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**Left running head:** Holcomb et al.

**Right running head:** Horses Response Shade or Sun

**Physiological, behavioral, and serological responses of horses to shaded or unshaded pens  
in a hot, sunny environment<sup>1</sup>**

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## ABSTRACT

Housing recommendations for horses invariably include providing access to shade on hot, sunny days but the potential benefits have not been scientifically studied. This experiment measured physiological, behavioral and serological responses of horses confined individually to completely shaded (SH) or completely unshaded (SUN) dry-lot pens during the summer in Davis, CA. Twelve healthy adult horses in a crossover design experienced both treatments for 5 d each. Rectal temperature, respiration rate, skin temperature, and sweat scores were recorded hourly from 1230 to 1730 h daily. Observations were recorded from 1200 to 1800 h for proximity to water, foraging, locomotion, and insect avoidance behaviors. Daily blood samples were obtained to measure cortisol, hematocrit, and neutrophil-to-lymphocyte ratio. Automated and handheld sensors were used to record environmental conditions. The mean ambient temperature from 1200 to 1800 h during the study was 30.6°C. Rectal temperature was greater for horses in SUN than for SH (mean 37.8°C and 37.5°C respectively, SE 0.06,  $P = 0.002$ ) as was respiration rate (25.5 and 20.5 breaths/min, SE 1.3,  $P = 0.008$ ), and skin temperature (35.6°C and 34.6°C, SE 0.1,  $P < 0.001$ ). Horses in SUN showed sweat in 51.4% of observations versus 1.1% for horses in SH. Horses in SUN spent more time than SH horses standing near their water source (34.0% of observations versus 20.2%, SE 0.3,  $P = 0.004$ ). No differences were observed for foraging, locomotion, or insect avoidance behavior ( $P > 0.05$ ). Cortisol concentrations were greater in SUN than SH (3.4 and 2.6 ug/dL, respectively,  $P < 0.001$ ) but remained within the normal range for resting horses. No treatment differences were observed for hematocrit or neutrophil-to-lymphocyte ratio ( $P > 0.05$ ). Horses exhibited treatment differences in the physiological measures first, most notably in rectal temperature at 1230 h corresponding to

peak solar radiation. Behavioral responses followed these physiological changes, with treatment differences in time standing near water becoming apparent at 1400 h as ambient and black globe temperature increased. Our results indicate that both the SH and SUN treatment groups exhibited thermoregulatory responses to these summer conditions and horses benefited from shade, as it mitigated these physiological and behavioral changes. These results are applicable in developing best management practices for the care of domestic horses.

**Key words:** animal welfare, animal well-being, behavior, horses, housing, shade use

## INTRODUCTION

Provision of shade for horses in hot, sunny environments is a common recommendation (Miller et al., 2011), but one that has not been scientifically studied. There is limited evidence available about heat tolerance and shade use in horses at rest. Anecdotally, horses have been reported to survive in environments with temperatures reaching 58°C (Hafez, 1968); however, that report did not describe mitigating factors such as access to shade and duration of exposure to extreme temperatures. Horses will use man-made shelters (Heleski and Murtazashvili, 2010), trees or other vegetation (Arnold and Grassia, 1982; Pratt et al., 1986; Stebbins, 1974), and in the case of foals, the shade cast by their dams (Siskova et al., 2006). Conversely, increased insect density has been suggested as a reason horses might avoid shade where vegetation is present (Keiper and Berger, 1982). Despite these observations, it is sometimes suggested by researchers, horse owners, and farm managers that horses do not need and rarely use available shade (Cymbaluk and Christison, 1990; Honstein and Monty, 1977).

While evidence about shade use in horses is anecdotal, there has been extensive research on the effects of hot weather on horses undergoing both strenuous exercise and road transport. These studies demonstrated that horses show thermoregulatory and stress responses including elevated rectal temperature, respiration rate, neutrophil-to-lymphocyte ratio, serum cortisol concentrations, and hematocrit percentages (Friend, 2000; Marlin et al., 1995; Schroter and Marlin, 1995; Stull and Rodiek, 2000). For exercising horses, shade has been recommended as a management practice to prevent heat stress (Jeffcott et al., 2009).

To understand how the biological and behavioral responses of horses to summer heat are affected by shade, we evaluated individually-housed horses that were either completely shaded or completely unshaded in a hot, sunny environment. We hypothesized that physiological,

behavioral, and serological responses of horses to these summer conditions would be favorably altered by shade provision.

## MATERIALS AND METHODS

Approval for this study was obtained from the University's Institutional Animal Care and Use Committee (protocol #16034).

### *Animals and Design*

The study was performed at the Center for Equine Health (CEH) facilities located on the University of California at Davis campus in Davis, California, during July of 2011. Twelve healthy adult horses, 5 mares and 7 geldings of Quarter Horse ( $n = 4$ ) and Thoroughbred ( $n = 8$ ) breeds, were used in the study. The mean age was  $10.5 \pm 3.2$  y, body weight was  $581 \pm 48$  kg, and body condition score (**BCS**) was  $5.8 \pm 1.3$  (Henneke, 1985).

A randomized crossover design with 2 treatments levels, completely shaded (**SH**) and completely unshaded (**SUN**) pens, was used in each of 2 trials. There were 3 pens/treatment and 1 horse/pen in each of the trials ( $n = 12$  horses). Each 12-d trial consisted of a 2-d acclimation period to adjust to this location and the individual housing, followed by a 10-d data collection period with 5 d per treatment in a crossover design. Order of exposure to treatments was balanced across horses. During the 2-d acclimation phase, horses were housed in shaded pens adjacent to the study pens.

The 3 completely shaded pens (**SH**) were constructed using 6-rail pipe livestock panels beneath an existing covered, open-sided, free-standing, corrugated metal roof structure that was oriented east-west on its long axis (18.3 m wide x 43.9 m long; the gable-style roof was 4.6 m above ground at center and 3.7 m above ground on each side with a 10% slope). All study pens

were 6.1 x 5.5 m in dimension and were located on the south side of the roofed area to prevent encroachment of direct sunlight into the pens as the summer progressed (Fig. 1). The 3 completely unshaded pens (SUN) were also constructed of 6-rail pipe panels in the same dimensions within an adjacent, uncovered paddock, and these pens did not receive shade from any structure or vegetation. The flooring in all pens was a packed dirt surface and no bedding was provided.

Horses were fed alfalfa hay at approximately 1.75% of their BW daily on an as-fed basis (hay analysis 20.2% CP, 93.7% DM). Hay was fed at ground level on the west side of all pens at approximately 0800 h and 1700 h daily. Water was provided to each horse in two, 18-L buckets secured to the fence on the northwest corner of the pens. Because cattle have been shown to stand near water in hot ambient temperatures (Widowski, 2001; Schütz et al., 2008), additional water buckets were placed outside the third pen in each treatment such that all horses were able to stand near their own water as well as near water in the adjacent pen (Fig. 1). A trace-mineralized salt block was available on the ground in each pen. Pens were cleaned daily at 1100 h by removing manure and any remaining hay.

### ***Data Collection***

***Weather Conditions.*** Weather factors were recorded during each of the 10 observation days of each trial by sensors with automated data loggers located in shaded and unshaded areas adjacent to the study pens in locations inaccessible to the horses. Factors recorded were ambient temperature ( $T_{amb}$ ) and relative humidity (**RH**; Model U23-002 with solar shields, Onset Computer Corporation, Bourne, MA; accuracy 0.2°C, resolution 0.02°C) and black globe temperature ( $T_{BGT}$ ; data logger models H08-031-08 and H08-004-02, Onset Computer

Corporation, Bourne, MA). These sensors were affixed to support poles at a height of 2.4 m. The locations were switched between shaded and unshaded areas on alternate days to control for potential instrument differences. Solar radiation was measured with 2 silicon pyranometers (S-LIB-M003 with H21-002 automatic logging microstation, Onset Computer Corporation, Bourne, MA; accuracy 5%). The pyranometers were affixed to support poles at a height of 1.8 m. Pyranometer locations were not rotated because of the precision required for installation. Data from all automated loggers were recorded at 5 min intervals for 24 h/d throughout and averaged by hour.

Wind speed was measured with a hand-held anemometer (Speedtech Instruments SM-18 SkyMate Wind Meter, Weathershock.com, Roanoke, VA; accuracy 5%) in the shaded and unshaded areas at a height of 1.8 m once each hour between 1200 to 1800 h.

Ground soil temperature ( $T_{\text{soil}}$ ) was measured with a handheld infrared thermometer (Raytek MT6, Raytek, Santa Cruz, CA, accuracy 1.5°C) at a height of 0.6 m in front of each pen once per hour from 1200 to 1800 h. Drinking water temperature ( $T_{\text{water}}$ ) was also measured with the infrared thermometer in 2 shaded and 2 unshaded buckets at a distance of 0.1 m from water surface once per hour from 1200 to 1800 h.

The temperature-humidity index (**THI**) was calculated from  $T_{\text{amb}}$  °C and RH using the Schütz et al. (2010) modification of the formula used by Kelly and Bond (1971):

$$\text{THI} = (1.8 \times T_{\text{amb}} + 32) - [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T_{\text{amb}} - 26)].$$

**Physiological Measures.** Rectal temperature (**RT**), respiration rate (**RR**), skin temperature (**SK**), and sweat score were measured hourly from 1230 to 1730 h. For these measurements a lead rope was clipped to the horse's halter and the horse was positioned to stand



with its head facing north. Rectal temperatures were taken with a lubricated digital livestock thermometer (Model L2214, GF Health Products, Inc., Atlanta, GA) inserted 7 cm into the rectum. Respiration rate was measured by observing flank movement and recording the elapsed time for 10 full breaths using a stopwatch (Model 470, Sportline, Yonkers, NY). The values were subsequently converted to breaths/min (**bpm**). Skin temperature was measured with a handheld thermometer (Model HH-25TC with Type T Thermocouple, Omega Engineering, Stamford, CT; resolution 0.1°C, accuracy 0.6°C) placed directly over a standardized location on the left and right triceps muscles. The skin area and thermocouple were shaded as needed to prevent the sun's radiant energy from affecting the measurement. Values for the 2 locations were averaged for each time point. The presence of visible, wet sweat was recorded in 5 specific regions of the body (neck, chest, girth, flank, and hindquarters) on a pre-printed drawing of the outline of a horse (Fig. 2). The sweat score was the total number of regions marked ranging from 0 for no sweat to 5 if all of the specified regions were involved (George, 2010). Daily water consumption was calculated by measuring the depth of water remaining in each bucket at 0900, 1100 and 1800 h and converting to liters of water consumed per 100 kg body weight (**L/100 kg BW**) using a regression equation. Buckets were refilled after measurement.

***Behavioral Measures.*** Behavioral measures consisted of recording observations on each horse daily during each 5-d observation period using instantaneous scan sampling and focal sampling (Martin and Bateson, 2007) following the definitions in Table 1. On d 1 to 5 during observation periods, instantaneous scan sampling was performed at 5-min intervals from 1200 to 1800 h to record standing near (within 1 m) or away from water bucket, drinking, locomotion, foraging, and recumbent or rolling behavior. Tape had been placed on the pipe panel rails 1 m

from buckets for accuracy of assessment of proximity to the water buckets. The order in which horses were scanned was randomized for each observation day. Behavioral data were not collected from any horse that was undergoing physiological measurements at the moment of a scan. Data were calculated for each horse as the percentage of observations in which the behavior was recorded during each observation hour.

Focal sampling was used to record insect avoidance behaviors for each horse for 1 min/h between 1200 and 1800 h on each observation day. Data for head movements and hoof stomps were combined as a count per d of total insect avoidance behaviors for each horse. To estimate the number of flying insects, adhesive fly strips (4x60 cm, Catchmaster #9144, AP&G Co, Inc., Brooklyn, NY) were suspended 1 m above the ground. One strip was placed in a shaded and unshaded area just outside the study pens. The number of winged insects trapped over each 24 h period was recorded and the strips replaced at approximately 1145 h daily, before the start of observations.

Ten student observers were trained before the study to perform on-site behavior observations. A single observer stood a minimum distance of 15 m from the pens to reduce the effect of observer presence on the horses' behavior. Student observers were tested for reliability before the start of observations (Martin and Bateson, 2007). Students observed at least 6 horses 10 times. For instantaneous scan sampling students averaged  $96.5 \pm 3.0\%$  (range 92.0 to 100%) agreement with the researcher (KEH). For focal sampling, the correlation coefficient between observers averaged  $0.97 \pm 0.01$  with a range of 0.95 to 0.98.

***Serological Measures.*** Two blood samples (5 mL each) were collected at 1800 h on d 0 and d 1 to 5 during each observation period by jugular venipuncture into evacuated collection

tubes. Samples were refrigerated overnight and processed at 0900 h the following day. One sample, collected with EDTA, was processed by the UC Davis Veterinary Medicine Teaching Hospital's hematology laboratory to measure hematocrit (**HCT**) and the number of neutrophils and lymphocytes (Advia 120 Hematology System, Siemens Healthcare Diagnostics, Deerfield, IL). The neutrophil-to-lymphocyte ratio (**N:L**) was subsequently calculated. The second sample tube, without anticoagulant, was centrifuged at 1500 x g for 10 min at room temperature (Marathon 6K unrefrigerated centrifuge, Fisher Scientific, Pittsburg, PA) and serum was stored at -80°C. Subsequently, serum cortisol was measured in these samples by the UC Davis Veterinary Medicine Teaching Hospital's chemistry laboratory using an automated chemiluminescent assay (Immulite 2000, Siemens Healthcare Diagnostics, Deerfield, IL; intra-assay CV 11%, sensitivity 0.3 ug/dL). All samples for cortisol analysis were submitted on the same day and measured in a single assay.

### *Statistical Analysis*

All behavior and weather parameter data within an hour were averaged to obtain values for statistical analysis. Rectal temperature, RR, SK, and sweat score were measured and analyzed by hour. Water consumption, serology variables, and count of insects were each analyzed on a daily basis.

Recumbent and rolling behavior was recorded in less than 1% of observations (7 of 720) and was eliminated from further analysis. In addition, because horses were fed at 1700 h and consequently were eating hay in 86% of observations from 1700 to 1800 h, behavioral data are not reported for that final hour, with the exception of insect avoidance behavior.

Initial analyses were run to test the effects of treatment and time along with trial, order, breed, BCS, and sex using Proc GLM in SAS (SAS 9.3, SAS Institute, Inc., Cary, NC). There was a significant effect of trial ( $P < 0.05$ ), and it was retained in all models. There was a significant effect of breed for RT and of order for SK ( $P < 0.05$ ), and these factors were retained in the models for those variables as specified below. There was no effect of BCS or sex on any variable ( $P > 0.05$ ). A t-test was used to evaluate the d 0 values of HCT, N:L, and cortisol to ascertain that there were no treatment differences between horses at baseline.

Three models were used in analysis. First, to test the effect of treatment (SH, SUN), hourly data were averaged by horse for each day and then analyzed using Proc Mixed in SAS. The model for RT, RR, SK, HCT, N:L, cortisol, and all behaviors included treatment (SH, SUN), trial (1, 2), day (1 to 5) and interactions, with horse nested in trial as a random term. Breed was included in the model for RT, as well, with horse nested in trial and breed. The model for count of insects included treatment, trial, day, and interactions. This model was then modified to include the daily weather covariates  $T_{amb}$ ,  $T_{soil}$ , solar radiation,  $T_{BGT}$  and THI, separately, as measured by sensors in the unshaded area, to look for potential effects and interactions with treatment using Type I analysis. Non-significant interaction terms ( $P > 0.05$ ) for treatment with the weather factor were removed from the model.

Second, to assess the effect of time of day, hourly data were averaged across days by horse and treatment. Times for physiological measures were 1230, 1330, 1430, 1530, 1630 and 1730 h; times for standing near water, locomotion, and foraging were the hours beginning at 1200, 1300, 1400, 1500, 1600 h; insect avoidance behavior also included the hour beginning at 1700 h. The model (Proc Mixed) for RT, RR, standing near water, locomotion, and foraging included treatment, time and trial, with horse nested in trial as the random term. Breed was

included in the model for RT, with horse nested in trial and breed. Order was included as well in the model for SK, with horse nested in trial and order.

Data were tested for homogeneity of variance using the Levene Test and for normality using the Shapiro-Wilk statistic (Sachs, 1984). Data for drinking were not normally distributed and were Winsorized from the top and bottom 5% (Dixon and Tukey, 1968). Cortisol data were not normally distributed; these were log transformed and then Winsorized from the top and bottom 5%. Back-transformed means are reported for cortisol. Least squares means for other variables were calculated using the Tukey-Kramer Least Squares Means adjustment (Day and Quinn, 1989) and are reported with their standard errors. For all models significance is reported at  $P < 0.05$ .

Third, potential correlation between sweat and  $T_{amb}$ , solar radiation,  $T_{soil}$ ,  $T_{BGT}$  and THI were analyzed using a MANOVA statement in Proc GLM in SAS, with trial, day, time, and horse nested within trial included in the model. The resulting error sums of squares and cross products (**SSCP**) matrices for horse nested within trial were used to calculate coefficients of variation (**R**).

## RESULTS

The average daily  $T_{amb}$  during the study was 22.3°C and average peak  $T_{amb}$  was 33.4°C, with 61.7% RH and no precipitation. Mean  $T_{amb}$ , RH, THI,  $T_{BGT}$ , solar radiation,  $T_{soil}$ , wind speed, and  $T_{water}$  averaged over 24 h and over the daily 6-h observation period during the study are shown in Table 2. In both SUN and SH, solar radiation peaked at approximately 1300 h,  $T_{BGT}$  peaked at 1500 h, and  $T_{amb}$  peaked at 1600 h, whereas  $T_{soil}$  peaked at 1400 h in SH and at 1500 h in SUN.

The means for physiological, behavioral, and serological variables, water consumption, and count of flying insects in each treatment are listed in Table 3. Mean RT, RR, and SK of horses in the SUN treatment were consistently greater than in the SH treatment (Fig. 3). There was an interaction of treatment with time for RT ( $P < 0.001$ ) with RT in SUN greater than in SH in early and midafternoon (Fig. 3). There was also an interaction trend for RR ( $P = 0.068$ ) with greater RR in SUN than SH at 1330 and 1530 h (Fig. 3). The differences between treatments increased with elevated  $T_{BGT}$ ,  $T_{soil}$ , and solar radiation for RT and SK and with elevated  $T_{BGT}$  and  $T_{soil}$  for RR ( $P < 0.001$ ). Horses in the SH treatment exhibited sweat in only 4 of the 360 observations (1.1%), whereas horses in the SUN treatment exhibited sweat in 51.4% of observations with a mean score of 1.0 ( $SD \pm 0.07$ ; Fig. 3). Because there were so few observations in the SH treatment, sweat score was not analyzed further for treatment differences, although the correlation of weather factors with sweat scores of horses in SUN was evaluated. There was a strong between-horse correlation for all weather factors and THI with the sweat scores of horses in SUN ( $R > 0.75$ ).

Several horses were observed to splash the contents of their water buckets resulting in missing data for 9 of 60 (15%) water consumption measurements in SUN and 11 of 60 (18.3%) measurements in SH. Horses in SUN stood within 1 m of water buckets during a greater percentage of observations and consumed more water than horses in SH ( $P < 0.05$ , Table 3), but they did not show more drinking behavior ( $P = 0.269$ , Table 3). Time of day affected treatments as horses stood near water more in SUN than in SH at 1400 and 1500 h (time x treatment  $P = 0.018$ , Fig. 4).

No treatment differences were observed for foraging or locomotion but there were effects of time ( $P < 0.05$ ) as all horses decreased activity from 1300 to 1500 h then markedly increased

activity during the hour before they were fed ( $P < 0.001$ ; Fig. 5). Although more flying insects were trapped in SUN than in SH ( $P < 0.001$ , Table 3), there was no difference between treatments for insect avoidance behavior. Similar to foraging and locomotion, there was an effect of time on insect avoidance behavior with a decrease observed at 1500 and 1600 h ( $P = 0.002$ ; Fig. 5).

Horses showed no differences of baseline samples (d 0) for HCT, N:L, or cortisol ( $P > 0.05$  for 2 tailed t-test). There were no differences between horses in the SH and SUN treatments for N:L or HCT for the 5-d treatment period; however, cortisol concentration was greater for horses in SUN than in SH (Table 3). In addition, there was an interaction between treatment and day for cortisol, with horses in SUN having slightly greater values than in SH on d 4 and 5 (d 4: 3.3 vs. 2.4 mg/dl and d 5: 3.1 vs. 2.2 mg/dl, respectively,  $P < 0.05$ ).

## DISCUSSION

### *Physiological Measures*

The thermoneutral zone is the range of ambient temperatures within which homeothermic animals need not expend energy above that needed for maintenance to maintain core body temperature (Curtis, 1983). The upper critical temperature (UCT) of the thermoneutral zone for horses has been estimated to range from 20°C, at onset of increasing evaporative heat loss, to 30°C when vasodilation of peripheral tissues is greatest (Morgan, 1998). Consistent with this range, horses in the SUN treatment had already activated thermoregulatory mechanisms at 1230 h when average  $T_{amb}$  was 29°C, as indicated by greater RR and SK than horses in SH. Horses in this study were housed in open-air pens and were free to move around. Estimation of UCT is based upon differences in metabolic rate at designated  $T_{amb}$  under closed, environmentally-controlled conditions, e.g. hygrometric tents with stationary horses (Morgan et al., 1997). Thus,

under outdoor conditions and when horses have some ability to regulate their own comfort,  $T_{amb}$  alone is not an adequate indicator of the upper threshold of the thermoneutral zone for horses. Instead, other potential indicators of comfort such as RT, RR, SK, and behavior may provide insight into when and how horses begin to respond to environmental conditions.

Body temperature is highly conserved in mammals to maintain the narrow range required for effective metabolic processes. A deviation of 0.2 to 0.4°C outside the normal range in humans is known to trigger a thermoregulatory response (Sessler, 1997). The mean RT for horses in the SUN treatment was on average 0.2°C greater than in the SH treatment, although still within the normal range for horses of  $37.7 \pm 0.5$  °C (Kahn et al., 2005). Rectal temperature increased throughout the 6-h sampling period for both treatments in agreement with known circadian rhythms (Piccione et al., 2002). Interestingly, the largest difference between treatments in RT in the present study was recorded during the first measurement at 1230 h. This suggests that horses had begun responding to increasing solar radiation several hours before the greatest ambient temperature was recorded in late afternoon, the time at which treatment differences in RT became non-significant. The consistently greater RT for horses housed in SUN from 1230 to 1530 h is indicative of the challenge to thermoregulatory mechanisms posed by their inability to seek relief from the sun. Similarly, increases in RT of 0.3 to 0.6°C have been noted in cattle without access to shade compared to those with shade (Brown-Brandl et al., 2005; Gaughan et al., 2010).

In an effort to prevent elevated body temperature, horses can dissipate heat via evaporative cooling by increasing respiration rate and sweating. In the present study, horses in the SUN treatment showed greater RR than those in the SH treatment at 1330 and 1530 h, and only horses in the SUN treatment exhibited appreciable sweat. The RR for horses in both SUN



and SH was above the normal reference range of 10 to 14 bpm under thermoneutral conditions (Kahn et al., 2005). These slightly elevated RR values are similar to other reports of equine RR at summer temperatures. A RR of 23 bpm was reported for ponies in environmental chambers at  $T_{amb}$  30°C (Kaminski et al., 1985), and RR of 31.7 bpm reported for nine horses measured outdoors while confined to stocks on days with an average  $T_{amb}$  of 37.9°C (Honstein and Monty, 1977). In comparison, RR reached 180 bpm in fit horses undergoing strenuous exercise on a treadmill in a climate controlled room at 30°C, 80% RH (Marlin et al., 1999), indicating that there is considerable range in the use of respiration to cope with thermal load in horses.

The mean SK of horses in SUN was greater than horses in SH at all time points and as  $T_{BGT}$ ,  $T_{soil}$ , and solar radiation increased the difference between the two treatments became larger. A black globe thermometer measures the combined effect of ambient temperature and solar radiation, whereas ground soil absorbs solar radiation as heat and also reflects heat back into the environment. The present study suggests that elevated SK of horses in SUN was a response to a combination of  $T_{amb}$  with radiant heat from both direct sunlight and the soil, in contrast to the SH treatment for which neither horses nor soil were exposed to direct sunlight. These differences in exposure were also likely responsible for horses sweating almost exclusively when they were in the SUN treatment. This study did not attempt to quantify the rate or amount of sweat. Rather, it was an estimate of the magnitude of the horse's response to the weather conditions, with a larger score indicating involvement of more body regions. Notably, sweat scores of horses in the SUN treatment peaked just after the time of maximum solar radiation and at approximately the same time as  $T_{soil}$ , and then decreased even as SK continued to rise.

As with SK, treatment responses for RR and RT interacted with weather factors. Horses in SUN showed greater RT at elevated levels of  $T_{BGT}$ ,  $T_{soil}$  and solar radiation and showed

greater RR with elevated  $T_{BGT}$  and  $T_{soil}$  than horses in SH. Again, because the shade structure blocked direct sunlight, horses in the SH treatment were not exposed to these same levels of  $T_{BGT}$ ,  $T_{soil}$  and solar radiation. These results further support the importance of shade at greater levels of solar radiation. Respiration rate did not interact with solar radiation as RT did. It is believed that different mechanisms control RR and RT, with respiration playing an active role in thermoregulation whereas RT is an outcome of thermoregulatory processes (Kabuga, 1992).

Horses in SUN consumed 30% more water than horses in SH even though their water was on average 7°C warmer than the water in SH. The daily water intake for horses at maintenance on a hay diet is 4 to 6 L/100 kg BW under thermoneutral conditions (NRC, 2007). Horses in SH consumed water at the upper level of this range, 6 L/100 kg BW. In contrast, control horses in a transport study consumed 7.9 L/100 kg BW per 24 h while confined to pens in full sun with maximum  $T_{amb}$  of 37°C (Friend, 2000), compared to the intake by horses in SUN of 7.2 L/100 kg BW in this study with an average maximum  $T_{amb}$  of 33.1°C. Lactating mares increased drinking frequency with increasing ambient temperature (Crowell-Davis et al., 1985), but there was no difference in volume consumed by dehydrated horses when offered water that was 20°C or 30°C (Butudom et al., 2004). Increased water intake could replace loss through respiration and sweating, and can cool tissues in the mouth and upper digestive tract when lower in temperature than the body.

### ***Behavioral Measures***

Horses in the SUN treatment stood within 1 m of their water source 1.7 times as often as those in SH even though drinking behavior (i.e., time spent with muzzle in bucket) itself was equivalent. Horses in SUN may have taken in a larger volume in each drinking bout or could

have consumed more during non-observation hours than horses in SH. In addition, the time of day for the largest treatment differences in standing near water coincided with the peak  $T_{amb}$  and  $T_{BGT}$ . Dairy (Schütz et al., 2010) and beef cattle (Widowski, 2001) without shade or with access to limited shade spend more time near water than those with ample available shade. Both horses (Cymbaluk and Christison, 1990) and cattle (Gaughan et al., 2008) increase consumption of water as  $T_{amb}$  rises. Standing in close proximity to water may be a direct reflection of this increased consumption or may indicate that the water source provides a favorable microclimate to animals without access to shade (Schütz et al., 2010). Several horses that splashed their water resulting in wet areas of skin may have also taken advantage of cooling via surface evaporation.

Foraging behavior consisted of horses sifting through matter in the dirt-packed floor in search of stray leaves and stems, because any remaining hay from the morning feeding was removed before daily observations. Foraging behavior decreased through the afternoon and then increased markedly, presumably in anticipation of feeding at 1700 h. The afternoon decrease may have been mediated by a number of factors including termination of the behavior due to lack of success, by reduced activity due to increasing  $T_{amb}$ , crepuscular feeding, or possibly a circadian pattern of afternoon dozing. Przewalski horses with available feed exhibited lower activity at the hottest part of the day (Berger et al., 1999; Boyd et al., 1988), suggesting that other factors, including the hot sun, may play a role in the variation of afternoon activity.

Although there were more flying insects in SUN, horses showed no treatment difference in insect avoidance behavior. Similarly, there was no difference in insect avoidance behavior between shaded and unshaded dairy cattle (Kendall et al., 2007). Insect avoidance is believed to take precedence over heat reduction in free-roaming horses, with horses spending more time in open, sunny areas lacking vegetation that have lower fly densities; although, those areas also

tend to have higher wind velocities that would aid thermoregulation (Duncan and Cowtan, 1980; Keiper and Berger, 1982). Wind speed did not differ between SUN and SH treatments in this study, and no vegetation was present in the environment of either treatment. All horses decreased insect avoidance behavior in midafternoon corresponding to the time when the least amount of locomotion and foraging activity also was observed.

### *Serological Measures*

Horses in this study remained within normal reference ranges for HCT of 32 to 48% (Kahn et al., 2005), N:L of 0.8 to 2.8 (Morris and Large, 1990), and cortisol with a normal diurnal range of 2.5 ug/dL in the evening to 6.5 ug/dL in the morning (Stull and Rodiek, 1988; Stull and Rodiek, 2002; Giannetto et al., 2011). An elevated HCT can be indicative of dehydration in hot weather if water consumed directly and in feed is not adequate to replace fluid lost through sweating, respiration, urination, and defecation. The lack of difference in HCT between treatments is likely a reflection of the increased water consumption by horses in SUN.

Stress-related increases in glucocorticoid concentrations induce migration of leukocytes resulting in an elevated N:L (Davis et al., 2008). Whereas cortisol has a short half-life (1 to 1.5 h) and shows a pulsatile release pattern (Evans et al., 1977; Lassourd et al., 1996), the N:L ratio shows a more gradual departure from and return to baseline concentrations, making both measures valuable for evaluating potential stressors (Davis et al., 2008). There is substantial literature showing that transportation (Linden et al., 1991; Friend, 2000; Fazio et al., 2008; Stull et al., 2008; Andronie et al., 2010) and human-directed exercise of horses (Linden et al., 1991; Williams et al., 2002; Gordon et al., 2007) are associated with markedly increased concentrations of cortisol, with concentrations as high as 12.0 ug/dL after transport reported by Stull et al.

(2008), and 13.0 ug/dL after exercise reported by Williams et al (2002). Similarly, increased N:L was associated with transportation stress, with a peak of 12.0 after 24 hour transport reported by Stull and Rodiek (2002). A study of heat stress in feedlot cattle showed a significantly lower N:L in shaded animals and the authors suggested that shade might have prevented “mild, chronic stress” caused by heat (Mitloehner et al., 2002). In our study, cortisol showed a significant treatment difference, yet in both treatments the values remained well within the normal reference range for sedentary, mature horses. This finding, combined with the lack of difference in N:L, suggests that environmental conditions experienced by horses in SUN were not of sufficient intensity to stimulate the hypothalamic-pituitary-adrenocortical axis to increase cortisol nor to subsequently alter the N:L ratio. Indeed, the greater RT and RR of horses in SUN, which were also within the normal clinical range for healthy horses at rest, as well as the differences in the other measured parameters, indicates the ability of healthy, mature horses to thermoregulate in this hot, sunny environment. Research at more extreme temperatures would be useful to determine under what conditions horses’ ability to thermoregulate is compromised.

Additional studies are required to examine the effects of humidity in combination with hot weather on the physiological and behavioral responses of horses to shade. Horses whose health is compromised by old age, illness, disease, or poor body condition may exhibit different responses to thermoregulatory challenges. Horses in this study were subject to the same housing and management, but varying factors such as pen size, type of forage, quantity of fiber in the diet, and group housing might also reveal different responses. Finally, empirical evidence that an environmental feature is beneficial to a species is not confirmation that the animals will utilize it. Thus, further research also is needed to ascertain whether or not horses will take advantage of available shade when offered a choice.

The results support our hypothesis that horses benefit from shade as it mitigated measurable physiological and behavioral effects of hot, sunny weather conditions. Physiological differences between the SUN and SH treatments were detected before behavioral differences and may have been a response to increasing solar radiation rather than ambient temperature. These findings can be used in developing best management practices for the care of domestic horses.

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**Table 1.** Description of criteria for recording behavioral data using instantaneous scan sampling for various behaviors and focal sampling for insect avoidance behaviors

Behavior	Definition
<b>Instantaneous scan sampling</b>	
Stand away from water	Upright posture with no forward motion, at least 1 m away from water bucket and performing none of the other behaviors
Stand near water	Within 1 m of water bucket, muzzle not in bucket
Drink water	Muzzle within and below top rim of water bucket
Foraging	Muzzle at ground level actively exploring matter on ground
Locomotion	Forward ambulatory motion at any speed
Recumbent or rolling	Lateral recumbency: one side of body in contact with ground including neck, hip and shoulder  Sternal recumbency: body in contact with ground not including neck, hip or shoulder  Rolling: movement from standing to sternal recumbency, folding legs and rotating from sternal to dorsal position 1 or more times
<b>Focal sampling for insect avoidance behavior</b>	
Head movement	Using muzzle or teeth to bite at or rub any part of body  Shaking whole head in side-to-side motion 3 or more times, vigorous enough that bottom of jaw and top of ears swing in opposite arcs  Tossing head such that nose goes above level of top of withers or nose flexes toward chest going behind vertical
Stomp	Rapidly lifting and then lowering hoof; hoof clearly leaving the ground and returning to approximately the same place; no forward motion

**Table 2.** Mean and maximum ambient temperature ( $T_{amb}$ ); mean relative humidity (RH), black globe temperature ( $T_{BGT}$ ), temperature-humidity index (THI), soil temperature ( $T_{soil}$ ), solar radiation and wind speed shown for the daily (24 h) and the observation period of 1200 to 1800 h during both trials in the shaded (SH) and unshaded (SUN) areas

Weather Variable	24 h		1200 to 1800 h	
	SH	SUN	SH	SUN
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
$T_{amb}$ , °C	22.1 (6.8)	22.5 (7.2)	30.0 (3.9)	31.1 (3.7)
Maximum $T_{amb}$ , °C	38.6	39.6	38.6	39.6
RH, %	61.7 (19.1)	61.7 (19.8)	38.4 (8.7)	39.5 (9.2)
$T_{BGT}$ , °C	23.7 (7.8)	27.0 (11.3)	33.4 (4.1)	40.9 (4.0)
Solar radiation, W/m <sup>2</sup>	9.7 (10.0)	312 (339)	18.5 (3.7)	734 (18.5)
THI	67.7 (7.9)	68.1 (8.4)	76.3 (3.7)	77.6 (3.3)
$T_{soil}$ , °C	n/a	n/a	30.5 (3.1)	55.4 (5.0)
$T_{water}$ , °C	n/a	n/a	25.6 (3.1)	32.6 (2.8)
Wind speed, m/sec	n/a	n/a	1.3 (0.6)	1.2 (0.6)

**Table 3.** Mean values for physiological, behavioral and serological variables for horses (n = 12) of completely shaded (SH) and completely unshaded (SUN) treatments

Variable	SUN	SH	SE	<i>P</i> value
Rectal temperature, °C	37.8	37.6	0.05	0.002
Respiration rate, bpm	25.5	20.5	1.3	0.008
Skin temperature, °C	35.6	34.6	0.13	< 0.001
Standing near water, % of observations	29.4	16.9	2.6	0.003
Standing away from water, % of observations	45.0	57.6	3.6	0.025
Drinking, % of observations	4.2	3.2	0.5	0.269
Foraging, % of observations <sup>1</sup>	9.9	11.5	1.0	0.268
Locomotion, % of observations <sup>2</sup>	10.6	10.6	2.5	0.994
Water Consumption, L/100 kg BW	7.8	6.0	0.6	0.046
Insect avoidance behaviors, number/min	6.2	5.4	0.6	0.190
Flying insects in treatment area, number/d	22.6	13.6	1.8	0.001
Hematocrit, %	34.9	35.2	0.5	0.674
Neutrophil:Lymphocyte	2.1	1.9	0.2	0.087
Cortisol, ug/dL <sup>3</sup>	3.4	2.6	-	< 0.001

<sup>1</sup>Analysis weighted by trial and day

<sup>2</sup>Analysis weighted by time

<sup>3</sup>Mean values for cortisol before back transformation were: SUN 0.48, SH 0.46, SE 0.05



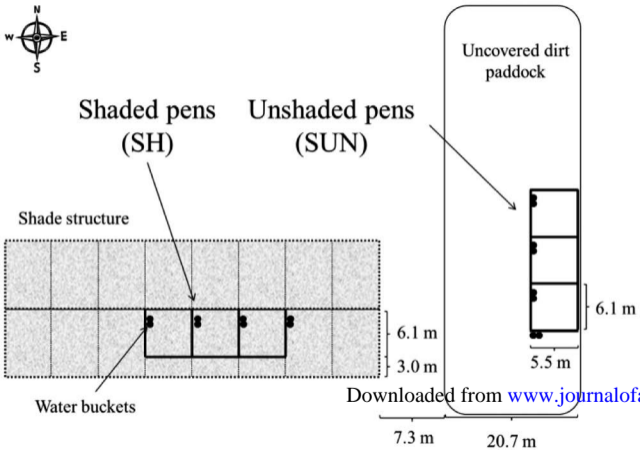
**Figure 1.** Pen design showing relative location, dimensions, and water bucket placement for 3 shaded (SH) and 3 unshaded (SUN) treatment pens.

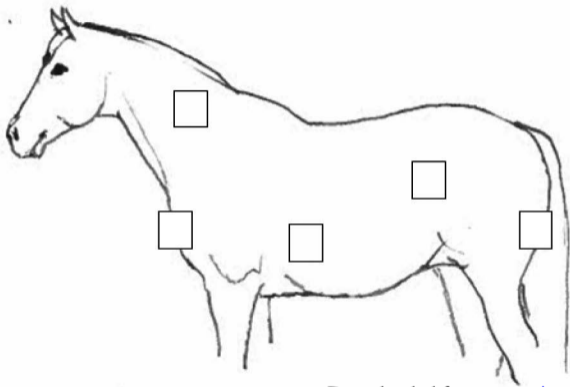
**Figure 2.** Diagram used to record visible, wet sweat in 5 body regions as indicated by boxes.

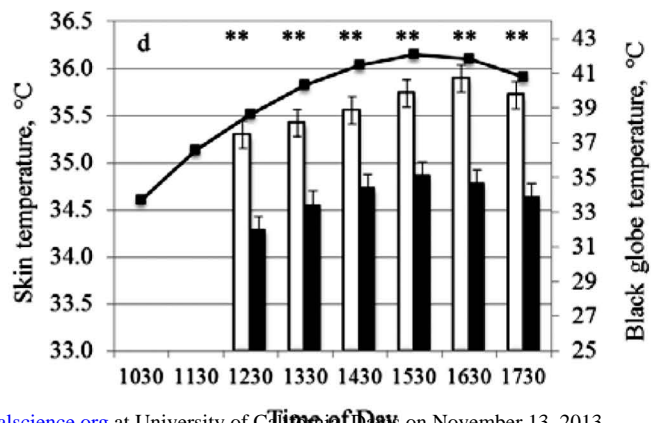
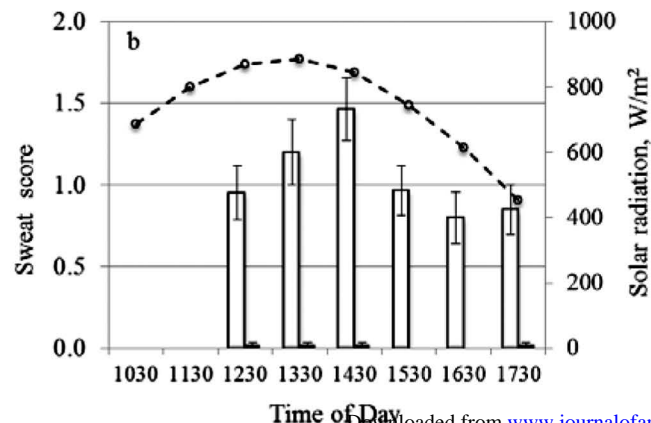
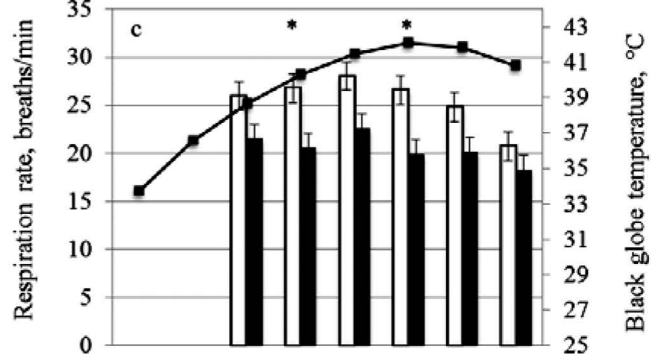
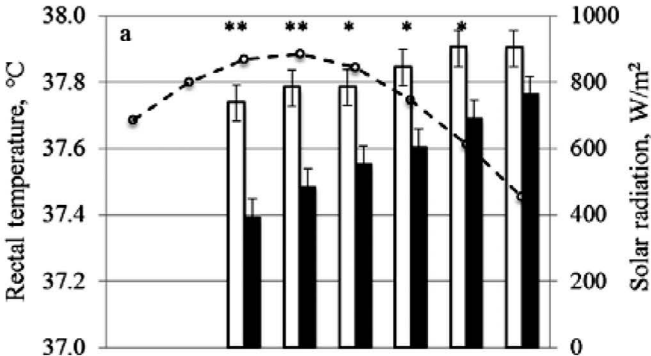
**Figure 3.** Physiological response variables by time of day for horses housed in the unshaded (SUN) and shaded (SH) treatments: (a) rectal temperature and (b) sweat score shown with solar radiation; (c) respiration rate, and (d) skin temperature shown with black globe temperature (Mean  $\pm$  SE. \*  $P < 0.05$ , \*\*  $P < 0.005$ ); treatment differences for sweat score were not analyzed due to limited observations of sweat in SH. [AU: please make the following edits to the figures and send revised files with your returned corrections: (1) remove internal grid lines (2) remove 'box' around graph (top and right-side 'axis' lines)]

**Figure 4.** Percent of observations in which horses were standing within 1 m of water buckets by treatment, shaded (SH) or unshaded (SUN), and time of day shown with solar radiation (Mean  $\pm$  SE; \*  $P < 0.05$ ; \*\*  $P < 0.005$ ). [AU: please make the following edits to the figures and send revised files with your returned corrections: (1) remove internal grid lines (2) remove 'box' around graph (top and right-side 'axis' lines)]

**Figure 5.** Foraging, locomotion and fly avoidance behavior of all horses by time of day, with black globe temperature; means without a common letter are different ( $P < 0.05$ ). [AU: please make the following edits to the figures and send revised files with your returned corrections: (1) remove internal grid lines (2) remove 'box' around graph (top and right-side 'axis' lines)]







□ SUN    ■ SH    - - - Solar Radiation

□ SUN    ■ SH    —●— Black Globe

