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The effect of a feeding stress-test on the behaviour and heart rate variability of control and crib-biting horses (with or without inhibition)

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ABSTRACT

Crib-biting is a form of oral stereotypy affecting 4–5% of horses. Once fixed, crib-biting is difficult to eliminate by behaviour therapy, however, its performance can be inhibited by collar or surgery treatment (modified Forssell's procedure). Although surgical intervention is widespread, the effects on stress coping in horses have not been studied.

In the present study we evaluated changes in behaviour response and heart rate variability in 9 control, 10 crib-biting, 10 collar and 11 surgically treated horses in a feeding stress-test, in which a feeding-bowl was placed in front but out of the reach of the horses, from which tidbits were given 3 times.

We found that stress triggers high oral activity, mainly cribbing in crib-biting horses, elevates other forms of oral activities in the inhibited groups and does not affect oral activities of controls. Instead of performing oral activities, control horses tended to target an unavailable feeding-bowl by pawing or head-tossing. Changes in stress level were indistinguishable in controls and crib-biters as heart rate variability returned to baseline values in both groups. In contrast, horses inhibited to perform crib-biting showed elevated stress level throughout the test period. Our results suggest that crib-biting may develop to cope with stress, and such coping function diminishes when inhibited.

1. Introduction

Stereotypies are repetitive, relatively invariant actions considered abnormal when they occur without any primary function (Mason, 1991). Since function of any behaviour is seldom obvious, Mason and Rushen (2006) redefined stereotypy as repetitive behaviour induced by frustration, repeated attempts to cope and/or central nervous system dysfunction. This new definition poses new challenges to research: the development of stereotypies is a long process and little is know about their origin (i.e. Nagy et al., 2008). Possible central nervous system dysfunctions are difficult to study (McBride and Hemmings, 2009) and coping function of a behaviour detrimental to the health of the animal can be questionably (Cooper and Albentosa, 2005). For example, crib-biting/wind-sucking, an abnormal stereotypy in horses with prevalence of 4–5% (Pell and McGreevy, 1999; Bachmann et al., 2003a; Albright et al., 2009) is associated with tooth-erosion, weight loss, altered gut function (McGreevy and Nicol, 1998a; Clegg et al., 2008), gastric inflammation/ ulceration (Nicol et al., 2002), and epiploic foramen entrapment colic (Archer et al., 2008). Its presence may decrease the economical value of the horse, and it is also considered an unsoundness (McBride and Long, 2001; Albright et al., 2009). In this study we attempt to dismantle chronic health effects from possible coping function of crib-biting.

Crib-biting/wind-sucking usually involves seizing a projection with the incisors or supporting the chin (crib-biting), contracting the strap muscles of the ventral throat region, and emitting a grunt (wind-sucking). The 'wind-sucking' component makes crib-biting behaviour typical

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and distinguishable from wood-chewing (McGreevy et al., 1995a,b; Albright et al., 2009). Some horses would wind-suck even without the use of a solid object.

Because crib-biting can be detrimental to the animal, several methods have been worked out to diminish its occurrence. The most common treatment is the application of crib-biting strap (collar), which makes flexion of the neck difficult and therefore the terminal grunting less easy to perform. Horses often adapt to the pressure applied by the collar, which is subsequently tightened, occasionally to the extent that skin trauma becomes apparent. McBride and Cuddeford (2001) found that wearing collar increased heart rate, beta-endorphin and plasma cortisol level both in control and crib-biting horses, indicating that collar wearing per se imposes severe stress to the horse. Moreover, the first day after fitting the collar frequency of crib-biting significantly increased suggesting that the motivation to perform wind-sucking rises when prevented (McGreevy and Nicol, 1998b).

Another approach, the modified Forrsell's operation designed to treat crib-biting has been gaining popularity among horse-owners. The procedure involves the removal of a 10 cm section of the ventral branch of the spinal accessory nerves (which innervate the sternomandibularis muscles), and 34 cm sections of the paired omohyoideus and sternothyrohyoideus muscles in order to reduce the distracting forces acting on the oesophagus. Operated horses are unable to draw the larynx caudally and consequently cannot emit the grunting sound (Auer and Stick, 2006). The main complications of the modified Forssell's procedure are the development of swan-like neck or laryngeal hemiplegia. The assessment of its success-rate has revealed inconsistent results varying between 30 and 100% (Turner et al., 1984; Hakansson et al., 1992; Schofield and Mulville, 1998; Delacalle et al., 2002). Previous studies on the success-rate of the modified Forssell's procedure relied usually on the owner's reports and focused solely or mainly on the degree of stereotypy inhibition, postoperative complications and side-effects. The implications of the treatment for the general welfare of the horse have not been studied until to date, however, Schofield and Mulville (1998) noted that stereotypy elimination may increase stress. The need to evaluate the effect of a treatment on the welfare of the animal has become more apparent (Christiansen and Forkman, 2007).

The aim of the present study was to assess the success-rate of the prevention of crib-biting by the modified Forssell's procedure compared to the collar treatment and to measure stress-related behavioural and physiological variables in order to evaluate the 'quality of life' of the horses following treatment. Stereotypies may develop to cope with frustration, however, they often persist even after environmental stress is reduced thus fixed abnormal stereotypic behaviour itself may impose stress to the animal. Conversely, if stereotypies are maintained as coping mechanisms, animals may show signs of stress in a frustrating situation if execution of the abnormal behaviour is blocked by prevention methods. We hypothesised that horses prevented from performing crib-biting may either persist with the stereotypy in a modified form, or may show behavioural and/or physiological indicators of distress.

2. Materials and methods

2.1. Animals and housing

We tested 52 horses of four groups: control, crib-biting, collar-treated and surgically treated groups. Data of 12 horses were excluded from the analysis. Heart rate data of 7 individuals were not reliable or missing because of technical problems. Horses younger than 4 years of age (3 cases) were excluded from the analysis because they exhibited high stress reaction when tied with rope therefore baseline values could not be established. Data of one mare were not analysed because the attention of that horse was distracted by her foal staying in the vicinity. One horse in the control group exhibited oral stereotypies (licking the wall throughout the test) and was therefore not included in the analysis.

Sample size of the four experimental groups decreased to 9 horses in the control group without stereotypic behaviour, 10 crib-biting horses (crib-biting group) performing stereotypic behaviour since 1–2 years ($N = 4$) or more than 2 years ($N = 6$), 10 crib-biting horses that had been wearing cribbing collar continuously for at least six months ($N = 1$), between 1 and 2 years ($N = 7$) or more than 2 years ($N = 2$) before the experiment (collar-treated group), and 11 horses operated with the modified Forssell's procedure at least six months ($N = 3$), between 1 and 2 years ($N = 3$) or more than 2 years ($N = 5$) prior to the experiment (surgically treated group).

Horses had different owners and stayed at different riding schools. Behavioural tests were conducted in the familiar home environment of the horses.

2.2. Experimental design

Behavioural and heart rate variables of horses were assessed in a crib-biting triggering stress-test. The stressor was a modified version of the arousal-inducing situation applied by Bachmann et al. (2003b). The test was introduced 1.5–2 h following the morning or noon feeding of concentrates. During the whole test the box door was open. Five minutes before the test the horses were tighten to their box with a rope long enough so they could reach the ground.

The test lasted for 20 min and responses were evaluated in 9 periods (Table 1). Baseline was established in the first 5 min without any stimulus. After that, the experimenter was walking up and down in front of the box and was making noise with a bowl filled with oats (feeding-bowl) to direct attention to the bowl. Seven minutes after the start the feeding-bowl was placed in front but out of the reach of the horse. The feeding-bowl stayed there for 8 min, meanwhile tidbits were given to the horses (~5 g oats was taken from the feeding-bowl into the feed bin with delivery duration of ~10 s) three times. Two minutes after the 3rd tidbit the bowl was removed, and for 5 min no stimulus was presented, however, the horses stayed tied up.

Behaviour was videotaped and heart rates of horses were recorded continuously throughout the test.

2.3. Behaviour

Mutually exclusive behavioural elements were defined and recorded continuously with precision of 1 s (continuous

Table 1
An overview of the test periods of the feeding stress-test.

Test period	1	2	3	4	5	6	7	8	9
Duration (min)	2.5	2.5	2	2	2	2	2	2.5	2.5
Description	No stimuli		Experimenter is making noise with a feeding-bowl filled with oats	Feeding-bowl is placed in front but out of the reach of the horse		Tidbits (5 g oats) are given in the beginning of these periods. At the end of the 7th period the feeding-bowl is removed.		No stimuli	

sampling method) by replaying and when necessary slowing the video record. Elements were assigned to 4 major behaviour categories.

- *Oral activity*: repetitive oral activities without overt nutritional function; such as tongue flicking (tip of tongue is briefly extended), biting or grasping stable fittings or repeated licking of stable fittings, as well as crib-biting or wind-sucking.

  Specific forms of oral stereotypy (e.g. wood-chewing, crib-biting, wall-licking) are sometimes difficult to consistently differentiate (Cooper et al., 2005), and horses treated by collar or surgery are not always able to perform the whole behavioural sequence of crib-biting. These horses may e.g. try seizing a projection and arching the neck but fail to emit the grunting sound. Consequently, behaviour elements listed as oral activities include not just the terminal grunting of this stereotypy, which is in focus of inhibition, but other behaviour elements as well, which are considered to be associated with crib-biting behaviour—e.g. crib-biters tend to lick fixed objects before wind-sucking (McGreevy et al., 1995a).
- *Motor activities*: vocalisation, snorting, kicking, pawing, head-tossing, head-circling, nodding or manipulation of the rope.

  Some of these behaviour elements may initially arise as anticipatory activities, but later may become a conditioned response to feeding cues (Cooper et al., 2005) and are thought to indicate arousal (Weeks and Beck, 1996; Bachmann et al., 2003b).
- *Feeding-related behaviour*: bedding directed activities; standing with muzzle in the feed bin; or muzzle closer than 10 cm to the bedding. Every individual consumed the tidbits rapidly and actual feeding was excluded from this category.
- '*Immobility*': standing still and not doing any of the above listed behavioural categories.

Reactions to unexpected stimuli which might have disturbed the horse (e.g. dog barking) and the 10 s periods of tidbit deliveries were marked as distraction and were left out from analysis.

For each test period, the time spent with behavioural categories was calculated.

Interobserver agreement for the four behavioural categories was assessed by means of parallel coding of 20% of the total sample by two independent observers. The intraclass correlation coefficients are 0.99 for *oral activities*, 0.97 for *motor activities*, 0.87 for *feeding related behaviour*, and 0.93 for *immobility*.

2.4. Heart rate and heart rate variability

Heart rate (HR) and heart rate variability (HRV) were chosen as physiological indicators of 'emotional' stress (Bachmann et al., 2003b; Rietmann et al., 2004). Beat-to-beat (R-R) intervals were recorded by Polar® Equine S810i heart rate monitor. Prior to HRV analysis, R-R interval data were pre-processed for excluding artefacts by automatic error correction (moderate filter power with minimum protection zone set at 6 bmp/min) of the Polar® Horse-Trainer 3.0 software (Polar Electro Oy, Finland). The smoothness priors method was used for detrending of the R-R series ('HRV-analysis software', Niskanen et al., 2004). Average heart rate in each period was compared to baseline value obtained as the average heart rate in the first 5 min of the test.

The autoregressive model of power spectral analysis of HRV resulted in two main spectral components.

One HRV variable was chosen for analysis: the ratio of the low-frequency component (LF) and the high-frequency component (HF) of the HRV (LF/HF ratio). The low-frequency component corresponds to vasomotor waves, whereas the high-frequency component to respiratory acts. LF/HF ratio is considered a good indicator of the cardiac sympatho-vagal balance representing a measure of stress level. All analyses were carried out with a threshold set at 0.05–0.15 Hz for LF and 0.15–0.4 for HF (Rietmann et al., 2004).

The R-R segments of each test periods were selected and analysed. The beginning and the end of the test periods were time-matched with the video analysis of the behaviour (e.g. 5th period started when the first tidbit was delivered and finished when the 2nd tidbit was given). R-R segments corresponding to 'distraction' were left out from the HRV analysis.

2.5. Questionnaire survey

The owners were asked questions of a predetermined survey sheet (Appendix 1) and responses were recorded by the experimenter. Questions were about the age, breed, gender, current use (sport or leisure), access to pasture and amount of exercise per week, current feeding program as well as details of the crib-biting behaviour of the horse and severity of the stereotypy as judged by the owner.

2.6. Statistical analysis

All analyses were carried out by R 2.7.2. Statistical Software (R Development Core Team, 2007). The significance level was set at $p < 0.05$ and an effect was considered a trend when p was between 0.05 and 0.10. For hypothesis testing, general linear mixed model was fit to the data (Pinheiro and Bates, 2000) with random effects for each horse and fixed effects for the different groups, test periods and their interaction. The given HR, HRV and behavioural parameters served as response variables in the different models. Square root transformation of frequencies of behaviour categories was applied to satisfy the normality and variance homogeneity assumptions of the models. Due to the large inter-individual variability in the absolute levels of HR and HRV, changes in HR and HRV for a given horse in the test periods were calculated by subtracting the baseline values (average rate of the 1st and 2nd test periods). Baseline values for HR and HRV were included as covariates into the fitted models. Because of multiple comparisons p-values were corrected according to Tukey–Kramer. In each group, differences in HRV between the first and last 5 min of the test was calculated by using paired t-test.

3. Results

3.1. Animals

Groups did not differ significantly regarding age, breed, gender, housing and management conditions or the usage of the horse (Fisher's tests, $p > 0.100$, in all cases). The pre-treatment proportion of moderate and severe crib-biters did not differ significantly between collar-treated, surgically treated crib-biting groups (Fisher's test, $p = 0.413$). Altogether 9 stereotypic horses (with or without inhibition) were categorised by the owners as moderate and 22 as severe crib-biter.

3.2. Reliability of the questionnaire survey

History and severity of crib-biting as evaluated by the owners were compared to crib-biting elicited by the test. The prevalence of severe crib-biting were significantly lower among horses that had a history of this abnormal behaviour for less than 1 year (Fisher's test, $p < 0.001$), used only one or two types of cribbing surface (Fisher's test, $p = 0.049$), or performed crib-biting usually following consumption of concentrates rather than continuously throughout the whole day (Fisher's test, $p < 0.001$).

The stress-test successfully triggered crib-biting in all non-prevented crib-biters and to a certain degree in some of the collar or surgically treated horses. Even among control horses *oral activities*, e.g. grasping, could be observed occasionally. Horses that were categorised by the owners as moderate crib-biters spent $3 \pm 1\%$ (mean $\pm$ S.E.) of the total time with *oral activities* whereas horses categorised as severe crib-biters performed it significantly longer, $16 \pm 4\%$ ($t_{25} = -3.329$, $p = 0.003$).

3.3. Behaviour

Groups did not differ significantly regarding the baseline values (average of 1st and 2nd test periods) of the percentage of time spent with any of the behavioural categories ($p > 0.1$ in all cases). Immobility increased, motor activity decreased in the 3rd test period, when the bowl with oats was presented.

3.3.1. Oral activities

Throughout the test, crib-biting horses performed significantly more *oral activities* than controls ($p < 0.001$), whereas levels of the two inhibited groups were in between them. Values of collar and surgically treated horses did not differ from each other ($p = 0.305$). Controls exhibited significantly less oral activities than collar-treated horses ($p = 0.016$), but did not differ from surgically treated horses ($p = 0.520$). The frequency of *oral activities* increased remarkably after the first tidbit was presented (5th test period), especially in crib-biting horses (Table 2, Fig. 1a). Tongue flicking was observed in 3 collar-treated horses.

3.3.2. Motor activities

Control horses spent significantly more time with *motor activities* compared to crib-biting ($p = 0.003$), or surgically treated horses ($p = 0.011$). There was a trend for difference between control and collar-treated horses as well ($p = 0.064$). Crib-biting, collar-treated and surgically treated horses did not differ from each other. Placing the bowl in front of the horses elicited high motor activities, especially in control horses (Table 2, Fig. 1b).

3.3.3. Feeding-related behaviour

Frequency of *feeding-related behaviour* sharply declined when pre-feeding activities were imitated by

Table 2
Test statistics and significance level of the treatment, test period and their interaction in the case of the given behavioural categories, heart rate (HR) and LF/HF ratio according to the final significant general linear mixed models.

	Treatment		Test period		Interaction of treatment and test period	
	$F_{3,36}$ value	p-Value	$F_{1,316}$ value	p-Value	$F_{3,316}$ value	p-Value
Oral activities (% of time)	4.649	0.008	69.784	<0.001	16.144	<0.001
Motor activities (% of time)	5.648	0.003	7.731	0.006	n.s.	n.s.
Consummatory behaviour (% of time)	n.s.	n.s.	60.416	<0.001	n.s.	n.s.
Immobility (% of time)	2.998	0.043	32.745	<0.001	2.666	0.048
Changes in HR (bpm)	n.s.	n.s.	6.185	0.013	n.s.	n.s.
Changes in LF/HF ratio (n.u.)	3.953	0.016	n.s.	n.s.	6.605	<0.001

bpm: beat per minutes, n.u.: normalised units, and n.s.: not significant.

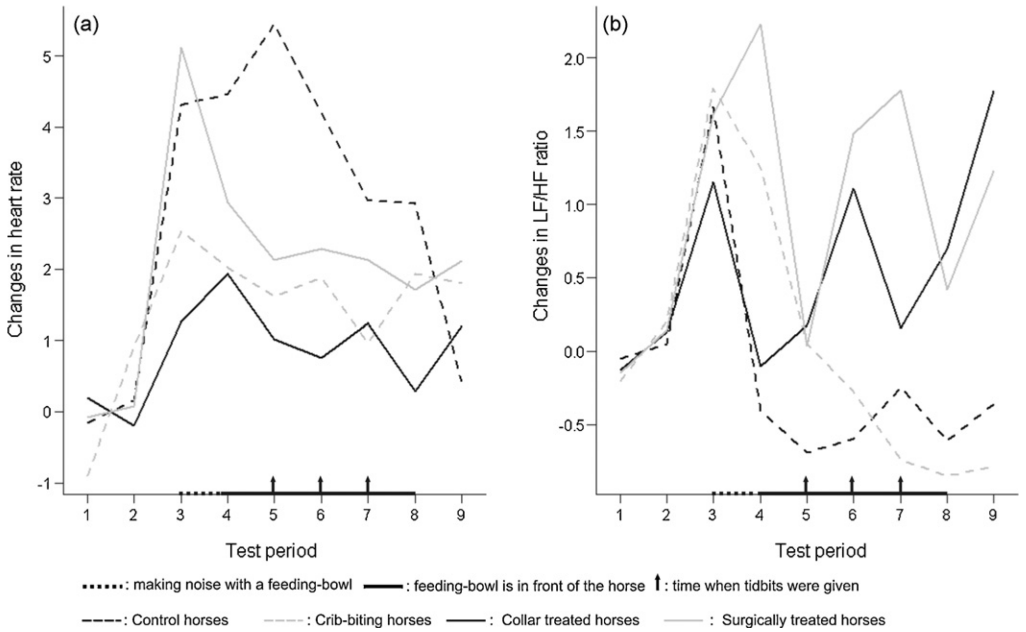

Fig. 1. Mean percentage of time spent with oral (a) and motor (b) *activities* during the test periods.

the experimenter, and increased above base-line levels following tidbit presentation. Controls exhibited somewhat lower feeding-related activity, however, differences among groups were not significant (Table 2, Fig. 2a).

3.3.4. Immobility

Throughout the test, crib-biting horses spent significantly less time with *immobility* compared to control horses ($p = 0.047$), however, none of the other comparisons reached level of significance (Table 2, Fig. 2b).

3.4. Heart rate and heart rate variability

Baseline HR value (first 5 min of test) were similar in the four groups ($F_3 = 0.757$, $p = 0.526$, Table 3). Heart rate of most horses increased during pre-feeding imitation and remained high in control horses. However, changes in heart rate did not differ among groups (Table 2, Fig. 3a).

Regarding the baseline value of LF/HF ratio there was a trend for differences among groups ($F_3 = 4.594$, $p = 0.050$, Table 3) control and crib-biting horses having somewhat

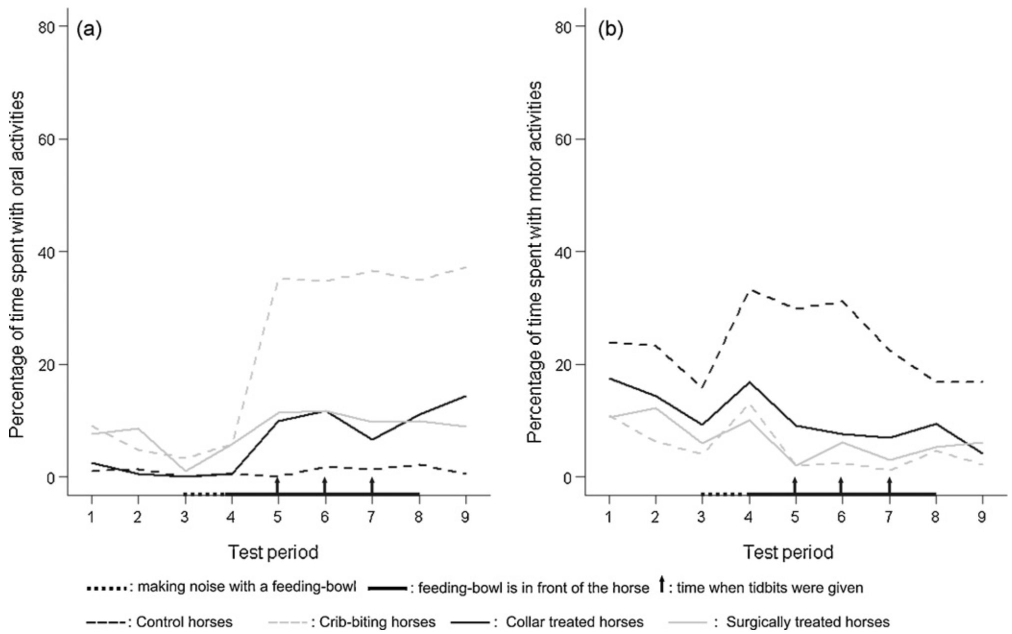

Fig. 2. Mean percentage of time spent with feeding-related behaviour (a) and immobility (b) during the test periods.

Table 3
Mean baseline values (±standard error) of heart rate (HR) and heart rate variability (LF/HF ratio) during 1st and 2nd test period; and the significance level of their comparison (using general linear models).

	Control horses (N = 9)	Crib-biting horses (N = 10)	Collar-treated horses (N = 10)	Surgically treated horses (N = 11)
HR (bpm)	36.3 (0.8)	36.1 (1.7)	38.8 (2.4)	38.5 (0.8)
LF/HF ratio (n.u.)	3.54 (0.37)	3.58 (0.57)	2.35 (0.16)	2.43 (0.32)

bpm: beat per minutes; n.u.: normalised units.

higher values than the inhibited groups. Throughout the stress-test the changes in LF/HF ratio differed significantly among groups (Table 2, Fig. 3b). The time dependence of LF/HF ratio of crib-biting horses differed significantly from collar-treated ($p = 0.004$) and surgically treated horses ($p < 0.001$) but did not differ from control horses ($p = 0.989$). Control horses differed significantly from collar-treated ($p = 0.014$) and surgically treated horses ($p = 0.004$). Collar and surgically treated horses did not differ from each other ($p = 0.986$).

Heart rate variability increased in all groups during pre-feeding imitation. Difference in HRV between the first and last 5 min of the test was significant in the collar and surgery treated groups ($t_9 = 2.348$ $p = 0.044$; $t_{10} = 2.498$, $p = 0.032$, respectively), whereas HRV levels returned to baseline in the control and crib-biting groups ($t_8 = -1.644$, $p = 0.139$; $t_9 = -1.103$, $p = 0.299$).

4. Discussion

We are aware of certain limitations of the present study. Most importantly, effects of surgery or collar treatment on individual horses could not be estimated. A self-control design comparing reaction of horses before and after surgery or collar treatment would be a good option for validation. Unfortunately, that was impossible to conduct, since most of the horses arriving at the clinic for the surgery had been wearing collars. Removal of the collar for pre-treatment evaluation was not an option as horses following collar treatment perform crib-biting behaviour more vigorously than usual, as the result of the post-inhibitory rebound (McGreevy and Nicol, 1998b). Observation of few horses arriving without collar indicated that their behaviour was not consistent in the novel environment which is known to alter the occurrence of stereotypic behaviour (McBride and Cuddeford, 2001). However, the design in which we compared reaction of control, crib-biting, collar and surgically treated horses can be considered a relevant tool for evaluating the effect of the given treatments (Mason and Rushen, 2006; Christiansen and Forkman, 2007). The suitability of our stress-test was further corroborated by the significant relationships between pre-treatment history and intensity of crib-biting behaviour as reported by the owners with the total time spent with *oral activities* throughout the test. Our finding that the assessment by the owners was a reliable indicator of cribbing is important in itself, because report of the owners is the only tool to test post-treatment effects in most studies (Turner et al., 1984; Hakansson et al., 1992; Schofield and Mulville, 1998; Delacalle et al., 2002).

In our experiment horses were exposed to a single stress-test and only once. This is another limitation of the present

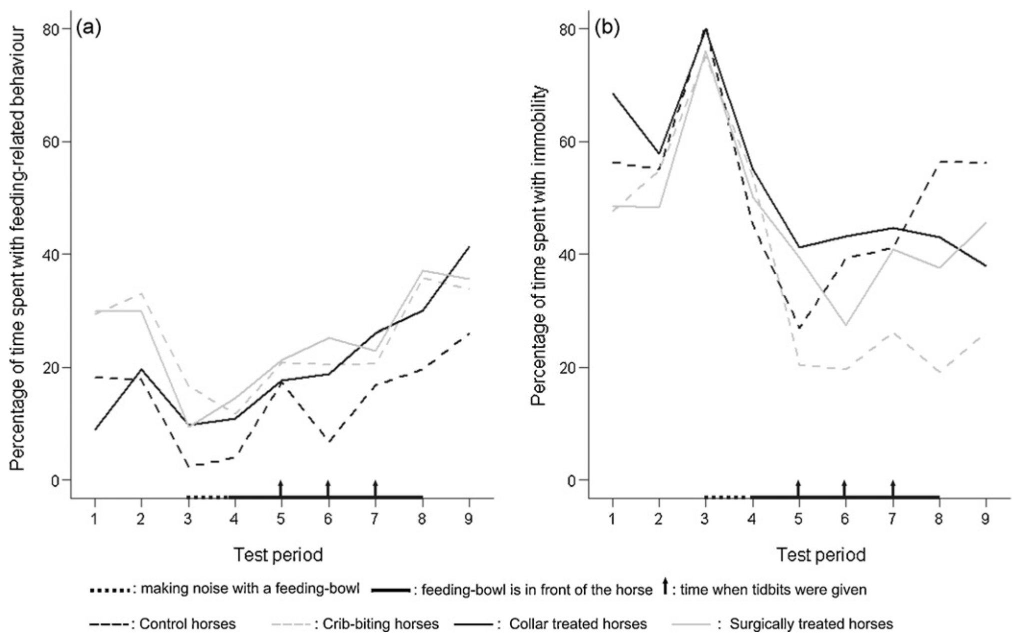

Fig. 3. Changes in mean heart rate (a) and LF/HF ratio (b) interpreted as the sympatho-vagal balance or stress level of horses during the test periods.

study as coping style is defined as consistent reaction to stress over time and across situations (Koolhaas et al., 1999; Koolhaas, 2008). By analysing the data from a single exposure it is impossible to tell whether high variance of most responses represented consistent individual differences or reflected the variability of the behaviour of individuals. To dismantle the two possible sources of variance 10–12 repetitions would have been required. Our test effectively elicited strong stress reactions and oral responses in most horses; therefore repetitions of the stress situation could not be considered independent and might have aggravated the problematic behaviour. Therefore, both statistical and ethical considerations led us to drop the option of repeated measurements. Nevertheless, responses significantly different among treatment groups can be considered consistent effect of the treatment.

Although stress level as indicated by heart rate variability was indistinguishable between control and crib-biting horses, their behaviour was strikingly different. Most control horses made attempts to reach the feeding-bowl by pawing or head-tossing and either kept trying or stood still, whereas motor activity as well as immobility of cribbers were low because instead of targeting the bowl crib-biting horses engaged in *oral activities* (crib-biting) throughout the entire test. Control horses thus adapted active coping by fighting for the food, while crib-biting horses exhibited a flight response to frustration, a strategy which can be labelled proactive coping.

Similarly to crib-biters and contrary to controls, inhibited horses performed little motor activity and few attempts to act upon the stressor (reaching the feeding-bowl). Their oral activity was elevated by the stress, however, remained significantly lower that that of crib-biting horses. On the other hand, stress level as assessed by heart rate variability remained high in inhibited groups throughout the test even when the stressor was removed. Previous studies revealed that collar treatment inhibits crib-biting, however does not extinguish the motivation for such stereotypy (McGreevy and Nicol, 1998b). Motivations for cribbing in operated horses cannot be directly studied as surgery is irreversible, however, the similarities in reactions between the two inhibited groups suggests that collar treatment and surgery have similar effects on crib-biting tendencies. Taking these data together we suggest that crib-biting is a coping strategy, which when inhibited leads to high stress levels in frustrating situations.

Our results do not allow to evaluate any differential effects of collar as compared to surgery treatment, nor to draw general conclusions on possible benefits or risks of the inhibition of crib-biting. On one hand, inhibition of cribbing may elevate stress level in frustrating situation, however, it may be beneficial regarding the overall health of the horses. Further studies are needed to disentangle long term positive and negative effects of both inhibition methods so that owners could make an informed decision on the treatment of crib-biting.

5. Conclusions

Crib-biting is probably the most detrimental abnormal stereotypy in horses, prevention of its development is crucial. Once fixed, crib-biting is difficult to eliminate by behaviour therapy, therefore several methods to inhibit its performance have been worked out. However, if crib-biting is a strategy to cope with stress, the effects of inhibition should be carefully estimated. In the present study we found that crib-biting horses spent more time with oral activities, primarily with cribbing than controls or inhibited horses, whereas their stress level as indicated by heart rate variability were indistinguishable from controls and significantly lower than that of the inhibited groups. Overall our results suggest that performance of oral stereotypies in a stress situation successfully diminishes subjective stress, while inhibition of such stereotypy elevates it, thus crib-biting may be a true coping strategy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.applanim.2009.09.008.

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