03±0.67	75.07±0.78	82.11±0.60	75.93±0.81	**	**	N.S.
Crude protein (% on DM[2])	98.25±0.10	98.32±0.11	98.17±0.09	98.40±0.11	N.S.	N.S.	***
Ashes (% on DM[2])	1.75±0.09	1.67±0.11	1.85±0.09	1.56±0.11	N.S.	**	**

N.S. - not significant;** P < 0.01;*** P< 0.001; [1]WB = Wet basis;[2]DM = Dry Matter

The hoof of Anglo Arabian Horse and Monterufoli Pony had different Al, Ca, K, Li, Mg, Mn, Ni, P, Pb, Zn content; these minerals, except K, were in higher content in the Anglo-Arabian hoof (table 3).

The greatest content in crude protein was in sole of Monterufoli Pony and in wall of Anglo-Arabian Horse; the lowest content was in wall of Monterufoli Pony (table 4). The greatest ash content was in wall of Monterufoli Pony and in sole of Anglo-Arabian Horse; the lowest ash content was in sole of Monterufoli Pony (table 4).

Table 4. Interaction between breeds and hoof regions for chemical and mineralogical content

parameters	Anglo-Arabian Horse		Monterufoli Pony	
	Sole	**Wall**	**Sole**	**Wall**
Crude protein (%)	98.14^{bc}±0.15	98.37^{ab}±0.11	98.67^{a}±0.17	97.97^{c}±0.15
Ashes (%)	1.86^{ab}±0.15	1.65^{bc}±0.11	1.28^{c}±0.17	2.07^{a}±0.14
Ca (ppm)	1088.1^{a}±133.1	1284.9^{a}±117.4	372.9^{b}±143.8	1295.5^{a} ±143.8
Li (ppm)	0.17^{b}±0.05	0.66^{a}±0.04	0.05^{b}±0.05	0.01^{b}±0.05
Mg (ppm)	323.8^{a}±324.8	290.0^{a}±30.7	142.5^{b}±37.6	278.6^{a}±37.6
Ni (ppm)	0.9^{a}±0.7	6.0^{a}±0.6	0.4^{b}±0.8	2.1^{b}±0.8
P (ppm)	217.1^{a}±20.6	175.5^{ab}±18.2	91.6^{c}±22.3	151.7^{bc}±22.3
Zn (ppm)	117.2^{b}±6.0	138.0^{a}±5.3	72.2^{c}±6.5	137.8^{a}±6.5

a, b, c: values in rows with different letters differ significantly (P<0.05)

The table of the interactions (table 4) showed that the wall of Anglo-Arabian hoof had the higher content in Ca, Li, Mg, Ni, P, Zn. The sole of Monterufoli Pony hoof showed the lowest content in these minerals.

In table 5 many correlations between minerals were shown, and the majority were positive, especially for Ca and Zn. Only K and Na have shown significant negative correlation: K with Fe, Li, Ni, Pb, Zn, and Na with Zn.

The PCA identified 10 significant components at Bartlett test (table 6): the first five components covered enough 80% of the total variability, constituted by 15 parameters concerning the mineral composition in hoof. Over the 5, the components eigenvalues were lower of 1, and were not significant for the interpretation of PCA results (Podani, 2007).

Table 6. Eigenvalue, cumulative percentage of variance and Bartlett test

Eigenvalue

Number	Eigenvalue	percentage	20 40 60 80	Cumulative percentage	Chi-square	DF	Prob>Chisq
1	5,1509	34,339		34,339	296,884	102,380	<,0001*
2	2,3233	15,488		49,828	217,341	96,912	<,0001*
3	1,6834	11,223		61,050	182,697	86,619	<,0001*
4	1,5438	10,292		71,343	156,475	75,780	<,0001*
5	1,2459	8,306		79,648	126,649	65,187	<,0001*
6	0,8004	5,336		84,985	97,648	54,959	0,0003*
7	0,7048	4,698		89,683	79,212	45,273	0,0013*
8	0,4147	2,765		92,448	58,374	36,491	0,0122*
9	0,4012	2,675		95,122	47,966	28,304	0,0119*
10	0,2636	1,757		96,880	33,288	21,047	0,0437*
11	0,2051	1,367		98,247	23,370	14,777	0,0712
12	0,1343	0,895		99,143	13,028	9,508	0,1910
13	0,0706	0,471		99,613	4,403	5,236	0,5249
14	0,0339	0,226		99,839	0,381	1,997	0,8258
15	0,0242	0,161		100,000	.	.	.

Eigenvalue ≤ 1,0 - not significant

Table 3: Mineral composition (ppm) in order of Breed and of Hoof Region

	BREED		HOOF REGION		Significance level		
	Anglo-Arabian Horse	Monterufoli Pony	Wall	Sole	Breed	Hoof Region	Breed × Hoof Region
Al	463.4±105.2	25.7±120.9	134.3±109.3	354.6±116.3	**	N.S.	N.S.
Ca	1186.5±88.7	834.2±101.7	1290.2±92.8	730.5±98.0	*	N.S.	***
Cu	5.0±1.0	4.6±1.1	6.9±1.0	2.7±1.1	N.S.	**	N.S.
Fe	1609.1±260.8	819.6±299.9	2120.3±271.1	308.5±288.5	N.S.	***	N.S.
K	1412.0±199.5	2346.8±229.4	1757.8±207.4	2000.9±220.7	***	N.S.	N.S.
Li	0.4±0.003	0.03±0.003	0.3±0.03	0.1±0.03	***	***	***
Mg	306.9±23.2	210.6±26.6	284.3±24.3	233.2±25.6	***	N.S.	**
Mn	93.1±23.1	11.5±26.6	98.0±24.1	6.6±25.6	**	**	N.S.
Na	384.7±44.1	366.2±50.7	360.5±45.9	390.4±48.8	N.S.	N.S.	N.S.
Ni	3.4±0.5	1.2±0.5	4.0±0.5	0.6±0.5	***	***	*
P	196.4±13.7	121.7±15.8	163.6±14.4	154.4±15.2	***	N.S.	*
Pb	2.3±0.3	0.3±0.4	1.9±0.3	0.6±0.4	***	**	N.S.
Se	0.7±0.2	0.2±0.2	0.6±0.2	0.2±0.2	N.S.	N.S.	N.S.
Sr	3.2±0.3	2.6±0.3	4.1±0.3	1.7±0.3	N.S.	***	N.S.
Zn	127.6±4.0	105.0±4.6	138.0±4.2	94.7±4.4	***	***	***

N.S. not significant; * P<0.05;** P< 0.01; ***P< 0.001

Table 5: Pearson correlation coefficient among minerals in both breeds

	Al	Ca	Cu	Fe	K	Li	Mg	Mn	Na	Ni	P	Pb	Se	Sr	Zn
Al	**1**														
Ca	-	**1**													
Cu	-	-	**1**												
Fe	-	-	0.454 **	**1**											
K	-	-	-	0.412 *	**1**										
Li	-	-	-	0.523 **	-0.457 **	**1**									
Mg	-	0.784 ***	-	-	-	-	**1**								
Mn	-	-	-	0.447 **	-	0.615 ***	-	**1**							
Na	0.439 **	-	0.471 **	0.841 ***	-	-	-	-	**1**						
Ni	-	-	-	-	-0.490 **	0.658 ***	-	0.475 **	-	**1**					
P	-	0.560 **	-	0.494 **	-	-	0.561 **	-	-	0.514 **	**1**				
Pb	-	-	-	-	-0.469 **	0.518 **	-	-	-	-	-	**1**			
Se	-	-	-	0.363 *	-	-	-	0.491 **	-	-	-	-	**1**		
Sr	-	0.749 ***	0.520 **	0.412 *	-	0.349 *	0.457 **	-	-	0.565 **	-	0.548 **	-	**1**	
Zn	-	0.675 ***	0.346 *	0.596 **	-0.335 *	0.338 *	0.529 **	0.442 **	-0.392 *	0.514 **	0.530 **	-	0.371 *	0.570**	**1**

- not significant;* P<0.05; ** P < 0.01;*** P< 0.001

In table 7 the component loading matrix after Varimax rotation for mineral characteristics of Anglo-Arabian Horse hoof and Monterufoli Pony hoof was shown.

The strongest component, PC1, is especially strongly linked with 6 out of 15 mineral elements: 4 macroelements (Ca, Mg, Na, P), 1 microelement (Zn), and one extraneous (Sr). The PC2 reflects primarily the microelements and oligoelements.

The PC1 covers 34.3% of the variability, and identify the majority of the considered minerals. As shown in figure 1, these minerals are more highly correlated more they are close together and far from zero; Na, K, and partially Al, head in opposite direction, in the negative part of PC1. Na and K move independently respect the other minerals, and K has shown a negative correlation with potentially dangerous minerals (Al, Li, Ni, Pb). The PC1 quite clearly separated the two breeds (Figure 1). Anglo-Arabian hoof was identified by macro elements Ca, Mg, P, by microelements Mn, Zn, and some extraneous minerals, Li and Sr, and Na, having osmoregulation activity, identified Monterufoli Pony hoof.

The PC2 explains 15.5 % of the variability and showed a more homogeneous distribution among minerals that were placed in both parts, positive and negative, of PC2 (figure 1). The PC2 was strongly linked with two microelements, Cu and Fe, an oligoelement (Ni) and two extraneous minerals, Pb and Sr. In PC2 a separation between breeds was still confirmed, with a distinct group of Monterufoli Pony in the positive part (figure 1).

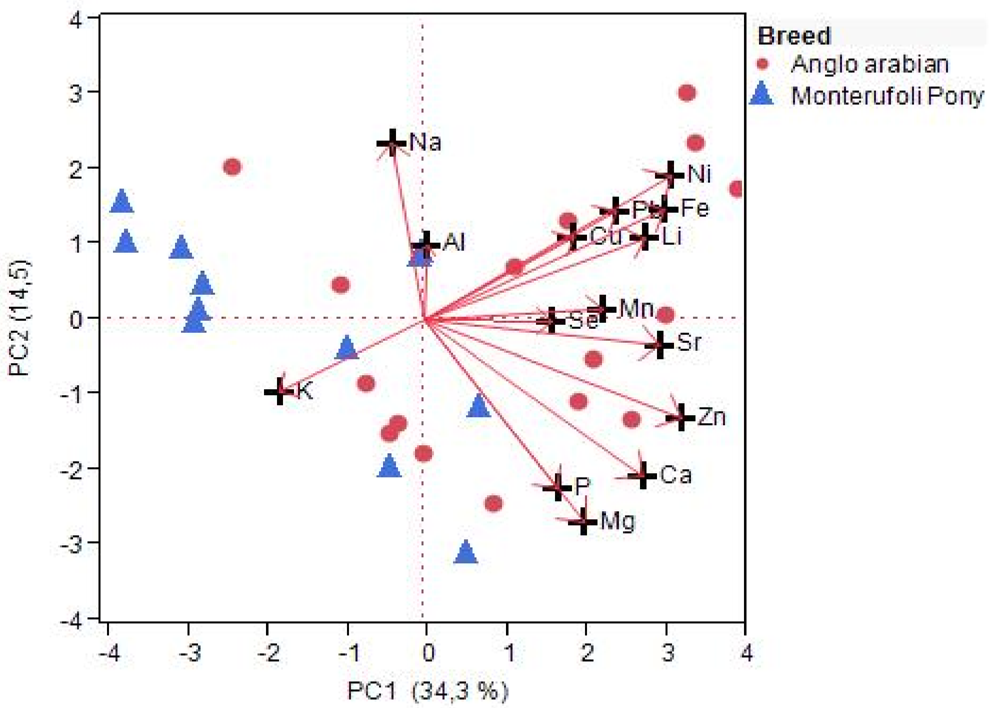

Figure 1. Rohlf Biplot for standardized PCA of Anglo-Arabian and Monterufoli Pony mineral in hoof

DISCUSSION

The visual analysis on hoof met the equine standard literature. The lower crown and plantar face ratio (conicity index) in Monterufoli Pony hoof confirmed as ponies and small horses hooves are smaller and compact (Catalano, 1984).

The greatest hoof hardness in Anglo-Arabian confirmed a high quality: a good hoof is strong and hard (Bertram and Gosline, 1987). The hoof of both breeds was harder than literature data; these latest were very variables, because considered in different breeds and in different hoof regions, with different shore (Albarano and Warzecha, 1994; Coenen and Spitzlei, 1996; Spitzlei, 1996; Hinterhofer *et al.*,1998; Schmitt, 1998; Frohnes, 1999; König and Budras, 2003; Patan and Budras, 2003; Schnitker, 2004; Pütz, 2006). Tocci (2010) found, with shore A, that Maremmano and Haflinger hoof was softer, both in wall and in sole. Zencher (1991) claimed that only hardness cannot indicate hoof of good quality: a too hard nail can be fragile, with consequent cracks and fractures. The expected lower values in white line are justified because this region is the conjunction point among wall, sole and internal hoof structures. This region is the softest and the more vulnerable to bacterial and fungal infections (Budras *et al.*, 1998; Faravelli *et al.*, 2004).

The hoof hardness changes with season, and is higher during the spring and the summer time respect to the cold period (Patan, 2001; Mac Callum *et al.*, 2002; Patan and Budras, 2003; Schnitker, 2004; Pütz, 2006), especially in autumn-winter time, when the terrain is wet and muddy.

The wall thickness in Anglo-Arabian hoof has shown lesser value than literature (Stachurska *et al.*, 2008).

The withe line thickness in Anglo- Arabian was greater than that of Monterufoli Pony hoof was close to the optimum value (3 mm=1.8 inch) indicated as an average value for the horses (Farcus and Alloway, 2010).

Moisture content, conditioned by seasonal effects (Scheuplein and Blank, 1971), geo-pedological characteristics, and husbandry systems (Pütz, 2006), acts on foot mechanical properties.

The moisture determines the nail quality and its water-soluble substances. The sole moisture conditions the absorption capacity of this region, in particular near the white line; this region shows less storage capacity (Bertram and Gosline, 1987).

The moisture values in this study were lower than the literature moisture percentages (Butler and Hintz, 1977; Bertram and Gosline, 1987; Douglas *et al.*, 1996; Patan and Budras, 2003; Landers, 2006; Pütz, 2006), concerning Continental European ponies. The Mediterranean environment is characterised by dry periods conditioning the nail moisture. Some authors claim that at the moisture decrease, corresponds to a hardness increase (Bertram and Gosline, 1987; Zenker, 1991; Douglas *et al.*, 1996; Spiezlei, 1996; Stern, 2000; Patan and Butras, 2003). The negative correlation between hardness and moisture found in this study seemed to confirm this theory. Other authors say that a nail with an average moisture content is elastic (Bertram and Gosline, 1987), while a very dry or too hydrated hoof show less elasticity (Bertram and Gosline, 1987; Leach and Zoarb; 1995; Douglas *et al.*, 1996), with consequent nail damages (Warren Evans, 1992).

The crude protein, essentially constituted by keratin, has shown very high values (over 90% on dry matter) and higher than the literature values (Hunt, 1994; Faria *et al.*, 2005; Kellon, 2008).

The ashes content in hoof was comparable with that of horses reared in Tuscany (Tocci *et al.*, 2010), and lower than that of Mangalarga Marchador and Pantaneiro breeds (Faria *et al.*, 2005).

The minerals having different content in hoof of both breeds were macroelements, Ca, P, K, that play a major role in the life of animals (El hag *et al.*, 2014) Mg, microelements, Cu, Mn, Ni, Se, Zn, and extraneous and potentially toxic minerals, Al and Pb. The Ca content, wich has been shown to be important in the integrity of the hoof wall (Harper, 2005), in Anglo-Arabian hoof showed similar value of those found by Abdin-Bey (2007) in Arabian pure breed. The Ca content in Monterufoli Pony hoof has shown an intermediate value between the hoof of Arabian horse (Abdin-Bey, 2007) and those of Draft horses found by Sasimowski *et al.*, (1987), and Mangalarga Marchador and Pantaneiro horses (Faria *et al.*, 2005). The P content in Anglo-Arabian hoof was slightly lower of that of Arabian horse hoof (Abdin-Bey, 2007).

The Cu content has shown similar values to those of Sasimowski *et al.,* (1987) and of Faria *et al.*, (2005) for Mangalarga Marchador horse hoof. Zn content had higher content in Anglo-Arabian Horse hoof, and similar to the values found by Abdin-Bey (2007), Weiser *et al.*, (1965), Sasimowski *et al.*, (1987) and Faria *et al.*, (2005) for the Pantaneiro horse hoof. Ca, Cu, Zn and Mg are important in relation to claw affection (Johnson and Schugel, 1994).
The Anglo-Arabian horse hoof tends to cumulate minerals in nail, except K that showed in Anglo-Arabian hoof enough half-content respect the Monterufoli Pony foot.

As found by Tocci (2011), the Monterufoli Pony seemed to cumulate in hoof less quantity of minerals, also dangerous. With regard to this topic it needs to remember that the foot is a dense network arteriovenous that allows nutrition and disposal of toxic waste. The blood circulation on foot is fundamental, and many foot diseases are due to a poor or incorrect blood circulation (Roghi, 1995).

Zn, which had significant correlations with many minerals (table 5), was in high content in skin and in skin appendages (Henzel *et al.*, 1970). K and Na, having negative correlations with many minerals, are biological osmoregulators.

The negative correlation found in PCA of K with some potentially dangerous minerals (Al, Li, Ni, Pb) confirmed as found by Tocci for Monterufoli Pony hoof (2011).

CONCLUSION

This study delineated the morphological, physical, chemical, and mineralogical characteristics of good quality hooves belonging to two breeds appreciated for this characteristic. The hoof morphology was similar between breeds, the Anglo-Arabian horse hoof has shown hardest hoof, and greater content in dry matter. The crude protein and the ash content in hoof was similar between breeds. The mineral content in nail was different between breeds. Through the PCA was possible to see as a lot of important minerals, as Ca, Mg, P, Mn, Zn, were strongly linked with the Anglo-Arabian hoof, while the MP hoof was linked with Na, that probably excretes the minerals that otherwise would accumulate in nail. In Anglo-Arabian hoof there was a higher bioaccumulation of minerals, while the Monterufoli Pony hoof quickly remove the minerals, probably through the nail consumption, that is softer than that of Anglo-Arabian.

ACKNOWLEDGEMENTS

The Authors want to thank the Corpo Forestale dello Stato of Siena. This work was supported by Regione Toscana. Dir. Gen. Sviluppo economico. Progetto per la caratterizzazione ed il recupero produttivo di razze equine; Grosseto and Pisa Provinces: VAGAL (Valorizzazione dei genotipi animali autoctoni) - Programma di cooperazione transfrontaliera Italia Francia “Marittimo 2007-2013”.

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