

JOURNAL OF ANIMAL SCIENCE

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J Anim Sci 2000. 78:1458-1466.

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Physiological responses of horses to 24 hours of transportation using a commercial van during summer conditions¹

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ABSTRACT: Fifteen mature horses (mares, n = 6); geldings, n = 9) were used to assess the physiological responses of 24 h of transport in a commercial van under California summer conditions. The study was conducted on four consecutive days, and data were collected on d 1 and d 2 to obtain baseline values and to determine any diurnal variation in the individual measurements. Travel commenced on d 3 at 0800 for 24 h, with a total of 1,622 km traveled. Blood samples were collected at 0800, 1100, 1400, 2000, and 0200 each day. Horses were weighed and rectal temperatures recorded at 0800 each day and at 2000 each day except d 3. Body weight, rectal temperature, serum cortisol, serum lactate, and white blood cell (WBC) counts exhibited diurnal variation ($P = .0001$) on d 1 and d 2. Body weight immediately after unloading showed a 6% loss. At 24 h following transit, a 3% deficiency in body weight loss remained. The WBC counts showed a progressive increase with duration of travel and peaked at the termination of transport. Dehydration measures of hema-

tocrit and total protein increased during transport and returned to baseline during the posttransport period. Serum concentrations of lactate, creatine kinase and aspartate aminotransferase increased during transport and in the early posttransit period, but returned to baseline values at the conclusion of the 24-h posttransport period. Glucose concentration increased with the initiation of transport and did not decrease to baseline concentration at the end of the 24-h posttransport period. Plasma cortisol and neutrophil:lymphocyte ratio increased with duration of transit and returned to baseline during the posttransport period. These data clearly showed physiological responses of horses undergoing 24 h of transport including changes in muscle metabolism, stress indices, dehydration and immune parameters, and body weight. These responses may increase disease susceptibility and influence energy availability for athletic performance following long-term transport of horses.

Key Words: Horses, Lymphocytes, Stress, Transport, Well-being

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J. Anim. Sci. 2000. 78:1458–1466

Introduction

Studies on stress and well-being during transportation of agricultural animals have primarily been directed toward the cattle and swine industries, and little information on the effects of long-distance transportation of horses is available. Research examining long-distance transportation of cattle clearly shows physiological and behavioral signs of stress (Mormede et al., 1982; Agnes et al., 1990; Tarrant et al., 1992). Potential

stressors for horses during transportation may parallel these affecting other agricultural animals and originate from a combination of sources including physical factors such as space, noise, and road conditions; psychological stressors including social regrouping or unfamiliar environments; climatic factors such as air temperature and relative humidity; and health status.

Limited studies with horses have reported posttransit physiological responses. The effects of 9 h of transportation showed increased concentrations of cortisol and progesterone in early-gestation mares, but early embryonic death rate was not affected (Baucus et al., 1990). In racehorses, short-term (300 km) transportation or an exercise bout of cantering 1,500 m effected similar serum enzyme and metabolic changes (Codazza et al., 1974). In healthy horses traveling 36 h (1,100 km) in a trailer, the number of alveolar macrophages and their bactericidal function were decreased and cortisol concentration was elevated 1 wk after transport (Laegreid et al., 1988). Respiratory disease and even

¹Partially supported by a Cooperative Agreement from USDA, APHIS, Veterinary Services. Technical assistance of Renee D'Ambrosi, Kim Crawford, Jon Mitts, Coralie Munro, Chad Stewart, Travis Thayer, Kelly Weaver, and Neil Willits is gratefully acknowledged.

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Received September 10, 1999.

Accepted January 4, 2000.

death have been reported in horses following transport (Anderson et al., 1985; Oikawa et al., 1994; Austin et al., 1995). The objective of this study was to assess the physiological responses in mature, healthy horses during 24 h of transport and a posttransport period using a commercial van under summer conditions typical of California.

Materials and Methods

Horses and Study Design

Fifteen horses (mares, $n = 6$; geldings, $n = 9$) from the California State University-Fresno riding program were used. Horses ranged in age from 4 to 18 yr old and included American Saddlebred, Hackney Horse, Paint, Quarter Horse, Thoroughbred, and Warmblood breeds. The study was conducted on four consecutive days in August 1998 at Fresno, California. On d 1 and 2 horses were housed individually in stalls (3.7×6.1 m) and data were collected to obtain baseline values and to determine any diurnal variation in individual measurements. On d 3 between 0700 and 0800, horses were loaded onto a commercial van. Travel commenced at 0800 for a 24-h period. Horses were unloaded at 0800 on d 4 and housed individually in stalls for a 24-h posttransport period concluding on d 5 at 0800. Water was always available to the horses in the stalls. Alfalfa hay (approximately 1.5 to 2% of body weight daily) was provided at 0700 and 1700 during d 1, d 2, d 4, and d 5. On d 3, alfalfa hay was placed in hay nets accessible to each horse during the 24-h transit period. The research protocol was approved by the University of California Animal Care and Use Committee.

A commercial van (1986 Morton-Davis, air ride) designed to haul a total of 15 horses and pulled by a semi-tractor (1991 Freightliner, 3 axle, cab over engine) was used for the 24 h of transport. Horses were randomly arranged in groups of three parallel to the length of the van. Six horses faced the direction of travel, and nine horses faced the opposite direction. Horses were cross-tied in individual stalls ($.76 \times 1.67$ m) and provided with alfalfa hay throughout the trip. Bedding (sawdust or straw) was placed on rubber mats for secure footing. Windows and vents were open fully to allow for ventilation; air movement was not measured. Travel was initiated at 0800 on August 11, 1998, and continued for 24 h, covering a total distance of 1,622 km. The van stopped five times for blood sample collections and to provide water for each horse, usually a period of approximately 40 min. Water was hauled from the University to the site of sampling during transit to ensure that the horses were offered water that was familiar in taste. All horses had previous trailering experience; however, 1 wk prior to the study, horses were loaded into and unloaded from the van to ensure that all horses loaded without hesitation.

Temperature and relative humidity in the stable and van was monitored continually by microcomputer data

logger units (Models WTA32 and SRHA32, respectively; Onset Instruments, Pocasset, ME) placed near the center of the barn during d 1, d 2 and d 4, and approximately 5 cm from the center of the ceiling in the van during transport (d 3).

Collection of Physiological Data

Similar data collection procedures were used at each sampling time. Horses were weighed on d 1, d 2, and d 4 at 0800 and 2000 and at 0800 on d 5 using an electronic portable scale (Model H90-3042, Fairbanks, St Johnsbury, VT). On the day of transport (d 3), horses were weighed prior to loading between 0700 and 0800. Rectal temperature was recorded using the same schedule as weighing. Blood was collected at 0800, 1100, 1400, 2000, and 0200 each day. Additionally, on the day of transport, a blood sample was collected at 0700 prior to loading. On the posttransport day (d 4), a blood sample was also collected at 0930. Horses were gently restrained with a halter during blood collection. Blood samples (20 mL) were collected into evacuated glass tubes via venipuncture of the jugular vein. Blood samples analyzed for cortisol, lactate, glucose, α_1 -acid glycoprotein (**AGP**) and total protein were immediately placed on ice and allowed to clot. Serum was obtained and frozen at -56°C . White blood cell (**WBC**) and differential counts and hematocrit (**hct**) measurements were performed with samples collected containing EDTA at 0800 and 2000 each day, at 0700 and 1100 on the day of transport (d 3), at 0930 and 1100 on the posttransport day (d 4), and at 0800 on d 5. Serum for aspartate aminotransferase (**AST**) and creatine kinase (**CPK**) activities was collected on the same schedule as blood for cell counts and refrigerated until analyses were performed on d 5.

Laboratory Analyses

Glucose and lactate were determined by use of an autoanalyzer (YSI 2300 STAT Plus, Yellow Springs, OH). Cortisol was determined in duplicate by a microplate enzyme immunoassay technique (Munro and Stabenfeldt, 1984; Munro and Lasley, 1988). Intra- and interassay coefficients of variation were less than 10 and 15%, respectively. A commercially available enzymatic colorimetric assay (Procedure 541, Sigma Diagnostics, St. Louis, MO) was used to determine total serum protein. The AGP concentration was evaluated with a commercially available radioimmunoassay plate (Development Technologies International, Frederick, MD). Serum activities of AST and CPK were determined using a clinical autoanalyzer (Hitachi 717, Boehringer Mannheim, Indianapolis, IN) by the Veterinary Medicine Teaching Hospital (Davis, CA). White blood cell counts and hct were determined by an automated cell counter (System 9000, Serono-Baker Diagnostics, Allentown, PA). Whole blood was used for determination of WBC differential counts using standard

Table 1. Minimum and maximum environmental temperature and relative humidity for each day of the study

Study day	Temperature, °C		Relative humidity, %	
	Minimum	Maximum	Minimum	Maximum
1	16.9	38.5	21.2	86.6
2	18.6	40.6	14.1	84.6
3	19.0	38.5	26.6	81.2
4	26.0	42.8	9.0	74.0

laboratory techniques (Heckner et al., 1988) and the neutrophil:lymphocyte ratio (N:L) was calculated.

Statistical Analyses

To establish any diurnal effects, data collected for weight, rectal temperature, and blood measurements on d 1 and d 2 were evaluated using repeated measures ANOVA (PROC GLM; SAS, 1989) with no grouping factors and one within-factor (sampling event). The effects of transportation and measurements taken after transport were evaluated using daily means for d 2, d 3, and d 4 using repeated measures ANOVA (PROC GLM; SAS, 1989) with no grouping factors and one within-factor (day). Dunnett comparisons were used to compare time points of 0700, 0800, and 1100 on d 3 to show the response of loading and transportation on blood measurements. Significance was claimed whenever $P < .05$.

Results

The maximum and minimum temperature and relative humidity values are shown in Table 1. The ranges in temperature and relative humidity in the barn on d 1, d 2, and d 4 was 16.9 to 42.8° C and 9.0 to 86.8%, respectively. During transport (d 3), the temperature and relative humidity ranges were 19 to 38.5° C and 26.6 to 81.2%, respectively.

Body Weight and Rectal Temperature

Body weight and rectal temperature were not recorded during transport. Rectal temperature fluctuated within the normal range (37.5 to 38.5° C) for the resting horse throughout the study (Merck Veterinary Manual, 1986) (Table 2). Both measurements exhibited ($P < .0001$) a diurnal rhythm on d 1 and d 2 with increases at 2000 compared to 0800. Body weight immediately after unloading at 0800 (508 ± 43 kg) on d 4 showed a 6% loss, but at 3 h after transit (1100, 528 ± 45 kg), with ad libitum water access, there was only a 2.4% loss. However, body weight at 24 h after transport (524 ± 45 kg) had not returned to pretransit values (539 ± 45 kg) (Table 3).

Table 2. Daily means (± SD) and P -values for diurnal variation and comparisons between days of body weight, rectal temperature, and blood measurements

Item	Study day				Diurnal	P - value			
	1	2	3	4		D 1 and 2	D 2 and 3	D 2 and 4	D 3 and 4
Weight, kg	541 ± 46	539 ± 45	539 ± 44	508 ± 43	.0001	.09	.78	.0001	.0001
Temperature, °C	37.2 ± .3	37.4 ± .3	37.5 ± .2	37.6 ± .4	.0001	.02	.23	.20	.56
White blood cells, × 10 ³ /μL	8.2 ± 1.7	8.6 ± 1.3	10.5 ± 1.8	12.3 ± 2.8	.0001	.05	.0001	.0001	.007
Hematocrit, %	37 ± 2	37 ± 2	40 ± 3	41 ± 3	.59	.31	.0003	.0001	.33
Total protein, g/dL	7.5 ± .3	7.2 ± .3	7.6 ± .3	7.4 ± .3	.0001	.0012	.0001	.0105	.05
Glucose, mg/dL	74 ± 3	78 ± 5	94 ± 14	95 ± 11	.12	.0002	.0004	.0001	.78
Lactate, mg/dL	7 ± 2	7 ± 2	8 ± 2	11 ± 3	.0001	.80	.12	.0001	.0001
Aminotransferase, IU/L	247 ± 40	249 ± 42	255 ± 40	267 ± 40	.09	.23	.008	.0001	.001
Creatine kinase, IU/L	224 ± 105	229 ± 114	243 ± 122	317 ± 144	.35	.74	.39	.03	.008
Cortisol, ng/mL	25 ± 7	23 ± 5	66 ± 15	48 ± 27	.0001	.12	.0001	.016	.001
Neutrophil:lymphocyte ratio	2.4 ± .7	3.0 ± 1.1	4.9 ± 1.6	8.9 ± 5.4	.55	.10	.0007	.0011	.012
α ₁ -Glycoprotein, μg/mL	175 ± 30	177 ± 29	184 ± 30	182 ± 32	.99	.72	.10	.31	.29

Table 3. Mean values (\pm SD) of measurements and *P*-values of comparisons between 0800 on baseline day (d 2) and following a 24-h posttransport period (0800, d 5)

Item	Mean \pm SD		<i>P</i> -values
	0800, d 2	0800, d 5	
Weight, kg	539 \pm 45	524 \pm 45	<.0001
Temperature, °C	37.4 \pm .3	37.6 \pm .8	.24
White blood cells, $\times 10^3/\mu\text{L}$	8.3 \pm 1.3	9.8 \pm 2.7	.06
Hematocrit, %	36 \pm 2	35 \pm 3	.30
Total protein, g/dl	7.4 \pm .4	7.3 \pm .4	.98
Glucose, mg/dL	79 \pm 5	93 \pm 12	.008
Lactate, mg/dL	5 \pm 1	6 \pm 1	1.000
Aminotransferase, IU/L	251 \pm 47	246 \pm 38	.74
Creatine kinase, IU/L	216 \pm 121	216 \pm 80	1.000
Cortisol, ng/mL	40 \pm 10	26 \pm 13	.32
Neutrophil:lymphocyte ratio	3.0 \pm 1.3	4.1 \pm 1.9	.99
α_1 -glycoprotein, $\mu\text{g/mL}$	183 \pm 37	162 \pm 36	.18

Blood measurements

General Health and Dehydration. The WBC counts exhibited a diurnal variation with a slight increase at 2000 on d 1 and d 2 (Table 2) but remained within the reference range (5.5 to $14.3 \times 10^3/\mu\text{L}$) for healthy horses during the 4-d study (Jain, 1993). The mean daily WBC counts during transportation (d 3) and the posttransport period (d 4) were elevated over baseline (d 2). The WBC response increased following loading ($P = .007$) and after the initial 3 h of transport ($P = .0009$) (Table 4). The WBC count ($12.7 \pm 3.0 \times 10^3/\mu\text{L}$) response peaked at 0800 h on d 4 at the termination of 24 h of transport.

Estimates of water consumption were recorded only during transit; horses consumed 22.7 ± 6.4 L during the 24-h transportation period; 91% of the water was consumed after 12 h of transport. All mean hct values were within the normal range of 32 to 53% (Jain, 1993) (Table 2). Hematocrit did not show a diurnal variation ($P = .59$) or any difference in daily means on d 1 and d 2 ($P = .31$). However, hct increased throughout transport to a peak of $47 \pm 4\%$ at the conclusion of transport. The daily means for days of transport (d 3, $40 \pm 3\%$) and the posttransport period (d 4, $41 \pm 3\%$) were elevated ($P < .001$) compared to baseline (d 2, $37 \pm 3\%$). Loading of

the horses onto the van elevated ($P = .003$) the hct, but no difference ($P = .34$) was shown in the initial 3 h of transport (Table 4). The total protein concentration remained in the reference range of 5.2 to 7.9 g/dL (Kaneke et al., 1997) throughout the study with a slight decrease ($P = .0012$) between d 1 and d 2, and that measured on the day of transport (d 3) and in the posttransport period (d 4) was increased ($P < .02$) over mean daily baseline (d 2) values. Peak total protein concentration ($7.8 \pm .5$ g/dL) occurred following 12 h of transport (Figure 1). A slight decrease in total protein concentration occurred with loading but returned to preloading levels after 3 h of transport (Table 4).

Serum Metabolites and Enzymes. Mean daily glucose and lactate concentrations for the study were within reference range for the resting horses (71 to 104 and 5 to 10 mg/dL, respectively) (Brobst and Parry, 1987; Stull and Rodiek, 1995) (Table 2). No diurnal effect was shown for glucose; however, the daily baseline mean of d 2 (78 ± 5 mg/dL) was greater ($P = .0002$) than that of d 1 (74 ± 3 mg/dL). A diurnal effect ($P < .0001$) was exhibited during d 1 and d 2 for lactate concentrations, but no difference ($P > .05$) in daily baseline means was shown for d 1 (7 ± 2 mg/dL) and d 2 (7 ± 2 mg/dL). Neither glucose nor lactate was affected ($P > .05$) by

Table 4. Mean values (\pm SD) of blood indices sampled at 0700 (prior to loading), 0800 (following loading, prior to transport), and 1100 h (transported for 3 h), and *P*-values for comparisons of 0700 and 0800, and 0800 and 1100

Item	Mean \pm SD			<i>P</i> -value	
	0700	0800	1100	0700 and 0800	0800 and 1100
White blood cells, $\times 10^3/\mu\text{L}$	8.7 \pm 1.6	9.7 \pm 1.7	11.4 \pm 2.8	.007	.0009
Hematocrit, %	37 \pm 4	41 \pm 5	40 \pm 5	.003	.34
Total protein, g/dL	7.5 \pm .5	7.2 \pm .3	7.5 \pm .4	.05	.001
Glucose, mg/dL	75 \pm 6	78 \pm 6	89 \pm 12	.12	.0006
Lactate, mg/dL	5 \pm 2	5 \pm 1	6 \pm 2	.13	.017
Aminotransferase, IU/L	249 \pm 44	245 \pm 39	256 \pm 38	.13	.002
Creatine kinase, IU/L	203 \pm 96	204 \pm 102	250 \pm 130	.68	.0007
Cortisol, ng/mL	27 \pm 9	40 \pm 12	68 \pm 19	.002	.0001
Neutrophil:lymphocyte ratio	3.1 \pm 1.1	3.3 \pm .8	6.3 \pm .5	.61	.03
α_1 -Glycoprotein, $\mu\text{g/mL}$	188 \pm 35	172 \pm 30	188 \pm 32	.056	.04

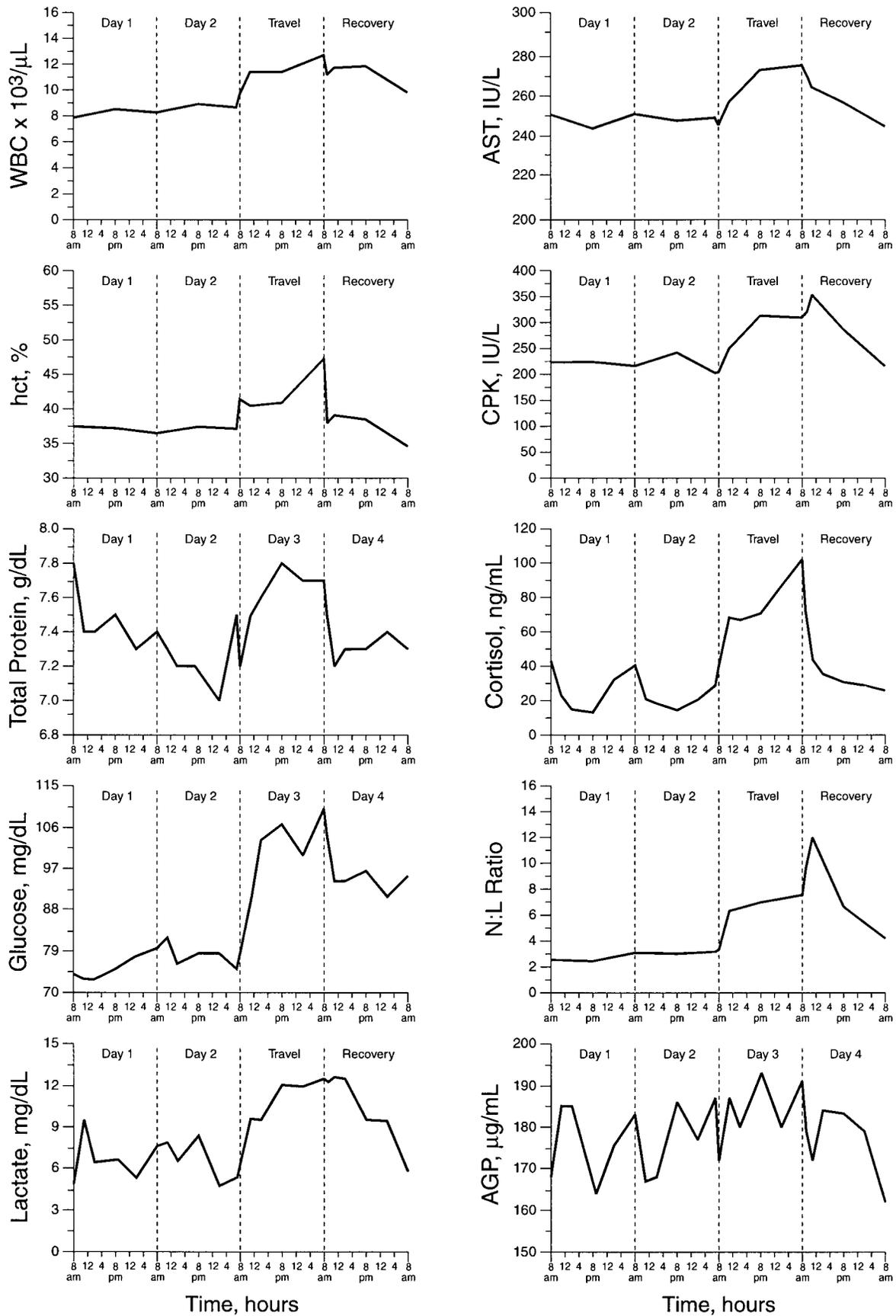


Figure 1. Response of white blood cell counts (WBC), hematocrit (hct), total protein, glucose, lactate, aspartate aminotransferase (AST), creatine kinase (CPK), cortisol, neutrophil:lymphocyte (N:L) ratio, and α_1 -acid glycoprotein (AGP) during baseline days (d 1 and 2), travel (d 3), and the posttransport period (d 4).

loading on d 3, but the initial 3 h of transportation (1100) elevated ($P < .05$) the concentrations of glucose and lactate over the preloading sample collected at 0700 (Table 4). The daily glucose means during transportation (d 3, 94 ± 14 mg/dL) and the posttransport period (d 4, 95 ± 11 mg/dL) were elevated ($P < .001$) compared to the baseline mean (d 2, 78 ± 5 mg/dL). Glucose peaked (105 ± 21 mg/dL) at the termination of 24 h of transport, whereas lactate peaked 3 (12.4 ± 4 mg/dL) to 6 h (12.4 ± 4 mg/dL) into the posttransport period (Figure 1). The daily mean lactate concentration during the posttransport (d 4, 11 ± 3 mg/dL) was increased ($P = .0001$) over baseline (d 2, 7 ± 2 mg/dL). Neither serum concentrations of CPK nor of AST exhibited ($P > .05$) diurnal variation or difference ($P > .05$) between daily baseline means of d 1 and d 2 (Table 2). During transportation (d 3) and the posttransport period (d 4), mean AST concentrations were elevated ($P < .05$) over baseline (d 2), whereas serum CPK was elevated ($P = .03$) over baseline only during the posttransport period. Serum AST activity peaked (275 ± 39 IU/L) at the termination of transport at 0800 on d 4 (Figure 1) but remained in the normal reference range (138 to 409 IU/L; Veterinary Medicine Teaching Hospital, University of California, Davis) throughout the study. The increase in CPK activity was elevated above the reference range (119 to 287 IU/L; Veterinary Medicine Teaching Hospital) following 12 h of transport and remained elevated for 12 h after transport. Peak CPK activity (353 ± 162 IU/L) occurred at 1100 on the posttransport day (d 4) (Figure 1). Both serum enzyme concentrations showed no difference ($P > .05$) during loading (0700 and 0800, d 3) but increased ($P < .01$) with the initial 3 h of transit (0800 and 1100) (Table 4).

Stress Indices. No difference ($P > .05$) in AGP concentrations was shown between any of the daily means, diurnal variation, or in response to loading, but AGP concentrations increased during the initial 3 h of transport (Tables 2 and 4). However, all daily mean AGP concentrations were elevated over the normal range (99 ± 26 μ g/mL) established for healthy, adult horses (Taira et al., 1992). A circadian rhythm in cortisol concentrations has been documented in horses with peak levels (65 ± 2 ng/mL) in the morning (0600 to 1200) and low values (25 ± 2 ng/mL) in the evening (1300 to 1600), with a mean daily concentration of 45 ± 1 ng/mL in resting horses (Stull and Rodiek, 1988). A diurnal rhythm was exhibited in cortisol during baseline days (d 1 and d 2), as expected (Table 2), but there was no difference ($P > .05$) in mean daily (d 1 and d 2) concentration prior to transport. Transportation increased ($P = .001$) cortisol concentrations over baseline values (d 2), and the peak concentration of 101 ± 41 ng/mL was measured at the termination of transport (d 4, 0800). Cortisol increased ($P = .02$) with loading and again during the first 3 h of transport ($P = .0001$) (Table 4). During the posttransport period, cortisol concentrations progressively decreased; however, the daily mean concentration for d 4 was elevated ($P < .002$) above

baseline (d 2). Transportation (d 3) also increased ($P = .0007$) daily mean N:L values over baseline (d 2) (Table 2). No difference ($P > .05$) in N:L was shown between baseline days (d 1 and 2), and no diurnal effect was observed. During both transportation and the posttransport period, N:L values exceeded the normal range established for healthy horses of .8 to 2.8 (Morris and Large, 1990) (Figure 1). Loading did not ($P = .61$) affect the N:L ratio; however, an increase occurred ($P = .03$) during the initial 3 h of transport. The peak (11.9 ± 12.2) in N:L values was exhibited 3 h following unloading during the posttransport period (Figure 1).

Discussion

Heat stress during the summer months contributes to transportation-induced stress. In studies examining the effects of thermal stress on breathing and body temperature of ponies acclimated to summer environmental conditions, the thermoneutral control group experienced ambient temperatures of 21°C and the heat-stressed treatment group was exposed to ambient temperatures of 30°C (Kaminski et al., 1985). At ambient temperatures between 40 and 43°C, horses markedly increased sweating to maintain homeothermy without increasing respiration or heart rate (Honstein and Monty, 1977). In the present study, maximum temperature during each of the four study days exceeded 30°C (Table 1). The ability to dissipate heat is further impaired if humidity levels exceed 50%. The daily maximum humidity levels did exceed 50%, and usually these decreased when environmental temperatures rose.

Diurnal variation in individual physiological parameters must be recognized in study designs when measuring responses over time such as pre- and posttransit measurements. Body weight, rectal temperature, cortisol, lactate, and WBC indices exhibited diurnal variations on d 1 and d 2. The daily fluctuation in rectal temperature was within the normal diurnal variation (.5 to 1.0°C) expected (White, 1990). The diurnal increase in body weight at 2000 (549 ± 45 kg) as compared to 0800 (540 ± 46 kg) on both d 1 and d 2 may partially be attributed to the amount of gut fill. Horses were fed two meals per day, scheduled at 0700, 1 h prior to weighing (0800), and at 1700, which was 3 h prior to weighing (2000). Thus, body weight recorded at 2000 may partially be attributed to the consumption of the 1700 meal. Circadian rhythms of cortisol have been reported in horses, with the highest values exhibited in the morning hours and the lowest in the evening hours (Evans et al., 1977; Stull and Rodiek, 1988). The circadian rhythm of cortisol was reflected in baseline measurements taken on d 1 and 2; peak concentrations occurred at the 0800 sampling and the lowest concentration at 2000. Lactate concentration exhibited diurnal variation with lower values at 0800 and peaks at 1400, but values were within established resting values of approximately 5 to 10 mg/dL (Stull and Rodiek, 1995). The WBC counts were evaluated at only two sampling

points (0800 and 2000) on d 1 and 2, and the diurnal variation exhibited less than an 8% increase ($1 \times 10^3/\mu\text{L}$) in WBC at 2000.

Several mean daily measurements differed on the two pretransit days (d 1 and 2) (Table 2). These differences may be due to many undetermined factors, but horses may have been less familiar and experienced on d 1 than on d 2 with the procedures performed. Thus, the data collected on d 2 were used for baseline comparisons with transport and posttransport data.

Body weight, rectal temperature, and WBC were measured as general indicators of health and the ability of the horses to handle the heat during transport. The 6% weight loss immediately following transit may be due to heat dissipation, sweat loss, and decreased gut fill during transit. After 3 h of posttransport recovery time in stalls with ad libitum water availability, the weight loss was only 2.4%, even though horses were offered water from buckets and consumed 22.7 ± 6.4 L while in transit. Following a 24-h posttransport period, there remained a 3% deficiency in body weight loss (Table 3). Weight loss has been documented at 4% in horses undergoing commercial transportation to slaughter in summer conditions with duration of transport from 6 to 30 h (Stull, 1999). Although rectal temperature was not recorded during transit, it was within the normal range for a healthy horse (37.5 to 38.5°C) immediately following transport and during the posttransport day. This may support the notion that horses respond to heat stress during transit through respiration and sweating mechanisms. The WBC counts showed a progressive increase with the duration of travel and peaked at the termination of travel. Even though not significant, the final WBC count (d 5, 0800) ($9.8 \pm 2.7 \times 10^3/\mu\text{L}$) was elevated ($P < .06$) over baseline (d 2, 0800) ($8.3 \pm 1.3 \times 10^3/\mu\text{L}$) (Table 3).

Hematocrit and total protein concentration are often used as indicators of dehydration in horses. Hematocrit and total protein showed similar results, with incremental increases during transit and a decline to baseline levels during the posttransport period. Peak levels of both measurements occurred during the last 12 h of transport but were within the reference range for a normal horse. Interestingly, during the last 12 h of transport, when hct exhibited the largest elevation (41 ± 4 to $47 \pm 4\%$), 91% of water offered was consumed. The increase of hct during loading is probably due to splenic contraction from sympathetic stimulation (Carlson, 1990) rather than to dehydration.

Lactate produced by anaerobic metabolism was measured as an indicator of muscle fatigue. Lactate concentrations showed diurnal variation on d 1 and d 2, with slight increases occurring in the afternoon and evening. The resting range for lactate concentration in horses is 5 to 10 mg/dL (Stull and Rodiek, 1995), and levels over 200 mg/dL have been reported following racing (Snow et al., 1983). Daily means of d 1, d 2, and d 3 were within the established resting lactate range of 5 to 10 mg/dL, and lactate concentration on d 4 was slightly

above this range. Peak lactate concentration (12.4 ± 4 mg/dL) occurred 3 to 6 h after transport. This indicates that there was minimal muscular fatigue due to transportation in these horses.

Two serum enzymes with high activity in skeletal muscle and evaluated clinically in horses with muscular diseases are CPK and AST (Hodgson, 1990). Moderate increases in CPK (400 to 500 IU/L) are associated with moderate exercise or initiation of a training program, whereas more strenuous and fatiguing exercise such as 3-d events or endurance competitions may evoke increases over 1,000 IU/L. However, myopathies such as exertional rhabdomyolysis are diagnosed with activities from several to hundreds of thousands of international units per liter. In this study, the CPK peaked at 353 ± 161 IU/L following transport, which is slightly elevated over the reference range but less than in horses undergoing moderate exercise. The activity of AST remained in the reference range but rose in response to transport and returned to baseline in the 24-h posttransport period. The limited increase in the two serum enzymes indicates minimal muscular insult due to 24 h of transport.

During stressful situations such as exercise or transportation, activation of the hypothalamic-pituitary-adrenal axis results in an increase in plasma cortisol. Plasma cortisol concentration exhibited diurnal variation, increased during loading and the first 3 h of transport, and continued to rise throughout the 24-h cycle to peak (101 ± 41 ng/mL) at the termination of transport. After the stressor (i.e., transportation) ceased, cortisol dramatically declined. This is probably due to cortisol's relatively short half-life of 1 to 1.5 h (Lassourd et al., 1996). The peak in cortisol concentration was greater than posttransit cortisol levels measured in slaughter horses traveling in groups in two-tiered or straight-deck trailers for up to 30 h (Stull, 1999) but similar to concentrations in moderately exercised horses (Stull and Rodiek, 1995).

Cortisol release due to stress may lead to neutrophilia and lymphopenia, and thus increase the N:L ratio. Gross and Siegel (1983) reported that the N:L ratio may be a more reliable indicator of stress than cortisol concentration. The N:L peak (11.9 ± 12.2) was comparably greater than N:L values reported for exercising horses (Stull and Rodiek, 1995) and N:L values for slaughter horses following transportation for up to 30 h (Stull, 1999). This elevation in the N:L ratio may be due to the long-term effect of the stressor on the release of cortisol. The individual partitioning of horses and the restricted movement of the head and neck due to cross-ties during transport in the study van may have contributed to increased stress compared to that of slaughter horses which were transported loose in small groups without any head restrictions under similar environmental conditions (Stull, 1999).

Glucocorticoids may also initiate parts of the acute phase response, such as production of acute phase reactive proteins such as AGP (Stone and Mauer, 1987;

Itoh et al., 1992; Taira et al., 1992). The acute phase response can be initiated by disease, inflammation, surgery, and other types of stress. The function of AGP is not completely known, but it is reported to have immunosuppressive effects and aids in tissue repair (Taira et al., 1992). Although not significant ($P = .10$), transportation tended to increase the daily mean AGP compared to baseline values (d 2) with a significant ($P = .04$) rise during the initial 3 h of transport. The AGP, WBC, and N:L responses to transportation suggest that the immune system may have been compromised, which could increase susceptibility of the horses to infectious diseases. Published research articles on posttransit respiratory disease in horses are numerous (Laegreid, et al., 1988; Crisman et al., 1992; Oikawa et al., 1994).

Implications

Horses undergoing 24 h of transportation in hot, summer conditions showed physiological responses that included changes in stress indices, serum metabolites, dehydration and immune indices, body weight, and rectal temperatures. The metabolite responses appeared to be minimal compared to those in moderately exercised horses and were limited to the period of transport. Effects of dehydration due to high environmental temperatures and limited water consumption during transport diminished remarkably within a few hours of the cessation of transport. However, the large incremental rise in cortisol concentration during transport may have influenced the immune system, exhibited by an increase in white blood cell count and elevated neutrophil:lymphocyte values. This may contribute to disease susceptibility following long-term transport. Additionally, elevated glucose concentration following transport may alter energy metabolism during an athletic endeavor performed within 24 h after transit.

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