THE EFFECT OF EXERCISE DEPRIVATION ON THE BEHAVIOUR AND PHYSIOLOGY OF STRAIGHT STALL CONFINED PREGNANT MARES

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The purpose of this experiment was to investigate the welfare of pregnant mares kept in straight stalls and given only limited exercise, conditions that are similar to those encountered in the pregnant mare urine industry. Sixteen pregnant mares (eight in each of two years) were randomly assigned to two groups: Ex (exercised in a paddock for 30 min per day) or NoEx (exercised for one 30 min period every 14 days). The horses were housed in straight (or 'tie') stalls for six months and had ad libitum *access to grass hay. Each horse's behaviour was recorded on videotape once per week for 24 h. The major behaviours were eating hay, standing, and stand-resting (head down and one hind limb flexed). There was no difference between the behaviours or the number of foot lifts per min of the Ex and NoEx groups in their stalls. Nine of 16 mares were not observed in recumbency throughout the whole of the six-month observation period, suggesting that horses with no previous experience in straight stalls may be reluctant to lie down. Thirteen of 16 mares dropped to their knees at least once, probably when they were REM sleeping while standing. There were no significant differences between the Ex and the NoEx mares in baseline plasma cortisol levels or in cortisol response to ACTH. Following 30 min of exercise, NoEx mares showed an increase in cortisol from 5.0 to 5.4 $\mu g\ dL^{-1}$, whereas Ex mares showed a decrease from 4.6 to 3.6 $\mu g\ dL^{-1}$. The NoEx horses that had been confined for two weeks trotted more (NoEx = 22 [6–38; median and range]% of time; Ex = 2.4 [0–8.7]%) and galloped more (NoEx = 6 [2–8]%; Ex = 0 [0–4]%) than the Ex that were released daily, but walked less (NoEx = 17 [10–26]%; Ex = 35 [20–40]%) and grazed less (NoEx = 0%; Ex = 3 [0–12]%). Confined horses show rebound locomotion — that is, a compensatory increase — when released from confinement, indicating a response to exercise deprivation.*

Keywords: *animal welfare, behaviour, cortisol, exercise restriction, horse, restraint, welfare*

Introduction

There has been considerable attention paid by the public to the condition of the pregnant mares whose urine is used as a source of oestrogen for treatment of postmenopausal women (pregnant mare urine [PMU]). Because the horses are confined in straight stalls (also called 'tie stalls') for variable periods of time without exercise, concern has arisen over their welfare.

Although the American Association of Equine Practitioners has issued a statement that PMU production is humane (King *et al* 1997), animal rights groups and humane organisations continue to declare, both in print (Paulhus 1998) and electronically, that the mares are not treated humanely. A major concern is their housing. The mares are housed in straight stalls to accommodate the urine-collection devices. Although they can walk forward or backward several steps and lie down, they cannot turn around. They may not be released from confinement for some weeks. There are several abstracts (Lamb *et al* 1996; Flannigan & Stookey 1998) and two recent reports (Freeman *et al* 1999; McDonnell *et al* 1999) relating to PMU production, but the behavioural and physiological responses of pregnant mares to confinement in straight stalls without exercise have not been evaluated quantitatively.

The main question to be addressed concerns whether or not there are indications of acute or chronic stress (measured as cortisol level, cortisol response to exogenous ACTH stimulation, and changes in behavioural time budget) in stall-confined mares given the opportunity to exercise only once every 14 days, compared to similarly confined mares given the opportunity to exercise daily. Horses in the PMU industry have restricted access to water (Freeman *et al* 1999). Half of the horses in this study had a similar intermittent schedule. Ancillary questions concern the change in cortisol level as a result of access to exercise, which might be a stressful event after confinement and lack of direct contact with other horses. Hematocrit, plasma osmolality, and plasma protein in mares given access to water only for 5 min every 2 h were compared to those values in mares allowed free access to water, in order to determine whether the mares were dehydrated when water was available only intermittently.

Methods

The study was undertaken over the course of two years. Sixteen mares in the 3rd to 4th month of pregnancy (in two replicates of eight different mares studied in each year: six Belgiums, four Thoroughbreds, two Hanoverians, and one each of Rhinelander, BelgiumWarmblood, Anglo-Trakehner and Belgium/Thoroughbred cross), 14.6 ± 5 years old, and weighing 618 ± 84 kg, were placed in straight (ie 'tie') stalls from October until March. Assignment to exercise (Ex) and non-exercise (NoEx) groups was random in Year 1, but resulted in lighter horses being assigned to the Ex group. In Year 2, the heavier horses were assigned to the Ex group. The prior stalling history of these horses was unknown. Presumably, the lighter horses had not been confined in straight stalls. The straight stall measured 1.2 x 3.6 m, as recommended by the Animal Industry Branch of the Agricultural Services Complex (Anonymous 1990). The stalls were constructed of pipe rail so that the mares had visual, auditory, and olfactory — but not tactile — contact with the other horses. Each year, there were four horses on each side of the aisle of the barn: six of eight horses had horses on each side; two had only one. All faced four other horses (there were additional horses at each end of the aisle). Stalls used for Ex horses in Year 1 were used for NoEx horses in Year 2. The mares were restrained with a halter and lead rope. The lead rope was attached through a ring to a wooden weight, which allowed the horse to move back and forth in the stall without the danger of a slack rope in which she might become entangled. The mares could put their head and neck out of the stall, or could back up to the wall behind them approximately three steps. The concrete flooring was covered with rubber matting, with wood shavings (0.2 m^3 $stall^{-1}$) as bedding. The lights in the barn were left on at all times to facilitate videotaping. Half of the mares (Ex; n = 4 each year) were walked to a paddock and allowed to exercise at will in groups of four for 30 min daily. The others (NoEx; n = 4 each year) remained in their stalls for 14 days and then were allowed to exercise at will in groups of four for 30 min. The group composition remained constant. The mares were weighed every 14 days immediately after the exercise period to determine whether confined mares gained more weight than those exercised daily.

Water was supplied from automatic drinkers. Water was freely available at all times in half of the stalls (F). The other waterers were controlled by a timer such that water was available for 5 min every 2 h (T). The mares could drink as much as they wanted during the 5 min. The waterers held 2.9 kg H_2O and would automatically refill at a rate of 10 kg min^{-1}. Mares had *ad libitum* access to non-legume grass hay and each was fed approximately 1 kg of a 14 per cent protein mixed grain (corn, barley, oats, soybean meal) twice daily (Agway Horse Feed).

The design was a 2 x 2 factorial. There were four groups of four mares each (two mares of each group each year): those exercised daily with free water (ExF), those exercised daily with timed water (ExT), those with free water exercised once every 14 days (NoExF), and those with timed water exercised every 14 days (NoExT).

Physiological tests

Six of the mares in Year 1 and two in Year 2 were blood-sampled (direct venipuncture) on pasture and immediately after being transported to the experimental barn, but before confinement in straight stalls. The other horses were not available for sampling before arrival. They were initially housed in box stalls (3.6 x 3.6 m). Samples were taken both before and after transport and change in housing to validate the measurement of acute stress: Mal *et al* (1991) have shown that movement from pasture to a box stall increases cortisol in horses; it was also important to determine when cortisol fell again to normal before the ACTH stimulation test (see below). During the experiments, all of the mares were blood-sampled by direct venipuncture every four weeks just before the exercise period at approximately 1100h. In addition to the pre-exercise samples, blood samples for cortisol analysis were also taken four times in Year 1 and five times in Year 2 immediately after exercise and weighing to determine whether exercise after a period of restraint causes an increase in cortisol. Cortisol was measured by radioimmunoassay using a commercial kit (Immunlite, Diagnostic Products Corp). This method has been validated for horses (Reimers *et al* 1996). Plasma protein and osmolality were measured. Plasma protein concentration was estimated to within ± 0.1 g dL^{-1} with a hand-held refractometer (Veterinary Refractometer, AO Veterinary Instruments). Plasma osmolality was determined in duplicate on a 2 ml plasma sample using a freezing point osmometer (Osmette A, Precision Systems Inc). This instrument has a precision of ± 0.5 milliosmols kg^{-1} H_2O.

A complete blood count was performed on the samples before confinement and every four weeks just before the exercise period to determine if the white blood counts indicated a stress reaction. Although a rise in cortisol has been used as an indicator of stress (Rushen 1991), another more sensitive test is the response to ACTH (adrenocorticotrophic hormone). A decreased response to ACTH indicates adrenal exhaustion, whereas an elevated response (Terlouw *et al* 1997) indicates adrenal hypertrophy. Before confinement and after six months of confinement, an ACTH stimulation test was performed on 15 mares. A catheter was inserted in the jugular vein the day before the ACTH stimulation test to avoid the stress associated with repeated venipuncture. Blood samples were taken at 1100h immediately before, and then again four hours after, 1 unit kg^{-1} intravenous ACTH administration (Yr 1, when the ACTH gel was not available; n = 8) or 200 units intramuscular ACTH gel administration (Yr 2; Beech 1987; n = 7: one mare had developed extensive ventral oedema after six months of confinement and was not tested to avoid stimulating premature parturition).

Behavioural tests

Each mare was video-recorded once a week for 24 continuous hours. The video-recording machine was in a separate room of the barn. Tapes were changed in the late morning. The tapes were scored using instantaneous behaviour (Martin & Bateson 1986) samples each minute. The behaviours recorded were mutually exclusive; that is, the horse could only be doing one thing at a time. The behaviours recorded were: eat hay; stand rest; stand; eat grain; drink; defecate; groom self; urinate; paw; interact with humans; and lie sternal (recumbent in an upright position with the forelegs flexed under the body). Weave (walk in place and shift body from side to side) and stamp (lift limb and replace in same spot without forward momentum) were also recorded. In addition to the instantaneous sampling, all occurrences of lying down or dropping to the knees were recorded. Every hour, the tape was played back in real time for 30 s and the number of foot-lifts per 30 s was calculated to determine if there was an increase in restlessness or in stall exercise by the NoEx mares in comparison to the Ex mares.

The mares in the NoEx condition were videotaped in real time for 30 min when they were released into a paddock after two weeks of stall confinement. The mares in the Ex condition were also videotaped during their regular daily turnout on the same day and in the same paddock as the NoEx mares. The groups were released separately. The time spent standing, grazing (the paddock contained no grass, but the mares put their heads down and foraged, sometimes ingesting weeds, leaves, dirt, snow or faeces), walking, trotting, galloping, grooming, and rolling was calculated. Grooming (both auto- and allo-grooming) was combined with rolling as 'comfort behaviour'. Footlifts were measured in real time and expressed as median min^{-1}. These figures were used to compare the behaviour of exercise-restricted horses with that of exercised horses to determine if there was a compensatory increase in locomotion or other differences between the two groups.

Statistics

The data appeared to be skewed, sample sizes were small, and within-mare patterns through time were inconsistent. Therefore, non-parametric (rank-based) analyses (including secondary analyses stratified on potential sources of variation) were used to test differences between groups (based on each mare's median across time, unless otherwise specified; Siegel 1956). For each behaviour, the percentage of time each mare spent performing each behaviour across all study-weeks combined was calculated; these percentages were used in the statistical tests (rank-sum or Kruskal-Wallis tests). The median blood value for each mare across all sampling times was calculated for each treatment group and condition; these values were used in the statistical tests. Significance was interpreted at $P \leq 0.05$. Unless year explicitly was a stratification factor, data were combined across years for analyses.

Results

All values are given as medians (range).

Timed versus free water

There were significant effects of timed (T) versus free (F) water availability on red blood cell count, hemoglobin, hematocrit, and plasma protein (Table 1). The red-blood-cell count was significantly higher in F (7.6 [6.3–9.5] millions μL^{-1}) than T horses (7.1 [5.3–8.7] millions μL^{-1}; $P < 0.002$). Hematocrit and hemoglobin were also significantly higher in F horses. There was a significant interaction between exercise status and water status ($P < 0.001$). NoExT horses had a lower red blood cell count than all other groups (Table 1). Plasma protein was higher in the T horses (6.8 [6.1–8.7]) than in the F horses (6.6 [5.9–8.0]; $P < 0.02$). There were no significant differences among groups in total white blood cell count, number of lymphocytes or ratio of neutrophils to lymphocytes.

Table 1 Effect of exercise and water status on blood count and fluid balance (median [minimum–maximum]).

	Hematocrit %	Hemoglobin g/μL	Red blood cells M/μL	Total Protein g/dL	Osmolality milliosmol/kg
ExF	39[a] (31–43)	13.9 (11.4–15.5)	7.5 (6.3–9.1)	6.8 (6.0–7.3)	285 (277–291)
ExT	35[a] (32–41)	12.5 (11.2–14.5)	7.5 (6.6–8.7)	6.8 (6.1–8.7)	286 (249–292)
NoExF	37[a] (32–45)	13.1 (10.9–16.5)	7.8 (6.5–9.5)	6.4 (5.9–8.0)	286 (256–292)
NoExT	34[b] (28–41)	12.0 (10.0–14.3)	6.7 (5.3–8.5)	6.9 (6.2–7.5)	284 (263–290)

[a,b] Medians with different superscripts are significantly different.
ExF = exercised daily, free water (continuous access).
ExT = exercised daily, water available every 2 h for 5 min.
NoExT = confined for 14 days, water available every 2 h for 5 min.
NoExF = confined for 14 days, free water (continuous access).

Cortisol

There was a significant rise ($P < 0.02$) in cortisol corresponding with the move from pasture (5.1 [2.1–11.2] $\mu g\ dL^{-1}$) to box stall (8.9 [3.5–23] $\mu g\ dL^{-1}$). Within two weeks, cortisol had fallen (to 5.3 [3.8–12] $\mu g\ dL^{-1}$) and remained low (pre-exercise) in both Ex and NoEx horses (Table 2).

Table 2 Cortisol (μg/dL) responses of pregnant mares to ACTH and exercise (median and minimum–maximum).

Exercise Group	Condition	Pre-ACTH	Post-ACTH
Ex	ACTH	5.3 (3.3–9.5)	10.9 (8.3–23.8)
NoEx		4.5 (3.4–8.4)	12.3 (7.8–22.4)
		Pre-exercise	*Post-exercise*
Ex	Exercise	4.6 (1.4–11.2)	3.6[a] (0.74–12.6)
NoEx		5.0 (2.4–13.0)	5.4 (.9–14.1)

[a] The decrease for Ex mares between pre-exercise and post-exercise cortisol differs significantly from the increase seen in NoEx mares ($P < 0.05$).
Ex: turned out for 30 min every day
NoEx: turned out for 30 min once every 14 days

There was no sign of either adrenal exhaustion or hypertrophy. The Ex and NoEx horses showed almost identical responses to ACTH stimulation (an increase of 5 to 6 $\mu g\ dL^{-1}$) at both the beginning and the end of the six-month confinement period (medians shown in

Table 2). There were no differences in the median responses in the ACTH stimulation test between Ex (6.2 [4.3–14.4] μg dL^{-1}) and NoEx (5.6 [3.6–14.0] μg dL^{-1}) horses.

Preliminary analysis showed that there was no consistent pattern through time between each mare's pre-exercise and post-exercise cortisol levels even when examined within year or treatment group (data not shown). Therefore, modelling the patterns of change through time (eg as slope) was considered unreasonable. However, the nine tests stratified on study-year and sampling-month showed that the NoEx (by inspection) had greater pre-exercise to post-exercise differences than Ex mares on seven out of nine tests ($P < 0.08$). Each mare's differences were summarised as the median of her differences and both years were combined.

Cortisol decreased in the Ex mares (–0.84 [–3.4–4.1] μg dL^{-1}) from before to after exercise, but increased (0.39 [02.2–6.6] μg dL^{-1}) in the NoEx mares ($P < 0.05$). The differences were small because of the variability among mares.

Behaviour in the straight stall

Each mare was video-recorded for at least 17 nights, during which time nine of 16 horses never lay down. Two of the Ex horses and five of the NoEx horses were observed to lie down in the straight stalls. Thirteen of the 16 horses dropped to their knees in the stalls (usually during the night); their knees buckled, and they fell on their knees or sometimes onto their chests.

There were no differences in most behaviours between the two groups (Ex and NoEx). The behaviours of the mares in straight stalls are given in Table 3 for both years combined. The only significant behavioural difference was that Ex horses spent more time drinking (0.62 %) than the NoEx horses (0.48 %). In the straight stalls, there were no differences in forefoot lifts (0.95 per 30 s by Ex horses and 0.85 per 30 s by NoEx horses [$P = 0.1$]). In all cases, the horses moved their forefeet more frequently than their hind feet.

Table 3 **Behaviour of pregnant mares in straight stalls: median percent of time (and minimum–maximum).**

Behaviour	Exercise	No Exercise	Rank-sum test *P* (1-tailed)
Eat Hay	47 (38–54)	42 (39–51)	0.38
Stand Rest	39 (34–50)	38 (34–48)	0.42
Stand	7.4 (4.9–15.2)	11.1 (7.6–150)	0.09
Eat Grain	1.2	1.4	0.30
Drink	0.6 (0.4–1.2)	0.5 (0.07–0.6)	0.01
Defecate	0.1 (0–0.22)	0.1 (0.07–0.2)	0.12
Groom Self	0.1 (0–0.8)	0.1 (0–0.8)	0.50
Urinate	0.08 (0–0.15)	0.07 (0–0.14)	0.12
Paw	0.04 (0–0.9)	0 (0–0.1)	0.05
Interact With Humans	0 (0–0.4)	0.1 (0–0.4)	0.32
Lie Sternal	0 (0–2.3)	0 (0–7.8)	0.27

There were differences between the two study years (data not shown). The horses in Year 2 groomed themselves more often ($P < 0.02$), and they stood more ($P < 0.05$), but spent less time eating grain ($P < 0.0008$). In addition, people were interacting with the horses more often in Year 2 ($P < 0.02$).

One horse in the Ex group had a history of stereotypic behaviour when she arrived and she continued to weave, spending 13 per cent of her time in this behaviour. Another exercised horse spent 0.2 per cent of her time weaving. None of the NoEx mares showed any stereotypic behaviour.

Behaviour in the paddock

The behaviours of the horses in the paddock are shown in Table 4. The paddock provided an opportunity for social interaction as well as exercise, and some foraging although the paddock contained no grass. There was a difference in behaviour between horses that had been confined for the two previous weeks (NoEx) and those that were turned out daily (Ex). During the 30 min period, the NoEx horses trotted more. (There was a year effect in that the NoEx trotted more in the first year than in the second [$P < 0.005$]). The NoEx horses also galloped more and walked less in the paddock. NoEx horses exhibited a significantly greater number of foot lifts than Ex horses (NoEx 20.6 [4.9–99.2] min^{-1}; Ex 13.6 [1.7–50.1] min^{-1}; [$P < 0.0003$]).

Table 4 Behaviour of pregnant mares in a paddock (median percent of time and minimum–maximum).

Behaviour	Exercise	No Exercise	Rank-sum test *P* (1-tailed)
Stand	39 (31–49)	32 (21–44)	0.17
Walk	35 (20–40)	17 (10–26)	0.001
Trot	2.4 (0–8.7)	22 (6–38)	0.001
Gallop	0 (0–4)	6 (2–8)	0.001
Comfort	3.2 (0–19)	3.5 (0–5.7)	0.1
Graze	3 (0–12)	0 (0–0)	0.03

Physical findings

The NoEx mares gained 13.7 per cent body weight during the six months and the Ex mares gained 9.7 per cent body weight, but the difference was not significant. The mares of Year 1 (n = 8) were examined after six months of confinement by an equine medicine clinician (Dr D Ainsworth), who was unaware of the horses' exercise status; all had some limb oedema. There was a greater frequency of moderate or extensive oedema in the NoEx mares (see Table 5). In Year 2, one non-exercised horse had to be released into a box stall two weeks before the end of the experiment because of the extent of ventral oedema. Two of the other three NoEx horses and one Ex horse also had ventral oedema. This is not a normal condition of late pregnant mares. The usual treatment is to increase exercise. Although the mares had limb oedema earlier, the ventral oedema did not appear until the end of the study.

Table 5 Effect of exercise restriction on moderate to extensive oedema formation in pregnant mares.

	No Exercise	Exercise
Year 1	3 oedema	1 oedema
Year 2	3 oedema	1 oedema

Discussion

Perhaps the most interesting result is that the confined mares were not physiologically stressed and did not develop stereotypic behaviour, although previously acquired stereotypies were exhibited. The presence of *ad libitum* forage probably is responsible for the normal behavioural time budget and lack of cortisol elevation. McGreevy *et al* (1995) have noted that the incidence of stereotypies in horses is lower in those that have more access to forage. Flannigan and Stookey (1998) surveyed PMU farms and found that horses given limited amounts of hay had a higher incidence of stereotypic behaviour than those with *ad libitum* access to hay. Sows in farrowing crates, which also prevent the animal from turning around, have a lower rate of bar chewing and other stereotypies if roughage is made available (Terlouw *et al* 1997).

The novel finding is that some of these older horses were never observed to lie down. This was not a function of stall size because the largest draft mares did lie down. It may be an experiential factor. Horses that have not been housed in straight stalls when young may be reluctant to lie down when first introduced to them at age ten or greater. Because the horses had been donated to Cornell University, their early history was unknown. McDonnell *et al* (1999) reported that all the mares on the PMU farm they studied did lie down, but these were probably younger horses that had learned to lie down in a narrow space.

The fact that nine of 16 horses were not seen lying down is unusual because horses usually do lie down at some time during each 24 h period. Horses in metabolism crates spent four per cent of their time lying down (Willard *et al* 1977), whereas pregnant mares in box stalls spent six per cent of the night lying down (Shaw *et al* 1988). In the present study, no electroencephalographic recordings were taken, but Ruckebusch (1972) found that during a 24 h period, nine per cent of the time (2 h) was spent in slow-wave sleep and three per cent (45 min) in rapid eye movement (REM, or 'paradoxical') sleep. All REM sleep and most slow-wave sleep occurred while the horses were recumbent. Although Ruckebusch (1975) found that horses in a new environment would not lie down on the first night, our horses did not lie down for months. One hundred and eighty days without recumbency or REM sleep would be expected to have a deleterious effect, but we noted neither physiological nor behavioural differences between those horses that lay down and those that did not.

The behaviour of collapsing onto the knees resembled that seen in narcoleptic horses (Sweeney & Hansen 1987). In our study, the collapse is apparently caused by sleep deprivation. The horses probably enter REM sleep, which results in complete muscle relaxation — therefore, they collapse. It is interesting to us that this happens and that, in some cases, deprivation of lying for up to three months was necessary before the horses collapsed.

Cortisol levels increased with an acute change in housing, but not with chronic restraint. The lack of difference in white blood cell numbers and ratio also indicated the lack of a stress response. There were differences between Ex and NoEx mares in response to exercise. NoEx mares exhibited an increase in cortisol after exercise. Cortisol did not rise to levels that indicate stress (ie a doubling of the level at pasture [Rushen 1991]). Nevertheless, the NoEx mares were affected physiologically by the 'novel' experience of exercise and both positive and negative social interactions such as allo-grooming and fighting. Galloping and trotting alone may have led to the increased cortisol in these unfit mares (Church *et al* 1987). Lamb *et al* (1996) had also noted that exercise increased urinary cortisol levels in PMU mares.

The NoEx horses were more active than Ex horses when released into a paddock. These results indicate that the NoEx horses were showing compensatory locomotor activity when released. A similar result was found when calves that had been confined in crates were released onto pasture (Dellmeier *et al* 1990). Apparently, there is a motivation to exercise that is greater after 14 days of confinement than after 23 h of confinement. The more severe oedema in NoEx mares indicates that lack of exercise may compromise fluid dynamics when mares are in late pregnancy.

There were few other differences in behaviour between the two groups in the stable. Straight-stall confinement and lack of the opportunity to exercise had little effect on the behaviour of these pregnant mares. The exercised horses probably pawed in anticipation of their daily release.

Horses that exercised for 30 min per day would have a greater demand for water because they were probably losing more water as sweat, even in these relatively cool conditions. NoEx horses would only experience an increased water-demand on one day every two weeks. The scheduling of water delivery was relatively frequent and the amount of water that each mare could drink was large (50 L per 5 min); therefore, the mares showed no signs of physiological dehydration nor marked differences in behaviour. McDonnell *et al* (1999) also found no differences in behaviour between intermittently and continuously watered horses. The horses with freely available water had higher hematocrits and plasma protein. The effects of more severe water restriction on mares are discussed in Houpt *et al* (2000).

There was an increase in cortisol levels when the horses were moved from pasture to the barn. This is similar to the findings of Mal *et al* (1991) that showed increased cortisol when horses were moved from pasture to stall, and those of Smith *et al* (1996) showing increased cortisol when horses were transported. This validated our method of measuring physiological stress. Our results indicate that a change in environment is stressful to horses but that they quickly habituate. This is similar to the physiological reactions of calves placed in veal crates (Stull & McDonough 1994). Freeman *et al* (1999) also reported no increase in cortisol in confined mares under PMU conditions.

There was no difference in response to ACTH after six months of confinement. This finding is in contrast to that of Ladewig and Smidt (1998), who found that the cortisol response to ACTH decreased in bulls after five weeks of tethering. There appears to be a species-specific difference between bulls and mares in response to tethering (restraint); alternatively, the differences may be attributable to details of the imposed restraint and the more frequent measurement of cortisol in the cattle study.

Mares that have been confined for two weeks without exercise in a straight stall tended to show more fluid accumulation than horses allowed to exercise daily, and also exhibited compensatory locomotion when first released from confinement. There was no physiological indication of stress, and no new abnormal behaviours developed for the first time in confined mares. Some mares would not lie down in the straight stalls and fell to their knees occasionally. An amount of exercise sufficient to prevent fluid accumulation — especially late in pregnancy — should be provided. Thirty min day^{-1} was not enough to prevent limb oedema. Longer periods of release, forced exercise or a stall that allowed the mares to walk might prevent this. In the presence of *ad libitum* hay, pregnant mares do not appear stressed behaviourally or physiologically by straight-stall confinement, but do show compensatory locomotion when released.

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