Palatability assessment in horses in relation to lateralization and temperament

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Palatability assessment in horses in relation to lateralization and temperament

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Highlights

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

Abstract

Compared to other domestic animals, little is known about dietary preferences and feed palatability in horses. Furthermore, it is known that horses exhibit a marked lateralization, that is a preference for one side over the other, and that each individual differs in temperament. However, there is a gap of knowledge regarding influence of lateralization and temperament in palatability tests. The aim of the study was to understand the preference for odour and taste of different flavours using palatability tests, taking into consideration both temperamental characteristics and lateralization response. Twelve ponies were randomly enrolled. Three behavioural tests were carried out (arena test, novel object test and person test) to assess individual temperament and lateralization. Behavioural responses and movements within the arena were recorded. Two choice tests were carried out to assess palatability using first-cut chopped hay 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 consumption of the feed (Provenza, 1995). Furthermore horses generally eat little quantities of feed when it is offered for the first time. This could be due to an innate herbivore survival strategy to prevent the excessive consumption of toxic plants in nature (Hinch et al., 2004; Provenza, 1995; van den Berg et al., 2016d; van den Berg and Hinch, 2016). Commercial horse feed industry uses different feed flavours to overcome horses' neophobic nature. The restricted variety of flavours used in horse commercial feedstuffs is probably a consequence of the small number of published studies on the acceptance of flavours.

It is known that behaviour has also a direct impact on feed consumption and diet selection, but aspects such as temperament/personality and individual lateralization are rarely taken into account in horses' feeds selection mechanism. Temperamental traits (Momozawa et al., 2003; Visser et al., 2001) and personality (Ijichi et al., 2013; König v, 2013; Lloyd et al., 2007) are defined by the characteristics of horses' behaviour, both genetically inherited and influenced from the subsequent experiences. Previous works have focused mainly on the possibility to understand the association between personality and welfare. To our knowledge, previous studies have never considered the possible influence of behavioural traits on mechanism of feed selection. However, it is known that animals respond individually to challenges and the results of each test could be influenced by individual response. It is reported that horse's reactivity (or emotionality or nervousness), intended as "an exceeded state of arousal" (McCall et al., 2006) can affect also eating and drinking behaviours (McGreevy, 2004). Besides, lateralization of vertebrates, the asymmetric regulation of right and left hemispheres involved in some behaviours, has received more attention over the last years (Leliveld et al., 2013; Macneilage et al., 2009; Rogers, 2014; Rogers and Andrew, 2002; Vallortigara and Rogers, 2005). Many studies (Austin and Rogers, 2014, 2012, 2007; Baragli et al., 2011; De Boyer Des Roches et al., 2008; Farmer et al., 2018; Larose et al., 2006; McGreevy and Rogers, 2005; McGreevy and Thomson, 2006; Sankey et al., 2011; Savin, 2015; Warren-Smith and McGreevy, 2010) demonstrated that equid exhibit a preference for one side over the other in different contexts. However, to our knowledge, no studies emphasized the influence that a lateralized response could also have on preference tests. In fact, different stimuli might be cause of different lateralized responses in animals (De Boyer Des Roches et al., 2008; Larose et al., 2006). During preference tests, it is not clear if horses select the one feed over the other due to a smell/taste preference or because they independently select the feed on one side. The last could be due to an innate lateralization and or specific temperamental characteristics instead of preference for a given feed based on its organoleptic characteristics. The aim of the study was to assess the preference for four different flavors using palatability test, bearing in mind the influence of temperamental characteristic and individual lateralization response on the choice in preference test. The study was divided in two parts. In the first part three behavioural tests (arena test, novel object test, person test) were carried out in order to assess temperamental characteristics and lateralization (left, L; right, R; none, N), while in the second part four palatability tests were carried out.

2. Materials and methods

2.1 Animals and management

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

2.2 Testing procedures

2.2.1 Behavioural test

Three behavioural tests were carried out over a 7-day period during the morning, each horse being tested just once per day. Tests lasted 5 min each. Firstly, the arena test and then the novel object test were carried out in a testing area of 5x3 m set inside an uncovered paddock. For the person test, a pen of 15x18 m was used. Both the testing areas were virtually divided in four equal zones. For the novel object test, we placed the novel object (a blue rucksack and two pillows) inside the testing area in the opposite side of the entrance. During the person test, an unfamiliar person to the ponies was positioned in the middle of the area. A 1-m semicircle around the object/person was considered in order to assess the distance between 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**
Behavioural variables	
Quiet standing	
Slow walk	Walking slowly, the neck is horizontal or lower in quiet condition of exploration.
Sustained walk	Walking actively and looking in front or around (from Wolff *et al.*, 1997).

Trot-gallop	A two/three beat gait.
Vigilance	Standing still, the neck is elevated, head and ears are orientated with attention (from Visser *et al.*, 2001).
Whinnying	Vocalization
Passage (prancing)	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 *et al.*, 1997).
Raised tail	Tail up (tail root above horizontal line).
Snorting	Snorting ("forceful expulsion of air through the nostrils incidentally preceded by a raspy inhalation sound", from Visser *et al.*, 2001).
Focus novelty	Focusing on novel object (ears, eyes and head pointed in direction of novel object) (from Visser *et al.*, 2001).
Exploring object/person	Exploring the novel object/person (<2m), nose under the belly line, ears and eye pointed to the object.
Pawing	Striking a vertical or horizontal surface/the air with a forelimb (from Seaman *et al.*, 2002).
Defecation/ Urination	Elimination of faeces/urine.
Rolling	Rolling on the ground.
Exploring	Exploring area (out of 2 m from novelty, nose below the belly-line, ears, eyes and head not pointed towards the novel object) (Visser et al., 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 et al., 2008; Larose et al., 2006) (Figure. 1)
Nostril	Sniffing the object with the right/left nostril (Larose et al., 2006; McGreevy and Rogers, 2005)
Forelimb leg	Standing with forelimb leg right or left in front.

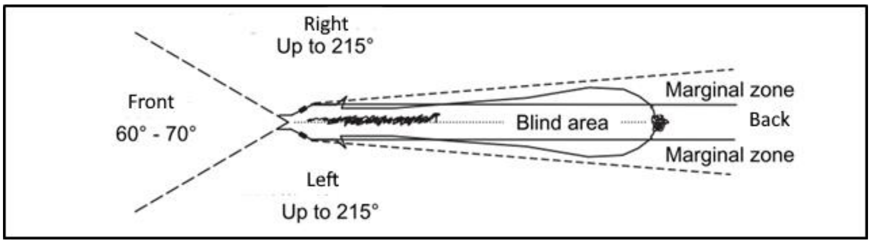

FIGURE 1: Vision in horses: panoramic visual field (adapted from Larose et al., 2006)

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 (Ø = 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

All data have been analyzed using R (v 3.6.1). Each individual horse was set as experimental unit. Normal distribution of the data was tested by Shapiro Wilk test. The K-means cluster analysis on lateralizated behavior was performed to detect different lateralization groups (left, L; right, R; none, N). The ANOVA and Kruskal-Wallis procedures were used for checking differences on previous identified groups in normal and not normal distributed parameters respectively. Significant effects ($P < 0.05$) were than compared between time points with a Tukey and Wilcox test in parametric and no parametric tests respectively. Results are reported as mean and standard deviation or median and quartiles for normal and not normal distributed parameters, respectively. Principal Component Analysis (PCA) was carried out to assess temperamental characteristics. The PCA was used to analyse behavioral variables, indexes of activity of animals (latency of axis, latency of area) and time go near (time to go near) or to interact (time to approach) with the object or the person. Components were interpreted considering high positive (+, > 20%) and negative (-, <-20%) coefficients. Sign test was applied to evaluate the buckets approached first (both from side that for flavour point view). Chi-squared and sign one sample test were used to evaluate correspondence of group detected from PCA from clusters detected.

3. Results

3.1 Behavioural test

3.1.1 Lateralization

Results concerning the novel object test and the person test are reported in Supplemental File 1. K-means cluster analysis for lateralized behaviour showed that the ponies were divided in 3 groups that were defined as left (L) N=4, right (R) N=6 and none (N) N=2. Significant differences between the groups were observed during the novel object test but not during the person test. Significance differences were showed between group N and R (P = 0.02) for the frequency of the body placed with axis on the right respect to the 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 were recorded in L group. The R group tended also to spend more time with body axis placed on the right side with a median of 149.3 (131.1-188.7) seconds that differed significantly (p = 0.01) from group N (0; 0-0.3). The L group was not different from both N and R groups. The L group spent 140.3 (66.1-226.9) seconds with body axis on the left that was significantly different (P = 0.05) from group N (0; 0-0). The R group instead did not present differences for this variable. N group did not spent time with body axis placed on right or on the left with the median equal to 0 (0-0) in both cases. This result significantly differed ($P < 0.05$) 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 respectively on the right or on the left axis.

3.1.2 Temperament/Personality

Four components were extracted by the PCA analysis from the data of the behavioural variables and activity variables of the 12 ponies during the three behavioural tests. Together the components explained 72.2% of the total variance. The items loading for the four components are shown in Table 2. Each component was interpreted by examining the contribution of behavioural and activity variables (Table 2 in 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.

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

***Average latency body axis* [4]**	-5.34%	**25.39%**	**23.71%**	7.87%
Cumulative Variance	25.32%	44.94%	62.32%	72.21%
Standard deviation	2.01	1.77	1.67	1.26

[1]Slow walking+ sustained walking

[2]Frequencies

[3]Average latency of movement between the different areas

[4]Average 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

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

3.2 Palatability tests

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

3.3 Interaction of personality traits, lateralization and palatability test

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

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

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
X squared: P value = 0.08		

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*
X squared: P value < 0.001		

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 +++

4. Discussion

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

4.1 Lateralization

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

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

4.2 Temperament/Personality

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

Lloyd *et al.* (Lloyd et al., 2007) defined as excitable a horse that “over reacts to any change” and is “easily excited, highly strung”. The excitatory component found in their research had a negative correlation with standing position. This is in agreement with the results found in our research, in which *excitable component* had a negative correlation with quiet standing variable and a positive correlation with trot-gallop. 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).

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

4.4 Interaction of personality traits, lateralization and palatability tests

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

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

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

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

Conflict of interest statement

None.

Ethical statement

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

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