Doi: 10.1002/jsfa.8334

Running title: Plant extract and sensory evaluation of *Equidae* meat

Effect of dietary plant extract on meat quality and sensory parameters of meat from *Equidae*.

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ABSTRACT

BACKGROUND: Plant extracts as *Lippia spp.* have been proven antioxidant properties. Recent studies have been shown that dietary supplementation with plant extracts are able to enhance meat quality parameters. Studies regarding meat quality in *Equidae* are limited.

RESULTS: The effect of dietary plant extract (PE), containing verbascoside, on meat quality, oxidative stability and sensory parameters of *Longissimus Lumborum* (LL) muscle in *Equidae* was studied. Dietary treatment did not affect (P>0.05) pH, colour indices and chemical parameters of muscle in both donkey and horse. Dietary PE improved (P<0.01) oxidative stability in donkey muscle during refrigerated storage. Sensory characteristics of LL muscle were positively affected (P<0.05) by dietary PE in both donkey and horse. In particular, colour, taste and texture were enhanced in LL muscle from animals fed PE. Oxidative stability was lower (P<0.05) in LL muscle of horse than donkey.

CONCLUSION: Dietary plant extract, containing verbascoside, can be considered as a natural source of antioxidants, able to improve oxidative stability of donkey meat and to affect sensory attributes of *Equidae* meat.

Keywords: *Equidae*, Meat quality, Nutrition, Plant extract, Antioxidant, Sensory parameters.

INTRODUCTION

Meat is subjected to a quality deterioration and one of the main causes is related to oxidative phenomena. It is affected by several factors such as fatty acids composition, content and activity of pro and antioxidants, temperature, oxygen pressure, surface area in contact of oxygen and water activity. [1] Dietary supplementation with antioxidant in farm animals is an effective strategy for preventing meat oxidation.[2,3] Several compounds exert antioxidant activities, improving colour, flavour and oxidative stability in meat.[4] The attention in dietary plant extract, containing polyphenols, has been increasing in recent years. In fact, polyphenols, extracted from many plants and spices, showed a high antioxidant activity.[5,6] In *Verbenaceae* family, Verbascoside (VB) is the most abundant phenolic compound, with higher antioxidant activity compared with the other substances.[7,8] Several studies in farm animals, reported the *in vivo* antioxidant properties of VB.[9,10] Recent studies reported an improvement of meat quality parameters of broiler and pig fed plant extract, containing verbascoside. [3,11,12]

Equidae meat have been an important food source, particularly, in some European countries, where it is traditional consumed.[13] In general, the management practices for horses are traditional, sustainable and in harmony with local environment. [14] Even if donkey breeding is less popular than horse, its meat represented an interesting product that can increase the income of local farmers. [15] Meat derived from these species has excellent nutritional properties, rich in bioavailable iron, and low in fat and cholesterol. [16-18] The fatty acid profile, with a higher omega 3 fatty acids content compared to beef and pork, make this product more suitable for human health.[19] Moreover, this meat is appreciated for its slightly sweet taste, due to the high content of glycogen. [20]

In literature, data on sensory characteristics of horses meat are limited, [21] and no previous study reported the sensory profile of donkey meat. Moreover the effect of dietary supplementation with natural antioxidant on meat sensory characteristic and quality parameters in *Equidae* was evaluated. The aim of the present study is to evaluate the effects of dietary supplementation with plant extract,

containing verbascoside, on meat quality parameters, oxidative stability and sensory characteristics in *Equidae*.

MATERIALS AND METHODS

Animals, diets and sampling

Procedures involving animals were carried out in accordance with the European Communities Council Directive (2010/63/EU) and approved by the Italian Ministry of Health (DL 26, 2014 march 4th).

Twelve weaned male donkeys of the Martina Franca breed and twelve weaned males Avelignese horses were selected and individually reared indoor in two different farms. The animals were randomly divided in two experimental groups. The control groups of horses (CH) and donkeys (CD) fed a control diets. The other groups of horses (PEH) and donkeys (PED) fed the same diets supplemented with plant extract. The plant extract supplement contained a water-soluble extract of *Verbenaceae* (*Lippia spp.*) leaves. The dietary integration provided 0.5 mg verbascoside per kg^{-1} of metabolic weight ($LW^{0.75}$). This dosage was chosen on the basis of our previous study. [3] The horses and donkeys were fed *ad libitum* oat hay and wheat straw (1:1 ratio) with concentrate feed, that was gradually increased, to meet the requirement for all nutrients. Commercial feed was composed by corn, wheat, soybean meal, wheat by-products, field beans (*Vicia faba minor*) and mineral/vitamin premix (crude protein, 183 g kg^{-1}, crude fibre 38 g kg^{-1}, ether extract 35 g kg^{-1}). The experimental diets were administered for 6 months. The animals were slaughtered at 12 months of age. After an on-farm fasting period of 8 hours, the animals were transported to the slaughterhouse. The average slaughter weight: was 540 ± 25 kg for horses and 295.6 ± 8.6 kg for donkeys. The animals were laired for 4 hours with free access to water. The animals were stunned with a captive bolt, slaughtered, skinned and eviscerated according to current European Union regulations (Council Directive 95/221EC). After slaughtering, from the right side of the carcasses approximately 400 g of muscle was taken from Longissimus Lomborum (LL) of donkey (CD n=6; PED n=6) and horse (CH n=6;

PEH n=6) between the 9th and the 10th rib. The samples were cut crosswise to the fibres in slices of 15 mm and each slice was vacuum packed in coded plastic bags free from flavour transmission and immediately stored at -20 C° for 7 days until laboratory analyses. The day before assessment all samples were transferred to a cooling chamber with a temperature of 4° C, pending analyses.

Meat physical and chemical parameters

The pH measurement was performed using a pH meter (HI 9023 microcomputer, Hanna Instruments, Vila do Conde, Portugal). Colour measurements were determined, using a CR-300 Chroma Meter (Minolta Camera, Co., Osaka, Japan). The instrument was calibrated on the CIE LAB colour space system using white calibration plate (Calibration Plate CR-A43, Minolta Cameras). The colorimeter had an 8-mm measuring area and was illuminated with a pulsed Xenon arc lamp (illuminat C) at 0° viewing angle. Reflectance measurements were obtained at a viewing angle of 0° and the spectral component was included. The measurement values were given in the color spectrum Commission Internationale d'Eclairage (CIE), where L* is lightness; a* is redness; and b* is yellowness. Each data point is the mean of three replications measured at the chop surface. Samples of LL were analysed for dry matter, crude protein, ether extract and ash according to Association of Analytical Chemists methods.[22]

Lipid oxidation in relation to storage time (0 and 4 days) at 4°C was determined by the thiobarbituric acid reactive substances (TBARS) method.[23] The absorbance at 532 nm was measured with spectrophotometer and compared with a standard curve of malonaldehyde prepared by hydrolysis of tetraethoxypropane. The results were expressed as mg of malondialdehyde (MDA) per kg^{-1} of meat.

Sensory analysis, sample preparation and evaluation

The slices of 15 mm were cooked for 4 min at greatest power (200 °C) on double-plated grills. A thermocouple (Pentronic AB, 198 Gunnebobruk, Sweden) was inserted in the centre of each piece of meat to register the core temperature. The core temperature was not allowed to exceed 68°C and

116 therefore the horse and donkey meat was removed from the oven at approximately 60 to 65°C to
117 avoid post-heating rise. Afterwards, the meat of both species was sliced into 15-mm cubes for
118 presentation to panelists.[24] Each panelist received four cubes of each sample, in white plastic plates,
119 labeled with three-digit random codes and covered with lids. Unsalted crackers and water were
120 provided for panelists to clean their palates between samples.

121 A selected and highly trained sensory panel, consisting of 8 members (four man and four
122 women) with a testing experience with several food products, in particular meat and meat products,
123 was chosen. All assessments were carried out in an equipped sensory laboratory according to ISO
124 8598 recommendations.[25]The training period of the judges lasted for 2 months. The aim of this
125 training session was to develop a common vocabulary to describe the *Equidae* meat samples. The list
126 of descriptors for *Equidae* meat with the relevant definitions is reported in Table 1. The judges
127 evaluated separately the two couple of samples (CD vs PED and CH vs PEH) in triplicate. The
128 sessions were performed on three different days. Prior to tasting, panelists determined colour intensity
129 on fresh meat and subsequently, panelists evaluated intensities of each attribute established in the
130 orientation phase and scored them on a 10 cm unstructured line scale with two anchors, with 0
131 meaning none and 10 meaning extremely strong, according to ISO 4121.[26] They were asked to score
132 texture, flavor and taste during chewing Within each session the design was balanced for order and
133 carry over effects.[27]

134

135 ***Statistical analysis***

136 All statistical procedures were computed using SPSS 22.0 (IBM SPSS Statistics, Italy). Data
137 on physical and chemical parameters were analyzed by two-way analysis of variance (ANOVA)
138 where diet and specie were the main factors. Data related to oxidative stability during storage time
139 were assessed by repeated measures ANOVA, to evidence the effects of treatment, time, and their
140 interactions. The sensory data for each attribute were submitted to Analysis of Variance (ANOVA)
141 with dietary treatment (CD *vs* PED and CH *vs* PEH), judges, replicates and their interactions as

142 effects. The significance of these effects was tested with F test. Means were compared according to
143 Duncan test with a level of significance at $P < 0.05$. The means of the dietary treatment averaged
144 across judges and replicates were submitted to Principal Component Analysis (PCA) in order to
145 interpret sensory differences among LL muscles. Correlations between variables were determined by
146 Pearson's linear correlation coefficient.
147

148 **RESULTS and DISCUSSION**

149 ***Meat quality parameters***

150 The pH, colour indices and nutrient content of LL muscle from donkeys and horses are
151 reported in Table 2. No effect (P>0.05) of dietary plant extract supplementation on meat quality
152 parameters were observed in *Equidae* meat. Specie significantly affected (P<0.001) the yellowness
153 (b*) of meat. No interaction (P>0.05) between factors was observed. The available literature on meat
154 quality parameters of *Equidae* meat is limited. In particular, no studies reported the effect of dietary
155 supplementation with plant extract on meat quality in both horses and donkey. In our study, no effect
156 of PE dietary supplementation on pH and colour indices in *Equidae* were observed. The pH values
157 were within the range expected for horse and donkey meat and the mean values are comparable with
158 those reported in literature.[18,28] In the present study, muscle colour indices were not affected by PE
159 supplementation, according with previous studies in pig LD muscle. [3,29]. A higher b* values was
160 observed in horses meat than donkeys. This difference between specie could be explained by the
161 different meat fatty acid composition of the intramuscular fat according to Mancini and Hunt. [30] In
162 fact, as previously observed in horse meat by Lee *at al.* [19], the b* values were strictly linked to the
163 intramuscular fat polyunsaturated fatty acids (PUFA) content.

164 The chemical parameters of LL muscle from Martina Franca donkey and horses are in line
165 with the results obtained in *Equidae* muscle. [15,31] In Figure 1 are shown the oxidative stability of LL
166 muscle stored at 4°C for 4 days in relation to dietary treatment. In donkey, dietary inclusion of PE
167 decreased LL muscle (P<0.001) oxidative phenomena during refrigerate storage at 4°C (Figure 1a).

168 As expected storage time negatively affected oxidative stability in LL muscle (P<0.001).
169 There are no previous studies regarding TBARS values in donkey LL muscle. The present findings
170 indicated that meat from donkey fed PE had a higher protection from lipid oxidation, in agreement
171 with several studies reporting a lower TBARS concentration in muscle of animals fed plant
172 antioxidants, containing polyphenols. [3,11,32]

173 The TBARS values in horse meat showed no difference (P>0.05) between PE and control
174 group (Figure 1b). The present data are in line with the values reported in horse fillet. [33] The reason
175 for the different susceptibility to lipid oxidation in the two species is probably due to the higher PUFA
176 content of horse meat (210-415 g kg^{-1}) than donkey (130-251 g kg^{-1}). [21,34,28] The oxidative stability
177 of meat and meat products depends on the balance of anti- and prooxidants and the substances
178 susceptible to oxidation including PUFA. A high content of these fatty acid negatively affected the
179 oxidative stability because of their greater susceptibility to oxidative breakdown. So, the addition of
180 a proper amount of an antioxidant was requested to slow down the oxidative processes.[35-37]

181

182 ***Sensory evaluation***

183 The spider plots for sensory traits of LL muscle are reported in Figure 2. In donkey LL muscle
184 (Table 3), the F values for replicates were not significant for all the descriptors, except for metallic
185 flavour (P<0.05). The interactions between samples and judges were significant only for sweet taste
186 (P<0.01), while judges for replicate were significant for typical aroma and sweet taste (P<0.01) and
187 metallic aroma (P<0.05). Judges presented differences for all the descriptors (P<0.001), except red
188 colour. In horse LL muscle (Table 4), the F values for replicates were not significant for all the
189 descriptors, except for typical aroma (P<0.05). The interactions between samples and judges were
190 significant only for typical aroma and salty taste (P<0.05), while judges for replicate were significant
191 for typical sweet taste (P<0.05). Judges presented differences for all the descriptors (P<0.001). The
192 difference between judges is a common condition in sensory evaluation due to the use of different
193 parts of a scale when making judgments. [38]

194 Red colour, typical aroma, sweetness and tenderness resulted higher (P<0.05) in LL muscle
195 of donkey fed PE and fibrousness resulted lower (P<0.05) in PED than CD (Figure 2a). The red colour
196 resulted higher in muscle from PE treatment than control (above 8), according to previous studies
197 reporting a preserving effect of antioxidant from plant extracts on meat colour. [39,11] Furthermore, this
198 result is also related to the lower TBARS content observed in meat from PE supplemented donkey.
199 In fact oxidative processes in meat contribute to the degradation of colour pigments. [40]
200 Concerning the aroma, a higher score was observed in LL muscle from animal fed PE than
201 controls. This parameter resulted important for the consumers eating habits and it is strictly linked
202 with consumers expectation of meat quality. [41]
203 The texture sensory descriptors valuated by trained judges resulted higher for tenderness (above 7)
204 and a lower for fibrousness (above 5) in LL muscle from PE treatment than controls. The higher
205 values of tenderness in the antioxidant group might be due to the protection exerted against oxidation
206 process as observed in lambs by Moran *et al.*[42]. The authors reported that antioxidants play an
207 important role in the protection of μ-calpain and m-calpain against oxidation, during the aging
208 process. In fact, as reported by Huff-Lonergan and Lonergan [43] the enhancement of protease
209 functionality may positively affect meat tenderness that represents one of the most important meat
210 quality characteristic for the consumers.
211 The Pearson correlation revealed that juiciness was positively related to fat content (r= 0.754
212 P=0.042). This data, observed also in horse LL muscle, are in agreement with those reported in
213 literature in different species.[44,45] No significant correlations were found between the other sensory
214 properties and the chemical parameters.
215 In horse, the sensory descriptors related to typical and metallic aroma, saltiness and tenderness
216 resulted higher (P<0.05) in LL muscle from animal fed PE than control (Figure 2b). Moreover a less
217 fibrousness (P<0.05) was observed in LL muscle from PEH group than CH. Regarding taste, a high
218 scores for sweet in PEH (above 8) and a low scores for salty in CH (above 4) was observed. Also for
219 the aroma, typical (above 7) and metallic aroma (above 6) was higher in LL muscle from PEH than

220 controls. The textural parameters are affected by PE dietary treatment, in fact tenderness resulted
221 higher (above 8) and fibrousness (above 5) resulted lower than controls. No differences between
222 treatments was observed for juiciness. The texture parameters, tenderness and fibrousness, are
223 enhanced in Equidae meat by dietary supplementation with PE, according to other authors. [46,47] In
224 fact, they found that supplementing pig diets with garlic had a positive effect on meat texture. [46,47]

225 Lorenzo *et al*.[21] reported that in horse the LL muscle was the tenderest muscle in disagreement
226 with Tateo *et al.*[18]. A high variability in LL muscle chemical composition was observed, in particular
227 regarding fat content, that is related to the foal breed.[16,13,18,48-50]

228 The Pearson correlation proved that juiciness was positively related to fat content (r=0,754,
229 P<0.05) and water content (r=0.860, P<0.05). However, no significant correlations were found
230 between the other textural sensory properties and nutritional parameters.

231 The results indicated that the mean scores for each descriptor could be assumed to be
232 satisfactory for the sensory profile of *Equidae* meat. In particular, sensory evaluation showed that PE
233 dietary supplementation affect *LL* aroma and texture in both donkey and horse.

234 In literature, only Lorenzo *et al*.[21] reported sensory parameters in horse meat. No data are
235 available for donkey meat sensory characteristics. The present results showed that the sensory ratings
236 of individual products obtained by ANOVA allows us to state that there are differences in describing
237 and perceiving meat samples from a qualitative point of view. Our findings are in agreement with
238 data observed by Webb and O'Neill in contemporary consumers, reported that one of the main
239 sensory criteria associated with meat quality is tenderness. [51] This sensory descriptor is able to
240 discriminate the LL muscle from PE treatment in *Equidae* meat.

241

242 **Principal component analysis**

243 Both donkey and horse sensory descriptors were averaged across assessors and submitted to
244 Principal Component Analysis (PCA) in order to evaluate the results from a multidimensional point

245 of view. A multidimensional space based on significant sensory data is reported in the Bi-Plot (Figure
246 3). The variance explained by the first two principal components (PC) was 88%.

247 Principal component 1 (PC1) was the most important variable in terms of differences among
248 type of specie as it accounted for 61% of the total variability. CH and PEH muscles were in the
249 positive side of PC1 and PED and CD were in the negative side. So, from left to right along the first
250 component (PC1) donkey and horse meat samples were well distinguished. On the other hand,
251 principal component 2 (PC2) accounted for 27 % of the total variability. Along PC2 it can be seen
252 that the samples were separated according to dietary treatments. PED and PEH were in the positive
253 side of PC2 and CD and CH were in the negative side.

254 From left to right along the first component (PC1) donkey and horse meat samples were well
255 distinguished as specie, while along PC2 it can be seen that the samples were separated according to
256 dietary treatments. Bi-plot also showed the relationship between the sensory attributes and their
257 distribution in space. In fact, texture attributes, sweet taste and metallic (aroma and flavour) are
258 located along PC1 in the right hand panes and in opposition salty taste, red colour and typical aroma
259 and flavour are located in the left hand panes. In PC2, that accounts for less variation, tenderness and
260 typical aroma are located at positive side of the plot and fibrousness is located in the negative site.
261 The PED meat resulted redness, saltiness and had high typical aroma than control group (CD). Also
262 PEH meat resulted more tender, juicy and had high metallic aroma and flavour than CH group.

263 The PCA model well described the *Equidae* samples in relation to dietary treatment (control
264 vs plant extract). Moreover, the samples assumed a well-defined position in the space for both donkey
265 and horse meat. This data indicated that the judges had the same perception of the sensory aspects of
266 the *Equidae* samples and were able to categorize them.

267

268 **CONCLUSION**

269 The present data show that dietary PE supplementation in donkey are able to reduce levels of
270 lipid oxidation biomarkers during refrigerated storage. The sensory parameters are affected by PE

dietary supplementation in both specie. Sensory characteristics of donkey meat could be useful for future studies on this type of meat. These results suggest the opportunity to enhance the eating quality of donkey and horse LL muscle. Further studies in *Equidae* are needed to confirm the present data and to determine the PE dietary concentration and length of time needed for improving meat quality.

ACKNOWLEDGMENT

This research was supported by grants from the Italian Ministry of University and Scientific Research (PRIN 2008 project n°20084B3LRA).

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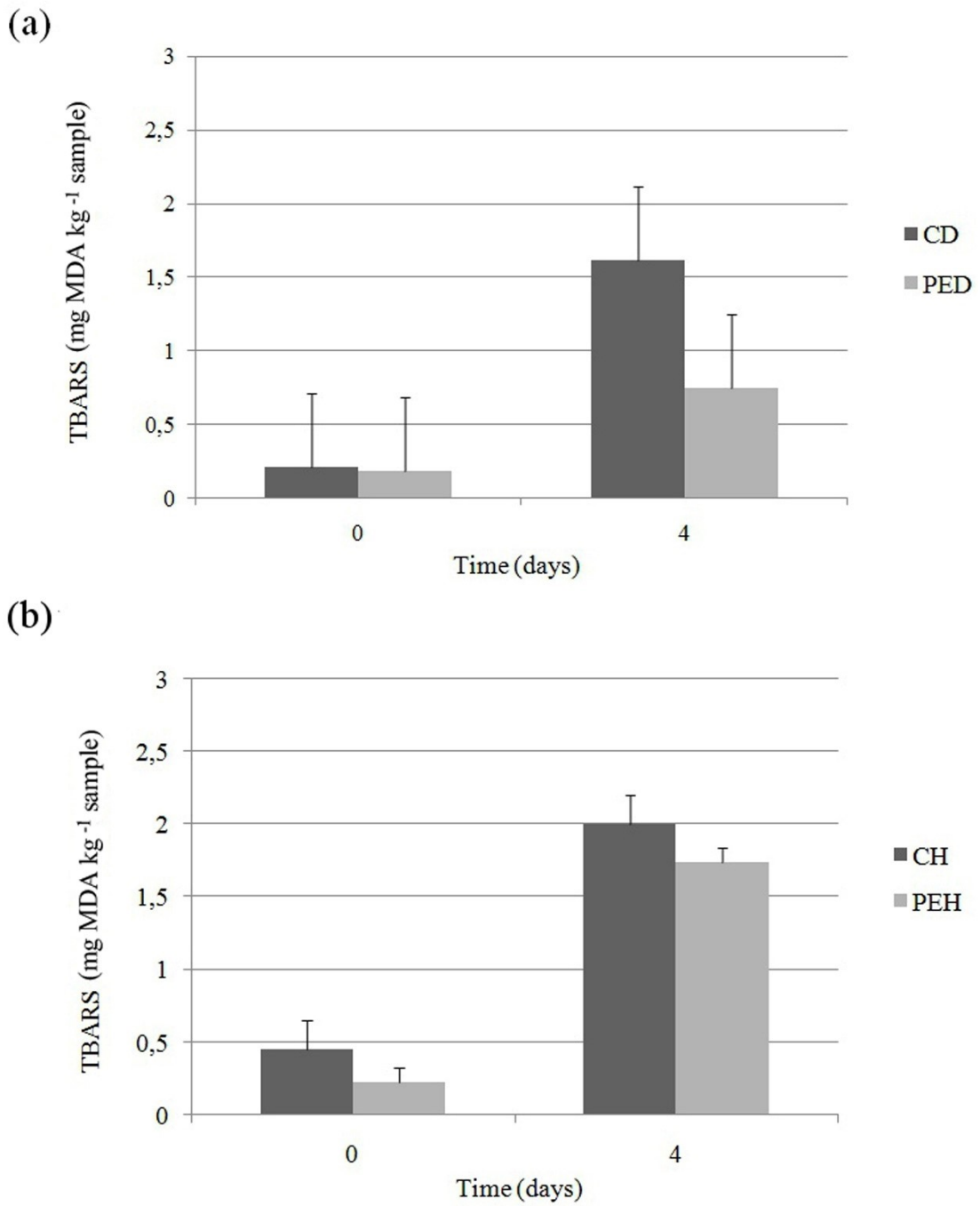
400

401 **Fig. 1.** Oxidative stability during refrigerated storage at 4 °C of *Longissimus Lomborum* muscle from
402 (a) donkey fed control (CD) or plant extract supplemented diet (PED); n = 6; data are reported as
403 mean ± SEM. Effects of treatment, P <0.001; time, P < 0.001; treatment∗time, P < 0.001; and (b)
404 from horse fed control (CH) or plant extract supplemented diet (PEH); n = 6 : data are reported as
405 mean ± SEM. Effects of treatment, P > 0.05; time, P < 0.001; treatment∗time, P >0.05.



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408 **Figure 2.** Spider plot of the sensory profile of LL muscle from (a) donkey fed control diet (CD) or
409 diet supplemented with plant extract (PED) and (b) horse fed control diet (CH) or diet supplemented
410 with plant extract (PEH).*Values are different for P<0.05. b).

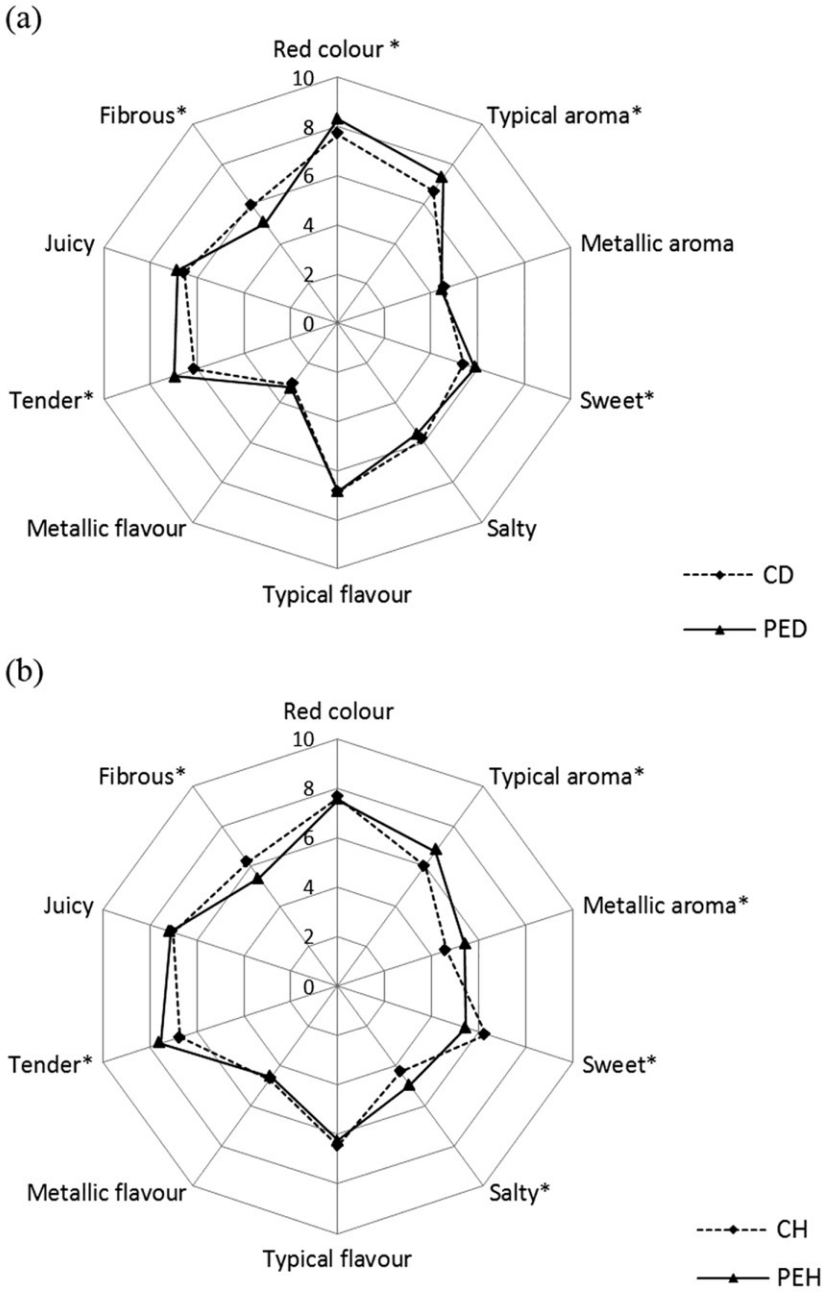

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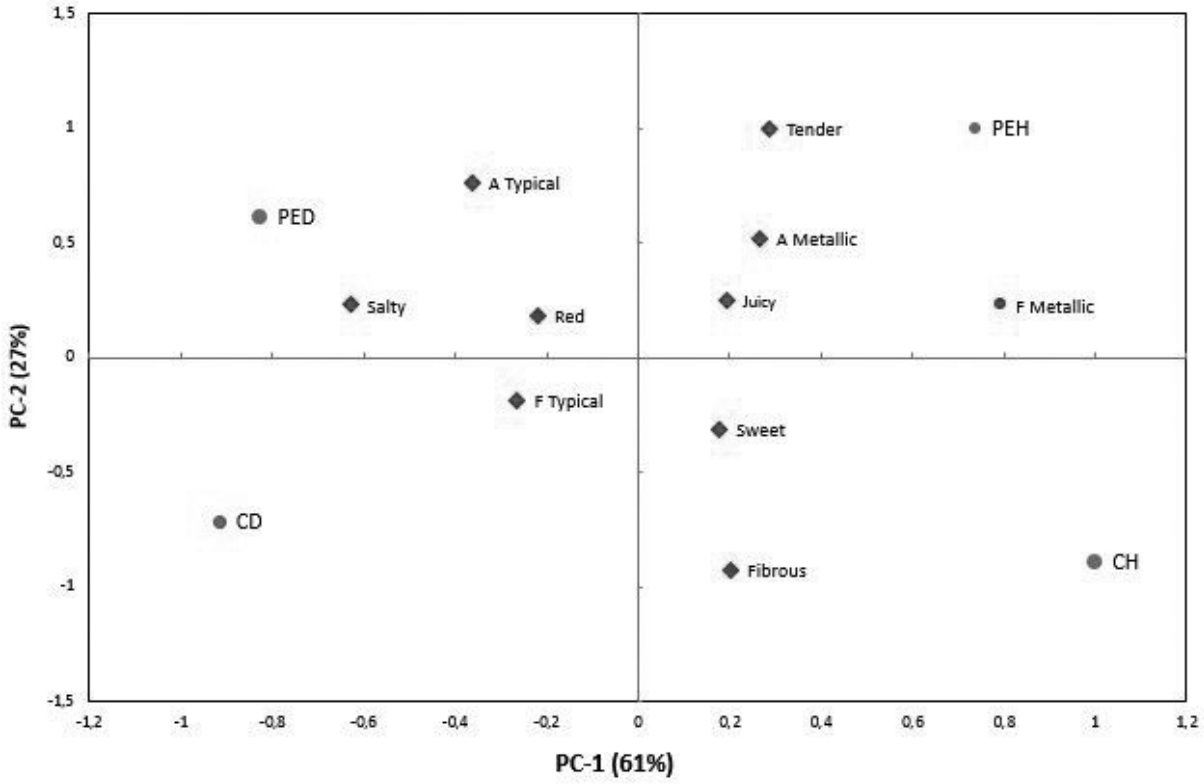
Figure 3. Bi-plot obtain by PCA model of donkey and horse meat sensory data. • CD, donkey receive a control diet; • CH, horse receive a control diet; • PED, donkey receive supplemented diet with plant extract; • PEH, horse receive supplemented diet with plant extract; ♦ Sensory attributes (F, Flavour; A, Aroma).



420 **Table 1.** Attributes and definitions of sensory profile for both donkey and horse *Longissimus*
421 *Lumborum* muscle.
422

Attibutes		Definition
Appearance		
	Colour	Intensity of red colour
Aroma		
	Typical	Typical aroma associated with cooked meat
	Metallic	Aroma associated with blood or rare meat
Taste		
	Sweet	One of the four basic tastes caused by water solutions of various substances perceived on the tip of the tongue
	Salty	One of the four basic tastes caused by water solutions of various substances perceived on the tip of the tongue
Flavour		
	Typical	Typical flavour, resulting from placing cooked meat in the mouth, involving taste in water solution and smell at the moment of swallowing
	Metallic	Flavour associated with blood or rare meat
Texture		
	Tender	The force needed to masticate the meat ready for swallowing (chewing 5 times)
	Juicy	Wet sensation in the mouth caused by a product after compression between the teeth
	Fibrous	Presence of fibers during chewing

423
424

425 **Table 2**. Influence of dietary plant extract and *Equidae* specie on meat quality parameters of

426 *Longissumus Lumborum* muscle.

	Donkey[a]		Horse[a]		*P*	
Item	C	PE	C	PE	*Treat*	*Specie*
pH	5.72 ±0.01	5.69 ± 0.01	5.71 ± 0.01	5.63 ± 0.02	0.231	0.489
Colour indices:						
L*	58.31 ± 2.81	56.72 ± 1.98	59.46 ± 0.84	57.57 ± 1.95	0.532	0.527
a*	13.66 ± 1.61	12.44 ± 0.90	13.8 ± 1.58	13.1 ± 0.89	0.464	0.731
b*	-3.81 ± 0.83	-3.49 ± 0.85	1.28 ± 0.82	1.39 ± 0.59	0.664	<0.001
Chemical composition [b]:						
Dry matter	289.8 ± 5.8	283.4 ± 6.7	277.2 ± 7.9	271.4 ± 7.7	0.548	0.237
Protein,	200.3 ± 4.8	206.8 ± 3.9	206.9 ± 5.7	211.6 ± 2.4	0.346	0.332
Fat[b]	48.7 ± 10	51.4 ± 8.7	36.2 ± 10	39.4 ± 07.1	0.823	0.138
Ash	9.7 ± 0.1	9.9 ± 0.1	9.9 ± 0.1	9.8 ± 0.1	0.651	0.696

427

428 [a]Data are reported as mean values ± SEM; n= 6; C, Control animals; PE, Plant extract supplemented

429 animals.

430 [b] Data are expressed as g kg^{-1}

Table 3. Sensory evaluation in donkey meat: F value and statistical significance of samples (n =6 CD and PED), judges (n = 8), replicates (n = 3) and their interaction for each sensory attributes.

Attributes	F value					
	Samples	Judge	Replicates	S∗J	S∗R	J∗R
Red colour	8.89***	2.46 n.s.	3.74 n.s.	2.26 n.s.	3.49 n.s.	1.41 n.s.
Typical aroma	11.49**	45.32***	3.46 n.s.	2.23 n.s.	0.17 n.s.	5.55**
Metallic aroma	0.10 n.s.	33.49***	3.01 n.s.	0.18 n.s.	2.10 n.s.	2.53*
Sweet	8.50*	88.42***	2.76 n.s.	7.14**	15.28***	4.62**
Salty	1.76 n.s.	52.34***	2.23 n.s.	1.89 n.s.	2.12 n.s.	1.83 n.s.
Typical flavour	0.00 n.s.	40.97***	1.15 n.s.	1.11 n.s.	2.60 n.s.	1.59 n.s.
Metallic flavour	0.50 n.s.	82.76***	6.24*	0.49 n.s.	0.21 n.s.	0.68 n.s.
Tender	4.89 *	6.90**	3.45n.s.	1.04 n.s.	1.54 n.s.	1.03 n.s.
Juicy	2.17 n.s.	28.90***	3.32 n.s.	0.89 n.s.	4.25 n.s.	2.67 n.s.
Fibrous	6.28*	16.01***	3.01 n.s.	1.36 n.s.	0.09 n.s.	1.80 n.s.

S∗J, Sample∗Judge; C∗R, Sample ∗ Replicates; G∗R= Judge ∗Replicates

*** Significant at P < 0.001; ** Significant at P < 0.01; * Significant at P < 0.05; n.s. = no significant.

449 **Table 4**. Sensory evaluation in horse meat: F value and statistical significance of samples (n = 6 CH
450 and PEH), judges (n = 8), replicates (n = 3) and their interaction for each sensory attributes.

451

Attributes	F value					
	Samples	Judges	Replicates	S∗J	S∗R	J∗R
Red colour	0.48 n.s.	16.68***	2.08 n.s.	0.68 n.s.	2.37 n.s.	2.00 n.s.
Typical aroma	6.66*	28.76***	5.73*	3.79*	3.03 n.s.	2.27 n.s.
Metallic aroma	5.06*	21.00***	0.38 n.s.	2.08n.s.	2.29 n.s.	0.49 n.s.
Sweet	15.72**	46.24***	2.54 n.s.	2.72 n.s.	1.35 n.s.	3.04*
Salty	5.61*	29.94***	0.50 n.s.	3.79*	0.51 n.s.	2.37n.s.
Typical flavour	0.86 n.s.	31.56***	3.36 n.s.	1.05 n.s.	0.49 n.s.	2.05 n.s.
Metallic flavour	0.12 n.s.	24.30***	0.79 n.s.	0.64 n.s.	1.89 n.s.	1.75 n.s.
Tender	11.34**	8.86***	1.19 n.s.	1.24 n.s.	2.94 n.s.	1.16 n.s.
Juicy	0.15 n.s.	8.07***	1.47 n.s.	1.27 n.s.	3.49 n.s.	0.77 n.s.
Fibrous	5.19*	27.27***	1.82 n.s.	1.88 n.s.	1.74 n.s.	1.73 n.s.

452

453 S∗J, Sample ∗ Judge; C∗R, Sample ∗ Replicates; G∗R= Judge ∗ Replicates

454 *** Significant at P < 0.001; ** Significant at P < 0.01; * Significant at P < 0.05; n.s. = no significant.

455