A preliminary investigation into the effect of *ad libitum* or restricted hay with or without Horslyx on the intake and switching behaviour of normal and crib biting horses

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Abstract

In an attempt to reduce 'fibre–belly' and prevent obesity in horses many owners restrict access to hay in the stable. Such restrictions can lead to digestive disturbances and promote the development of stereotypic behaviours.

The objectives of this experiment were to determine if *ad libitum* or restricted forage with or without the molasses based lick, Horslyx, would alter the behaviour in a group of normal and confirmed stereotypic horses.

Two Randomised Block Design trials were conducted simultaneously. Group A consisted of 3 crib-biters and 1 normal horse, while group B contained 4 non-stereotypic (normal) horses. Horses were individually housed in 10 x 12 foot boxes and bedded on dust-extracted shavings with water available *ad libitum*. Diets were *ad libitum* hay, *ad libitum* hay + Horslyx, restricted hay, and restricted hay + Horslyx. For two days of each collection period every horse was individually observed, and an ethogram completed for ½ hour 3 x /day = 6 observation sessions for each horse. Switching behaviour and data for hay and lick intakes were averaged across the 5 days of collection and subjected to Friedman's non-parametreic ANOVA with horse, diet and behaviour as fixed factors.

Ad libitum or restricted forage or the presence of a Horslyx had no significant impact on horse behaviour. Crib-biting horses tended to consume less hay 8.81 (± 3.60) kg/d and more Horslyx 1.10 (± 0.38) kg/d compared with normal horses who consumed more hay at 11.72 (± 4.59) kg/d and less Horslyx at 1.01 (±0.45) kg/d respectively, but there was no significant differences between the groups. Crib-biting horses switched behaviour (eating, licking, cribbing, drinking, looking over the door resting) an average of 40 times more during the thirty minute observation sessions than normal horses. Crib-biting horses also licked the Horslyx 1.5 times more than normal horses.

These results confirmed that stereotypic animals are addicted to the reward of the dopamine release, achieved by the action of crib biting, and are thus not influenced by *ad libitum* forage or access to a stable lick. The 4 fold increase in switching behaviour and additional licking by the crib-biting horses suggests an increased transmission of the neurotransmitter dopamine and in this regard licking may promote coping in certain environmental circumstances.

The results of this study suggest that providing a lick in the stable for crib-biting horses gives them another activity to the normal forage consumption and resting actions and may provide another mechanism for dopamine release and thus enhance their 'coping' strategy when confined in stables.

Key words: Stereotypy, Horslyx, Behaviour, Feed intake, Horse

Introduction:

In order to reduce 'fibre–belly' in race horses and prevent obesity in pleasure horses, many owners restrict access to hay in the stable. Ellis et al. (2010) reported that horses spend 12.5 ± 2.5 hours per day eating, which is essential for maintaining both digestive and mental health. Any restriction of this normal behaviour can cause digestive disturbances and promote the development of stereotypic behaviours (Ellis et al. 2012). This is supported by McGreevy et al. (1995) who reported a positive association between stereotypic behaviour in Thoroughbreds when fed less than 6.8 kg of fibre /day. Furthermore, the feeding of concentrates after weaning (a feeding regimen often associated with low forage provision) led to a 4-fold increase in the initial development of crib-biting (Waters *et al.* 2002), and has also been shown to elicit post-development increases in crib-biting activity (Gillham *et al.* 1994). Concentrate feed induced elevations have been attributed to neurotransmission of dopamine (Roberts *et al.* 2015), and more complex fluctuations in neuro-active molecules such as leptin and Ghrelin (Hemmann *et al.* 2013). Forage on the other hand, elicits a depression in crib-biting intensity (Hemmings and Hale 2013) although the precise schedule of forage provision does not impact upon locomotor activity (stereotypic or otherwise) in a 24 hour period (Piccione *et al.* 2013). Therefore, in order to prevent development and lower stereotypy rate in habitual cribbers, increased forage provision would appear to be essential, although the pattern of provision is not important. Finally, it should be noted that in rodent species stereotypy development has a strong genetic component whereby mice of the inbred DBA/2 strain reliably manifest stereotypy following feed restriction, whereas the C57/b strain do not (Cabib and Bonaventura 1997). As such, alongside environmental factors such as feeding, genotypic predisposition may play an important role in stereotypy manifestation. Indeed, limited pedigree analysis suggests a heritable component of crib-biting, weaving and box-walking (Vecchiotti and Galanti 1986) , although a more recent study into the molecular basis of stereotypy development refute this notion(Hemmann *et al.* 2014). However, the latter work employed simple candidate gene approach, and undoubtedly extended investigation featuring genome wide analysis techniques is warranted to properly test the genotypic predisposition hypothesis.

From a perceptual angle, stereotypies are commonly regarded as undesirable traits in performance horses as owners believe that these behaviours can have detrimental effects on health status, reflected in lower body condition scores, and increased susceptibility to certain types of colic (Scantlebury et al. 2011). The negative health aspects of stereotypies is further demonstrated by the fact that equine veterinary examinations class stereotypic behaviours as vices, leading to financial depreciation of the animal by up to 50% (McBride and Long 2001). From the perspective of training and performance (Parker *et al.* 2009) reported that crib-biting horses demonstrated a bias towards habitual responding in a two choice Tolmans maze. Cognitive inflexibility such as this leads to problems in competition disciplines (i.e dressage) where refinement and adaptation of previously learned responses is required. Learning deficits also extend to simple instrumental tasks not involving locomotion. Hausberger et al. (2007) demonstrated that 70% of crib-biting horses compared with 15% of normal horses were unsuccessful in oral manipulation of a hinged lid for a food reward. Finally, McGreevy (2004) has also noted that crib-biters spent less time resting than normal horses, whereas in other studies bouts of stereotypy are observable at times when control animals would otherwise be sleeping (Hausberger et al. 2007). It is therefore possible that the cognitive deficits cited above could be a consequence of altered patterns of sleep.

Stereotypy is likely to be self-reinforcing , due to the reward aspect of dopamine release (Hahn, 2004) and there is increasing evidence that stereotypic behaviours are coping mechanisms in stressed stable horses (Hemmings et al. 2004, Nagy et al. 2009). As stereotypies are not observed in feral horses indicating lack of stimulus for coping mechanisms, domestic management regimes should provide an environment that will ameliorate the stress of confinement by offering sufficient forage so that horses can live as stress-free as possible.

The objectives of this study were to determine if *ad libitum* or restricted forage with or without the molasses based lick, Horslyx, would alter the behaviour in a group of 5 normal and 3 confirmed stereotypy (crib-biting) performing horses

Method

Experimental Design:

Two replicated 4 x 4 Latin Square designed trials were conducted simultaneously. Group A consisted of 4 horses with the confirmed stereotypy of crib-biting and group B 4 non-stereotypic (normal) horses (see Table 1). The 4 x 7 day periods were divided into 2 days dietary adaptation and 5 days data collection. The periods were set on the fact that the content of the basal diet i.e., hay did not alter, the only variations being in amount of hay and access to a Horslyx lick. Duration of the trial was 28 days in total.

Horses:

All eight horses were geldings and ranged in size from 15hh to 17hh. Four horses were geldings and typical 'hunter-type' being Thoroughbred X Irish Draught or Thoroughbred X Connemara breed. They were in moderate to good body condition ranging from 420 to 789 kg LW and ranged from 8 to 14 years old. Four of the geldings were Thoroughbred polo ponies which were under light training, being ridden 4 times per week for approx. 30 minutes at trot and slow canter. Before commencing the trial, all the hunt horses were out at pasture on their summer rest after the hunting season. They received some supplementary mixed species meadow hay once / day hay while at grass. Polo ponies were out at grass all morning and stabled overnight, also receiving *ad libitum* rye-grass hay when stabled. The normal horses (non-stereotypy) consisted of 3 hunter-type horses and 1 polo pony while the stereotypy (crib-biters) consisted of 3 polo ponies and 1 hunter-type.

Feed:

All horses were on fibre diets i.e., hay + pasture before the trial. The hay fed throughout the trial was medium cut perennial rye grass (*Lolium perenne)* hay that was conserved locally at Foss Hill Farm Coates, Cirencester, in summer 2011. The hay was well conserved and baled in big square bales weighing approx. 350 kg each and stored in an open-sided Dutch barn at Foss Hill Farm. Diets consisted of 1 or 2 components. Hay with or without the molassed-based vitamin and mineral supplement block called Horslyx. The Horslyx Original contained: Dehydrated Molasses, Mono-Calcium Phosphate, Pure Vegetable Oil, Hipro Soya, Sodium Chloride, Calcium Carbonate and Magnesium Oxide with a nutrient analyses of: Oil 6%, Protein 6.5%, Fibre 0.25%, Sugar 33%, Calcium 2.5%, Phosphorus 1.6%, Magnesium 0.4%, Manganese 800mg/kg, Zinc 1200mg/kg, Copper 600mg/kg, Iodine 6mg/kg, Vitamin B12 220mcg/kg, Selenium 5mg/kg, Vitamin A 25,000iu/kg, Vitamin D3 4,000iu/kg, Vitamin E 200mg/kg Biotin 2mg/kg http://horslyx.co.uk/. *Ad libitum* amounts were determined during the initial two adaptation days on each diet and based on weekly live weight measurements taken by a Spillers horse weigh-tape. All hay was offered in conventional hay nets. Diet 1 was *ad libitum hay* (H), Diet 2 *ad libitum* hay + Horslyx (H+), Diet 3 restricted hay (RH) based on 1.5% of LW in dry matter (DM) per day and Diet 4 restricted hay + Horslyx (RH+). See Table 1 for diet and horse sequence.

Animal Management:

Animals were fed twice per day at 9 am and 5 pm. Daily mucking out started at 10 am during which time each horse was put on the horse walker for approximately 30 minutes walking exercise. On 4 days per week the polo ponies were exercised under saddle for 30 minute in the afternoon while the other horses were put on the walker for 30 minutes. Post daily exercise all horses were fed their evening ration at approximately 5pm. Observations were done by the same person for all horses throughout the trial and took place after morning mucking out, at midday and just before evening feeding. The observer sat 10 meters away in an elevated position opposite the front of the stable to enhance the view of all activities performed by the horse during the 30 minute period. A new ethogram was completed per observation period. Four of the horses (Polo ponies) were given light ridden exercised each afternoon and so were not put on the walker during the evening ‘skipping out’ period. Those horses not ridden at all were put in the all-weather turn-out arena twice per week for a period of free exercise.

Measurements:

For two days of each collection period every horse was individually observed for ½ hr 3 x /day = 6 observation periods for each horse. Specifically designed ethograms were filled-in which mapped the frequency and duration of their activities. Feed refusals and licks were collected and weighed each morning for the previous 24 hour period.

Data Analyses:

One of the horses in group A did not display the stereotypy of crib-biting during the complete trial period, thus the data could not be analysed as a repeated Latin Square. The data was divided into randomised blocks of 5 normal horses and 3 crib-biting horses and subjected to Friedman's non-parametric ANOVA (Genstat 12, 2012). Data for hay and lick intakes were averaged per horse across the 5 days of collection for each period; switching behaviour was averaged across the 6 x 30 minute repetitions with horse (8), diet (4) and behaviour (2) as fixed factors. Differences between treatments were determined by least significant difference test (LSD) where LSD = $t_{\text{(error degree of freedom)}}$ x s.e.d.

Results.

No invasive health checks were done on any horse before commencing the trial, however all the horses were checked for any abnormal skin conditions, had their feet trimmed by a farrier and passports checked to ensure vaccination cover was current. For the short period of this trial all horses remained healthy (alert displaying normal behaviour, shiny coats and normal droppings) and neither stabling nor diet had any significant impact on their body weights or their normal behaviour. Although crib biting horses tended to consume less hay 8.81 (± 3.60) and more Horslyx 1.10 (± 0.38) kg/d compared with normal horses who consume 1.72 (± 4.59) kg/d of hay and 1.01 (±0.45) kg/d of Horslyx respectively, there was no significant difference between the groups.

Ad libitum or restricted forage or the presence or absence of a Horslyx had no significant impact on the number of times the horses switched behaviour or licked the Horslyx (Table 2). When measuring the number of times that all horses licked the Horslyx when offered *ad libitum* or restricted amounts of hay, those on the restricted diets tended to lick more, but this was not significantly different form the number of licks when *ad libitum* hay was offered (Table 2).

Observations on horse activity in the stable showed that crib-biting horses, independent of diet, switched behaviour (eating, licking, cribbing, drinking, looking over the door etc) an average of 40 times more ($P<0.005$) during the 30 minute observation periods than normal horses (Table 3). Crib biting horses also licked the Horslyx 1.5 times more than normal horses ($P<0.008$).

Table 4 shows the effect of diet on the number of switches and crib-biting across both normal and stereotypy horses. Restricting hay or the addition of a Horslyx did not significantly influence the number of switches performed by all the horses. Restricted hay and the presence of Horslyx induced the highest level of crib-biting in the stereotypy horses which was double that noted when *ad libitum* hay + Horslyx was offered. Restricted hay alone also produced high levels of crib-biting behaviour but the variation across the 8 horses was high and thus no significant differences were detected.

Discussion.

The fact that normal and crib biting horses had similar intakes of hay and lick per day demonstrated that oral stereotype did not interfere with voluntary food intake in horses. The consumption of approximately 1 kg of molasses – based lick per 24 hours as noted in both normal and stereotype horses, may suggest a high intake of sugar per day. However, Horslyx contains only 33% sugar thus a horse consuming 1 kg of lick would be consuming only 330g sugar in 24 hours. The water soluble carbohydrate content of hay ranges from 100- 310 g/kg (Longland et al. 2009), so a horse would consume similar amounts of sugar from 1 kg of high WSC hay and 3 kg of low WSC hay. Furthermore, the glycaemic impact from Horslyx would be negligible as it was consumed gradually by licking at a rate of 6.37 to 8.48 licks per hour thereby tricking WSC into the stomach over a 24-hour period.

Access to *ad libitum* hay or a stable lick did not significantly alter crib biting behaviour in this experiment which is in agreement with Hemmings et al. (2004) who concluded that confirmed stereotypic animals are addicted to the reward of the dopamine release obtained by the action of crib biting and are thus not influenced by environmental enrichment. The significant additional number of short-duration licks by the crib biting horses of the molassed-based Horslyx indicates that crib-biting horses have an increased desire for a sweet-feed. These results enforce previous research that crib-biting horses tend to perform cribbing of a higher intensity during the consumption of, and directly after provision of a sweet feed (Gillham*, et al.* 1994). From a mechanistic standpoint, highly palatable sweet feeds bring a widespread release of opioid peptides in the CNS, which subsequently bind and activate the brain centres responsible for stereotypy performance (Cabib 1993). The significant increase in licking behaviour, maybe serves alongside crib-biting to bring dopamine release. Indeed, it lends weight to the notion of increased transmission of the neurotransmitter dopamine in this group of horses.

On this basis providing a lick in the stable for crib-biting horses gives them another activity and may provide another mechanism for dopamine release and thus enhance their ‘coping’ strategy when stabled.

Diet i.e., *ad libitum* or restricted hay with or without the presence of Horslyx did not alter the switching behaviour performed by either normal or crib biting horses. However, the crib-biting horses as a group performed switching behaviour 4 times more frequently than the normal horses. This increased switching in the crib-biting group provides us with useful evidence regarding the aetiology of equine oral stereotypy and is in agreement with the finding of Roberts et al. (2015) who found that a group of 8 crib biting horses performed significantly more switching behaviours that 8 normal horses. These results lend weight to the notion of increased transmission of the neurotransmitter dopamine (an important pleasure neurotransmitter) in this group of horses. As such, these behaviours no doubt have addictive properties and like any addiction will be somewhat pervasive and difficult to inhibit even though the environment has been enriched to afford a greater foraging opportunity.

Conclusions

The results from this preliminary trial indicate that stereotype horses are more active in the stable and switch behaviours more frequently than non-stereotype horses.

Furthermore in both normal and stereotype horses this behaviour was not altered by continual access to forage or the provision of a molasses-based lick. Stereotype horses did not alter crib biting frequency throughout the trial indicating that the behaviour is established and that the reward aspect of crib biting is a more potent initiator of behaviour than the action of eating. Providing a molasses lick to all stabled horses offers them another activity to engage with but does not alter innate behaviour and cannot be used as a means of alleviating stress in a stabled horse.

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Table 1. Latin square design employed for groups A (horses 1 to 4) and B (horses 5 to 8) detailing horse, diet and period

Diet	Hay *Ad libitum*	Hay restricted	Hay restricted + Horslyx	Hay *ad libitum* + Horslyx
Period 1	Horses 1A, 5B	Horses 2A, 6B	Horses 3A, 7B	Horses 4A, 8B
Period 2	Horses 4A, 8B	Horses 1A, 5B	Horses 2A, 6B	Horses 3A, 7B
Period 3	Horses 2A, 6B	Horses 3A, 7B	Horses 4A, 8B	Horses 1A, 5B
Period 4	Horses 3A, 7B	Horses 4A, 8B	Horses 1A, 5B	Horses 2A, 6B

Table 2. The influence of diet on the average switching frequency and number of licks of the molasses-based Horslyx in 5 normal and 3 crib biting horses

Diet	*Ad lib* hay	*Ad lib* hay + horslyx	Restricted hay	Restricted hay + horslyx	s.e.d	Sig
Number of switches	34	28	24	27	6.6	NS
Number of licks of Horslyx	0	3.2	0	4.2	0.81	NS

Table 3. The average number of times normal vs crib biting horses switched behaviour and licked the Horslyx across 4 different forage-based diets

	Crib-biting horses	Normal horses	s.e.d	Sig
Number of switches	53a	14b	8.9	0.005
Number of licks	2.9a	1.2b	0.42	0.008

ab values in the same row not sharing common letters differ significantly (P<0.008)

Table 4. The effect of 4 different diets on switching behaviour and the number of cribs performed by 3 crib-biting horses

	Ad lib hay	*Ad lib* hay + horslyx	Restricted hay	Restricted hay + horslyx	sed	Sig
Number of switches	70	48	41	52	16.3	NS
Number of cribs	60	48	71	92	18.6	NS

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