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Behavioral and Physiological Responses of Horses to Initial Training: The Comparison Between Pastured Versus Stalled Horses

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Behavioral and Physiological Responses of Horses to Initial Training: The Comparison Between Pastured Versus Stalled Horses

By

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Abstract

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There are evidences that learning ability may be impaired in animals housed in social isolation or barren environments. Responses to initial training may be affected by housing conditions. Sixteen 2-yr-old Arabian horses were kept on pasture (P) (n=8) or in individual stalls (S) (n=8). Twelve horses (6P and 6S) were subjected to a standardized training procedure, carried out by two trainers in a round pen, and 4 horses (2P and 2S) were used as control. On sample collection day 0, 7, 21 and 28, behaviors were recorded, blood samples were drawn and heart rates were monitored. Total training time for the stalled housed horses was significantly higher than total time for the pastured horses. The stalled group required more time to habituate to the activities occurring from the start of training to mounting. Frequency of undesirable behaviors such as bucks and jumps was higher in the stalled horses. On day 0 and day 21, basal plasma cortisol levels were lower when compared to basal plasma cortisol levels on day 7 and day 28. Pastured horses tended to have higher basal heart rates on day 0.

While the physiological data failed to identify differences among housing groups, the behavioral data suggests that pasture-kept horses adapt easier to training than stalled horses.

This thesis is dedicated to my parents
Enrique Rivera and Delia Diaz
and my son Pork Chop
for their love, support and encouragement.

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Introduction

Horses kept in stalls are deprived of opportunities for social bonding and the performance of natural behaviors (Hogan et al., 1988). Housing horses on pasture or in box stalls are accepted management techniques in the horse industry. Pasture sizes will vary according to the owners' available land, while box stalls are typically 9 m^2 or 13 m^2 in size, possibly with a small window and/or dividing bars between stalls (Evans et al., 1990). Humans are accustomed to seeing horses in box stalls and may not perceive their housing condition as negatively as they might a calf in a veal crate. Housing horses in stalls is different from their natural, pasture-like environment. In the wild, horses have a structured social environment. Harem bands, averaging five to seven horses, are typically comprised of fillies, a few yearlings and foals and one stallion (Kirkpatrick and Francis, 1994). These cohesive bands may roam over vast areas of land and stay together even in the absence of the stallion. For the convenience of feeding and accessibility, humans stable horses inhibiting some behavior patterns and social interactions.

Cunningham (1991) suggests that environmental conditions contribute to 65% of a horse's performance potential while 35% of their potential is genetically inherited. Trainability affects the monetary value of horses since the horse must be handled for racing, showing and pleasure riding. If the environment negatively affects the animal's learning ability, trainability along with the value of the horse may decrease. Environmental variables include, but are not limited to, nutrition, trainer experience, degree of handling, temperature, lighting and housing. An animal's ability to learn, solve problems or survive stressful situations is influenced by the environment they are raised in. The environmental condition a horse is exposed to does not only affect its learning ability, but may affect its welfare. If the environment causes the horse to make significant adjustments in behavior and physiology, the housing situation may be deemed as stressful (Fraser, 1992).

Animals may respond to environmental challenges or stressors imposed on them through a variety of physiological, biochemical, anatomical, immunological and behavioral adaptation mechanisms (Ewbank, 1985). Behavioral and physiological measures have become widely used to determine stress levels and welfare in animals (Broom and Johnson, 1993).

Abnormal behaviors that are not commonly observed in pastured horses have been attributed to stabling of horses. Weaving, cribbing and stall walking are a few of the behavior abnormalities linked to stalling horses (Kiley-Worthington, 1990). Physiological measures such as plasma cortisol (Boulton et al., 1997) and heart rate (Baldock and Sibling, 1990) have been associated with activation of the hypothalamus-pituitary-adrenal axis system, which is triggered during stressful situations in animals.

Based on this information, the hypothesis of this study is that pastured yearlings will acclimatize more readily to initial training than individually stalled horses. The objective of this study is to determine if housing conditions may have an effect on behavioral and physiological measures in horses that are subjected to a standardized training procedure. In this study, stress is a response to stressors, the environment in which the animals are housed and the training regimen. The long-term goal of the study is to improve the welfare and housing conditions of horses.

Literature Review

Housing

Housing environments can create behavioral problems as well as interfere with naturally occurring behaviors. Stalling weaned foals altered their behavior qualitatively and quantitatively when compared to foals weaned in a paddock (Heleski et al., 1999). In this study, paddock-housed foals spent the majority of their time standing in close contact with the other horses, interacting with the horses or eating. The stalled horses, could not perform some of the behaviors observed in the paddock housed foals (i.e. grazing, social interactions). In addition, the stalled foals performed more uncommon behaviors such as licking, chewing and kicking the stall walls. Rearing, bucking, bolting and head throwing in horses have been associated with some environmental variables such as a barren environment with insufficient stimuli, restriction in movement and isolation from conspecific or social partners (Kiley-Worthington, 1990).

In addition to altering behaviors, barren environments can affect learning. Heird et al. (1986) demonstrated that young horses in a richer environment perform better during complex tasks. Similarly, group housed rats raised with various toys learned a radial maze more quickly and accurately than rats raised in an isolated environment (Juraska et al., 1983). Moore and Spear (1995) reported that memory retention was decreased in rats housed with anesthetized dams and siblings as well as those housed in social isolation. However, when housed with foster dams, sires and littermates, these rats showed no alteration in their olfactory memory. Isolated male rats during an active avoidance test could not learn to avoid an electric shock when warned by an indicator light (Viveros et al., 1990). Forgay and Read (1962) demonstrated rats provided with wooden and metal objects to enrich their environment made fewer errors in a maze than rats housed in a barren environment. Housing conditions reflected through behavior and various trial experiments have influenced an animals' learning ability which may affect trainability.

Training in Horses

The value of a horse is greatly increased by its trainability. Training relies on manipulating the horses' environment into stimuli and reinforcements in order to attain a desired response (Yeates, 1994). Learning processes such as habituation, classical and operant conditioning, shaping, positive and negative reinforcements are used for training horses (Fraser, 1992). Habituation is defined as the decrease or cessation of a physiological or behavioral response as a result of repeated exposure to the stimulus (Voith, 1986). Horses refusing to load into a trailer will gradually habituate, with practice over time, and easily load into the trailer (Houpt, 1986).

Classical conditioning is using unconditioned stimuli and unconditioned responses and pairing them with conditioned stimuli (Schmajuk, 1997). With time, the unconditioned response is associated with the conditioned stimuli and the unconditioned response becomes a conditioned response. Leading a stallion towards a customary service area (conditioned stimulus) can act as a sexual stimulus (unconditioned response) (Fraser, 1992). Sexual stimulation (unconditioned response) in the stallion becomes associated with the service area and the stimulation becomes the conditioned response.

Operant conditioning is the process of modifying the frequency of a behavior as a result of an association between an environmental stimuli, an unconditioned stimuli and a response (Schmajuk, 1997). With horses, the most common use of operant conditioning is avoidance learning which rewards the horse by removing something unpleasant (Houpt, 1986). In order to cue a horse for forward motion, riders will press with their legs (stimulus), if the horse has learned to avoid the pressure from the legs (unconditioned stimulus), it should move forward on cue (operant response). Shaping, a form of operant conditioning, is teaching the horse small parts of a large maneuver over time (Houpt, 1986). Shaping begins by initially rewarding the animal when it performs a behavior that is similar to the desired response, then rewarding when successive approximations of the desired behavior are achieved until only the precise behavior is rewarded (Fraser, 1992).

Reinforcements during training can be primary or secondary and positive or negative. Primary reinforcements are natural situations for the horse, such as food or returning to the herd. Secondary reinforcements are learned such as a pat on the neck or vocal praises (Waring, 1983).

Positive and negative reinforcements develop a stronger link between a stimulus and a desired response in horses, increasing the chance of repeating its correct response (Tarpy, 1982). Positive reinforcements can be primary or secondary and are referred to as reward training. Dougherty and Lewis (1991) during discrimination training, demonstrated that horses learned quickly to press a lever once the correct picture was displayed resulting in a food reward. Haag et al. (1980) demonstrated ponies that promptly learned to avoid a negative reinforcement, an electrical shock also, promptly learned a maze test that used positive reinforcement. Avoidance and punishment are methods of negative reinforcements which use aversive stimuli (Yeates, 1994). Using the reins to apply light contact on the mouth for the horse to back up is an avoidance responses since there is a possibility for a stronger application of the reins if the response is not reached (Yeates, 1994). However, this stimulus can also be used as a cue. Reinforcements increase the chance the response will occur again while punishment works to suppress or eliminate a response (Tarpy, 1982). The purpose of punishment is to eliminate or weaken a response (Yeates, 1994). Both punishment and negative reinforcement use aversive stimuli. Punishment as well as any positive or negative reinforcement, must be paired closely to the response in order to be effective (Fraser, 1992; Yeates, 1994). Kratzer et al. (1977) observed fewer errors in Quarter Horses after presentation of an aversive stimulus; however a greater amount of time was spent on deciding which side of the maze to go through.

Learning, a change in the brain in response to an event "outside of the brain", will occur with or without human intervention, throughout the life of an animal (Broom and Johnson, 1993). Every encounter with a stimulus and/or response has the chance to become memorized and influence future behaviors and physiological functions (Broom and Johnson, 1993). "Learning from learned" means a horse is capable of incorporating what they have learned and apply it to the task at hand, suggesting future tasks will be easier to complete (Fiske and Potter, 1979). In the studies conducted by McCall et al., (1981) and Baer et al. (1983) using discrimination learning and Hebb-Williams Closed Field Maze animals which had the opportunity to observe the correct response were more efficient in solving the problems themselves. Time between training sessions can also influence learning. Ponies that were trained once a week, in fewer sessions reached the highest level of performance when compared to ponies trained daily and biweekly (Rubin et al., 1980).

Stress Response

In order to survive, animals maintain homeostasis, which is defined by Broom and Johnson (1993) as the "relatively steady state of a body variable, which is maintained by means of physiological or behavioral regulation". The physical and chemical properties of body fluids and tissues will remain constant despite the changes that are occurring. Any stimulus or adverse condition that can alter or disturb this balance can be considered a stressor. When confronted with a stressor, the human and animal body will undergo adaptation to maintain or regain homeostasis (Broom and Johnson, 1993). Stressors may be physical, psychological or psychosocial in nature (Mason, 1975). Physical stressors include, but are not limited to, injuries (Boyce et al., 1998), changes in body temperature (Geor et al., 1998; Friend et al., 1998), electric shock (Pynoos et al., 1996; Brundige, 1998) and changes in the environment such as confinement (Boyce et al., 1998). Psychological stressors are considered emotional or mental, such as fear or anxiety (Boissy et al., 1998). Psychosocial stress, as the term describes, refers to social interactions or lack of social interactions, for example isolation (Asterita, 1985).

Although there are different forms of stressors, stress responses can activate three major pathways; autonomic nervous system, neuroendocrine system and behavior (Moberg, 1985).

Behavior

Behavioral observations can determine the response of an individual to a stressor. Variations in intensity, frequency and duration of behavioral responses from the normal level can indicate a disturbance (Broom and Johnson, 1993). Behavioral observations are inexpensive, relatively easy and do not subject the animals to additional stressors such as blood sampling (Moberg, 1985). Behavioral measurements can provide an indication of short- term and long-term problems and if an animal is having difficulty in coping with a problem (Broom and Johnson, 1993).

Coping is defined as "having control of mental and bodily stability" (Fraser and Broom, 1990). Coping with problems may not necessarily be harmful to the animal. Coping can be divided into three levels. In the first level, problems are tolerable and coping is easy (i.e. moderate exposure to cold or heat exposure). In the second level, the animal copes but with difficulty (i.e. stomach ulcers) and in the third level, the animal fails to cope and may collapse or die (Broom and Johnson, 1993).

Short-term behavioral responses include situations of human intervention, handling, certain training techniques, transport, and pre-slaughter procedures. In horses, changes in ear, nostril, tail positions and posture, pressing their heads against the wall or staring at the abdomen can be evidence of short-term pain or discomfort (Fraser, 1969). In other species pawing, rolling, staring at flanks, groaning, frequent lying down and getting up, sitting in a dog position are indicators of pain (Waring, 1983). During a freeze and hot-iron branding study, cattle experiencing the hot-iron branding exhibited greater avoidance reactions (jumping away and kicking) toward the procedure (Lay et al., 1992).

Long-term behavior responses deal with situations where the animal lacks control of their interactions with their environment (Broom and Johnson, 1993). Stereotypies are repetitive, invariant sequences of movements that have no obvious function (Mason, 1991). They are amongst the most important indicators of long-term welfare problems (Fraser and Broom, 1990). Inadequate or improper environmental conditions, isolation, and overcrowding are a few examples of causative factors leading to stereotypies (Mason, 1991). Pawing, in horses, was interpreted as a "response to frustration, a displacement activity that originated from the activity of uncovering food buried under snow" (Odberg, 1973). Pawing has been noted to occur in different situations, such as movement limitations, eating grain, anticipation of feed and trying to get a lying foal to stand (Houpt, 1986). Cribbing is when the horse grabs an object with its incisor teeth and force swallows gulps of air (Evans et al., 1990). Cribbing may lead to gastric upsets or colic. Weaving is when the horse weaves its head back and forth while rocking side to side (Evans et al., 1990). In 1995, McGreevy et al., using questionnaires, studied 1750 horses in different equestrian disciplines and revealed a higher percentage of cribbing and weaving in dressage and event horses than in horses used in endurance riding. There was also a positive correlation, both within and between the dressage and eventing horses, in the amount of time spent in a stall to the occurrence of stereotypic behavior. Although wood chewing occurs in horses housed both in restricted environments and pasture, Sambraus (1985) determined the behavior is aggravated in close confinement. Piglets which were reared in a "poor environment" (farrowing crate) compared to piglets reared in a "rich environment" (outdoor pasture with half-open crates) displayed more aggressive behaviors (DeJonge et al., 1996). In addition, the subordinates in the poor environment showed symptoms of chronic social stress exposure by delayed onset of puberty, smaller weight gain and elevated cortisol levels. Environmental stress and welfare of animals can be determined using long and short-term behavioral observations.

Physiological Indicators of Stress

Hans Seyle was the first to report the neuroendocrine response to stress in 1956. Seyle (1956) reported that animals who undergo challenges to their homeostasis such as heat, cold, and infection will elicit morphological reactions in the body, such as decrease in size of the thymus and lymphatic structures, occurrence of ulcers in the gastrointestinal tract and adrenocortical stimulation. Seyle concluded this triad of reactions represents a non-specific reaction to almost all-harmful stimuli and refers to this syndrome as the "general adaptation syndrome". Selye hypothesized the general adaptation syndrome, through a series of nervous and endocrine (neuroendocrine system) pathways can help the body to adapt to the stress.

The general adaptation syndrome is divided into three stages (Seyle, 1956). Alarm reaction, the initial response, is the body's first defense mechanism. In this stage, the adrenal cortex will release glucocorticoids into the bloodstream, depleting its stores. If the animal does not die during the alarm phase, the stage of resistance follows. During the resistance stage the body counteracts the alarm reaction by coping with the stressor and returning the body to normal conditions. If the stressor persists, the third phase of exhaustion is reached. At this stage, the animal or human is no longer able to adapt, leading to death.

Three main criticisms have been argued against Selyes' concept of stress (Broom and Johnson, 1993; Mason, 1975). First, the adrenal cortex response does not always occur or may decrease during stressful situations. Second, there are other physiological measures that may occur during a stressful situation. Finally, Seyle has been criticized for his inconsistent usage of the word stress. Nonetheless, Selyes' work has stimulated decades of research on stress and the neuroendocrine system (Mason, 1975).

Broom and Johnson (1993) define stress as an "environmental effect on an individual which overtaxes its control systems and reduces its fitness or appears likely to do so". The term stress means many things to different people. It is a term that has been applied to a stimulus, response and an interaction between stimulus and response (Barnett and Hemsworth, 1990). Lazarus (1971) suggested that scientists avoid using the term loosely by clearly defining the manner in which the concept is used.

Physiological indicators of stress, such as the hormones released by the hypothalamic-pituitary-adrenal axis (Fig. 1) and heart rate measurements can be used to assess animal welfare.

Hypothalamic-Pituitary-Adrenal Axis

The hypothalamus is responsible for regulating body functions (i.e. thirst, hunger, pleasure and pain) and releasing neurohormones, which affect endocrine function in the body (Asterita, 1985). Stimulation of body functions and neurohormones can occur by visual, auditory, tactile (pain), or physiological disturbances leading to a change in homeostasis. Once stimulation has occurred, the hypothalamus releases corticotrophin-releasing factor (CRF). The paraventricular nucleus (PVN) and the anterior paraventricular nuclei (PVA) are the primary locations for the synthesis of CRF (Brown, paraventricular nuclei (PVA) are the primary locations for the synthesis of CRF (Brown, 1994). Corticotropin releasing factor is the dominant regulator of adrenocorticotropic hormone (ACTH) release from the pituitary gland.

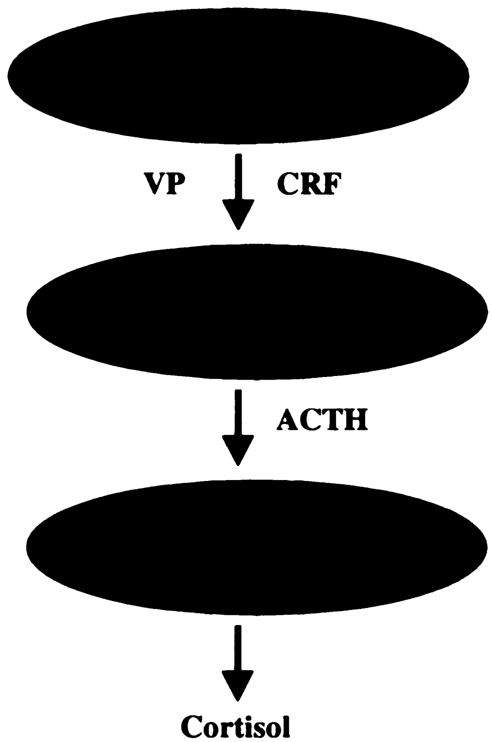

Fig. 1. Hypothalamus – Pituitary – Adrenal Axis system diagram

The pituitary gland connects to the hypothalamus at the base of the brain via the hypophyseal stalk (Brown, 1994). The gland's primary function is to regulate neuroendocrine function. Adrenocorticotropic hormone is produced in the corticotroph cells of the anterior lobe and melanotroph cells in the intermediate lobe (adenohypophysis) of the pituitary gland (Brown, 1994). Adrenocorticotropic hormone is derived from a prohormone, proopiomelanocortin (Eipper and Manis, 1980) and is hormones. Proopiomelanocortin is a large molecule that, once enzymatically cleaved, releases the sequence for seven pituitary peptides, including ACTH (Brown, 1994). The distal lobe of the pituitary gland produces oxytocin and vasopressin. The intermediate lobe synthesizes melanocyte-stimulating hormone, while the anterior lobe produces growth hormone, thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, prolactin, and ACTH.

Simultaneously and independently, under stressful situations, both CRF and VP can trigger the release of adrenocorticotropic hormone (ACTH) from the pituitary gland in horses (Redekopp et al., 1986; Livesey et al., 1988). Vasopressin is an anti-diuretic hormone that promotes water absorption in the kidneys and increases blood pressure. During exercise, horses displayed a significant increase in vasopressin and CRF secretions remained unchanged (Alexander et al., 1991). In all farm animal species studied, CRF and VP combined magnified the secretion of ACTH (Minton and Parssons, 1993; Familari et al., 1989).

Once the pituitary gland is activated, the adrenal cortex is stimulated to release steroid hormones; mineralcorticoids (aldosterone), sex steroids (androgens) and glucocorticoids. Glucocorticoid levels increase during many short-term situations. Glucocorticoid measurement allows for valuable information on the welfare of the animals (Broom and Johnson, 1993). Glucocorticoids influence blood glucose levels, carbohydrate and protein metabolism. In addition, glucocorticoids have been found to inhibit formation of antibodies, lower lymphocyte counts and delay the growth of new tissue (Vining and McGinely, 1986). Glucocorticoids can maintain or inhibit the production of ACTH and CRF (Broom and Johnson, 1993). Antoni et al. (1990) showed increases in CRF and VP in adrenalectomized rats and that the increases could be blocked by replacement glucocorticoids. Furthermore, ACTH and glucocorticoid responses to stress can be blocked by prior administration of glucocorticoids (Keller-Wood and Dallman, 1984). McFarlane (1995) demonstrated in sheep that low concentrations of cortisol inhibited ACTH secretion responses to exogenous CRF and AVP. However, a combination of CRF and AVP was able to override the feedback system, stimulating ACTH release. In rats, Castro and Moreira (1996) observed that CRH secretion was inhibited by ACTH administration in a negative, dose-dependent manner, demonstrating a short-loop feedback mechanism for CRH.

Plasma Cortisol

Cortisol is the primary glucocorticoid produced by the adrenal cortex in horses (Zolovick et al., 1966). Most of the circulating cortisol is bound to plasma proteins. Cortisol binding globulins bind to 80-90% of circulating cortisol and 5-10% is loosely bound to albumin (Brody et al., 1994). Cortisol levels participate in the stress response by potentiating the activities of the sympathetic nervous system, increasing hepatic gluconeogenesis and glycogenolysis, increasing protein catabolism, inhibiting ACTH secretion (negative feedback mechanism), maintaining blood pressure by sensitizing arterioles to the action of noradrenaline and increasing renal excretion (Brody et al., 1994).

In horses, circadian rhythms have been observed in cortisol (Lebelt et al., 1996). The age and gender of a horse does not have an affect on the circadian rhythm (Hoffis et al., 1970). Discrepancies in the literature have been reported on the occurrence of circadian rhythms in horses. Some studies have reported changes (Hoffis et al., 1970; Evans et al., 1977) in the circadian rhythms of horses while others did not record any changes (Elier et al., 1979). Typically, circadian cortisol activity peaks in the morning (0600-0900) and nadir at night (0700-2100) (Lebelt et al., 1996; Larsson et al., 1979). Irvine et al. (1994) concluded that a circadian cortisol rhythm in horses occurs when the animals are left undisturbed. However, any minor disruption, such as environment change and training, can alter the rhythm. Although the circadian rhythm could be lost, Irvines' et al. (1994) study also showed horses could adapt to their environment, reestablishing the circadian rhythm.

Slone et al. (1983) examined the disappearance rate of cortisol in bilateral adrenalectomized horses. Cortisol was observed having a biphasic disappearance. Phase one, the redistribution phase, is the rapid (0 to 30 min) liner decrease of free and bound cortisol from the blood. Phase two, elimination phase, is a slow (2 to 12 h), progressive linear decrease to non-detectable levels. During this study, the half-life of cortisol was measured at 2.1 ± 0.6 h.

Plasma cortisol and environment

Plasma cortisol has been examined under various, and possibly stressful, environmental conditions such as housing isolation, barren or complex environments and temperature variability. Pseudopregnant gilts, injected with prostaglandin $F2\alpha$, in which nest building behavior was induced, showed a greater level of cortisol when housed in a farrowing crate versus a pen (Boulton et al., 1997). In addition, the amount of time spent inactive linearly correlated with the cortisol concentrations in both penned and crated gilts. Siberian dwarf hamsters displayed high level of cortisol when separated from their mates (Castro and Matt, 1997). Contrary to the gilts examined by Boulton et al. (1997) the Siberian dwarf hamsters did not display a correlation with cortisol and inactivity time. Alexander et al. (1988) showed mean cortisol levels were not significantly altered during a ten-min isolation period, however the horses displayed agitation, vocalization, hyperventilation and sweating. In squirrel monkeys, a 32% increase in cortisol from basal was observed in monkeys housed without their companions (Lyons et al., 1995). Al-Gahtani et al. (1991) determined that sheep isolated from their herdmates had higher levels of cortisol. These studies show separation, barren environments and isolation accompanied by an increase in hypothalamic-pituitary-adrenal axis function can be perceived as stressful in several species.

Plasma cortisol, training and exercise

The role of glucocorticoids, such as cortisol, is to stimulate gluconeogenesis thereby mobilizing amino acids and fats. Gluconeogenesis increases liver conversion of amino acids into glucose, which increases liver glycogen and blood glucose (Tharp, 1975). Mobilization of amino acids from tissues results in a protein catabolic effect in muscle and leaves the animal in negative nitrogen balance. Mobilization of fatty acids from adipose tissues increases free fatty acids. From these roles one can hypothesize that these changes during exercise can be helpful by providing greater blood supplies of compounds used for energy and synthesis of needed cellular compounds (Tharp, 1975).

Plasma cortisol increases can be used to predict exercise-induced stress in horses (Linden et al., 1990). Linden et al. (1990) measured plasma cortisol after a physiological and pharmacological stressor occurred. The physiological stressor was a cross-country competition, while the pharmacological stressor was the administration of ACTH one-week after the competition. After the occurrence of the pharmacological and physiological stressors a relative increase of plasma cortisol was observed, suggesting each individual has a specific adrenocortical response independent of the type of stress (Linden et al., 1990).

Submaximal exercise studies in horses have varied in results. Glucocorticoid 11-hydroxycorticosteriod was found to increase progressively during and after a ten week training study conducted by Snow and Mackenzie in 1977. During a short standardized exercise test, Church et al. (1987) determined exercise produced a highly significant increase in plasma ACTH and cortisol followed by a decrease post exercise. In contrast, during light exercise, cortisol levels were decreased throughout the study and during the recovery period (Davies and Few, 1973). Horses swimming over 15 min had higher cortisol levels immediately after swimming than horses swimming less than 15 min (Garcia and Beech, 1986).

During maximal exercise, as the workload increases, studies have clearly demonstrated elevated cortisol levels. Snow and Rose (1981) demonstrated high levels of plasma cortisol (440 ± 15 nmol / l) during a long distance exercise which did not decrease (343 ± 11 nmol / l) until thirty min post exercise. Lucke et al. (1980) examined endurance riding in Arabian horses competing in a two day, long distance ride (80 km followed by a 40 km). They demonstrated that mean cortisol levels were higher in both the 80 km ride (pre-ride: 202 ± 1 nmol l; post-ride: 574 ± nmol l) and 40 km ride (pre-ride: 236 ± 19.8; post-ride: 401.7 ± 43.6). Lucke et al. (1980) examined Arabian horses during a marathon race (42 km) and found a significant rise in blood glucose, plasma free fatty acid and glucagon associated with a rise in plasma cortisol (pre-ride: 151 ± 14.8 nmol / l; post-ride: 445 ± 23).

Distance has an effect on glucocorticoid levels. Additionally, speed, recovery rates and level of fitness in the animal can be correlated with glucocorticoids. Rose et al. (1983) found that the fast group (234 m min) of horses which completed a 160 km endurance ride had significantly higher cortisol values post-ride (540 ± 50 vs. 284 ± 19 nmol l) when compared to the slower group (144 m min). Trotting horses at 5m/sec on a treadmill for 55 min resulted in increases of plasma cortisol levels while post-exercise cortisol levels continued to increase (Valberg et al., 1989). In addition, at increasing speeds, cortisol levels continuously rose (Valberg et al., 1989). Dybal et al. (1980) conducted a two-year study on plasma corticosteroids in horses at pre-ride, mid-ride and post-ride during a 160-km endurance ride. In the first year of this study, post-ride levels returned to pre-ride levels, however, in the second year, using different horses, post-ride levels remained significantly elevated. A study comparing cortisol concentrations in trained and untrained Arabian horses demonstrated that both groups exhibited elevated cortisol levels post exercise. However, the cortisol levels in the trained group returned to basal levels quicker during a standardized test (Hower and Wicker, 1989).

Overall, there are many variables that may affect levels of cortisol in animals. Increased speed, intensity, duration, environmental temperature and prior training experience have lead to variable cortisol values. Researchers have hypothesized that the reason for such variation in results can be due to psychological stress and/or physiological stress (Tharp, 1975). However, it is important to emphasize that increase of cortisol and ACTH is not synonymous with stress.

Heart rate

Sympathetic and para-sympathetic neural pathways are included in the autonomic nervous system. The interaction of these two pathways is commonly referred to as the "fight or flight" response (Cannon, 1929). This response provides the body with the necessary physiological measures needed to handle a dangerous situation when detected. Release of catecholamines from the adrenal medulla during sympathetic stimulation intensifies the sympathetic activity (Swenson and Vogel, 1983). Sympathetic activity during a stressful situation increases body activities needed, such as heart rate, circulation and oxygen uptake while inhibiting activities, i.e. gut mobility, that are not necessary for survival at the time.

Heart rate can be a useful indicator for recognizing acute stress in animals (Marchant et al., 1995). Cardiac measurements using radiotelemetry have been widely used in animal behavior studies (Stohr, 1988). Radiotelemetry provides a non-invasive procedure to measure heart rate and heart rate variability in horses (Evans and Rose, 1986).

In general, during stressful situations, the parasympathetic nervous system decreases (bradycardia) and the sympathetic nervous system increases (tachycardia) heart rate. Although the heart has a greater innervation of sympathetic nerves compared to parasympathetic nerves, there are cases where bradycardia occurs more readily (Hindell and Lea, 1998). Tachycardia was exhibited in sheep during visual isolation, introduction to a new flock, human and canine interaction (herding the flock) and transportation (Baldock and Sibly, 1990). However, this study also showed no increase in heart rate when the sheep were isolated with a conspecific or confined in a stationary trailer. Morton and Griffith (1985) reported that elevated heart rates are associated with greater pain sensation in animals.

Horse trailer transportation studies revealed travelling heart rates are higher when compared to non-travelling heart rates (Clark et al., 1993; Smith et al., 1994). Vibration, noise and handling were stressors used during a transportation study in pigs (Stephens and Rader, 1983). Throughout the study, heart rates remained increased but handling and restraint caused the maximal disturbance. Brundige (1998) demonstrated that the use of an electrical prod when moving pigs for loading increased heart rates 1.7 times that of basal levels. Increased heart rates were observed in cattle when restrained in a novel breeding box compared to restraining the cattle in a breeding chute to which they were typically exposed (Lay et al., 1992). Pollard et al. (1993) showed that deer stags confined with unfamiliar animals had higher heart rates than when confined with familiar deer stags. During a study examining the effects of twitching horses, heart rates were monitored (Lagerweij et al., 1984). In this study, painful stimuli were applied with and without twitching the horse. While twitching, the release of endogenous opiods helped to decrease the pain, lowering heart rates by 8% whereas, without twitching the stimuli caused a 22% increase in heart rate. In addition, the percent changes in heart rate were related positively to the reactive behaviors to the painful stimuli. Overall, handling, management techniques and environments can be stressful as demonstrated by the increases in heart rate in various species.

Heart rate and training

Pre-exercise studies have demonstrated an anticipatory affect by heart rates in horses. Hall et al. (1976) revealed an increase in mean heart rates in Thoroughbred horses while moving between their home stall to a preparation shed and from the preparation shed to the race track, prior to exercise.

Cardiovascular measurements can provide information on determining if training is capable of inducing adaptations and greater tolerance to the exercise (Gottlieb et al., 1995). Heart rates during sub-maximal exercise have been found to be lower after training (Bayle et al., 1983). Untrained Standardbred horses in poor physical fitness demonstrated higher resting heart rates, higher heart rates during exercise and a slower recovery or resting heart rate when compared to highly trained horses (Marsland, 1968). Foreman et al. (1990) examined Thoroughbred horses with previous training experience in a standardized test on a racetrack. In this study, recovery heart rates decreased indicating an increase in cardiovascular fitness. Heart rate monitoring can help prevent a trainer from excessively exercising subjects that cannot otherwise communicate fatigue (Ivers, 1982).

Summary

This review examined the effects of housing environments on behavior and physiological responses and welfare of horses. Barren environments can hinder the acquisition of learning concepts and may induce the development of behavior abnormalities. Raising animals in a challenging and complex environment can help decrease the occurrence of abnormal behavior, increase learning abilities and improve the level of welfare for many species.

Changes in behavior, as well as activation of the autonomic and neuroendocrine systems, occur under physical, psychological or psychosocial stress. These changes when monitored can indicate if an animal is having difficulty coping with a situation thereby, classifying the situation as stressful. Physiologically, during a stressful situation activation of the hypothalamic-pituitary-adrenal axis (Fig. 1) will occur. Once activated, the hypothalamic-pituitary-adrenal axis influences the release of glucocorticoids. Glucocorticoid levels have increased during many short-term situations allowing for assessment on the welfare of animals. Isolation and barren housing conditions have induced higher cortisol levels when compared to animals housed in a richer environment. During sub-maximal exercise studies, increases and decreases in plasma cortisol concentrations have been revealed. While undergoing maximal exercise, most studies have demonstrated elevated cortisol levels. Heart rate measurements using radiotelemetry is a non-invasive method in recognizing acute stress in animals. The sympathetic nervous system will increase in the presence of a stressor such as transportation, handling, restrainment and electric prodding.

Materials and Methods

Animals

Sixteen Arabian yearlings, owned by Michigan State University, were used in this experiment. The experimental group was comprised of castrated males (n=11) and intact females (n=5), with an average age of 18.6 month (range 16.6 -19.9). Average weight for the stalled and pastured horses at the start of the project was 349 ± 10 kg and 342 ± 10 kg, respectively.

Housing

All animals were housed at the Michigan State University Horse Teaching and Research facility. The horses were randomly assigned to three treatment groups according to housing, training and age: pasture (P) with training (n=6) or stalled (S) with training (n=6) and a control (C) group with no training (n=4). The control group was assigned into the pasture (n=2) or stalled (n=2) treatment group. Two weeks into the experiment, due to an accidental injury, one of the pastured horses was removed from the project. Therefore, only five horses were in the pasture group for the remainder of the study.

Pasture horses were housed on approximately 29,166 m^2 (seven acres) of land with a water dispenser, hay trough and grain trough in the field. Maximum temperatures on training days were 3 °C, 9 °C, 5 °C and 7 °C, respectively.

The 10 m^2 box stalls used for housing had dividing walls constructed with wooden panels on the bottom and steel guards on the top, allowing limited social interaction between the horses. Each stall had a window along with a hay trough, grain bucket and water bucket. Stalls were bedded with wood shavings or straw. Natural sunlight and barn lights provided lighting during the day. At night (19:00 h) illumination in the corridor were turned off and individual stall lights were turned on.

Animal Management

The study was conducted during the winter when minimal nutrients were available from pasture grazing. All treatment groups were fed a diet recommended by NRC (1989) for long yearlings and two-year-olds (Table 1). Each horse was fed 1.8 kg, per day, of a commercial concentrate (Strategy™, Purina Mills, Lansing, MI) divided into two equal feedings, at 06:00 and 18:00 h. In addition, horses had unlimited access to mixed alfalfa-grass hay. Stalls were cleaned daily at 16:00 h and all horses had their hooves trimmed and were dewormed routinely. Vaccinations against rhinopneumonitis, tetanus, influenza and equine encephalomyelitis were provided to all horses.

Table 1

Calculated nutrient content of total ration as-fed

Nutrient	Units	Ration
DE	Mcal/kg	2.169
CP	%	14.7
Ca	%	.75
P	%	.29

Hoekstra, 1998

Trainers

Two trainers were involved in the training procedure. The novice trainer (female, 50.0 kg) was a graduate student with no prior experience in the initial training of horses. The second trainer had ten years of horse experience (male, 62.3 kg). In the interest of human safety, horses that were subjectively determined as "easier" to train were assigned to the novice trainer. On day 0 of training, the experienced trainer trained eight horses (4 pasture and 4 stalled) while the novice trainer trained four (2 pasture and 2 stalled). For the remainder of the study, including the loss of the pastured horse, the experienced trainer trained five horses (2 pasture and 3 stalled) while the novice trainer trained six (3 pasture and 3 stalled).

Training Arena

All training sessions were conducted at the MSU Horse Teaching and Research facility. Training took place in an indoor round pen (Fig. 2). The round pen area was approximately 15 m in diameter. The flooring material throughout the arena was a mixture of sand and clay. The round pen had two sliding doors that were used. The north sliding door was the entrance where the experimental horses were lead from their respective housing situation. The second door, which faced west, lead to a breeding room where the video recording equipment was stored. Adjacent to the breeding room was another room used as a laboratory, for immediate processing of samples. The animals had no contact with the adjacent rooms.

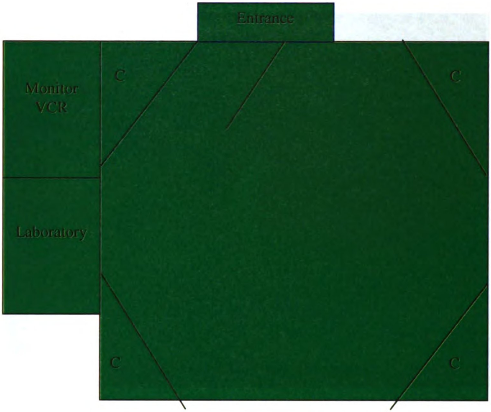

Fig. 2. Schematic diagram of round pen used in the study.
C = cameras

Equipment

Training tack

Equipment used for initial training of the horses included a lead rope, halter, saddle pad, bridle with a jointed snaffle bit and reins (Fig. 3). The lead rope was 1.8 m long and was attached to a ring on the halter with a clip. In this study, the lead rope was used for a variety of purposes including, controlling the horses while walking and standing, encouraging forward movement and as reins for the first day of training. The halter is designed to fit on the horses' head so the trainers can control the horses as they lead them. The saddle pad is placed on the horses' back before the saddle.

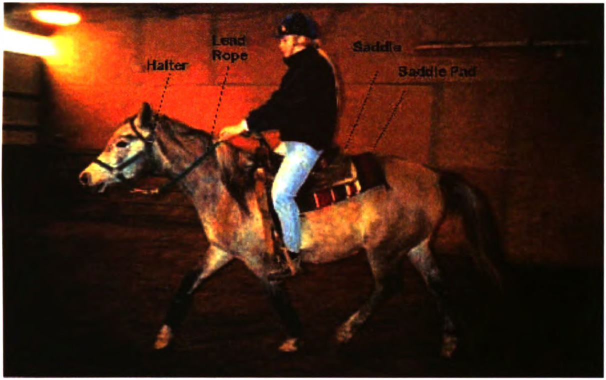

Fig. 3. Photograph of equipment used during training.

The pad is placed near the withers and extends down towards the center of the shoulder (C.H.A., 1996). The purpose of the saddle pad is to prevent chaffing or discomfort from the saddle while riding. Both trainers used a western style saddle.

Bridles are used for steering or directing the horses. The snaffle bit allows the trainer to communicate with and control the horses through pressure (C.H.A., 1996). A drawing of a bridle with a snaffle bit can be found in Fig. 4. The snaffle bit consists of two rings joined by a smooth mouthpiece, which is jointed in the center. The snaffle bit applies pressure on the mouth of the horses. Reins are run through the rings of the bit, which are controlled by the trainer.

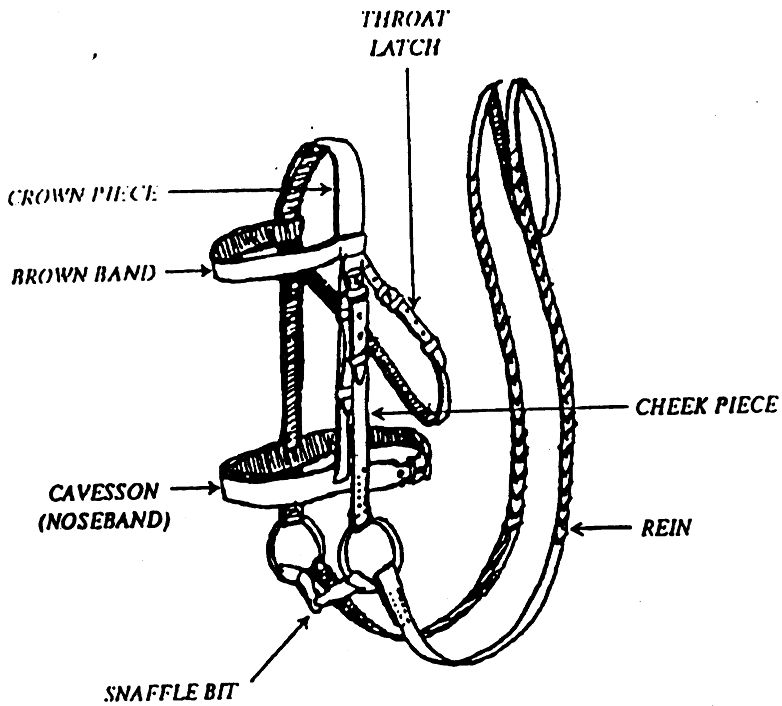

Fig. 4. Picture of a bridle and snaffle bit, similar to the one used in the study. (C.H.A., 1996)

Video observations

Four Panasonic CCTV WV-BP310/ WV- BP314 cameras, fitted with wide angled lens, were used to record the entire round pen during the training sessions. For training days three and four, only two cameras were used at opposite ends of the round pen. All horses were videotaped on collection days using continuous recording. Video cameras were wired to a Panasonic AG-6730 VCR. The VCR was connected to a Panasonic WV-

CM146 multiplexer monitor, with split mode capabilities allowing for simultaneous viewing on all four cameras. The television monitor divided the television screen into four sections. Each camera view was represented in a section allowing for viewing of every angle.

Blood collection preparation

Prior to collection days, a total of six, 1-ml microcentrifuge flat top/graduated tubes and EDTA vacutainer tubes per horse were labeled with the horse number and blood sample number.

Heart rate monitors

The Polar Vantage NV™ was used to monitor heart rates in the horses. The monitor was equipped with a Polar coded transmitter, elastic strap and wrist watch receiver (Fig. 5). The strap provided was adequate for human use but not for horses and was consequently modified using a 1.3 x .55 m piece of elastic. Each end of the elastic band was sewn onto a snap. The snaps were attached to the transmitter and to fortify the area, black electrical tape was wrapped around the connection.

To improve the conductivity between skin and electrodes, K-Y® lubricating gel was used to moisten the area where the transmitter electrodes touched the girth of the horse. In addition, the wrist watch receiver was tied onto the elastic strap near the Polar coded transmitter.

Fig. 5. Photograph of heart rate monitor.

Training

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|--------------------------------------------------------------|---|---------------------------|
1                                                              84  0                           28
                 Pre-training                                            Training
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Fig. 6. Timeline (days) for the study.

Procedure

Prior to the start of the experiment, a pre-training period occurred for 84 days (Fig. 6). During this time, pastured horses were given free access to exercise and the stalled horses were walked daily, for one h, on a mechanical walker (Fig. 7). A mechanical walker leads horses in a circle at a slow pace and is typically used after training as a cool down period. Training occurred five days a week, for 28 days with sample collections occurring on days 0, 7, 21 and 28. On off-days, as their stalls were cleaned, stalled horses were exercised, one h a day, on a mechanical walker.

Fig. 7. Photograph of the hot walker used for the stalled horses during the study.

The objective for the first day of training was to habituate the horse to the trainer, having a saddle on their back, being mounted, ridden and dismounted. Depending on the procedure, the main objective for each technique was to either have the horse stand still or move forward when asked. Horses have monocular vision, 215° field for each eye, and binocular field of vision (60 – 70 degrees) in front of them (Evans,1990). Therefore, it was important to perform all techniques on the left and right side of the horses so a crossover of the information can occur in the horses' brain, registering the experience of the stimuli from both sides. For analysis purposes, training was divided into two periods, groundwork and riding. The groundwork period encompassed all the training procedures that occurred up to the first mount. The riding period began once the trainer was mounted on the horse and ready to ride.

Groundwork

Training began with suppleness exercises that were accomplished by gently applying pressure on the lead rope, which was attached to the halter. Suppleness training was performed on both sides until the horses would slightly turn towards the pressure. Upon acclimating to the pressure the horses were released in the round pen. Once released the trainer would move towards the hindquarters of the horses while swinging the arms and/or lead rope. When running, horses will not look at what is chasing them. Eventually the horses will glance at the stimulus, in this case the trainer, and at that moment the trainer must stop moving towards the horses hindquarters and swinging the arms and/or lead rope (positive reinforcement). The trainer then approached the horses and an association between the stimulus (trainer) and positive reinforcement (stopping) was attained. The trainer then attached the rope to the halter and resumed suppleness exercises. Again, once acclimatized to the pressure on both sides, a new training procedure began.

The horses were habituated to the equipment by gently tossing the lead rope, saddle pad and saddle, repeatedly onto the horse's back until the horses stood still. The objective of this habituation was to make the horses comfortable with the tack such that they would stand still when the equipment was presented. Habituation training was performed for the saddle pad and for the saddle. The lead rope was also tossed and/or rubbed along the bodies of the horses' to accustom them to stimuli in those areas. Once the saddle and pad were placed on the horses' back and the horses were standing still the trainer tightened the girth. With the saddle on, the horses were once again released into the round pen. This procedure continued with an additional tightening of the cinch until a subjective decision based on how the saddle was laying on the back of the horses was made by the trainer to end this procedure. The trainer then approached the horses and snapped the rope to the halter. Suppleness exercises often occurred again before the final procedures were performed for mounting.

Following the saddling procedure, the trainer placed a foot in the stirrup while holding the rope, slightly turning the horses' head. Turning the horses' head added a safety measure for the trainer. In this position, the horses were semi-restricted to moving only in a small circle. The trainer stood with a foot in the stirrup until the horses remained still. Using the same procedure, the trainer would next place a foot in the stirrup and in addition, add bodyweight by hoisting and laying the upper body onto the back of the horse. Once the process was done on each side and the horses were standing still, the trainer would lift the free leg over the horses and gently lower the body onto the saddle. The trainer was now mounted on the horse. Many of the groundwork procedures were not performed by days 21 and 28 of training, when the trainer would immediately mount the horse and begin the riding portion of training.

Riding

Once the trainer had mounted the horse, the riding period began. The primary objective during this period was forward motion of the horse. Using hands, legs and body weight as cues along with positive and negative reinforcements at the walk, trot and lope, the trainer encouraged the horses to move forward. For the remainder of the study, horses were walked, trotted and galloped.

Control group

The control group horses experienced the same protocol as the trained group without the actual training procedures. Control horses were lead from their respective housing situations but instead of training horses were to be released into the round pen and allowed to roam the environment for thirty min, freely. To protect the video cameras, a human observer was asked to stand behind a gate in the arena and have no contact with the horses. However, the human observer interacted with the horses, outside of the gate, leading to behavioral differences between the control treatment groups.

Data Collection

Heart rate

Heart rates were recorded and data was stored in the watches continuously beginning 60 min before the horse entered the round pen until 75 min post-training. Heart rate data were downloaded from the wristwatches using the Polar Advantage Interface™ and stored in the Polar Precision Performance™ Software for Windows (Version 2.0) for future analysis.

Blood sampling

Blood samples were collected four times per horse for each training session: once before entering the round pen, at the completion of training, as well as 15 min post training (15 pt) and 75 min post training (75 pt). Seven ml of blood per tube was collected using 20 gauge x .038 m, single sample needles and purple-topped Vacutainer™ (Becton Dickinson, Franklin Lakes, NJ) tubes. Samples were immediately placed on ice for five min and transported to the laboratory, located adjacent to the round pen. Samples were centrifuged at 1340 x g for fifteen min at 4 °C and 1 ml of plasma was pipetted into the 1.5-ml polypropylene bullet tubes for the physiological assay.

Preliminary data analysis

Behavior data / Program configuration

Preliminary analysis of the behavior data was accomplished using the Observer™ (Noldus Information Technology Inc., Sterling, VA) program. A configuration file was created within the Observer™ program that specified the research design, independent variables and subjects under observation. The independent variables were trainer (n=2) and training days (n=4). Tapes were pre-viewed to record and categorize behaviors that occurred most frequently. These behaviors were defined and assigned two-letter codes that identified the behaviors within the configuration file. Behavior categories and definitions can be found in Tables 2 a, b, c & d.

Behaviors were categorized in the Observer™ configuration file both as a horse or trainer and as an event or state. "Events" are classified as behavior patterns of short duration, i.e. vocalizing, while "states" are behavior patterns of long duration, i.e. sleeping, (Martin and Bateson, 1993). Classification of the behavior categories as events or states and two-letter codes can be found in Appendix A.

Event recording

Once the configuration file was designed, tapes were reviewed and behavior was decoded using the two-letter codes. Chronological order of behavior was filed in the event recorder program of the Observer™ software. The researcher decoded all tapes within ten days.

Table 2

Horse and trainer behavior categories used in study for the groundwork training period.

Horse Categories	
Circling	Trainer applies a stimuli and the horses respond by circling.
Standing still	During a training procedure, horses do not move in any direction.
Pull head away from pressure	While pressure is applied in either direction with the reins or lead rope, horses move their heads away from the pressure.
Trainer Categories	
Chase	Trainer encourages the horses to move forward by positioning the body towards the hindquarters of the horses while swinging lead rope and/or arms.
Sack with lead rope	Rope is gently tossed and/or rubbed onto the horses' bodies and removed.
Swinging rope / arms	Trainer will swing the lead rope and/ or arms to encourage the horses to move forward.
Hit with rope	Trainer using the rope will make physical contact with the horses to encourage the horses to move forward.
Sacking with saddle pad	Pad is gently tossed onto the horse's back and removed.
Sacking with saddle	Saddle is gently tossed onto the horses' back and removed.
Pressure on the reins	Trainer applies pressure to the right or left rein.
Foot in stirrup	Trainer places foot in stirrup.
Weight on back	Trainer places foot in stirrup and lays body across the horse's back.
Mount	Trainer lifts the leg over the horse's back and gently lowers the body onto the saddle.

Table 2b

Horse behavior categories and definitions that occurred during the riding portion of training

Looking towards the wall	While horses are moving, they will turn and look towards the wall.
Head / Neck extension up	While riding, horse's neck is stiffened and head is extended upward.
Head / Neck extension down	While riding, horse's neck is stiffened and head is extended downward.
Head / Neck extension straight out	While riding, horse's neck is stiffened and head is extended straight out.

Table 2c

Behavior categories and definitions that occurred during both the groundwork and riding periods of training.

Normal tail	Tail is not elevated or switching.
Raised tail	Tail is elevated.
Bucks and jumps	Horse's four legs are elevated off the ground while the hind legs are extended outward.
Tossing head	Flinging or shaking of the head up and down abruptly.

Table 2d

Descriptions of training periods.

Groundwork	Duration of time between the horses entering the arena without a saddle, until the time the saddle is placed on the horses and cinched.
Riding	Duration of time, which begins at the point when the trainer is mounted on the horses until the trainer dismounts.

Data Analysis

Behavior data

Data analysis of the observational data was performed using The Observer™ elementary statistics. The elementary statistics provided total duration of the training session, percent total duration for states, frequency for events and latency between two events or states, for each horse.

Some categories that are mentioned in appendix A were used to record behaviors but were not statistically analyzed. Behaviors such as "trainer standing still", "not moving", "dismount" and "other" were used to continue the chronological recording, on the Observer™ program, by ending or starting a state. Additional behaviors (i.e. "leg tie", "backing up", "stroking", "ear positions", "heavy on the reins") were not analyzed statistically if they occurred infrequently between the treatment groups or became difficult to record, due to camera positioning, by days 21 and 28.

Reliability tests

To ensure consistency in decoding the behavior data, a fifteen-min segment of tapes was decoded prior to the start of observations for that day. Analysis was performed, with a 15-second margin of error, using the Observer™ software. The range in reliability scores were 60% to 78%, with an average of 66% accuracy.

Cortisol Assay

Plasma cortisol samples were processed using a Coat-A-Count® kit from Diagnostic Products Company® (Los Angeles, California) designed to measure cortisol (hydrocortisone) in serum, urine, and plasma. All samples were processed in one day using the radioimmunoassay protocol procedure recommended by the kit. The procedure is a solid-phase radioimmunoassay. ^{125}I- labeled cortisol competes with the sample cortisol for antibody sites for a set amount of time. The antibody sites are immobilized to the wall of the polypropylene tubes. Decanting the supernatant ends the competition for the antibody sites and isolates the antibody-bound fraction of the radiolabeled cortisol. Samples were counted using a 1290 Gamma Trac™ counter (Elk Grove Village, Illinois) for one min.

Heart rate data

All heart rate data were reviewed and edited. Editing consisted of replacing any zero values with means from heart rate values before and after a loss in connection. The average, minimum and maximum heart rate values during the time between blood sampling were chosen for final analysis.

Statistical Analysis

The behaviors were recorded as count data and were analyzed assuming Poisson distribution (Steel et al., 1997). Since all behaviors were recorded as count data, a square root transformation (y = sqrt (x + 1)) was used to normalize the distribution. A linear model based on the factors of housing (with two levels) and trainer (with two levels) with repeated measures over 4 days was used to analyze the data. This model also allowed for all possible two-way interactions. Least-square means were computed and backtransformed as point estimates and as 95% confidence intervals on the scale of observation.

A linear model based on the factors housing (with two levels) the random effect of horse nested within housing with repeated sampling time measures over four days, was used to analyze cortisol. All two-way interactions between these factors were also included in the model. Least-square means were used for comparing means responses.

For individual horse cortisol concentrations over time, area under the curve using the procedure reported by Matthews et al. (1990) were computed. Correlation between the area under the curve cortisol values and horse and trainer behaviors was assessed.

All behavioral and physiological data were analyzed using SAS Proc Mixed and Proc Corr. Results were considered highly significant at $p \leq .01$ and significant at $p \leq .05$ while $p \leq 0.1$ were considered trends.

Results

Training Times

Overall, stalled horses required a greater amount of time (min) to complete the entire training procedure than the pastured horse (S: 26.4 ± 1.5 min; P = 19.7 ± 1.1; p = 0.032). Analysis of the house by day interaction did not reveal a difference on day 0 between the treatment groups however, on day 7 (p = 0.081), day 21 (p = 0.069) and day 28 (p = 0.079) the stalled group tended to require greater amount of time to complete training (Fig. 8). Overall, the experienced trainer spent a greater amount of time with the entire training process on the stalled treatment horses (S: 26.2 ± 2.3; P: 16.5 ± 2.3 min; p = 0.001). No difference in training was observed between horses kept in stalls and pastured horses handled by the novice trainer (S: 26.6 ± 3.2; P: 23.3 min; p = 0.399).

Overall, during the groundwork portion of training the stalled group required more time to habituate to the various training procedures (S: 11.4 ± 0.96; P: 7.3 ± 0.75 min; p = 0.007). Further analysis revealed no differences between treatment groups on day 0 (S: 28.8 ± 3.3; P: 22.9 ± 2.6; p = 0.162) and day 21 (S: 3.5 ± 1.0; P: 1.7 ± 0.8; p = 0.122). However, the stalled horses took longer to complete the groundwork portion of training than the pastured horses on day 7 (p = 0.039) and a tendency for longer training times was revealed on day 28 (p = 0.072) (Fig. 9). The experienced trainer spent a greater amount of time with the stalled treatment group compared to the pasture group (S: 11.6 ± 1.1; P: 6.2 ± 1.6 min; p = 0.005). No difference was observed between the treatment groups trained by the novice handler (S: 11.3 ± 1.5; P: 8.4 ± 1.0; p = 0.125).

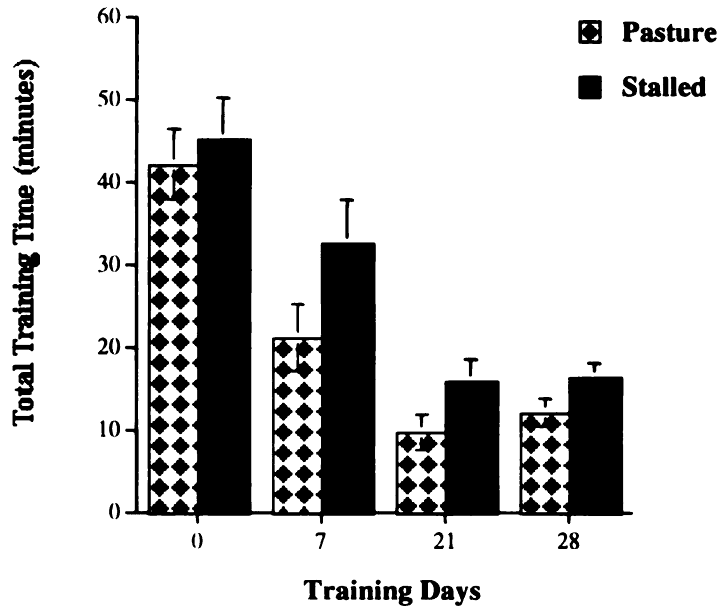

Fig. 8. Differences (mean ± SEM) in total training times between treatment groups on training days. Time began once horses entered the roundpen until the trainer dismounted.

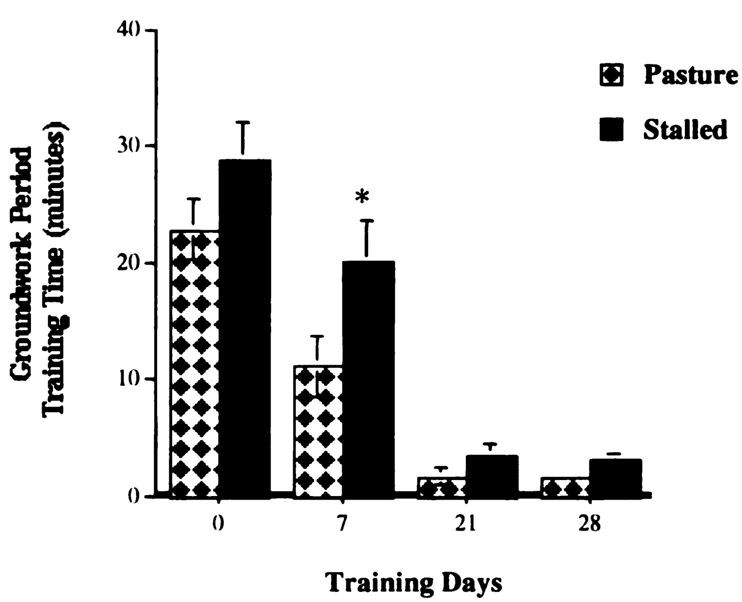

Fig. 9. Latency (mean ± SEM) of time between horses entering the roundpen until the trainer mounted the horses for the first time. * $p \leq 0.05$

Overall, there was no effect of housing during the riding portion of training, time interval between mount to dismount observed (S: 9.7 ± 1.6; P: 8.5 ± 0.8 min; p = 0.33). No differences were observed on day 0 (S: 13.0 ± 2.2; P: 12.8 ± 2.1; p = 0.162), day 7 (S: 9.3 ± 1.7; P: 7.3 ± 1.5; p = 0.176), day 21 (S: 6.2 ± 1.4; P: 6.9 ± 1.6; p = 0.656) and day 28 (S: 10.5 ± 1.8; P: 6.2 ± 1.5; p = 0.211) between housing treatments (Fig. 10). No differences were revealed in the interactions between the trainers and their treatment groups (Experienced; S: 8.0 ± 0.9; P: 7.6 ± 1.0; p = 0.714; Novice; S: 11.4 ± 2.0; P: 9.5 ± 0.5; p = 0.279).

Behavioral Results

Horse behaviors during groundwork training

Overall, the stalled treatment group, stood still for longer periods of time during the groundwork training (S: 7.5 ± 0.6; P: 5.3 ± 1.0; p = 0.05). On day 21, the stalled group stood still for longer periods of time than the pastured horses (p = 0.02) (Fig. 11). The experienced trainer stalled horses remained stationary longer than the pastured treatment group (p = 0.01). No difference between groups for the novice trainer was observed (Table 3).

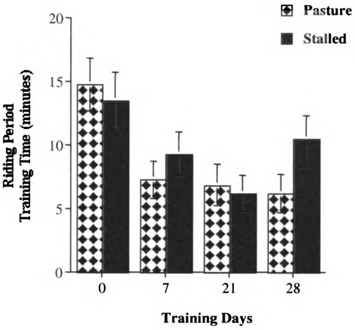

Fig. 10. Latency (mean ± SEM) of time between the trainer mounting the horses until the trainer dismounted, on training days.

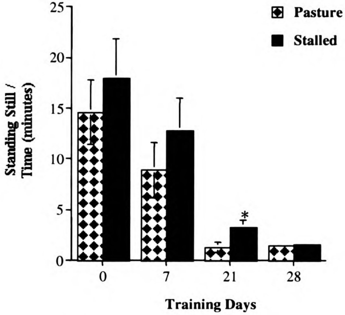

Fig. 11. Duration (mean ± SEM) of time horses spent standing still on training days. * $p \leq 0.05$

Table 3

Duration (mean ± SEM) of time spent standing still, during the groundwork period, by the treatment groups according to the experience of their trainers.

Category	Trainer	Housing	mean ± SEM	p-value
Standing Still	Experienced	Pasture	4.8 ± 0.83	0.012
		Stalled	8.0 ± 0.9	
	Novice	Pasture	5.8 ± 0.71	0.398
		Stalled	6.7 ± 0.99	

Housing did not affect the occurrence of circling behavior (S: 15.0 ± 3.2; P: 11.9 ± 2.8; p = 0.468). However, a day effect was observed (p = 0.0001). Stalled group circled more than the pasture group on day 7 (p = 0.039), 21 (p = 0.005) and 28 (p = 0.006) (Fig. 12).

Trainer behaviors towards the horses during groundwork training

Over the entire training period trainers sacked the stalled horses more times with the blanket compared to the pasture treatment group (S: 23.0 ± 3.5; P: 8.6 ± 2.5; p = 0.045). On day 0, trainers sacked the stalled group more times compared to the pastured group before the desired response of standing still was obtained (p = 0.031) (Fig. 13). The experienced trainer tended to sack the stalled horses more with the blanket when compared to the pastured horses (p = 0.072) (Table 4).

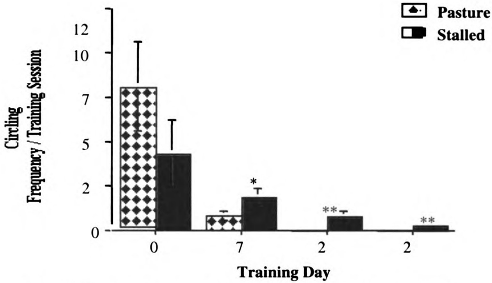

Fig. 12. Frequency (mean ± SEM) of circling behavior performed by the horses during the groundwork period, over training days. $*p < 0.05$ $** p < 0.01$

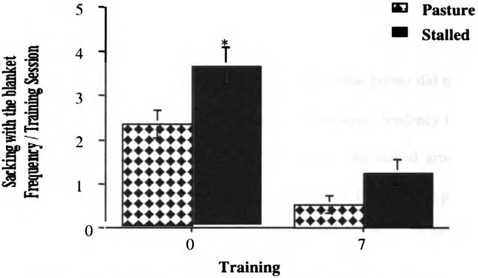

Fig. 13. Frequency (mean ± SEM) of sacking the horses with a blanket, during the groundwork period, for training days. $*p < 0.05$

Table 4

Frequency (mean ± SEM) of sacking with the blanket by the experienced and novice trainers.

Category	Trainer	Housing	mean ± SEM	p-value
Sack w/blanket	Experienced	Pasture	9.4 ± 3.2	0.07
		Stalled	19.0 ± 3.5	
	Novice	Pasture	17.0 ± 3.0	0.1
		Stalled	27.8 ± 6.0	

Overall, housing did not affect the frequency of placing the saddle onto the horses' backs (S: 0.87 ± 0.05; P: 0.76 ± 0.06; p = 0.197). On day 0, the stalled horse's required more occurrences, by the trainers, in placing the saddle onto their backs before habituating (S: 3.7 ± 0.2; P: 2.9 ± 0.2; p = 0.042). No difference was observed on day 7 between treatment groups (S: 1.0 ± 0.1; P: 1.0 ± 0.1; p = 0.966).

Overall, the application of the trainer weight onto the backs of the horses did not reveal a housing effect (S: 1.4 ± 0.5; P: 1.9 ± 0.2; p = 0.161). There was a tendency for an interaction between treatments and day (p = 0.083). On day 0, the stalled group needed less time of weight on back before they stood still (S: 2.2 ± 0.3; P: 3.9 ± 0.4; p = 0.025). No difference was observed on day 7 (S: 0.63 ± 0.3; P: 0.43 ± 0.2; p = 0.557). The experienced trainer tended to leave his weight on the backs of the pastured horses for a longer time (S: 0.55 ± 0.08; P: 0.82 ± 0.11; p = 0.080). There was no difference observed for the two treatment groups by the novice trainer (S: 0.61 ± 0.12; P: 0.67 ± 0.11; p = 0.408).

Horse behaviors during the riding portion of training

On day 7 stalled horses looked towards the wall more often than the pastured horses ($p = 0.05$). No difference was observed on day 0, day 21 and day 28 (Table 5). The stalled horses had a tendency to look towards the wall while riding with the experienced trainer. No difference was revealed between the treatment groups of the novice trainer (Table 6).

Table 5

Frequency (mean ± SEM) of looking towards the wall, during the riding period, for training days.

Category	Day	Housing	mean ± SEM	p-value
Looking towards the wall	0	Pasture	0.27 ± 0.39	0.147
		Stalled	2.5 ± 1.4	
	7	Pasture	0.47 ± 0.64	0.055
		Stalled	4.4 ± 1.9	
	21	Pasture	0.46 ± 0.56	0.366
		Stalled	1.6 ± 0.98	
	28	Pasture	1.4 ± 1.5	0.345
		Stalled	3.8 ± 2.0	

Table 6

Frequency (mean ± SEM) of looking towards the wall, during riding portion of training, for treatment groups according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
Looking towards the wall	Experienced	Pasture	0.53 ± 0.48	0.07
		Stalled	3.8 ± 0.75	
	Novice	Pasture	0.70 ± 0.84	0.375
		Stalled	2.3 ± 1.4	

Head and neck extension upward displayed a significant effect due to the housing treatment ($p = 0.0008$). Stalled horses extended their head and neck upward more than pastured horses for day 7 ($p = 0.0004$), day 21 ($p = 0.010$) and day 28 ($p = 0.007$) (Fig. 14). The stalled horses for both trainers exhibited a higher occurrence of head and neck extension upward by the horses (Table 7).

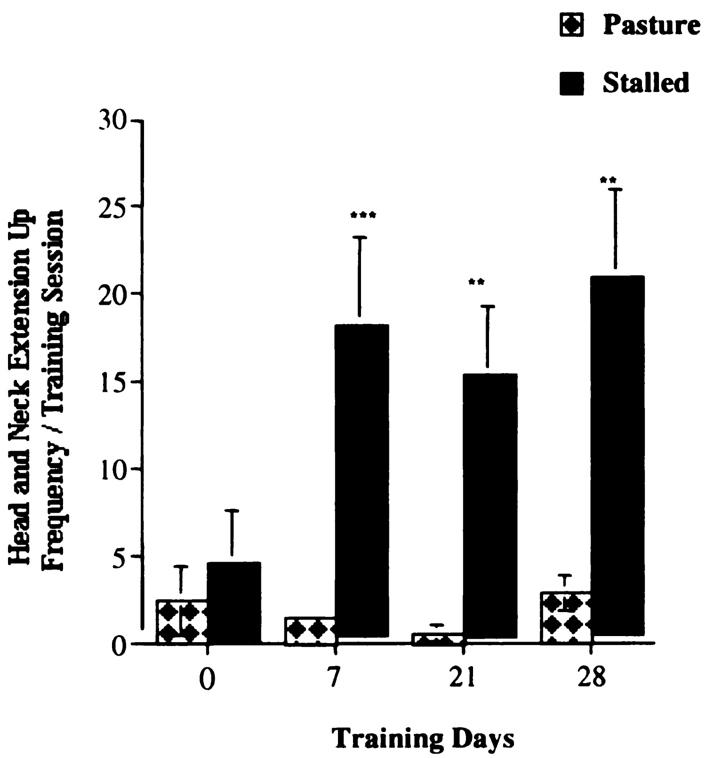

Fig. 14. Frequency (mean ± SEM) of head and neck extension up behavior performed by the horses, during the riding period, over training days.
**p < 0.05 ***p < 0.001

Table 7

Frequency (mean ± SEM) of the head and neck extension upward behavior performed by the treatments groups, during the riding period, according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
Extension upward	Experienced	Pasture	2.1 ± 0.8	0.006
		Stalled	13.0 ± 2.5	
	Novice	Pasture	1.5 ± 1.3	0.004
		Stalled	15.0 ± 4.0	

Overall, the stalled horses stiffened their neck and extended their head straight out during the riding period 8.6 ± 1.3 times while the pastured horses displayed the behavior only 2.2 ± 1.0 times ($p = 0.002$). The stalled group extended their heads and necks during riding more often on day 7 ($p = 0.004$), day 21 ($p = 0.001$) and day 28 ($p = 0.007$) (Fig. 15). The occurrence of head and neck extension straight by the horses according to their trainer can be found in Table 8.

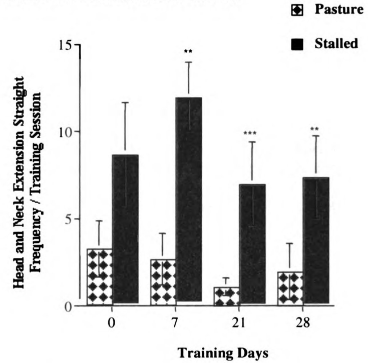

Fig. 15. Frequency (mean ± SEM) of head and neck extension straight performed by the horses, during the riding portion of training, over training days. **$p < 0.05$ *** $p < 0.01$

Table 8

Frequency (mean ± SEM) of the head and neck extension straight behavior performed by the treatments groups, during the riding period, according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
	Experienced	Pasture	1.7 ± 1.0	0.009
Extension straight		Stalled	7.6 ± 2.0	
	Novice	Pasture	2.8 ± 1.3	0.016
		Stalled	9.6 ± 2.4	

Overall, no difference in head and neck extensions down between stalled and pasture treatment groups was observed (S: 8.3 ±1.4; P: 6.3 ± 1.5; p= 0.421). On day 21, stalled horses tended to extend their head and neck down more than the pastured horses (p = 0.075). The stalled treatment group extended downward more frequently when compared to the pastured group trained by the experienced trainer (p = 0.038). No differences were found between the treatment groups trained by the novice trainer (Table 9).

Table 9

Frequency (mean ± SEM) of the head and neck extension down behavior, performed by the treatment groups, during riding portion of training according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
	Experienced	Pasture	4.2 ± 2.2	0.03
Extension down		Stalled	11.1 ± 2.2	
	Novice	Pasture	8.9 ± 2.5	0.372
		Stalled	5.9 ± 2.3	

Horse behaviors during groundwork and riding portions of training

Overall, the stalled horses bucked and jumped more frequently than the pastured group (S: 8.0 ± 2.0; P: 2.2 ± 1.0; p = 0.020). The frequency of bucking and jumping between treatment groups was different on day 7 (p = 0.003), day 21 (p = 0.026) and day 28 (p = 0.0001) (Fig. 16). The interaction between housing treatments and trainer was significant (p = 0.001). The experienced trainer stalled horses exhibited higher occurrences of bucking and jumping (p = 0.001) (Table 10).

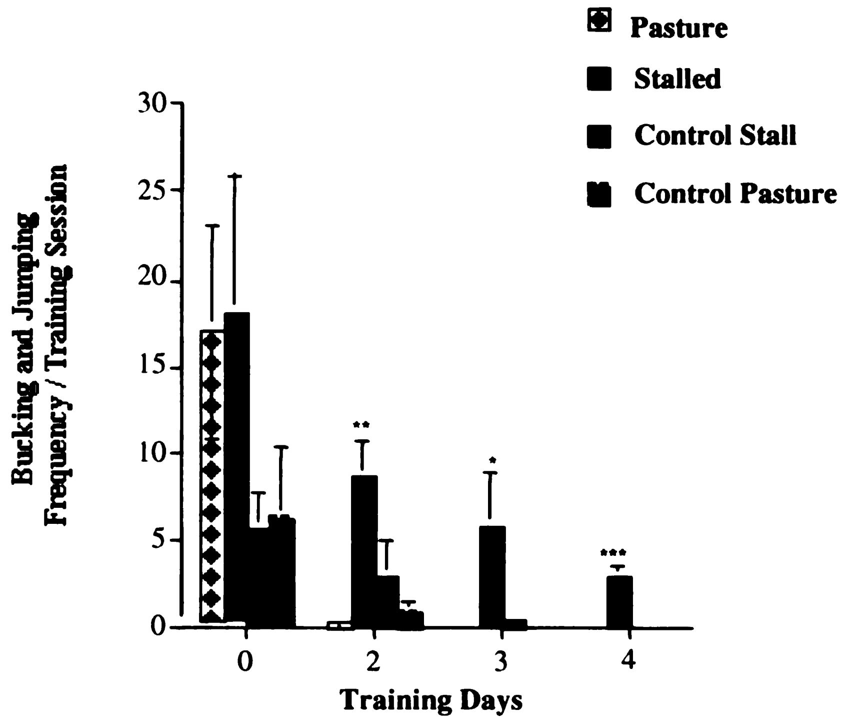

Fig. 16. Frequency (mean ± SEM) of bucking and jumping by the treatment groups, over training days.
* p < 0.05 **p < 0.01 ***p < 0.001

Table 10

Frequency (mean ± SEM) of bucking and jumping, performed by the treatment groups, during the groundwork and riding period, according to the experience of their trainer.

Category	Trainer	Housing	Mean ± SEM	p-value
Bucks and Jumps	Experienced	Pasture	2.1 ± 1.2	0.001
		Stalled	14.4 ± 3.0	
	Novice	Pasture	2.2 ± 1.3	0.506
		Stalled	3.5 ± 1.8	

The duration of time (min) of horses carrying a normal tail setting was not affected by housing treatment (S: 8.6 ± 1.7: P: 5.2 ± 1.2; p = 0.137). Pastured horses carried a normal tail setting more frequently than stalled horses on day 21 (p = 0.007) (Fig. 17). The experienced trainers' stalled treatment group tended to consistently carry their tail with a normal tail setting than the pasture group (p = 0.082) (Table 11).

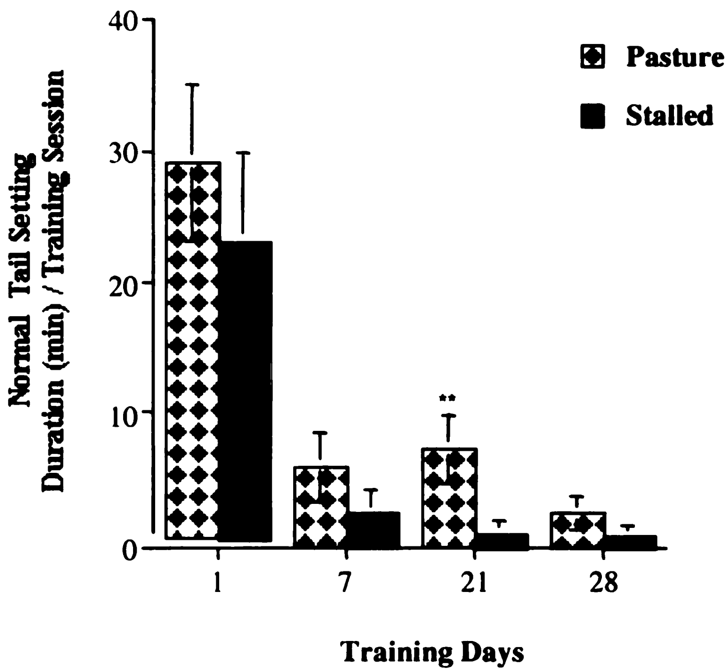

Fig. 17. Duration (mean ± SEM) of normal tail setting during the groundwork an riding periods of training, over training days.
** p < 0.01

Table 11

Duration (mean ± SEM) of time spent carrying a normal tail setting, by treatment groups, during the groundwork and riding periods, according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
Normal tail setting	Experienced	Pasture	4.3 ± 1.7	0.08
		Stalled	9.7 ± 2.2	
	Novice	Pasture	6.1 ± 2.0	0.636
		Stalled	7.6 ± 2.5	

Physiological Data

Cortisol

Overall, plasma cortisol concentration was not affected by housing conditions ($p = 0.525$). The main effects of days ($p= 0.0001$) and time ($p= 0.0001$) were highly significant. A three way interaction between house and time and days failed to reveal a difference ($p= 0.507$). No differences in cortisol concentrations were observed between housing treatments during training days (Fig. 18).

Differences were revealed, among combined treatment groups, between cortisol concentrations and time sampling periods for training days (Fig. 19). A response to training revealed cortisol concentrations at the completion of training were higher than basal cortisol concentrations on day 0 ($p = 0.003$) and day 7 ($p = 0.0001$). No differences were observed on day 21 ($p = 0.623$) and 28 ($p = 0.339$).

With the exception of day 21 and day 28, cortisol concentrations required more than 15 min to return to basal levels. On day 0 ($p = 0.0001$) and day 7 ($p = 0.0008$), cortisol levels at the 15 min post-training (pt) recovery levels were higher than basal cortisol levels. No differences were observed on day 21 ($p = 0.623$) and day 28 (p =

0.233). However, by 75 min pt, cortisol levels were significantly lower than cortisol levels at basal. On day 7 ($p = 0.044$) and day 28 ($p = 0.0006$), 75 pt recovery cortisol levels were significantly lower than cortisol levels when compared to the basal cortisol levels. On day 0 ($p = 0.689$) and day 21 ($p = 0.774$) cortisol levels between basal and 75 pt samples were similar.

Basal plasma cortisol levels revealed differences between training days. On day 7 and day 28 basal cortisol levels were higher than basal cortisol levels for day 0 and day 21. The differences in cortisol levels were significant for day 0 and day 7 ($p = 0.0001$), day 21 and day 28 ($p = 0.0001$), day 7 and day 21 ($p = 0.0001$) and day 21 and day 28 ($p = 0.0001$) (Fig. 20).

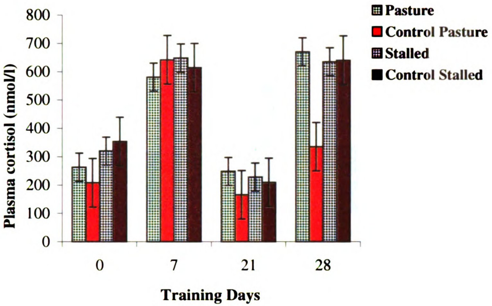

Fig.18. Plasma cortisol concentrations on training day, for treatment groups.

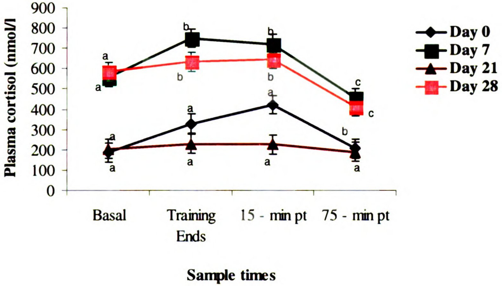

Fig. 19. Plasma cortisol concentration at time sampling period, for training days.
[a, b, c] Sample times with different subscripts differ ($p < 0.05$)

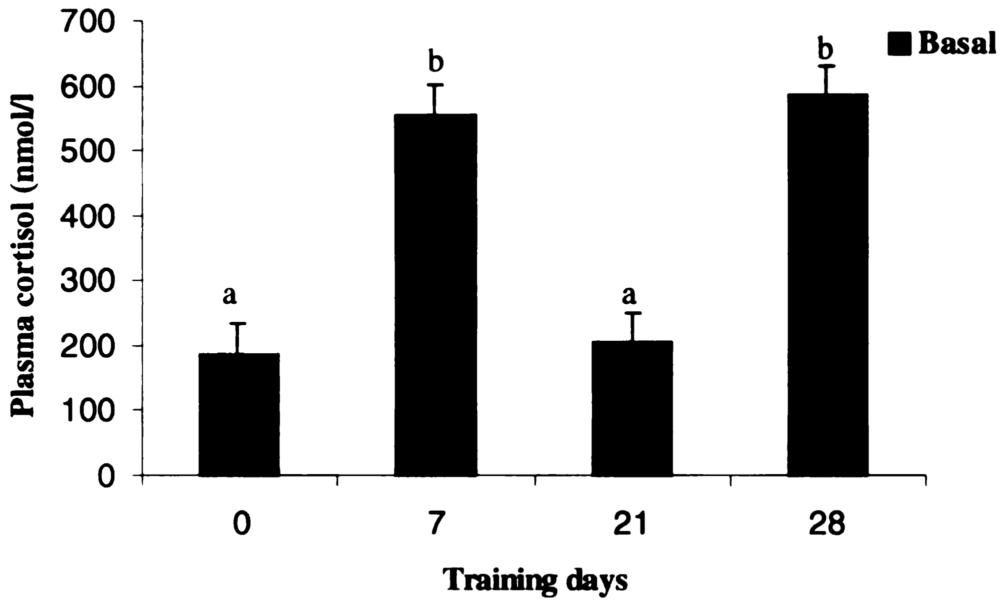

Fig. 20. Basal plasma cortisol concentrations on training days.
[a, b] Training days with different subscripts differ ($p < 0.001$)

Heart rate

Heart rate was affected by time (p = 0.0001) and training days (p = 0.001). Stalled horses tended to have a lower basal mean heart rate value when compared to the pastured horses (p = 0.07) (Fig. 21). For the other three sampling times, no significant differences were observed between the pasture and stalled treatment. However, a difference was observed between the control groups. Once the stalled control horses were allowed to explore the round pen, the stalled control group demonstrated higher mean heart rate values when compared to the control pastured group (p = 0.0007).

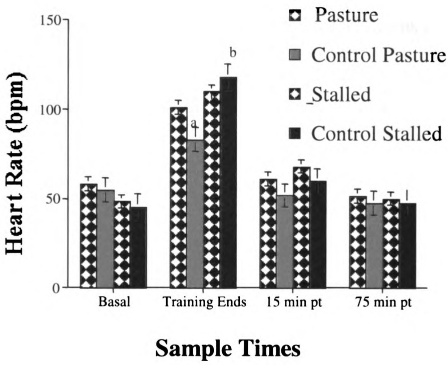

Fig. 21. Heart rate values at sample collection times.

Correlation among horse behaviors and plasma cortisol

A correlation using the area under the curve horse cortisol concentrations and each behavior category performed during the entire training procedure, by trainers and horses, was performed. Correlation and p - values for horse behaviors and cortisol are represented in Table 12. Cortisol was negatively correlated with the horse's behavioral responses side stepping, circling and bucking and jumping. A positive correlation was found with cortisol increasing with the occurrence of cantering by the horses.

Examining the correlation between behaviors performed by the trainers on the horses during training and cortisol can be found in Table 13. Out of the seven categories, only one, tugging on the reins, did not reveal a significant correlation. Sacking with the saddle revealed a negative tendency with cortisol. Stimuli categories such as sacking with the blanket, swinging rope/arm, kicking and hitting with the rope were negatively correlated with cortisol.

Table 12

Correlation analysis for horse behaviors performed throughout the study and cortisol.

Category	Cortisol	
	r	P-value
Stop	-0.2021	0.1282
Defecate	-0.2073	0.1203
Smell	-0.1842	0.1664
Side step	-0.4256	**0.0044**
Circle	-0.3347	**0.0282**
Ears back	-0.1023	0.5139
Ears switching	0.0578	0.6663
Cow kick	-0.045	0.7744
Away from pressure	-0.2525	0.1022
Slowdown	0.18977	0.2229
Speed up	0.2507	0.1049
Extension up	0.0986	0.5294
Extension down	-0.0239	0.8584
Extension straight	-0.0038	0.9807
Tossing head	-0.0563	0.6747
Look towards wall	0.2109	0.1745
Pawing	-0.2442	0.0647
Buck and jumps	-0.2495	**0.059**
Walk	-0.1511	0.2491
Trot	0.1798	0.1691
Lope	0.3705	**0.0036**
Stand	-0.1016	0.4395

Table 13

Correlation analysis of categories performed by the trainers and cortisol.

Category	Cortisol	
	r	P-value
Sack with saddle	-0.2931	**0.0828**
Swing rope	-0.3509	**0.0359**
Tugging reins	-0.1663	0.3324
Weight on back	-4167	**0.0115**
Sack with blanket	-0.3407	**0.042**
Hit with rope	-0.3507	**0.0359**
Kicks	-0.5731	**0.0003**

Discussion

Training a young horse for the first time requires the trainer to relax the horse, attain the confidence of the horse and develop a willingness to learn in the horse. The concept is to eliminate the fear of the trainer within the horse. Round pen training provides the environment for these lessons to take place. The trainers in this study used round pen techniques similar to the horse trainers John Lyons and Ray Hunt. There are different training styles used to train horses, however, documentation of the frequency and duration of behaviors that occur during a training session have never been documented. Moreover, to my knowledge no one has examined how housing may help or hinder adaptation by a horse during training. Studies examining housing conditions with horses focus mainly on stereotypic behaviors and its effects on the welfare of the horse. The results of this study demonstrated that young stalled horses, undergoing their first training experience, required more time to adjust to the training procedures than young pastured horses. The animal's behavioral responses to training were affected by their housing conditions.

Training Times

Total training time in the arena, on average was four min less for the pastured group. Horse owners may own only one to three horses for pleasure. If this is the case, the time difference may not seem important when training. However, there are trainers whose livelihood depends on the number of horses they train in one day. For these trainers the time difference may result in a loss of income.

The stalled horses had a tendency to require more time to complete the entire training procedure. On day 0, the training procedure was new to the horses possibly explaining the similarity between the groups. For the rest of the training schedule, the pastured group tended to complete the training regimen faster than the stalled horse's. This tendency may be explained by the differences observed in the behavioral responses to the groundwork training techniques. Many of the training techniques, i.e. sacking with the blanket, took longer for the stalled horses to habituate to. During the riding period, no differences were revealed in training times. It appeared as if the stalled horses were expending excess energy during the groundwork period and by the start of the riding period they were more receptive to training.

Studies on the free-roaming Camargue horse (Duncan, 1980), Przewalski horses (Hogan et al., 1988) and pasture reared Quarter Horse foals (Heleski et al., 1999) showed that the horses spent a high percentage of their time grazing. For stalled horses, their restricted environment hinders the opportunity for them to walk around and graze. As a result, a shorter amount of time is needed before the horses reach satiety. Hogan et al. (1988) suggested that the lack of opportunity to graze results in the horses having extra time and unspent energy. In addition, the unspent energy was displayed through the occurrence of abnormal behaviors such as pacing and milling (Hogan, 1988). Milling was defined as walking bouts interrupted by stops and numerous direction changes with no consistent path followed (Hogan et al., 1988). Energy expenditure was not measured in this study. However, the stalled horses did perform many of the negative, unwanted behavioral responses. An increase in activity levels, displayed by the increase frequency of behaviors such as bucking and jumping, looking towards the wall, circling, head and neck extension up, down and straight may be a result of unspent energy due to stalling. Pasture housing may have provided the exercise, which relaxes the horses allowing them to be more receptive during training.

Horse and trainer behaviors during groundwork training

Interestingly, the stalled horses stood still for longer periods of time during the groundwork period. Standing still was the desired response for many of the training techniques applied such as suppleness training, sacking with the blanket, placing a foot in the stirrup and weight on the back of the horse.

During many of the groundwork procedures, the trainers would hold onto the lead rope while slightly turning the horse's head restricting their movement to small circles. On day 0, pastured and stalled horses displayed similar frequencies of circling. However, by day 7 the pastured horses had habituated to the procedures and by day 21 and 28, circling did not occur with the pastured horses. The performance of the circles was an escape response by the horses to the pressure or stimuli that was being applied. While play fighting, foals will bite at each other's hindlegs resulting in circling (Waring, 1983). Circling has also been reported to occur before parturition by a mare as a sign of uneasiness (Waring, 1983).

Repeatedly tossing the blanket and saddle onto the horse's back as well as placing weight onto the horse's back were training techniques involving habituation. The horses had to acclimatize to the repetitive exposure of various stimuli such as the blanket, saddle and weight on their back. Both the saddle pad and saddle took longer for the stalled horses to stand still for. However, by day 7, no differences in the amount of time for habituation was observed between the treatment groups. The results may be explained by the richer environment experienced by the pasture kept horses. Enriched environments provide challenges to animals in which the experience may be used to adapt to situations, i.e. locating food or shelter (Sheperdson et al., 1993). In contrast, barren environments have impaired learning ability in animals. Isolated rat pups, compared to rat pups housed with parents and littermates, experienced difficulty in memory retention (Arnold and Spear, 1995).

Habituation, in this study, was the primary learning concept used during the groundwork period. The horses had to acclimatize to repetitive exposure of various stimuli such as the blanket, saddle and weight on their back. The daily challenges from the pasture environment may have helped the horses acclimatize easier to a stressful situation. This was the case for rats, when housed with other rats and provided with different toys everyday (Juraska et al., 1983). The experience gained by the rats housed in complex environments appeared to have changed their problem-solving strategies and perhaps, learning abilities (Juraska et al., 1983). The opportunity to explore objects in the environment and play with other horses is an option for pastured horses. These opportunities may have influence on learning. In kittens, increased exploration with objects was suggested to involve learning about stimuli properties, while play-exploration involves learning about perceptual-motor properties and play involves learning to coordinate movements (West, 1977). In another study with domestic cats, observations revealed that littermate-deprived kittens did not learn social communication skills suggesting that social play may be more complex than the element of object play (Guyot et al., 1980).

On pasture, horses have more opportunities to differentiate between stimuli in the environment that may be harmful or harmless. This experience in differentiation may be learned on their own or through play with a conspecific. Play is important as exercise for young horses and is a major role player in the behavioral, social and physiological development for all horses (Waring, 1983). It functions as a way of practicing and perfecting adult behavior skills that may be necessary in life for a wide range of actions (Fraser, 1992). Fagan (1981) suggested playing among animals helps develop cognitive skills necessary for behavioral adaptability, flexibility, inventiveness or versatility. Social play, such as chasing and mock fighting is most common in young horses (Fraser, 1989). During mock fighting, horses attempt to bite the head and neck, rear and strike each other and bite at the forelegs often causing the opponent to lose balance or drop to their knees (Waring, 1983).

Interestingly, the training procedure of applying weight on the horse's back took longer for the pasture horses to adapt to. If the environment is indeed helping the pastured horses to determine differences in stimuli, perhaps the idea may be taken further. Maybe responses can vary with the degree of technique being applied. Having weight on the horses' back can be perceived as a threatening situation such as experiencing an attack by a predator or as a playful act. Pastured horses may be more responsive to tactile stimuli, making it more difficult for the pastured horses to adapt.

Horse behaviors during the riding portion of training

It should be mentioned that behaviors observed during the riding period and even the groundwork period are not necessarily abnormal. The behaviors observed were typical for young horses, which have never been trained. It would be abnormal if the horses would ignore their natural fight or flight response and stand still while something or someone is touching its body. Although the results of looking towards the wall were not significant except for day 7, the stalled group consistently performed the behavior more often on training days. Looking can also be referred to as staring in horses. Horse's will look at their abdomen as a sign of discomfort and may look at a stimulus in order to determine its potential as a threat (Waring, 1983). Looking towards the wall is considered a lack of attentiveness by the horses (Lyons, 1989).

Head and neck extension up and straight were observed more frequently, during the riding period, with the stalled horses. On day 0, the trainers used the halter with a lead rope to direct the horses while riding. By day 7, the trainers began using a bridle and snaffle bit (see page 28). The jointed snaffle bit applies direct pressure backwards or sideways on the mouth of the horse (C.H.A., 1996). When the trainer applied pressure to the reins for the horses to move in a direction, the horses would respond by trying to evade the bit and move their heads either up, down and straight. Avoiding the pressure was not unusual however, it was evident that the stalled horses were having difficulty dealing with the trainers commands using the reins. The stalled horses consistently extended their head and neck throughout the entire training procedure.

Bucking and jumping may occur under different circumstances. In this study, the horses bucked and jumped primarily while being chased with or without a saddle on their back and occasionally during the riding period. The stalled horses bucked and jumped more often than the pastured horses on days 7, 21 and 28. A horse may buck and jump in the presence of pain, if the horse is confused, startled or is feeling playful (Fraser, 1992).

Although bucking and jumping may be a playful act and some trainers may find it challenging, it may become dangerous if the trainer is thrown from the saddle as a result. The trainers may tolerate bucking and jumping. However, a decrease in the chance of an occurrence may be safer for the trainer.

Perhaps the change in the relationship between the horses and the humans was difficult for the horses to adapt to, resulting in the observed behaviors during riding. At first for the stalled horses, the relationship was a positive one, since humans were a source of food, water and an opportunity for exercise. Once training began, the human role switched from a passive role to an active one. Grandin et al. (1994) revealed a tendency for cattle to resist change once they were accustomed to a treatment associated with a specific side of an Y maze. Stalled horses may have had a similar experience. It may have been difficult during the training to acclimatize to the changes that were occurring in the human - animal relationship.

A horse's tail setting will commonly accompany facial, neck and leg expressions (Waring, 1983). In most breeds of horses, a relaxed horse carries its tail down while an elevated tail can indicate aggression, alarm or when a horse is not comfortable with a handler (Waring, 1983). However, in the Arabian breed of horse's a slightly elevated tail is desirable and considered a normal, relaxed tail setting. In this study, the pastured horse's tended to carry a normal tail setting. It was demonstrated that the pastured group acclimatized to the training procedures by their infrequent performance of unwanted behaviors, resulting in a tendency for less time in training. The normal carriage setting is further indication of the ease with the training procedures.

Trainers

In this study, there was an experience and gender difference between the trainers. It was interesting to find that the male, experienced trainer's stalled horses required more attention and time while performing more of the unwanted behaviors. The responses observed in their horses according to the experience of their trainer were interesting however, it is important to mention that various variables might have played a factor. On the first day of training, the novice trainer trained the horses regarded as "safer". The number of horses on day 0 were not equal, eight (experienced trainer) versus four (novice trainer). On day 7, day 21 and 28, the novice trainer was allowed to train two of the experienced trainer horses. This exchange between horses and trainers may have created a bias due to the differences in training styles and their initial perception on the horse's trainability. There is the possibility that after the first day of training the trainers had an opinion on which horses were going to be difficult to train, which may have affected their attitude when during the next training sessions.

Cortisol

Overall, cortisol concentrations were high when compared to other studies. Mean cortisol values for horses housed in stalls and pasture, with no training have been reported at 163 ± 14 nmol l while trained horses exhibited cortisol levels ranging between 90 to 190 nmol l (Irvine, 1994). Plasma cortisol concentrations in this study ranged from 180 to 750 nmol l. When compared to horses undergoing a marathon race, which demonstrated mean cortisol levels of 151 ± 14 nmol l before race time and 445 ± 23 nmol l after the race was completed, the cortisol levels in the present study were higher.

This study revealed an increase in cortisol concentrations due to exercise with a significant decrease by 75 min post-training. The increase after exercise is comparable to many studies conducted with horses when cortisol rose after exercise (Church et al., 1987; Lucke et al., 1980). The fifteen-min post-training cortisol concentrations were still elevated however by 75 min post-training the levels had decreased. This is comparable to other cortisol where cortisol levels will decrease by thirty min post exercise (Snow and Mackenzie, 1981; Rose et al., 1983).

Contrary to the hypothesis, there were no differences between housing treatments in cortisol concentrations over training days. Studies have shown that cortisol may increase (Hennessy and Weinberg, 1990; Boulton et al., 1997) or not change (Freeman, 1985) in response to a stressor. Dallman et al. (1987) suggested that the threshold for adrenocortical system responses might be considerably higher than for behavioral systems. Linden et al. (1990) demonstrated a strong correlation between the post-ACTH and post-exercise cortisol concentration increase suggesting that each individual has a specific stress-induced response independent of the type of stress. Since day one and seven of the study focused on habituation training with limited riding for physical exercise, it is possible, that with a more strenuous or longer training regimen a different treatment adrenocortical response may have been observed.

It was surprising to find an increase followed by a decrease in basal cortisol levels according to training days. On both days one and twenty-one basal cortisol concentrations were lower for each sample collection time compared to the training days seven and twenty-eight. This trend can not be compared to any other study in the literature. The differences between basal cortisol levels over training days warrants further research

Heart rate

No effect of housing on heart rate values was observed. It was surprising, considering the potentially different levels of physical fitness, resulting from the difference in exercise opportunities between treatment groups, that differences were not observed.

Marsland (1968) suggested an untrained horse with poor physical fitness during submaximal exercise would exhibit higher heart rates at rest and during exercise. In this study, mean resting (basal) levels averaged 58.3 beats per min for pasture and 48.5 beats per min for the stalled treatment groups. These heart rates were higher than the reported range of 25 to 40 beats/min (Evans et al., 1994) exhibited by idle horses. Anticipation has been shown to affect resting heart rates in horses moved from their home stall, to preparation shed and track (Hall et al., 1976). This scenario is similar for the pastured group who incidentally exhibited higher mean resting heart rates. The pastured group was removed from the pasture and lead into a stall one h prior to training. First the horses were equipped with the heart rate monitors. Then towards the end of the h, before or after entering the training arena, horses were tacked and the first blood samples were drawn. With the pasture group, the increased activity that occurred prior to the start of training may have elicited an anticipatory effect, reflected through the increase in basal heart rates.

The dual innervation between the parasympathetic and sympathetic mechanisms causes variability in heart rate and the variability may serve as an important mechanism for adaptability, during a stress response (Porges, 1992). In this study, measures of heart rate variability may have provided important information. Due to technical difficulties these data were not analyzed.

Correlation among horse behaviors and plasma cortisol

Plasma cortisol was inversely correlated with frequency of sacking with the blanket and saddle, weight on the backs of the horses, swinging the lead rope and/or arms, hitting with the rope and kicking while riding. With the exception of kicking, these different stimuli were used by the trainers during the groundwork period. Horse cortisol concentrations were also negatively correlated with horse behaviors sidestepping, circling and bucking and jumping. These behaviors were responses, which may have occurred at any point in the training session. Studies have examined individual variability in response to different stressors. Hessing et al. (1994) suggested that animals have different ways of coping with stress and each can be associated with different physiological, endocrine and immunological responses. Bohus et al. (1987) suggested that pigs that react to a stressor passively, will react to the stress with an increased parasympathetic nervous activity while pigs that actively react to stress primarily elicit a sympathetic nervous stress response. Mendl et al. (1992) demonstrated that pigs that were successful during aggressive interactions had lower levels of salivary cortisol in response to ACTH. In contrast, pigs that were unsuccessful showed the highest peak levels of cortisol in response to ACTH. Considering there were no physiological differences observed however, many behavioral differences were noted. It is possible that the some of the horses in this study coped with stress using a behavior pathway instead of the autonomic or neuroendocrine pathways.

Conclusion

Housing conditions are an important aspect that should be considered when training young horses for the first time. Research over the last decade has proven that barren housing conditions can affect an animal's behavior and learning abilities. The physiological data in this study did not identify differences between housing although, the data did reveal initial training as a stressful event. The present study did however provide the first in depth assessment of the effects of housing conditions on the responses of horses to training.

Although this is the first study of its kind, the results presented provide initial data towards increasing the welfare of a horse during training. Land for pasture housing may not be accessible or feasible to every horse owner. Nonetheless, horse owners should try to provide horses a more enriched environment, if not through pasture housing, perhaps increasing the amount of time a house is released in a paddock. Future studies examining partial pasture turnout versus stalled housing conditions on training behaviors and physiological measures may demonstrate further that increased opportunities for social interaction or the chance to release excess energy can be beneficial in increasing the trainability of the horses. In addition, examining young horse in the partial pasture condition, either isolated or with other horses, may help to determine which factor, exercise or social interaction, is more critical for improving trainability of the young horse.

Appendix A

Glossary

Glossary

Bucking and jumping – horse arches its back with the head and neck lowered is called bucking. By leaping off the ground at the same time is called a buck and jump

Circadian rhythm – the regular reoccurrence of cortisol during a 24 h interval

Cribbing – horse will grab at an object with its incisor teeth and then pull it neck back in a rigid arch as it swallows air

Head tossing – horse abruptly tosses its head up and down

Pawing - horse will repeatedly strike the ground with its foreleg

Rearing - horse's hindlegs will remain on the ground while the forequarters are raised high into the air

Sacking – repeatedly tossing an object onto the horse's back

Stall walking – horse constantly paces or circles around the stall

Twitch - A loop of rope or chain on the end of a stout wooden shaft. The loop is placed around the upper lip of the horse and the handle or shaft, is twisted to snare the lip firmly

Weaving- horse stands in place but weaves its head and neck back and forth as it rocks from side to side

Appendix B

Behavior categories and codes

Periods	Code	Event / State
Riding	s e	State
Groundwork	n s	State

Trainer Categories	Code	Event / State
Chase	c h	State
Walk	h w	State
Approaches horse	a h	Event
Swing lead rope/arms	s r	Event
Stroking	r n	Event
Tug on lead rope/reins	p r	Event
Sack with blanket	s b	Event
Sack with saddle	s a	Event
Foot in stirrup	f s	Event
Weight on back	w b	Event
Mount	m t	Event
Not moving	n m	Event
Steady	s y	Event
Kick	l k	Event
Posting	p o	State
Hit with rope	h r	Event
Other	o t	Event
Dismount	o f	Event

Horse Categories	Code	Event / State
Circling	h c	State
Stop	s t	Event
Smelling	n g	Event
Normal tail	n t	Event
Raised tail	r t	Event
Ears back	e b	Event
Ears forward	e f	Event
Ears switching	e s	Event
Defecate	p p	Event
Stand still	s s	Event
Approach trainer	a t	Event
Bucks and jumps	b j	Event
Cow kick	c k	Event
Tossing head	t h	Event
Side stepping	s d	Event
Pawing	p w	Event
Pull away from pressure	a p	Event
Walk	h w	Event
Trot	h t	Event
Lope	h l	Event
Slow down	s l	Event
Speeding	s p	Event
Looking towards wall	l r	Event
Head / neck extension up	x u	Event
Head / neck extension down	x d	Event
Head / neck extension straight	x s	Event
Backs up	b u	Event
Horse falls	h b	Event

Appendix C

Supplemental horse and trainer behavior categories

Horse behaviors during groundwork training

Table 14

Frequency (mean ± SEM) of applying pressure on the lead rope or reins by the trainers, for training days.

Category	Day	House	mean ± SEM	p-value
Pressure on lead rope/reins	0	Pasture	57.0 ± 12.0	0.7
		Stalled	63.9 ± 14.0	
	7	Pasture	6.3 ± 2.8	0.05
		Stalled	16.8 ± 4.5	
	21	Pasture	3.7 ± 1.5	0.6
		Stalled	4.7 ± 2.6	
	28	Pasture	3.8 ± .9	0.8
		Stalled	4.1 ± .9	

Table 15

Frequency (mean ± SEM) of pulling away from the pressure of the reins by treatment groups, per training day.

Category	Day	Housing	mean ± SEM	p-value
Pulling Away From Pressure	1	Pasture	0.67 ± .29	0.01
		Stalled	1.9 ± 4.0	
	7	Pasture	0.13 ± 0.21	0.5
		Stalled	0.29 ± 0.25	

Categories performed by the trainer on the horse during groundwork training

Table 16

Duration (mean ± 1 SEM) of chasing (min) by trainers on treatment groups per training day.

Category	Day	Housing	mean ± SEM	p-value
	1	Pasture	5.0 ± 3.7	0.8
Chasing		Stalled	6.4 ± 1.4	
	7	Pasture	1.0 ± .35	0.02
		Stalled	0.11 ± 0.11	

Table 17

Frequency (mean ± SEM) of swinging rope/arms performed by the trainers, on the treatment groups, per training day.

Category	Day	House	mean ± SEM	p-value
	0	Pasture	53.0 ± 7.0	0.1
Swing Rope / Arms		Stalled	71.0 ± 9.0	
	7	Pasture	24.0 ± 6.5	0.6
		Stalled	28.6 ± 7.5	
	21	Pasture	0.72 ± 0.82	0.1
		Stalled	2.8 ± 1.2	
	28	Pasture	0.42 ± 0.55	0.5
		Stalled	0.99 ± 0.65	

Table 18

Frequency (mean ± SEM) of swinging rope/arms performed by the trainers according to the experience of the trainer.

Category	Trainer	Housing	mean ± SEM	p-value
	Experienced	Pasture	9.3 ± 3.1	0.04
Swing Rope/Arms		Stalled	20 ± 3.7	
	Novice	Pasture	16.0 ± 4	0.8
		Stalled	15.1 ± 4	

Table 19

Frequency (mean ± SEM) of hitting the horses with the rope, per training day.

Category	Day	Housing	mean ± SEM	p-value
	1	Pasture	7.1 ± 4.0	0.9
Hit with rope		Stalled	6.3 ± 4.5	
	7	Pasture	0.77 ± 0.35	0.4
		Stalled	0.01 ± 0.1	

Horse behaviors during riding portion of training

Table 20

Frequency (mean ± SEM) of head tossing performed by the treatment groups, during the groundwork and riding portion for training days.

Category	Day	Housing	means ± SEM	p-value
Head tossing	0	Pasture	6.4 ± 2.3	0.1
		Stalled	2.2 ± 1.4	
	7	Pasture	0.43 ± 0.49	0.2
		Stalled	1.4 ± 0.8	
	21	Pasture	0.53 ± 0.85	0.5
		Stalled	2.1 ± 2.1	
	28	Pasture	0.002 ± 0.26	0.1
		Stalled	0.89 ± 0.51	

Table 21

Frequency (mean ± SEM) of head tossing, performed by the treatment groups, during the groundwork and riding portion of training for treatment groups, according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
Head tossing	Experienced	Pasture	2.1 ± 1.0	0.9
		Stalled	1.9 ± 1.0	
	Novice	Pasture	0.62 ± 0.7	0.5
		Stalled	1.3 ± 1.1	

Table 22

Duration (mean ± SEM) of walking (min) by treatment groups for training days

Category	Day	House	mean ± SEM	p-value
	0	Pasture	14.8 ± 2.8	0.3
Walking		Stalled	18.7 ± 3.4	
		Ctrl(P)	11.0 ± 2.1	0.7
		Ctrl(S)	10.5 ± 2.0	
	7	Pasture	9.4 ± 2.3	0.3
		Stalled	12.8 ± 2.7	
		Ctrl (P)	3.5 ± 1.7	0.04
		Ctrl (S)	9.5 ± 1.9	
	21	Pasture	1.3 ± 0.53	0.02
		Stalled	3.6 ± 0.82	
		Ctrl (P)	3.1 ± 1.8	0.9
		Ctrl (S)	3.2 ± 1.8	
	28	Pasture	1.6 ± 0.39	0.8
		Stalled	1.7 ± 0.37	
		Ctrl (P)	4.3 ± 1.4	
		Ctrl (S)	2.8 ± 1.0	0.4

Table 23

Duration (mean ± SEM) of walking (min) by the treatment groups according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
	Experienced	Pasture	5.8 ± 0.90	0.5
Walking		Stalled	7.0 ± 0.71	
	Novice	Pasture	6.4 ± 0.7	0.08
		Stalled	8.7 ± 1.1	

Table 24

Duration (mean ± SEM) of trotting (min) by treatment groups, per training day.

Category	Day	House	mean ± SEM	p-value
	0	Pasture	3.6 ± 1.5	0.9
Trotting		Stalled	3.7 ± 1.6	
		Ctrl(P)	2.6 ± 1.5	0.2
		Ctrl(S)	6.1 ± 2.4	
	7	Pasture	4.7 ± .93	0.4
		Stalled	5.7 ± 1.2	
		Ctrl (P)	4.3 ± 1.6	0.9
		Ctrl (S)	4.4 ± 1.3	
	21	Pasture	3.5 ± 0.64	0.09
		Stalled	5.1 ± 0.74	
		Ctrl (P)	1.1 ± 0.5	0.006
		Ctrl (S)	4.6 ± 1.2	
	28	Pasture	4.5 ± 1.0	0.5
		Stalled	5.5 ± 1.0	
		Ctrl (P)	0.23 ± 0 .3	0.006
		Ctrl (S)	4.3 ± 1.5	

Table 25

Duration (mean ± SEM) of walking (min) by the treatment groups, according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
	Experienced	Pasture	3.0 ± 0.89	0.2
Trotting		Stalled	4.6 ± 0.82	
	Novice	Pasture	5.2 ± 0.86	0.9
		Stalled	5.1 ± 1.1	

Table 26

Duration (mean ± SEM) of cantering (min) performed by the treatment groups, per training day.

Category	Day	House	mean ± SEM	p-value
	0	Pasture	1.2 ± 0.73	0.9
Cantering		Stalled	0.9 ± 0.73	
		Ctrl(P)	0.3 ± 0.3	0.8
		Ctrl(S)	0.17 ± 0.5	
	7	Pasture	1.4 ± 0.24	0.0001
		Stalled	4.0 ± 0.43	
		Ctrl (P)	0.11 ± 0.14	0.5
		Ctrl (S)	3.1 ± 0.26	
	21	Pasture	1.1 ± 0.2	0.007
		Stalled	2.1 ± 0.26	
		Ctrl (P)	0	0.06
		Ctrl (S)	0.4 ± 0.31	
	28	Pasture	2.3 ± 0.64	0.2
		Stalled	3.4 ± 0.74	
		Ctrl (P)	0	0.1
		Ctrl (S)	0.62 ± 0.5	

Table 27

Frequency (mean ± SEM) of stopping, speeding and slowing down behaviors performed by the treatment groups for training days.

Category	Housing	mean ± SEM	p- Value
Stop	Pasture	8.4 ± 1.5	0.4
	Stalled	10.5 ± 1.6	
Speed	Pasture	2.9 ± 1.2	0.1
	Stalled	4.8 ± 1.0	
Slow down	Pasture	1.3 ± 0.25	0.7
	Stalled	1.4 ± 0.24	

Table 28

Frequency (mean ± SEM) of stopping behavior performed by the treatment groups for training days .

Category	Day	Housing	mean ± SEM	p-value
Stop	0	Pasture	33.6 ± 4.3	0.6
		Stalled	31.0 ± 4.0	
	7	Pasture	8.5 ± 2.2	0.2
		Stalled	14.1 ± 3.0	
	21	Pasture	1.0 ± 1.0	0.1
		Stalled	3.5 ± 1.6	
	28	Pasture	3.2 ± 1.8	
		Stalled	2.9 ± 1.5	0.8

Table 29

Frequency (mean ± SEM) of speeding performed by the treatment groups for training days.

Category	Day	Housing	mean ± SEM	p-value
Speed	0	Pasture	6.9 ± 2.2	0.1
		Stalled	12.1 ± 3.0	
	7	Pasture	1.6 ± 1.2	0.2
		Stalled	4.0 ± 2.0	
	21	Pasture	1.7 ± 1.6	0.6
		Stalled	2.5 ± 1.5	
	28	Pasture	2.8 ± 2	0.9
		Stalled	2.8 ± 1.6	

Table 30

Frequency (mean ± SEM) of slowing down performed by the treatment groups for training days.

Category	Day	Housing	Mean ± SEM	p-value
Slowdown	0	Pasture	1.1 ± 0.63	0.3
		Stalled	2.1 ± 0.8	
	7	Pasture	0.87 ± 0.59	0.6
		Stalled	1.2 ± 0.60	
	21	Pasture	0.99 ± 0.71	0.07
		Stalled	0.54 ± 0.55	
	28	Pasture	2.1 ± 1.1	0.9
		Stalled	2.1 ± 0.84	

Table 31

Frequency (mean ± SEM) of stopping, speeding and slowing down performed by the treatment groups, according to the experience of their trainer,

Category	Trainer	Housing	mean ± SEM	p-value
	Experienced	Pasture	8.5 ± 2.0	0.2
Stopping	Novice	Stalled	11.5 ± 2.1	
	Experienced	Pasture	8.5 ± 2.1	0.7
	Novice	Stalled	9.4 ± 2.2	
Speeding	Experienced	Pasture	2.8 ± 1.4	0.1
	Novice	Stalled	3.0 ± 1.1	
	Experienced	Pasture	5.2 ± 1.1	0.4
	Novice	Stalled	4.3 ± 1.3	
Slowdown	Experienced	Pasture	1.0 ± 0.25	0.8
	Novice	Stalled	1.5 ± 0.35	
	Experienced	Pasture	1.1 ± 0.26	0.6
	Novice	Stalled	1.8 ± 0.40	

Table 32

Frequency (mean ± SEM) of side stepping performed by the treatment groups for training days.

Category	Day	Housing	mean ± SEM	p-value
Side stepping	0	Pasture	33.1 ± 9.0	0.3
		Stalled	23.0 ± 7.0	
	7	Pasture	4.4 ± 1.4	0.2
		Stalled	7.0 ± 1.7	
	21	Pasture	0.60 ± 0.82	0.07
		Stalled	4.5 ± 1.7	
	28	Pasture	1.5 ± 1.4	0.3
		Stalled	3.7 ± 1.3	

Table 33

Frequency (mean ± SEM) of sidestepping by the horses according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
	Experienced	Pasture	4.2 ± 2.3	0.0384
Sidestepping		Stalled	1.1 ± 2.2	
	Novice	Pasture	8.9 ± 2.4	0.3729
		Stalled	5.9 ± 2.3	

Trainer behaviors during the riding portion of training

Table 34

Frequency (mean ± SEM) of kicking by the trainers on the treatment groups on training days.

Category	Day	Housing	mean ± SEM	p-value
Kicks	0	Pasture	54.0 ± 25.0	0.7
		Stalled	41.5 ± 24.3	
	7	Pasture	6.3 ± 2.2	0.1
		Stalled	2.4 ± 1.6	
	21	Pasture	1.8 ± 1.4	0.9
		Stalled	2.0 ± 1.7	
	28	Pasture	3.4 ± 1.3	0.03
		Stalled	0.54 ± 0.57	

Table 35

Frequency (mean ± SEM) of kicking by the trainers on the treatment groups.

Category	Trainer	Housing	mean ± SEM	p-value
Kicks	Experienced	Pasture	6.7 ± 3.5	0.5
		Stalled	9.4 ± 3.8	
	Novice	Pasture	16.0 ± 4.5	0.06
		Stalled	4.5 ± 3.0	

Horse behaviors during groundwork and riding portions of training

Table 36

Frequency (mean ± SEM) of smelling the environment performed by the treatment groups on training days.

Category	Day	Housing	mean ± SEM	p-value
Smell	0	Pasture	39.3 ± 13.5	0.3
		Stalled	23.0 ± 10.5	
	7	Pasture	7.8 ± 2.6	0.4
		Stalled	5.3 ± 2.3	
	21	Pasture	2.2 ± 1.3	0.6
		Stalled	3.0 ± 1.5	
	28	Pasture	4.1 ± 2.4	0.7
		Stalled	3.2 ± 2.2	

Table 37

Frequency (mean ± SEM) for the smelling the environment performed by treatment groups, according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
Smell	Experienced	Pasture	5.3 ± 2.5	0.8
		Stalled	6.1 ± 2.1	
	Novice	Pasture	16.1 ± 6.0	0.1
		Stalled	8.0 ± 3.1	

Table 38

Frequency (mean ± SEM) of defecation by treatment groups, for training days.

Category	Day	Housing	mean ± SEM	p-value
Defecation	0	Pasture	4.8 ± 1.1	0.9
		Stalled	4.8 ± 1.1	
	7	Pasture	2.0 ± 0.65	0.5
		Stalled	0.94 ± 0.56	
	21	Pasture	1.0 ± 0.29	0.2
		Stalled	0.34 ± 0.21	
	28	Pasture	0.43 ± 0.14	0.06
		Stalled	0.55 ± 0.15	

Table 39

Frequency (mean ± SEM) of cow kicking performed by the treatment groups on training days.

Category	Day	Housing	mean ± SEM	p-value
Cow kicking	0	Pasture	0.96 ± 0.5	0.2
		Stalled	3.1 ± 1.6	
	7	Pasture	0.39 ± 0.4	0.6
		Stalled	0.77 ± 0.58	
	21	Pasture	0.19 ± 0.11	0.1
		Stalled	0.004 ± .08	
	28	Pasture	0.12 ± 0.14	0.2
		Stalled	1.1 ± 0.5	

Table 40

Frequency (mean ± SEM) of cow kicking, performed by treatment groups, according to the experience of their trainer.

Category	Trainer	Housing	mean ± SEM	p-value
Cow kicking	Experienced	Pasture	0.08 ± 0.22	0.1
		Stalled	0.89 ± 0.46	
	Novice	Pasture	0.91 ± 0.5	0.6
		Stalled	1.2 ± 0.62	

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