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This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1765747> since 2021-01-03T21:58:46Z

Published version:

DOI:10.1016/j.applanim.2020.105110

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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

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

Keywords

Palatability; Flavour; Horses; Lateralization; Personality; Temperament

Abbreviations:

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

1. Introduction

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

Animals use two interrelated pathways to assess feedstuffs preferences: pre-ingestive feedback, that includes the oro-sensory characteristics of the feed detected by the animal before the ingestion, and post-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

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

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

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

2.2.2 Palatability tests

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

2.3 Flavours preparation

A total of 48 trials were made (four trials for each pony, one for each flavour). The flavours tested were carrot (C), vanilla (V) milk protein (MP) and milk protein with sugar (MS) in four consecutive trials. Hay was mixed with each flavour in a 6:1 ratio. Each flavour was tested against a control, which consisted on hay mixed with water (W) in order to offer hay at the same moisture level.

Fifty grams of hay for each bucket were used. MP aroma was a testing flavour provided by a private company. Both F that V aroma were previously diluted with water in order to present a 2% concentration on the total weight (60 g of feed as fed bases). MS flavour was prepared using a 30% moisture solution of milk protein (80% on DM) and sugar (20% on DM). The preparation was mixed on a heating stirrer for 5 minutes and then it was diluted as described previously. For the C aroma and the control bucket it was used 10 g of commercially available undiluted carrot juice and 10 g of water respectively.

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 Wilcoxon 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%
<i>Sing one test: P value<0.0001</i>		

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

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

4.3 Palatability tests

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

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

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

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).

384 **Acknowledgements**

385 This work was supported by Kemin Nuitsurance SRL (grant nuber XXX).

386 **References**

387 Altmann, J., 1974. Observational study of behavior: Sampling methods. Behaviour 49, 227–267.

388 <https://doi.org/10.1002/zamm.19730531271>

389 Arnold, G., Boer, Es., Boundy, C., 1980. The influence of odour and taste on the food preferences and food
390 intake of sheep. Aust. J. Agric. Res. 31, 571. <https://doi.org/10.1071/AR9800571>

391 Austin, N.P., Rogers, L.J., 2014. Lateralization of agonistic and vigilance responses in Przewalski horses
 392 (*Equus przewalskii*). *Appl. Anim. Behav. Sci.* 151, 43–50.
 393 <https://doi.org/10.1016/j.applanim.2013.11.011>
 394 Austin, N.P., Rogers, L.J., 2012. Limb preferences and lateralization of aggression, reactivity and vigilance in
 395 feral horses, *Equus caballus*. *Anim. Behav.* 83, 239–247.
 396 <https://doi.org/10.1016/j.anbehav.2011.10.033>
 397 Austin, N.P., Rogers, L.J., 2007. Asymmetry of flight and escape turning responses in horses. *Laterality*. 12,
 398 464-474. <https://doi.org/10.1080/13576500701495307>
 399 Bailey, D.W., Gross, J.E., Laca, E.A., Rittenhouse, L.R., Coughenour, M.B., Swift, D.M., Sims, P.L., 1996.
 400 Mechanisms That Result in Large Herbivore Grazing Distribution Patterns. *J. Range Manag.* 49, 386-
 401 400. <https://doi.org/10.2307/4002919>
 402 Baragli, P., Vitale, V., Paoletti, E., Sighieri, C., Reddon, A.R., 2011. Detour behaviour in horses (*Equus*
 403 *caballus*). *J. Ethol.* 29, 227–234. <https://doi.org/10.1007/s10164-010-0246-9>
 404 Basile, M., Boivin, S., Boutin, A., Blois-Heulin, C., Hausberger, M., Lemasson, A., 2009. Socially dependent
 405 auditory laterality in domestic horses (*Equus caballus*). *Anim. Cogn.* 12, 611–619.
 406 <https://doi.org/10.1007/s10071-009-0220-5>
 407 Braccini, S.N., Caine, N.G., 2009. Hand preference predicts reactions to novel foods and predators in
 408 marmosets (*Callithrix geoffroyi*). *J. Comp. Psychol.* 123, 18–25. <https://doi.org/10.1037/a0013089>
 409 Burton, J.H., Price, D.J., Aspinall, J., 1983. The effect of feed flavour and feed consumption in horses, in: 8th
 410 Equine Nutrition and Physiology Symposium. Lexington, Kentucky, Usa, pp. 27–32.
 411 De Boyer Des Roches, A., Richard-Yris, M.A., Henry, S., Ezzaoui, M., Hausberger, M., 2008. Laterality and
 412 emotions: Visual laterality in the domestic horse (*Equus caballus*) differs with objects' emotional
 413 value. *Physiol. Behav.* 94, 487-490. <https://doi.org/10.1016/j.physbeh.2008.03.002>
 414 Dohi, H., Yamada, A., 1997. Preference of sheep and goats for extracts from high-grain concentrate. *J.*
 415 *Anim. Sci.* 75, 2073-2077. <https://doi.org/10.2527/1997.7582073x>
 416 Farmer, K., Krüger, K., Byrne, R.W., Marr, I., 2018. Sensory laterality in affiliative interactions in domestic
 417 horses and ponies (*Equus caballus*). *Anim. Cogn.* 21, 631–637. <https://doi.org/10.1007/s10071-018->

418 1196-9

419 Forrester, G.S., Hopkins, W.D., Hudry, K., Lindell, A., 2018. Cerebral Lateralization and Cognition:
 420 Evolutionary and Developmental Investigations of Behavioral Biases, Cerebral Lateralization and
 421 Cognition, first ed. Elsevier, London.

422 Goodwin, D., Davidson, H.P.B., Harris, P., 2007. Responses of horses offered a choice between stables
 423 containing single or multiple forages. *Vet. Rec.* 160, 548–51. <https://doi.org/10.1136/VR.160.16.548>

424 Goodwin, D., Davidson, H.P.B., Harris, P., 2005. Selection and acceptance of flavours in concentrate diets
 425 for stabled horses. *Appl. Anim. Behav. Sci.* 95, 223–232.
 426 <https://doi.org/10.1016/j.applanim.2005.04.007>

427 Hanggi, E.B., 2010. Short-term Memory Testing in Domestic Horses: Experimental Design Plays a Role. *J.*
 428 *Equine Vet. Sci.* 30, 617–623. <https://doi.org/10.1016/J.JEVS.2010.10.004>

429 Harrison, K.E., Byrne, R.W., 2000. Hand preferences in unimanual and bimanual feeding by wild vervet
 430 monkeys (*Cercopithecus aethiops*). *J. Comp. Psychol.* 114, 13–21. [https://doi.org/10.1037/0735-](https://doi.org/10.1037/0735-7036.114.1.13)
 431 [7036.114.1.13](https://doi.org/10.1037/0735-7036.114.1.13)

432 Hinch, G.N., Nolan, J. V, Lynch, J.J., Hills, J., 2004. Familiar odour and flavour cues reduce feed neophobia in
 433 sheep. *Anim. Prod. In Aust.* 25, 97-99. <https://doi.org/10.1071/SA0401025>

434 Ijichi, C., Collins, L.M., Creighton, E., Elwood, R.W., 2013. Harnessing the power of personality assessment:
 435 Subjective assessment predicts behaviour in horses. *Behav. Processes* 96, 47–52.
 436 <https://doi.org/10.1016/j.beproc.2013.02.017>

437 Illius, A.W., Gordon, I.J., 1990. Constraints on Diet Selection and Foraging Behaviour in Mammalian
 438 Herbivores, in: *Behavioural Mechanisms of Food Selection*. Springer Berlin Heidelberg, Berlin,
 439 Heidelberg, pp. 369–393. https://doi.org/10.1007/978-3-642-75118-9_19

440 Janczarek, I., Wilk, I., Pietrzak, S., Liss, M., Tkaczyk, S., 2018. Taste Preferences of Horses in Relation to Their
 441 Breed and Sex. *J. Equine Vet. Sci.* 64, 59–64. <https://doi.org/10.1016/j.jevs.2018.02.010>

442 König v, B., 2013. Assessing and influencing personality for improvement of animal welfare: a review of
 443 equine studies. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* 8, 1-27.
 444 <https://doi.org/10.1079/pavsnnr20138006>

445 Larose, C., Richard-Yris, M.A., Hausberger, M., Rogers, L.J., 2006. Laterality of horses associated with
 446 emotionality in novel situations. *Laterality* 11, 355–367. <https://doi.org/10.1080/13576500600624221>
 447 Leliveld, L.M.C., Langbein, J., Puppe, B., 2013. The emergence of emotional lateralization: Evidence in non-
 448 human vertebrates and implications for farm animals. *Appl. Anim. Behav. Sci.* 145, 1-14.
 449 <https://doi.org/10.1016/j.applanim.2013.02.002>
 450 Lloyd, A.S., Martin, J.E., Bornett-Gauci, H.L.I., Wilkinson, R.G., 2008. Horse personality: Variation between
 451 breeds. *Appl. Anim. Behav. Sci.* 112, 369–383. <https://doi.org/10.1016/J.APPLANIM.2007.08.010>
 452 Lloyd, A.S., Martin, J.E., Bornett-Gauci, H.L.I., Wilkinson, R.G., 2007. Evaluation of a novel method of horse
 453 personality assessment: Rater-agreement and links to behaviour. *Appl. Anim. Behav. Sci.* 105, 205-
 454 222. <https://doi.org/10.1016/j.applanim.2006.05.017>
 455 Lovrovich, P., Sighieri, C., Baragli, P., 2015. Following human-given cues or not? Horses (*Equus caballus*) get
 456 smarter and change strategy in a delayed three choice task. *Appl. Anim. Behav. Sci.* 166, 80–88.
 457 <https://doi.org/10.1016/J.APPLANIM.2015.02.017>
 458 Macneilage, P.F., Rogers, L.J., Vallortigara, G., 2009. Evolutionary Origins of Your Right and Left Brain The
 459 division of labor by the two cerebral hemispheres-once thought to be uniquely human-predates us by
 460 half a billion years. Speech, right-handedness, facial recognition and the processing of spatial relat.
 461 *Sci. Am. Mag.* 24, 1-6.
 462 Mars, L.A., Kiesling, H.E., Ross, T.T., Armstrong, J.B., Murray, L., 1992. Water acceptance and intake in
 463 horses under shipping stress. *J. Equine Vet. Sci.* 12, 17–20. [https://doi.org/10.1016/S0737-](https://doi.org/10.1016/S0737-0806(06)81378-X)
 464 [0806\(06\)81378-X](https://doi.org/10.1016/S0737-0806(06)81378-X)
 465 McCall, C.A., Hall, S., McElhenney, W.H., Cummins, K.A., 2006. Evaluation and comparison of four methods
 466 of ranking horses based on reactivity. *Appl. Anim. Behav. Sci.* 96, 115-127.
 467 <https://doi.org/10.1016/j.applanim.2005.04.021>
 468 McGreevy, P., 2004. *Equine Behaviour*. Fourth ed. Elsevier, London.
 469 McGreevy, P.D., Rogers, L.J., 2005. Motor and sensory laterality in thoroughbred horses. *Appl. Anim. Behav.*
 470 *Sci.* 92, 337–352. <https://doi.org/10.1016/j.applanim.2004.11.012>
 471 McGreevy, P.D., Thomson, P.C., 2006. Differences in motor laterality between breeds of performance

472 horse. *Appl. Anim. Behav. Sci.* 99, 183–190. <https://doi.org/10.1016/J.APPLANIM.2005.09.010>

473 McLean, A.N., 2004. Short-term spatial memory in the domestic horse. *Appl. Anim. Behav. Sci.* 85, 93–105.

474 <https://doi.org/10.1016/J.APPLANIM.2003.09.009>

475 Meagher, R.K., Weary, D.M., von Keyserlingk, M.A.G., 2017. Some like it varied: Individual differences in

476 preference for feed variety in dairy heifers. *Appl. Anim. Behav. Sci.* 195, 8–14.

477 <https://doi.org/10.1016/J.APPLANIM.2017.06.006>

478 Michelena, P., Sibbald, A.M., Erhard, H.W., McLeod, J.E., 2009. Effects of group size and personality on

479 social foraging: the distribution of sheep across patches. *Behav. Ecol.* 20, 145–152.

480 <https://doi.org/10.1093/beheco/arn126>

481 Momozawa, Y., Ono, T., Sato, F., Kikusui, T., Takeuchi, Y., Mori, Y., Kusunose, R., 2003. Assessment of

482 equine temperament by a questionnaire survey to caretakers and evaluation of its reliability by

483 simultaneous behavior test. *Appl. Anim. Behav. Sci.* 84, 127–138.

484 <https://doi.org/10.1016/j.applanim.2003.08.001>

485 Moreira, C.G., Bueno, I.C., Menezes, M.L., Mota, T.P., Souza, A.D., Tavares, A.F., Augusto, L.S., Brandi, R.A.,

486 2017. Palatability and digestibility of horse diets containing increasing levels of citrus pulp. *Rev. MVZ*

487 *Córdoba* 20, 4544–4555. <https://doi.org/10.21897/rmvz.57>

488 Müller, C.E., Udén, P., 2007. Preference of horses for grass conserved as hay, haylage or silage. *Anim. Feed*

489 *Sci. Technol.* 132, 66–78. <https://doi.org/10.1016/j.anifeedsci.2006.02.013>

490 Neave, H.W., Weary, D.M., von Keyserlingk, M.A.G., 2018. Review: Individual variability in feeding

491 behaviour of domesticated ruminants. *Anim.* 12, s419–s430.

492 <https://doi.org/10.1017/S1751731118001325>

493 Provenza, F., Villalba, J., Dziba, L., Atwood, S., Banner, R., 2003. Linking herbivore experience, varied diets,

494 and plant biochemical diversity. *Small Rumin. Res.* 49, 257–274. [https://doi.org/10.1016/S0921-](https://doi.org/10.1016/S0921-4488(03)00143-3)

495 [4488\(03\)00143-3](https://doi.org/10.1016/S0921-4488(03)00143-3)

496 Provenza, F.D., 1995. Origins of Food Preference in Herbivores. *Natl. Wildl. Res. Cent. Repellents Conf.*

497 1995, pp. 81–90.

498 Redgate, S.E., Cooper, J.J., Hall, S., Eady, P., Harris, P.A., 2014. Dietary experience modifies horses' feeding

499 behavior and selection patterns of three macronutrient rich diets. *J. Anim. Sci.* 92, 1524–1530.
500 <https://doi.org/10.2527/jas.2012-5579>

501 Rice, M., Jongman, E.C., Butler, K.L., Hemsworth, P.H., 2016. Relationships between temperament, feeding
502 behaviour, social interactions, and stress in lambs adapting to a feedlot environment. *Appl. Anim.*
503 *Behav. Sci.* 183, 42–50. <https://doi.org/10.1016/J.APPLANIM.2016.07.006>

504 Rogers, L.J., 2017. A matter of degree: Strength of brain asymmetry and behaviour. *Symmetry (Basel)*. 9, 1-
505 14. <https://doi.org/10.3390/sym9040057>

506 Rogers, L.J., 2014. Asymmetry of brain and behavior in animals: Its development, function, and human
507 relevance. *Genesis*. 52, 555-571. <https://doi.org/10.1002/dvg.22741>

508 Rogers, L.J., 2010. Relevance of brain and behavioural lateralization to animal welfare. *Appl. Anim. Behav.*
509 *Sci.* 127, 1–11. <https://doi.org/10.1016/j.applanim.2010.06.008>

510 Rogers, L.J., Andrew, R., 2002. *Comparative Vertebrate Lateralization*, first ed. Cambridge University Press,
511 Cambridge.

512 Sankey, C., Henry, S., Clouard, C., Richard-Yris, M.A., Hausberger, M., 2011. Asymmetry of behavioral
513 responses to a human approach in young naive vs. trained horses. *Physiol. Behav.* 104, 464-468.
514 <https://doi.org/10.1016/j.physbeh.2011.05.009>

515 Savin, H., 2015. The effects of lateralisation on detour based problem solving in horses (*Equus caballus*).
516 Pearl, University of Plymouth.

517 Schork, I.G., Schetini de Azevedo, C., Young, R.J., 2018. Personality, abnormal behaviour, and health: An
518 evaluation of the welfare of police horses. *PLoS One* 13, 1-18.
519 <https://doi.org/10.1371/journal.pone.0202750>

520 Seaman, S.C., Davidson, H.P.B., Waran, N.K., 2002. How reliable is temperament assessment in the
521 domestic horse (*Equus caballus*)? *Appl. Anim. Behav. Sci.* 78, 175–191.
522 [https://doi.org/10.1016/S0168-1591\(02\)00095-3](https://doi.org/10.1016/S0168-1591(02)00095-3)

523 Senft, R.L., Coughenour, M.B., Bailey, D.W., Rittenhouse, L.R., Sala, O.E., Swift, D.M., 1987. Large Herbivore
524 Foraging and Ecological Hierarchies. *Bioscience* 37, 789–799. <https://doi.org/10.2307/1310545>

525 Sibbald, A.M., Erhard, H.W., Hooper, R.J., Dumont, B., Boissy, A., 2006. A test for measuring individual

variation in how far grazing animals will move away from a social group to feed. *Appl. Anim. Behav. Sci.* 98, 89–99. <https://doi.org/10.1016/J.APPLANIM.2005.08.015>

Sorvano, V.A., Rainoldi, C., Bisazza, A., Vallortigara, G., 1999. Roots of brain specializations: preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behav. Brain Res.* 106, 175–80. [https://doi.org/10.1016/S0166-4328\(99\)00105-9](https://doi.org/10.1016/S0166-4328(99)00105-9)

Toscano, B.J., Gownaris, N.J., Heerhartz, S.M., Monaco, C.J., 2016. Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. *Oecologia* 182, 55–69. <https://doi.org/10.1007/s00442-016-3648-8>

Triebe, C., Van Den Berg, M., Brown, W., 2012. Tagasaste versus Golden Bamboo: which do horses prefer?, in: *Austrlian Equine Sc. Symp.* p. 40.

Vallortigara, G., Rogers, L.J., 2005. Survival with an asymmetrical brain: Advantages and disadvantages of cerebral lateralization. *Behav. Brain Sci.* 28, 575–633. <https://doi.org/10.1017/S0140525X05000105>

van den Berg, M., Giagos, V., Lee, C., 2016a. Spatial memory or food cues; which do horses use to locate preferred forage patches? *J. Vet. Behav.* 15, 85. <https://doi.org/10.1016/j.jvneb.2016.08.032>

van den Berg, M., Giagos, V., Lee, C., Brown, W.Y., Cawdell-Smith, A.J., Hinch, G.N., 2016b. The influence of odour, taste and nutrients on feeding behaviour and food preferences in horses. *Appl. Anim. Behav. Sci.* 184, 41–50. <https://doi.org/10.1016/j.applanim.2016.08.015>

van den Berg, M., Giagos, V., Lee, C., Brown, W.Y., Hinch, G.N., 2016c. Acceptance of novel food by horses: The influence of food cues and nutrient composition. *Appl. Anim. Behav. Sci.* 183, 59–67. <https://doi.org/10.1016/j.applanim.2016.07.005>

van den Berg, M., Hinch, G.N., 2016. Effect of flavour change on food intake by horses. in: *Recent Advances in Animal Nutrition - Australia Conference*. Armidale, Australia, pp. 83-84.

van den Berg, M., Lee, C., Brown, W.Y., Hinch, G.N., 2016d. Does energy intake influence diet selection of novel forages by horses? *Livest. Sci.* 186, 6–15. <https://doi.org/10.1016/j.livsci.2015.07.022>

Van Tien, D., Lynch, J.J., Hinch, G.N., Nolan, J. V., 1999. Grass odor and flavor overcome feed neophobia in sheep. *Small Rumin. Res.* 32, 223-339. [https://doi.org/10.1016/S0921-4488\(98\)00185-0](https://doi.org/10.1016/S0921-4488(98)00185-0)

Villalba, J.J., Manteca, X., Provenza, F.D., 2009. Relationship between reluctance to eat novel foods and

553 open-field behavior in sheep. *Physiol. Behav.* 96, 276–281.

554 <https://doi.org/10.1016/J.PHYSBEH.2008.10.010>

555 Visser, E.K., Reenen, C.G. Van, Rundgren, M., Zetterqvist, M., Morgan, K., Blokhuis, H.J., 2010. Responses of

556 horses in behavioural tests correlate with temperament assessed by riders. *Equine Vet. J.* 35, 176–

557 183. <https://doi.org/10.2746/042516403776114108>

558 Visser, E.K., Van Reenen, C.G., Hopster, H., Schilder, M.B.H., Knaap, J.H., Barneveld, A., Blokhuis, H.J., 2001.

559 Quantifying aspects of young horses' temperament: Consistency of behavioural variables. *Appl. Anim.*

560 *Behav. Sci.* 74, 241-258. [https://doi.org/10.1016/S0168-1591\(01\)00177-0](https://doi.org/10.1016/S0168-1591(01)00177-0)

561 Warren-Smith, A., McGreevy, P., 2010. The use of pedometers to estimate motor laterality in grazing

562 horses. *J. Vet. Behav. Clin. Appl. Res.* 5, 177–179. <https://doi.org/10.1016/j.jveb.2009.12.023>

563 Wolff, A., Hausberger, M., Le Scolan, N., 1997. Experimental tests to assess emotionality in horses. *Behav.*

564 *Processes* 40, 209–221. [https://doi.org/10.1016/S0376-6357\(97\)00784-5](https://doi.org/10.1016/S0376-6357(97)00784-5)

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