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The Relationship of Dominance, Reproductive State and Stress in a Non-Cooperative Breeder, The Domestic Horse (*Equus caballus*)

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THE RELATIONSHIP OF DOMINANCE, REPRODUCTIVE STATE AND STRESS
IN A NON-COOPERATIVE BREEDER, THE DOMESTIC HORSE (*EQUUS*
CABALLUS)

A Thesis
Presented to
The Faculty of the Department of Biology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Carly Anne Sinderbrand

August 2011

THE RELATIONSHIP OF DOMINANCE, REPRODUCTIVE STATE AND STRESS
IN A NON-COOPERATIVE BREEDER, THE DOMESTIC HORSE (*EQUUS*
CABALLUS)

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Animals that live in a social group are often organized in a hierarchy with rank determining access to resources. Maintaining a dominant position requires a high rate of energetically expensive aggressive displays and physical exertion. Lab based winner-loser studies, often conducted with individuals from non-social species, have shown that subordinates have higher stress hormone levels than dominant individuals (subordinate-stress hypothesis). However, in carnivorous animals that are cooperative breeders, displays of aggression are associated with elevated stress hormone levels (dominance-stress hypothesis). The effect of reproductive state on dominance and stress is not addressed within either of these hypotheses. The purpose of this study was to examine stress level in relation to dominance rank and reproductive state in a non-cooperative breeder and herbivore, the domestic horse. As rank and reproductive state can affect behavior, I examined activity budgets, behavioral patterns, and social interactions, as well as the proximity and identity of neighbors in the social group. At two facilities in Kentucky, I recorded the social interactions and measured fecal glucocorticoids in pastured, female horses that were either lactating or non-lactating. While fecal glucocorticoid level did not differ between reproductive state and rank, activity behavior

demonstrated significant differences between reproductive states. Higher energetic requirements of lactation were reflected in significantly longer bouts of eating and significantly less time spent alert and socializing. The non-cooperative social nature of horses does not limit their reproduction or resource acquisition based upon rank, and therefore does not fit with the dominance-stress hypothesis or subordinate-stress hypothesis and instead offers the alternative of an independent-stress hypothesis.

Introduction

Stress is defined as a change in internal state that is induced by an external condition (Creel 2001). Animals physiologically react to stress with endocrine responses. A primary response to stress is an activity increase in the hypothalamic-pituitary-adrenocortical axis, which causes an increase in the concentration of adrenal glucocorticoids, as well as activation of the sympathetic nervous system (Alexander et al. 1998, Reeder and Kramer 2005). These responses usually inhibit physiological processes that are not immediately required (Abbott et al. 2003). Harmful consequences, if the stressor is not eliminated and glucocorticoid levels remain high, include immune suppression, loss of muscle mass, reproductive suppression, and brain atrophy (Sapolsky 1996, DeVries et al. 2003). When confronted with a stressor, animals mount physiological and behavioral adaptations that serve to neutralize its effects. Behavioral reactions to a stressor include escape or avoidance behaviors, altered cognition and attention span, increased awareness, altered sensory threshold, sharpened memory, inhibited feeding behavior, and suppressed reproductive behavior (Reeder and Kramer 2005). Reacting to a stressful situation requires the animal to expend energy, which can ultimately decrease its overall fitness (Sapolsky 1996).

Stressors that cause an increase in physiological activity can be either or both physical and psychological (Sapolsky and Ray 1989). Physical stressors can be internal, such as anoxia and hypoglycemia, or external, such as a change in environment or injury. Psychological stressors include stimuli that affect emotions such as fear, anger, anxiety, or frustration. While stressors have been mostly studied in lab animals, they are equally applicable to domestic and free-ranging species. Individuals of social species are

particularly susceptible to psychological and physical stressors because of their constant interaction with other animals (Blanchard et al. 2001, Reeder and Kramer 2005).

One type of study used to examine stress response during social interactions involves pairing animals in an arena, noting the winner and measuring the stress hormone levels of each contestant (Bronson and Eleftheriou 1964, Louch and Higginbotham 1967, Martinez et al. 1998, Schuett and Grober 2000). Because losers often have the higher stress hormone levels, low ranking animals were hypothesized to show higher stress than high ranking individuals, giving rise to the subordinate-stress hypothesis (Abbot 2003). However, in these situations, both winner and losers show high levels of stress hormones (reviewed in Creel 2001). The inability of animals to escape from their unnatural surroundings makes the circumstances of these studies unlikely to occur in nature, where animals often have enough space to avoid aggressive interactions. Therefore, these studies do not reflect the consequences of being a subordinate individual in a naturally occurring social group. In contrast to the winner-loser studies, the stress of dominance hypothesis predicts that dominant individuals will demonstrate greater physiological stress than subordinate animals (Creel 2001).

Social animals are often organized in a hierarchy composed of dominant, middle, and subordinate individuals. Significant positive correlations have been found between aggression and dominance rank within a hierarchy (Weeks et al. 2000). These agonistic interactions can be a source of chronic physiological stress, especially in dominant individuals (Creel 2001). Dominant animals gain benefits from their social status such as access to resources and mates, but they also are subjected to costs associated with their high-rank. In social mammals such as the hyena (*Crocuta crocuta*) and the mole rat

(*Heterocephalus glaber*), dominant individuals have higher levels of stress hormones than lower ranking animals (Creel et al. 1996, Clarke et al. 1998). In bison bulls (*Bison bison*) glucocorticoid level is positively correlated with dominance rank and copulatory success (Mooring et al. 2005). Dominance rank is positively correlated with urinary cortisol within a stable dominance hierarchy in wild chimpanzees (*Pan troglodytes*) (Muller and Wrangham 2004). The increase in stress hormones with dominance ranking suggests that being the dominant individual is not without physiological consequences. Maintaining a dominant position requires a high rate of energetically expensive aggressive displays and physical conflict that is associated with elevated stress hormone levels (Mooring et al. 2005). The dominance-stress hypothesis also states that although dominant individuals often have priority access to necessities, the hidden physiological costs could provide an indication as to why subordinates accept a lower status in the hierarchy (Morell 1996).

A number of studies have examined the association between stress hormone levels and dominance of animals from a variety of taxonomic groups (Table 1). Of these studies, only a few have detected higher levels of stress hormones in subordinate animals (Schwable et al. 1988, Sapolsky and Ray 1989, Lidgard et al. 2008). Another subset of studies found no detectable difference in the stress hormone levels of dominants and subordinates (Bercovitch 1995, Bales et al. 2005). The largest number of studies found base stress hormone levels to be significantly higher in dominant animals (Schoech et al. 1991, Creel 1996, Creel et al. 1997, Smith and French 1997, Saltzman et al. 1998, Cavigelli 1999, Muller and Wrangham 2004, Sands and Creel 2004). Of the studies that found dominant individuals to have significantly higher stress levels, the majority of the

species were cooperative breeders or social primates (Creel 2001, Muller and Wrangham 2004).

Cooperative breeders are defined by species in which individuals that are not the parents of the offspring will provide care such as feeding, allosuckling, grooming, and protection (Jennions and Macdonald 1994). This type of behavior is exhibited in numerous mammals ranging from rodents to elephants. Another characteristic often found within cooperative breeders is a high reproductive skew, where only dominant animals breed and subordinates undergo reproductive suppression (Macdonald 1983). Although dominant individuals in social, non-cooperative breeders have priority access to mates (Creel 2005), the skew is much lower and true reproductive suppression is rare (Jennions and Macdonald 1994). The dynamics of cooperative breeding social groups could lead to dominant individuals having higher stress levels because of characteristics that are not prevalent among the less examined, non-cooperative social species.

The foraging strategies of social groups may also play a role in stress response throughout the hierarchy. Many of the species in which dominant animals show higher cortisol levels are carnivorous such as the dwarf mongoose (*Helogale parvula*), African wild dog (*Lycaon pictus*), and the gray wolf (*Canis lupus*) (Creel 2005). Resource distribution throughout the hierarchy could be highly skewed within a carnivorous species, as only a few individuals can consume a kill at the same time (Macdonald 1983). In contrast to carnivores, herbivores are socially tolerant of each other because of wide resource distribution and lack of competition while foraging (Rubenstein 1994). This is evident in the plains zebra (*Equus quagga*), where group size has no significant effect on the proportion of time spent foraging, and aggression while foraging is rare (Rubenstein

1981). Thus, it may follow that herbivores would not show the rank related stress response observed in carnivores.

Similar to alterations in dominance status, the shifting of reproductive states also could cause a change in physiological stress. Stress levels could be elevated as a response to the highly energetic process of reproduction (Weingrill et al. 2004). Because lactation is the most energetically expensive reproductive state (Hamel and Cote 2008), there should be a corresponding increase in glucocorticoid levels compared to other conditions (e.g., non-lactating states). For example, when hyenas (*Crocuta crocuta*) are in a stable social group, lactating females have a higher corticosteroid concentration than non-lactating females (Goymann et al. 2001). The increased energetic demands of lactation should prompt animals to strive for a position in the social hierarchy that provides priority access to resources (Barrette and Vandal 1986). However, it is unclear whether lactating females increase their rates of aggression in order to gain priority access to resources. In the plains zebra (*Equus quagga*), lactating females often initiate more harem movements than non-lactating females (Fischhoff et al. 2007), although no direct changes in the social hierarchy are evident after females give birth (Pluhacek et al. 2006). Lactating females are already in a physiologically demanding state, and the aggression required to maintain a dominant position would compound the amount of stress the animal must endure (Creel 2005). Theoretically, these costs would be outweighed by the benefits of having access to resources, which could allow a higher likelihood that their offspring will survive, and therefore increase the individual's reproductive success (Hamel and Cote 2008).

The combined effect of dominance status and reproductive state has not been examined in social, non-cooperatively breeding herbivores, where neither the conditions of winner-loser studies nor wild studies on cooperative breeders are met. Feral and free-ranging horses form harems that are matriarchal and consist of enduring relationships between mares and their offspring until they are of reproductive age. Though there is often a stallion in a harem, the association between the mares remains consistent in the absence of a stallion (Goodwin 1999). In the wild, living as a herd is an important anti-predator strategy. While an individual horse can flee or when necessary fight a predator with teeth and hooves, herd living likely evolved in part because defense by a group was more successful in minimizing predation (Rubenstein 1994). Because of this, the social behavior of the horse has developed to minimize conflict within the group and promote herd stability. Individual dominance order is unidirectional, but may not be linear throughout the group (Haupt et al. 1978). Age is often positively correlated with dominance status (Clutton-Brock et al. 1976, Weeks et al. 2000, Rho et al. 2004, Powell 2008). The existence of a dominance order in an established group is better observed through avoidance behaviors than aggressive encounters, and given that there is adequate space, subordinate individuals will deliberately avoid moving close to dominant ones (Goodwin 1999). Despite provisioning and care by humans, domestic horses also form stable hierarchies, which determine access to resources that may be limited at times in pastures such as shade and water (Berger 1977, Rubenstein 1994, Bennett and Hoffmann 1999, Vervaecke et al. 2006). When in groups of two or more, horses develop and maintain dominance relationships where the dominant individuals have priority access to resources such as food, water, and shelter (Rubenstein 1981, Weeks et al. 2000).

Following an eleven-month gestation, parturition in horses occurs during times of reasonable weather and plentiful resources, providing forage for the lactating mare and thus adequate nutrition to the foal (Crowell-Davis 2007). Nevertheless, lactation in horses as with other mammals is an energetically expensive endeavor (Duncan et al. 1982). Mares are seasonally polyestrous with estrous cycles beginning in mid to late winter and culminating in early fall (Bennett and Hoffmann 1999). Postpartum mares will experience estrus during the first week after birth and may conceive at that time (Aurich 2011). During spring, summer, and early fall months, horses may be lactating or still pregnant (non-lactating), providing the opportunity to examine the effect of lactation on dominance and stress hormone levels.

The purpose of this study was to examine the relationship between stress and dominance of a social, non-cooperative breeding species, specifically the domestic horse. If subordinate animals do not have access to resources such as food, water, and shelter, then they should have higher levels of stress hormones than dominant animals (subordinate-stress hypothesis). However, if being a dominant horse requires high rates of aggression in order to maintain a high-ranking position, dominant animals would have higher stress levels than subordinates (dominance-stress hypothesis). Stress hormone levels could be independent of status in a social, non-cooperative herbivore (rank independent stress hypothesis) if resource limitation and access to mates drives the rank-stress relationships observed in cooperative breeders as measured by glucocorticoid levels. It is also possible that reduced access to resources in subordinates, and high rates of aggression in dominant horses balances costs and benefits throughout the hierarchy, thereby also creating an independent relationship between stress and rank (Fig.1).

Because lactation requires additional energy and time investments compared to other reproductive states, female horses may exhibit higher stress hormone levels. Dominance is determined through the outcome of aggressive interactions, but it also may influence activity budgets, behavioral patterns, and social interactions such the proximity and identity of neighbors in the social group. I also examined these aspects of social behavior in the domestic horses under study. Abiotic factors such as temperature can cause variation in stress level and behavior (Huber et al. 2003, Weingrill et al. 2004, Hamel and Cote 2008), therefore the effect of season also was examined.

Methods

Study Population

I studied horses at two locations, Westwind Farm and 3T Farm (Table 2). Westwind Farm is a thoroughbred breeding facility located in Bowling Green, Kentucky. Females with male and female offspring and non-lactating females were maintained in separate fields. Hence, per field, horses were only in a single reproductive state. Horses were brought into stalls in the morning and in the afternoon to be fed grain, but otherwise they remained turned out in their designated paddocks. All but three horses at Westwind were impregnated during the spring 2010, therefore the horses were categorized according to lactation status (lactating or non-lactating).

3T Farm is an American saddlebred breeding facility in Scottsville, Kentucky. The herds consisted of one stallion, non-lactating mares, lactating mares, colts, and fillies. Thus, in contrast to Westwind, lactating and non-lactating horses were together in the same field. As females had constant access to a stallion, it was not possible to determine whether mares were pregnant or cycling. For this reason again horses were categorized by lactation status. These horses remained in their fields at all times and did not have any additional food supplementation to grass. In May 2011, Ms. Hillary Whitaker, manager of 3T Farm, informed me as to which females had conceived over the time of the study, as well as their estimated date of parturition.

Behavioral Observations

All of the methods outlined in this study were approved by Western Kentucky University's Institutional Animal Care and Use Committee (Welfare Assurance #A3558-

01). Behavioral data were recorded from May 17, 2010 through August 4, 2010. All data were collected between the hours of 8:00am and 1:00pm. I rotated observation periods at the two farms throughout the study period spending approximately two weeks at the site during each rotation. For a particular field at a farm, I also rotated the time of observation to avoid time of day becoming a confounding variable. In late June I took a one week hiatus from collecting data in order to examine the methods of my study. The average minimum and maximum temperature for each week of study was collected retroactively via weather history data from the local airport (Fig. 2). This hiatus and the concomitant differences in temperature before and after motivated the analysis of the data by season, demarcated as early (May 17 – June 28) and late (July 3 – August 4) summer.

I observed horses from approximately 15 m away within the pastures, as this distance provided good visibility without disturbing the herd. I identified individuals via coat color, leg and face markings, as well as other distinguishing characteristics. Several horses at both farms also could be identified by a nametag on their halter. To determine a time budget, 12 ten-minute focal animal samples with continuous recording were performed for a total of 2 h of behavioral observation on each individual (Martin and Bateson 2007). To establish social behavior patterns within the herds, nearest neighbor data were recorded at 5min intervals for an hour during each session for 12 h of data on each herd. To estimate the distance between individuals for the nearest neighbor measure, I used the distance between fence posts (1.5 m) or walking strides (0.5 m/stride) as a reference.

All-occurrence data were collected on agonistic behaviors to help determine a dominance hierarchy. Agonistic encounters include aggressive contact, aggressive non-

contact, and aggressive positional. Aggressive contact included biting, kicking, nipping, push-body, push-head, and rear-end behaviors. Aggressive non-contact behaviors were bite threats, kick treats, ears back and chasing. Displacing and supplanting were categorized as positional aggressive behaviors. Non-aggressive behaviors included play fight, touching, or grooming. Submissive behaviors were turning away or avoiding the aggressor (Table 3).

The dominance hierarchy of each herd was determined using all aggressive behaviors in which there was a clear winner and loser. The loser was defined as the first horse to show submissive behavior such as avoidance or turning away (Table 3). The Clutton-Brock Index (CBI) (Clutton-Brock et al. 1979) was used to establish the dominance hierarchies of horses in each field. This index is commonly used in vertebrate studies, and it is a suitable equation for animal societies in which multiple agonistic interactions are uncommon (Bayly et al. 2006, Bang 2010). Because agonistic interactions are potentially very costly in large mammals, such as ungulates, these behaviors often do not occur at high rates. For this reason, the CBI has been frequently used in studying species such as horses, cows, and deer (Clutton-Brock et al. 1976, Freeman et al. 1992, McElligot et al. 2001). To establish each horse's CBI score, the following equation was applied to all individuals: $CBI\ Score = (B+b+1)/(L+l+1)$,

where B = number of individuals whom the subject dominates, b = number of individuals whom those dominated by the subject in turn dominate, L= number of individuals who

dominate the subject, ℓ = number of individuals who dominate those dominating the

subject (Bang 2010). To determine the relationship between stress level and position in the dominance hierarchy without the effect of varying scores for dominant animals from different herds, the CBI scores from each field were standardized by giving the most dominant animal a score of one. The standardized CBI score for the rest of the hierarchy was established by dividing their position in the herd by the total number of horses in the herd.

Fecal Sampling

Glucocorticoid metabolites in feces have been shown to accurately reflect adrenal activity and therefore can be used to assess stress (Von der Ohe and Servheen 2002, Mooring et al. 2005, Franceschini et al. 2008). Hormone levels in feces reflect hormone secretion over a longer period of time than a single blood sample, making fecal sampling a better representation of individual daily hormonal levels (Millspaugh and Washburn 2004). Fecal sampling is also a noninvasive way to monitor hormone levels in wild or captive animals that does not interfere with a behavioral study (Millspaugh et al. 2001). For hormone analysis I collected 222 fecal samples from 75 horses (217 fecal samples from 73 mares; 5 fecal samples from 2 stallions) with one to five samples per individual, based on opportunity throughout the study. Fecal samples were identified by noting on a map where a particular horse defecated. The sample was collected after the animal moved away from the site. Samples were collected by hand using an inverted bag to pick up the sample and then sealing it. I acquired 5-6 fecal pellets in order to assure

that the samples include a measureable quantity of hormones. Samples were placed in a cooler filled with ice until they could be stored in a -20C freezer within 4 hours.

Extraction of Steroids

The hormone analysis portion of this study was completed at Saint Louis Zoo's Endangered Species Research Center in September 2010. Samples were thawed overnight in a refrigerator before beginning the extraction procedure. The weight of empty 20 mL plastic scintillation vials was recorded. Approximately 0.5 g wet feces were added to each vial avoiding obvious foreign material such as straw and soil. Five milliliters of working Fecal Extraction Buffer II (50% methanol [Sigma M 1775]) was added to each vial which was then tightly capped. Each vial was vortexed until the sample was well dispersed. Samples were shaken overnight (approximately 16 h) at 200 RPM. Samples were allowed to settle for one hour, and then liquid was decanted from each vial into a 12 x 75 polypropylene tube. The samples were centrifuged for one hour at 4,000 RPM. The supernatant was decanted into clean cryovial and frozen at -70°C until the day of assay (Sheidler et al. 1993).

Assays

Although cortisol is the primary circulating corticoid in the blood of most mammals, it is excreted as a mixture of corticoid metabolites (Wasser et al. 2000). Fecal glucocorticoid (FGC) levels were determined by radioimmunoassay using the ImmuChem Double Antibody Corticosterone kit from MP Biochemicals LLC (Irvine,

CA; formerly ICN). Assays were performed according to manufacturer's protocols with the exception that the matrices were equalized by adding assay diluent to the fecal samples, and fecal extraction buffer (containing 50% methanol) to the standards. Concentrations were determined as ng/mL, and then divided by the dry weight of feces extracted to give the results as fecal glucocorticoids ng/g feces. All samples were assayed in duplicate. The assay was repeated for any sample in which the duplicates differed by 15% or greater.

Validation

Inter-assay coefficients of variation for low and high QC pools were 11.3% and 5.1 %, respectively. Serial dilutions of horse fecal extracts for parallelism yielded O/E values of $88.9 \% \pm 8.72\%$ (mean \pm SD). Addition of known amounts of corticosterone at several levels to horse fecal extracts resulted in recovery of $104.0\% \pm 6.0$ (mean \pm SD). Assay sensitivity (minimum detectable dose) was 7.7 ng/mL. The cortisosterone antibody cross-reacts as follows: desoxycorticosterone 0.34%, testosterone 0.1%, cortisol 0.05%, aldosterone 0.03%, progesterone 0.02%, androstenedione 0.01%, 5 α -dihydrotestosterone 0.01%, and all other steroids tested <0.01%. The manufacturer supplied the cross-reactivity data.

Statistical Analysis

Of the 75 horses sampled during the study, only the 73 females were included in the data analysis. Average minimum and maximum temperature from May 12 to June 28 and July 3 to August 4 was analyzed to determine differences between seasons. Data

were examined for location and season effects between early summer and late summer. For comparison of activity between seasons, only horses that were present in both early and late summer were used in analyses (n=61). Of the 61 horses present for both seasons, fecal samples were only available from 30 horses in both early and late summer. To determine the effect of season on fecal glucocorticoid level, matched t-test was performed on horses that had samples present in both seasons (n=30). For the analysis of fecal glucocorticoid concentration, the mean value was calculated for each individual in order to prevent horses with more samples from being overly represented in the data set. Aggression and activity were measured by obtaining the rate of event behaviors and the duration of state behaviors, respectively. Rates for each individual were obtained by dividing the frequency of event behaviors by total duration of focal length in minutes. Focal animals were always visible for the entire 10 minute sampling period. The behavioral states of 'other' and 'drinking' were omitted from the analysis because of low rates of occurrence. When calculating the bout duration of state behaviors, the first and last behaviors of each focal were not included because the beginning and end times of the behavior were unknown. In order to avoid pseudo-replication, I calculated the mean duration of all individual behaviors for each horse and used the mean value throughout analysis so each horse only contributed one value for a particular behavior.

Statistical analyses were performed using Microsoft Excel 2007 and Statistica (Statsoft Inc., Tulsa, OK). I performed a regression of fecal glucocorticoid level and CBI score to determine the relationship between hormone level and dominance rank without standardization. The CBI score for the most dominant horse in each herd ranged from three to eight. To adjust for variation between herds, I also performed a regression of

fecal glucocorticoid level using a standardized CBI score. Horses also were placed into categories of dominant, middle, and subordinate using the standardized CBI score, with dominant horses having standardized CBI scores higher than 0.67, middle horses scoring between 0.67 and 0.33, and subordinate horses having scores below 0.33 (Table 4 and 5). A two-way analysis of variance (ANOVA) was used to analyze the difference between fecal glucocorticoid level of dominant (N = 22), middle (N = 28), and subordinate (N = 23) females, lactating (N = 33), and non-lactating females (N = 40), and any interaction between rank and reproductive state. Other measures such as state behaviors and rates of specific events were analyzed similarly. Season was used as a repeated measure in analysis of all activity behavior.

The effect of age on dominance and fecal glucocorticoid level was examined via linear regression. A chi-square test was used to compare the number of dominant and subordinate horses in each reproductive state at 3T Farm. A G-test was used to compare the number of horses in each rank and reproductive state that performed aggressive behavior. The G-test was chosen over a chi-square test for this analysis because of the small sample size produced when horses were categorized by rank and reproductive state. The proportion of scans in which lactating and non-lactating females were nearest neighbor to a same or different reproductive state was analyzed via chi-square test. Position in reference to the stallion also was analyzed. These analyses were not performed with the data from horses at Westwind farm because horses in the same reproductive state were pastured together. Mean proximity to a nearest neighbor was analyzed via two-way ANOVA using horses from both facilities. At 3T Farm, where females were in a pasture with a stallion, the number of the horses in each rank that

conceived over the study period was analyzed using a chi-square test. The number of horses by rank that conceived in early versus late summer was evaluated similarly. A p-value of 0.05 was used to determine significance for all analyses. Mean \pm standard error values are presented for most data.

Results

Fecal Glucocorticoid Level

The variable of season was examined in addition to analyzing mean fecal glucocorticoid level with reproductive state and rank. Although the minimum temperature did not significantly differ between seasons (t-test assuming unequal variances: $t=1.82$, $df=9$, $p=0.27$), maximum temperature was significantly different between early and late summer (t-test assuming unequal variances: $t=1.83$, $df=9$, $p=0.01$); therefore, the potential for fecal glucocorticoid to be affected by temperature was considered (Fig. 2). Fecal glucocorticoid was compared between samples using only horses for which I was able to collect fecal samples in both early and late summer, which were not significantly different ($n = 30$; early summer: 14.6 ± 8.6 ng/g; late summer: 16.5 ± 7.8 ng/g) (matched pair t-test: $t = 1.7$, $df = 29$, $p = 0.33$).

In order to examine the relationship between fecal glucocorticoid concentration and dominance rank, I created a dominance hierarchy based upon aggressive interactions. Using horses that were present in both seasons ($n = 61$), rate of aggressive behavior differed dramatically in early (0.09 events/minute) compared to late (0.02 events/minute) summer with an approximately 78% reduction in overall aggression in the late summer (matched pair t-test: $t = 7.45$, $df = 60$, $p < 0.001$). This trend was consistent at both locations and across reproductive state and rank. Because there was a low rate of aggressive behavior in late summer, it was not possible to build distinct hierarchies for each season. However, it is likely that the hierarchy established by each horse herd in early summer held throughout the study, as there were not high rates of aggressive interactions later in the study that would indicate shifts in the hierarchy.

As age is often a cofactor of dominance, I also examined whether there was a relationship between age, dominance, and fecal glucocorticoid concentration. Horses ranged from 4 to 26 years (Table 4 and 5). Examining all of the horses from both facilities, no relationship was found between age and dominance (linear regression: $y = 0.15x + 11.8$, $R^2 = 0.001$, $p = 0.76$), nor was any relationship found between age and mean fecal glucocorticoid level (linear regression: $y = -0.015x + 17.1$, $R^2 = 0.009$, $p = 0.94$). Age was therefore excluded as a covariate in further analyses.

Using the raw CBI dominance hierarchy score for each horse across both seasons, fecal glucocorticoid level did not vary with dominance rank (linear regression: $y = 0.56x + 16.39$, $R^2 = 0.006$, $p = 0.72$; Fig. 3; Table 6). A regression using the standardized CBI score also showed no significant relationship between rank and fecal glucocorticoid level (linear regression: $y = 2.29x + 15.71$, $R^2 = 0.006$, $p = 0.51$; Fig 4; Table 6). Mean fecal glucocorticoid level did not significantly differ between dominant, middle, and subordinate ranks with values of 16.4 ± 6.5 ng/g, 14.8 ± 6.3 ng/g and 17.4 ± 8.5 ng/g, respectively (ANOVA: $F_{2,70} = 0.35$, $p = 0.71$; Fig. 5). The mean fecal glucocorticoid level of lactating females was 15.7 ± 7.9 ng/g and the mean value of non-lactating females was 16.6 ± 7.9 ng/g, which were not significantly different ($F_{1,69} = 0.20$, $p = 0.65$). In addition, there was no significant interaction of reproductive state and rank ($F_{1,69} = 0.28$, $p = 0.60$). The effect of rank on fecal glucocorticoid also was analyzed independently at 3T Farm and Westwind Farm. This additional analysis did not reveal any significant differences in fecal glucocorticoid by rank (3T Farm: $F_{2,30} = 0.78$, $p = 0.56$; Westwind Farm: $F_{2,21} = 0.78$, $p = 0.47$). Additionally, the effect of reproductive state on fecal

glucocorticoid level of the horses at each location was analyzed, but no significant effects were evident (3T Farm: $F_{1,30} = 0.34$, $p = 0.91$; Westwind Farm: $F_{1,21} = 0.02$, $p = 0.90$).

Activity Behavior

The total and bout duration of alert, eat, locomotion, rest, and social behaviors collected by focal animal sampling were analyzed to compare the activity patterns by rank and reproductive state. Three of these behaviors differed between early and late summer for total and bout duration (Table 7). Alert behavior occurred in longer bouts and for more total time in late summer, while the reverse was true of locomotion (Tables 8 and 9). Resting demonstrated an interesting pattern of longer total duration in the early summer but longer bouts in the late summer. In early summer, alert behavior accounted for approximately 23% of the horses' time budget, but increased to about 33% in late summer; whereas, resting behavior went from 27% to 19% from early to late summer. However, time spent eating remained approximately 40% across both seasons. Overall, eat, alert, and rest comprised 90-92% of the activities across both early and late summer; the variation in alert, rest, and locomotion motivated the analysis of rank and reproductive state by season using only individuals that were present throughout the entire study ($n = 61$).

Using season as a repeated measure, the effect of rank and reproductive state were analyzed on total and bout duration of activity behaviors (Table 10). For all these behaviors, rank had no effect on the total duration of the behaviors, except when it had a significant interaction with reproductive state (Table 11). This interaction only occurred in the total duration of rest ($F_{1,55} = 4.67$, $p = 0.01$). Middle-ranked, non-lactating females

(3.76 ± 2.38 minutes) had longer durations in early summer than middle-ranked, lactating females (1.72 ± 1.73 minutes) (t-test assuming unequal variances: $t=1.73$, $df=17$, $p=0.03$). This trend was also apparent in late summer where lactating females performed 0.85 ± 0.90 minutes of rest behavior compared to non-lactating females who performed 2.44 ± 1.81 minutes ($t=1.76$, $df=14$, $p=0.02$). The bout duration of behaviors did not reveal any differences between dominant, middle, and subordinate individuals (Table 12). A significant interaction between rank and season in locomotion was found ($F_{2,55} = 3.29$, $p = 0.04$). This had very little impact on the activity budget, as locomotion only accounted for approximately 5% of the horses' total time budget. Overall, without the influence of reproductive state and season, rank had very little effect on the behavior of the horses.

Unlike rank, the total and bout duration of behavior differed with reproductive state of the horses (Fig 6; Table 13 and 14). Non-lactating females had longer total durations of socializing ($F_{1,55} = 9.4$, $p = 0.003$), as well as alert behavior ($F_{1,55} = 16.65$, $p = 0.001$). Lactating females, on the other hand, had significantly longer total durations of eating ($F_{1,55} = 9.42$, $p = 0.003$) and locomotion ($F_{1,55} = 5.65$, $p = 0.02$). From the 12 ten-minute focal periods that I conducted per horse, I summarized the differences in activity that occurred between lactating and non-lactating females. Within the activity budget of a two hour long period, lactating females spent approximately twenty more minutes eating than non-lactating females; whereas, non-lactating females spent about ten more minutes being alert. Total eating duration showed a significant interaction between farm and reproductive state ($F_{1,57} = 4.49$, $p = 0.04$) with lactating horses from Westwind Farm having longer durations (5.1 ± 1.5 minutes) than lactating females at 3T Farm (3.6 ± 1.4 minutes) (t-test assuming unequal variances: $t=1.7$, $df=23$, $p=0.01$). Although there was

not a significant difference in total duration of resting between lactating and non-lactating horses, a significant three-way interaction did occur between location, reproductive state and season ($F_{1,57}=5.48$, $p=0.04$). This interaction was driven by significant differences between 3T Farm and Westwind Farm in early summer ($F_{1,57}=9.31$, $p=0.003$), where lactating females at 3T Farm (3.75 ± 2.31 minutes) had significantly longer durations of resting than lactating females at Westwind Farm (1.25 ± 1.06 minutes) (t-test assuming unequal variances: $t=1.77$, $df=13$, $p=0.006$). This difference is likely motivated by variation within field rather than by differences in location, as there were also significant differences in rest behavior between the lactating horses in two fields (one with male offspring and the other with female offspring) at Westwind farm (t-test assuming unequal variances: $t=1.76$, $df=14$, $p=0.02$). Bout duration of behaviors was reflective of total duration with non-lactating females having longer bouts of socialization ($F_{1,55} = 8.25$, $p = 0.006$) and alert ($F_{1,55} = 4.80$, $p = 0.03$), while lactating females had longer bouts of eating ($F_{1,55} = 9.75$, $p = 0.003$) and locomotion ($F_{1,55} = 5.37$, $p = 0.02$). Location and reproductive state showed an interaction in eating behavior ($F_{1,55} = 4.84$, $p = 0.03$), where lactating females at Westwind Farm exhibited longer bouts of eating (2.5 ± 1.1 min) than lactating females at 3T Farm (1.8 ± 0.5 min) in the latter half of the summer. Locomotion was significantly different by location with horses at 3T Farm showing longer bouts than those at Westwind Farm. The horses at 3T Farm also exhibited longer bouts of social behavior than horses at Westwind Farm ($F_{1,55} = 8.46$, $p = 0.005$). Locomotion and social behavior together only accounted for approximately 8% of total activity behavior, so these differences in location had little overall effect on the activity behavior of the horses.

To examine whether reproductive state had an effect on dominance rank, the number of lactating and non-lactating horses in each rank were compared. This was only tested at 3T Farm, as Westwind Farm separated horses of each reproductive state into different fields. The relative number of dominant, middle, and subordinate horses in each reproductive state did not differ (chi square test: $\chi^2 = 0.08$, $df = 5$, $p = 1.0$; Fig.7). Aggressive behavior was divided into the categories of contact, non-contact, and position to compare the number of horses in each rank and reproductive state that performed specific types of aggression (Table 3). A G-test revealed no significant differences between the number of dominant, middle, and subordinate horses that performed each type of aggression, nor did reproductive state have an effect on the number of horses that performed the behaviors (Table 15; Figs. 8 and 9).

Aggressive behavior was also compared between reproductive states as aggression is a correlate of dominance. Using season as a repeated measure, non-lactating females had higher rates of aggressive behavior at the rate of 0.11 events/minute compared to lactating females at 0.06 events/minute ($F_{1,55} = 8.57$, $p = 0.005$), but an interaction between reproductive state and season was present ($F_{1,55} = 9.08$, $p = 0.004$). This interaction was driven by significant differences between lactating and non-lactating females in early summer ($F(1,59) = 10.98$, $p = 0.002$), as there was no difference in aggression in late summer ($F(1,59) = 0.15$, $p = 0.70$).

The nearest neighbor choice of lactating and non-lactating females, as well as average distance to another individual in the herd, revealed differences only when the presence of a stallion was considered. Average proximity to a nearest neighbor did not differ between rank and reproductive state, nor was there a significant interaction (All $F <$

1.25, all p values > 0.29 ; Fig. 10). At 3T Farm, non-lactating females had a lactating nearest neighbor only 18% of the time, which was significantly different than expected, and instead had the nearest neighbor of the stallion three times more than expected ($\chi^2 = 6.57$, $df = 2$, $p = 0.037$). Lactating females did not differ in their nearest neighbor preference between other females (lactating or non-lactating) or the stallion. Twenty-seven horses became pregnant at 3T Farm during the course of the study, but subordinate, middle, and dominant animals did not differ in the relative number of individuals that conceived ($\chi^2 = 2.35$, $df = 2$, $p = 0.31$). There also were no significant differences in whether they conceived in early summer ($\chi^2 = 1.38$, $df = 2$, $p = 0.50$) or in late summer ($\chi^2 = 4.53$, $df = 2$, $p = 0.10$).

Discussion

In the horses examined in this study, fecal glucocorticoid level had no relationship to either CBI score or hierarchy ranking, clearly demonstrating that dominance and subordination do not cause a stress response. Activity behavior between the ranks also did not differ, leading to the conclusion that rank had little effect on the physiological stress response or general activity patterns of individuals in each herd. While lactation was not related to higher fecal glucocorticoid levels, the increased energetic needs of lactating females were evident in their activity behavior. A trade-off was apparent in which lactating females spent significantly more time foraging and in locomotion, while non-lactating females spent more time being alert and social. It appears that in this social system, rank has little effect on behavior and stress level while reproductive state creates the greatest differences between the activity of individual females within a herd.

The results of this study indicate that the domestic horse represents a situation in between the strict hierarchy of carnivorous animals that are cooperative breeders, and solitary species commonly examined in winner-loser studies (Table 1). Instead, horses represent a social but non-cooperative herbivore in which fecal glucocorticoid level is independent of dominance status. There are several possible reasons why these results do not support the subordinate-stress or the dominance-stress hypothesis. The subordinate-stress hypothesis was often tested using unfamiliar individuals in a closed arena (as reviewed in Creel 2001). Unlike winner-loser studies, the individuals examined in this study were familiar with each other and a hierarchy was already established. Because a hierarchy was in place, aggressive interactions were less severe than if it was a first encounter. The horses also had space to avoid aggressive advances.

The social structure of horses has several differences from that of carnivorous animals that exhibit cooperative breeding. Cooperative breeding groups often have a high skew in reproductive success with subordinates rarely having the opportunity to breed (Jennions and Macdonald 1994). Horses, while social, do not often perform helping behaviors associated with cooperative breeding societies. Although there is a skew in reproductive success associated with dominance rank, subordinate horses are not forced into reproductive suppression (Rubeinstein 1994, Pluhacek et al. 2006, Powell 2007, Rubeinstein and Nunez 2009). It is possible that the stakes of being a dominant animal in a cooperatively breeding group are higher than in a non-cooperative group. This in turn could increase stress levels in dominant animals more than in species with a lower reproductive skew, as evident by the trends in fecal glucocorticoid shown by the studies listed in Table 1. Although dominant females have priority access to mates, the social system of the horse is not so strict as to physiologically prevent subordinate animals from reproducing. At 3T Farm, where horses were allowed to breed freely, individuals throughout the entire hierarchy conceived during the period of this study. There were also no obvious differences in the time of year that each rank was permitted to mate, and therefore foal the following year. Consequently, maintaining dominance does not infer the same cost as within cooperatively breeding societies. This is reflected in the independence of fecal glucocorticoid and dominance rank seen in this study's population.

Differences in foraging behavior of cooperative carnivores and social herbivores could also play a role in the varying relationship between fecal glucocorticoid and dominance. Equine species rarely compete while foraging and overt aggression during feeding is rare (Rubenstein 1994). The horses in this study spent a large component of

their activity budget foraging (approximately 40%), and therefore that portion of the day is spent in a mostly peaceable social situation. As hindgut fermenters, horses can extract nourishment from low quality resources, making competition for the richest patches less necessary (Illius and Gordon 1992). As a result, the foraging success of horses is limited by the time available for grazing, but not by the quality of food available and therefore competition within the social structure is limited (Rubenstein 1994). Low rates of aggression were visible in this population (on average 0.03 acts/h/female), and the most common type of aggression performed was non-contact and positional with relatively few instances of contact aggression. This is consistent with feral horses where aggressive behavior occurs at approximately two acts an hour (Sigurjonsdottir et al. 2003). In contrast to the tolerant foraging nature of horses, there is a higher skew in a carnivorous species in regards to who consumes resources. As only a few animals can feed at a kill simultaneously, aggression rates are higher than seen in herbivores (Macdonald 1983). In wild wolf packs, dominant pack members feed first and control when subordinate individuals feed, making a high rank necessary to obtain quality resources (Mech 1999). Because extreme aggression and competition is thought to stimulate a physiological stress response (Alexander et al. 1998), it would then make sense that the dominance-stress hypothesis is supported in carnivorous species, but fecal glucocorticoid is independent of dominance rank in social herbivores that have little competition when resources are abundant.

The hypothesis that lactating mares will have higher fecal glucocorticoid levels as a result of higher physiological requirements was not supported. Although lactating females have higher energetic needs than non-lactating females (Hamel and Cote 2008),

which was evident by an increase in longer periods of eating, continuous access to vegetation diminishes any stress that would be prevalent from a lack of resources. A previous study on Jeju ponies indicated that reproductive state has a differential effect on aggressive behavior that correlates with dominance rank (Rho et al. 2004), but the current study found no differences in the proportion of lactating and non-lactating females in each rank that performed aggressive behavior. The study by Rho et al. was conducted from early spring through fall so it is likely that resources were not abundant during the early and late parts of the study. It is possible that the difference in aggression occurred during seasons when resources were less abundant. Vegetation was widely available throughout the present study; so apparently, the need for the horses to compete for resources to satisfy their energetic needs was less than the study by Rho et al. The additional drive to behave like dominant animals diminished as resources were equally available to middle and subordinates in the herds. Therefore, lactating females can satisfy their energetic requirements while holding any position in the hierarchy.

The activity behavior demonstrated in the current study indicates that reproductive state had a larger influence on behavior than dominance ranking. Dominance had little effect on total and bout duration of behaviors. In some social groups, subordinate individuals would be expected to spend longer periods of time foraging than dominant animals, although subordinates often have more interruptions while foraging which would be detected in bout duration (Hamel and Cote 2008, Kolowski et al. 2007). However, in the current study reproductive state had more of an influence on time spent foraging than rank. Lactating females spent approximately 15% more time in the active behaviors of eating and locomotion than non-lactating females.

As a trade-off, lactating horses spent significantly less time being alert or socializing. Lactating females from Westwind Farm showed longer durations of eating, while at 3T Farm lactation was associated with significantly longer durations of resting. Both of these behaviors support the increased energetic needs of lactation and the differences were likely due to variation within each field as seen by differences between the lactating females at Westwind Farm. Some work has been done comparing investment by female horses in sons and daughters (e.g., Cameron and Linklater 2000), which could be relevant at Westwind where lactating females were separated based on the sex of their offspring. However, this topic was not the focus of the current study. The differences in activity behavior by reproductive state reveal that females compensate for the increased physiological demands of lactation by allocating more time towards resource consumption or rest at the cost of being social and alert.

The abiotic factor of season affected the behavior of the horses. Differences in activity behavior and aggression were detected between early summer and late summer, however, no differences were seen in fecal glucocorticoid level. Differences in stress level by season was detected in other studies when temperatures were extremely low and when there was short duration of daylight (Huber et al. 2003, Weingrill et al. 2004). Although maximum temperature increased from early to late summer, the extremes in temperature and associated variables that may contribute to a stress response did not occur during the present study. Non-lactating females showed significantly more aggression in early summer. Since non-lactating females spent more time being social and alert, it is likely that these behaviors lend themselves to more aggressive encounters than eating, which is how lactating females spent the majority of their time. Overall,

horses spent more time in locomotion during early summer and more time alert and resting in the second half of the summer. This can likely be attributed to the change in temperature throughout the study. When the horses were alert or resting, they were often in a shady area that provided relief from the heat. The differences that were detected between the two farms in association with season are likely attributed to different behavior patterns in each field. For example, at Westwind Farm, field 2 had an open barn that non-lactating females used for shade, while the horses in field 3 preferred to spend time around a large pond during hot periods rather than seek shade. Hence, the location difference was based more on the dynamics of the horses in their fields rather than differences in breeds or management styles between 3T Farm and Westwind Farm.

While most studies on horses have indicated age as a correlate of dominance (Rubenstein 1981, Rho et al. 2004, Powell 2007), this study did not find a significant effect of age on behavior. Studies that found this relationship were examining feral populations, and these naturally formed herds might have different social dynamics than horses placed together by humans. The herds used in this study were together for a relatively short amount of time before data were collected (approximately 2 months), while the feral horses used in other studies spent most of their lifetime in the same herd. It is possible age has a more significant effect with a more established group than in a herd of less familiar individuals.

It is interesting to note that despite little differences between the ranks, a hierarchy was still evident in each of the herds. Although there was an abundance of grass, it is likely that some areas of the field are more desirable than others (Hamel and Cote 2008); in addition, the access to other resources such as shade or water may warrant

the maintenance of a hierarchy (Berger 1977, Powell 2007). Most of the aggressive acts recorded occurred when there was a limited area of shade or drinking space. At 3T Farm, where a stallion was present in each field, access to a mate could be a factor in hierarchy formation. Although my sample size was too small for statistical analysis on nearest neighbor and rank, in both fields, the stallions' most consistent nearest neighbor was a dominant mare. Females that form stable relationships with a stallion have improved fecundity, making distance from the stallion a valuable resource (Linklater et al. 1999). The conflict produced as a result to limited resources was probably sufficient to keep a hierarchy stable, but since all of the horse's other necessities were satisfied, physiological stress differences by rank was not evident through the measurement of fecal glucocorticoid concentrations.

The domestic horse provides an example of a social system that does not fit the most well known hypotheses of dominance-stress and subordinate-stress. Instead, the results of this study indicate that the relationship between rank and fecal glucocorticoid level is highly dependent on several variables. The social structure of a species will have a great effect on the roles of dominant individuals within the society (Creel 2001). Although reproductive state did not directly influence stress, it had an impact on activity behavior; as such, reproductive state should be considered when studying the relationship between dominance and activity. Resource consumption is also a driving factor in a dominance hierarchy and varying food sources between species can change the dynamics within the hierarchy. The hierarchy in domestic horses provides benefits to the dominant individuals, such as primary access to shade, water, or the dominant individual (e.g., stallion), but such benefits may be less pronounced than in carnivores that show

cooperative breeding. The more subtle hierarchy of the domestic horse with the lack of any relationship between rank and fecal glucocorticoid concentration provides support for an independent-stress hypothesis in which position in the hierarchy does not drive differences in fecal glucocorticoid level or activity behavior. Instead, it appears that in this social system, reproductive state creates the greatest differential between the behaviors of individual females within a herd.

Table 1. Information on previous studies looking at the relationship between dominance and fecal glucocorticoid level.

Common Name	Scientific Name	Sex	Glucocorticoid Pattern	Social System	Citation
Subordinate-Stress Hypothesis (* indicates Winner-Loser study)					
Mouse	<i>Mus musculus</i>	Male	Sub>Dom	Solitary	Louch et al. 1967*
Rat	<i>Rattus norvegicus</i>	Female and Male	Sub>Dom	Solitary	Blanchard et al. 1993*
Domestic Pig	<i>Sus scrofa</i>	Female	Sub>Dom	Solitary	Mendl et al. 1992
White throated sparrow	<i>Zonotrichia querula</i>	Female and Male	Sub>Dom	Pairs	Schwabl et al. 1988
Olive Baboon	<i>Papio anubis</i>	Male	Sub>Dom	Social	Sapolsky and Ray 1989
Grey seal	<i>Halichoerus grypus</i>	Male	Sub>Dom	Social	Lidgard et al. 2008
Dominance-Stress Hypothesis					
Common marmoset	<i>Callithrix jacchus</i>	Female	Dom>Sub	Social and cooperative	Saltzman et al. 1998
Florida scrub jay	<i>Aphelocoma coerulescens</i>	Female	Dom>Sub	Social and cooperative	Schoech et al. 1991
Dwarf mongoose	<i>Helogale parvula</i>	Female	Dom>Sub	Social and cooperative	Creel 1996
Ring-tailed lemur	<i>Lemur catta</i>	Female	Dom>Sub	Social and cooperative	Cavigelli 1999
African wild dog	<i>Lycaon pictus</i>	Female and Male	Dom>Sub	Social and cooperative	Creel 1997
Wolf	<i>Canis lupus</i>	Female and Male	Dom>Sub	Social and cooperative	Sands and Creel 2004
Iberian wolf	<i>Canis lupus</i>	Female and Male	Dom>Sub	Social and cooperative	Barja et al. 2008
Black tufted-ear marmoset	<i>Callithrix kuhli</i>	Female	Dom>Sub	Social and cooperative	Smith and French 1997
Rock hyrax	<i>Procavia capensis</i>	Male	Dom>Sub	Social and cooperative	Koren et al. 2008

Chimpanzee	<i>Pan troglodytes</i>	Male	Dom>Sub	Social	Muller and Wrangham 2004
Bison	<i>Bison bison</i>	Male	Dom>Sub	Social	Mooring et al. 2005
Independent-Stress Hypothesis					
Rhesus macaque	<i>Macaca mulatta</i>	Male	Dom=Sub	Social	Bercovitch 1995
Golden lion tamarins	<i>Leontopithecus rosalia</i>	Female	Dom=Sub	Social	Bales et al. 2005

Table 2. Information on reproductive states of groups of horses from Westwind and 3T horse farms in the greater Bowling Green, KY region.

Location	Total	Lactating	Non-lactating	Notes
Westwind Farm	27	17	10	Horses separated by RS*
Field 1	9	9	0	
Field 2	10	0	10	
Field 3	8	8	0	
3T Farm	46	16	30	Mixed herds with stallions†
Field 4	26	9	17	
Field 5	20	7	13	

*Reproductive State

†One stallion per field

Table 3. Descriptions of state and event behaviors for domestic horses recorded via focal observations. Modified from McDonnell 2003.

States	Behavior	Definition
Activity	Drink	Imbibing water
	Eat	Head to ground consuming grass
	Rest	On ground with body on side or upright, or standing in one position with no movement in any direction often with one leg resting
	Social	Interaction with another horse, including active touching or more passive head to tail alignment.
	Locomotion	Moving in any gait for more than 5 seconds
Events	Behavior	Definition
Aggressive Contact	Bite	Open and rapid closing of the jaws with the teeth grasping the flesh and ears usually pinned
	Kick	One or both hind legs lift off the ground and rapidly extend backwards toward another horse, with apparent intent to make contact.
	Nip	Similar to a bite, but with the mouth less widely opened and the teeth closing on only a small piece of flesh.
	Push-body	Pressing of the shoulder, chest, or body against another
	Push-head	Pressing of the head or neck against another
Rear-end	Pressing of the rump against another	
Aggressive Noncontact	Bite threat	Similar to a bite, except no contact is made. The neck is stretched and the ears pinned back.
	Chase	One horse pursuing another, in an apparent attempt to overtake, directs the movement of, or catches up with another. Chaser typically pins the ears, exposes the teeth, and bites at the pursued individual.
	Ears back	Ears pressed caudally against the head and neck.
	Kick threat	Similar to a kick, but without sufficient extension or force to make contact.
Aggressive Positional	Displace	Horse A causes horse B to move but A does not occupy B's original location.
	Supplant	Horse A takes the spot of horse B.
Non Aggressive	Play fight	Interaction between horses where no real aggressive behaviors are displayed
	Touch	Includes sniffing, mutual grooming, etc., between two horses
Submissive	Avoid	The recipient of the aggression moves away from the aggressor
	Head turn	The recipient of the aggression turns its head away from aggressor

Table 4. Information of aggressive behavior and dominance hierarchy from female horses at Westwind Farm.

Name	Age (y)	Total Wins	Total Losses	CBI	S CBI*	Rank	RS**
Barbette	7	3	5	0.23	0.25	Sub	Lac
BB	9	8	6	0.73	0.63	Mid	Lac
Big'n	7	5	14	0.21	0.30	Sub	Non-Lac
Black	16	28	1	2.25	1.00	Dom	Non-Lac
Blue	6	0	5	0.05	0.10	Sub	Non-Lac
Chris	15	9	0	5.00	1.00	Dom	Lac
Dark	19	5	2	1.00	0.89	Dom	Lac
Delil	14	2	2	0.67	0.50	Mid	Lac
Duct	6	3	3	0.57	0.38	Mid	Lac
Flaxy	18	2	9	0.11	0.20	Sub	Non-Lac
Gold	15	1	3	0.40	0.44	Dom	Lac
Hobble	18	12	3	1.75	0.90	Dom	Non-Lac
Jail	5	7	25	0.26	0.40	Mid	Non-Lac
Left	7	6	5	0.38	0.22	Mid	Lac
Leslie	17	0	10	0.13	0.11	Mid	Lac
Lime	21	5	15	0.33	0.50	Mid	Non-Lac
Liver	6	3	1	0.83	0.75	Dom	Lac
Med	13	1	1	0.50	0.56	Mid	Lac
New	8	0	0	1.00	0.78	Sub	Lac
None	16	6	7	0.55	0.60	Mid	Non-Lac
Phone	7	2	9	0.15	0.13	Sub	Lac
Rock	10	3	4	0.40	0.33	Sub	Lac
Scrawny	17	2	0	3.00	1.00	Dom	Lac
Socks	20	11	12	0.58	0.70	Dom	Non-Lac
Tail	6	30	15	0.90	0.80	Dom	Non-Lac
Thunder	12	0	0	1.00	0.67	Sub	Lac
Yes	17	4	1	1.00	0.88	Dom	Lac

*Standardized CBI

** Reproductive State

Table 5. Information of aggressive behavior and dominance hierarchy from male (two stallions) and female horses at 3T Farm.

Name	Age (y)	Total Wins	Total Losses	CBI	S CBI*	Rank	RS**
Autumn	14	1	13	0.07	0.04	Sub	Non-Lac
Bay	24	0	8	0.08	0.1	Sub	Non-Lac
BC	8	1	0	2	0.89	Dom	Lac
Bone	6	0	0	1	0.75	Dom	Non-Lac
Chocolate	?	11	2	0.75	0.68	Dom	Lac
Chore	16	2	6	0.19	0.2	Sub	Lac
Crooked	16	2	13	0.17	0.18	Sub	Lac
Day	6	4	0	4	1	Dom	Non-Lac
DB	10	0	0	1	0.82	Dom	Lac
Diag	20	10	1	2.25	0.93	Dom	Non-Lac
Dock	24	1	1	0.67	0.55	Mid	Lac
Eye	17	2	3	0.29	0.29	Sub	Non-Lac
Face	10	3	5	0.29	0.3	Sub	Lac
Five	12	0	1	0.2	0.25	Sub	Non-Lac
Flaw	8	7	3	0.44	0.54	Mid	Non-Lac
Flax	21	2	7	0.17	0.14	Sub	Lac
Four	8	5	0	5	0.95	Dom	Non-Lac
Garlique	10	9	3	0.67	0.61	Mid	Lac
Grate	7	4	4	0.45	0.45	Mid	Lac
Grey	14	5	3	0.83	0.71	Dom	Non-Lac
Guy	11	0	4	0.1	0.12	Sub	Non-Lac
Lies	11	4	4	0.33	0.39	Mid	Non-Lac
Lip	6	2	5	0.42	0.4	Mid	Non-Lac
Loner	26	0	0	1	0.70	Dom	Non-Lac
Mange	?	1	4	0.17	0.15	Sub	Non-Lac
Manly	6	4	4	0.27	0.21	Sub	Stallion
Miss	8	6	2	0.75	0.64	Mid	Lac
Moon	?	2	6	0.43	0.46	Mid	Lac

Night	10	3	4	0.31	0.32	Sub	Non-Lac
One	14	4	1	2.5	0.9	Dom	Non-Lac
Pen	18	0	0	1	0.7	Dom	Lac
Plain	16	4	1	0.83	0.6	Mid	Non-Lac
Precious	6	2	3	0.43	0.43	Mid	Non-Lac
Pretty	4	1	9	0.1	0.07	Sub	Non-Lac
Princess	13	0	7	0.08	0.05	Sub	Non-Lac
Radiant	5	5	4	0.56	0.57	Mid	Non-Lac
Red	?	5	3	1.5	0.86	Dom	Non-Lac
Right	16	5	3	0.44	0.5	Mid	Non-Lac
Riva	?	0	0	1	0.75	Dom	Lac
Sight	14	4	2	1.25	0.8	Dom	Lac
Star	6	4	2	0.5	0.5	Mid	Non-Lac
Stud	12	6	1	2.5	0.85	Dom	Stallion
Thick	?	4	5	0.33	0.36	Mid	Non-Lac
Three	8	0	0	1	0.65	Mid	Non-Lac
Thrill	?	6	2	2.33	0.96	Dom	Non-Lac
Tight	5	9	0	8	1	Dom	Non-Lac
Unique	9	1	2	0.33	0.35	Mid	Lac
War	9	2	2	0.29	0.25	Sub	Non-Lac

*Standardized CBI Score

**Reproductive State

Table 6. Regression of mean fecal glucocorticoid, CBI Score and Standardized CBI Score for each field (see Figures 3 and 4 for scatterplots of the relevant data).

CBI Score	Field	R²	Y equation	P value
	1	0.01	1.0x+19.0	0.83
	2	0.10	1.3x+9.4	0.37
	3	0.02	1.9x+13.5	0.72
	4	0.04	0.2x+17.7	0.31
	5	0.01	-0.9x+19.3	0.70
Standardized CBI Score	1	0.28	11.8x+11.7	0.14
	2	0.28	17.8x+10.9	0.11
	3	0.02	-6.2x+26.4	0.74
	4	0.02	-2.4x+13.3	0.51
	5	0.01	-1.9x+20.1	0.71

Table 7. Activity duration of each behavior in early and late summer at 3T Farm and Westwind Farm.

Activity by Season						
	Behavior	N	d.f	F	p value	Direction
Total Duration	Alert	61	1,55	28.29	<0.001**	July/Aug>May/June
	Eat	61	1,55	0.63	0.43	
	Locomotion	61	1,55	14.1	<0.001**	May/June>July/Aug
	Rest	61	1,55	7.98	0.006**	May/June>July/Aug
	Social	61	1,55	0.53	0.46	
Bout Duration	Alert	61	1,55	27.23	<0.001**	July/Aug>May/June
	Eat	61	1,55	0.04	0.83	
	Locomotion	61	1,55	9.01	0.004**	May/June>July/Aug
	Rest	61	1,55	9.52	0.003**	July/Aug>May/June
	Social	61	1,55	2.93	0.09	

** Indicates significance $p < 0.05$

Table 8. Means and standard deviations of total activity behavior at 3T Farm and Westwind Farm by season.

Comparison of Total Activity					
		3T Farm		Westwind Farm	
	Behavior	Mean (s)	SD (s)	Mean (s)	SD (s)
May/June	Alert	130.24	±61.58	139.09	±65.24
	Eat	179.43	±117.55	282.47	±113.27
	Locomotion	48.97	±29.26	48.41	±26.64
	Rest	209.51	±126.08	102.12	±66.76
	Social	25.31	±43.65	8.99	±15.38
July/Aug	Alert	211.52	±73.09	175.17	±75.84
	Eat	229.32	±98.68	257.28	±126.80
	Locomotion	25.41	±15.21	38.23	±35.31
	Rest	120.95	±97.32	106.57	±113.18
	Social	14.89	±24.35	13.11	±37.32

Table 9. Means and standard deviations of bout duration behavior at 3T Farm and Westwind Farm by season.

Comparison of Bout Duration					
		3T Farm		Westwind Farm	
	Behavior	Mean (s)	SD (s)	Mean (s)	SD (s)
May/June	Alert	57.75	±71.66	62.57	±31.63
	Eat	108.46	±74.75	97.37	±41.49
	Locomotion	28.53	±24.82	20.53	±6.32
	Rest	86.07	±72.38	44.49	±39.63
	Social	26.40	±46.73	5.20	±6.07
July/Aug	Alert	129.51	±38.14	96.76	±52.35
	Eat	91.63	±33.48	6.09	±14.35
	Locomotion	17.22	±10.61	16.46	±12.68
	Rest	90.15	±35.23	105.83	±81.05
	Social	11.68	±18.47	4.99	±17.28

Table 10. ANOVA statistics comparing the total and bout duration of activity behaviors between reproductive state and rank at 3T Farm and Westwind Farm.

Comparison of Activity						
Total Duration	Behavior	N	d.f	F	p value	Direction
Rank	Alert	61	2,55	2.29	0.11	
	Eat	61	2,55	0.39	0.67	
	Locomotion	61	2,55	0.20	0.82	
	Rest	61	2,55	1.75	0.18	
	Social	61	2,55	0.75	0.47	
Reproductive State	Alert	61	1,55	16.65	<0.001**	Nonlactating>Lactating
	Eat	61	1,55	9.42	0.003**	Lactating>Nonlactating
	Locomotion	61	1,55	5.65	0.02**	Lactating>Nonlactating
	Rest	61	1,55	0.96	0.32	
	Social	61	1,55	9.48	0.003**	Nonlactating>Lactating
Bout Duration						
Rank	Alert	61	2,55	0.54	0.58	
	Eat	61	2,55	1.34	0.27	
	Locomotion	61	2,55	0.54	0.58	
	Rest	61	2,55	0.12	0.88	
	Social	61	2,55	1.06	0.35	
Reproductive State	Alert	61	1,55	4.79	0.03**	Nonlactating>Lactating
	Eat	61	1,55	9.75	0.003**	Lactating>Nonlactating
	Locomotion	61	1,55	5.36	0.02**	Lactating>Nonlactating
	Rest	61	1,55	1.19	0.28	
	Social	61	1,55	8.25	0.005**	Nonlactating>Lactating

Table 11. Mean and standard deviation of total activity duration of dominant, middle, and subordinate horses at 3T Farm and Westwind Farm.

Comparison of Total Duration					
		May/June		July/August	
	Behavior	Mean (s)	SD (s)	Mean (s)	SD (s)
Dominant	Alert	154.68	±64.91	216.36	±49.05
	Eat	233.80	±116.77	209.85	±81.34
	Locomotion	45.51	±26.08	36.10	±33.12
	Rest	136.99	±98.62	104.28	±77.81
	Social	23.54	±35.81	20.43	±41.16
Middle	Alert	125.51	±69.13	189.91	±74.76
	Eat	227.11	±145.10	268.86	±109.85
	Locomotion	44.74	±25.76	33.88	±24.63
	Rest	167.11	±137.85	100.89	±97.88
	Social	19.15	±30.27	11.98	±22.65
Subordinate	Alert	123.18	±48.90	185.01	96.40
	Eat	202.93	±112.89	237.70	±132.75
	Locomotion	56.81	±31.94	21.32	±16.50
	Rest	192.07	±108.38	142.99	±129.22
	Social	13.06	±42.15	10.54	±25.11

Table 12. Mean and standard deviation of bout duration of dominant, middle, and subordinate horses at 3T Farm and Westwind Farm.

Comparison of Bout Duration					
		May/June		July/August	
	Behavior	Mean (s)	SD (s)	Mean (s)	SD (s)
Dominant	Alert	63.64	±52.51	120.52	±40.73
	Eat	100.59	±58.42	79.79	±46.43
	Locomotion	23.12	±18.61	16.29	±12.81
	Rest	63.72	±68.14	96.70	±67.07
	Social	23.94	±45.23	9.76	±19.27
Middle	Alert	58.79	±57.96	121.42	±51.05
	Eat	112.05	±67.05	118.45	±63.29
	Locomotion	24.68	±11.34	16.79	±9.59
	Rest	74.16	±70.99	87.34	±50.60
	Social	19.83	±40.82	10.30	±19.37
Subordinate	Alert	56.95	±66.71	105.20	±48.31
	Eat	97.38	±64.89	103.57	±44.16
	Locomotion	28.07	±28.04	17.68	±12.50
	Rest	68.14	±53.21	107.64	±59.23
	Social	8.93	±21.26	6.46	±16.08

Table 13. Mean and standard deviation of total activity duration of lactating and non-lactating horses at 3T Farm and Westwind Farm.

Comparison of Total Duration					
		May/June		July/August	
	Behavior	Mean (s)	SD (s)	Mean (s)	SD (s)
Lactating	Alert	113.82	±57.78	163.54	±65.06
	Eat	273.18	±120.89	269.57	±128.14
	Locomotion	53.58	±29.37	38.43	±30.58
	Rest	136.61	±121.57	112.48	±124.28
	Social	7.74	±17.37	5.73	±15.69
Non-lactating	Alert	150.88	±62.53	224.69	±73.65
	Eat	177.94	±114.04	216.35	±88.74
	Locomotion	44.63	±26.51	24.07	±19.43
	Rest	190.02	±110.57	117.24	±83.77
	Social	27.85	±44.02	21.31	±37.04

Table 14. Mean and standard deviation of bout duration of lactating and non-lactating horses at 3T Farm and Westwind Farm.

Comparison of Bout Duration					
		May/June		July/August	
	Behavior	Mean (s)	SD (s)	Mean (s)	SD (s)
Lactating	Alert	52.21	±52.21	101.87	±50.91
	Eat	113.79	±113.79	131.06	±60.29
	Locomotion	29.29	±29.29	20.26	±10.59
	Rest	49.95	±49.95	99.26	±78.86
	Social	6.04	±6.04	4.09	±14.86
Non-Lactating	Alert	66.10	±70.12	128.15	±40.33
	Eat	95.53	±70.53	76.93	±33.15
	Locomotion	21.83	±14.85	14.07	±11.45
	Rest	85.22	±69.40	94.31	±33.75
	Social	27.62	±47.41	13.05	±19.83

Table 15. G-test statistics and p- values for aggressive behavior of dominant, middle, and subordinate horses in each reproductive state at 3T Farm and Westwind Farm. See Table 3 for descriptions of behavior.

Comparison of Rank				
Aggressive Behavior	N	df	G	p-value
Contact	73	2	0.18	0.91
Non-contact	73	2	0.49	0.78
Position	73	3	0.90	0.82

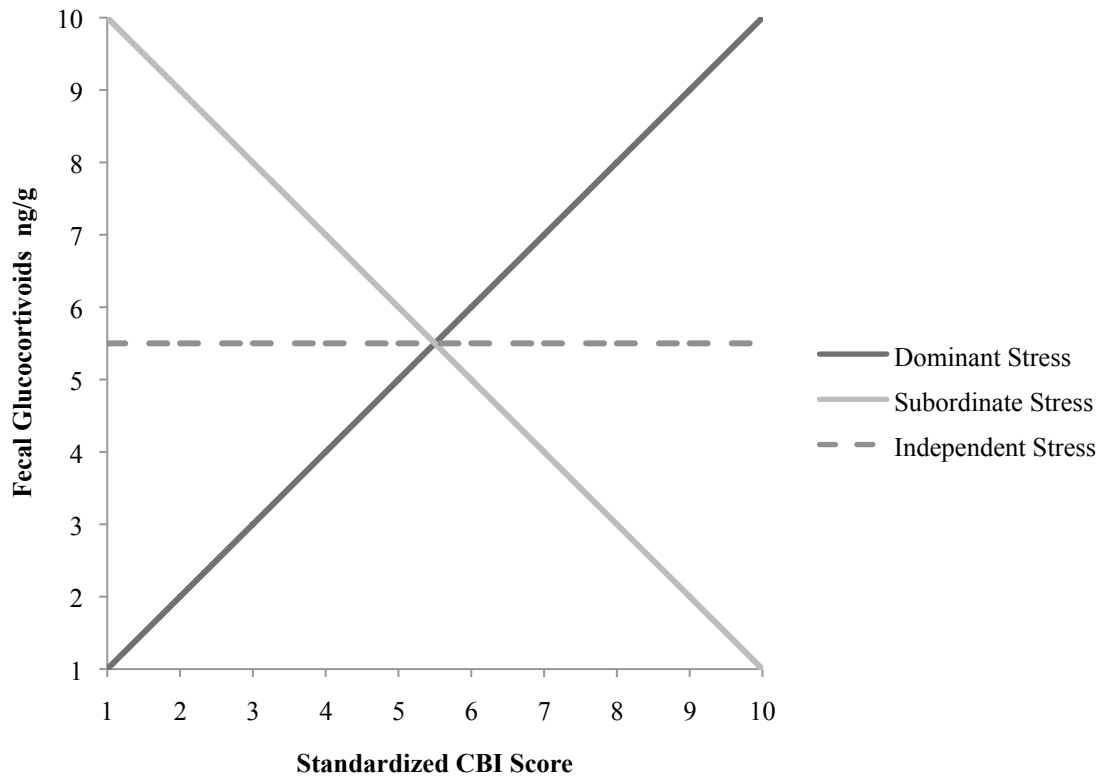


Figure 1. Hypothesized stress level and dominance rank in the dominant-stress hypothesis, subordinate-stress hypothesis, and independent-stress hypothesis.

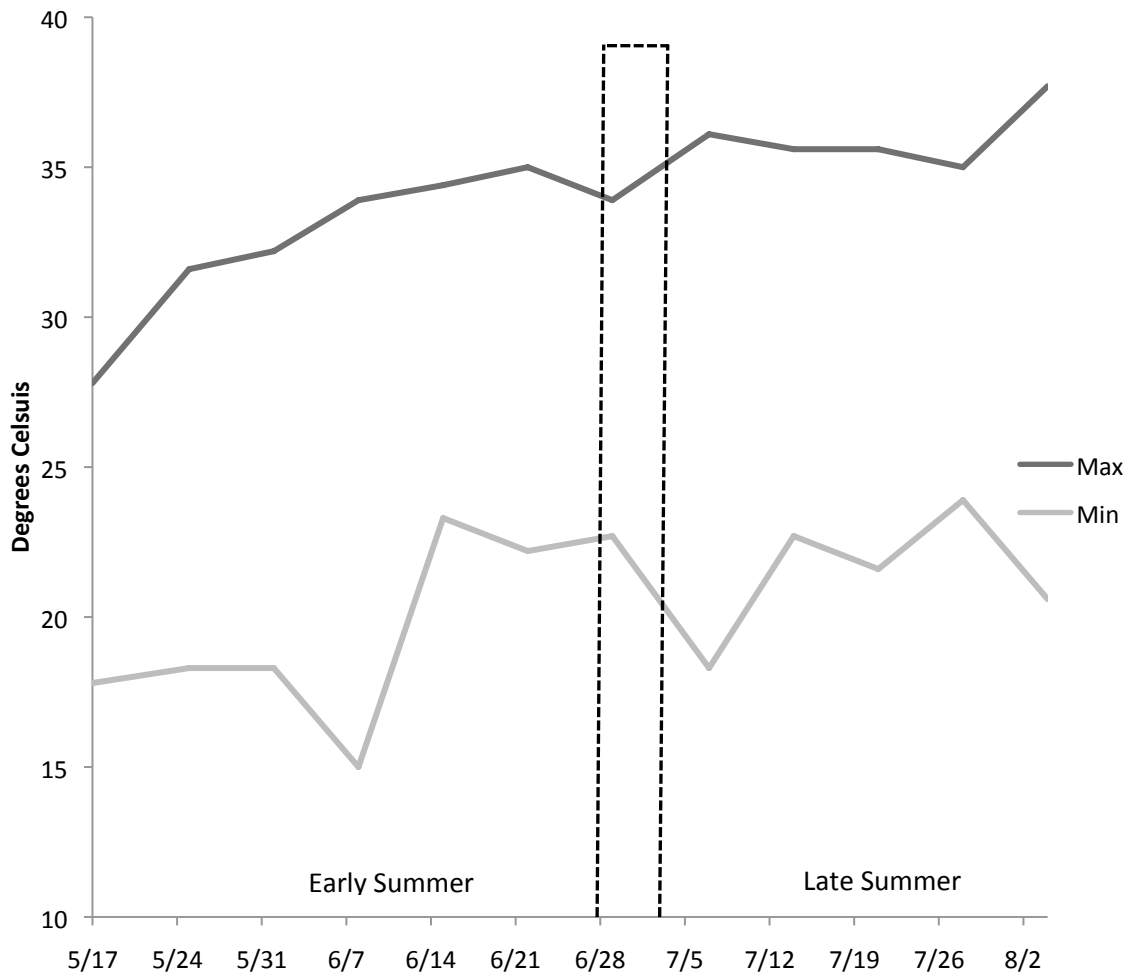


Figure 2. Mean minimum and maximum temperature (°C) for each week of this study May 16-August 4, 2010. The dotted line represents the point in which there was a break from data collection (June 28-July 3).

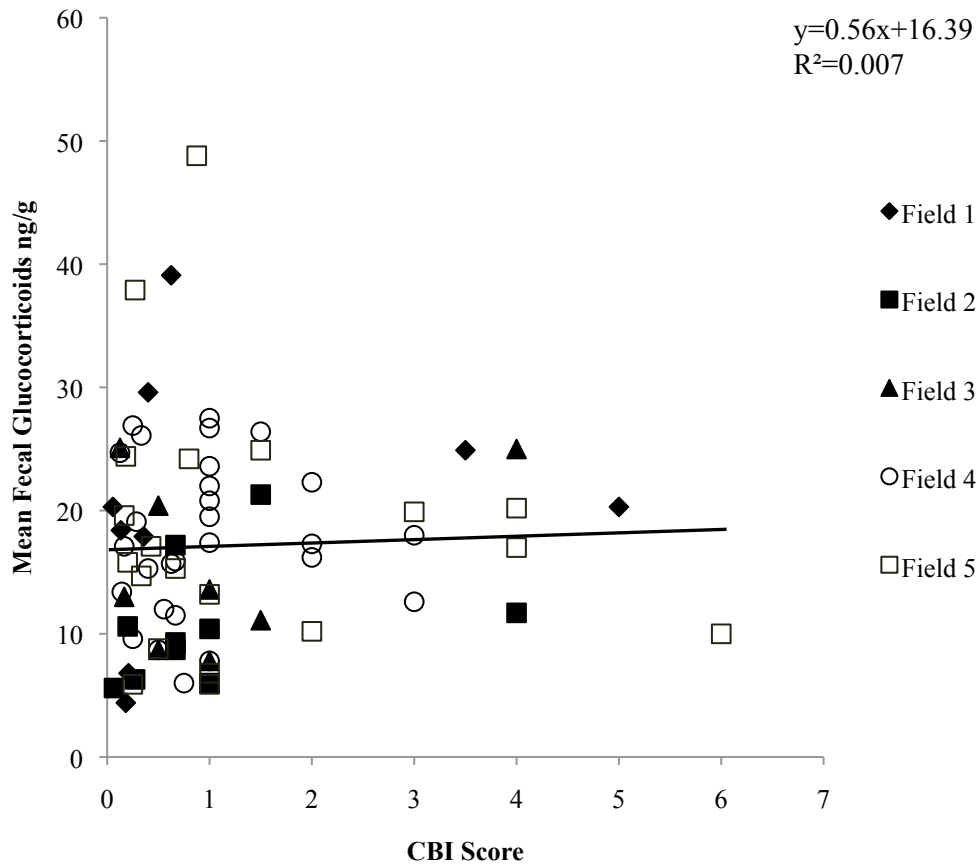


Figure 3. Regression of mean fecal glucocorticoid level and CBI score per horse at both facilities. Closed shapes represent Westwind Farm and open shapes represent 3T Farm. No significant relationship was found and the regression line is shown only to indicate the absence of a relationship between cortisol and CBI score.

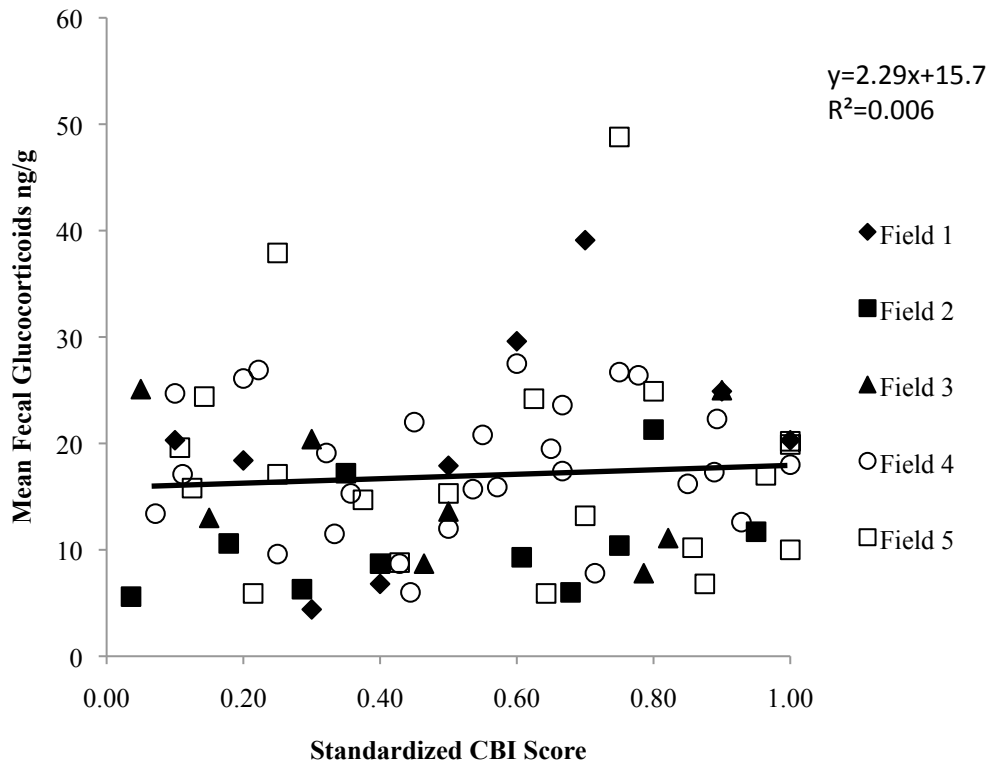


Figure 4. Regression of mean fecal glucocorticoid level and standardized CBI score per horse at both facilities. See methods for how the standardized CBI was calculated. Closed shapes represent Westwind Farm and open shapes represent 3T Farm. No significant relationship was found and the regression line is shown only to indicate the absence of a relationship between fecal glucocorticoid and standardized CBI score.

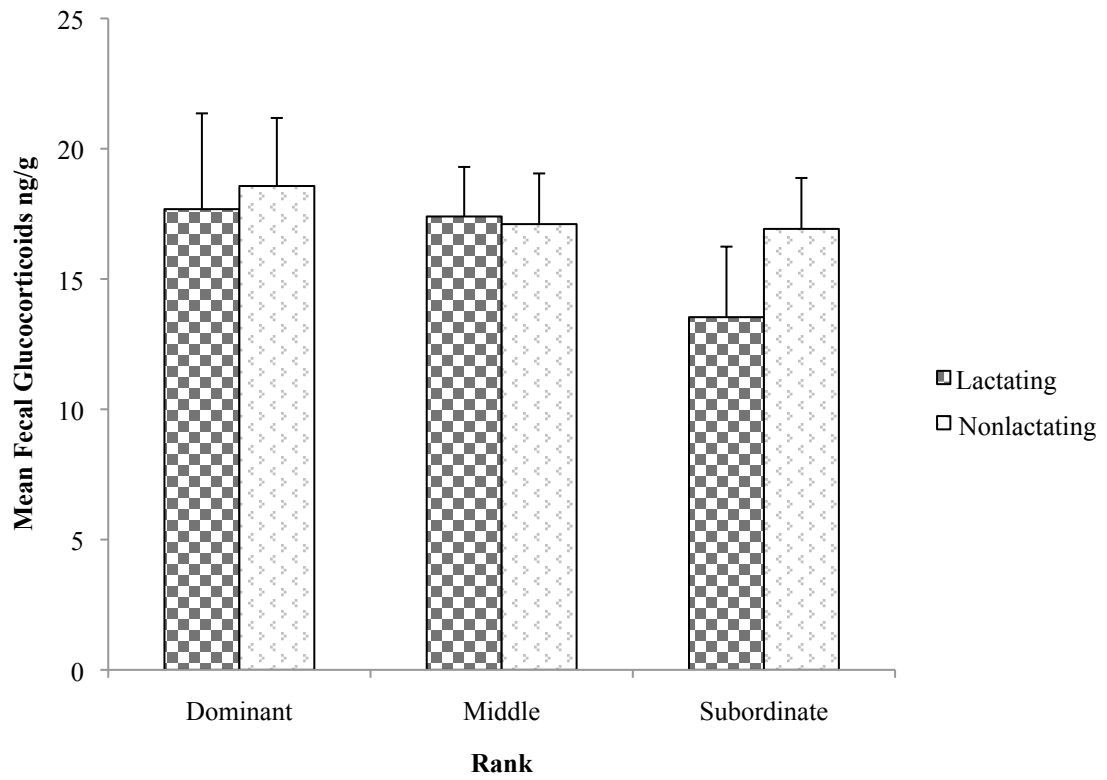


Figure 5. Mean fecal glucocorticoid level (\pm SE) of lactating and non-lactating horses in each rank at 3T Farm and Westwind Farm.

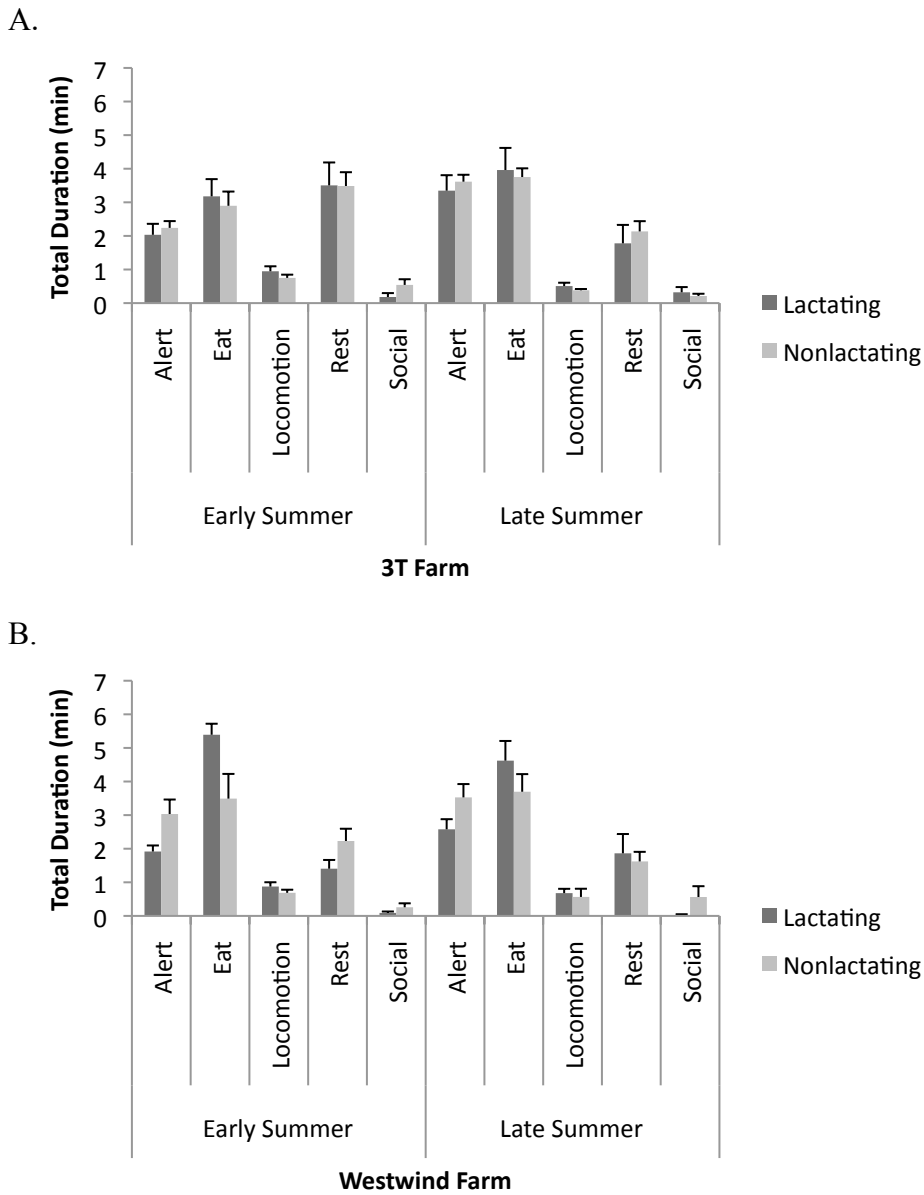


Figure 6. Total duration (\pm SE) of behavior of lactating and non-lactating horses. Figure A depicts data from 3T Farm by season, while Figure B depicts data from Westwind Farm.

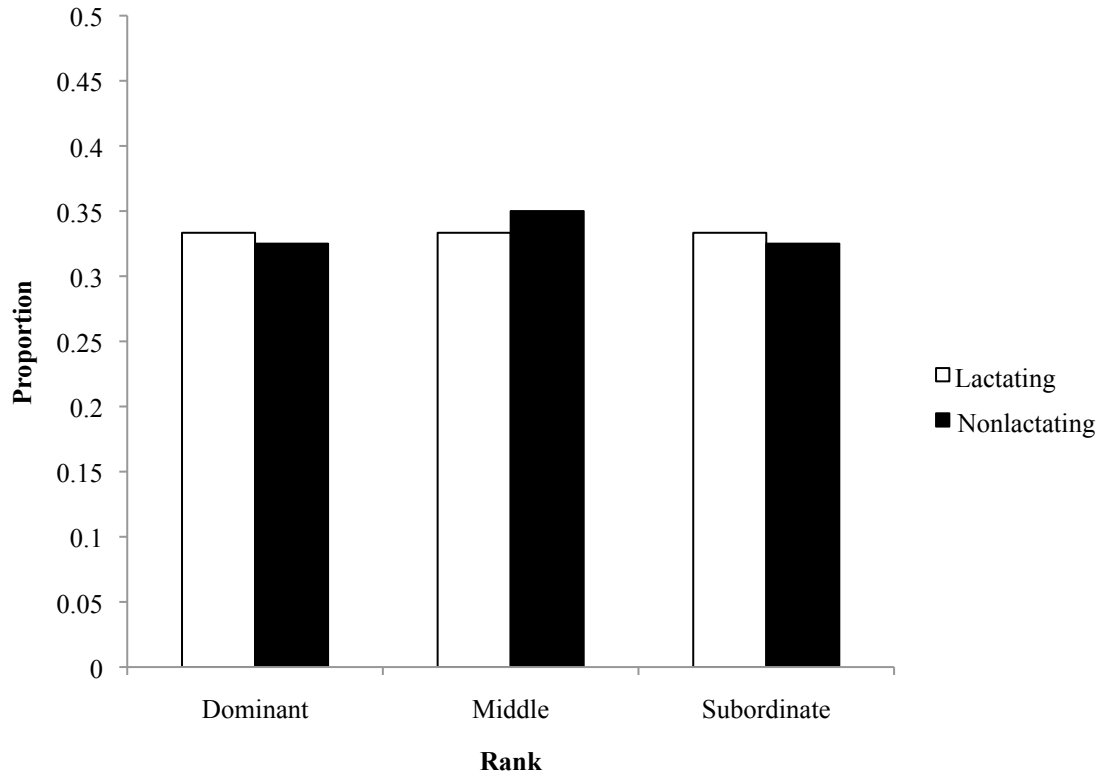


Figure 7. Proportion of lactating and non-lactating and dominant, middle, and subordinate horses at 3T Farm.

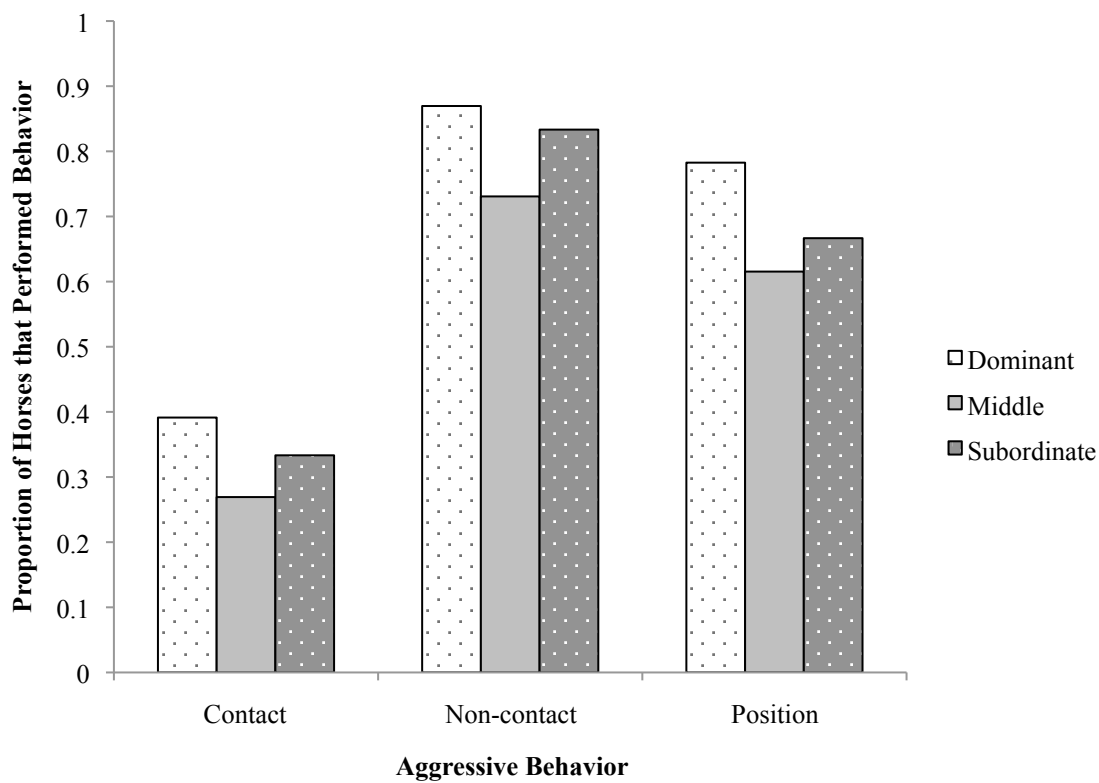


Figure 8. Proportion of horses within each rank that performed aggressive behavior at 3T Farm and Westwind Farm.

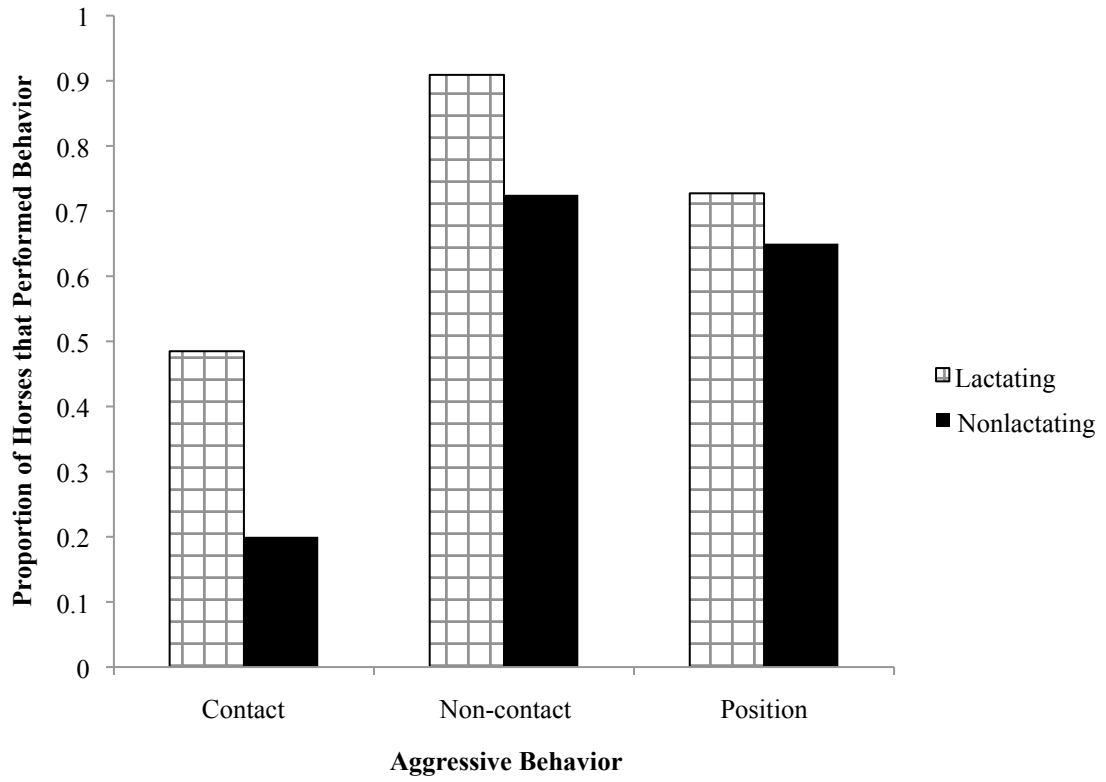


Figure 9. Proportion of horses within each reproductive state that performed aggressive behavior at 3T Farm and Westwind Farm.

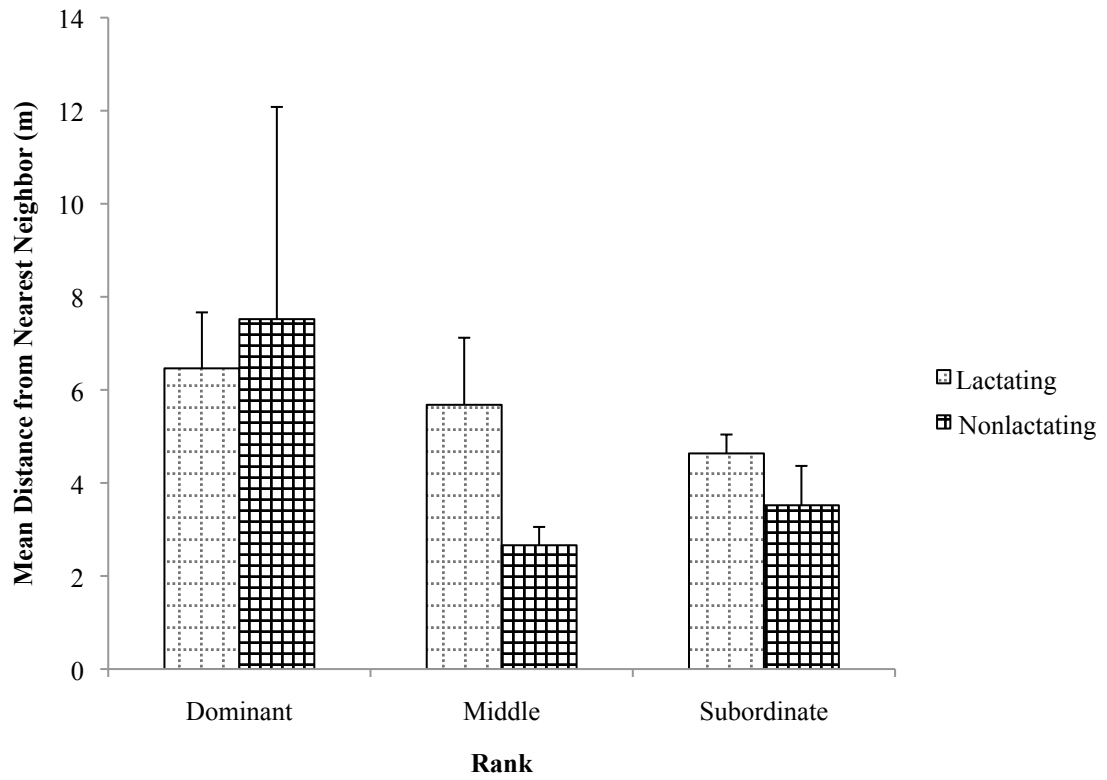


Figure 10. Mean (\pm SE) distance from nearest neighbor within rank and reproductive state at 3T Farm and Westwind Farm.

Appendix

In addition to the data that was collected and analyzed above, I attempted to determine the effect of rank and reproductive state on herd movement. I did not establish my sampling rules firmly enough while collecting data, and therefore did not use the information to draw conclusions. Horses were designated as being in either the front, middle, rear, or outside of the herd. I determined how to categorize horses based on overall orientation of the herd. The horses that were towards the front of the herd and seemed to be leading movements were classified as being in front. Horses that were at least five horse lengths behind that group were in the middle group. Any individuals that were more than five horse lengths away, but in the same orientation as the herd were designated as being in the rear. Horses that were at least ten horse lengths away from any of the three main groups and were in a different orientation than the herd were recorded as being outside of the herd. Although these data were not used in this study, they could be a point of reference for future studies. The table below provides details on the average proportion that each horse was in the front, middle, rear, or outside position. The table also provides the reproductive state and rank of each individual.

Name	Reproductive State	Rank	Front	Middle	Rear	Outside
Autumn	Non-Lac	Sub	0.00	0.50	0.50	0.00
Barbette	Lac	Sub	0.23	0.68	0.10	0.00
Bay	Lac	Sub	0.27	0.37	0.36	0.00
BB	Lac	Mid	0.48	0.12	0.40	0.00
BC	Lac	Dom	0.00	0.52	0.48	0.00
Big'n	Non-Lac	Sub	0.28	0.29	0.43	0.00
Black	Non-Lac	Dom	0.41	0.57	0.02	0.00
Bone	Non-Lac	Dom	0.63	0.15	0.06	0.15
Chocolate	Lac	Mid	0.38	0.62	0.00	0.00
Chore	Lac	Sub	0.01	0.71	0.27	0.00
Chris	Lac	Dom	0.27	0.49	0.24	0.00
Crooked	Lac	Sub	0.00	0.38	0.62	0.00
Dark	Lac	Dom	0.25	0.13	0.62	0.00
Day	Non-Lac	Dom	0.64	0.36	0.00	0.00
DB	Lac	Dom	0.00	1.00	0.00	0.00
Delil	Lac	Mid	0.22	0.29	0.49	0.00
Diag	Non-Lac	Dom	0.47	0.17	0.36	0.00
Dock	Non-Lac	Mid	0.12	0.19	0.03	0.66
Duct	Lac	Mid	0.52	0.42	0.06	0.00
Eye	Non-Lac	Sub	0.08	0.81	0.11	0.00
Face	Lac	Sub	0.19	0.79	0.02	0.00
Five	Non-Lac	Sub	0.25	0.40	0.19	0.15
Flaw	Non-Lac	Mid	0.04	0.87	0.09	0.00
Flax	Lac	Sub	0.00	0.38	0.62	0.00
Flaxy	Non-Lac	Sub	0.15	0.00	0.85	0.00
Four	Non-Lac	Dom	0.25	0.38	0.21	0.15
Garlique	Lac	Mid	0.28	0.57	0.15	0.00
Gold	Lac	Mid	0.38	0.00	0.13	0.50
Grate	Lac	Mid	0.08	0.73	0.19	0.00
Grey	Non-Lac	Mid	0.08	0.57	0.36	0.00
Guy	Non-Lac	Sub	0.64	0.36	0.00	0.00
Hobble	Non-Lac	Dom	0.47	0.50	0.03	0.00
Jail	Non-Lac	Sub	0.00	0.00	1.00	0.00
Left	Lac	Sub	0.08	0.88	0.04	0.00
Leslie	Lac	Sub	0.72	0.28	0.00	0.00
Lies	Non-Lac	Mid	0.25	0.49	0.26	0.00
Lime	Non-Lac	Mid	0.08	0.92	0.00	0.00
Lip	Non-Lac	Mid	0.18	0.32	0.50	0.00
Liver	Lac	Dom	0.52	0.48	0.00	0.00
Mange	Non-Lac	Dom	0.11	0.74	0.15	0.00
Manly	Non-Lac	Sub	0.21	0.25	0.55	0.00
Med	Lac	Mid	0.23	0.64	0.13	0.00

Miss	Lac	Mid	0.00	0.74	0.26	0.00
Moon	Lac	Dom	0.40	0.34	0.26	0.00
New	Lac	Sub	0.66	0.23	0.04	0.08
Night	Non-Lac	Mid	0.15	0.60	0.25	0.00
None	Non-Lac	Dom	0.28	0.64	0.08	0.00
One	Non-Lac	Mid	0.24	0.76	0.00	0.00
Pen	Lac	Sub	0.12	0.00	0.23	0.65
Phone	Lac	Mid	0.31	0.41	0.28	0.00
Plain	Non-Lac	Mid	0.18	0.24	0.58	0.00
Precious	Non-Lac	Sub	0.28	0.47	0.25	0.00
Pretty	Non-Lac	Sub	0.00	0.91	0.09	0.00
Princess	Non-Lac	Mid	0.45	0.54	0.01	0.00
Radiant	Non-Lac	Dom	0.28	0.68	0.04	0.00
Red	Non-Lac	Mid	0.17	0.43	0.40	0.00
Riva	Non-Lac	Dom	0.06	0.43	0.51	0.00
Rock	Lac	Dom	0.00	1.00	0.00	0.00
Scrawny	Lac	Dom	0.65	0.13	0.22	0.00
Sight	Lac	Sub	0.04	0.40	0.54	0.02
Socks	Non-Lac	Mid	0.08	0.92	0.00	0.00
Star	Lac	Mid	0.48	0.11	0.42	0.00
Stud	Non-Lac	Dom	0.24	0.45	0.15	0.15
Tail	Non-Lac	Mid	0.57	0.43	0.00	0.0
Thick	Non-Lac	Dom	0.17	0.60	0.23	0.00
Three	Non-Lac	Mid	0.27	0.54	0.04	0.15
Thrill	Non-Lac	Dom	0.25	0.31	0.44	0.00
Thunder	Lac	Mid	0.04	0.26	0.70	0.00
Tight	Non-Lac	Sub	0.04	0.58	0.38	0.00
Unique	Lac	Dom	0.17	0.61	0.19	0.02
War	Non-Lac	Sub	0.25	0.65	0.10	0.00
Yes	Lac	Dom	0.45	0.34	0.21	0.00

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