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The Effect of Living Conditions on Stress and Behavior of Horses

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ABSTRACT

Providing an adequate environment for horses is important to minimize the level of stress for domesticated horses. The objectives of this study were 1) to evaluate the effect of living conditions on stress level of horses, 2) to observe the effect of one month confinement on self-maintenance behavior and stereotypic behavior of horses. The experiment was conducted at National Institute of Animal Science, Equine Field Station (Seonghwan-eup, Korea). Horses were staying in the paddock prior to the experiment. On day 1, five horses were randomly selected and housed in metal fence panels stall. Six horses remained in the same paddock. The ratio of neutrophil to lymphocyte (on day 15) and cortisol (on day 1 and 29) from stalled horses were significantly higher than horses in the paddock. Duration or frequency of self-maintenance behaviors such as feeding, drinking, resting, walking was not significantly different between day 1 and day 29. However, the frequency of urination significantly decreased ($p<0.05$) on day 29 compared with day 1. The frequency of stereotypic behaviors was not different between day 1 and 29. Our data indicate that horses may be more stabled when they are staying in the paddock rather than staying in the stall, but the stress level of horses in the stall during one month confinement was not effective for horses to adapt stereotypic behavior. In conclusion, providing an adequate environment and stress-less horse management techniques can minimize the stress level of horses.

(**Key words :** Horses, Stress, Behavior, Stall, Paddock)

INTRODUCTION

Providing stress free living conditions is critical to minimizing environmental stressors for domesticated horses. Previous studies have suggested that the level of stress and type of environmental stressors horses are exposed to can be determined based on the type (Dallaire, 1986) and design of living conditions (Cooper et al., 2000). Severe weather, physical confinement, fear, and social isolation are considered environmental stressors for animals (Mason et al., 2007). Few studies have evaluated the effects of living conditions on stabilization of horses (Cooper et al., 2000; Dallaire, 1986). A study conducted to compare the resting behavior of horses housed in stalls and pastures (Dallaire, 1986) revealed that horses in separate stalls had slightly more total sleep (4% REM sleep and 13% slow-wave sleep) than those kept at pasture (2% REM sleep and 11% slow-wave sleep) (Dallaire, 1986). Additionally, horses kept in the stall (8%) showed less drowsiness than those at pasture (13%). Although the results of this study showed different patterns of resting behavior between living conditions, the difference in stress levels among environments was not elucidated. Stereotypic behaviors are physical movements that are repetitive, invariant and functionless (Mason, 1991), such as weaving, wind-sucking, stall walking, and pawing (McBride and Cuddelford, 2001). Such behavior may cause health problems in horses, reducing their value. In other spices, it has been suggested that the main cause of stereotypic behavior is living conditions (Morgan, 1973 Odberg, 1986). Cooper and coworkers (2000) demonstrated that the incidence of stereotypic behaviors significantly decreased when horses were housed in stalls with increasing visual horizons, indicating that providing adequate living conditions is important to minimize stress for horses (Cooper et al., 2000). Taken together, these studies suggest that stabilization of horses is strongly dependent on their living conditions. Thus, it is essential to conduct research to provide information about stress free environments and horse management techniques to the horse industry.

The effects of prolonged exposure to the stable on

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adapting stereotypic behavior and the level of stress have yet to be elucidated. Moreover, it is still necessary to identify stress free housing conditions among stalls, paddocks, and pastures in different environments because preferred housing conditions may vary depending on environment. Therefore, this study was conducted 1) to evaluate the effects of living conditions on horse stress levels, 2) to observe the effects of one month of confinement on self-maintenance behavior and stereotypic behavior of horses. The results of this study may be applied to advance horse management techniques for use in further development of the horse industry in Korea.

MATERIALS AND METHODS

1. Horses and Study Design

The experiment was conducted at National Institute of Animal Science, Equine Field Station (Seonghwan-eup, Chungcheongnam-do, Korea) from Oct. 23, 2012 to Nov. 23, 2012. A total of eleven horses were used in this experiment. The breeds of the horses were Jeju, Thoroughbred, and Apaloosa (Table 1). Horses were fed a mixture of forage [Italian ryegrass (30%), tall fescue (40%), and orchard grass (30%)] on an ad libitum basis. Additionally, a concentrate [crude protein (11%), crude fat (3%), crude fiber (15%), crude ash (10%), calcium (0.6%), phosphorus (1%)] was provided at approximately 1.5% body weight twice daily (09:00~09:30, 17:30~18:00). Throughout the experiment, horses had free access to an automatic water system.

Prior to the experimental period, horses were placed in an approximately 7,332 m^2 paddock. On day 1, five horses were randomly selected and housed in approximately 345 cm (width) × 306 cm (length) metal fence panels stalls, while six horses remained in the paddock. The assigned stall allowed horses to view the neighboring horses and engage in social behaviors. Additionally, the stall was bedded with shavings (pine sawdust) and the feces and contaminated bedding were removed daily. The stall group of horses was exercised daily on the round field ground from 17:00 for 30 min during the experimental period.

2. Blood collection

Blood was collected from the jugular vein into a glass tube using the vacutainer technique (BD Vacutainer, Serum, REF 367820, BD Franklin Lakes, NJ, USA) at 16:00, prior to exercise, on days 0, 1, 2, 3, 10, 15, 20, and 29. During blood collection, horses were gently restrained with a halter and moved to the laboratory to draw blood samples. Extra care was taken to minimize possible stress from the procedure. The whole blood sample was used to count neutrophils and lymphocytes (N:L). After centrifugation of the whole blood sample at 3,000 rpm for 10 min, the plasma was collected and frozen at −20°C for subsequent cortisol analysis.

3. Stress level analysis

As parameters for stress level, the ratio of N : L orthe cortisol levels was used to determine the horse stress level. The counting of N:L in the horse blood were performed using an automated veterinary analyzer (Vet Scan HM5,

Table 1. The basic information of horse by group of living condition, breed, sex, age and weight

Group	Horse ID	Breed	Sex	Age (year)	Weight (Kg)	
					Day 0	Day 29
Stall	S1	Appaloosa	Gelding	12	450	428.2
	S2	Thoroughbred	Gelding	12	541.7	536.2
	S3	Jeju-horse	Gelding	7	420.7	421.4
	S4	Jeju-horse	Mare	11	433.9	371.8
	S5	Jeju-horse	Colt	1	198	217.7
Paddock	P1	Jeju-horse	Mare	6	465.9	452.2
	P2	Jeju-horse	Gelding	7	448.9	431
	P3	Jeju-horse	Mare	8	372.1	346.9
	P4	Jeju-horse	Mare	1	204.6	245.2
	P5	Jeju-horse	Mare	12	344.5	345.1
	P6	Jeju-horse	Mare	1	180.1	204.2

Abaxis Europe, Darmstadt, Germany). Cortisol levels were evaluated using an Immulite 1000 Cortisol competitive chemiluminence enzyme immunoassay on an Immulite 1000 Analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA).

4. Observation of horse behavior

Behavior of stalled horses was video recorded on day 1 and 29 using a camera (RSC-34P, RDS, Korea) placed on a pole in each stall. The angle of the camera was wide enough to observe the behavior of the horses at any place in the stall. Upon completion of the experiment, the video was reviewed and behavior was analyzed by five well-trained observers. Behavior was observed from 08:00 to 16:00 each experimental day. As self-maintenance behavior, 1) feeding, 2) drinking, 3) resting (standing and lying), 4) walking, 5) playing, and 6) eliminative behavior (defecation, and urination) were observed, while 1) pawing, 2) kicking, 3) weaving, and 4) cribbing behaviors were observed as stereotypic behaviors. The duration of behaviors was recorded using the momentary time sampling method in every minute and observed for feeding, drinking, resting, and walking behaviors. The frequency of behavior was recorded for playing, eliminating, pawing, kicking, weaving, and cribbing. Each type of behavior was recorded on the event recorder sheet and transferred to a computer for further analysis.

5. Statistical analysis

The ratio of N : L and the cortisol levels were statistically analyzed using repeated measures ANOVA (Proc ANOVA; SAS 9.2, SAS Institute Inc., 2009). The model for the ratio of N : L was:

$$y_{ijkl} = \mu + DL_i + stage_j + sex_k + e_{ijkl}$$

where, μ is the overall population mean, DL_i is the fixed effect of the i^{th} date of measurement and living condition, stage is the j^{th} stage of age (1 = young horses (younger than 1 year), 2 = adult horses (older than 6 years)), sex is the k^{th} sex categories (1 = gelding, 2 = mare), y_{ijkl} is the l^{th} observation on the i^{th} date of measurement and living condition in the j^{th} stage of age with k^{th} sex categories, and e_{ijkl} is the random error. Significant differences ($p < 0.05$) between the level of DL, stage of age and sex were identified using the PDIFF option in SAS. However, there were no significant differences in the cortisol levels ($p > 0.05$) by stage of age and sex; thus, these factors were excluded from the model. Significant differences in the frequency of stereotypic behavior between day 1 and day 29 were identified using a paired t-test.

RESULTS

1. Effects of living condition on stress levels of horses

The N : L ratio and plasma cortisol levels were used to determine the stress level of horses in paddocks and stalls on day 0 (before separation), 1, 2, 3, 10, 15, 20 and 29 (Fig. 1 and 2). The N : L ratio on day 15 was significantly higher (p=0.0013) in the stalled group than in the paddock group (Fig. 1). The N:L ratio appears to be higher in stalled

Table 2. Self-maintenance behaviors of stalled horses

Types of behavior	S1		S2		S3		S4		S5		Mean ± S.E.M	
	D1	D29	D1	D29	D1	D29	D1	D29	D1	D29	D1	D29
Feeding (min.)	181	190	222	252	176	256	241	250	239	323	211.8 ± 14.01^a (44%)	254.2 ± 21.07^a (53%)
Drinking (min.)	40	9	9	9	13	4	25	4	21	12	21.6 ± 5.4^a (5%)	7.6 ± 1.57^a (2%)
Standing (min.)	210	274	228	153	266	218	172	151	150	77	205.2 ± 20.48^a (43%)	174.6 ± 33.40^a (36%)
Lying (min.)	0	0	17	66	17	0	39	58	64	68	27.4 ± 11.05^a (7%)	38.4 ± 15.77^a (8%)
Walking (min.)	10	2	5	1	5	1	4	18	7	1	6.2 ± 1.07^a (1%)	4.6 ± 3.36^a (1%)
Playing (freq.)	1	3	4	5	15	7	0	2	0	0	4 ± 2.85^a	3.4 ± 1.21^a
Urinating (freq.)	3	0	5	1	3	2	2	1	7	3	4 ± 0.89^a	1.4 ± 0.51^b
Defecating (freq.)	3	4	5	6	5	6	4	3	9	6	5.2 ± 1.02^a	5 ± 0.63^a

[a-b] Values within the raw with different superscipts are significantly different (p<0.05).

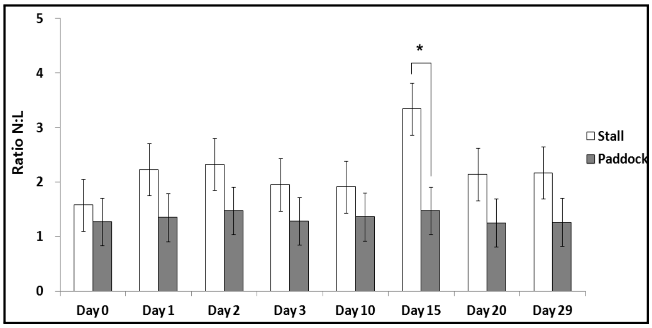

Fig. 1. Changes in the ratio N:L in horses stayed in the stall (n=5, white-bar) and in paddock (n=6, grey-bar). Bars represent lsmean of ratio N:L in each group. On Day 0, all horses were staying in the paddock, and randomly selected 5 horses were housed in the stall from Day 1 for 29 days. The asterisk indicates the level of statistical significance (P<0.05).

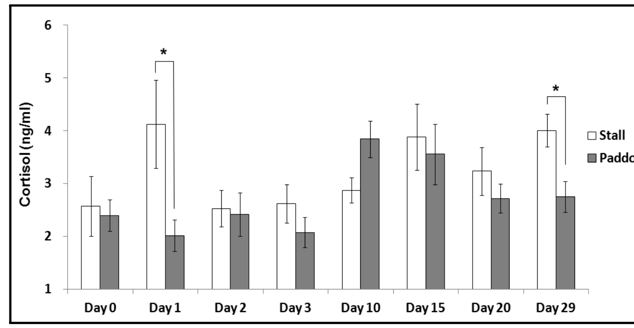

Fig. 2. Changes in cortisol from horses stayed in the stall (n=5, white-bar) and in paddock (n=6, grey-bar). Bars represent mean of cortisol (ng/ml) in each group. On Day 0, all horses were staying in the paddock and randomly selected 5 horses were housed in the stall from Day 1 for 29 days. The asterisk indicates the level of statistical significance (P<0.05).

horses on day 1 (p=0.06) and day 29 (p=0.0093), but the difference was not statistically significant.

Similar results were observed when the cortisol levels between the two living conditions were compared (Fig. 2). Observation of cortisol level on each sampling day indicated that the levels were higher in the stalled group on day 1 (p=0.009) and day 29 (p=0.0416).

2. Effect of one month of confinement on behavior of horses

Changes in self-maintenance and stereotypic behaviors of horses in the stall groups were examined in the stalled group for one month. On day 1, the proportion of time budgeted for feeding, drinking, standing, lying and walking was 44%, 5%, 43%, 7% and 1%, while on day 29, these values were 53%, 2%, 36%, 1% and 1%, respectively (Table 2). No significant differences in the duration of feeding, drinking, standing, lying, and walking behavior were observed after one month of confinement. On day 1, the mean incidences of playing, defecating, and urinating behavior were 4±2.85, 4±0.89 and 5.2±1.02 times during 480 min of observation, while on day 29, these values were 3.4 ± 1.21, 1.4 ± 0.51, and 5±0.63 times during 480 min of observation, respectively (Table 2). The frequency of playing and defecating behavior did not differ significantly between day 1 and day 29, however, the frequency of urination decreased significantly on day 29 when compared with day 1 (p<0.05) (Table 2). Moreover, the mean incidence of pawing was 0.6 ± 0.4 and 2.8 ± 1.71 on day 1 and 29, respectively, but this difference was not significant (Table 3). No kicking or weaving was observed on day 1 or day 29 of confinement.

DISCUSSION

In this study, the N : L ratio and cortisol from horses in the paddock were lower than those of horses in stalls, suggesting that horses may be more stable when housed in a paddock than a stall. These findings are comparable to those

Table 3. Stereotypic behavior of stalled horses

Types of behavior	S1		S2		S3		S4		S5		Mean ± S.E.M	
	D1	D29	D1	D29	D1	D29	D1	D29	D1	D29	D1	D29
Cribbing (freq.)	0	0	1	0	0	0	0	0	0	0	0.2±0.2 [a]	0 [a]
Weaving (freq.)	0	0	0	0	0	0	0	0	0	0	0 [a]	0 [a]
Kicking (freq.)	0	0	0	0	0	0	0	0	0	0	0 [a]	0 [a]
Pawing (freq.)	1	0	2	0	0	9	0	4	0	1	0.6±0.4 [a]	2.8±1.71 [a]

[a] Values within the raw with samesupersciptisnot significantly different (p>0.05).

of a previous study showing that horses in a pasture had less sleep than those in a stall because they required high levels of exposure to the external environment (Dallaire, 1986). However, the results of the present study suggest that being exposed to the external environment may not affect the overall stress levels of horses in the paddock. Generally, confinement of horses in a stall limits their movement, field of view, and social activity. In this study, the specific stress factor for stalled horses was not clearly defined. However, the limitation of movement was likely the main stressor for horses in the stall since the design of the stall used in this study did not limit the field of view and allowed horses to engage in social behaviors through the pole between stalls.

Interestingly, the date at which a significant difference in N : L ratio and cortisol levels was observed was not the same for the paddock and stall. For the cortisol levels, horses in the stall showed a significantly higher level of cortisol on day 1 and day 29 when compared with paddock group, but the N : L ratio in the stalled group was significantly higher than in the paddock group on day 15. This difference may have occurred because the stress parameters have different release mechanisms and half-lives. Cortisol is involved in stress response (Mostl and Palme, 2002), and its levels in plasma of horses increase during stressful situations such as exercise or transportation (Stull and Rodiek, 2000). However, immediately after the stressors disappear, the level of cortisol decreases dramatically because of its short half-life of 1 to 1.5 h (Lassourd et al., 1996; Stull and Rodiek, 2000). The N : L ratio may respond to stressors more slowly than cortisol because elevation of N : L ratio is followed by a cortisol release. Since elevation of the N : L ratio appears to be due to the long-term effects of stressors on cortisol secretion, the N : L ratio has been suggested as the more reliable parameter (Stull and Rodiek, 2000). The different release mechanisms of both hormones suggests that the N:L ratio may be the optimal parameter for monitoring of stress levels of horses in response to environmental stressors over long term periods. On day 1, the cortisol levels of confined horses were dramatically elevated, however, they decreased to the baseline level on day 2. This hormonal change may have been due to a sudden change of living conditions from the paddock to stall. Indeed, a previous study indicated that the level of glucocortocosteroids could be elevated in response to unpredicted environmental change (Wingfield and Kitaysky, 2002).

For healthy horses, the normal range of the N : L ratio is 0.8~2.8 (Morris and Large, 1990). Previous investigations monitoring the stress levels of horses during 24 h of transportation showed that the N:L ratios during the transportation and 24 h-post transportation were 10 ± 3.6 and 3.2 ± 0.5, respectively, while the ratio of stabled horses before transportation was 1.7 ± 0.5 (Stull and Rodiek, 2000). In the present study, the range of N : L ratios of horses in the paddock (1.25~1.57) was within the normal range for healthy horses. However, the range of N : L ratios from horses in the stall (1.91~3.34) was outside the normal range, suggesting that horses in the stall were exposed to an environmental stressor. Since the level of feed consumed was not monitored in this study, it is not possible to determine if there was a correlation between feed intake and N:L ratios of horses under different living conditions. Nevertheless, previous studies have indicated that changes in quality and quantity of concentrate had no influence on leukocyte count or hematocrit levels in mares (Rupic et al., 2001), suggesting that the significant difference in the N : L ratio is not due to the difference in feed intake.

In the present study, 29 days of horse confinement did not change the length of feeding, drinking, standing, lying, and walking, nor the frequency of playing and defecating. Interestingly, the frequency of urinating was significantly lower on day 29 than on day 1. This change may have occurred because horses spent more time drinking water on day 1 (21.6 ± 5.4 min) than on day 29 (7.6 ± 1.57 min). This behavioral change appears to have been due to a change in stall temperature during the experimental period. Specifically, the temperature of the stall at noon on day 1 and day 29 was 16°C and 6°C, respectively. It should be noted that the behavior of horses in the stalls and paddock were not compared because the camera in the paddock was not set up properly to monitor behavior of horses throughout the area. Thus further studies with a longer experimental period and no weather effect that enable comparison of behaviors between the stalls and paddock are warranted to fully understand horse maintenance behavior under different living conditions.

In this study, although stalled horses showed higher stress levels than those in a paddock, stall confinement for 29 days did not increase the incidence of stereotypic behaviors. This may have been because the stress level was not high enough to induce horses to adopt stereotypic behaviors. During the experiment, horses exercised regularly for 30 min per day. Exercising horses for at least 30 min per day is a widely used technique to maintain horse body condition when they are stalled. The design of stalls used in this study may also have been a critical factor preventing a dramatic increase in stress level and the incidence of stereotypic behaviors of horses during confinement. Stalled horses in this study were allowed to view external environments without any blockage. Additionally, since each stall was divided with three level poles, horses were able to engage in social interactions with horses in neighboring stalls. A previous study showed that increasing the visual horizons of stabled horses by changing the view outside of the stall and/or horses in the neighbor decreased the incidence of weaving of horses (Cooper et al., 2000). It is also possible that confinement for one month is not sufficient for horses to adapt stereotypic behavior. Thus exercising horses for 30 min daily and providing a stall with an open design may inhibit elevation of stress levels during one month of confinement.

In conclusion, providing an adequate environment and stress free horse management techniques can minimize stress levels in horses. The results of this study should be applied to development of horse management techniques used at horse farms and riding and breeding facilities.

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