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1 **Palatability assessment in horses in relation to lateralization and temperament**

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12 **Highlights**

- 13 • Sweet flavours seem to enhance palatability in ponies.
- 14 • Lateralization is to be taken into consideration when setting palatability studies.
- 15 • Temperament is to be taken into consideration when setting palatability studies.

16 **Abstract**

17 Compared to other domestic animals, little is known about dietary preferences and feed palatability in
18 horses. Furthermore, it is known that horses exhibit a marked lateralization, that is a preference for one
19 side over the other, and that each individual differs in temperament. However, there is a gap of knowledge
20 regarding influence of lateralization and temperament in palatability tests. The aim of the study was to
21 understand the preference for odour and taste of different flavours using palatability tests, taking into
22 consideration both temperamental characteristics and lateralization response. Twelve ponies were
23 randomly enrolled. Three behavioural tests were carried out (arena test, novel object test and person test)
24 to assess individual temperament and lateralization. Behavioural responses and movements within the
25 arena were recorded. Two choice tests were carried out to assess palatability using first-cut chopped hay
26 with or without the addition of the following flavours: carrot (C), vanilla (V), milk protein (MP), and milk

27 protein with sugar (MS). Each flavour was tested simultaneously with a control (water, W). The evaluated
28 variables were first feed approached (flavoured or not), first bucket approached (left or right), voluntary
29 intake. Three groups were defined based on lateralization (left N=4, right N=6, none N=2) and four
30 components were extracted by the PCA from behavioural variables. All the ponies accepted new flavours,
31 excepting for MP. As regard first choice, horses tended to prefer V (P = 0.06) and MS (P = 0.06) and
32 significantly choose MP as first choice (P < 0.05). No significant differences were seen concerning the intake
33 for C, V, and MS flavours against W; whereas intake was significantly higher for W against MP. In general,
34 there was a tendency to choose the appetizers (P < 0.001) as first choice. Taking into account the total
35 sample there was a preference to choose right bucket as first choice. In conclusion, new odours seems to
36 enhance palatability in ponies, however the preference for a new odour is not necessarily synonymous of a
37 greater intake. Moreover, lateralization and temperaments needs to be taken into consideration during the
38 set of palatability studies.

39 **Keywords**

40 Palatability; Flavour; Horses; Lateralization; Personality; Temperament

41 **Abbreviations:**

42 C: carrot flavour; V: vanilla flavour; MP: milk protein flavour; MS: milk protein with sugar flavour; W: water,
43 negative control; E: Excitable component; NE: Non excitable component; L: left; R: right; N: none

44 **1. Introduction**

45 Among all behavioural activities, horses spend more time eating. However, compared to other domestic
46 and companion animals, currently little is known about horses' dietary preferences and feed selection.
47 Preference tests are often used to assess taste preferences and palatability (Goodwin et al., 2007, 2005;
48 Mars et al., 1992; Moreira et al., 2017; Müller and Udén, 2007; Redgate et al., 2014; Triebe et al., 2012; van
49 den Berg et al., 2016d, 2016a; van den Berg and Hinch, 2016). The methods to assess feed preference in
50 equines are not yet standardized and big divergences are present in test protocols.

51 Animals use two interrelated pathways to assess feedstuffs preferences: pre-ingestive feedback, that
52 includes the oro-sensory characteristics of the feed detected by the animal before the ingestion, and post-
53 ingestive feedback, that includes all the metabolic consequences -positive or negative- after the

54 consumption of the feed (Provenza, 1995). Furthermore horses generally eat little quantities of feed when
55 it is offered for the first time. This could be due to an innate herbivore survival strategy to prevent the
56 excessive consumption of toxic plants in nature (Hinch et al., 2004; Provenza, 1995; van den Berg et al.,
57 2016d; van den Berg and Hinch, 2016). Commercial horse feed industry uses different feed flavours to
58 overcome horses' neophobic nature. The restricted variety of flavours used in horse commercial feedstuffs
59 is probably a consequence of the small number of published studies on the acceptance of flavours.

60 It is known that behaviour has also a direct impact on feed consumption and diet selection, but aspects
61 such as temperament/personality and individual lateralization are rarely taken into account in horses' feeds
62 selection mechanism. Temperamental traits (Momozawa et al., 2003; Visser et al., 2001) and personality
63 (Ijichi et al., 2013; König v, 2013; Lloyd et al., 2007) are defined by the characteristics of horses' behaviour,
64 both genetically inherited and influenced from the subsequent experiences. Previous works have focused
65 mainly on the possibility to understand the association between personality and welfare. To our
66 knowledge, previous studies have never considered the possible influence of behavioural traits on
67 mechanism of feed selection. However, it is known that animals respond individually to challenges and the
68 results of each test could be influenced by individual response. It is reported that horse's reactivity (or
69 emotionality or nervousness), intended as "an exceeded state of arousal" (McCall et al., 2006) can affect
70 also eating and drinking behaviours (McGreevy, 2004). Besides, lateralization of vertebrates, the
71 asymmetric regulation of right and left hemispheres involved in some behaviours, has received more
72 attention over the last years (Leliveld et al., 2013; Macneilage et al., 2009; Rogers, 2014; Rogers and
73 Andrew, 2002; Vallortigara and Rogers, 2005). Many studies (Austin and Rogers, 2014, 2012, 2007; Baragli
74 et al., 2011; De Boyer Des Roches et al., 2008; Farmer et al., 2018; Larose et al., 2006; McGreevy and
75 Rogers, 2005; McGreevy and Thomson, 2006; Sankey et al., 2011; Savin, 2015; Warren-Smith and
76 McGreevy, 2010) demonstrated that equid exhibit a preference for one side over the other in different
77 contexts. However, to our knowledge, no studies emphasized the influence that a lateralized response
78 could also have on preference tests. In fact, different stimuli might be cause of different lateralized
79 responses in animals (De Boyer Des Roches et al., 2008; Larose et al., 2006). During preference tests, it is
80 not clear if horses select the one feed over the other due to a smell/taste preference or because they

81 independently select the feed on one side. The last could be due to an innate lateralization and or specific
82 temperamental characteristics instead of preference for a given feed based on its organoleptic
83 characteristics. The aim of the study was to assess the preference for four different flavors using
84 palatability test, bearing in mind the influence of temperamental characteristic and individual lateralization
85 response on the choice in preference test. The study was divided in two parts. In the first part three
86 behavioural tests (arena test, novel object test, person test) were carried out in order to assess
87 temperamental characteristics and lateralization (left, L; right, R; none, N), while in the second part four
88 palatability tests were carried out.

89 **2. Materials and methods**

90 *2.1 Animals and management*

91 Twelve ponies (6 mares and 6 geldings, 12.1 ± 5.3 years old, body condition score from 6.7 ± 1.3) in good
92 health were enrolled in this study. The animals were selected and held in the same equestrian centre
93 throughout the entire duration of this study (April and May 2019). Ponies were fed with meadow hay, three
94 times a day, and they were kept in outside paddock during the day and in single or double boxes during the
95 night . The care and use of the animals followed the guidelines set by the University of Turin Animal Ethics
96 and Welfare Committee (Prot.n. 655 13/03/2019).

97 *2.2 Testing procedures*

98 *2.2.1 Behavioural test*

99 Three behavioural tests were carried out over a 7-day period during the morning, each horse being tested
100 just once per day. Tests lasted 5 min each. Firstly, the arena test and then the novel object test were
101 carried out in a testing area of 5x3 m set inside an uncovered paddock. For the person test, a pen of 15x18
102 m was used. Both the testing areas were virtually divided in four equal zones. For the novel object test, we
103 placed the novel object (a blue rucksack and two pillows) inside the testing area in the opposite side of the
104 entrance. During the person test, an unfamiliar person to the ponies was positioned in the middle of the
105 area. A 1-m semicircle around the object/person was considered in order to assess the distance between
106 the object/person and the animal. The behaviour was recorded by two observers, standing motionless in

107 the border of the area farthest from the entrance door, while a video camera was positioned on the
108 midline comparing to the object/person. The video recording was made using a Sony® camera (Handycam®
109 HDR - CX240E). Video analysis was carried out using commercially available software, Solomon Coder
110 software® version beta (19.08.02) (<https://solomoncoder.com/>). Each pony was set free into the testing
111 area exactly in front to the object/person when present. The time required to touch the objects or to go
112 near the person was recorded. The sensorial and motor laterality of the animal was evaluated considering
113 sniffing the object/person with the right or left nostril (expressed as % of time), the forelimb leg each 10 s
114 (expressed as % of time) and the position of the object/person compared to the pony's head axis (on the
115 left, on the right, in front, behind; Figure 1, adapted from Larose *et al.*, 2006). The average latency of ponies
116 body axis shift (s), the frequency, the total and relative time (s) spent in each axis position was assessed.
117 The different movements and the average latency of movement (s) between the different areas were
118 calculated.

119 Behavioural responses and movements within areas were recorded using “*Instantaneous scan sampling*” at
120 10 s interval (30 frames per animal). “*All occurrences*” of less frequent activities were recorded (defecation,
121 urination, whinnying, pawing and snorting). The two sample methods were described by Altmann (1974)
122 and used in other horse temperament studies (Larose *et al.*, 2006; Wolff *et al.*, 1997). An ethogram profile
123 was developed considering behavioural parameters and variables linked to lateralization (Table 1; De
124 Boyer Des Roches *et al.*, 2008; Larose *et al.*, 2006; McGreevy and Rogers, 2005; Seaman *et al.*, 2002; Visser
125 *et al.*, 2010, 2001; Wolff *et al.*, 1997). Average latency time of areas of the set and body axis position
126 changes were calculated.

TABLE 1: Behavioural variables of ethogram profiles in horses recorded.

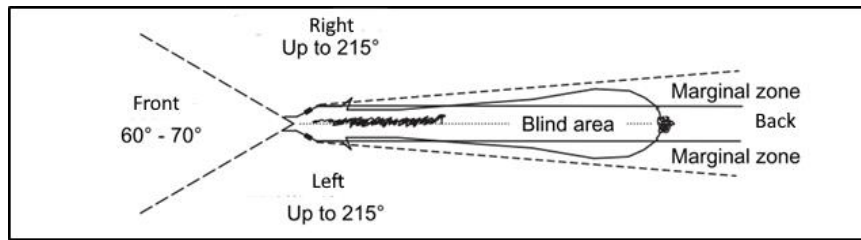
| Variable | Definition |
|------------------------------|--|
| <i>Behavioural variables</i> | |
| <u>Quiet standing</u> | |
| <u>Slow walk</u> | Walking slowly, the neck is horizontal or lower in quiet condition of exploration. |
| <u>Sustained walk</u> | Walking actively and looking in front or around (from Wolff <i>et al.</i> , 1997). |

| | |
|---------------------------------------|---|
| <u>Trot-gallop</u> | A two/three beat gait. |
| <u>Vigilance</u> | Standing still, the neck is elevated, head and ears are orientated with attention (from Visser <i>et al.</i> , 2001). |
| <u>Whinnying</u> | Vocalization |
| <u>Passage (prancing)</u> | Form of the trot where the legs are raised with more elevation. It is often associated with audible hoof contact with the ground (from Wolff <i>et al.</i> , 1997). |
| <u>Raised tail</u> | Tail up (tail root above horizontal line). |
| <u>Snorting</u> | Snorting (“forceful expulsion of air through the nostrils incidentally preceded by a raspy inhalation sound”, from Visser <i>et al.</i> , 2001). |
| <i>Focus novelty</i> | Focusing on novel object (ears, eyes and head pointed in direction of novel object) (from Visser <i>et al.</i> , 2001). |
| <i>Exploring object/person</i> | Exploring the novel object/person (<2m), nose under the belly line, ears and eye pointed to the object. |
| <i>Pawing</i> | Striking a vertical or horizontal surface/the air with a forelimb (from Seaman <i>et al.</i> , 2002). |
| <i>Defecation/Urination</i> | Elimination of faeces/urine. |
| <i>Rolling</i> | Rolling on the ground. |
| <i>Exploring</i> | Exploring area (out of 2 m from novelty, nose below the belly-line, ears, eyes and head not pointed towards the novel object) (Visser <i>et al.</i> , 2001). |

In the novel object test and person test lateralized behaviour were evaluated:

Lateralization variables

| | |
|---------------------|---|
| Axis | Position of the object compared to the axis of its head (De Boyer Des Roches <i>et al.</i> , 2008; Larose <i>et al.</i> , 2006) (Figure. 1) |
| Nostril | Sniffing the object with the right/left nostril (Larose <i>et al.</i> , 2006; McGreevy and Rogers, 2005) |
| Forelimb leg | Standing with forelimb leg right or left in front. |



128

129 **FIGURE 1:** Vision in horses: panoramic visual field (adapted from Larose et al., 2006)

130 *2.2.2 Palatability tests*

131 Two choice preference test was set up using first cut chopped meadow hay (the same included in horses'
 132 diet) with or without the testing flavours. These were offered in two yellow buckets simultaneously, placed
 133 inside black tires ($\varnothing = 26$ cm). Ponies were trained to eat from the buckets twice prior the start of the trial.
 134 The order of the first flavours tested alone was randomly chosen for each pony. The buckets were on the
 135 opposite side respect the entrance door. The distance between the two tires was 0.5 m. The pony was
 136 released into the testing area in front of the buckets. The first bucket approached (with/without flavours)
 137 and voluntary intake were evaluated. It was also evaluated the side of the first bucket approached, in order
 138 to assess if ponies presented a lateralized response that could influence the choice during the preference
 139 tests. The position of the flavoured hay on the right or on the left bucket was randomly defined each time.

140 *2.3 Flavours preparation*

141 A total of 48 trials were made (four trials for each pony, one for each flavour). The flavours tested were
 142 carrot (C), vanilla (V) milk protein (MP) and milk protein with sugar (MS) in four consecutive trials. Hay was
 143 mixed with each flavour in a 6:1 ratio. Each flavour was tested against a control, which consisted on hay
 144 mixed with water (W) in order to offer hay at the same moisture level.
 145 Fifty grams of hay for each bucket were used. MP aroma was a testing flavour provided by a private
 146 company. Both F that V aroma were previously diluted with water in order to present a 2% concentration
 147 on the total weight (60 g of feed as fed bases). MS flavour was prepared using a 30% moisture solution of
 148 milk protein (80% on DM) and sugar (20% on DM). The preparation was mixed on a heating stirrer for 5
 149 minutes and then it was diluted as described previously. For the C aroma and the control bucket it was used
 150 10 g of commercially available undiluted carrot juice and 10 g of water respectively.

151 *2.4 Statistical analysis*

152 All data have been analyzed using R (v 3.6.1). Each individual horse was set as experimental unit. Normal
153 distribution of the data was tested by Shapiro Wilk test. The K-means cluster analysis on lateralized
154 behavior was performed to detect different lateralization groups (left, L; right, R; none, N). The ANOVA and
155 Kruskal-Wallis procedures were used for checking differences on previous identified groups in normal and
156 not normal distributed parameters respectively. Significant effects ($P < 0.05$) were then compared between
157 time points with a Tukey and Wilcox test in parametric and non parametric tests respectively. Results are
158 reported as mean and standard deviation or median and quartiles for normal and not normal distributed
159 parameters, respectively. Principal Component Analysis (PCA) was carried out to assess temperamental
160 characteristics. The PCA was used to analyse behavioral variables, indexes of activity of animals (latency of
161 axis, latency of area) and time to go near (time to go near) or to interact (time to approach) with the object or
162 the person. Components were interpreted considering high positive (+, > 20%) and negative (-, <-20%)
163 coefficients. Sign test was applied to evaluate the buckets approached first (both from side that for flavour
164 point view). Chi-squared and sign one sample test were used to evaluate correspondence of group
165 detected from PCA from clusters detected.

166 **3. Results**

167 *3.1 Behavioural test*

168 *3.1.1 Lateralization*

169 Results concerning the novel object test and the person test are reported in Supplemental File 1. K-means
170 cluster analysis for lateralized behaviour showed that the ponies were divided in 3 groups that were
171 defined as left (L) N=4, right (R) N=6 and none (N) N=2. Significant differences between the groups were
172 observed during the novel object test but not during the person test. Significance differences were showed
173 between group N and R ($P = 0.02$) for the frequency of the body placed with axis on the right respect to the
174 object, R groups had a median of 2.5 (1.2-4.5), respect the group N that had a median of 0. No differences
175 were recorded in L group. The R group tended also to spend more time with body axis placed on the right
176 side with a median of 149.3 (131.1-188.7) seconds that differed significantly ($p = 0.01$) from group N (0; 0-
177 0.3). The L group was not different from both N and R groups. The L group spent 140.3 (66.1-226.9) seconds
178 with body axis on the left that was significantly different ($P = 0.05$) from group N (0; 0-0). The R group

179 instead did not present differences for this variable. N group did not spent time with body axis placed on
 180 right or on the left with the median equal to 0 (0-0) in both cases. This result significantly differed ($P < 0.05$)
 181 from the other two groups. In fact, the R group spent 20.6% (14.3-37.6) and 5.50% (3.9-8.5) of time
 182 respectively on the right or on the left axis.

183 3.1.2 Temperament/Personality

184 Four components were extracted by the PCA analysis from the data of the behavioural variables and
 185 activity variables of the 12 ponies during the three behavioural tests. Together the components explained
 186 72.2% of the total variance. The items loading for the four components are shown in Table 2. Each
 187 component was interpreted by examining the contribution of behavioural and activity variables (Table 2 in
 188 bold font). Components were characterized as active, low active, fearful, excitable.

Table 2: Variables loading for each component with reported cumulative variance and standard deviation.

| <i>Behavioural and activity variable</i> | Component 1 | Component 2 | Component 3 | Component 4 |
|--|--------------------|---------------------|--------------------|--------------------|
| | (Active) | (Low active) | (Fearful) | (Excitable) |
| <i>Exploring</i> | -6.33% | -45.51% | 15.89% | 16.30% |
| <i>Trot-gallop</i> | 30.42% | 5.32% | 26.95% | 44.61% |
| <i>Pawing</i> | -25.91% | 17.75% | 11.56% | 10.33% |
| <i>Rolling</i> | 27.40% | 14.47% | -35.51% | 17.42% |
| <i>Snorting</i> | 16.78% | -27.78% | 29.83% | -27.32% |
| <i>Quiet standing</i> | -23.55% | 7.72% | -26.24% | -37.45% |
| <i>Raised tail</i> | 25.64% | 18.42% | -22.93% | 16.99% |
| <i>Vigilance</i> | 24.50% | -0.80% | 45.17% | -12.83% |
| <i>Walking</i>¹ | 23.21% | 2.54% | -25.39% | -13.91% |
| <i>Whinnying</i> | -9.04% | 41.04% | 24.79% | -3.40% |
| <i>Focusing on the object</i> | -32.91% | 22.40% | 27.38% | 4.51% |
| <i>Time to approach the object</i> | 28.70% | 11.87% | 29.55% | -36.22% |
| <i>Time to go near the object</i> | 21.46% | 34.34% | -5.95% | -47.66% |
| <i>Fr.² near the object</i> | 24.19% | 38.77% | 1.61% | 29.09% |
| <i>Average latency area</i>³ | -43.79% | 23.32% | 0.05% | 3.82% |

| | | | | |
|---|--------|---------------|---------------|--------|
| Average latency body axis ⁴ | -5.34% | 25.39% | 23.71% | 7.87% |
| <i>Cumulative Variance</i> | 25.32% | 44.94% | 62.32% | 72.21% |
| <i>Standard deviation</i> | 2.01 | 1.77 | 1.67 | 1.26 |

¹Slow walking+ sustained walking

²Frequencies

³Average latency of movement between the different areas

⁴Average latency of shift of ponies' body axis

Bold font: contributing behavioural and activity variables to each component

Between brackets: descriptive tag indicating of the different components

189 The behaviour variables that scored for the first component (**Active**) were *average latency area* (- 43.79%),
190 *focusing on the object* (-32.91%), *trot-gallop* (+30.42%), *time to approach the object* (+28.70%), *rolling*
191 (+27.40%), *pawing* (-25.91%), *raised tail* (+25.64%), *vigilance* (+24.50%), *frequency near the object*
192 (+24.19%), *quiet standing* (-23.55%), *walking* (+23.21%), *time to go near the object* (+21.46%). Component
193 2 (**Low active**) was characterized by *exploring* (-45.51%), *whinnying* (+41.04%), *frequency near the object*
194 (+38.77%), *time to go near the object* (+34.34%), *snorting* (-27.78%), *average latency body axis* (+25.39%),
195 *average latency area* (+23.32%), *focusing the object* (+22.40%). Component 3 (**Fearful**) included *vigilance*
196 (+45.17%), *rolling* (-35.51%), *snorting* (+29.83%), *time to approach the object* (+29.55%), *focusing the object*
197 (+27.38%), *trot-gallop* (+26.95%), *quiet standing* (-26.24%), *walking* (-25.39%), *whinnying* (+24.79%),
198 *average latency body axis* (+23.71%), *raised tail* (-22.93%). Behaviour variables *time to go near the object* (-
199 47.66%), *trot-gallop* (+44.61%), *quiet standing* (-37.45%), *time to approach the object* (-36.22%), *snorting* (-
200 27.32%) were grouped together to explain component 4 (**Excitable**).

201 3.2 Palatability tests

202 All the ponies accepted the C, V and MS flavours, but no ponies accepted the MP flavour. Comparing all the
203 flavoured respect to unflavoured hay there was a preference to choose the appetizers (56.25%, $P < 0.001$;
204 Table 3) as first choice. Analysing first choice for each single flavour there was not a significant effect for
205 the C flavour, but there was a tendency for the V flavour ($P = 0.06$) and the MS flavour ($P = 0.06$). Horses
206 significantly chose MP flavour as first choice ($P < 0.005$). No significant differences were seen concerning

207 the intake for C, V, MS flavours against the negative control (W), whereas the intake was significantly
 208 higher for W against MP flavour ($P < 0.01$).

Table 3: Number of choices between flavoured or unflavoured hay as a first choice, considering all the trials together (C+V+MS+MP).

| | Number of choices | |
|-------------|-------------------|--------|
| | N | % |
| Unflavoured | 21 | 43.75% |
| Flavoured | 27 | 56.25% |

Sing one test: P value < 0.0001

209

210 3.3 Interaction of personality traits, lateralization and palatability test

211 Taking into account the total sample, there was a preference to choose as first choice the right bucket
 212 (64.48%, $P < 0.001$), but this was not influenced from that lateralization found with the behavioural test (L, R
 213 and N group). Considering temperament, among all the personality traits detected from PCA analysis, only
 214 the component 4 (excitability) influenced the results of palatability test. Instead, no significant differences
 215 between ponies were observed considering the influence of the other three components on palatability. Six
 216 ponies were characterized as excitable (E) (ponies that scored for component 4 in a positive way) and six as
 217 non-excitable (NE) (ponies that scored negatively for component 4, Table 4).

218 There was a tendency for the NE ponies to be in R group ($P = 0.059$) detected from K means cluster analysis,
 219 while E ponies showed a predisposition to be in N or L group. The NE group had the highest preference to
 220 choose as first choice the flavoured hay ($P = 0.08$, Table 5) and the right bucket ($P < 0.001$, Table 6). No
 221 significant differences were seen between E and NE as regarding the preference of each single flavour. In
 222 Table 7 it is reported a summary of differences between E and NE regarding lateralization, preferred flavour
 223 and first bucket approached.

Table 4: Characterization of pony temperament based on component 4 (Excitable).

| Pony no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|----------------------------|------|------|------|-------|------|-------|-------|-------|-------|------|------|-------|
| Component 4 (Excitable) | 0.69 | 0.23 | 0.22 | -2.06 | 1.97 | -0.47 | -1.07 | -0.04 | -0.39 | 2.19 | 0.24 | -1.52 |

224

Table 5: Number of ponies that choose flavoured or unflavoured hay as first choice, considering all the trials together.

| | Unflavoured | Flavoured |
|----------------------------------|-------------|-----------|
| Excitable | 14 | 10 |
| Non excitable | 7 | 17 |
| <i>X squared: P value = 0.08</i> | | |

225

Table 6: Number of ponies that selected the right or the left bucket as first side, considering all the trials together.

| | Right | Left |
|--------------------------------------|-------|------|
| Excitable | 15 | 9 |
| Non excitable | 16 | 8 |
| Total: | 31 | 17 |
| <i>X squared: P value < 0.001</i> | | |

226

Table 7: Summary differences between excitable (E) and non-excitable (NE) ponies.

| | Excitable (E) | Non excitable (NE) |
|----------------------------|---------------|--------------------|
| Lateralization (group) | Left or None | Right |
| Bucket preference – flavor | Not flavored | Flavored |
| Bucket preference – side | Right | Right +++ |

227

228

229 **4. Discussion**

230 Previous research studies on horse feed preference are very limited and have not taken into consideration
231 the interaction of horse behaviour and lateralization on feed preference. The hypothesis of this study was
232 that it is possible an influence of lateralization and personality traits during palatability test. Literature
233 highlighted the complexity of mechanisms involved in feed selection in horses, consequences of
234 interrelations between feed cues, post-ingestive consequences (Provenza et al., 2003; Provenza, 1995) and
235 individual basis variations (Neave et al., 2018; Toscano et al., 2016). Our study provides a different
236 approach to conduct behavioural and palatability tests.

237 *4.1 Lateralization*

238 In our study, ponies were be divided in three groups considering sensory and motor laterality: right, left
239 and no lateralized animals. In previous studies it was found that individual differences in laterality are
240 possible and could be related to a widespread range of unfamiliar stimuli (animate and/or non-living feed
241 and potential predators) (Forrester et al., 2018). Moreover, it was shown that different breeds of
242 performance horses manifested a different motor laterality (McGreevy and Thomson, 2006) and that a
243 more emotional breed, such as French Saddlebred, displayed a more evident sensory laterality in a novel
244 object test than a calmer breed (Trotter) (Larose et al., 2006). In our study, the same prevalence of use of
245 one side for the entire group was not found. In literature, a preference for using the right eye was reported
246 for viewing an emotionally neutral novel object (De Boyer Des Roches et al., 2008), while the right ear was
247 preferentially used in the processing of familiar conspecifics sounds (left hemisphere use) (Basile et al.,
248 2009). On the other hand, it was reported that horses left-side (right hemisphere) is linked to higher
249 emotionality (Larose et al., 2006) and to a greater response to surprising stimuli (Austin and Rogers, 2007).
250 Horses were normally more sensitive when a new stimulus was presented on their left side and responded
251 in a more reactive way when they turned to the left. The right hemisphere is used for both positive and
252 negative emotions in a variety of species (Austin and Rogers, 2012; Leliveld et al., 2013; Rogers, 2017, 2010;
253 Rogers and Andrew, 2002), facilitating population aggressive responses even if the reasons are still
254 unknown (Austin and Rogers, 2014). In literature is reported a greater importance for the strength of
255 laterality than the direction (Rogers, 2017), with differences among individuals (Macneilage et al., 2009;

256 Rogers and Andrew, 2002; Sorvano et al., 1999; Vallortigara and Rogers, 2005). Therefore, it is possible that
257 the laterality observed in our study during the behavioural tests was just an index of the attention applied
258 from the animal and a quantification of the emotional involvement felt, independently from the direction,
259 as it has been supposed during affiliative interactions (Farmer et al., 2018). It has been shown that laterality
260 in vervet monkeys (*Cercopithecus aethiops*) tend to increase with the difficulty of the chore and the
261 focusing on it (Harrison and Byrne, 2000).

262 4.2 Temperament/Personality

263 The four temperament components, namely active, low active, fearful and excitable, assessed in our study
264 derived from terms adopted in previous research (Lloyd et al., 2008, 2007; Schork et al., 2018; Seaman et
265 al., 2002). *Active component* in our research was found to be positively correlated with trot gallop,
266 vigilance, walking and negatively correlated with standing and average latency of shift in the areas,
267 meaning that the ponies move inside the set frequently. *Passive component*, instead, was positively
268 associated with high time required to approach the object/person, high latency of shift in the areas and of
269 body axis, which means that these ponies took long time before move themselves. in a previous study of
270 Seaman *et al.*, (2002) horses with high activity were described by component with a strong association
271 between sustained walk, trot, vigilance, vocalisation and defecation. On the contrary the same authors
272 reported that passive horses exhibited high standing behaviour during person and object tests associated
273 with high approach times. These descriptions are in agreement with our components “Active” and “Low
274 Active”. *Fearful component*, in our study, was linked to behaviour that express stress situations in horses
275 (vigilance, snorting, whinnying) and additionally, high time was required for this animal to go near the
276 object/person. According with Lloyd *et al.* (Lloyd et al., 2008, 2007), “fearful” was linked with anxiousness
277 component and with horses that show high insecurity and suspiciousness and apprehension.

278 Lloyd *et al.* (Lloyd et al., 2007) defined as excitable a horse that “over reacts to any change” and is “easily
279 excited, highly strung”. The excitatory component found in their research had a negative correlation with
280 standing position. This is in agreement with the results found in our research, in which *excitable component*
281 had a negative correlation with quiet standing variable and a positive correlation with trot-gallop.
282 Furthermore, this component was also linked with a greater and an earlier investigation of novel

283 object/person during the behavioural test, suggesting their reactive response to the changes in the
284 environment. Among all the personality traits detected from PCA analysis, only the component 4
285 (excitability) had some influence on lateralization. In particular, we found that animals with non-excitable
286 temperament tended to be part of the right group and vice versa. Also Larose *et al.* (Larose et al., 2006)
287 described that novelty associated with high arousal level was managed by the right hemisphere, confirming
288 our founding that excitability behaviour is linked to left lateralization. The component “Excitable” was the
289 only component suggesting to influence also palatability tests.

290 4.3 Palatability tests

291 In our study, all the ponies accepted the new odours without showing a neophobic response, except for MP
292 flavour that seemed to be disliked. In literature, instead, an initial large difference in individual intake is
293 reported when introducing new odours/flavours. Differences in feed neophobia may be the cause of the
294 variability in feeding behaviour. For instance, intraspecific differences in feed neophobia may lead to minor
295 feeding times in some lambs considered as “shy feeders” (Rice et al., 2016). According with van den Berg
296 and Hinch (2016), an adaptation of 3-4 days seemed to be necessary to reduce the variability following the
297 introduction of a new odour in horses. These results were in accordance with another study of the same
298 researchers (van den Berg et al., 2016c). Similar conclusion were found by Hinch *et al.* (Hinch et al., 2004)
299 showing that the association of a novel feed (wheat) with a familiar odour or flavour (alfalfa) seemed to
300 decrease the variability in intake and to increase the total intake of the novel feed compared to the control
301 group. In our study, the ponies tended to approach as first choice the bucket with the flavour addition. This
302 is in agreement with previous researches, where non-nutritive flavours had been effectively used to
303 encourage intake of water and medicated foods in horses, in order to overcome the horses’ neophobia
304 (Burton et al., 1983; Goodwin et al., 2005; Mars et al., 1992). Also the impact of plant odours had been
305 demonstrated on foraging behaviour and feed preferences in sheep (Arnold et al., 1980). Van Tien *et al.*
306 (1999) showed that a grass odour that is familiar to the animal and a mixture of taste and odour added to a
307 novel feed (rice bran) can increase the intake in sheep. Furthermore, sheep and goats appeared to
308 consume more gladly a less palatable hay when feed was covered with an extract of pleasant high-grain
309 concentrate (Dohi and Yamada, 1997).

310 In our research sweet flavours seemed to enhance palatability in ponies, as it was found in previous studies
311 (Goodwin et al., 2005; Janczarek et al., 2018). As reported by Janczarek *et al.* (2018), a typical behaviour of
312 the horse during the two choice palatability test is the immediate approach to one bucket, then starting to
313 sniff it and finally eating it. Janczarek et al., (2018) sustains that first preference behavior regarding one
314 feed over the alternative should not be always be considered as an evidence of horse's preference. In our
315 trials, ponies tended to sample from both hay alternatives (flavoured and water) and to select an equal
316 proportion of feed from both buckets, whenever new flavours were accepted. As reported by Van den Berg
317 (2016a, 2016b, 2016d) horses display patch foraging behavior sampling from all foods offered and an equal
318 proportion of familiar and new feed eaten during multiple choice test (van den Berg et al., 2016d). The
319 same authors showed also a similarity for the time spent walking towards different "forage zones". This
320 seems to suggest that horses avoid the use of short-term spatial memory to recognize familiar and
321 preferred patches (van den Berg et al., 2016a) which would be necessary in an excessive quantity to
322 remember each feeding station (Bailey et al., 1996; Senft et al., 1987). Researchers suggested that grazing
323 herbivores and mainly ruminants may depend more on visual and oro-sensory characteristics than on
324 memory of spatial cues (Illius and Gordon, 1990). However, in horses there are different hypothesis
325 regarding the short-term memory during foraging activity, probably due to variability in studies in sample
326 size and differences in design (Hanggi, 2010; Lovrovich et al., 2015; McLean, 2004). Oro-sensory features,
327 trial and error seem to be essential components of foraging behavior and diet selection in horses? (van den
328 Berg et al., 2016d).

329 *4.4 Interaction of personality traits, lateralization and palatability tests*

330 Diet and behaviour were assumed to be highly plastic within individuals (Toscano et al., 2016) and it is still
331 not widely understood why individuals within a same species and/or herd differ in their feeding behaviour
332 (Neave et al., 2018). Based on literature we hypothesized an influence of personality traits and
333 lateralization on palatability and preference test in horses. We found that horses tended to choose right
334 bucket as first choice, making them right-sided (use of the left hemisphere) during palatability test. Their
335 right choice seemed to be independent from the position of the flavoured hay and from their lateralization
336 assessed previously with the behavioural tests. Right side is often associated with a less fear response

337 (Austin and Rogers, 2007; Sankey et al., 2011). In a marmoset species (*Callithrix geoffroyi*) tested with novel
338 feeds (vegetables and nuts) Braccini and Caine (2009) showed that right-handed subjects tended to sniff
339 and taste novel feeds in shorter time than left-handed ones, indicating that left-handers are more fearful
340 and less prone to explore novel feeds than right-handers. In our study, horses went preferentially on right
341 side, independently of being prone to the right or to left side during the behavioural test and they tended
342 to taste the feed without sign of neophobia, except for MP flavour that seemed to be disliked. Differences
343 in temperament and personality may condition diet choices in grazing and in confined systems and it may
344 affect also how the animals are able to cope with diets (and environment) shift (Neave et al., 2018). Our
345 results displayed that only PCA component 4 (excitability) showed an influence on preference test. In
346 particular, the ponies classified as “non-excitable” (negative assessment of component 4) seemed to select
347 easier the flavoured buckets. It is known that some subjects are more capable in exploring and sampling a
348 different feeds (Neave et al., 2018). In particular, in literature is reported that feed neophobia seems to
349 reflect fearfulness assessed during behavioural tests. For instance, Villalba *et al.* (2009) showed that lambs
350 presenting a higher neophobia versus feed were also more fearful in a novel arena. Individuals
351 characterized by a higher exploratory behaviour, through expression of a greater examination of novel
352 objects or feed, may adopt a more dangerous foraging strategy. Literature reports that animals that are
353 faster to reach a novel feed also tend to spend more time consuming the alternative feed and to change
354 more frequently between offered bins/bucket (Neave et al., 2018). For example, more exploratory sheep
355 were also more prone to divide into smaller groups and to graze away from conspecific, exploring more of
356 their feeding area but losing the possibility to stay together, as a cohesive group (Michelena et al., 2009;
357 Sibbald et al., 2006). Also in cattle, it was shown (Meagher et al., 2017) that heifers that had a tendency to
358 spent more time near to a novel object during behavioural test, tended to pass more time exploring and
359 consumed a larger variety of feed.

360 These results are in contrast with our study, in which the non-excitable group went preferentially on the
361 new flavours as first choice, but it was composed by the ponies that went in a more reluctant way near to
362 the object during the behavioural test.

363 **5. Conclusions**

364 In conclusion, this study underlined the importance of oro-sensory features and behavioural individual
365 characteristics in feed preferences and intake in ponies. It also shown that individual differences exist
366 concerning lateralization response during behavioural tests and that they may be affected by personality
367 traits, in particular by the level of excitability of the animals.

368 Our findings support the idea that novel sweet flavours seem to enhance palatability in ponies. However
369 feed preference for new flavours was not accompanied by increased feed intake.

370 Furthermore, it is clear the difficulty to properly set up a palatability test in horses and, to our knowledge,
371 this is the first study showing that lateralization and temperament are factors that need to be taken into
372 consideration during the set of palatability studies' design and checking them before doing each tests
373 should always be done. The current study was limited by the difficulty to correctly analyse animals'
374 behaviour, which is plastic by definition. Another limitation was to correctly assess the feed preferences in
375 horses due to their patch foraging strategy. This lead ponies to eat both feed alternatives in an almost
376 equal proportion. Further data collection with other flavours and a higher sample of ponies is required to
377 better determine exactly how laterality and temperament affects palatability of feed and diet selection in
378 horses. Despite this we believe our work could be the starting point for future projects.

379 **Conflict of interest statement**

380 None.

381 **Ethical statement**

382 The care and use of the animals followed the guidelines set by the University of Turin Animal Ethics and
383 Welfare Committee (prot.n. 665 13/03/2019).

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