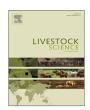
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Grazing behaviour of Miranda donkeys in a natural mountain pasture and parasitic level changes



M. Couto ^{a,b}, A.S. Santos ^{b,c,*}, J. Laborda ^d, M. Nóvoa ^d, L.M. Ferreira ^e, L.M. Madeira de Carvalho ^a

- a CIISA, FMV, Lisboa, Portugal
- ^b Escola Universitária Vasco da Gama, Coimbra, Portugal
- ^c CITAB-UTAD, Vila Real, Portugal
- d Associação para o Estudo e Proteção do Gado Asinino, 5225-011 Atenor Miranda do Douro, Portugal
- e CECAV-Departamento de Zootecnia, Universidade de Trás-os-Montes e Alto Douro, PO Box 1013, 5001-801 Vila Real, Portugal.

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ABSTRACT

The Miranda donkey (Equus asinus) has an important role in the maintenance of rural landscape and traditions in inland of Portugal. Breeding and keeping of these animals rely on mountain pasture areas, known for their characteristic botanical diversity. However, their grazing behaviour in these areas has not been described and this knowledge is important to set the best management practices that promote an efficient use of these particular feed resources that could be related with parasitic level of these animals. In this way, this study aimed to assess feeding behaviour, diet selection, and relate the total phenolic and tannin content of ingested plants with the parasite load in a group of Miranda donkeys under free ranging system in natural mountain pastures. For this purpose, eight adult non-pregnant Jennies from the Miranda donkey breed were managed under continuous grazing on a 1.6 ha natural mountain pasture from late May to late July. Body condition score (BCS) and grazing behaviour of the Jennies was assessed in two different occasions. Additionally, the level of gastrointestinal nematode infections of the herd was assessed throughout the study. Results showed that animals spent 75.6% of total observation time in search/prehension activities corresponding to an average of 16 h/day. Jennies showed a preference for herbaceous species, although they were able to incorporate in their diet up to 30% of shrubs, suggesting that they can be used as biological tool for controlling and reducing shrub encroachment in these mountain areas. Parasite level increased from 0% positive samples in June to a maximum of 25% in July. The increase of EPG may be related with the observed decrease in the shrub consumption throughout the observation period. Having in account the presence of phenolic and condensed tannins in shrubs, their potential as natural anti-parasitic compounds and the possibility of taking part in donkey diet, these preliminary results can be of the utmost importance regarding an integrated approach of Miranda donkey parasite control.

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1. Introduction

The Miranda donkey is one of the two native Portuguese donkey breeds. These animals are an integrating part of the local communities of the Trás-os-Montes region in the inland north of Portugal, they play an important role in maintaining rural communities and sustainable farming practices, and are a part of the local genetic biodiversity, being a key player in rural development of these territories nowadays. The Miranda donkey is well-

E-mail address: anasofiasantos@euvg.pt (A.S. Santos).

conformed and characterized by a "rustic" appearance. The main coat is dark brown with lighter shades on the sidewall and bottom surface of the trunk, white muzzle and around the eyes, with hirsutism accented in broadsides, face and edges of ears and extremities, and abundant manes. Their average withers height is 1.35 m (Quaresma et al., 2005). Depending the season and the feed availability, nutritional requirements of these animals will vary along the year and changes in body condition score (BCS) may occur if proper supplementation is not given (McDonalds et al., 2002).

These animals are still mostly used as working animals, both in farming work and more recently in equestrian tourism. They are traditionally bred under extensive systems often together

^{*}Corresponding author at: Escola Universitária Vasco da Gama, Coimbra,

with cattle, where pasture supply is the basis of their feeding regimes. Grazing areas mostly used by the Miranda donkey are the traditional mountain pastures, commonly known as "lameiros". "Lameiros" are semi-permanent natural mountain pastures characterized by a high local botanical diversity including a considerable amount of shrub areas. These semi-permanent natural mountain pastures have been mostly abandoned, due to exodus of rural population. However, recently a new concern has arose to maintain these areas as a part of an integrated, selfsufficient production system since they have an incalculable environmental and landscape value and their conservation is an important objective to the maintenance of high levels of biodiversity in mountain areas. The establishment and development of livestock systems in less favoured areas can provide an environmental and economic benefit, contributing to the improvement of living conditions of rural population (Ferreira et al., 2013, Garcia et al., 2013). Both the Miranda Donkey and the lameiros play a role in its agroecosystem.

Parasitic infections, especially by gastrointestinal parasites, particularly nematodes, are common in donkeys and are considered one of the major animal health and welfare problems, having an influence on their body condition and daily work routine (Yoseph et al., 2005). Infections occur by the ingestion of freeliving third stage larvae (also called L3), often when grazing in pastures. There is an increasing evidence of nematode resistance to conventional anthelmintic products in equids and therefore, natural alternative and/or complementary antiparasitic treatments are being pursued (Cernea et al., 2010; Payne et al., 2013).

Recent evidence (Villalba et al., 2006) suggests that animals can self-medicate, and if so medicinal plants on diet could provide an important tool for parasite control. The use of plant secondary metabolites may be an effective way to treat animals, as much as phenolic compounds such as condensed tannins can provide beneficial medicinal effects to herbivores as they act against infectious conditions, such as parasitism (Juhnke et al., 2012). The "Lameiros" complex and biodiverse botanical composition, may allow animals to select specific plants that may have an influence in what concerns their natural resistance to gastrointestinal parasites, namely on the donkeys parasite resistance. However, information concerning grazing behaviour of donkeys on these types of pastures is very scarce and knowledge on how animals interact and select this specific botanical diversity could present interesting results to both natural parasite control in donkeys, as well as to the management of these type of biodiverse mountain pastures. In this way, the objective of this study was to observe the feeding behaviour, assess diet selection and relate the total phenolic and tannin content of ingested plants with the parasite load in a group of Miranda donkeys under free ranging system in these natural pastures, during the summer grazing season.

2. Material and methods

2.1. Experimental site, animals and study design

The study was carried out in 2013 on a single plot of 1.6 ha pasture located at 651 m above sea level in the northern inland of Portugal, at Atenor, Miranda do Douro (41°24′48.12′′N; 6°29′15.38′′O). The climate characteristic of the region has high Mediterranean influence. Mean annual rainfall was 554.7 mm, and rainfall was persistent until the end of May, leading to a high pasture availability in the beginning of June. Mean monthly temperature recorded at the site during the study months was 15.3 °C in June and 23.4 °C in July. The plot used in this study is characterized by vegetation dominated by a mosaic of pasture grass sub-divided by shrub. At the beginning of the study, the lameiro plot was

dominated by pasture with an average height of 50 cm, composed of several herbaceous species (grasses: *Bromus* sp., *Dactylis glomerata*, *Lolium* sp., *Briza* sp., *Calamagrotis* sp., *Avena* sp., *Cynosurus* sp., *Agrostis* sp., legumes: *Lathyrus* sp., *Trifolium* sp., *Ononis* sp., *Anthylis* sp., *Vicia* sp. and other non identified genera of herbaceous plants), and shrubs (*Quercus* sp. dominated: *Quercus* robur; *Q. ilex*; *Q. pyrenaica*, *Q. faginea*, and also Narrow-leafed ash: *Fraxinus angustifolia*, hawthorn: *Crataegus monogyna*, ulmus: *Ulmus* L., and gorse: *Cytisus* spp.).

A total of 8 adult non-pregnant jennies from the Miranda donkey breed were managed under continuous grazing from late May to late July. Jennies' BCS was assessed on a scale of one to five (one=poor, five=obese) according to Smith and Wood (2008), at the beginning and end of the study.

2.2. Grazing behaviour and diet selection

The study was distributed in two observational periods, period 1 carried out in June (1–4 June) and period 2 July (16–19 July) of 2013. These two close observation periods where chosen in order to allow the collection of data in two different situations: high and good quality pasture availability (period 1) and low quality and amount of pasture availability (period 2). Since climatic conditions in this area are characterize by a rapid temperature elevation together with an inexistence of rainfall in the summer months (from June to August), this leads to rapid changes in vegetation type and availability, and very often, pastures availability goes from high, to very low within a month time span.

Each observational period (11 days) comprised a seven-day for adaptation of animals to the diet and experimental conditions; followed by four days of sample collection: faeces and diet components (herbaceous and shrub), and feeding behaviour observation: time spent grazing, by each animal, was assessed by recording the grazing activity every 15 mins during the light period of the day (from sunrise to sunset) on two consecutive days in two observation periods (2–3 June and 17–18 July).

In the same periods, diet selected by each animal was estimated using the n-alkane markers (Dove and Mayes, 2006). For that, individual faecal samples were collected twice a day for four days in each observation period (i.e. 1–4 June and 16–19 July). Simultaneously, samples of the main herbaceous components found in the plot and shrub vegetation were collected. Since the plant species within the herbaceous vegetation and within the shrub vegetation presented resemblances in their alkane profile, only two diet components were considered: i.e. herbaceous and shrub. This procedure allowed us to discriminate these two distinct feed resources that was the main objective of this study.

Diet composition was estimated for each animal using a nonnegative least-squares procedure in the "EatWhat?" software (Dove and Moore, 1995) which minimizes the discrepancies between the observed concentrations of each n-alkane $(C_{25}-C_{33})$ in the faeces and the estimated proportions of plant components in the diet.

Alkane faecal concentrations of animals were not adjusted as previous results (Ferreira et al., 2007) showed that faecal recovery of these markers in equids is unaffected by carbon-chain length.

2.3. Parasitological evaluation

All animals had been previously dewormed in January of 2013, by subcutaneous administration of ivermectin 2% (1 ml per 50 kg body weight). During the whole study periodic analysis of faecal samples was performed to control the parasitic load in the group between June and July, namely on the 2nd June, 2nd July, and on the 22nd July. Spot samples of donkey faeces were individually collected directly from the rectum to assess gastrointestinal

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