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Behavior and physiologic responses of mares to short-term isolation

Sarah C. Strand[a], Stefan Tiefenbacher[b], Melissa Haskell[a], Trina Hosmer[c], Sue M. McDonnell[d], Douglas A. Freeman[a,*]

[a]*Department of Veterinary and Animal Sciences, University of Massachusetts at Amherst, Amherst, MA 01003, USA*

[b]*New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772, USA*

[c]*Data Analysis Group, Lederle Graduate Research Center, University of Massachusetts at Amherst, Amherst, MA 01003, USA*

[d]*Equine Behavior Lab, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348, USA*

Abstract

The aim of this study was to evaluate the behavior and physiologic responses of mares to removal from an established pasture herd and to isolation in a pasture setting for 6 h (Group I, $n = 5$). Responses of mares in Group I were compared to mares that were transported and returned to the herd (Group T, $n = 5$) and to mares moved to the isolation pasture with a companion (Group C, $n = 5$). Behavior was recorded continuously for 6 h on the day before the isolation procedures (baseline, Day 0) and again on the day of the procedure (test, Day 1). Plasma cortisol, white blood cell count (WBC), neutrophil:lymphocyte ratio (N:L), and hematocrit (HCT) were measured once on Day 0 (a.m.) and twice on Day 1 (a.m. and p.m.). Heart rate (HR) was monitored continuously during Day 0 and Day 1. Intradermal response to phytohemagglutinin (PHA) injection was measured 18 h following injection, which was administered at the end of Day 1.

Average time spent standing alert increased ($P < 0.05$) in Groups I and C and average time spent grazing decreased ($P < 0.05$) in Group C from Day 0 to Day 1. Also, there was a significant difference between groups (based on a calculated χ^2-square value) in the proportion of mares that autogroomed, defecated, urinated, rolled, and whinnied on Day 1. Activity shift rate (ASR) and temperament scores increased significantly in Groups I and C from Day 0 to Day 1 ($P < 0.05$). Plasma cortisol increased significantly in all groups from Day 0 to Day 1, a.m. ($P < 0.05$) and decreased significantly from Day 1, a.m. to Day 1, p.m. ($P < 0.05$). HCT significantly increased in all three groups from Day 0 to Day 1, a.m. ($P < 0.05$). WBC significantly increased in Group T from Day 0 to Day 1, a.m. ($P < 0.05$). N:L ratio significantly increased in Groups I and C from Day 0 and Day 1, a.m. to Day 1, p.m. ($P < 0.05$).

* Corresponding author. Present address: Department of Veterinary and Microbiological Sciences, North Dakota State University, Van Es Hall, Fargo, ND 58105, USA. Tel.: +1-701-231-8504; fax: +1-701-231-7514.
E-mail address: douglas.freeman@ndsu.nodak.edu (D.A. Freeman).

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A variety of measures did indicate a response to removal from the pasture group, however, the overall, short-term response was minimal. Since the responses of Groups I and C were similar, the effects of isolation versus a novel environment or separation from the established herd could not be differentiated.

Keywords: Animal welfare; Equine behavior; Equine physiology; Social isolation; Novel environment; Transportation

1. Introduction

Horses are housed and managed in a variety of settings which provide various levels of contact with other horses. In many cases, contact between horses is limited or absent. However, the horse is a highly social species in both wild and domestic conditions (Wood-Gush and Galbraith, 1987), and the social environment may influence their well-being (Mal et al., 1991a,b). Environmental enrichment, including the addition of social companions (Newberry, 1995), is one method used to improve a barren environment. A barren environment, including a lack of social stimuli, may result in a high frequency of abnormal behaviors, or stereotypies. Therefore, the effects of social environment are an important area of study.

Social preferences were compared in horses in small pens placed together in pairs, separate but in contact, or alone (Houpt and Houpt, 1989). Isolated horses were three times more active, based on increased time spent walking and trotting, and spent 10% less time eating, versus horses in pairs or horses separated but in contact. Mares kept isolated for 72 h performed more energetic activities when released from confinement than mares allowed social contact (Mal et al., 1991a). The isolated mares traveled farther, and they trotted more often and for longer periods of time. Physiologic measures indicative of stress were also compared in horses under increasing levels of confinement and isolation in box stalls (Mal et al., 1991b). Changes characteristic of a stress response were observed in relation to increasing isolation, including a significant increase in hemoglobin and a trend to increase in white blood cell count and neutrophil:lymphocyte ratio. Additional physiologic indicators of stress which have been used in horses include an increase in plasma cortisol (Alexander and Irvine, 1998) and a decrease in the local immune response to intradermal injection of phytohemagglutinin (Dimock and Ralston, 1999; Mal et al., 1991b).

Currently many privately owned horses are isolated in paddocks or on pasture. Minimal information is available regarding effects of social isolation on behavioral and physiologic measures of well-being in horses, particularly in a pasture or large paddock setting. The specific objective of the current study was to evaluate behavior and physiologic responses of mares to temporary (6 h) isolation in a small pasture setting.

2. Materials and methods

2.1. Animals and pastures

The study was conducted during June and July 2000, using 15 adult, mixed-breed mares. Except for brief, periodic removal, either alone or in groups for research purposes, the

mares were maintained for 3 years in a static herd on a 10 ha pasture. For the duration of this study, a sub-section of the 10 ha pasture was created using temporary electric fencing. It was approximately 1.5 ha in size (0.1 ha per mare) and included a 160 m^2, three-sided shed (home pasture). At a separate facility, a pasture was constructed of wood planks and metal pipe panels and was approximately 0.1 ha, including a 55 m^2, three-sided shed (isolation pasture). No other domestic animals were present at the isolation pasture facility during the time of the study.

2.2. Design of study

The mares were randomly assigned to one of three groups and to the order of evaluation within each group. The study consisted of five consecutive weekly replicates. Table 1 details the schedule of procedures and measures for each replicate. Each mare was evaluated on a baseline day (Day 0) and the following test day (Day 1). Evaluation consisted of 6 h behavior observation, with collection of samples for physiologic measures occurring before and after the observation periods. Each replicate required 6 days and included one mare from each of the three groups.

On Day 0, mares were evaluated in the home pasture. On Day 1, mares in the isolation group (Group I, $n = 5$) were transported alone to the isolation pasture and returned to the home pasture after a period of 6 h. Transportation required approximately 20 min each

Table 1
Outline of the schedule of procedures and measures for each replicate, based on behavior observation from 8 a.m. to 2 p.m.

Day 0	Day 1
Isolation group (I)	
(1) Physiologic sample (a.m.)	(1) Trailer to isolation paddock (20 min)
(2) Behavior observation (in group, 6 h)	(2) Physiologic sample (a.m.)
	(3) Behavior observation (in isolation, 6 h)
	(4) Physiologic sample (p.m.)
	(5) Return to group pasture
	(6) PHA injection (measure site 18 h later)
Trailer group (T)	
(1) Physiologic sample (a.m.)	(1) Trailer (20 min)
(2) Behavior observation (in group, 6 h)	(2) Physiologic sample (a.m.)
	(3) Behavior observation (in group, 6 h)
	(4) Physiologic sample (p.m.)
	(5) PHA injection (measure site 18 h later)
Companion group (C)	
(1) Physiologic sample (a.m.)	(1) Trailer to isolation paddock with companion (20 min)
(2) Behavior observation (in group, 6 h)	(2) Physiologic sample (a.m.)
	(3) Behavior observation (with companion, 6 h)
	(4) Physiologic sample (p.m.)
	(5) Return to group pasture
	(6) PHA injection (measure site 18 h later)

way. As a control for effects of transportation, mares in the transport group (Group T, $n = 5$) were transported approximately half of the distance along the route between the two pastures and returned to the Home Pasture. Mares in the companion group (Group C, $n = 5$) were transported with a companion mare (the completed test mare from Group T) to the isolation pasture and returned to the home pasture after a period of 6 h. Group C controlled for the effect of exposure to a novel environment and separation from the established herd.

2.3. Behavior measures

Measurements of behavior (duration and/or frequency) were recorded from 8 a.m. to 2 p.m. via continuous observation using a stopwatch and a customized, time-based behavior check sheet designed to record the occurrence of specific behaviors (defined in Table 2). The check sheet was developed from preliminary observations and previous reports (McDonnell et al., 1999). Observations were conducted from within a motor vehicle along the perimeter of the pastures to minimize effect of the observer. An overall assessment was made by the observer after each 6 h behavior observation period using a series of 10-point rating scales representing calm (1) to agitated (10), calm (1) to fidgety (10), calm (1) to anxious (10), quiet (1) to active (10), non-aggressive (1) to aggressive (10), comfortable (1) to uncomfortable (10), based upon work by McDonnell et al. (1999). An assessment of the ease of handling of each mareÂwas made once on Day 0 and twice on Day 1 based on behavioral response to blood collection, heart rate (HR) monitor application, and transport using a 5-point rating scale (1 representing most compliant and 5 representing most difficult). In addition to specific behaviors, activity shift rate (ASR; McDonnell et al., 1999) was measured.

2.4. Physiologic measures

2.4.1. Blood collection and analysis

Jugular blood samples were obtained on Day 0, on Day 1, a.m. (immediately after transport), and on Day 1, p.m. (immediately after behavior observations were completed) using Vacutainer® tubes containing either EDTA or heparin anti-coagulants. EDTA samples were shipped for complete blood count (CBC) analysis to a commercial veterinary laboratory (AnTech Diagnostic Laboratory, Farmingdale, NY). Plasma harvested from heparinized samples by centrifugation was stored at −80 °C until assayed for cortisol (RIA, Diagnostic Products Corporation, Los Angeles, CA).

2.4.2. Heart rate (HR)

A HR monitor (VMAX® Heartbelt™) and receiver (Polar® Accurex Plus™ Heart Rate Monitor) were attached to the mare on Day 0 for continuous remote recording of HR. The data were downloaded at the end of Day 1 to a PC using Training Advisor™ software provided by Polar®.

2.4.3. Phytohemagglutinin (PHA) skin test

Mares were injected intradermally with 0.1-ml PHA (Sigma®) on the left neck and with 0.1-ml saline (negative control) on the right neck. PHA was dissolved in saline at a

Table 2
Definitions of specific behaviors

Behavior	Definition
Eat	Mouth and/or chew hay or leaves (for 5 s or longer)
Graze	With head lowered to ground, take grass into mouth and chew (for 5 s or longer)
Drink	Lower head; put lips in water trough or pond in posture typical of ingesting water in horses (for 5 s or longer)
Stand alert	Stand with eyes and ears focused forward (for 5 s or longer)
Stand rest	Stand with eyes either down or forward, ears soft (for 5 s or longer)
Rest recumbent	Lie on ground either sternally or laterally (for 5 s or longer)
Sleep (post priori)	Stand with head lowered, in posture typical of rest with eyes closed and ears relaxed (for 5 s or longer)
Walk	Move forward with a slow, four-beat gait (for 5 s or longer)
Trot/canter	Move forward with a two- (trot) or three-beat gait (canter) (for 5 s or longer)
Gallop	Move forward with a fast, four-beat gait (for 5 s or longer)
Spook	Move abruptly in any direction in a manner typical of avoidance or removal from an area
Flehmen	Elevate head, extend neck and evert upper lip to expose the upper incisors and adjacent gums, drawing air into the mouth and nasal cavity
Affiliate	Allogroom, touch nose-to-nose, or nuzzle (for 5 s or longer)
Initiate/receive aggression	Initiate or receive kick, threaten to kick (orienting the hind quarters toward an individual with hind limb raised), bite or threaten to bite (ears laid back, head in line with neck and stretched out)
Whinny	Vocalize with a loud, high-pitched sound
Investigate	Sniff or touch with muzzle objects on or areas of the ground (for 5 s or longer)
Paw	Lift forelimb from ground slightly and extend forward quickly, drag toe backward against ground in digging motion repeatedly
Wood chew	Chew on woody vegetation or wooden fence (for 5 s or longer)
Defecate	Eliminate solid waste (feces)
Urinate	Eliminate fluid waste (urine)
Autogroom	Rub body against stationary object (i.e. fence post, etc.), nip body with teeth or scratch with hoof (for 5 s or longer)
Swat flies	Swing head, hitting insects on the body or limbs, with ongoing activity interrupted for 5 s or longer
Roll	Drop to knees, roll on to back and/or roll onto other side, and return to feet

concentration of 1 mg/ml. The length and width of the intradermal response was measured 18 h post-injection using a caliper and a ruler.

2.4.4. Body weight (BW) and body condition score (BCS)

BW was estimated using a calibrated equine girth tape and BCS was assessed using the method of Henneke et al. (1983).

2.5. Statistical analyses

Both SAS (SAS, 2001) and SPSS (SPSS, 2001) were used to analyze the behavior and physiologic data. Twenty specific behavior measures were observed in a small percentage of mares and were therefore dichotomized based on their frequency distributions and analyzed between groups for each day using Fisher's Exact Test (behaviors listed in Table 3). Four behaviors that were performed by the majority of mares were analyzed between groups for each day (Day 0 and Day 1) using parametric ANOVA, Scheffé and Dunnett's T3 tests as well as non-parametric Kruskal–Wallis, and Median One-Way. Specific behavior measures were also each analyzed within each group across days using the parametric repeated measures test and single degree of freedom contrasts.

Table 3
Dichotomous behavior measures compared between groups ($n = 5$ per group), for each day

Dichotomous[a]	Day 0			Day 1		
	Group I	Group T	Group C	Group I	Group T	Group C
Eat	2	0	1	0	0	2
Drink	3	3	4	2	2	4
Rest recumbent	0	1	0	0	0	0
Sleep	0	1	0	0	0	0
Trot/canter	3	2	3	5	5	5
Gallop	1	0	0	0	0	1
Spook	0	0	1	2	0	1
Flehmen	0	1	0	1	0	2
Affiliate	1	1	2	n/a	1	2
Initiate aggression	1	3	3	n/a	3	3
Receive aggression[b]	2 (−1.67)	4 (0.33)	5 (1.33)	n/a	5	2
Whinny[c]	1	0	0	5 (2.00)	0 (−3.00)	4 (1.00)
Investigate	4	3	4	5	4	5
Paw	1	1	0	1	0	0
Wood chew[b]	4 (2.33)	1 (−0.67)	0 (−1.67)	3	2	1
Defecate[c]	3	5	4	4 (1.67)	3 (0.67)	1 (−1.33)
Urinate[c]	1	1	1	4 (1.33)	1 (−1.67)	3 (0.33)
Autogroom[c]	3	3	3	1 (−0.33)	3 (1.67)	0 (−1.33)
Swat flies	1	0	1	2	0	1
Roll[c]	2	3	2	4 (1.00)	1 (−2.00)	4 (1.00)

[a] Dichotomous data listed as number of mares (out of 5) performing behavior per group (observed − expected in parentheses).
[b] Significant differences between groups on Day 0 (Fisher's Exact Test).
[c] Significant differences between groups on Day 1 (Fisher's Exact Test).

ASR was calculated by adding all frequencies per hour of each behavior each day and calculating an average for each group. ASR was analyzed between groups for each day using parametric ANOVA, Scheffé and Dunnett's T3 tests and non-parametric Kruskal–Wallis and Median One-Way. ASR was also analyzed within each group across days using the parametric repeated measures test and single degree of freedom contrasts.

Temperament and handling scores were analyzed between groups for each day with parametric ANOVA, Scheffé and Dunnett's T3 tests and the non-parametric Kruskal–Wallis and Median One-Way. Temperament and handling scores were also analyzed within groups across days using the parametric repeated measures test and single degree of freedom contrasts.

Cortisol, HCT, WBC, N:L ratio, and the PHA skin test measures were analyzed between groups for each time period (Day 0, Day 1 a.m., and Day 1 p.m.) and HR was analyzed between groups for each day. These analyses were performed using parametric ANOVA, Scheffé and Dunnett's T3 tests as well as non-parametric Kruskal–Wallis and Median One-Way. Cortisol, HCT, WBC, N:L ratio and HR measures were also analyzed within each group across time periods using the parametric repeated measures test and single degree of freedom contrasts and the non-parametric Kruskal–Wallis and Median One-Way tests.

3. Results

3.1. Behavior measures

The dichotomous analyses of proportions of mares exhibiting certain behaviors are detailed in Table 3. Overall, there were some significant differences ($P < 0.05$). On Day 0, fewer Group I mares than expected received aggressive interactions, while a greater proportion of Group I mares were observed wood chewing than expected. Also on Day 0, a greater proportion of Groups T and C mares received aggressive interactions, and fewer than expected were observed wood chewing. On Day 1, a greater proportion of Groups I and C mares than expected whinnied, urinated, and rolled, while fewer than expected Group T mares whinnied, urinated, and rolled. On Day 1, fewer than expected Groups I and C mares autogroomed and a greater proportion of Group T mares autogroomed; a greater proportion of Groups I and T mares defecated, while fewer than expected Group C mares defecated.

Table 4 summarizes the remaining behavior measures. For remaining behavior measures, differences between groups on Day 0 were not significant (Table 4). There were significant differences between groups on Day 1 in the average time spent standing alert and grazing ($P < 0.05$) with Groups I and C spending more time standing alert than Group T and with Group I spending less time grazing than Group T. There was a significant increase within Groups I and C in average time spent standing alert from Day 0 to Day 1 ($P < 0.05$, Table 4). There was also a significant decrease within Group C in average time spent grazing and a significant increase in average time spent walking from Day 0 to Day 1 ($P < 0.05$, Table 4).

For ASR, temperament, and handling, there were no significant differences between groups on Day 0 (Table 5). However, on Day 1, ASR was significantly greater in Groups I

Table 4
Behavior measures compared within and between groups ($n = 5$ per group), mean (S.D., min)

	Day 0	Day 1
Group I		
Standing rest	88.5 (60.40)	49.5 (53.93)
Standing alert	64.1 (39.67) a	198.6 (88.85) b,x
Walk	25.6 (6.62)	46.6 (42.25)
Graze	124.7 (111.87)	68.6 (57.51) x
Group T		
Standing rest	80.3 (44.52)	99.8 (79.60)
Standing alert	24.5 (19.03)	17.4 (15.11) y
Walk	22.2 (7.42)	23.1 (8.74)
Graze	224.8 (63.94)	216.9 (83.54) y
Group C		
Standing rest	73.6 (58.00)	42.2 (67.04)
Standing alert	33.8 (17.01) a	163.6 (86.92) a,x
Walk	24.3 (6.17) a	61.3 (33.97) b
Graze	222.7 (79.24) a	98.4 (49.79) b,x,y

Different letters (a and b) denote significant differences ($P < 0.05$; repeated measures, single d.f. contrasts) within a group from Day 0 to Day 1. Different letters (x and y) denote significant differences ($P < 0.05$; ANOVA with Scheffé and Dunnett's T3 comparisons; Kruskal–Wallis or Median One-Way) between groups for each day.

Table 5
Activity shift rate (ASR), temperament, and handling scores compared within and between groups ($n = 5$ per group), mean (S.D.)

	Day 0	Day 1
Group I		
ASR per hour	37.8 (7.02) a	80.7 (31.44) b,x
Temperament	2.8 (0.77) a	5.5 (2.04) b,x
Handling	1.9 (0.37)	1.8 (0.45)
Group T		
ASR per hour	35.9 (8.71)	34.2 (7.30) y
Temperament	1.7 (0.38)	1.9 (0.43) y
Handling	1.9 (0.74)	1.9 (0.74)
Group C		
ASR per hour	36.5 (3.04) a	109.4 (37.99) b,x
Temperament	2.4 (0.75) a	6.2 (2.01) b,x
Handling	2.0 (0.64)	2.1 (0.53)

Different letters (a and b) denote significant differences ($P < 0.05$; repeated measures, single d.f. contrasts) within a group from Day 0 to Day 1. Different letters (x and y) denote significant differences ($P < 0.05$; ANOVA with Scheffé and Dunnett's T3 comparisons; Kruskal–Wallis or Median One-Way) between group for each day.

and C ($P < 0.05$, Table 5). Similarly, temperament score was significantly greater (less calm, less comfortable, less quiet, and more aggressive) in Groups I and C ($P < 0.05$, Table 5). There was also a significant increase within Groups I and C in ASR and temperament scores from Day 0 to Day 1 ($P < 0.05$, Table 5).

3.2. Physiologic measures

3.2.1. Cortisol

There were no differences in plasma cortisol between groups for each time period (Table 6). Within all three groups, plasma cortisol significantly increased from Day 0 to Day 1, a.m. ($P < 0.05$) and significantly decreased from Day 1, a.m. to Day 1, p.m. ($P < 0.05$, Table 6). The concentrations of plasma cortisol detected in this study ranged from 3.04784 to 14.59667 μg/dl and were measured in a single assay. The minimal detectable concentration for the plasma assay was 0.2 μg/dl, and the highest standard was 50 μg/dl. The intra-assay CV was 7.23% based on a single pooled blood sample.

3.2.2. CBC measures

There were no differences in HCT between groups for each time period (Table 6). Within all three groups, HCT increased significantly from Day 0 to Day 1, a.m. ($P < 0.05$) and decreased from Day 1, a.m. to Day 1, p.m. ($P < 0.05$, Table 6). There were no differences in the number of white blood cells (WBC) between groups for each time period (Table 6). Within Group T, WBC increased significantly from Day 0 to Day 1, a.m. ($P < 0.05$,

Table 6
Physiologic measures compared within and between groups ($n = 5$ per group), mean (S.D.)

	Day 0	Day 1 (a.m.)	Day 1 (p.m.)	Overall average[a]
Group I				
Plasma cortisol (μg/dl)	5.4 (1.82) a	9.1 (1.70) b	7.0 (2.12) a	7.1 (2.34)
HCT (%)	39.3 (3.70) a	46.2 (2.21) b	40.2 (2.74) a	41.9 (4.40)
WBC (10^3/μl)	9.0 (2.04)	10.1 (2.40)	10.3 (1.99)	9.8 (2.30)
N:L ratio	1.7 (0.36) a	1.4 (0.31) a	2.7 (0.58) b	1.9 (0.73)
PHA − LL[b] (mm)				51.1 (21.35)
PHA − LW[b] (mm)				34.1 (17.23)
Group T				
Plasma cortisol (μg/dl)	5.5 (1.17) a	10.6 (0.82) b	4.7 (1.52) a	6.9 (2.91)
HCT (%)	40.2 (4.40) a	46.7 (4.25) b	40.2 (5.40) a	42.4 (5.83)
WBC (10^3/μl)	8.7 (0.83) a	10.7 (0.79) b	9.7 (2.02) a,b	9.7 (1.62)
N:L ratio	1.6 (0.35)	1.5 (0.38)	1.9 (0.49)	1.7 (0.46)
PHA − LL[b] (mm)				43.8 (10.59)
PHA − LW[b] (mm)				31.0 (10.03)
Group C				
Plasma cortisol (μg/dl)	6.4 (1.14) a	10.8 (2.95) b	7.4 (2.36) a	8.2 (2.85)
HCT (%)	42.0 (3.81) a	47.6 (6.08) b	43.0 (4.96) a	43.9 (5.69)
WBC (10^3/μl)	10.9 (2.18)	12.5 (2.27)	12.5 (3.54)	11.9 (2.98)
N:L ratio	1.7 (0.38) a	1.6 (0.42) a	2.8 (0.71) b	2.1 (0.82)
PHA − LL[b] (mm)				58.6 (14.42)
PHA − LW[b] (mm)				33.8 (7.49)

Different letters (a and b) denote significant differences ($P < 0.05$; repeated measures, single d.f. contrasts) within a group from Day 0 to Day 1.

[a] Denotes group averages across time periods for comparison between groups.

[b] One measurement taken 18 h post-injection; LL: left length; LW: left width.

Table 6). There were no differences in N:L ratio between groups for each time period (Table 6). Within Groups I and C, N:L ratio increased significantly from Day 0 and Day 1, a.m. to Day 1, p.m. ($P < 0.05$, Table 6).

3.2.3. PHA skin test

There were no differences between groups in the response to intradermal PHA injection (Table 6).

3.2.4. Heart rate

On Day 0, the average HR was significantly greater in Group C than Group T ($P < 0.05$; 43.9 ± 5.3 and 35.5 ± 3.3 beats/min, respectively), but Group I (42.3 ± 6.1 beats/min) was not different from Groups T and C. On Day 1, the average HR for Group C was significantly greater than Groups I and T ($P < 0.05$; 66.1 ± 12.8, 54.1 ± 12.2, and 41.0 ± 8.1 beats/min, respectively). Within Group C, HR increased significantly from Day 0 to Day 1 ($P < 0.05$).

3.2.5. Body weight and body condition score

There were no significant differences between Groups I, T, and C for BW (443, 428, and 414 kg, respectively) and BCS (6.8, 6.4, and 7.0, respectively).

4. Discussion

The purpose of this study was to evaluate a set of behavior and physiologic responses of mares to temporary removal from a pasture group to an isolation pasture, either alone or with a companion mare. Previous studies of isolation have had a longer period of isolation and have evaluated horses in a barn or small pen environment rather than in a pasture setting (Mal et al., 1991a,b; Houpt and Houpt, 1989). While some of the responses observed in this study were similar to studies of longer isolation periods, mares demonstrated less response than one might expect. Additionally, the presence of a companion mare in the isolation pasture did not appear to affect response. This may be due to our short test time (6 h) or to previous experience of the mares to separation and return to pasture.

The distribution of mares receiving aggressive interactions between the three groups on Day 0 was most likely due to the position in the dominance hierarchy, despite the random group assignments. Furthermore, more mares than expected exhibited wood chewing on Day 0. These mares may simply have a higher propensity toward wood chewing.

The increase in the number of mares that urinated, rolled, and whinnied on Day 1 may indicate an increase in anxiety, in particular because these behaviors were not significantly different between groups on Day 0, which suggests that the isolation procedure had an effect on these behaviors. This is further supported by the increase in average time spent standing alert in Groups I and C and the decrease in the average time spent grazing in Group C on Day 1. These behavior results are similar to those in previous reports of mares that were isolated or separated for longer durations (Houpt and Houpt, 1989). However, contrary to other studies, walking was not significantly different between groups. This may be because mares exhibited movement by means of trotting or cantering on Day 1. Since many mares did not exhibit trotting and cantering on Day 0, this behavior was analyzed

according to the number of mares that exhibited trot/canter bouts. However, on Day 1, number of bouts of trotting and cantering in isolated mares (Group I) and mares with companions (Group C) were much greater than mares that stayed with the herd (Group T; 202, 245, and 6, respectively). Therefore, while average time spent walking was similar in all three groups on Day 1, raw frequency of trotting and cantering was much greater, in Groups I and C. An observed increase in movement in isolated horses is consistent with previous reports (Mal et al., 1991a; Houpt and Houpt, 1989).

Environmental changes may cause a change in the rhythm of behavior (Blokhuis et al., 1998; Buré, 1983). Isolated mares (Group I) and mares with a companion (Group C) demonstrated an increase in ASR and temperament scores that may have been a result of increased vigilance and/or agitation, however handling scores were not different between or within groups. This most likely indicates that the results were not affected by the mares' response to handling.

The morning baseline concentrations of cortisol in this study were consistent with those in previously reported work (Stull and Rodiek, 1988). The rise in cortisol concentrations from Day 0 to Day 1, a.m. are consistent with previous work indicating that transport is a significant stressor (Dimock and Ralston, 1999; Stull, 1999; Smith et al., 1994; Cregier, 1982). Plasma cortisol reportedly follows a circadian pattern of release with the peak in the morning and the nadir in the afternoon (Stull, 1999; Irvine and Alexander, 1994; Stull and Rodiek, 1988). Therefore, if the decrease in cortisol concentrations observed from Day 1, a.m. to Day 1, p.m. in this study was due to circadian rhythm, we would have expected to observe a higher cortisol concentration during Day 0 compared to Day 1, p.m. This was not the case with the current data. Minor perturbations in the environment of the horse results in an increase in the afternoon cortisol to a concentration similar to the morning concentration (Irvine and Alexander, 1994). Therefore, the hypothalamic–pituitary–adrenal (HPA) axis was most likely still activated due to the conditions of isolation and/or exposure to the new environment.

The significant increase in HCT from Day 0 to Day 1, a.m. most likely resulted from splenic contraction due to transport. This is further supported by the significant decrease in HCT from Day 1, a.m. to Day 1, p.m. Despite the increase, values stayed within the normal range of 32–53% (Stull, 1999). WBC count also remained within the normal range of (5.5–14.3) $\times 10^3/\mu l$ (Stull, 1999) despite the increase observed in Group T. An increase in the N:L ratio is indicative of stress in animals (Stull, 1999; Baker et al., 1998). This increase may result from the negative effect of cortisol on lymphocyte proliferation, and the subsequent decrease in lymphocytes (Houpt, 1991). Overall, however, N:L values stayed within the normal range of 0.8–2.8 (Stull, 1999).

Phytohemagglutinin (PHA) is a mitogen that, upon intradermal injection, causes T-cells to migrate to the area of the injection and proliferate (Baker et al., 1998). In a healthy immune system, the area of injection develops a large wheal, whereas in an impaired immune system, the wheal is small or absent (Dimock and Ralston, 1999; Baker et al., 1998; Mal et al., 1991b). Dimock and Ralston (1999) found a significant decrease in the size of the wheal in horses after a transport period of 6 h, a time frame similar to this study. Other studies in horses (Mal et al., 1991b; Targowski, 1976), sheep (Sevi et al., 2001), water buffalo (Grasso et al., 1999), and chickens (Regnier and Kelley, 1981) that used the PHA skin test did so after test periods of much longer than 6 h. Also, in pigs, frequent

measurements provided more thorough characterization of response in the PHA skin test than single measurements (Ekkel et al., 1996). While more frequent measurements may have improved the characterization of the PHA response in the current study, based on our data, it is most likely that the test conditions had no effect on T-lymphocyte proliferation.

The quantity of HR data recorded on the continuous HR monitor was sporadic, and for many mares there were large portions of the collection period where little or no HR data were recorded. For this analysis, blocks of time where sufficient HR data was collected for all three groups were used for the comparisons. With an improved collection procedure, additional differences may have been detected.

5. Conclusion

In this study of short-term, pasture isolation, a variety of measurements were evaluated. While some of the responses may indicate a degree of disturbance, similar to studies of longer isolation periods (Mal et al., 1991a,b; Houpt and Houpt, 1989), the overall effect of moving mares from an established group in a home pasture to an isolation pasture for 6 h, either alone or with one companion, was minimal. In addition, based on the similarity of responses between mares placed in the isolation pasture and mares placed in the isolation pasture with a companion, it was difficult to determine whether the responses observed were due to isolation, separation from the established herd, or exposure to a novel environment. Future studies should further define responses to isolation and to a new environment.

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