



The effect of two different housing conditions on the welfare of young horses stabled for the first time

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Abstract

The effect of stabling for the first time on the behaviour and welfare of young and naïve horses has not yet been studied in detail. In this study we examined the effect of two typical housing systems on their subsequent behavioural and physiological responses upon first time stabling. Thirty-six 2-year-old Dutch warmbloods, 18 geldings and 18 mares were included in the study. Half of the horses were stabled in individual stables (10.5 m²) and the other half in pair housing (48 m² for two horses). The study lasted 12 weeks. At the end of the study the physiological and temperamental responses of the horses on the different treatments was tested using a CRF challenge test (to test the HPA-axis function) and a Novel Object test (to test temperamental differences) respectively. Especially in the first week after stabling pair housed horses spent more time eating whereas individually housed horses spent more time either standing vigilant or sleeping. Stress-related behaviours like neighing, pawing, nibbling and snorting were all displayed significantly more frequently in the individually housed horses ($P < 0.01$). At the end of the study 67% of the individually housed horses was seen performing one or more stereotypies ($P < 0.01$). The cortisol response and ACTH response on the CRF challenge test were lower for horses in the individually housed boxes. It is suggested that this depression in socially isolated animals is caused by a desensitisation of the HPA axis in response to stress-induced elevations in ACTH and cortisol. In general there was no effect of the treatment on the reactivity of the horses during the Novel Object test. However, there were significant relations between the responses of horses in the Novel Object test and in the stable environment. It is concluded that sudden isolated stabling is stressful to young and naïve horses, resulting in a high prevalence of stereotypies and abnormal behaviours. This study also provided some support for the notion that social stress in horses may be associated with a blunted adrenocortical response to CRF challenge. The finding that responses of horses to a behavioural test are correlated with home environment behaviours suggests that individual horses exhibit consistent

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behavioural traits across different contexts, and opens the possibility of using behavioural tests in horses to predict more general underlying behavioural characteristics.

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1. Introduction

Feral horses are social herd animals which are free-roaming and pasture-grazing. In their natural environment they have to adapt to environmental changes and challenges for their survival. Compared to their feral relatives, the diversity of behaviours observed in stabled horses has been dramatically altered due to the confining nature of the husbandry systems (Flannigan and Stookey, 2002). The equine industry often ignores the biological need of the horse to adapt to its environment, and sometimes ‘human standards’ are applied to assess quality of daily husbandry methods. Nevertheless there is growing awareness that the way we manage, house and feed horses is suboptimal for this species. Confinement, controlled exercise regimes, social isolation and restricted feeding regimes may all contribute to the development of abnormal behaviour patterns and stereotypies, like weaving, box walking, cribbing and wind sucking (McGreevy et al., 1995a; Cooper and Mason, 1998; Nicol, 1999). Recent studies suggest that the performing of stereotypic behaviour might be a beneficial adaptation for the animal within an ‘abnormal’ (e.g. unnatural) situation (Cooper and Albertosa, 2005), and, therefore may represent an indicator of a present or past reduced welfare state of the animal. A likely pathogenesis of stereotypic behaviour is that animals generally develop the stereotypy in times of ‘trauma’ although they often do not discontinue the abnormal behaviour once the traumatic situation has been resolved.

In several countries the quantitative impact of abnormal behaviours on the horse population has been assessed by means of surveys. These surveys have found prevalence of horses showing stereotypic behaviours between 5 and 15% of the populations studied (e.g. McGreevy et al., 1995b; Luescher et al., 1998) and up to 35% for abnormal behaviour patterns (Waters et al., 2002).

It is generally accepted that husbandry practices have a significant effect on the development of abnormal behaviour patterns and stereotypies in stabled horses (Haupt and McDonell, 1993; Simpson, 1998). In order to improve the welfare state of individually housed horses some countries have made recommendations for stable sizes. In Denmark, for example, the stable area (in m²) is recommended to measure at least twice the height at the withers squared, and the shortest side of the stable should be at least 1.5 times the height at the withers (1.6 m horse: 10.2 m × 5.7 m). In the United Kingdom the British Horse Society recommends a box size of 3.6 m × 3.6 m for ‘horses’ (Raaybymagle and Ladewig, 2004).

Other than box size, restricted possibilities to interfere with their conspecifics are thought to contribute to the development of stereotypies. Several studies of feral and pasture-kept animals have highlighted the importance of interspecific social contacts, exercise and lengthy grazing times (Crowell-Davis et al., 1985; Haupt et al., 1986; Van Dierendonck et al., 1996).

Confinement and social isolation restrict horses in movement and behavioural options, reduce environmental stimulation (Kiley-Worthington, 1990; Mills and Clarke, 2002) and may cause elevated levels of stress (Mal et al., 1991a,b). The effects of long- or even short-term isolation and confinement can lead to abnormal behaviour, increased heart rate, vocalizations, defecation, and feeding disturbances (Kiley-Worthington, 1990; Bagshaw et al., 1994).

Most research on the effect of husbandry practices includes physiological and behavioural measurements of stress. Elevated cortisol levels, increased heart rate and decreased heart rate variability are interpreted in terms of an increased level of stress (Kiley-Worthington, 1990; Bagshaw et al., 1994). An altered time budget, abnormal behaviour patterns and incidences of stereotypic behaviours are suggested to reflect a coping mechanism to a less optimal environment (Kiley-Worthington, 1989; Waran, 1997).

Previous studies on management systems in horses have focussed on different housing conditions in adult horses. For the stabled horses there are two major social challenges capable of eliciting a stress response characterised by adaptive behaviours that may be quantified to assess the degree of stress (Hoffman et al., 1995). These events are weaning (usually between 4 and 9 months) and first time confinement in a stables (usually between 2 and 4 years of age) (Houpt et al., 1984; McCall et al., 1985, 1987). Unfortunately individual stabling for the first time often coincides with the traumatic process of weaning.

Limited research exists regarding different weaning methods and the effect on stress and welfare in horses. Nicol et al. (2005) studied the effect of different diets on stress during the weaning period and Heleski et al. (2002) studied the effect of housing (paddock versus stable) in weanlings. Most previous studies of domestic horses have assessed behavioural differences between stabled horses and horses maintained on pasture; less information is available on how different degrees of physical and social confinement affect behaviour of horses stabled for the first time.

Hoffman et al. (1995) comprehensively studied the stress response, both behaviourally and physiologically, during weaning of foals. They measured not only basal levels of stress hormones but also challenged the hypothalamus-pituitary-adrenal (HPA) axis to measure adrenocortical responsiveness in the weanlings. In their study they challenged the HPA axis using an ACTH challenge with a corticotrophin injection. The response blood sample was taken five hours after the challenge. Harewood and McGowan (2005) performed one of few studies regarding the stress responses of first time confinement in a stable. They investigated the response of horses to confinement and isolation in an individual stable for the first time using behavioural variables, heart rate, and salivary cortisol measurements. Although they found marked behavioural stress responses, these were not reflected in increased heart rates or salivary cortisol concentrations.

In the present study, we examined the level of stress induced by first time confinement in a stable of naïve 2-year-old horses in two different housing systems with different degrees of physical and social confinement. We combined several behavioural and physiological measures that were used previously to evaluate stress responses in horses.

2. Materials and methods

All procedures involving animal handling and testing were approved by the Animal Care and Use Committee of the Animal Sciences Group of Wageningen University and Research Centres in Lelystad, the Netherlands.

2.1. Animals, housing, handling and management

A total of 36 Dutch Warmblood horses, 18 geldings and 18 mares were used in this study. All horses were two years old and had the same background in housing and management. Twenty-seven horses were born at the experimental farm and an additional nine horses were bought from other farms three months before the experiment started. All horses had been weaned at six months of age and had been housed in a large group either at pasture or in an indoor group housing system until the start of the experiment. Before the start of the

experiment all horses were checked physically. None of the horses were reported to possess abnormalities in health status, or performed abnormal behavioural patterns.

After blocking for sex all horses were randomly allocated to one of two treatment groups: in one group, 18 horses were housed individually in boxes of 3 m × 3.5 m, and in another group, 18 horses were housed in 9 pairs with each pair having access to an area of 48 m². The individual boxes had three solid walls (without grid windows in the side walls) and one half-grid door at the front. These horses were able to hear their neighbours and were able to hear and see their neighbours opposite their own boxes. The horses housed in pairs also had the possibility to interact with the neighbouring pairs through the feeding corridor and through the grids in the side walls.

During the experiment horses were fed roughage only, 13–17 kg per horse per day, according to bodyweight, which was distributed over the day in three portions per day. Additionally to the roughage horses received a vitamin and mineral supplement. Water was ad libitum available for the horses at all times.

The bedding material for both the horses in the individual boxes as well as the horses in the group housing boxes consisted of straw which was renewed once a week and new straw was added daily. During the first three weeks of the experiment horses were not turned out, apart from the time the bedding in the boxes was renewed. After the third week, all horses received a limited level of exercise in a trainingsmill for 20 min a day.

2.2. Measurements

At the first day of the experiment, at the end of the summer, horses were brought in from pasture, weighted, health checked and assigned to one of the two treatment groups. The total experiment lasted 12 weeks. In weeks 1–3 behaviour of the horses was observed, in week 10 horses were exposed to a corticotropin releasing factor (CRF) challenge test and in week 12 horses were tested for emotional reactivity during a Novel Object test.

The behaviour of each horse was quantified from direct observations. Behavioural observations were performed from 9 a.m. to 5 p.m., on working days, for 3 weeks in total. In week 1 behaviour was continuously recorded for 10 min per hour, 8 h a day. In the second and third week the behaviour was recorded using a scan sampling method. Every five minute the observers walked randomly through the stable and recorded each horse's behaviour. The behavioural categories used for these observations are listed in Table 1.

Between the 4th and the 10th week horses were handled according to a standardized handling procedure in their boxes twice a week to habituate to handling procedures, humans and heart rate equipment. This habituation included petting and gently brushing all parts of the body, picking up feet, fastening a soft elastic belt for heart rate measurement and standing tied up. This habituation period was necessary to minimize the extra handling stress during the CRF challenge test and the Novel Object test.

On the morning of the CRF challenge test, horses were equipped with intravenous catheters, which were filled with heparinized physiological saline (125 IU/ml). Horses received an intravenous injection with 0.1 µg bovine CRF, administered via the catheter. Blood samples were collected prior to ($t = 0$) and at 15, 30, 45, 60, 75, 90 and 120 min after the administration of CRF. Horses were challenged simultaneously in groups of two, i.e. either two horses belonging to the same pair, or two horses in individual boxes housed opposite to each other. The first group of two horses started at 11 a.m., the second pair at 01:00 p.m. and the third pair at 03:00 p.m. in order to minimize possible effects of elevated night levels of cortisol. Between blood sampling there was no interaction with the humans and horses were free to move around.

In the 12th week horses were exposed to a Novel Object test. In the stable horses were fitted with heart rate electrodes and recording devices (Polar S810). For several minutes the stable heart rate was monitored and recorded. Next, the horse was taken to the test environment which consisted of an indoor arena of 25 m × 35 m. The other half of the paired horses was left alone in its stable. The experimental horse was left by itself and the observers recorded the behaviour of the horse during the test with a video recorder hidden in one corner. Neither the observers nor the video recorder was visible to the horse. After two minute a white and blue umbrella was lowered from the ceiling. After an additional five minute the horse was caught and brought back to its stable.

Table 1

Definition of behaviours recorded during the first three weeks of stabling and used in both the continuous sampling as well as the scan sampling (D, duration; F, frequency)

Behavioural category	Description	Variable type
Eat silage	Head low, nose moving through straw and silage while chewing	D
Locomotion	Horizontal movement of the body, four-time gait	D
Stand vigilant	Stand attentively with head and neck raised, eyes wide open and ears picked or mobile	D
Groom/scratch	Scratch oneself with hooves or teeth, possibly using stable materials	D
Stand rest	Standing inattentively, with head and neck lowered, eyes partially or fully closed and ears relaxed	D
Lie down	Lying down with head and neck lowered, eyes partially or fully closed and ears relaxed	D
Negative social	A threat or an aggressive interaction with another horse	D
Positive social	Affiliative behaviour, including licking, sniffing, grooming or resting head on another horse	D
Not seen	Not visible or not defined behaviour	D
Neighing	Open mouth, high-frequency sound	F
Snorting	Closed mouth, wide open nostrils, raspy noise	F
Kicking	Kicking walls or door	F
Pawing	Repeatedly forward–backward movement of one foreleg	F
Rearing	Two forelegs off the ground	F
Nibbling	Nibbling the walls or the bucket	F
Defecating	Defecating	F
Flehmen	Curling up the upper lip, head upwards	F

2.3. Data analysis

Behaviour was analysed using the Observer Software system 3.1. Behavioural variables scored in the Novel Object are listed in Table 2.

To summarize the hormonal responses to the CRF challenge test, integrated areas under the plasma ACTH and plasma cortisol curves were calculated. The cortisol/ACTH ratio after injection of exogenous CRF was calculated by dividing the area under the plasma cortisol curve by the area under the plasma ACTH time curve. This ratio represents a measure of adrenocortical sensitivity to endogenous ACTH (Janssens et al., 1995; Veissier et al., 1999)

Heart rate recordings were downloaded on a computer and incidental artifacts were corrected manually by visual inspection.

Table 2

Definition of behaviours recorded during the Novel Object (NO) test for young horses after being stabled for 12 weeks either individually or pairwise

Behavioural category	Definition
Snorting	Frequency of making a raspy noise, with mouth closed and nostrils wide open
Defecating	Frequency of defecating
Tail switching	Frequency of sudden vertical or horizontal movements of the tail
Leg circle	Number of times horse puts at least one leg in the circle around the NO. The diameter of the circle is two horselengths
Touch NO	Number of times horse touches the NO with its nose
Latency leg circle	Latency time for the horse to first time entering the circle with one leg
Latency NO	Latency time for the horse to first time touching the umbrella
Locomotion	Total duration of trotting or cantering

To test for treatment effects, general linear models were used, which included main effects for housing (individual versus pair housing) and sex (mare versus gelding), and their interaction. The measure for heart rate variability (rMSSD) was log transformed prior to analysis, to obtain homogeneity of variance. Behaviours expressed as percentages of time were analysed as fractions with a logistic regression model, comprising a multiplicative overdispersion factor with respect to the binomial variance function. Frequencies of behaviours, i.e. count data, were analysed using a log linear model comprising a multiplicative dispersion function relative to the Poisson variance function. Analyses of logistic and log linear models were based on quasi maximum likelihood (McCullagh and Nelder, 1989). Since there were neither any effects of sex nor any significant interactions between housing and sex (results not shown), we will report only results on the effects of housing.

To analyse correlations between behaviour and physiology in the home environment and responses in the Novel Object test, a Spearman rank correlation test was applied on residuals obtained after fitting the appropriate model (described above). The differences between treatment groups in the prevalence of horses exhibiting stereotyped behaviours was analysed with Fisher's exact test. Statistical analysis was performed with Genstat 7.0 (GenStat Committee, 2000), and in all analyses stables, with either one or two horses, were considered as the experimental unit.

3. Results

3.1. Effect of social isolation

3.1.1. Behaviour

Due to the different methods of behavioural recordings in the home environment (continuously recording during the first week and scan sampling recording during the second and third week) these results were analysed separately. Grooming, social and antagonistic behaviours (if appropriate) were grouped into the category 'other'.

Results showed that all horses spent between 50 and 65% of the total time eating and browsing. In the first week after stabling the horses housed in pairs spent significantly more time eating compared to the individual stabled horses ($P < 0.01$, see Fig. 1). Conversely, the time horses were standing vigilantly and were sleeping or dozing was significantly higher for the individually housed horses ($P < 0.05$). In the second and third week after stabling the individual stabled horses were significantly more vigilant compared to the pair housed horses ($P < 0.02$).

The frequencies of most behaviours were significantly higher for the individual stabled horses compared to the pair housed horses (Fig. 2). Individually housed horses were found to nibble more on the wall and food basket and to neigh and snort more in all 3 weeks after stabling ($P < 0.001$). Moreover, the first week after stabling the individually housed horses defecated, reared and flehmed significantly more ($P < 0.02$); the second and third week they pawed and kicked significantly more ($P < 0.02$).

At the end of the 12-week period the presence or absence of stereotypies was evaluated for each horse. It appeared that four out of 18 horses in the individual stabling had developed a crib-biting stereotypy. Eight other horses in individual stabling showed weaving of which four horses also displayed box-walking behaviour. Whereas in total 67% of the horses housed individually developed a stereotypy (12 out of 18), none of the horses in pair housing developed a stereotypy (0 of 9 pairs) (Table 3). This difference was statistically significant ($P < 0.001$, Fisher's exact test).

3.1.2. Physiology

At the start of the experiment horses weighed between 482 and 651 kg, with a mean of 549 kg. The mean weight in the 6th week after stabling was 554.0 kg (range 484–635 kg) and the mean

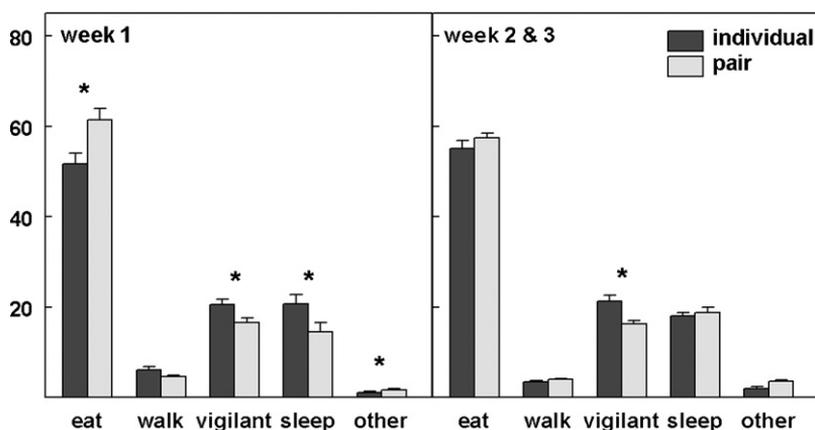


Fig. 1. Percentage of behaviour of total observation time displayed by young horses stabled individually or in pairs during the first, second and third week after stabling. Asterisk (*) indicates significant ($P < 0.05$) differences between treatment groups.

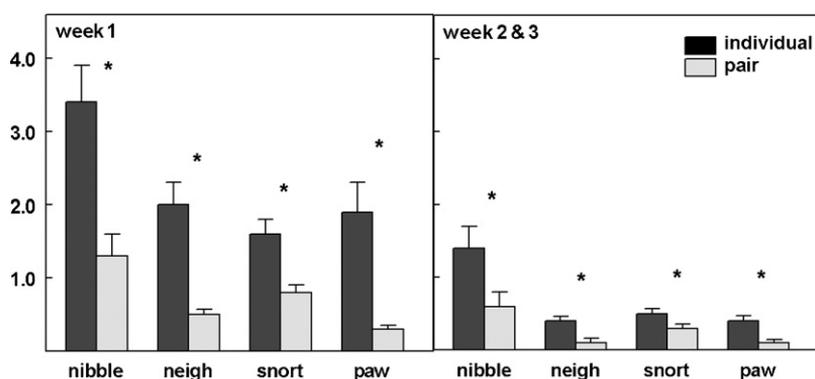


Fig. 2. Average daily frequencies of behaviour per horse for young horses stabled individually or in pairs during the first, second and third week after stabling. Asterisk (*) indicates significant ($P < 0.05$) differences between treatment groups.

weight in the 12th week was 564 kg (range 494–638 kg). The total weight gain between the first and 12th week, and the weight gain between sixth and the 12th week was significantly higher for the horses in the pair housed boxes compared to the individually housed horses (Table 4, $P < 0.001$).

Table 3

Number of horses (% of horses) that was seen performing one or more stereotypies at least twice by the researchers at the end of the 12-week housing period

	Individual	Pair	Significant, P
Weaving	8 (44%)	0	<0.001
Box walking	4 (22%)	0	<0.001
Cribbing	4 (22%)	0	<0.001
Total abnormal behaviour	12 (67%)	0	<0.001

Table 4

Weight gain of young horses housed individually or pairwise over the first 6 weeks, over the second 6 weeks and over the entire period of 12 weeks

Week	Individual (kg)	Pair (kg)	Significant, <i>P</i>
1st to 6th	3.8 ± 2.5	6.9 ± 1.6	n.s.
6th to 12th	9.1 ± 1.4	15.6 ± 1.8	<0.001
1st to 12th	12.8 ± 3.1	23.0 ± 1.5	<0.001

The average basal (before challenge test) plasma cortisol level for all horses 10 weeks after stabling was 129.2 nmol and the average basal level of ACTH was 17.1 nmol (Fig. 3). There was no difference between treatment groups before the challenge test. The peak of the plasma cortisol response on the administration of bovine CRF was at 60 min after administration. The peak level of ACTH was at 30 min after administration. At 120 min after administration the final sample was taken, both the ACTH and cortisol levels had not returned to basal levels at this time. The cortisol response and ACTH response were lower for horses in the individually housed boxes. This difference was significant for the cortisol response at 45, 60 and 75 min after administration ($P < 0.05$). The area under the curve did not differ significantly between the two treatment groups. Also the ratio of the area under the curve for cortisol to the area under the curve for ACTH did not differ between treatment groups.

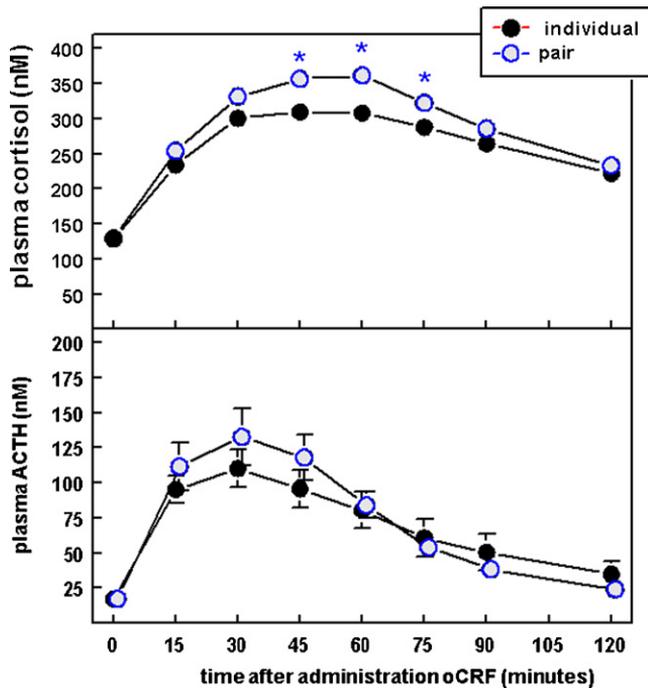


Fig. 3. Jugular plasma cortisol and plasma ACTH concentrations in horses that have been stabled individually or pairwise for 12 weeks with samples were taken 15, 30, 45, 60, 75, 105 and 120 min after oCRF administration. Asterisk (*) indicates significant ($P < 0.05$) differences between treatment groups.

3.2. Novel Object test

The effect of different housing systems on the reactivity and emotionality of horses was studied with a Novel Object test. In general, there were no distinct effects of the housing systems on the emotionality of the horses tested in the Novel Object test. One behavioural variable differed significantly between treatment groups: the time horses were trotting and galloping was higher for the pair housed horses compared to the individually stabled horses (114 ± 18 s versus 62 ± 8 s, $P < 0.05$). There was no difference in latency times to touch the umbrella and no differences in frequencies of snorting, defecating, tail switching, and touching the umbrella.

There was no difference in heart rate variables between housing groups during the Novel Object test. However, there was a significant difference in mean heart rate in the stable between the treatment groups (individual 40.1 ± 1.7 versus pair 34.4 ± 1.0 , $P < 0.01$). There were no significant differences in mean heart rate and heart rate variability measures during the Novel Object test. The mean heart rate during the Novel Object test was 128 beats per minute, and in the stable this was 37 beats per minute. The heart rate variability measure rMSSD during the Novel Object test was 23 ms and in the stable this was 86 ms.

Spearman rank correlations revealed that there were relationships between the reactions of horses in the Novel Object test and their behaviour in the stable. Horses that approached and touched the Novel Object soon and frequently after the exposure showed a high frequency of pawing in the stable ($R_{SP} = 0.44$; $P < 0.05$) and a high frequency of nibbling the wall ($R_{SP} = 0.39$; $P < 0.05$). Likewise horses that defecated frequently during the exposure to the Novel Object, had a high-mean heart rate and a low-mean heart rate variability in the stable ($R_{SP} = 0.36$; $P < 0.05$).

4. Discussion

Behavioural observations showed clear differences between horses stabled in pairs and the individually stabled horses in the first 3 weeks after first time stabling. Individually stabled horses were more vigilant and showed more stress related behaviours like vocalisations, defecations, pawing and nibbling at the stable doors. These results are in concordance with similar studies. In the study of Hoffmann et al. (1995) it was shown that paired horses had a significantly lower behavioural score (were less vigilant), compared to single housed horses. Heleski et al. (2002) found that aberrant behaviours, such as licking, bucking, rearing, kicking, pawing were observed more frequently in the stabled weanlings than the paddock weanlings. The fact that the pair housed horses had more space per horse might also have contributed to the more aberrant behaviours found in the single housed horses; some of these behaviours, like pawing, can be regarded as a motivation to move around.

Although several studies have reported effects of breed and sire on the behaviour of offspring (e.g. Hausberger and Ricard, 2002), with 15 different sires in this study it was impossible to experimentally test the sire effect.

While a somewhat higher prevalence of stereotypical behaviours among the individually stabled horses was anticipated, the relatively high percentage (67%) of individually housed horses with a stereotypy by the end of the 12-week period was unexpected. Surveys in different countries indicated prevalence of horses exhibiting stereotyped behaviours to be between 5 and 15% of the populations studied (e.g. Luescher et al., 1991; McGreevy et al., 1995b). However, these prevalences predominantly refer to older horses who may have developed an established (or

later stage) stereotypy in which the behaviour had become emancipated from its original cause. At this state a change in environment would not bring about a change in the behaviour (Mills and Nankervis, 1999). It appeared that in our study stereotypic behaviour was still reversible at 12 weeks of stabling in a considerable proportion of the horses showing stereotypic behaviour (data not shown). Weaving had the highest incidence in our study. Weaving behaviour involves the repetitive lateral swaying of the head, neck, forequarters and sometimes hindquarters of the horse (McGreevy et al., 1995b; Nicol, 1999) suggested that weaving may be derived from frustrated attempts to gain social contact with other horses. Also Cooper et al. (2000) found increased incidences of weaving when horses were denied social contact with neighbouring horses. Crib biting, the second largest proportion in this study, is thought to reduce arousal in stabled horses (McGreevy and Nicol, 1998). It is likely that the crib-biters in our study also developed this behaviour due to high-arousal levels, as there was no shortage of forage, eliminating the other possible cause of replacing feed intake behaviour.

The average weight gain of the individually stabled horses was significantly lower compared to the pair housed horses (respectively 12.8 kg versus 23.0 kg). This clearly shows that there was no competition for food within the pairs. Only one other study reported data on bodyweight as a result of different types of stabling. Heleski et al. (2002) studied the effect of different housing systems during the weaning period and found after 56 days no differences in increased weight between treatment groups.

The mean resting heart rate in the stable was significantly higher in the individual stabled horses compared to the pair housed (individual 40.1 ± 1.7 versus pair 34.4 ± 1.0 , $P < 0.05$). In the study of Harewood and McGowan (2005) fillies that were stabled for the first time did show a drop in mean heart rate compared to the pasture measurements, but they could not detect a difference between stabling treatment groups. On the other hand, Bagshaw et al. (1994) did detect a comparable increase in mean heart rate for individuals.

In line with behavioural and other physiological measurements, the response in the CRF challenge test in our study did not reveal clear treatment differences. There was no significant difference between individually and pair housed horses in the pre-CRF challenge plasma cortisol levels. The HPA-axis response to CRF challenge caused a significantly higher level of plasma cortisol at 45, 60 and 75 min after CRF administration in pair housed horses compared to individually stabled ones. However, there were no differences between the two housing treatments in the areas under the ACTH and cortisol curves, or in the cortisol/ACTH ratio. Hoffman et al. (1995) performed an ACTH challenge test to assess the weaning stress for individual weanlings and paired weanlings. In this latter study it was found that the pair housed weanlings displayed a higher pre-ACTH plasma cortisol level compared to single foals. Other studies in which plasma cortisol was considered did not include a challenge test, but measured plasma cortisol levels at different times during the day. These studies show variable results. In older horses, Mal et al. (1991b) did not find significant differences between the plasma cortisol levels of horses placed in pasture, confinement or isolation. On the other hand, Houpt et al. (2001) found a marked increase in plasma cortisol levels following initial confinement. Additionally, a study of Alexander and Irvine (1998) showed that plasma cortisol levels of isolation and confinement stress did not always reflect the animal's behavioural responses. Our results confirm this latter result. The behavioural responses of the horses showed marked differences between the treatment groups: individually housed horses, with a high level of confinement and social isolation, showed more vigilant and stress related behaviour than horses housed in pairs. At first glance, these behavioural findings do not seem to correspond with the outcome of the CRF challenge test,

since putatively distressed horses in the individual boxes showed a slightly weakened adrenocortical response compared to the horses in pair housing. However, similar depressions in adrenocortical reactivity to an ACTH challenge tests, relative to group-housed controls, have been observed in bulls subjected to individual confinement on a slatted floor after previous group housing on deep litter (Ladewig and Smidt, 1989), and in socially isolated calves tested 4 weeks after social isolation (Van Reenen et al., 2000). It has been suggested that a depression in adrenocortical reactivity to exogenous ACTH in socially isolated animals is caused by a desensitisation of the hypothalamo-pituitary-adrenal axis (HPA axis) in response to stress induced rises in ACTH and cortisol (Ladewig and Smidt, 1989; Sanchez et al., 1998). A similar mechanisms may also have operated in individually housed horses, and may therefore explain the present results of the CRF challenge test.

In general, there was no effect of housing treatment on the reactivity of the horses during the Novel Object test. Individually housed horses were not found to behave more or less reactive to the Novel Object compared to the pair housed horses. Pair housed horses showed a higher level of locomotion during the test, but this may not be associated with a difference in emotionality or fearfulness since it was not accompanied by any change in the latency time to first contact or the frequency of contact with the umbrella. The number of studies in which the effect of management factors on temperament of horses is studied is very limited. There is some evidence that horses that are being handled more frequently early in life are less reactive in Novel Object situations (Visser et al., 2001).

Significant relations between the responses of horses in the Novel Object test and in the stable were found. Horses showing a high frequency of defecating during the behavioural test, which might reflect fearfulness, were also the ones showing levels of heart rate and heart rate variability in the stable that are likely indicative of stress. On the other hand, putatively bold horses in the Novel Object test, i.e. those exhibiting a high level of contact with the umbrella, appeared to be restless and very active in the stable. Such relationships may help us to interpret responsiveness of horses in terms of stress or reactivity, and may enables us to predict behaviour in one context, for example the stable, based on behaviour in another, for example a standard behavioural test.

In conclusion, this study clearly shows that sudden isolated stabling is stressful to young and naïve horses, resulting in a high prevalence of stereotypies and abnormal behaviours. This study also provided some support for the notion that, in agreement with other species, social stress in horses may be associated with a blunted adrenocortical response to CRF challenge. More work, however, is necessary to validate HPA-axis reactivity tests, as well as the HPA-axis reactivity patterns they produce, as tools for the diagnosis of stress in horses. The finding that responses of horses to a behavioural test are correlated with the frequency of some behaviours in the stable suggests that individual horses exhibit consistent behavioural traits across different contexts, and opens the possibility of using behavioural tests in horses to predict more general underlying behavioural characteristics.

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