

Preference for heights of feeding troughs in mares: a pilot study

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Abstract

Preferences for the height of feeding troughs of seven mares were evaluated and, as awareness of surroundings when feeding is adaptive for horses, the relationship between preference responses and attention behaviours was also assessed. First, feeding troughs at four heights (0, 25, 50, and 75 cm) were provided for 16 days, and the amount of time animals ate from each height option was measured daily. These data were used to determine the preferred/non-preferred options for each individual. An overall preference for heights of 50 and 75 cm was detected, but responses showed significant individual variation. The same animals were subsequently observed, when feeding from preferred (four days) or non-preferred (four days) trough heights, while their behaviours were recorded. Moving ears/head (attention behaviours) occurred more frequently when feeding from preferred heights of feeding troughs. We concluded that to ensure better welfare conditions for the mares in this study, it was important to consider the individual variation of such preferences if possible, however, by using troughs positioned at 50 or 75 cm, the general preference could be assured. Moreover, when feeding from their preferred heights, the study mares paid more attention to their surroundings than when feeding from their non-preferred height and thus it is likely that they defended their preferred options more. However, further studies are needed to evaluate the generalisability of these findings. We have demonstrated a methodology to assess mares' preferences and provided some preliminary data on the relationship between preferences/non-preferences and the possible emotional states of mares.

Keywords: animal welfare, attention, feeding, mare, Preference Index, trough

Introduction

Identifying potential welfare indicators can be ambiguous (for a review, see Volpato *et al* 2007, 2009), and selecting which one is preferable is difficult. Dawkins (2006, 2008) has proposed more attention be paid to animals' needs, ie that the "point of view" of animals should be considered and Volpato *et al* (2009) have suggested welfare is enhanced when animals find themselves in a situation that has been chosen freely. There are numerous examples in the literature of preference tests that allow animals to choose freely between two (pairing tests) or more options (multiple-choice tests). For example, preference for sexual partners (Ryan 1980; Gonçalves & Oliveira 2003; Liao & Lu 2009), substrates (Webster & Hart 2004; Galhardo *et al* 2009; Matsui *et al* 2009), and environmental temperatures (Girguis & Lee 2006; Holcomb *et al* 2014).

While there are studies that have sought to evaluate welfare indicators and conditions for horses (Waran & Randle 2017), their preferences have only ever been investigated

superficially. For example, while there have been studies into horses' preferences for different feed types (McCann & Hoveland 1991; Cairns *et al* 2002; Müller & Udén 2007; Allen *et al* 2011), they have been mostly concerned with preferences for commercial foodstuffs. Moreover, horses' preferences for different food trough height are yet to be studied and no consensus exists as to the best way of feeding horses using troughs. Luz *et al* (2015) tested the influence of distance, proportion, and height of troughs on agonistic behaviours in horses and found that longer distances and greater height reduced the frequency of kicks regardless of the social stability. A possible explanation for this result is that troughs positioned at a greater height and longer distances allow for a wider field of vision, which may create less of a sense of vulnerability during feeding. On the other hand, Wheeler (2006) posited such positioning of the trough to be unnatural, as the horse is a grazing animal. If true, this would justify positioning troughs at ground level; however, horses in a breeding environment

are accustomed to supplementation via feeding troughs and, therefore, may prefer troughs to be elevated.

Behavioural patterns associated with the paying of constant attention to surroundings (eg head and ear movements), especially when feeding (Sillar *et al* 2016), are most likely evolutionary adaptations that improve the survival of these animals as prey species in their natural habitat (Granger 1908; Feist & McCullough 2010; Rochais *et al* 2017). Therefore, an increased level of such behaviours could be related to an improved environmental perspective as well as viewing possible competitors.

The aim of this pilot study was to evaluate seven mares' preferences for different feeding trough heights and study the influence of feeding at preferred heights on attention behaviours. Preference and non-preference responses were determined by calculation of the Preference Index (PI; Maia & Volpato 2016), which allows the determination not only of the preferred and non-preferred options but also considers the intensity of such responses at an individual level. We hypothesised that since study mares in our facility would be familiar with feeding troughs positioned at a high height, they would show a distinct preference for this and, in monitoring competition during feeding from these preferred options, they would express attention behaviours more frequently.

Materials and methods

Ethical note

All procedures followed the ethical principles of animal experimentation and were approved at CIAEP/CONCEA n° 01.0115.201 by the Ethics Committee on the Use of Animals (CEUA) of FMVZ, UNESP, Botucatu, São Paulo, Brazil.

Study animals and holding conditions

We tested seven unbred mares (mix of Appaloosa, Arab, and American Quarter breeds) that were aged 5 to 10 years (mean [\pm SD]: 7.50 [\pm 0.98]), weighing 340 to 380 kg (360.00 [\pm 21.15] kg; CV = 4.34%), with a height at the withers from 1.50 to 1.60 m (1.56 [\pm 0.033] m; CV = 2.11%). Animal availability in the test facility led to these mares being utilised for data collection (see below). As our sample size was small, it was used to demonstrate a methodology that can be applied to assess mares' preferences, as well as providing preliminary data related to preference behaviours of mares. More studies are required to generalise the findings beyond this small sample.

Prior to this study, the animals were housed in a semi-intensive system, where they had access to pasture with mineral salt supplementation and received a commercial grain ration only sporadically, ie when animals were used for farm work or during drought periods. These seven mares lived as a group prior to this study. However, occasionally, they were separated for brief periods in order to be put to use in various farm management projects, eg feeding commercial grain ration, herding cattle, and for other experiments or practical lessons. Since these mares

were born and raised in the same environment and always managed by the same handler, who was also actively involved in the study, stress levels were minimised during the study. None of these animals had any previous experience of feeding from troughs positioned above 10 cm but they were used to the occasional commercial grain ration and feeding management.

Test facilities

The study was performed at the Equine Production of Teaching, Research, and Extension Farm (FEPE) of the University of Veterinary Medicine and Animal Science (FMVZ), UNESP, Botucatu, São Paulo, Brazil. The experiment was conducted in a closed, round pen (diameter: 7 m). A closed circular area was chosen for the procedure in order to mimic a stable environment. Care was taken to avoid corners, which could influence a possible side-bias on preference response, such as lateralisation effects, ie a preference for a particular side of the body movement (Larose *et al* 2006) that could influence the preference for troughs positioned at different sides.

Pre-pilot study test

Based on the National Research Council guidelines (NRC 2007), horses' daily consumption of commercial grain, in conditions of maintenance, must not exceed 1% of their living weight. A total of 1 kg of such grain was used in the daily tests, which corresponded to around 0.3% of the average weight of the tested mares. In order to determine the average amount of time the study mares spent feeding on the total amount of commercial grain (1 kg) provided in the troughs in each test, an experiment was conducted prior to the pilot study. This involved utilising concrete feed bunks (placed around 10 cm above ground level) as adapted feeding troughs for the horses. These troughs differed from those used in the subsequent experiment and, additionally, were placed at a different height in order to avoid the risk of bias originating from animals' previous experiences. In this pre-pilot test, we added 1 kg of commercial grain to the concrete feed bunks and recorded the time for each mare to consume all the grain. Each mare was evaluated individually under such conditions and the average time spent feeding was 15 min.

Procedures

First study

The seven study mares' height preferences as regards feeding troughs were evaluated using individual multiple-choice tests. Four height options for each animal: 0, 25, 50, 75 cm (Figure 1) were simultaneously provided. Troughs were spaced equally apart (0.5 m; Figure 1). Each (four) was identical in shape (35 \times 30 \times 31.5 cm; length \times width \times height) and contained the same type and amount (250 g totalling 1 kg) of commercial grain ration. They were attached to the round pen wall and able to be moved to various heights on the wall. To avoid habituation, the position of each feeding trough was randomised (each

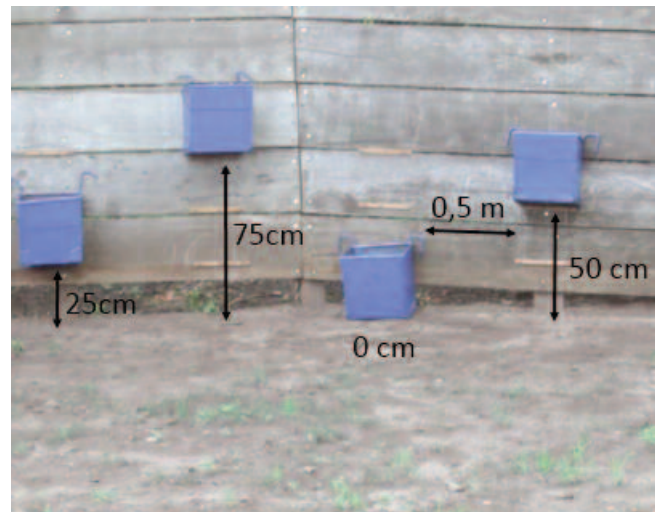
was numbered and drawn) per animal on each day of the treatment and, following the test each day, mares were provided with free access to pasture.

The mares were led individually, always by the same handler and in a random order (defined by the daily draw of the animals, which were numbered), to the test area (Figure 2). They were then held by two handlers, one on each side, who simultaneously released the mare from the halter to allow her to feed from the troughs. This procedure was implemented as a way of avoiding side-bias (eg lateralisation effects). The distance between the animals and all trough options was kept the same and mares were filmed by the same observer (one of the two handlers mentioned above) throughout the entire experimental period (15 min, which was sufficient to allow the animals to consume the commercial grain ration, see *Pre-pilot test*). The observer always stood a safe distance (3 m) outside of the round pen to avoid influencing the mares' behaviour. A semi-professional Sony camera was used and, from the recordings, we were able to establish the total feed consumption time for each animal at each trough option. This procedure was repeated for 16 consecutive days, during which time the height and position of feeding troughs were randomly alternated on each test day to avoid positioning biases in preference responses.

Second study

The trough that we used was structurally identical to those in the first study and was filled with the same type and quantity of feed. We evaluated the same seven mares feeding from their most preferred or most non-preferred height options on alternating test days. The experimental procedures related to the handling of mares were as described in the first study except animals were only tested on eight consecutive days and with just one feeding trough per day. The mares were tested individually for four days with their individual most preferred option and for four days with their individual most non-preferred option and test days were alternated between preferred and non-preferred options of feeding troughs per animal. From the recordings, we elucidated the frequencies of attention behaviours (moving ears, moving head) and pawing, which is frequent during the feeding time. We used a focal-animal sampling method, recording for a specified time-period (Altmann 1974). The behavioural categories 'moving ears' and 'moving head' are described in the equid ethogram (McDonnell 2003; Ransom & Cade 2009). The animal elevates its neck and head (moving head behaviour) or ears (one or both; moving ears behaviour) directed to a specific sound or other stimulus that catches its attention as an alert response while standing at attention. Pawing is described as a scratching of the foreleg (right or left) along the substrate (McDonnell 2003), which is interpreted as a frustration behaviour and occurs when a horse is prevented from reaching a desired goal or resource (Odberg 1973).

Figure 1



The four height options of feeding troughs: 0, 25, 50, or 75 cm above the ground.

Figure 2



The two handlers released the horse from the halter simultaneously.

Table 1 Preference Index calculation.

Test number	Step 1 Raw Frequency (RF) ¹	Step 2 Cumulative Frequency ²	Step 3 Area ³	Step 4 Cumulative Area ⁴	Step 5 Expected Area ⁵ (ExA)	Step 6 Variation of cumulative area from the ExA ⁶	Step 7 Preference Index ⁷
1	140	140	70	70	107	-26.13	-26.125
2	514	654	771	841	429.5	424.63	398.5
3	49	703	122.5	963.5	967	128.38	526.87
4	385	1,088	1,347.5	2,311	1,574.25	685.38	1,222.25
5	545	1,633	2,452.5	4,763.5	2,495.625	2,132	3,354.25
6	606	2,239	3,333	8,096.5	3,719.375	4,659.25	8,013.5
7	258	2,497	1,677	9,773.5	4,999.875	4,488.63	12,502.13
8	232	2,729	1,740	11,513.5	6,608.625	4,456.75	16,958.88
9	696	3,425	5,916	17,429.5	8,446.75	8,479.38	25,438.25
10	263	3,688	2,498.5	19,928	10,527.25	8,854.63	34,292.88
11	306	3,994	3,213	23,141	12,632.5	9,762.88	44,055.75
12	261	4,255	3,001.5	26,142.5	15,007.25	10,018.75	54,074.5
13	388	4,643	4,850	30,992.5	17,729.13	12,503.13	66,577.63
14	598	5,241	8,073	39,065.5	20,695.75	17,552.13	84,129.75
15	24	5,265	348	39,413.5	23,468.88	15,369.88	99,499.63
16	145	5,410	2,247.5	41,661	25,654.38	14,246.13	113,745.8

This procedure is carried out for each height of feeding trough. Example based on data from mare 2 and for trough positioned at a height of 50 cm.

¹ Registered frequencies obtained in each test day;

² $\sum_{t1}^m RF$;

³ Areas calculated above the cumulative-frequency line;

⁴ $\sum_{t1}^m Area$;

⁵ Mean of cumulative areas from each height of feeding trough in the respective number of test day and mare. In this table, we show data only for the height of 50 cm; the ExA is calculated from data of all tested heights at tn for mare 2;

⁶ Data obtained subtracting Step 5 from Step 4;

⁷ Cumulative data of Step 6.

Data analysis

First study

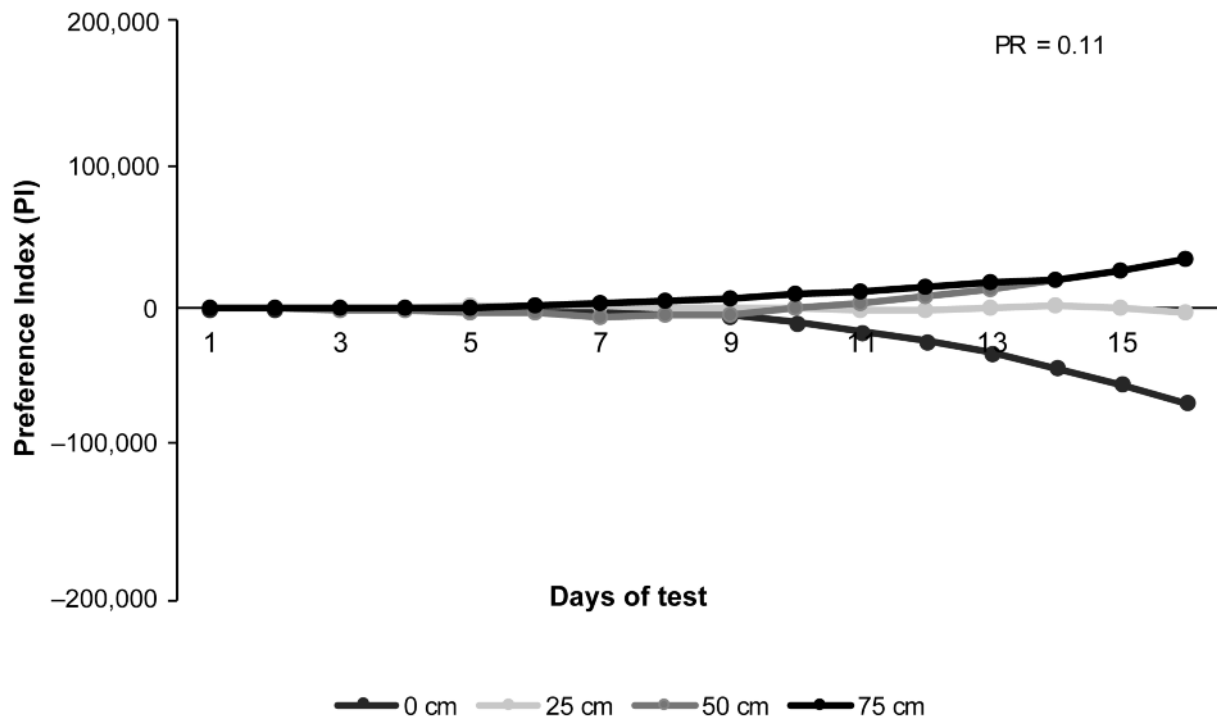
The Preference Index (PI) as described by Maia and Volpato (2016) was used to assess the preferences/non-preferences and the intensities of these responses by incorporating individual data values into the Preference Index valuations. This method minimises the influence of animals' initial decisions compared to subsequent choices since more recent choices receive more weighting in the PI calculations. The assumption here is that animals' initial choices may be prone to change since subjects are not used to the experimental apparatus and procedures, unlike subsequent choices.

Using this index, the cumulative time of feeding from each trough over the days of testing was calculated first, such that more recent periods of time spent feeding were given greater weighting (higher values) to represent a more accurate response, while also considering the history of

animal choices (for more information, see Maia & Volpato 2016). Then, areas above the cumulative timeline were calculated and summed over time, obtaining cumulative areas, which were subtracted from expected areas (calculated as whether none of the choice options were preferred). Positive Preference Index (PI) values indicated preference responses while negative values indicated non-preferred options. The individual value of PI represented the intensity of a preferred or non-preferred response of each animal, ie how motivated an animal was to reach an option under a free-choice situation as recently demonstrated (Maia *et al* 2017; Maia & Volpato 2018). Details of step-by-step calculations of PI are given in Table 1.

Individuals expressing two or more preferences are qualitatively different from single-preference individuals; the latter may represent a group of more decisive animals, ie individuals which consistently select just one option from all the available ones. Therefore, when

Figure 3



Preference (positive PIs) and non-preference (negative PIs) responses of the group of mares during the feeding time over the test days. The PR value is also indicated as the group showed more than one preference.

mares expressed more than one preference, we calculated the preference rate (PR), which is a correction for PI values when two or more preferences are expressed (Maia & Volpato 2016). The PR indicates the strength of the response of the first preference in relation to the other preferred options. High PR values indicate that the first preference is highly preferred compared to the other preference responses, while low PR values indicate that the first preference is very similar to the other preferred options.

We compared the frequencies of preferences or non-preferences for different feeding trough heights by using Goodman's proportion test (1965; within multinomials). Significance was settled at $\alpha = 0.05$. Additionally, coefficients of variation (CVs) of the intensity responses of preferences or non-preferences among individuals were calculated in order to evaluate individual variation of intensity of preference or non-preference.

Second study

When data were not normally distributed (Kolmogorov-Smirnov test; $P < 0.05$) and/or heteroscedastic (Levene test; $P < 0.05$), which was the case for moving ears behaviour, they were transformed ($\text{root}[x + 0.5]$) to achieve parametric assumptions. The dependent *t*-test (one-tailed) was used to compare the frequency of occurrence of each attention behaviour or pawing between treatments (preferred vs non-preferred height options). Significance was settled at $\alpha = 0.05$.

Results

First study

The mares as a group preferred (positive PI values) the trough options positioned at 50 and 75 cm, thus non-prefering (negative PI values) both ground-level and 25-cm high options (Figure 3). Troughs that were 50-cm high were the most preferred ones (the highest positive PI value) for the group, but the strength of response for this option as compared to the second preferred one (75 cm) was lower (PR value; Figure 3).

Despite the group preference, significant variation was seen among animals' preferences for the different heights of troughs (five preferences for 25, 50, or 75 cm and one preference for 0 cm; Goodman's proportion test, 1965; $P > 0.05$, high limit = 0.63 and low limit = -0.13). The intensity of individual preferences indicated different positive PI values that also varied (CV = 60.80%) between individuals (Table 2). The troughs placed at ground level were preferred by only one individual (mare 2, representing 14.29% of mares), and it was the most non-preferred option by five horses (71.42%; Table 2). Moreover, the individually non-preferred heights of feeding troughs also varied, both considering the non-preferred options (based on Figure 4, six non-preferences for 0 cm and two non-preferences for 25, 50, or 75 cm; Goodman's proportion test, 1965; $P > 0.05$, high limit = 0.91 and low limit = -0.25), as well as the intensity of such responses indicated as negative PI values (CV = 30.18%)

Table 2 Individual intensities of preference (positive values) and non-preference (negative values) responses for the height positioning of the feeding troughs indicated by PI calculations.

Height of feeding trough (cm)	Mares						
	1	2	3	4	5	6	7
0	<i>-146,607</i>	27,025	<i>-34,563.9</i>	<i>-116,939</i>	<i>-118,262</i>	<i>-91,086.9</i>	<i>-73,904.3</i>
25	<i>-45,682.6</i>	6,996	34,210.13	19,790.75	<i>-60,589.6</i>	46,034.13	55,360.75
50	121,042.9	15,559	50,835.63	60,066.25	<i>-39,031.1</i>	<i>-36,071.9</i>	1,338.25
75	71,246.88	<i>-49,580</i>	<i>-50,481.9</i>	37,082.25	217,882.9	81,124.63	17,205.25

Values in bold = the most preferred options (the highest positive PI value for each mare). Values in italics = the most non-preferred option (the highest negative PI value for each mare).

(Table 2). PR values also varied between individuals that expressed more than one preference response, indicating variation in the strength of the first preference response compared to the other preferences (Figure 4).

Second study

With respect to attention behaviours, we found higher frequencies of head movements during the feeding time at troughs positioned at the preferred height (mean values [\pm SD]: 21.28 [\pm 17.30] and 12.28 [\pm 12.49] for preferred and non-preferred heights, respectively; dependent *t*-test = 2.30; *P* = 0.03; Figure 5). Moreover, ear movements were also more frequent when mares were feeding from troughs positioned at the preferred height (9.044 [\pm 5.74]), compared to the non-preferred (5.93 [\pm 3.29]); dependent *t*-test = 2.63; *P* = 0.02 (Figure 6). On the other hand, pawing behaviour was not affected by the height positioning of the feeding trough (11.71 [\pm 13.03] and 19.57 [\pm 15.75] for preferred and non-preferred heights, respectively; dependent *t*-test = 1.76; *P* = 0.06).

Discussion

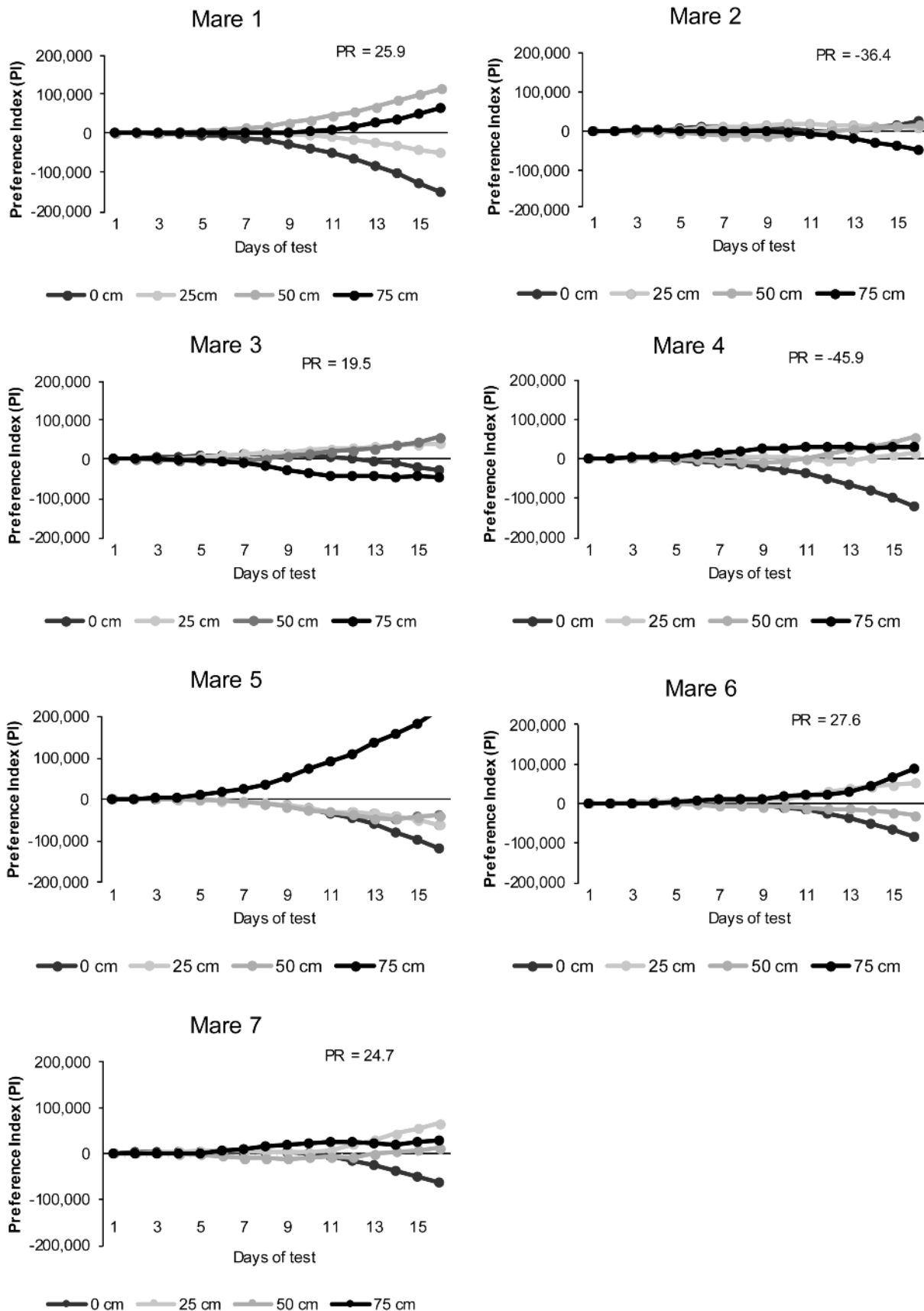
When the mares in this study selected different feeding trough heights to feed from, there was a general preference for troughs positioned at 50 and 75 cm, although individual preferences differed. Conversely, although non-preference responses also varied among study mares, such responses appeared more standardised as most avoided feeding troughs positioned at ground level. For these mares, it could be argued that it would be better to provide higher feeding troughs or, at least, avoid providing feeding troughs at the ground level, however, whenever possible, individual preferences should be taken into account. Despite our small sample size (*n* = 7), this is the first time, as far as we know, that preference and non-preference responses of mares for feeding trough height has been demonstrated. Furthermore, the study mares expressed more attention behaviours when feeding from troughs at preferred heights. These findings are suggestive of our study mares being more concerned about their general surroundings when feeding from troughs positioned at preferred heights. Future studies could investigate the extent to which these findings are generalisable for mares of different sizes, breeds, backgrounds, etc. Our findings

also provide preliminary data on the relationship between mares' preferences and their possible emotional states.

The general consensus is that the best way to provide grain feed to horses is to seek to mimic the natural feeding habits of such animals. In the case of horses, a large herbivore, it is natural to eat from ground level (Wheeler 2006). However, since domestic horses tend to be accustomed to feeding from troughs positioned at higher levels, our original hypothesis was that higher troughs would be preferred. In fact, at a group level, the troughs arranged at 50 or 75 cm were both strongly preferred (see PI and PR values in Figure 3). Furthermore, troughs at 25 cm and, especially, troughs at 0 cm, had negative PI values, ie were avoided by mares (Figure 3). Similarly, even at the individual mare level, troughs positioned at ground level were avoided by most individuals (Table 2, Figure 4). It is worth also noting that feeding losses are significantly higher when horses are fed on the ground, and there is an increased risk of contamination (Lewis 1995). Our study mares also increased attention behaviours when feeding at preferred trough heights, suggesting that such preferences are, in fact, important to them. Future studies should aim to explore the implications of such management on horse welfare and improve the generalisability of our results.

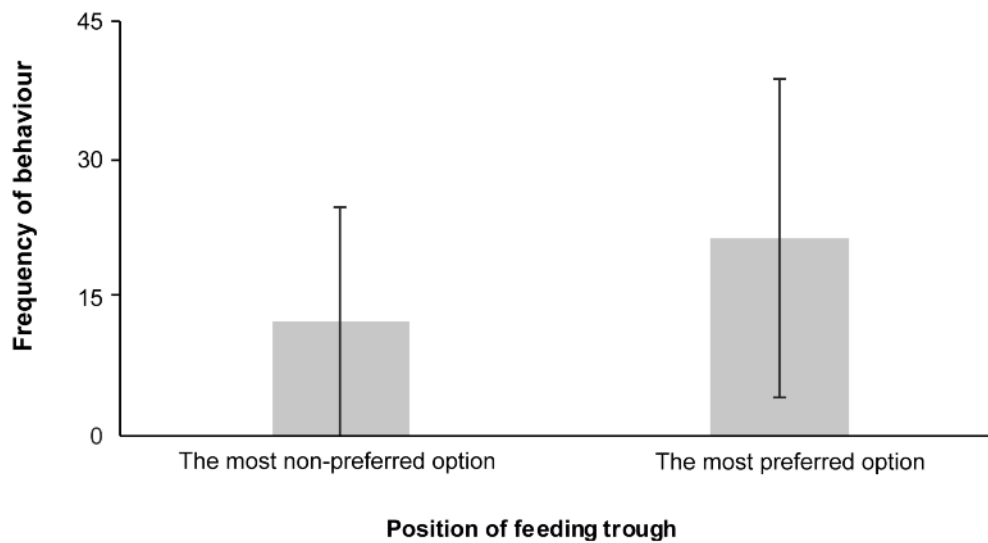
The large variation in individual preferences/non-preferences of study mares for feeding trough height highlights the importance of considering preferences at the levels of the individual to improve their welfare conditions, as suggested by Maia and Volpato (2016). This is even more important when we consider that the preference/non-preference responses varied not only in terms of preferred (positive PIs) or non-preferred (negative PIs) options, but also in their intensity of response (PI and PR values). Future studies may better elucidate the cause of the variability seen here, but we should consider that individuals may express different strategies to cope with environmental adversities (Broom & Fraser 2010). However, as only seven mares were tested, it is possible that individual variation may be significantly minimised when evaluating larger groups.

Figure 4



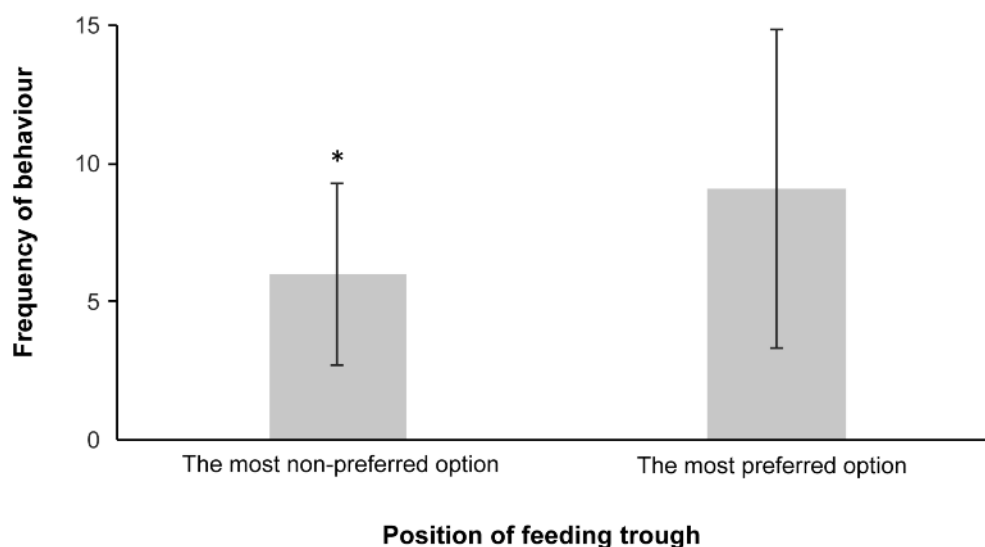
Individual profiles of preference (positive PIs) and non-preference (negative PIs) responses during the feeding time over the test days. The PR values are indicated for mares that expressed more than one preference response.

Figure 5



Effect of the height positioning of the feeding troughs (preferred vs non-preferred options) on moving-head behaviour. Data are presented as means (\pm SD) (the most non-preferred option = 12.28 [\pm 12.49]; the most preferred option = 21.28 [\pm 17.30]). * Indicates significant difference (one-tailed dependent *t*-test: = -2.30; *P* = 0.03; *n* = 7).

Figure 6



Effect of the height positioning of the feeding troughs (preferred \times non-preferred options) on moving-ears behaviour. Data are presented as means (\pm SD) (the most non-preferred option = 5.93 [\pm 3.29]; the most preferred option = 9.04 [\pm 5.74]). * Indicates significant difference (one-tailed dependent *t*-test = 2.63; *P* = 0.02; *n* = 7).

Animal height could have influenced mares' individual preferences for trough height, in which case, shorter individuals might have preferred lower troughs. This was unlikely to be a factor in our sample, given that the height and weight of the tested mares were very similar (CVs of 2.11 and 4.34%, respectively). Thus, we consider worthwhile to emphasise the need for greater consideration of preference studies at an individual level, especially since most studies ignore individual variation and infer preference at a group level (Soriguer *et al* 2002; Thomas 2002;

Matsumoto *et al* 2008; Burfeind *et al* 2009; Snowberg & Benkman 2009; Zizzari *et al* 2009).

In our second study, as in the first one, the mares were tested individually to avoid interference generated by the social context of dominance hierarchy during feeding, which may include, for example, resource competition by means of agonistic interactions (eg Luz *et al* 2015). Therefore, variation in head and ear movements could not be explained as communication with other horses. Further, as the tested mares were already used to the management procedures, the

test area, the handler, and also to being isolated under certain circumstances, changes in these behaviours also could not be explained by the stress response of animals. Such variations may reflect perception of surroundings, related, perhaps, to momentary emotional states arising from horses' attentional behaviour toward the environment (Ransom & Cade 2009; Rochais *et al* 2017). This may be an indication that study mares were paying greater attention to their surroundings and, consequently, felt more secure when feeding from their preferred trough height.

As regards pawing behaviour, no effect of preferred/non-preferred feeding troughs was found (see *Results*). However, our sample size was restricted (seven mares) and as the *P*-value approached significance caution is needed in interpreting such a result. Therefore, future studies including a greater number of mares should shed more light on this issue and determine whether or not there is an effect on pawing behaviour. We believe both results to be a possibility. Pawing can become a learned behaviour when associated with positive reinforcement to obtain feed (eg Baragli *et al* 2009). Thus, it may be related more to the motivation for the feed itself than the provision of different heights of the feeding troughs, which should explain a lack of effect on such behaviour. On the other hand, once domestic horses are confined in closed areas during feeding, restriction of movement may be detrimental and potentially worsen in a situation whereby horses are limited to non-preferred conditions. This should lead to more frequent pawing behaviour, which may be considered an indicator of frustration (Odberg 1973). However, as mentioned above, this requires further investigation.

Animal welfare implications and conclusion

Preference for feeding trough height differed significantly between our study mares. Despite the small sample size, individual preferences should be considered when providing suitable troughs in order to ensure better welfare conditions in management procedures for mares. However, when this is practically impossible, it seems that, as far as our sample mares were concerned, troughs 50 or 75 cm high were preferable to those placed lower. Moreover, when our study mares fed from the most preferred option, some attention behaviours were more prevalent. This probably indicates that our mares have been defending their preferred options when feeding from preferred heights of feeding troughs. As our sample size was small, further studies are needed to evaluate the generalisability of our findings. However, the methodology used can be applied to assess mares' preferences for welfare and practical purposes, and our findings provide a foundation for future research on preference/non-preference responses and emotional states of mares.

Acknowledgements

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