

Contents lists available at ScienceDirect

Applied Animal Behaviour Science



journal homepage: www.elsevier.com/locate/applanim

Urinating in transponder-controlled feeding stations – Analysis of an undesirable behaviour in horses

Chiara N. Ellerbrock^a, Margit H. Zeitler-Feicht^b, Marianne Cockburn^c, Michael H. Erhard^a, Miriam Baumgartner^{c,*}

^a Chair of Animal Welfare, Ethology, Animal Hygiene and Animal Husbandry, Department of Veterinary Sciences, LMU Munich, Veterinärstr. 13R, 80539 Munich, Germany

^b Chair of Organic Agriculture and Agronomy, TUM School of Life Sciences, Technical University of Munich, Liesel-Beckmann Str. 2, 85354 Freising, Germany

^c Agroscope, Swiss National Stud Farm, SNSTF, Les Longs-Prés 2, 1580 Avenches, Switzerland

ARTICLE INFO

Keywords: Displacement activity Horse behaviour Automated feeding system Equine welfare Horse husbandry Group housing

ABSTRACT

Urination in transponder-controlled roughage feeding stations is a widespread undesirable behaviour of grouphoused horses. Urination on hard surfaces, such as the floor of the stations, is contrary to the natural elimination behaviour of horses because they prefer to urinate on soft, absorbent surfaces, and it increases ammonia emissions around the stations. The following aspects were analysed: a) urination as potential displacement activity during feed anticipation, b) absence of appropriate elimination areas in the stable and c) ammonia odour as a trigger stimulus. We observed a group of 33 horses in three different situations: 1) baseline situation, 2) provision of elimination areas containing an absorbent substrate in front of the feeding stations and 3) neutralisation of ammonia odour in the feeding stations. In the baseline situation all horses were observed, regardless whether they urinated in the feeding station or not. In the other two situations, only the urinating-horses were observed. We analysed 5 h of video per day, recorded on 4 days from seven feeding stations in each situation. We used an information theory approach, calculating three different (generalized) linear mixed effects models and all according sub-models. In the baseline situation, the horses showed that the horses urinated often after 'ground exploration', and there was more ground exploration in the urinating-horses than in the non-urinating-horses. In addition, a higher percentage of the mares than of the geldings urinated during at least one station visit, and mares urinated more often per visit than geldings. Before urination, the horses in most cases lowered the head toward the hay container to trigger the sensor and cause the station to open the partition and make the hay accessible. The time span between lowering the head and access to feed could be perceived as too long by the horses (maximum duration: 30 s) and lead to urination as a displacement activity. In addition, urination never occurred when the hay was accessible, only when the hay was inaccessible, closed, opening or closing. This leads us to conclude that urination is related to feed anticipation. The frequency of urination bouts was not reduced by installing additional elimination areas or neutralising the urine odour, so additional elimination areas and urine odour do not seem to have a role in the undesirable behaviour. Further research is needed to investigate a displacement activity or a classical conditioning in more detail to prevent urination by horses in automated feeding stations.

1. Introduction

In group housing systems for horses, technology is increasingly used to optimise labour efficiency. Automated feeding methods provide benefits not only in terms of labour but also regarding animal welfare. In transponder-controlled feeding stations it is possible to feed individualised rations of hay multiple times per day without extended intervals between meals if enough feeding places are available, thus facilitating autonomous behaviour (Zeitler-Feicht, 2015; Kjellberg and Morgan, 2021). However, there may be risks associated, e.g. agonistic behaviour. Studies have so far focused on reducing the frequency of conflicts in the area around automated roughage feeding stations (Zeitler-Feicht et al., 2010; Baumgartner et al., 2022). Hence, automated feeding systems also bear unknown risks in terms of constraints in species-specific behaviour.

* Corresponding author. *E-mail address:* miriam.baumgartner@agroscope.admin.ch (M. Baumgartner).

https://doi.org/10.1016/j.applanim.2023.106015

Received 4 May 2023; Received in revised form 19 July 2023; Accepted 24 July 2023 Available online 26 July 2023

^{0168-1591/© 2023} The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

Another potential risk is that the horse has to wait for a partitioning board to open to get access to the roughage in the station. During this phase of feed anticipation, the emotional state of the horse can quickly turn from excitement to frustration (Hintze et al., 2017; Dietze et al., 2019).

For instance, group-housed horses in open barns with such feeding systems show an unnatural, although widespread, behaviour: they urinate on a solid surface in the automated roughage feeding station next to the hay container. However, horses prefer a soft, absorbent surface for urination (Feist and McCullough, 1976; Sambraus and Zeitler-Feicht, 2003; Zeitler-Feicht, 2015). Especially stallions and geldings avoid spraying their belly and legs with urine (Zeeb, 1992) and can withhold their urine for several hours if no adequate substrate is present (Sweeting et al., 1985). Thus, open barns with group-housed horses usually provide elimination areas covered with absorbent substrate (Fader, 2002). Nevertheless, the horses may urinate elsewhere and possibly in undesirable places. According to surveys in German language regions of Europe, this undesirable behaviour occurs with a prevalence of approximately 42% in group housing systems (Ellerbrock et al., in prep.). Urination in the feeding station leads to exposure to high and thus potentially harmful ammonia concentrations during the entire roughage intake. As a consequence, the risk of thrush (Brehm et al., 2017) and respiratory tract irritation (Gerber and Straub, 2016) is increased. Moreover, large urine puddles can form inside and in front of the stations (Fig. 1). Managers from affected farms have reported that the residues from the urine and thus also the ammonia odour become persistent with time and cannot be removed with usual cleaning measures. In transponder-controlled concentrate feeding stations, which operate similarly to the transponder-controlled roughage feeding stations, undesirable urinating has very rarely been observed.

In livestock farming, studies aiming to guide the urination behaviour of farm animals have been conducted because of the associated ecological and economic problems. For animal welfare and economic reasons it is highly important to reduce ammonia in the barn, but such work has not been done in horses (Varel, 2002; Leinker, 2007; Varel et al., 2007; Dirksen et al., 2020).

To date, it is unknown what causes urination on solid floor in automated roughage feeding stations. The aim of the present study was to identify possible causes of this behaviour. We considered the following factors: a) urination as displacement activity during feed anticipation, b) absence of appropriate elimination areas in the stable facility next to the feeding stations and c) ammonia odour as a trigger stimulus for urination.



Fig. 1. Feeding station where horses urinate frequently.

2. Animals, materials and methods

2.1. Animals and farm

The study took place on a horse farm near Munich (Germany), housing 33 leisure horses (21 geldings and 12 mares) of different breeds and ages (range: 7–30 years). They were kept in an open barn where the undesirable behaviour of urinating in automated feeding stations had occurred regularly for many years. The open barn included seven automated roughage feeding stations (HIT single- and double-haydosing units, HIT Active Stable®, Weddingstedt, Germany), of which six were arranged in double stations and one was a single station. Adjacent to the lying halls, which were bedded with rubber mats, elimination areas had been established for years. In addition to the automated roughage feeding stations, there were two transpondercontrolled concentrate feeding stations, in which no urination occurred during the observations. Straw was provided ad libitum in three panel feeding racks with 20 openings in total. The individual period of roughage availability per day in the automated roughage feeding stations was based on the body condition of the horses, judged by farm managers together with horse owners. One horse was given additional hay in a separate stall overnight because of his age, and one horse was ill throughout the period of the baseline situation, so he stayed in an individual stall.

2.2. Materials

Horses can enter transponder-controlled roughage feeding stations independently of their feeding time, which is checked via a sensor installed in the station in front of the hay container. When an incoming horse lowers the head toward the hay container and thus the sensor, the sensor detects the transponder that is implanted in the horse's neck, woven into the mane or attached with a neck collar (Fig. 2a) (Kjellberg and Morgan, 2021). If the horse has feeding time left, the rear gate at the station entrance closes, and the partitioning board to the hay container opens. It takes approximately 30 s from transponder detection to full feed access. The horse can then access the hay through the opening. An infrared sensor, which is installed on top of the station and directed at the horse's back (approximately at the withers), monitors the horse's presence in the station. If this sensor does not detect a horse for 2 min, the partitioning board to the hay container closes. The individual feeding duration in minutes throughout the 24-hour day can be programmed for each horse on a central computer. The program should ensure a species-appropriate roughage intake that is spread evenly throughout the day with only short intervals between meals (Zeitler-Feicht, 2015). The recommendation of about 10 meals per 24-hour day was met on the studied farm.

2.3. Methods

On randomly selected days between late September and late October 2021, we first assessed the baseline situation via video recordings. The recorded behaviours and events are listed in detail in Tables 1 and 2.

Thereafter, two mitigation trials were conducted between mid-January and early April 2022. In Trial 1 we examined the absence of appropriate elimination areas in the barn as a possible cause of urination in the feeding stalls. For this purpose, additional elimination areas, about 20 cm deep and filled with wood shavings, were installed in front of the automated roughage feeding stations (Fig. 2b). Thus, before entering the feeding station, the horses had to walk across a latrine with soft, absorbent substrate that is attractive for urination because horses avoid splashing their own body while urinating (Fader, 2002; Zeitler-Feicht, 2015).

In Trial 2 we examined the possible cause 'ammonia odour as trigger stimulus'. For this purpose, the solid floor inside the automated roughage feeding stations was neutralised by spreading granulate citric

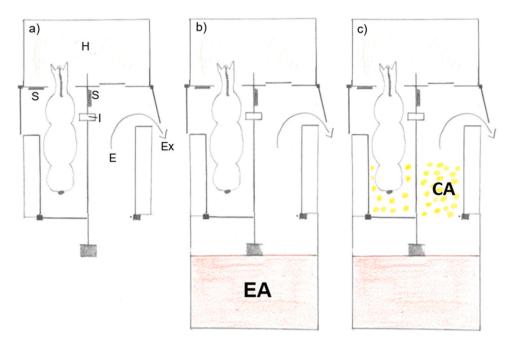


Fig. 2. Illustration of the transponder-controlled roughage feeding station for horses with two feeding places: a) baseline situation, b) Trial 1 (adding appropriate elimination areas next to the feeding stations), c) Trial 2 (adding citric acid to neutralise ammonia odour, which could be a trigger stimulus for urination); H = hay, S = sensor, I = infrared sensor, E = entrance, Ex = exit, EA = elimination area, CA = citric acid.

Table 1

Behaviours and events with description and unit or category of measurement assessed in the studied situations. All behaviours and environmental conditions were recorded during the baseline situation. During the two mitigation trials, only the frequency of urination bouts and the number of visits in the feeding stall were recorded for those horses that had shown urination in the baseline situation.

Behavioural category	Recorded event	Description	Levels of measurement
Elimination	Urination bouts Position of partitioning board to the hay container	Occurrence of urination bouts Position of the partitioning board at the beginning of urination	Number per station visit Closed, opening, open, closing
	Feeding attempt before urination	Lowering the head toward the sensor in front of the hay container maximally 30 s before urinating	Yes, no
Ingestion	Feeding bout	Lowering the head through the opening and lowering the head to the ground directly Infront of the hay container. These behaviours indicated eating. (Hay was available on the ground when a previous horse had taken some roughage out of the hay container)	Number of feeding bouts per visit
Emotional state	High arousal	Aroused: \geq 3 aggressive behaviours or pawing the ground indicating conflict- or frustration (seeTable 2)	Yes, no
Exploration behaviour	Ground exploration	Lowering the head toward the ground in the area between entrance and infrared sensor	Number of bouts per visit

Table 2

Ethogram with social behaviours for evaluating the emotional state (modified after Goldschmidt-Rothschild and Goldschmidt-Rothschild and von, Tschanz, 1978; McDonnell and Haviland, 1995; Burla et al., 2016; Baumgartner et al., 2022).

Behaviour	Description
Aggressive behaviours	
Facial threat expression	The ears are pinned backward to the side of the
	horse's neck to the rear.
Threat to bite	Threatening facial expressions where the mouth is
	opened, the head is turned to the side, the teeth can
	be shown temporarily.
Head swing threat	The horse moves its head and neck to the side with a
	threatening expression but closed mouth.
Attack	The horse moves in the direction of the exit (where
	potentially another horse stays) with a threatening
	gesture, the head is stretched forward.
Threat to kick with hind leg	The horse lifts one of the hind legs without
	extending it. At the same time, the horse shows a
	facial threat expression
Indicating conflict- or	
frustration-related behaviour	
Pawing the ground	The horse hits the ground with a foot more than
	once.

acid (citric acid monohydrate, Golden Peanut®, Garstedt, Germany; Fig. 2c). This treatment successfully aimed lowering the increased pH on the floor in the feeding stations and thereby reducing the colonisation by ubiquitous urease-producing bacteria (Leinker, 2007; Randall et al., 2016; Ray et al., 2018). In each trial, the horses had an adaptation period of 3 weeks, after which the assessments were made over a study period of 14 days.

For each situation (baseline situation, Trial 1, Trial 2), the observation period was 5 h per day (from 17:00 h to 22:00 h), and seven roughage feeding stations were evaluated on 4 days, resulting in 140 h of video material per situation. During the baseline situation, the aim was to investigate possible reasons for the urination. To this purpose, the urination behaviour was recorded in detail, as well as other behaviours and events that might be related to urination (see Table 1). Our goal was to evaluate the behavior immediately before the onset of the feeding stations opening mechanism. Therefore, the time-period for which behaviours were considered to be related to urination was set at 30 s before urination took place. This time span was chosen because the partitioning board to the hay container took about 30 s to fully open. Further, a pretest revealed that the preparation of the horses to urinate (tail raise, leg positioning) lasted up to 30 s, thus supporting this approach. Behaviours that indicate a high level of arousal, while a station visit, were additionally recorded (see Table 2). In the following, the horses that were observed urinating at least once in the baseline situation are described as the 'urinating-horses'. The horses that were not observed urinating at any time, in the baseline situation, are referred to as the 'non-urinating-horses'. During the two mitigation trials, only behaviours of the urinating-horses were recorded (number of station visits and frequency of urination bouts). If a horse did not enter any of the seven roughage feeding stations during the 5-hour observation period, the number of visits was recorded as zero, meaning that on the respective observation day, neither feed ingestion nor urinating was recorded for this horse. This was the case for three horses of the urinating-horses, once per horse, and for one horse of the non-urinatinghorses.

2.4. Statistical analyses

Data were analysed in R version 4.3.0 (R Core Team, 2023). We calculated two linear mixed effects models and one generalized linear mixed effect model from three individual data sets with the 'lmer' and 'glmer' function from the 'lme4' package (Bates et al., 2015). Two of these models were used to investigate potential influencing factors, using the data recorded in the baseline situation, whereas the third model tested the effect of the two mitigation measures (adding appropriate elimination areas in front of the feeding station and neutralising the ammonia odour with citric acid) on the frequency of urination bouts in the feeding stalls. One dataset included all horses including the non-urinating-horses (dataset: all horses), whereas a second dataset only included the urinating-horses in the baseline situation (dataset: urinating horses). A third dataset included the urinating-horses in the three different situations (baseline situation and two mitigation measures, dataset: urinating horses in the different situations). The computed models are shown in Table 3.

The parameters for restricted maximum likelihood was set to false. After fitting the model, the residuals were tested for normal distribution and homogeneity of variance. If this assumption was not fulfilled, the data were logit transformed. This was the case for 'frequency of

Table 3

Models created for the different data sets.

Model evaluating situation	Dataset	Used Model	Target Variable ^a	Fixed effects ^b	Random effects ^c
Baseline	All horses	glmer	UB	S + FA + EG + G	G/H + TD + FS
Baseline	Urinating horses	lmer	FU	S + FA + EG	H + TD + FS
Mitigation measures	Urinating horses in different situations	lmer	FU	MM	H + TD + FS

^a UB = urination bout during feeding station visit (factor with two levels), FU = frequency of urination bouts during feeding station visit (numeric)

^b S = Sex (factor with two levels), FA = feed availability (continuous), EG = exploration of the ground (continuous), G = classification of urinating- and non-urinating-horses (factor with two levels), MM = mitigation measures (factor with 3 levels), / = nested

 $^{\rm c}$ G = classification of urinating- or non-urinating-horses, H = horse (factor with 33 levels), TD = trial day (factor with four levels), FS = feeding station (factor with seven levels)

urination'.

As we followed an information theory approach, P-values are not provided because this approach to model selection is an alternative to the more common P-value-based hypothesis testing. Prediction models were calculated by bootstrapping with the 'boot' package (Davison and Hinkley, 1997; Canty and Ripley, 2022). The 'dredge' function ('MuMIn' package; (Burnham, 2002)) was used to find the best model based on the smallest Bayesian information criterion (BIC), smallest Akaike's information criterion (AIC) and largest model weight. The model weight can be interpreted as the probability that a specified model is optimal given the data in the set of models considered, where the model weights of all models in a given set add up to 1 (Symonds and Moussalli, 2011). The set was the maximum model described above and all simpler models including the null model. Although all models were run with the AIC and BIC, the BIC values were considered as the main result, because the BIC penalises complex models and as such selects the most functional model, whereas the AIC selects more complex models that best explain the data. The evidence ratio (ER) indicates how often the selected model is more likely to be true compared with the best model, and ER₀ indicates how often the selected model is more likely to be true compared with the null model. If the delta of the AIC and BIC between the first two models is below two, the simpler model should be chosen (Symonds and Moussalli, 2011; Cockburn et al., 2017). Furthermore, the data were plotted with the 'ggplot2' package (Wickham, 2016).

3. Results

In the baseline situation, the feeding stations were entered 379 times (258 times by urinating-horses) during the 4-day observation period, regardless of urination bouts. In 65.7% (n = 249 of 379) of the total station visits, the horses received hay from the hay container (62.4% of visits of urinating-horses, n = 161 of 258; non-urinating-horses: 72.7%, n = 88 of 121). In 48.5% (n = 184 of 379), the horses ate hay from the ground (51.6% of urinating-horses, n = 133 of 258; non-urinating-horses: 42,1, n = 51 of 121). On average, the horses triggered the sensor and consequently received feed from the hay container 1.40 times (1.42 times in urinating-horses) during a visit in the roughage feeding station (see Table 4).

At least one urination bout was observed in the automated roughage feeding stations in 19 horses (57.6%; N = 33). Among them were 11 of 12 mares and eight of 21 geldings. The non-urinating-horses were one mare and 13 geldings. The total number of urination bouts in the feeding stations was 193 (Fig. 3), occurring during 138 visits of horses that urinated. Thus, horses urinated in 53.4% of the visits. In the urinating-horses, the probability of urination during a visit was higher in mares ($X^- = 0.619$) than in geldings ($X^- = 0.314$; Fig. 4A).

If a horse showed the behaviour 'urinating in the automated roughage feeding station', it urinated on average 1.3 times during a visit. Mares were found more often than geldings to urinate more than once during a station visit (Fig. 4B). In addition, the mares urinating several times during a feeding station visit urinated several times per visit over the entire 4-week period. A high percentage (71.5%; n = 138 of 193) of the urination bouts occurred when the partitioning board to the hay container was in a closed position. In 26.4% (n = 51 of 193) of the station visits, horses urinated while the partitioning board was in the process of opening, and in 2.1% (n = 4 of 193) of the visits while the partitioning board was in the process of closing. When the partitioning board was open, urination behaviour did not occur. In 66.8% (n = 129 of 193) of the urination bouts, horses lowered their heads toward the sensor immediately before urination (see Table 5).

Regardless of urination behaviour, the horses explored the ground 108 times during 67 visits in total. In the non-urinating-horses, only one horse once performed this behaviour. In the urinating-horses, 62.6% (n = 67 of 107) of the cases of ground exploration were followed by urination behaviour. In comparison with the non-urinating-horses, we found that there were more urination bouts after exploring the ground

Applied Animal Behaviour Science 266 (2023) 106015

Table 4

Descriptive statistics of the recorded behaviours and events.

Variable	Sample	Average frequency per visit	Standard deviation per visit	Maximum number per visit	Number of visits	Total number
Hay from the hay contained	er					
	All horses	1.40	2.349	25	249	350
	Urinating horses	1.42	1.429	25	161	231
Hay from the ground						
	All horses	1.57	2.501	24	184	289
	Urinating horses	1.55	2.430	24	133	209
Urinating						
	Urinating Mares	1.49	1.140	8	108	161
	Urinating	1.07	0.254	2	30	32
	Geldings					
Ground exploration						
	All horses	1.61	1.154	6	68	108
	Urinating horses	1.60	1.169	6	67	107
Before urinating	Urinating horses	1.34	0.872	4	49	67

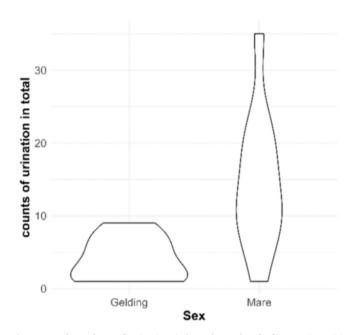


Fig. 3. Total numbers of urination independent of a feeding station visit separated by sex (N_{Geldings} = 32, N_{Mares} = 161).

(BIC_w: 0.153; ER: 0.452). The model with the smallest BIC value observed an effect of ground exploration and group, but it was not selected owing to the selection criteria; because the BIC difference between the best and the second-best model was < 2, the simpler model was chosen, which included an effect of ground exploration (see Table 6). The model with the smallest AIC value contained ground

Table 5

Descriptive statistics of the recorded behaviours and events involving urination. The sample included only the urinating-horses.

	ĩ				
Variable	Average frequency per urination	Standard deviation per urination	Maximum numbers per visit	Number of visits	Total number of urinations
Lowering the head (toward the hay container) before urinating Partitioning board at beginning of urination	1.21	0.579	5	107	129
Closed	1.39	0.946	7	98	138
Opening	1.04	0.455	4	48	51
Open	0	0	0	0	0
Closing	1.33	1.528	3	2	4

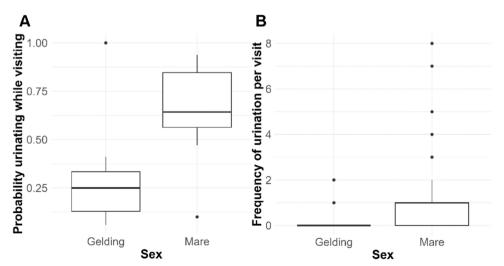


Fig. 4. (A) Probability of urinating in urinating-horses when visiting the station in the baseline situation, separated by sex ($N_{geldings} = 8$, $N_{mares} = 11$; e.g., the point at 1.0 indicates, that one gelding urinated each time while visiting the station). (B) Frequency of urination bouts per station visit in the baseline situation, separated by sex ($N_{visit/Geldings} = 102$, $N_{visit/Mares} = 156$). Boxplot with median, 25th to 75th percentile and extreme values (shown with dots). Numbers refer to the number of horses per sex (A) and the number of urination bouts per sex (B).

Table 6

Model descriptions of the best model for the comparison with the urinating and non-urinating-horses including the simplest Model; The selected model is marked in bold. If the difference in the BIC/AIC between a simpler model and the best model was < 2, the simpler model was selected.

		-		
	Model parameter ^a	1. model	2. model	3. model
BIC	Fixed effects ^b	EG + G	EG + S + G	EG
	Model	10	14	2
	complexity			
	Model	1	2	3
	hierarchy			
	BIC	297.8	298.4	299.4
	BICw	0.338	0.253	0.153
	Δ_{i}	0	0.57	0.158
	ER	1	0.749	0.452
	ER ₀	Infinite	Infinite	Infinite
AIC	Fixed effects ^b	EG + S	EG + S + FA + G	$\mathbf{E}\mathbf{G} + \mathbf{F}\mathbf{A} + \mathbf{S} + \mathbf{G}$
		+ G	+	+ FA *S
	Model	14	16	144
	complexity			
	Model	1	2	3
	hierarchy			
	AIC	266.9	268.1	268.5
	AICw	0.117	0.065	0.053
	Δ_{i}	0	1.18	1.59
	ER	1	0.556	0.453
	ER ₀	Infinite	Infinite	Infinite

^a Parameters used in the BIC- and AIC-based model selection. Model hierarchy = position in the BIC/AIC table, BIC/AIC = BIC/AIC value, BIC_w/AIC_w = Bayesian/Akaike's weight representing the probability that the given model is the best, Δ_i = differences in the BIC/AIC in comparison with the best model, ER = evidence ratio indicating how often the selected model is more likely to be true compared with the best model, $ER_0 =$ evidence ratio indicating how often the selected model is more likely to be true compared with the null model

^b EG = ground exploration, S = sex, G = group, FA = food availability, * = an interaction between variables

exploration, sex and group (AICw: 0.117; ER: 1) as fixed effects. Considering the 'urinating horses' dataset, an effect of ground exploration and sex could be observed in the AIC (AIC_w: 0.195; ER: 1). The same effect could also be observed in the model with the smallest BIC value, but the model was not selected owing to the selection criteria. Therefore, the best model had an effect only of ground exploration (BICw: 0.352; ER: 0.736; see Table 7).

The behavioural variables indicated high levels of arousal in 26.3% of the horses (five mares, one gelding). These horses showed high levels of arousal in 5.4% of the station visits (during 14 visits; the maximum was 27 times during one visit). Furthermore, we observed a horse that showed a posture typical of urination but was disturbed by another horse that was outside of the feeding station. The disturbed horse did not urinate and instead showed aggressive behaviours.

In the model comparing mitigation measures, the null model (no effects) was the best model under BIC model selection (BIC_w: 0.958; ER: 1), whereas the model under AIC selection indicated a mitigating effect for adding appropriate elimination areas in front of the feeding stations (AIC_w: 0.817; ER: 1).

4. Discussion

The present study is the first to investigate which causes could be responsible for the undesirable urination by horses in automated feeding stations and to what extend this behaviour could be prevented. The following factors were analysed as possible causes: a) urination as a displacement activity during feed anticipation, b) absence of appropriate elimination areas in the stable facility next to the feeding stations and c) ammonia odour as a trigger stimulus for urination.

The analyses revealed that the probability of horses that urinated during a station visit and the frequency of urination bouts per visit were

Table 7

Fixed effects of the best model from the model selection table for only consid-
ering the urinating-horses in the baseline situation; The selected model is
marked in bold. If the difference in the BIC/AIC between a simpler model and the
best model was < 2 , the simpler model was selected.

	Model parameter ^a	1. model	2. model
BIC	Fixed effects ^b	EG + S	EG
	Model complexity	6	2
	Model hierarchy	1	2
	BIC	423.0	423.6
	BICw	0.478	0.352
	Δ_{i}	0	0.61
	ER	1	0.736
	ERo	Infinite	Infinite
AIC	Fixed effects ^b	EG + S	$EG + S + EG^*S$
	Model complexity	6	22
	Model hierarchy	1	2
	AIC	398.1	398.8
	AICw	0.195	0.144
	Δ_{i}	0	0.61
	ER	1	0.738
	ER ₀	Infinite	Infinite

^a Parameters used in the BIC- and AIC-based model selection. Model hierarchy = position in the given BIC/AIC table, BIC/AIC = BIC/AIC value, BIC_w/AIC_w = Bayesian/Akaike's weight representing the probability that the given model is the best, Δ_i = differences in the BIC/AIC in comparison with the best model, ER = evidence ratio indicating how often the selected model is more likely to be true compared with the best model, $ER_0 = evidence$ ratio indicating how often the selected model is more likely to be true compared with the null model b EG = ground exploration, FA = food availability, S = sex

higher in mares than in geldings. In addition, 100% of urination bouts occurred while the partitioning board was in the closed position or in the process of opening or closing (i.e. while the hay was not yet or no longer accessible) and in 67% after the horses had lowered the head to get access to food. Ground exploration occurred more often in urinatinghorses than in the non-urinating-horses and more often in mares than in geldings. Only a few horses showed high arousal during a visit to the feeding station. The mitigation trials did not result in any improvement in urination behaviour in the feeding station, although a potential improvement was observed when an appropriate elimination area was provided (Trial 1).

The finding of more pronounced urination in mares (11 of 12) than in geldings (eight of 21) and mares urinating more frequently per visit could be related to male-specific urination behaviour on hard surfaces. Theoretically, according to Zeeb (1992), especially stallions and geldings avoid spraying their belly and legs with urine. Oestrous behaviour could also be a reason for the frequent urinations of the mares. However, our observations were made in autumn, a season when mares rarely show oestrus-related behaviours (Aurich, 2009). Moreover, the mares in our study that did so during a visit in the feeding station urinated several times over the entire study period of 4 weeks. This observation contradicts the hypothesis of oestrus-induced urination behaviour because horses show oestrous behaviour only for approximately 5 days (Engelhardt et al., 2015).

As no urination was observed while the hay was accessible, this suggests that urination is related with feed accessibility and potentially feed-retention related frustration. Furthermore, in a majority of cases the horses urinated after lowering the head toward the sensor in front of the hay container. Our study also revealed that the horses had to trigger the sensor more than once to receive feed in the roughage feeding stations. This was due to technical problems with the sensor, which in some cases failed to recognise a horse in the station even if the horse was still present. In the most extreme case, a horse had to re-register for access to hay 25 times during a single feeding period. This circumstance was likely associated with negative emotions such as frustration because in the phase of feed anticipation, the emotional state of horses can quickly shift from 'pleasant anticipation' to frustration (Hintze et al., 2017).

Ricci-Bonot and Mills (2023) set the period of food anticipation to 10 s with a period of one minute of frustration at waiting for a reward. This time period corresponds well to the common duration of the opening phase of the partition in our study. The negative emotional states (frustration and disappointment) were characterized by more abnormal behaviours like "biting feeder", "tongue show" and "chewing" (Ricci-Bonot and Mills, 2023). Horses in single housing systems show frustration-related behaviour while anticipating feed arrival (Dietze et al., 2019). In such a situation, horses can show spontaneous urination as a form of frustration or displacement activity (Zeitler-Feicht, 2015). In the present study, the horses could enter the automated feeding station anytime regardless of their allowed feeding time left. The length of the waiting period between two meals differed individually, and even for horses with feeding time, it took approximately 30 s until the feed was accessible.

The relatively long waiting period between detection by the sensor and feed receipt might also contribute to increased frustration in the roughage feeding stations. An additional indication for this is the finding that the horses in our study did not urinate in the two automated concentrate feeding stations although these operated with the same mechanism. A major difference was that in the concentrate feeding stations the horses directly accessed the feed and did not have to wait 30 s until the feed was accessible.

Based on our behavioural assessment 26% showed signs of high arousal. During high arousal, the sympathetic nervous system is activated in the body. Physiologically, the activity of the sympathetic nervous system inhibits the emptying of the bladder, whereas in restful situations the parasympathetic nervous system is activated and is said to promote body functions such as emptying of the bladder (Engelhardt et al., 2015). The fact that horses urinate only in a relaxed situation was illustrated by one of our studied animals. This horse was about to urinate when another horse approached the feeding station, whereupon the former interrupted the urination behaviour and instead displayed aggressive behaviour coinciding with increased arousal. In future studies, the level of high arousal could be assessed by measuring the heart rate and heart rate variability, which can provide an objective assessment to indicate if the sympathetic or the parasympathetic nervous system is being activated in certain situations (Scopa et al., 2018; Ketonen et al., 2022). Dietze et al. (2019) found that a delay in feed provision during the phase of feed anticipation induced an increase in the frequency of abnormal behaviours as well as increased changes between different abnormal behaviours. Scopa et al. (2018) suspects that abnormal behaviours, such as vacuum chewing, are calming behaviours used during or after stressful events. Because the feeding stalls were only observed from behind, we were not able to record facial expressions of the horses. The limited assessment of behaviours indicative of high arousal could be a reason why we found an increased level in only 26% of the horses. The delayed access to feed and, consequently, the emotional impact could impair the wellbeing of the horses in the transponder-controlled automated feeding stations and should be investigated in more detail. Installing additional cameras focused on the horses' head would help to reliably assess the emotional state. Therefore, the Horse Facial Action Coding System (EquiFACS) could be a useful tool (Carmo et al., 2023; Ricci-Bonot and Mills, 2023).

Because urinations mostly occurred after the lowering of the head toward the sensor and when the partitioning board was in the closed position, a classical conditioning is also conceivable. The first urination bout in a feeding station may have been a random behaviour caused by arousal or urine odour. During the next station visit (recurring situation) it is possible that the forthcoming access to feed subconsciously induced the urge to urinate. In this case, the horse does not consciously control the urination (Knipsel, 2007). Whereas with operant conditioning the horse may have learnt that urination is necessary to obtain food and it would urinate and expect to receive food afterwards (Kappeler, 2012). Both classical and operant conditioning or a combination of them are possible causes of urination. However, there are no research results on the conditioning of urination in horses in our study.

As a further hypothesis, we tested the effect of additional and adequate elimination areas with sufficiently absorbent substrate. They were installed in front of the entrance to the automated feeding stations. Therefore, the horses had to cross these elimination areas when they entered the stations. We found no relevant reduction in urination in the feeding stations, in contrast to the results of Sambraus and Zeitler-Feicht (2003). In their study, the horses urinated less frequently inside the feeding stations in two of the three open barns if an elimination area was present in front of the entrance to the station. Moreover, Muggenthaler et al. (2010) could observe that the horses urinated in ninety-five percent on a soft non splashing surface if there is one available.

Another hypothesis was that urine odour might be a trigger stimulus for urination. In the baseline situation the different results of the linear mixed effects models indicated an effect of ground exploration whereby more urination occurred after ground exploration. Moreover, whereas ground exploration was only shown once by one horse in the nonurinating-horses, 63% of the urinating-horses showed this behaviour before urination. Furthermore, mares did not only urinate more often than geldings but they also explored the ground more often. Thus, ground exploration was likely related to urination. In a further trial we treated the urine-contaminated floor area in the automated feeding station with citric acid to lower the pH and thereby inhibit the activity of the ubiquitous enzyme urease and neutralise the ammonia odour (Leinker, 2007; Troccaz et al., 2013; Randall et al., 2016). Although the treatment was successful in lowering the pH, the frequency of urination bouts in the feeding stations was not reduced by this mitigation measure. For several animal species, odorous substances in urine can be trigger stimuli for various behaviours, including urination (Nielsen, 2020). However, our results did not confirm the hypothesis that the ammonia odour stimulates the urination behaviour of the horses and thus the frequent urinations in the automated feeding stations. As we could only reduce the ammonia odour but not the other substances (e.g., pheromones), this could be a reason why the urination could not be reduced in the mitigation measures. Theoretically, the effect of ground exploration on urination might be associated with scent-marking behaviour, which is typical for stallions and possibly geldings (Ödberg and Francis-Smith, 1977; Tschanz, 1978; King and Gurnell, 2007). However, in the present study, mostly mares showed urination after ground exploration.

5. Conclusion

The results of our study show that many of the urination bouts in the automated feeding stations occurred in the context of feed anticipation and thus are probably caused by frustration. The time span from lowering the head to actual access to feed might be perceived as too long. In addition, our results suggest that various motivations can underly urination in the feeding station (e.g. frustration, absence of appropriate elimination areas, urine odour). In addition, conditioning of urination behaviour to the subsequent access to feed cannot be excluded. The neutralisation of ammonia odour and an adequate positioning of the elimination areas did not reduce the frequency of urination bouts in the feeding stations. Future studies on the prevention of urination behaviour in automated feeding stations should be conducted to assess the effects of automated feeding methods on the psychological and physical wellbeing of horses. Such studies could include measurements of heart rate, heart rate variability and salivary cortisol as physiological indicators of arousal or stress as well as more cameras to observe the whole area around the stations. A useful approach would be to re-engineer the feeding station in a way that the horses can access the roughage directly and do not have to wait 30 s until they receive feed. Comparing a fast versus a slow opening partitioning board would help to further assess the causes of urination.

Ethical statement

All provided data were stored in anonymised form and are available on a public repository (https://zenodo.org/record/8226012) according to the General Data Protection Regulation. The Animal Welfare Officer of the Faculty of Veterinary Medicine declared that the study is no animal experiment according to the German Animal Welfare Act and EU Directive 63/2010. In addition, the non-invasive nature of the study complies with the Guidelines for the Ethical Treatment of Animals in Applied Animal Behaviour and Animal Welfare Research (ISAE Ethics Committee, 2017). Thus, ethical review and approval were not required for the animal study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank the German Animal Welfare Federation (Stiftung des Deutschen Tierschutzbundes) for the financial support of the project. We are grateful to the farm manager and owners for allowing us to include their horses in our study.

References

- Brehm, W., Gehlen, H., Ohnesorge, B., Wehrend, A. (Eds.), 2017. Handbuch Pferdepraxis, 4th ed. Enke Verlag, Stuttgart, 1239 pp.
- Reproduktionsmedizin beim Pferd. Enke Verlag. In: Aurich, C. (Ed.), 2009. Stuttgart. https://doi.org/10.1055/b-004-129751.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using. lme4. J. Stat. Soft. 67. https://doi.org/10.18637/jss.v067.i01.
- Baumgartner, M., Erhard, M.H., Zeitler-Feicht, M.H., 2022. Which animal-to-feedingplace ratio at time-controlled hay racks is animal appropriate? Preliminary analysis of stress responses of horses. Front. Vet. Sci. 9, 1005102. https://doi.org/10.3389/ fvets.2022.1005102.
- Burla, J.-B., Ostertag, A., Patt, A., Bachmann, I., Hillmann, E., 2016. Effects of feeding management and group composition on agonistic behaviour of group-housed horses. Appl. Anim. Behav. Sci. 176, 32–42. https://doi.org/10.1016/j. applanim.2016.01.011.

Burnham, K.P., 2002. Model Selection and Multimodel Inference: A Practical

- Information-Theoretic Approach, 2nd ed. Springer New York, New York, NY, 512 pp. Canty, A., Ripley, B., 2022. boot: Bootstrap R (S-Plus) Functions. R package version 1.3–28.1.
- Carmo, L.G., Werner, L.C., Michelotto, P.V., Daros, R.R., 2023. Horse behavior and facial movements in relation to food rewards. PloS One 18, e0286045. https://doi.org/ 10.1371/journal.pone.0286045.
- Cockburn, M., Schick, M., Maffiuletti, N.A., Gygax, L., Savary, P., Umstätter, C., 2017. Lower working heights decrease contraction intensity of shoulder muscles in a herringbone 30° milking parlor. J. Dairy Sci. 100, 4914–4925. https://doi.org/ 10.3168/jds.2016-11629.
- Davison, A.C., Hinkley, D.V., 1997. Bootstrap Methods and Their Application. Cambridge Univ. Press, Cambridge, p. 582.
- Dietze, S., Zeitler-Feicht, M.H., Baumgartner, M., 2019. Worauf deutet eine erhöhte Aktivität der Pferde vor der Kraftfuttergabe hin - Vorfreude oder Stress? Aktuel. Arb. Zur. artgemäßen Tierhalt. 101–112.
- Dirksen, N., Langbein, J., Matthews, L., Puppe, B., Elliffe, D., Schrader, L., 2020. Conditionability of 'voluntary' and 'reflexive-like' behaviors, with special reference to elimination behavior in cattle. Neurosci. Biobehav. Rev. 115, 5–12. https://doi. org/10.1016/j.neubiorev.2020.05.006.
- Engelhardt, W., von, Breves, G., Diener, M., Gäbel, G. (Eds.), 2015. Physiologie der Haustiere, 5th ed.. Enke Verlag, Stuttgart, p. 675.
- Fader, C., 2002. Ausscheide- und Ruheverhalten von Pferden in Offenlaufstall- und Boxenhaltung. Diss. Agr. 206.
- Feist, J.D., McCullough, D.R., 1976. Behavior patterns and communication in feral horses. Z. fur Tierpsychol. 41, 337–371. https://doi.org/10.1111/j.1439-0310.1976. tb00947.x.
- Gerber, V., Straub, R., 2016. Pferdekrankheiten: Innere Medizin, 2nd ed. UTB; Haupt Verl., Bern, 1 Online-Ressource.
- Goldschmidt-Rothschild, B., von, Tschanz, B., 1978. Soziale organisation und Verhalten einer Jungtierherde bei Carmargue-Pferd. Z. f
 ür Tierpsychol. 372–400.

- Hintze, S., Murphy, E., Bachmann, I., Wemelsfelder, F., Würbel, H., 2017. Qualitative behaviour assessment of horses exposed to short-term emotional treatments. Appl. Anim. Behav. Sci. 196, 44–51. https://doi.org/10.1016/j.applanim.2017.06.012.
- ISAE Ethics Committee, 2017. Ethical Treatment of Animals in Applied Animal Behaviour Research: Guidelines. (https://www.applied-ethology.org/). Accessed 3 May 2023.
- Kappeler, P.M., 2012. Verhaltensbiologie. Springer Berlin Heidelberg, Berlin, Heidelberg, p. 648. https://doi.org/10.1007/978-3-642-20653-5.
- Ketonen, E.E., Salonen, V., Lonka, K., Salmela-Aro, K., 2022. Can you feel the excitement? Physiological correlates of students' self-reported emotions. Br. J. Educ. Psychol., e12534 https://doi.org/10.1111/bjep.12534.
- King, S.R.B., Gurnell, J., 2007. Scent-marking behaviour by stallions: an assessment of function in a reintroduced population of Przewalski horses (Equus ferus przewalskii). J. Zool. 272, 30–36. https://doi.org/10.1111/j.1469-7998.2006.00243.x.
- Kjellberg, L., Morgan, K., 2021. Introduction to automatic forage stations and measurement of forage intake rate in an active open barn for horses. Animal 15, 100152. https://doi.org/10.1016/j.animal.2020.100152.
- Knipsel, H.H., 2007. Harninkontinenz. In: Jocham, D., Miller, K. (Eds.), Praxis der Urologie. Georg Thieme Verlag, Stuttgart.
- Leinker, M., 2007. Entwicklung einer Prinziplösung zur Senkung von Ammoniakemissionen aus Nutztierställen mit Hilfe von Ureaseinhibitoren. Dissertation agr. Univ. - und Landesbibl. Sachs. -Anhalt. https://doi.org/10.25673/ 2784.
- McDonnell, S.M., Haviland, J., 1995. Agonistic ethogram of the equid bachelor band. Appl. Anim. Behav. Sci. 43, 147–188. https://doi.org/10.1016/0168-1591(94) 00550-X.
- Muggenthaler, K., Zeitler-Feicht, M.H., Mühlbauer, A.-C., Kilian, E., Reiter, K., 2010. Sägespäne versus Liegematten - Untersuchungen zum Ausruh- und Ausscheideverhalten von Pferden in der Liegehalle von Mehrraumaußenlaufställen mit Auslauf. KTBL-Schr. 145–155.
- Nielsen, B.L., 2020. Olfaction in animal behaviour and welfare. KTBL-Schr. 520, 11–17.
- Ödberg, F.O., Francis-Smith, K., 1977. Studies on the formation of ungrazed eliminative areas in fields used by horses. Appl. Anim. Ethol. 3, 27–34. https://doi.org/10.1016/0304-3762(77)90068-2.
- R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.
- Randall, D.G., Krähenbühl, M., Köpping, I., Larsen, T.A., Udert, K.M., 2016. A novel approach for stabilizing fresh urine by calcium hydroxide addition. Water Res. 95, 361–369. https://doi.org/10.1016/j.watres.2016.03.007.
- Ray, H., Saetta, D., Boyer, T.H., 2018. Characterization of urea hydrolysis in fresh human urine and inhibition by chemical addition. Environ. Sci. Water Res. Technol. 4, 87–98. https://doi.org/10.1039/C7EW00271H.
- Ricci-Bonot, C., Mills, D.S., 2023. Recognising the facial expression of frustration in the horse during feeding period. Appl. Anim. Behav. Sci. 265, 105966 https://doi.org/ 10.1016/j.applanim.2023.105966.
- Sambraus, H.H., Zeitler-Feicht, M., 2003. Das Harnen von Pferden im Offenlaufstall. Pferdeheilkunde 521–524.
- Scopa, C., Palagi, E., Sighieri, C., Baragli, P., 2018. Physiological outcomes of calming behaviors support the resilience hypothesis in horses. Sci. Rep. 8, 17501. https://doi. org/10.1038/s41598-018-35561-7.
- Sweeting, M.P., Houpt, C.E., Houpt, K.A., 1985. Social facilitation of feeding and time budgets in stabled ponies. J. Anim. Sci. 369–374. https://doi.org/10.2527/ ias1985_602369x
- Symonds, M.R.E., Moussalli, A., 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behav. Ecol. Socio 65, 13–21. https://doi.org/10.1007/s00265-010-1037-6
- Troccaz, M., Niclass, Y., Anziani, P., Starkenmann, C., 2013. The influence of thermal reaction and microbial transformation on the odour of human urine. Flavour Fragr. J. 28, 200–211. https://doi.org/10.1002/ffi.3143.
- Tschanz, B., 1978. Sozialverhalten beim Camarguepferd Dokumentierverhalten bei Hengsten (Freilandaufnahmen).
- Varel, V.H., 2002. Carvacrol and thymol reduce swine waste odor and pathogens: stability of oils. Curr. Microbiol. 44, 38–43. https://doi.org/10.1007/s00284-001-0071-z.
- Varel, V.H., Wells, J.E., Miller, D.N., 2007. Combination of a urease inhibitor and a plant essential oil to control coliform bacteria, odour production and ammonia loss from cattle waste. J. Appl. Microbiol. 102, 472–477. https://doi.org/10.1111/j.1365-2672.2006.03120.x.
- Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer, Switzerland, p. 260. https://doi.org/10.1007/978-3-319-24277-4.
- Zeeb, K., 1992. Artgemäße Pferdehaltung und verhaltensgerechter Umgang mit Pferden. In: Thein, P. (Ed.), Handbuch Pferd. BLV-Verlagsgesellschaft, München, Wien, Zürich.
- Zeitler-Feicht, M.H., 2015. Handbuch Pferdeverhalten: Ursache, Therapie und Prophylaxe von Problemverhalten, 3rd ed. Verlag Eugen Ulmer, Stuttgart, Online-Ressource.
- Zeitler-Feicht, M.H., Streit, S., Dempfle, L., 2010. Tiergerechtheit von Futterabrufstationen in der Gruppenhaltung von Pferden. Tierarzt Prax. Ausg. G 38, 363–370. https://doi.org/10.1055/s-0038-1624008.