

**Short communication**

A preliminary study of the effects of enrichment on stereotypic and non-stereotypic stabled horses

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Abstract

Our preliminary study investigated: 1) differences of general behaviors between stereotypic (male, n=2, ST) and non-stereotypic (male, n=2, NST) stabled horses 2) the different reactions to enrichment items (hay nets, bedding straw and yoga ball) between ST and NST horses. The frequency of general behaviors, stereotypic behaviors and the reactions to enrichment items were recorded. The results showed that there was a significantly different frequency of ingestion, locomotion, standing alert, investigation and social interactions between ST and NST horses during 24 hours of observation ($P < 0.05$). The ST horses performed weaving and wind sucking. There were significant differences in reactions, such as sniffing, watching and rolling when using enrichment items ($P < 0.05$). The frequency of sniffing was the highest when bedding straw was used. The horses displayed watching behaviors when using yoga ball ($P < 0.05$) and rolling ($P < 0.05$) when using bedding straw. The frequency of sniffing straw bedding in NST horses was greater than that of ST horses ($P = 0.006$). The NST horses preferred watching the ball ($P < 0.001$). Only ST horses performed rolling when provided with straw bedding. In conclusion, the differences in general behaviors between ST and NST horses were established. Different reactions to enrichment items also occurred between ST and NST horses. A more extensive study with a larger sample size and a continuous recording of behavior for a longer period is recommended.

Keywords: Enrichment, Horse, Non-stereotypic, Stereotypic

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INTRODUCTION

The most common way of housing horses is still in individual tie-stalls or boxes inside barns (Cooper and McGreevy, 2002). Barren environments and social isolation have also been linked to many equine behavioral problems (Jørgensen et al., 2011). A stereotypy is a repeated, relatively invariant sequence of movements with no obvious function (Broom and Kennedy, 1993), which are thought to be a consequence of suboptimal environmental or housing conditions (Freymond et al., 2019). Equine stereotypies such as weaving, crib-biting and box-walking are frequently described as 'stable vices' or problem behaviors (reviewed by Nicol, 1999). Moreover, the management factors related to the time spent in the stable showed the strongest associations with stereotypic behavior as well (McGreevy et al., 1995).

Environmental enrichment is a concept which describes how the environments of captive animals can be changed for the benefit of the inhabitants (Young, 2003). Behavioral opportunities that may arise or increase as a result of environmental enrichment can be appropriately described as behavioral enrichment. There are different types of enrichment criteria, including social, occupational enrichment, physical enrichment, sensory and nutritional enrichment (Young, 2003).

Jørgensen et al. (2011) investigated the use of edible and non-edible items intended to provide enrichment during turnout for the non-stereotypic horses. Glunk et al. (2014) studied the effect of hay net design on the rate of forage consumption when feeding horses, however, it was not established whether the horses displayed any form of stereotypic behaviors in their study. Rochais et al. (2018) found that fourteen horses out of 28 expressed stereotypic and abnormal repetitive behaviors at least once during 9 weeks of observation. The horses increased their feeding time on hay in enrichment treatments such as hay bags and slow feeders compared to the hay on the stall ground treatment. The hay bag distribution was associated with an increase of frustration behaviors. These results emphasize the importance of identifying feeding strategies and/or devices that improve feeding distribution and improve horse welfare (Rochais et al., 2018).

Moreover, the bedding material used in stables is an important factor affecting the welfare of horses (Kwiatkowska-Stenzel et al., 2016). Research has shown that straw bedding encourages more bedding-directed behaviors, less standing behavior and a longer duration of recumbent behavior (Reviewed by Greening et al, 2013). Kwiatkowska-Stenzel et al. (2016) claimed that straw was the best in terms of fulfilling the behavioral needs of horses kept in boxes. Compared with peat moss with shavings, bedding and crushed pellets, the application of straw led to significantly longer duration and higher frequency of occupation with bedding and, in turn, shorter fractions of standing.

Some questions of interest are: What are the behavioral differences between stereotypic (ST) and non-stereotypic (NST) horses before using any enrichment items? Do ST horses and NST horse respond to the enrichment items in the same way? These questions are rarely answered in the literature. Therefore, the aims of this preliminary study were to investigate: 1) differences of general behaviors between stereotypic and non-stereotypic stabled horses 2) the different reactions to enrichment items (hay nets, bedding straw and yoga

ball) between stereotypic and non-stereotypic stabled horses. We hypothesized that differences in general behaviors and reactions to the enrichment items existed between stereotypic and non-stereotypic horses.

MATERIALS and METHODS

Animals and housing

The animals in this study comprised four ponies (*Equus caballus*), stallions of a Thai native breed, aged 8.00 ± 3.37 years. The horses were kept individually in stables ($3 \text{ m} \times 3 \text{ m}$) for a period of 24 hours during the experiment on a Saturday and Sunday. Each horse was kept in their usual stable during the trial with free access to grass and water. The floor surface of the stables was half exposed concrete and half concrete covered by rubber matting. The horses could see outside from the front feeding area and from the back door. Horses could visually communicate with neighbors only from the front feeder and back door areas as the left and right remaining walls of the stable were solid. The horses were fed 500 grams of commercial feed (MaxWin 001R) per day. Pellet feed was delivered at 9:00 am and 14:50 pm. Total three kg of hay delivered at 10:00 am and 15:00 pm. All the horses were used for research purposes. These horses had about 1 hour of exercise daily, except during the 24 hours' observation period. There were two ponies have been performed wind sucking and waving. The study site was at Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Enrichment items

Three enrichment items were provided in this study: hay nets, bedding straw and yoga ball. All of the enrichment items were affordable and easy to find from the local market. The hay-net was a hanging objects as external accessories which was a physical enrichment item (Young, 2003). The hay nets were nylon bags with multiple holes, and the hole sizes were of 10 centimeters openings and a length of 80 centimeters (modified from Rochais et al., 2018). Three kg of hay were placed into the hay nets at 1.5 kg per time and delivered at 10:00 am and 15:00 pm. The straw bedding was tactile stimuli which was a sensory enrichment item (Young, 2003). Straw was used as bedding materials (modified from Kwiatkowska-Stenzel et al., 2016). In one stable, 17 kilograms of straw (approximately 1 kilogram per square meter) were used. The yoga ball was a toy as temporary accessories which was an occupational enrichment item (Young, 2003). The yoga ball was blue and of 65 cm diameter (modified from Jørgensen et al., 2011).

Experimental design

The 4×4 Latin square design was used in this experiment. The enrichment items were hay nets, bedding straw and yoga ball. Control group did not use any enrichment items. The horses were given the enrichment items on Saturday and Sunday for 4 weeks until all the enrichment items treatments of the horses had been assessed. The experiment was conducted from 10:00 to 17:00.

Behavioral observation

All the observations took place between September and November 2019. There were two observers in charge of data collection who had experience in behavioral studies. An instantaneous time and scan sampling observation technique was used (Martin and Bateson, 1986). The data for the general

behaviors were collected every 10 min with a total of 144 times recorded for 24 hours in two days by CCTV camera (VStarcam-C21, WATASHI) before giving using the enrichment items. The reactions to the enrichment items were collected during 7 hours from 10:00 to 17:00, including the frequency of pushing an item, sniffing, biting, watching, kicking and rolling. The observation details of horse behaviors are shown in an ethogram (Table 1) (Jørgensen et al., 2011; McDonnell, 2003). Therefore, the data in control group without any enrichment item were not analyzed. The procedures of the experiment were performed followed the guidelines for the ethical use of animals in applied ethology studies (Sherwin et al., 2003). Animal cares followed the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council of Thailand.

Table 1 Ethogram

Behaviors	Definition
General behaviors	
Ingestion	Muzzle is lowered to ground/within bucket; lips grasp hay/bedding; licking the feeder, masticating, prehending or swallowing food/water.
Standing alert	Rigid stance with the neck elevated and the head oriented toward the object or animal of focus. The ears are held stiffly upright and forward, and the nostrils may be slightly dilated.
Locomotion	Locomotor activities, such as jumping and walking.
Resting	Standing rest or dozing, lateral or sternal recumbent rest/ sleep and yawning.
Investigation	Including sniff, lick and pawing
Social interactions	Contact with neighbor
Vocalization	Sounds produced by horse
Stereotypic behaviors	
Weaving	Abbreviated pacing involving rhythmic, repeated side-to-side shifting of the weight on the forelegs. The vigor and speed of movement vary among individuals and within or between episodes from slow to frenetic. The front feet may remain “planted” in position, or in an extreme form, the horse “throws” its fore body from one side to the other of a doorway or narrow stall, sometimes contacting the walls with each motion.
Head shaking	Repeated, rhythmic head movements.
Wind sucking	Grasping of surface with incisors while arching the neck and drawing a gulp of air into the throat and then expelling it, repeated rhythmically in bouts typically lasting from minutes to as long as an hour.
Pacing	Stylized repetitious locomotion at any gait, usually along a perimeter.
Reactions to the items	
Pushing	The horse is using its head or muzzle to push the item so that it visibly moves.
Sniffing	The horse is standing with head very close to the item and is sniffing or blowing air (identified by the characteristic sound).
Biting	Manipulating with open mouth, as if to touch or taste.
Watching	The horse is moving towards the item, with eyes fixed on the item.
Kicking	With an object as an apparent target, a foreleg is lifted off the ground slightly, ex-tended quickly in a forward direction, and followed by a backward, toe-dragging movement as if digging.
Rolling	Dropping from standing to sternal recumbency, then rotating one or more times from sternal to dorsal recumbency, tucking the legs against the body.
Other	The horse is showing other behaviors not described in the ethogram

(Reference from: Jørgensen et al., 2011; McDonnell 2003).

Data analysis

The SPSS software (version 16.0; SPSS Inc.; Chicago, IL, USA) was used for the statistical analysis. The frequency of ingestion, standing alert, locomotion, resting, investigation and weaving, head shaking, and wind sucking were analyzed using one-way ANOVA to compare the behavioral differences in 24 hours between ST and NST horses. The effects of treatment (hay nets, bedding straw and yoga ball) on the reactions to the enrichment items in ST and NST horses were analyzed by two-way ANOVA. The treatment and horses (ST and NST) were considered to be fixed effects. The general linear model (GLM) repeated the same measures. When there was an interaction between treatment and horse, all the means were compared. If the behavioral data were not normally distributed, they were transformed prior to analysis. Means were compared using Duncan's multiple-range test and the significance was determined at $P < 0.05$.

RESULTS

Behavioral observation in 24 hours

It was found that two horses performed stereotypic behaviors during 24 hours of observation. Table 2 below shows that the most frequent type of stereotypic behavior was wind sucking (25.5 ± 9.70), followed by weaving (21.75 ± 8.56). The frequency of ingestion ($df=14$, $t=2.16$, $P=0.049$), locomotion ($df=14$, $t=2.69$, $P=0.018$), standing alert ($df=14$, $t=2.89$, $P=0.012$), investigation ($df=14$, $t=3.97$, $P=0.001$) and social interactions ($df=14$, $t=5.56$, $P<0.001$) in NST horses was significantly higher than that of ST horses (Table 2). However, the frequency of resting in ST and NST horses was not significantly different ($P>0.05$).

Table 2 Comparison of general behaviors between Stereotypic (ST) and Non-Stereotypic (NST) horses in 24 h observation

Behaviors	ST (n=2)	NST (n=2)	P-value
Ingestion	29.75 \pm 5.23	36.75 \pm 5.26	0.049
Resting	43.50 \pm 4.68	47.00 \pm 1.60	ns
Locomotion	5.13 \pm 0.81	10.88 \pm 1.89	0.018
Standing alert	8.63 \pm 1.08	21.13 \pm 3.99	0.012
Investigation	9.50 \pm 1.69	25.00 \pm 3.52	0.001
Social interactions	0.13 \pm 0.13	4.63 \pm 0.78	0.00
Weaving	21.75 \pm 8.56	n/a	n/a
Head shaking	0.13 \pm 0.13	n/a	n/a
Wind sucking	25.5 \pm 9.70	n/a	n/a

Values are presented as Mean \pm SE. ns means not significant ($P>0.05$).

n/a means not applicable. ST means stereotypic horses. NST means non-stereotypic horses.

The reactions to enrichment items

The results of the reactions to enrichment items in stabled horses showed that when the animals were interested in the enrichment items, they performed various activities such as pushing, sniffing, biting, watching, kicking and rolling behaviors (Table 3). The frequency of pushing, biting, kicking and total reaction was not significantly affected by the horses or the treatment ($P>0.05$). There was no interaction between horses and treatment. There were significant differences between ST and NST horses when they performed watching ($P<0.001$) and rolling ($P=0.025$) behaviors. The frequency of sniffing, watching ($P<0.001$) and rolling were significantly affected by the treatment ($P<0.05$).

The frequency of sniffing was highest when straw bedding rather than hay nets and yoga ball was used ($P=0.06$). There was no difference in the frequency of sniffing when using hay nets or yoga ball ($P>0.05$). The NST horses ($P<0.001$) performed watching activities when using the yoga ball ($P<0.001$), rather than when using the hay netting or straw bedding. Therefore, there was an interaction between horses and treatment ($P<0.001$). The ST horses ($P=0.025$) also performed rolling activities when using straw bedding rather than when using hay netting or yoga ball ($P=0.01$). Therefore, there was an interaction between horses and treatment ($P=0.01$) (Table 3).

Table 3 The reaction to the enrichment items in Stereotypic (ST) (n=2) and Non-Stereotypic (NST) (n=2) horses during 10:00-17:00 h observation

Treatment (T)	Horse (H)	Pushing	Sniffing	Biting	Watching	Kicking	Rolling	Total reaction
Hay net	ST	24.75	0.50 ^a	3.75	0.00 ^a	0.00	0.00 ^a	29.00
	NST	3.25	0.25	1.00	0.00	0.00	0.00	4.50
Straw bedding	ST	2.75	9.75 ^b	0.00	0.00 ^a	0.00	1.00 ^b	13.50
	NST	19.50	27.50	0.00	0.00	0.00	0.00	47.00
Yoga ball	ST	0.50	3.25 ^a	0.25	0.25 ^b	0.00	0.00 ^a	4.25
	NST	1.75	7.25	5.50	13.75	0.25	0.00	28.50
P-value	H	ns	ns	ns	0.00	ns	0.025	ns
	T	ns	0.006	ns	0.00	ns	0.01	ns
	T×H	ns	ns	ns	0.00	ns	0.01	ns
SE	H	5.34	2.97	1.59	0.74	0.06	0.10	7.64
	T	6.54	3.64	1.95	0.90	0.07	0.12	9.36
		9.24	5.14	2.75	1.28	0.10	0.17	13.24

Values are presented as Mean \pm SE. ns means not significant ($P>0.05$).

ST means stereotypic horses. NST means non-stereotypic horses. H means horse. T means treatment. T×H means the interaction between horse and treatment.

^{a, b} means within the same column with different superscripts were significantly different at $P < 0.05$ among the treatments with hay nets, straw and yoga ball.

DISCUSSION

The behavioral differences between ST and NST horses

According to the results of our 24 hours of observation, the NST horses were more active and alert than the ST horses. The NST horses performed more investigation behaviors (Bulens et al., 2013) such as investigation. However, the resting needs in ST and NST horses might not be different. The NST horses had more visual contact with their neighbors. Nevertheless, the ST horses in this study displayed rare social interaction with other horses in the stables, although they could have had visual contact with other horses, therefore, the stereotypic behaviors in ST horses might not be socially related (Huo et al., 2021).

McGreevy et al. (1995) claimed that the risk of an increase in abnormal behavior in the horses might be due to the amount of forage falling below 6.8 kg/day. Although we did not investigate the history or causes of stereotypic behaviors in horses, according to the farm management in our study, the pony horses were fed 500 grams of commercial feed per day. The three kg of hay given per day might not be enough for the horses. Moreover, the floor surface of the stables was half exposed concrete and half concrete covered by rubber matting without straw bedding during the 24 hours observation, which might also be related to stereotypic behaviors in the ST horses.

Although farmers assume that there is a lower economic value for production animals that exhibit stereotypic compared with their non-stereotypic counterparts (Williams and Randle, 2017), the owners who have had direct experience of horses that exhibit stereotypical behavior maintain that stereotypical behaviors do not negatively affect performance and that performance-based measures and values are equitable to those of non-stereotypical horses (Nagy et al., 2010).

The reactions to enrichment items

Our results indicated that reactions differ significantly according to the type of enrichment used. Bulens et al. (2013) found that their NST horses showed less reaction to enrichment items when there was a lot of hay or bedding material in their stables. In general, pushing and biting of the items were the most common types of behavior. Watching and sniffing behavior occurred less frequently because this type of behavior has an exploratory purpose and horses will exhibit it less over time (Bulens et al., 2013).

These results of frequency of reaction to enrichment items agreed with those of Jørgensen et al. (2011) who found that both NST horses kept individually and in groups performed significantly more item-directed behaviors towards edible items. With regard to those horses which require large quantities of feed over a short period of time, Glunk et al. (2014) found that the medium (4.4 cm openings) and small (3.2 cm openings) hay nets may cause some frustration (i.e., biting and shaking the hay net) which did not occur with the large (15.2 cm openings) hay nets. Therefore, our study used hay nets with 10 cm openings as the large size of hay net which was easy to obtain from the market.

Straw has been found to be the best bedding with regard to fulfilling the behavioral needs of horses when compared to peat moss with shavings and wood pellets (Kwiatkowska-Stenzel et al., 2016). Environmental enrichment using straw might stimulate natural foraging behavior (Goodwin et al, 2002).

However, when no hay is present, horses are stimulated to search for other roughage sources. Bedding material could provide an alternative source of roughage (Bulens et al., 2015). When bedding types other than straw are used it might increase the risk of horses performing abnormal behavior (McGreevy et al., 1995). However, only the ST stabled horses performed rolling activities with rare frequency when provided with straw bedding. This might have been because of the observation taking place during day time (10:00-17:00). A further study might need to investigate behaviors in the night time.

In our study, NST horses preferred watching the yoga ball, while ST horses did not seem to be interested in non-edible items. Surprisingly, the NST horses might be interested in yoga ball in order to stare at them for exploratory purposes (Bulens et al., 2013). However, based on behavioral measures alone, the provision of toys or other non-edible items does not seem to be an obvious enrichment for horses, but no negative effects were discovered either (Jørgensen et al., 2011). This was possibly due to the horses having access to a sufficient supply of forage in the stables which may have helped to confirm their absolute innate need to express appetitive and consummatory behavior (The British Horse Society. 2018). Moreover, this behavior was also consistent with Bulens et al. (2013) who found that although their horses exhibited item related behavior, the objects used did not offer a food reward and aroused interest only to a very limited extent, which showed a limited enriching effect. When horses are appropriately reared, nonedible items are not useful for enrichment purposes (Bulens et al., 2015).

Few publications show preliminary behavioral data in studies of stabled horses, therefore, the behavioral differences between ST and NST horses still need to be investigated in a fundamental study. The sample size in this preliminary study was only four. We suggest that a larger sample size should be used, so that more robust results could be found. A study by Jørgensen et al. (2011) suggests that horses might play with objects outside the observation periods. Therefore, the observation periods might need to be extended. Our observation was only during the day time, so night time observation might also need to be conducted. Equine stereotypies can also be influenced by an individual's temperament and personality (Williams and Randle, 2017), which suggests that these factors should also be taken into account in a future study.

In conclusion, the differences in general behaviors between stereotypic and non-stereotypic horses were established in this study. In addition, different reactions to enrichment items occurred between stereotypic and non-stereotypic horses. A more extensive study with a larger sample size and a continuous recording of behavior for a longer period is recommended.

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AUTHORS' CONTRIBUTIONS

Xin Huo: Conceptualization, Methodology, Project administration, Software, Writing-original draft, Writing-Review & Editing, Supervision.

Sutida Yaemklang: Investigation, Data curation

Pornnapa Pimmai: Investigation, Data curation

Pakanit Kupittayanant: Resources, Writing-Review & Editing

Pongchan Na-Lampang: Writing-Review & Editing

CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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