SHORT COMMUNICATION

Preliminary studies on hoof characteristics in Amiata donkey

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Abstract

In this work the biometrical, physical and chemical characteristics of Amiata donkey hoof were studied. The Amiata donkey is a local endangered breed and derives from the homonym mountain in Tuscany. This donkey, which was once used as pack animal in farms and in mines, is now involved in trekking, onotherapy and milk production. The mean hoof biometrics and its standard deviation were calculated. The physical and chemical characteristics were also estimated, through ANOVA, considering the hoof region as fixed effect: wall, white line and sole for hardness, wall and sole for chemical characteristics. The small size and healthy hoof, was basically cylindrical (crown cir/Foot plantar cir. ratio=0.9) and it showed higher hardness in wall (H=126.5± 3.3), followed by sole (H=105.2±3.3), and white line (H=74.0±3.3). The wall has shown the lower moisture content (%=11.7±3.2) and the higher content in Al, Mn, Li, Ni, Pb, Se. The positive correlation between Al, Li, Pb and Hardness has shown the hoof high resistance to toxic elements. Very interesting has seemed the negative correlation between K vs Li and Pb, to indicate the tendency of K to remove potentially harmful elements.

Introduction

The Amiata donkey is a Tuscan endangered breed, listed in the Repertorio delle risorse genetiche autoctone toscane (Arsia, 2006) and entered in the Registro Anagrafico (R.A.) delle razze Equine ed Asinine a limitata diffusione (http://www.aia.it).

This donkey, native to the Amiata Mount in southern Tuscany is now used for onotherapy, trekking and milk production. This breed can also valorise marginal and minor historical and touristic sites, through the transport of materials and the garbage collection (http://www.scarlinoenergia.it/index.php?page=layout_news&id=2529&lang=it).

The Amiata donkey shows the features of the ancestrals Equus asinus africanus and Equus asinus somaliensis (Sargentini et al., 2009). The african origin of Amiata donkey can also favor studies and search on donkeys in undeveloped countries. In these countries, especially in arid and semi-arid areas, donkeys are now used for cultivation and transport of persons and material.

The aim of this paper is the study of the hoof because the feet health is of utmost importance in equine management. The foot health is conditioned by the nail quality (Tocci et al., 2010). A healthy and strong hoof shows solid and resistant nail, able to protect the inner foot from pathogens (Pütz, 2006), and from the soil roughness, reducing the pressures on animal body due to weight (Bossi, 1926). The hoof and foot care is essential during the various horse activities: the hoof diseases in undeveloped countries, as laminess and pedal sepsi, are caused by improper hoofcare (Luurt, 2004). Bolbol and Saleh (1987) detail the hoof problems of donkeys in Upper Egypt; most hind limb problems are caused by poor hygiene. A resistant hoof can promote the natural hoof care practice, restricting hoofcare operations to ordinary hoof shaving (Tocci et al., 2010).

The nail quality depends from endogenous and exogenous factors. The endogenous factors are the chemical composition, the corneal stratum structure, the amount and distribution of the intracellular fluid, and the keratin type, amount and disposition into the cells. The exogenous factors are the husbandry methods, the diet, the weather, the seasonal effects (Pütz, 2006).

Materials and methods

This study was performed on fifteen adult females reared in the Tocchi farm (Potatine - San Lorenzo a Merse - Siena) and managed by the Corpo Forestale dello Stato - Territorial Biodiversity Office (UTB) of Siena. A visual analysis on hooves was performed, as indicated by Bossi (1926) and Catalano (1984). A visual analysis on wall and a visual analysis on foot surface, to evaluate the longitudinal and transversal diameters, were performed. Were also evaluated: the sole convexity level, the nail quality, the frog development, the wall thickness, and the hoof whole evaluation, considering the shape, the size, the direction and at last the whole nail quality.

On the left front hoof, the following parameters were performed: wall and white line thickness, through digital caliper (Sama tools – IP67 L200); the wall, white line and sole hardness, through a portable durometer shore A (Sama tools, Digital hardness Tester HT 65 10 A). On hoof, the maximum length and width, and the crown circumference and the plantar surface circumference were measured, through digital caliper and meter rule. The foot concility was also calculated through the crown circumference and the plantar surface ratio (Catalano, 1984). Nail samples from wall and sole were taken. These samples, were accurately cleaned (Faria et al., 2005) and subjected to chemical analysis and mineralogram. The nail samples were submitted to pre-drying (60°/24 h), followed by the moisture recovery (24 h). The samples were previously crushed with an electric mill and later with an analitic mill IKA A 11 basic, grinding through a discontious shock rotating knife.

To determine the moisture in wall and sole, the samples were dried in stove (105°/4 h). Ashes were also determined through the official methodologies (First Commission Directive 71/250/EEC of 15 June 1971, Official [Ital J Anim Sci vol.11:e22, 2012]
Journal L 155/20, 12.7.1971), while the total crude protein was determined through the Kjeldahl CEE-ASPA method (Martillotti et al., 1987).

Some important elements in hoof were determined. The elements at high concentration were: aluminum (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), zinc (Zn). The elements at low concentration were: Copper (Cu), lithium (Li), nickel (Ni), lead (Pb), selenium (Se), strontium (Sr). On foot the mean and the standard deviation of maximum lengths and widths, the crown and plantar surface circumferences and their ratio (conicity index) were calculated.

For the chemical-physical and mineralogical characteristics of the nail, datas were submitted to ANOVA one way analysis of variance, through the proc GLM in SAS (SAS, 2002), considering the hoof region as fixed effect. The differences among means were compared with t-Student test, and with the HSD Tukey test, when the levels were more than 2. The Pearson correlation coefficient between the hoof hardness (whole hoof, wall, sole and white line) and the moisture content, and the hoof hardness (whole hoof, wall, sole and white line) and the moisture content, and the reciprocal correlation among minerals were calculated, through the Proc CORR of SAS (SAS, 2002).

Results and discussion

In this study the visual analysis did not show nail problems; the hoof was healthy and the wall did not show breaking or chipping symptoms.

The small foot, as shown by the crown circumference and plantar surface ratio (Table 1), has met the asinine standards (Bossi, 1926). The circumferences ratio (0.91) was higher than in the horses ideal standards (0.86) (Catalano, 1984); donkeys had a more compact and lesser conical hoof than that of horses. The lack of donkey hoof references data availability leads to consider the hoof horse parameters; in general, the donkey hooves are more upright, tougher, and more elastic than those of a horse. The bulbs of the donkey hoof are less developed and the fusion of the bulbs of the heel is less complete. The heels are naturally long. The pastern angles are greater than the horse. The frog of the donkey hoof is not meant to be weight-bearing (McClinchey et al., 2011).

The hardness (Table 2) was higher in wall, followed by sole and lastly by white line. The expected low values in white line are justified because this region is the conjunction point among wall, sole and internal hoof structures. This region is the softer and the more vulnerable to bacterial and fungal infections (Budras et al., 1998; Faravelli et al., 2004). In this study the hoof was healthy and didn’t show any symptoms of disease in nail.

The wall thickness (Table 3) has met the horse literature data (Kasapi and Gosline, 1998). The white line is the sensitive layer separating wall and sole; the thickness of this region was 3.98 mm, more than 3 mm (1/8 inch) indicated as mean value for horses (Farcus and Alloway, 2010). The hoof moisture is strongly conditioned by seasonal factors (Scheuplein and Blank, 1971), geo-pedological and husbandry systems (Pütz, 2006); all these parameters condition the mechanic properties of the hoof. The moisture determines the nail quality and its water-solubles substances content. The mean values have shown significant variations between wall and sole (Table 4). The sole moisture is connected to absorbing capacity of this region, in particular close the white line; this region is with less storage capacity (Bertram and Gosline, 1987). The moisture percentage was lower than the literature data (Butler and Hintz, 1977; Bertram and Gosline, 1987; Kung, 1991; Douglas et al., 1996; Patan and Budras, 2003; Landers, 2006; Pütz, 2006) relating to Northern European horses and ponies, but never to Mediterranean donkeys. About 16% and about 20% in moisture content on dry matter were found by Bossi (1926) and by Pütz (2006) respectively, while Douglas et al., (1996) founded to range between 20% and 35%.

The total crude protein content and the total ashes content did not differ between wall and sole (Table 4). The crude protein content, predominantly keratin, constituted more than 98% of dry matter, and it has shown higher values than the horses literature data: 93% in Huntingdon and Pollit, (2005) and 94% in Jackson (1996).

The ashes content was comparable with the hoof ashes content of Anglo-Arabian, Haflinger and Marammuno (Tocci et al., 2010) and lower if compared with Monterafoli pony hoof, that showed percentage values of 1.6 (Tocci et al., 2010) and with Mangalarga Marchador and Pantomene hoof that showed on average 1.9 and 2.1, respectively (Faria et al., 2005).

In Table 5 the elements at high concentration in wall and in sole are shown. The Al content in wall was significantly higher than in sole. This element can derive in hoof from the external environment, considering that Al is the third on the Earth’s crust, after silicon and oxygen (Merendi, 2006). The Ca content did not show significant variations between sole and wall, although Weiser et al. (1965) founded higher content in horse hoof wall. The Ca content was higher than the hoof literature data relating to horses: on average 653.6 ppm (Beaumont et al., 2009) and 232.7 ppm in Mangalarga Marchador and 567.6 ppm in Pantomene (Faria et al., 2005).

The Fe content was not different between hoof regions and it showed very high value if compared with references data (Butler and Hintz, 1977). This latest result may be due to the outdoor husbandry conditions. Probably, the high rainfall during the trial has promoted a highly acidic environment, with consequent Fe solubilisation, easier to adsorbe in nail; this is the forth element as concentration on Earth’s crust, after silicon, oxygen and Al. The Amiata donkeys are reared in areas characterized to heavy metal rich soils, in particular Fe (Guerrini, 1986).

The K content, similar between sole and wall, has shown higher concentrations than the 225 ppm related in Warren Evans (1992). The Mg content was higher than the corresponding reference data (68.5 ppm in Warren

Table 1. Hoof biometric characteristics (mean±SD).

| Characteristic          | Wall         | Sole         | White line  |
|-------------------------|--------------|--------------|-------------|
| Crown circumference, cm | 31.36±1.45   | 23.2±2.6A    | 74.0±3.3C   |
| Foot plantar circumference, cm | 34.43±1.45 | 90.2±3.3A    | 74.0±3.3C   |
| Crown circ./FP circ. ratio | 0.91±0.03 | 0.91±0.03    | 0.91±0.03   |
| Maximum hoof length, cm | 12.0±0.7     | 12.0±0.7     | 12.0±0.7    |
| Maximum hoof width, cm  | 8.78±0.5     | 8.78±0.5     | 8.78±0.5    |

Table 2. Hoof hardness (mean±SEM).

| Region       | Wall         | Sole         | White line  |
|--------------|--------------|--------------|-------------|
| Hardness, H  | 126.5±3.3A   | 105.2±3.3A   | 74.0±3.3C   |

Table 3. Hoof thickness (mean±SEM).

| Region       | Wall         | White line  |
|--------------|--------------|-------------|
| Thickness, mm| 9.0±0.3A     | 3.98±0.3A   |

Table 4. Hoof chemical composition (mean±SEM).

| Region       | Wall         | Sole         |
|--------------|--------------|--------------|
| Moisture, %  | 11.7±3.2A    | 23.2±2.6A    |
| Crude protein, % DM | 98.8±0.1   | 99.5±0.2    |
| Ashes, % DM  | 1.2±0.1      | 1.0±0.1     |
Evans, 1992), and it was significantly higher in the sole than in the wall. Many authors claim that the partially pigmented hooves show higher Mg content than the not pigmented hooves (Naumann et al., 1987). The Na content was not different between sole and wall.

The P content (Table 5), the main mineral in hoof (Kovacs and Szilagyi, 1973), was similar to data references (Pütz, 2006), and it was higher in sole than in wall. The Zn content was higher in sole than in wall. In Table 6 the elements at low concentration in wall and in sole are shown. The Cu content was comparable between the hoof regions. The obtained results were lower than the 12 ppm in Warren Evans (1992) and comparable with the Beaumont et al. results (2009) and with the Marchador horses results (Faria et al., 2006). Faria et al. (2006) have found higher values in Pantaneiro horse’s hoof. Weiser et al., (1965) have found higher content in wall than in sole.

The results of this study (average of 4.5 ppm) are comparable with the Cu content in Earth’s crust (Merendi, 2006), to indicate the probable extra-biotic origin of this element in hoof. Li, Ni, Pb and Se have shown higher content in wall than in sole. Ni showed high concentrations (23.4 ppm in wall and 3.7 ppm in sole); it can derive from diet (e.g. the whole oat and from rye seeds, that contain relatively high Ni content: 2-3 ppm), but also from the contaminated soil contact (Sharma, 2007). Pb, highly toxic heavy metal, has shown higher content in wall than in sole. Horses are Pb susceptible; a daily intake in diet of 1.7 mg/kg live body weight, in a trial during the winter time, was toxic (Aronson, 1972). Se, a microelement essential at low content (0.1 ppm), has shown higher concentration in wall than in sole. Se excesses lead to mane and hair loss, laminitis, ringing and chipping and nail loss in wall (Lewis, 1995; Subcommittee On Mineral Toxicity In Animals, 1980). Other authors claim that, in comparison with healthy hooves, altered hooves show a double Se content. In this study all donkeys have shown healthy hoof, and did not suffer the Se excesses. The high Se content in Amiata donkey hoof may be due to species characteristics or to particular geo-pedological conditions. The Sr content was comparable between wall and sole, showing a similar trend to Ca, with which this mineral has metabolic relationship (Mills, 1969). Reference datas were not found, but the not toxicity of this mineral protects from possible excesses. Sr can derive from diet or from soil contamination (Subcommittee On Mineral Toxicity In Animals, 1980).

In this work, the low moisture values and the high hardness values have never resulted in damaged hooves; hardness and moisture never been correlated (Table 7). Some authors claim that the hoof mechanical properties are conditioned by the moisture content; at the moisture decrease, corresponds to an hardness increment (Bertram and Gosline, 1987; Douglas et al., 1996; Naumann et al., 1987; Patan and Budras, 2003; Spitzlei, 1996; Stern, 2000; Zenker, 1991). A nail with a average moisture content is elastic (Bertram and Gosline, 1987), while a very dry or too hydrated hoof shows less elasticity (Bertram and Gosline, 1987; Douglas, 1996; Leach and Zoerb, 1983), with consequent compression or cut nail break (Butler K.D in Warren Evans, 1992).

High quantity of hydrogen bonds is found in hoof containing low moisture, leading to hardening of nail cells. A high hardness in hoof can indicate a dry nail, more prone to breakage. The hardness was positively correlated with Al, Ni and Pb, all potentially toxic elements (Table 8), which tended to accumulate mainly in wall (Table 6). These elements seemed to determine, together with the main elements in hoof (Weiser et al., 1965. Baggott et al., 1988; Bodurov et al., 1981), the resistance and maintenance of wall, the hardest hoof region; the same elements are than removed through the hoof consumption. The wall seemed to have a

| Table 5. High concentration mineral composition in hoof (mean±SEM). |
|---------------------------------------------------------------|
| **Wall** | **Sole** |
|-------------------------|-------------------------|
| Aluminum, ppm | 315.2±25.2<sup>a</sup> | 216.9±25.2<sup>b</sup> |
| Calcium, ppm | 1350.0±54.2 | 1354.6±54.2 |
| Iron, ppm | 437.8±65.5 | 589.2±65.5 |
| Potassium, ppm | 1067.7±112.8 | 1690.5±112.8 |
| Magnesium, ppm | 286.7±20.5<sup>c</sup> | 347.7±20.5<sup>c</sup> |
| Manganese, ppm | 54.8±6.3<sup>c</sup> | 35.2±6.3<sup>c</sup> |
| Sodium, ppm | 267.6±15.0 | 249.2±15.0 |
| Phosphorus, ppm | 193.5±12.8<sup>c</sup> | 234.3±12.8<sup>c</sup> |
| Zinc, ppm | 108.7±3.7<sup>c</sup> | 120.2±3.7<sup>c</sup> |

<sup>a,bP<0.05; A,BP<0.01.</sup>

| Table 6. Low concentration mineral composition in hoof (mean±SEM). |
|---------------------------------------------------------------|
| **Wall** | **Sole** |
|-------------------------|-------------------------|
| Copper, ppm | 4.4±0.6 | 3.5±0.6 |
| Lithium, ppm | 0.7±0.05<sup>a</sup> | 0.2±0.05<sup>b</sup> |
| Nickel, ppm | 23.3±4.9<sup>c</sup> | 3.7±4.9<sup>c</sup> |
| Lead, ppm | 3.6±0.2<sup>a</sup> | 0.9±0.2<sup>b</sup> |
| Selenium, ppm | 3.8±0.7<sup>c</sup> | 0.4±0.7<sup>c</sup> |
| Strontium, ppm | 4.4±0.9 | 3.2±0.9 |

<sup>a,bP<0.001; A,BP<0.001</sup>

| Table 7. Pearson correlation between moisture and hardness in hoof. |
|---------------------------------------------------------------|
| **N observations** | **Moisture** |
|-------------------------|-------------------------|
| Hoof | 25 | -0.37 |
| Sole | 15 | -0.08 |
| Wall | 10 | 0.03 |

| Table 8. Hoof mineral content and hoof hardness (H) Pearson correlation (N observations=30). |
|---------------------------------------------------------------|
| **H** | **Al** | 0.43<sup>a</sup> |
| | **Ca** | -0.19 |
| | **Cu** | 0.22 |
| | **Fe** | -0.16 |
| | **K** | -0.54<sup>b</sup> |
| | **Li** | 0.56<sup>b</sup> |
| | **Mg** | 0.26 |
| | **Mn** | 0.26 |
| | **Na** | -0.05 |
| | **Ni** | 0.33 |
| | **P** | -0.32 |
| | **Se** | 0.63<sup>b</sup> |
| | **Sr** | 0.44 |
| | **Zn** | 0.25 |
| | **Zr** | -0.45<sup>b</sup> |

<sup>a,bP<0.05; A,BP<0.01.</sup>
very important role in the foot protection and functionality, also through the element replacement and removing. The hardness was instead negatively correlated with K and Zn; this latter element was positively correlated with Al, Li and Pb (Table 9). The heavy metals seemed to condition the hoof hardness and the K promoted the hoof health through the osmoregulation. Considering the healthy hoof, Zn seemed to condition the protein component, that promote the resistance and the elasticity. Al, not only was positively correlated with Cu and Li, but also with Mn, Sr, and Zn, all minerals useful for the hoof quality. The positive correlation with Ni and Se was instead harmful, considering their toxicity at high concentrations. Li has shown positive correlation with Mn, Ni and Se, all essential elements at low concentration but toxic at high content, and with Pb, can be no favourable for the hoof quality. The negative correlation with Zn may be harmful for the hoof health, because Zn is an important element involved in the Zn-proteins building. The Li content was quite low (Table 6), and probably it has met the phisiological content of the asinine hoof. Se was positively correlated with Al, Li, Ni and Pb. This result seemed to indicate a positive reaction of this element towards toxic elements. Se is a potentially dangerous element, but it’s not involved in hoof hardness. The metabolic relationship between Sr and Ca, favors the hoof quality. Zn has shown negative correlation with Al, Li and Pb. These correlations may negatively influence the hoof quality. The observed feet did not show defects or chippings on hoof, confirming the harmful element accumulation high tolerance in this breed.

Conclusions

The Amiata donkey hoof was small and tendentially cylindrical; it has shown good quality and high hardness, in particular in wall. The low moisture content, higher in sole but lower than the literature data relating to horses, can be connected with the African origin of this species; in particular Amiata donkey has ancestors deriving from the Dancalia desert. The crude protein content was very high; it’s higher than the literature data. The very high accumulation of heavy metal (Al, Li, Ni, Pb e Se), especially in wall, didn’t involve healthy problems in hoof, indicating the high adaptation of this breed to a hostile pedological environment, naturally rich in these elements. The sole had high Mg, P and Zn content, showing inverse trend of references data concerning horses; these elements favor the hoof quality. The positive correlation between Al, Li, Pb and hardness confirmed the harmful elements tolerance of the Amiata donkey hoof. The negative correlation between K and hardness can be correlated with the high organic matter accumulation, that caused the hoof softening. The negative correlation between Zn and hardness can be explained because this element is involved in the keratine building (Zn-protein), but it’s not involved in hoof hardness. Very interesting has seemed the negative correlation between K with Li and Pb, to indicate the probably trend of K to remove harmful elements, during its osmotic activity. The Amiata donkey hoof seemed acts as bio-accumulator and as emunctory organ of harmful elements.

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Table 9. Correlation between hoof mineral content.

| Element | Al | Ca | Cu | Fe | K | Li | Mg | Mn | Na | Ni | P | Pb | Se | Sr | Zn |
|---------|----|----|----|----|---|----|----|----|----|----|----|----|----|----|----|
| Al      | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Ca      | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Cu      | 0.51** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Fe      | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| K       | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Li      | 0.73** | ns | ns | ns | ns | -0.49** | ns | ns | ns | ns | ns | ns | ns | ns |
| Mg      | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Mn      | 0.40* | ns | 0.38* | ns | ns | 0.61** | ns | ns | ns | ns | ns | ns | ns | ns |
| Na      | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Ni      | 0.41** | ns | ns | ns | ns | 0.49** | ns | ns | ns | ns | ns | ns | ns | ns |
| P       | ns | ns | ns | ns | ns | 0.53** | ns | ns | ns | ns | ns | ns | ns | ns |
| Pb      | 0.54** | ns | ns | ns | -0.50** | 0.81** | ns | ns | ns | ns | 0.53** | -0.46** | ns | ns |
| Se      | 0.43** | ns | ns | ns | ns | ns | 0.54** | ns | ns | ns | ns | ns | 0.96** | ns | 0.64** |
| Sr      | 0.47** | ns | 0.96** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Zn      | -0.48** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

ns, not significant; *P<0.05; **P<0.01.
