THESIS

A PILOT STUDY OF BEHAVIORAL AND PHYSIOLOGICAL MARKERS OF STRESS IN HORSES DURING EQUINE-ASSISTED LEARNING FOR YOUTH WITH SOCIAL-EMOTIONAL CONCERNS

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ABSTRACT

A PILOT STUDY OF BEHAVIORAL AND PHYSIOLOGICAL MARKERS OF STRESS IN HORSES DURING EQUINE ASSISTED LEARNING FOR YOUTH WITH SOCIAL-EMOTIONAL CONCERNS

Equine Assisted Learning (EAL) is an increasingly popular service in providing comfort and life skills for youth who have social-emotional concerns. There is mixed evidence in previous research that shows increased stress in horses in EASs who are interacting with the population of youth with social- emotional concerns. This pilot study aimed to look physiological and behavioral indicators of stress in horses involved in equine assisted learning for youth with social- emotional concerns. This within-subject design included 11 horses and 18 human participants. Indicators of equine stress included salivary cortisol, eye temperature, and equine behavior. On average, indicators of stress in horses were not significantly different while the horses interacted with youth with social- emotional concerns compared to a control condition. There was a significant condition x week interaction effect such that horses demonstrated higher levels of cortisol in week 3 while interacting with youth with social-emotional concerns compared to the control condition, but this difference did not exist by Weeks 5 and 7. These condition by week interaction effects did not exist in equine eye temperature or behavior. This study provides evidence that interacting with youth with social- emotional concerns was not more stressful for horses that interacting with another population that commonly participates in EAL.

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CHAPTER 1- REVIEW OF LITERATURE: STRESS IN HORSES DURING EQUINE ASSISTED SERVICES FOR YOUTH WITH SOCIAL- EMOTIONAL CONCERNS

The following research sought to observe signs of stress in horses participating in equine-assisted services for youth with social- emotional concerns. Equine assisted services (EASs) refer to multiple services in which professionals incorporate horses and other equines to benefit people (Wood, 2021). There are several different types of EASs; the focus of this thesis is one service called equine-assisted learning. Equine assisted learning (EAL) is an educational service that engages people of all ages in learning processes that focus on academic skills, character development, or the promotion of relevant life skills such as problem solving or critical thinking skills. Horses are integrated into equine-assisted learning to help participants achieve the designated educational outcomes (Wood, 2021).

The focus of this study is equine-assisted learning for youth with social- emotional concerns. Social-emotional competencies include: self-awareness, self-management, responsible decision making, social awareness, and relationship skills (Casel, n.d). It is increasingly recognized that many youths have limitations in these competencies. In the past, these youth have been referred to as “youth at-risk,” a term used to describe youth who are at risk for experiencing negative life outcomes as they transition to adulthood such as higher probability of negative developmental outcomes, poor academic success, and reduced mental health when they engage in or are exposed to adverse risk (Wilkie et. al., 2016). Among the many factors that increase youth’s risk of experiencing negative life outcomes are emotional and behavioral difficulties, family conflicts, maltreatment, low social competence, and/or low socioeconomic status (Kaiser et. al., 2006; Wilkie, 2016). The current paper uses the term “youth with social-emotional concerns” to be in line with most up-to-date terminology recommendations. EAL can focus on teaching social-emotional skills to youth with social- emotional concerns, which may serve as a protective factor to decrease the risk of youth experiencing negative life outcomes. This study focuses on examining stress in horses that are integrated into EAL focused on teaching social-emotional skills to youth with social- emotional concerns in the community.

First it is important to understand why stress should be examined in horses and how it affects their body. Stress serves an adaptive role in the body, but chronic stress can lead to poor health outcomes. When interacting with humans, horses are highly susceptible to work stressors related to physical constraints and/or to the need to control emotions (De Santis et. al., 2017). With chronic stressors, there are negative health concerns (Koolhaas et. al., 1999), decrease in performance (Peeters et. al. 2013) and welfare concerns. Some health concerns with the presence of stress in horses include ulcers and a compromised immune system (Koolhaas et. al., 1999). The sources of stress in horses during EASs include physical activities like being ridden, or anxiety and fear when novel stimuli are presented (De Santis et. al., 2017). Another possible source of stress in horses participating in EASs can also be related to the rider’s or handler’s emotional status (Keeling et. al., 2009) and if the rider has a physical disability (Kaiser et. al., 2006).

A growing body of research has examined equine welfare in riding horses and in horses during EASs. Most studies have demonstrated that there are not higher levels of stress in horses participating in EASs when compared to recreational riding (Arrazola & Merkies, 2020; Kaiser et. al., 2006; Nobbe, 2016). However, a few studies have demonstrated that EASs with humans with history of trauma or characterized as “at-risk” could cause behavioral and physiological changes in horses (Kaiser et. al., 2006; Merkies et. al., 2018). After reviewing the pertinent literature, I was only able to locate two research studies that observe equine stress during EASs with youth with social- emotional concerns, referred to by the authors as “youth at-risk” (Arrazola & Merkies, 2020; Kaiser et. al., 2006).

A study done by Kaiser et. al. (2006) found that horses in EASs demonstrated increased stress-behaviors when working with “youth at-risk” compared to any other population of riders served by EASs. This specific study was conducted at a therapeutic riding program in Michigan in the summer. Horses were observed during EASs with recreational able-bodied riders, “individuals with physical and psychological handicaps”, “youth at-risk”, and advanced riders. All groups were viewed equally using the same horses and coding the horses’ behaviors using an ethogram. The authors found that recreational riders, “physically handicapped”, and “psychologically handicapped” groups demonstrated no differences in the mean number of stress- related behaviors in horses. However, horses demonstrated significantly more stress-related behaviors during EASs with the “youth at risk” group and the advanced riders. The author states, “our casual observation of riders in the program suggested that youth at risk seemed to view the horses as tools, whereas the other groups seemed to develop a more meaningful relationship with their equine partner.” The authors observed that “youth at-risk” demonstrated subtle signs of impatience, aggressive disposition, or displeasure toward the horses they were working with (Kaiser, et. al., 2006, p.43). This study in the end recommends, “that the time each horse is being ridden by a youth at-risk be limited, both daily and weekly” (Kaiser, et.al., 2006, p.43).

The other study that examined equine stress during EASs with youth with social and emotional concerns was conducted by Arrazola & Merkies, (2020). These authors studied the effects of the youths’ attachment style on horses participating in EASs and collected behavioral and physiological data from the horses. The horses were equipped with heart rate monitors 5 minutes before the adolescent arrived and recorded continuously until the session ended. The occurrence of affiliative, avoidance, and oral behaviors from horses were observed and recorded in 2-minute intervals throughout the sessions. This study found that youths' attachment styles affected the frequency of affiliative behaviors in horses during grooming sessions and affected the HR and frequency of avoidance behaviors during riding sessions. The conclusion stated that therapy horses did not exhibit physiological or behavioral distress during EASs with "youth at-risk", but that human attachment style of the youth can influence the horse's response during the horse-human interaction (Arrazola & Merkies, 2020).

These studies have several limitations, limiting the strength of their conclusions. Kaiser et. al. (2006) mentions that there is only one observer that recorded all equine behaviors meaning no inter-rater reliability was used. No physiological biomarkers were used in this study to validate the behaviors. The youth in this study were not well-characterized (i.e., age, diagnosis, history of trauma, and social-emotional competencies). Therefore, there is a need for expansion with rigorous methods upon these preliminary studies to better understand if horses integrated into EASs with youths who have trauma experience increased stress. In order to address these gaps in the literature, my study will focus if horses demonstrate increased stress during EAL for youth with social- emotional concerns compared to EAL for a population without trauma. Next, I will first review literature related to measuring stress in horses.

Measuring Stress in Equine-Assisted Service Horses

Stress can be both physical and psychological and research studies have concentrated on measuring welfare in livestock with the use of physiological indicators of stress (e.g., heart rate, salivary cortisol, eye temperature measured through infrared thermography), and behavioral indicators of stress measured through ethograms. This literature review focusses on studies that have measured physiological and behavioral parameters of stress in horses, focusing on EASs horses when literature was available.

Stress in horses is regulated by the autonomic nervous system (ANS) which is a portion of the central nervous system. The ANS is important for the regulation of heart rate, blood pressure, respiration, gastrointestinal motility and secretion, urination, as well as several other important bodily functions. The ANS encompasses the balance of the sympathetic and parasympathetic activity (Gehrke et. al., 2010). Sympathetic activity, when used for survival mode, could also be known as the fight or flight response to a stressful situation and is associated with increased heart rate, blood pressure, and respiration rate. The parasympathetic (vagal) system is also known as the "rest and digest" system, and is marked by lower heart rate, blood pressure, and respiration. In both human and animal research, it has been found that there is a relationship between the ANS and various diseases and mental states (von Borell et. al., 2007). The physiological measures mentioned below are all controlled by the ANS and indicators of either sympathetic or parasympathetic activity.

Physiological Markers

Physiological measures that are most often used to measure stress in EASs horses are heart rate, heart rate variability, cortisol, and eye temperature measured with infrared thermography. I will review each in their appropriate sections below.

Heart Rate (HR)

Heart rate is often used as a measurement of stress in horses. A horse's normal heart rate ranges from 28-40 beats/minute (bpm) but can vary by age, breed, body weight, and if there are current health problems (De Santis et. al., 2017). To efficiently measure heart rate when a horse is in work, heart rate monitors (HRM) or Electrocardiograms (ECG) are used without interrupting the session. Heart rate monitors are user friendly and cheaper than ECG. HRM can be placed on a horse with the use of an electro strap, a transmitter, and a receiver. An electro strap is an elastic surcingle specifically designed for horses with built in electrodes dampened with water or lube and placed around the horses' heart girth, (Arrazola & Merkies, 2020), with or without tack preferably 1 hour before the session starts (Malinowski, et.al, 2018).

There is currently a growing interest of using heart rate monitors to measure stress in horses participating in EASs. A study done by Merkies et. al. (2018) measured equine stress in EASs horses using heart rate monitors. Horses were equipped with heart rate monitors 30 minutes before the session started and recordings of the heart rate began 10 minutes before the session started and collected in "5 s" intervals until the session ended. Horses were randomly broken into two groups of 4, working with either individuals with PTSD or neurotypical individuals (control group). Authors found that horses exposed to individuals with PTSD demonstrated an increase in heart rate compared to individuals in the control group. When the individuals with PTSD left the pen, the horses continued to have a higher heart rate compared to the control group. Thus, heartrate may be sensitive to stress in horses during EASs (Merkies et. al., 2018).

Another study done by Ayala et. al. (2021) measured equine physiological parameters including heart rate to study the influence of EASs on horses. Three human participants and two horses in EASs were apart of this study. The heart rate was obtained by palpating the facial artery of the horse. The heart rate was recorded in four stages: 1) the anticipatory phase, 2) horse-participant interaction on the ground, 3) horse-participant interaction on horseback, 4) the recovery phase. The heart rate of the horse was the highest in the anticipatory phase and the riding phase. The authors concluded that EASs did not produce increased stress in horses, but rather increased heartrate was “associated with activity and external stimuli” (Ayala et.al., 2021, p.13).

There are some strengths to using heart rate to measure stress in horses participating in EASs. Heart rate monitors are a non-invasive way to measure heart rate of horses. I have some experience using heart rate monitors on endurance horses in which they were user friendly and gave quick feedback. I was able to stay on the horse and monitor his heart rate to prevent over working.

Though many studies have used heart rate monitors for observing equine welfare in EASs, there are quite a few limitations for using the heart rate monitor for this study. Heart rate is impacted by exercise and therefore not a valid measure of stress in horses during physical activity (Parker et. al., 2010). Therefore collecting heart rate was not a good choice for this study as horses in the EAL program are physically active. Additionally, a study conducted by von Borell et. al. (2007) stated that placement of heart rate monitors is extremely important for the function and availability of data; T-waves (repolarization of ventricles) in the horse could be inaccurately pronounced with a dramatic increase in voltage resulting in false readings (von Borell et. al., 2007). Previous studies also had issues with heart rate monitors functioning (Contalbringo et. al., 2021; Mendonca et. al., 2019; von Borell et. al., 2007). Given these limitations, I did not use heart rate as a measure of stress in this thesis study.

Heart Rate Variability (HRV)

Heart rate variability (HRV) is a non-invasive physiological measure of the autonomic nervous activity that detects the natural variation in time between consecutive beats (Malinowski, et.al, 2018; Stucke et. al., 2015) and is used as an indicator of acute and chronic stress in animal welfare (von Borell, et. al., 2007). Malinowski, et. al. (2018) states that “the most common reason for these variations is a deviation from the normal autonomic function, thus making HRV one of the promising markers for increased sympathetic or reduced vagal activity” (2018). HRV is most often acquired by calculating the inter-beat-intervals (IBI) obtained by a Telemetric Electrocardiogram (ECG) (Frippiat, et. al., 2021). ECG monitors use 2-4 electrodes that are placed under the chest and around the girth area and the earth electrode can be placed anywhere on the girth (Stucke et. al., 2015). There are many types of electrodes used to collect HRV: clip, adhesive, and contact electrodes (using gel and fixed by a strap) (2015). Low frequency (LF) and high frequency (HF) are power spectra shown on ECGs where HF reflects parasympathetic nervous functions and LF reflects both sympathetic and parasympathetic nervous systems. This ratio of LF/HF shows an index of cardio sympatho-vagal balance (Malinowski, et. al., 2018; von Borell, et. al., 2007).

Previous research has found mixed results when evaluating the reliability of heart rate monitor’s (HRM) as opposed to ECG to measure IBIs for HRV analysis in exercising horses. For instance, Parker et. al. (2009) compared a Polar Electro HRM to an ECG in measuring HRV in horses. Six horses were used in this study in three conditions (standing, stable, and field) to obtain ECG and HRM recordings. Results included errors when using the HRM data due to lost contact with the electrodes as movement in the horses increased even when low levels of physical exercise is involved. IBI data differentiate significantly between the ECG and HRM when the horses were moving. Therefore, the authors of this study concluded that HRMs cannot be used to reliably collected HRV data unless the horses are stationary (Parker et. al., 2009). Another study by Lenoir et. al. (2017) assessed the reliability of HRM and ECGs when measuring HRM in 36 young endurance horses. The results stated that half of the data collected could not be used due to possible synchronization and messy data from the HRM and ECGs. The author noted ECGs is preferable if a study needs HRV data, but efforts need to be considered to make sure the monitor functions properly. One study in 2021, however, found that HRM compared to ECG demonstrated to be a reliable method to use when monitoring the training, health, and welfare in resting and exercising horses (Fripiat, et. al., 2021). However, the HRM system used in this study, Hylofit, has been discontinued and is no longer for sale. Therefore, the reliability of using HRMs to measure HRV is not fully established.

HRV has been used in studies to measure stress in horses participating in EASs. A study done by Genhrke et. al. (2010) used ECGs on nine active horses in EASs to study the effect EASs had on horses in their average day compared to established HRV in thoroughbred horses. The ECGs were used to monitor these horses for 24-hour recordings and the results demonstrated that HRV in horses involved with EASs did not differ from established HRV in thoroughbred horses, suggesting EASs does not increase stress in EASs horses. A study by Mendonca et. al. (2019) measured HRV of nine horses recruited from two different facilities that provide EASs to see if EASs had an impact on equine welfare. There was a total of 51 human participants in this study that were categorized into two groups: participants with psychiatric conditions (PSY; e.g., autism, attachment disorder, depression, and anxiety) and participants with more physical conditions (PHY; e.g., Down syndrome, developmental delay with locomotor disabilities). There were three phases that were observed in the horses: the resting phase, the preparation phase, and working phase. HRV was collected using Polar V800 Equine equipment and observed in all three phases. A decrease in HRV from the resting phase to the preparation phase and an increase from the resting phase to the working phase were observed and recorded (Mendonca et. al., 2019). The working phase had a higher increase in HRV observed indicating an increase of activity level. It is discussed that horses in EASs are used at a much lower level of activity in the working phase and the increase of HRV was still in normal range. The authors conclude that changes in HRV were higher in horses that were involve in EASs with participants who had both physiological and psychological disorders than the other participants who only had psychological disorders (Mendonca et. al., 2019).

Contalbrigo et. al. (2021) used HRV as a physiological indicator of stress in horses involved in EASs with children with autism spectrum disorder (ASD) compared to sessions involving typically developing children. The results for this study found that there was a higher vagal tone in horses working with children with ASD compared to typically developing children. LF/HF activity (which represents sympathetic activity) was also significantly lower with children with ASD compared to sessions for typically developing youth (Contalbrigo et. al., 2021). Therefore, this study concluded that horses involved with EASs for individuals with ASD were less stressed in comparison to the typically developing control sessions.

Overall, HRV is a very promising method of measuring stress in horse in EASs. However, researchers must consider the best way to gather HRV data. Research above demonstrates that HRMs do not always collect HRV data reliably. While ECGs may be used more reliably, limitations of ECG monitors are accurate placement of electrodes, false recordings, and the expense of the monitor (von Borell et. al., 2007). There are confounding factors that may influence the reliability and repeatability of HRV measurements; for instance, a recent study found that 8 out of the 19 horses they studied had incomplete dataset due to malfunctioning of monitors (Contalbrigo et. al., 2021). ECG monitors are expensive as well as time consuming to use. Having someone who is very knowledgeable about these devices is recommended when using. Due to these limitations, I did not measure HRV in my thesis study. There are many factors that could contribute to malfunction and invasiveness by putting HRMs on the horses while in sessions with the youth with social- emotional concerns such as: incomplete data, faulty monitors, human error in attaching the monitors incorrectly, and the monitors causing a distraction to the youth in the study. Importantly, HRMs have also not been demonstrated to be consistent and reliable in recording accurate measures of HRV in horses in non-stationary work.

Cortisol

Cortisol is a quantifiable physiological biomarker used to measure the extent of acute stress in equines and widely used to measure short-term stress in handling procedures (De Santis et. al., 2017). Cortisol reflects hypothalamic-pituitary-adrenal axis and sympathetic nervous system responses (Hellhammer et. al., 2008; Lewinski et. al., 2013; Peeters et. al., 2013) and is used to assess the responses of horses to potentially stressful situations (Aurich et. al., 2015). Cortisol is a time dependent measure with a circadian rhythm that is highest in the early morning and decreases throughout the day. The normal ranges of cortisol in healthy horses are < 20 nmol/L (0.725 *u*g/dL) (NationWide Specialist Laboratories) and with a half-life varying 1.1-3.4

hours (Held et. al., 2019). This can be affected by the horse's breeding status (brood mare verse stallion etc.), management style, sex, breed and exercise (De Santis et. al., 2017). Cortisol also takes 10 to 20 min to reach peak values after a stressor (De Santis et. al., 2017, p. 4). Measuring cortisol in horses have been done by collecting blood or by collecting saliva. Serum cortisol concentration in blood is used to measure stress and has been used for a long time to measure the free cortisol concentration level. Serum cortisol collection is invasive which is why studies have been done validating the use of salivary cortisol concentrations to measure acute stress (Malinowski et. al., 2018; Peeters et. al., 2011). Saliva has been demonstrated to be an effective noninvasive way to measure cortisol in horses by using cotton rolls or swabs gently placed on the tongue loosely until the swab is soaked (Lewinski et. al., 2013). Cortisol rapidly diffuses into the saliva, and changes in salivary cortisol concentrations have been found to directly reflect cortisol changes in blood plasma (Lewinski et. al., 2013). Lastly, a study by Aurich et. al. (2015) states that salivary cortisol follows a diurnal rhythm with the highest concentration being in the morning and decreases throughout the day.

A study by Peeters et. al., 2011 compared the differences between blood serum and salivary cortisol concentrations in adult horses 9 to 17 years of age. The objective of the study was to validate the use of salivary cortisol assays in horses as a more welfare-friendly method for collecting cortisol. Five horses were used for this study and all horses were handled by the same experimenter. Blood cortisol samples were collected by horses using venipuncture followed by catheter placement. Saliva was then collected using a Salivette swab on a metal clamp that was held in the horse's mouth for 30-40 seconds. On day one, five saliva samples and five blood samples were collected from each of the five horses. Samples were collected at time 0 minutes then 5 minutes, 10 minutes, 30 minutes, and 60 minutes later. During day two, 21 blood serum and 21 salivary cortisol samples were collected. The authors stated in their results that the saliva cortisol concentrations on average reached peak values at 124 +/- 8.9 min and in serum at 96 +/- 16.7 min. Cortisol concentration came back to the baseline values faster in saliva (180 min) than it did in serum (280 min). The baseline cortisol ranges were 0.037ug/dL +/- 0.0225ug/dL in the morning and 0.07ug/dL +/- 0.048ug/dL, 20 min after the competition. The results showed a time lag between serum cortisol and salivary cortisol variation with salivary cortisol increasing 20-30 min later than in serum. About 80% of the salivary cortisol concentration variability could be explained by the serum cortisol concentration and reciprocally showing that there is a strong association between salivary cortisol and serum cortisol concentrations. Below I will review studies that have collected either blood or saliva samples to measure cortisol as an indicator of stress in horses.

One study that measured salivary cortisol as an indicator of stress in horses participating in EASs specifically looked at horses exposed to individuals with post-traumatic stress disorder (PTSD) and neurotypical individuals on the ground. Merkies et. al. (2018) investigated if there was greater stress in horses exposed to individuals with PTSD compared to neurotypical individuals. Seventeen horses were used in this study. Salivary swabs of horses were collected 0 min prior to the trial starting and 30 min after the session ended. The results stated that there were no differences in salivary cortisol concentrations of the horses when exposed to individuals who are diagnosed with PTSD and the neurotypical individuals. The conclusion of the study states that the horses did not demonstrate increased stress during unmounted activities with individuals with PTSD compared to neurotypical participants (Merkies et. al., 2018).

Malinowski et. al. (2018) also examined equine stress indicators when participating in EASs. Nine horses participating in EASs and seven veterans who had been diagnosed with

PTSD were part of this study. Blood samples were collected at rest, 30 min prior to sessions, right before the session started, 10 min after session started, and 30 min after the session ended. The conclusion stated that cortisol levels did not change in horses involved in EASs with veterans with PTSD (Malinowski et. al., 2018).

McKinney et. al. (2015) compared salivary cortisol measures on horses who participated in traditional hunt seat lessons and therapeutic riding exercises in order to compare stress. Six horses participated in the study and were experienced in both disciplines that were offered at their current barn. There were two groups of riders that participated in this study; a hunt seat riding group that consisted of six young girls between 8-14 years old, and the second group was a therapeutic riding group which encompasses a variety of diagnoses which had 5 boys and 1 girl between 8-14 years old. The authors used a within-subjects design where the same horses participated in both groups. Saliva swabs were collected at rest, right after riders had mounted, 30 min into riding, and an hour into the session which marked the end of session. The results stated that salivary cortisol concentrations were not higher in horses during the therapeutic riding sessions compared to the traditional hunt seat sessions. Conclusion of this study stated that the level of stress experienced by horses in therapeutic riding sessions were not higher than stress experienced by horses during hunt seat sessions (McKinney et. al., 2015).

After reviewing the pertinent literature for blood and salivary cortisol concentrations there are many strengths of measuring salivary cortisol concentrations for the current study. The process of collecting saliva is a much less intrusive than collecting blood samples. Salivary cortisol correlates with the free cortisol concentrations in the blood (Hellhammer et. al., 2009) and mirror changes in cortisol concentration in blood plasma (Lewinski et. al., 2013) without the separation manipulation needed like when collection blood serum (Peeters et. al., 2011). There are several considerations when using cortisol as an indicator of equine stress. It is important to consider the time of day and reproductive state when analyzing cortisol levels of horses (Aurich et. al., 2015; Malinowski et. al., 2018). The diurnal rhythm in cortisol release should also be accounted for. Ambient temperature, exercise, time of day, weather, insects, and types of interactions with the horse are also other factors that could alter cortisol concentrations (Aurich et. al., 2014; De Santis et. al., 2017; McKinney et. al., 2015). In this study, all of these considerations were controlled for through the use of an active control group consisting of the same horses at the exact same time on a different day of the week, but with a different population of riders.

Collecting blood to measure plasma or serum cortisol has limitations that are valid enough for me to not use this method in the study. The limitations in collecting blood as a way to measure cortisol concentrations is the invasiveness required to take the blood sample as well as potential risks for complications using venipuncture or a catheter. Blood samples need to be collected in a quick fashion to prevent the elevation in cortisol due to fear of the needle (De Santis, et. al., 2017). It is important that the one collecting the blood is knowledgeable about the jugular veins of horses as well as comfortable around blood and needles. Malinowski et. al. (2018) collected blood plasma samples to measure cortisol levels in 9 horses participating in EASs with individuals with PTSD. Unfortunately, two horses were eliminated from the blood sample process due to adverse reactions to the collection process (Malinowski et. al. 2018). Given the positive strengths of salivary cortisol and the invasive limitations of collecting blood cortisol concentrations to measure stress in horses, I decided to use salivary cortisol for this study.

Infrared Thermography (IRT)

Another physiological measure used to measure acute stress in equines is called Infrared Thermography (IRT). "IRT is the recording of the infrared radiation emitted by a body surface using a thermography camera" (Sanchez et. al., 2016, p.59). Recent studies were conducted to noninvasively measure the surface body temperature using IRT which is also useful in detecting changes in horse's skin temperature due to stress- induced cardiovascular changes. This allows the user of IRT to operate at a distance without creating extra stress for the horse (De Santis et. al., 2017; Sanchez et. al., 2016; Sutherland et. al., 2020). Specifically in equine riding activities, using IRT for assessing equine eye thermal radiation is shown to be a more consistent measurement of stress than other parts of the body (Bartolomé et. al., 2013; Stewart et. al., 2007). Baseline values of eye temperature range from 29.4- 37.7 degrees C with a variation of 0.97% in which physiological stress developed. Baseline values also depend on the sex and breed of the horse and increase during exercise (Jansson et. al., 2021). IRT can detect changes in peripheral blood flow; greater the flow, higher the stress response (Stewart, et. al., 2007). Below I review studies that have used IRT to measure eye thermal radiation as an indicator of acute stress in horses, including the only study I could locate in horses participating in EASs and two studies in competition horses.

A recent study by Contalbrigo et. al. (2021) observed horses who participated in EASs for signs of distress or discomfort and used IRT as a tool to measure possible stress. Nineteen horses from four different riding facilities and 38 children between the ages of 6-12 years old were observed for this study. Nineteen of the children were on the autism spectrum and the control group consisted of nineteen children who were typically developing. Sessions included 1 hour of EASs with children with autism and 2 hours of riding school for typically developing children in one day. Eye thermal radiation was collected from each horse immediately before starting session with the rider, during grooming, mounting, riding exercises, stationary exercises, and when the horse was given a treat by the rider at the end of the session. All temperatures were recorded with a thermographic camera from a distance of one meter. The authors concluded that there were no significant differences in eye thermal radiation observed in sessions with children who were on the autism spectrum and typically developing children. However, horses demonstrated increased eye thermal radiation during the grooming phase in both sets of individuals. The conclusion stated that the stress responses in the horses involved were not negatively affected during these short EASs sessions (Contalbrigo et. al., 2021).

A study done by Sánchez et. al. (2016) evaluated the genetic parameters of the eye thermal radiation of horses in the Pura Raza Española (PRE) Breeding Program. Three hundred and forty-two PRE stallions were analyzed by recording their eye thermal radiation using a FLIR i7 camera in this study from the year 2012 to the year 2014. The horse's data was recorded in the three final dressage competitions all at the same barn in similar weather conditions. Thermal radiation of the eye was recorded three times during each competition in three stages of the competition: 3 hours before competition, just after the competition (< 5 minutes), and 3 hours after the competition when the horse was resting. Several other parameters were also measured, including the horse's dressage score records for 6 events and environmental effects (age, rider, training, etc.) Results indicated that eye thermal radiation was significantly correlated with environmental effects, indicating that older horses experienced less stress, and the rider, training, and length of trip all affected equine stress. Furthermore, authors found that the horses' dressage expected breeding value was significantly correlated with their eye thermal radiation just after exercise. Authors concluded "dressage performance was influenced by the level of physiological stress developed by the animals participating in competitions" (Sánchez, et. al., 2016, p. 64). This study supports that eye thermal radiation is a promising way to measure the acute stress in equines.

Bartolomé et. al. (2013) conducted a study that used IRT in 173 young horses to measure stress during show jumping competitions and see the relationship between possible stress and performance results. This study took place in 2010 and 2011 only in October at the same show barn. Thermal eye radiation samples were collected at 3 hours before competition, 5 minutes after the jumping warmup, and 3 hours after the competition when the horses were resting. A ThermaCam i70 0 camera was used one meter from the horses left eye at a 90-degree angle to capture the thermal radiation. Authors found that the lower the eye thermal radiation of the horse before the competition and during the warmup phase resulted in better competition results. Therefore, authors concluded that a relatively small rise in eye thermal radiation could be attributed to stress, which would increase the chances of poor performance in the competition (Bartolomé et. al., 2013).

There are several strengths and limitations of IRT as a method of measuring equine stress. In relation to strengths, substantial data supports IRT as a valid way to measure an acute stress response in horses (De Santis et. al, 2017). Yet, these cameras need an expert who is knowledgeable in their use. The major limitation in using IRT for measuring thermal radiation in the eye is that results are easily influenced by any dirt, water, fur, ambient temperature, or sun exposure (De Santis et. al., 2017; Sanchez et. al., 2016). A well-thought-out plan including consideration of the current ambient temperature when using this device is advised (von Borell, et. al. 2007) . It is recommended that all measurements captured with IRT be captured in the same indoor arena due to the influence of dust, dirt, humidity, and sun expose and to avoid the use of a mixture of indoor and outdoor arenas (De Santis et. al., 2017; Kim & Cho, 2021). For this study, I decided to measure eye temperature, which all measurements were recorded in an indoor arena, using an IRT camera as way to measure potential stress in horses participating in EASs. This physiological method is noninvasive to the horse and will not interrupt the session with the rider. Equine eye temperature is affected by exercise, but it is not known if it is affected by low- level physical activity like that which occurs during EAL. Therefore, in the current study we included a control group to account for possible effects of physical activity.

Behavioral Measures

Though physiological measures are one method of measuring equine stress, horses also show behavioral signs that parallel this internal stress which helps in deciphering if physiological markers are indicative of negative mental stress. Coding behaviors is important for measuring stress in equines and is complimentary with measuring physiological measures. Research has been published to evaluate stress in horses by using applied ethograms to evaluate equine welfare. An ethogram is a catalogue of behaviors observed within a certain species with precise definitions, and for the purpose of this study, we will be using an applied ethogram to measure stress in horses in EASs. Studies of EASs often use "author-created" applied ethograms to measure stress in horses in EASs since there has not been a universal applied ethogram validated to measure stress in this population of horses. Behaviors commonly included in applied ethograms to measure equine stress include but are not limited to: ears pinned, tail swish, lowering of head, raising head, biting, pawing, licking, chewing, yawning (jaw stretch), head toss, and in more extreme cases kicking and rearing (Ayala et. al., 2021; Contalbrigo et. al. 2021; Dyson, 2021; Torcivia & McDonnell 2021).

Ayala et. al. (2021) researched the influence of EASs on equine behavior. Two horses in EASs and three participants were part of this study: one individual had general congenital hypotonia, one patient had Duchenne muscular dystrophy, and the last participant had cerebral palsy. Each participant attended one 60-minute session per week for a total of 10 weeks. In the first 30 minutes of the session the patient interacted with the horse on the ground and the last 30 minutes the patients rode at ta walk. The control conditions occurred 2-3 days away from the testing sessions by observing the horses in their resting state. Four phases were studied in the experimental condition: the first phase (anticipatory phase), second phase (horse- participant interaction on the ground phase), third phase (horse- participant interaction on horseback), and fourth phase as the recovery phase. One researcher observed the behaviors of horses every 4-7 minutes during the sessions and recorded them in an ethogram. It is not stated if the same individual researcher also recorded the behaviors in the control group as well which could result in discrepencies. The authors results indicated that the horses exhibited the highest frequency of behaviors during the anticipatory phase. Behaviors observed in sessions included: change of postural expression, kicking the ground, head lowered, ears pinned back, ears in listening attitude, gentle head shake. The authors' conclusions state that the therapy horses did not show stress directly attributable to the EASs activities but more related to external factors in the environment (Ayala et. al., 2021).

Contalbrigo et. al. (2021) studied the behavior of horses in EASs working with children on the autism spectrum. Nineteen horses were recruited from four different riding centers and 38 children in which half were on the autism spectrum and the other half were neurotypically developing. The phases that composed each session were grooming, horse at hand mounting, riding exercises, stationary exercises, and closing dismount reward. Each horse was involved in two sessions: an EASs session with a child on the autism spectrum and a control session with a typically developing child. A behavior ethogram was designed using certain behaviors adapted from previous research, illustrated in the table below.

Table 1

Horse's Stress-related Behaviors Scored During the Sessions

Behavior	Description
Head Nodding	The horse repetitively moves its head vertically (>3 movements up and down)
Head Shaking	The horse tosses its head in sudden bouts
Head Tossing	Head lowered with the ears pinned back interrupted with momentary sharp tossing or rotating gestures of the head
Head Raised/high	Head held higher than the normal carriage with nose extended upward and with a slight extension of the neck
Head Down	The horse held its nose below its belly-line; neck may be stretched out with nose pushed forward
Ears Pinned Back	Ears pressed caudally against the head and neck
Snorting	Forceful expulsion of air through the nostril incidentally preceded by a raspy inhalation sound
Lip Play	The horse moves its upper lip up and down without making contact with an object, or the horse smacks its lips together
Tongue Play	The horse sticks out its tongue and twists it in the air
Chomping the Bit	Any mouth or tongue manipulation of the bit independent of the rider's use of the reins
Lip/ Teeth Rubbing	The horse rubs its upper lip or its upper teeth repetitively against the arena wall
Head Bumping	The horse bumps or attempts to bump its head against the side walker or the instructor
Biting Leads	The horse bites or attempts to bite the side walker/instructor the lead rope
Avoidance/ Halt	The horse stops walking; cessation of movement of all feet, or backward movement
Pawing	The horse hits the ground with the hoof
Tail Swishing	Any exaggerated movement of the tail, usually more of a wringing motion than a rhythmic or directed swishing (no insect present)

Note. Adapted from "Equine-Assisted Interventions (EAIs) for Children with Autism Spectrum Disorders (ASD): Behavioural and Physiological Indices of Stress in Domestic Horses (Equus caballus) during Riding Sessions," by Contalbrigo, Borgi, M., De Santis, M., Collacchi, B., Tuozzi, A., Toson, M., Redaelli, V., Odore, R., Vercelli, C., Stefani, A., Luzi, F., Valle, E., & Cirulli, F. (2021 *Animals (Basel)*, *11*(6), 1562–. https://doi.org/10.3390/ani11061562

Results indicated that there were no differences in behaviors between groups, but there were differences observed in each phase. Mounting and dismounting phases showed an increase in behaviors observed compared to the other phases of the sessions. The authors concluded that horses in EASs with children on the autism spectrum compared to the controlled sessions with the neurotypical children did not demonstrate an increase in stressful behaviors. The equine ethogram complimented the physiological measures used in this study, providing further evidence that EASs did not cause increased stress in horses (Contalbrigo et. al. 2021).

Mendonca et. al. (2019) studied the behavior of horses in EASs to determine if EASs and the human participants cause stress to horses and used an ethogram to code the behaviors of the horses. Nine horses participating in EASs from two different barns and 51 human patients with different diagnoses were recruited for this study. The sessions included a resting phase, a preparation phase, and a working phase. The resting phase consisted of recording certain behaviors the horses performed in their normal living conditions. The preparation phase consisted of controlled interactions between the patient and the horses at the tie rail. This included: petting, brushing, saddling, picking the feet, and feeding the horses. The working phase consisted of the patients either riding or hand walking the horse. Each phase lasted 20-30 minutes and behaviors were recorded by a single observer. Behaviors commonly seen and included in the ethogram were: ears pinned back, head lateral movement (HLM), snort, and defecation. Results found no significant differences in equine behavior between phases. However, there was a significant interaction effect such that horses demonstrated greater head lateral movement during the preparation phase in participants with psychological diagnoses (i.e. autism, anxiety, ADD, attachment disorders, depression) as opposed to diagnoses associated with more physical impairments (i.e. developmental delay with locomotor disability, Down's

Syndrome). The authors' concluded that the horses did not exhibit abnormal range of stress and further research needs to be done to focus on improving equine welfare in EASs (Mendonca et. al., 2019).

Merkies et. al. (2018) did an observational study with seventeen horses in EASs, four individuals with PTSD and 4 neurotypical individuals were part of this study. Each horse was used eight times for the purpose of this study. All sessions were in a round pen with 2 video cameras on either side of the round pen. Any behavior the horses demonstrated (e.g.: vocalization, chewing, head height, ear and body position relative to human if applicable, and type of gait) was recorded by two independent observers in five second sampling intervals. The observers were blinded to which group the individual was in that entered the round pen with the horse. The baseline period consisted of just the horse in the round pen for five minutes. Then a human entered the round pen and the two-minute test period started. The human exited the round pen after 2 minutes and the post test period of the horse was observed for five minutes. The study results stated that there were some minor behavioral differences in the horses exposed to individuals with PTSD and neurotypical individuals, however horses alone in the round pen displayed higher stress behaviors than when any individual was in the round pen with the horse. When any individual with experience around horses entered the round pen, the horses approach the individual quicker than a non-experienced individual. The authors' concluded that the horses were less stressed when any individual was in the round pen but were more attentive towards the individuals with horse experience. The behavior ethogram is a promising way to measure stress in horses in EASs and compliments the physiological measurements (Merkies, et. al., 2018).

Kaiser et. al. (2006) observed the behavior of 14 horses in EASs at a therapeutic riding center for a year and developed an ethogram to code the behaviors. Each session was 1 hour long, and each horse was not used more than 3 hours in a day. There was a total of 126 riders that were classified into 5 groups: recreational riders with no handicaps, physically handicapped riders, psychologically handicapped, special education children, and youths who are at risk. The youth who were at risk were defined by poor performance/ failure in school due to a low socioeconomic status, living in a single parent home, or disciplinary action. With help from previous literature, the ethogram was developed by one individual researcher who coded all equine behaviors observed in 15 sessions. In each session, each horse was coded for 2 minutes, and after all the horses were coded for 2 minutes, the observer repeated this process going in the same order. Ears pinned, raising the head, turning head to the left or the right independent of actions of rider, tossing the head, shaking the head, holding head down, and defecating were seven specific stress and frustration related behaviors observed in this study. The results stated that the mean number of stress related behaviors were significantly higher in the group with youths who are at risk than any other group with individuals with disabilities. The conclusion states that EASs with individuals with physical or psychological disabilities are no more stressful for horses than being ridden in the same setting by recreational riders; but that EASs for "youth at-risk" may cause increased stress in horses (Kaiser et. al., 2006).

Outside of the literature on EASs, several authors have attempted to create ethograms of equine behaviors that are indicative of equine stress, pain or discomfort. Those studies are reviewed below. These applied ethograms may be pertinent for horses in EASs.

The Ridden Horse Pain Ethogram (RHpE)

The Ridden Horse Pain Ethogram (RHpE) is a collection of 24 distinct and previously defined behaviors seen more in horses that have underlying musculoskeletal issues which could result in observable in lameness. Seeing eight or more of these behaviors could indicate musculoskeletal pain in the horse. With a high occurrence of lameness in ridden horses, researchers have developed the RHpE which is informed by a series of studies (Dalla Costa et. al., 2014; Dyson et. al., 2017, 2018; Mullard et. al., 2017). Some factors and limitations of the RHpE include but are not limited to the rider skill level (beginner, intermediate, experienced, or professional), the horses tack fit (i.e. saddle or bridle fit), and the discipline that the horses is being asked to do (i.e. jumping, dressage, reining). The author concluded that the RHpE is a powerful tool to assess ridden horses for the identification of musculoskeletal pain and increase equine welfare (Dyson, 2021).

Facial Expressions in Ridden Horses (FEReq)

Mullard et. al. (2017) developed the ethogram called Facial Expressions in Ridden Horses (FEReq) to measure pain and stress in the facial expressions of riding horses. The strategy for the study was based off comparing 150 close-up photographs of either lame or non-lame ridden horses' facial expressions. Thirteen trained researchers each evaluated 27 photographs and used a training manual if needed to grade the photos. Each researcher was asked to complete the ethogram of the photos they were given and check yes, no, or cannot see if the presence of a feature was shown. Below is an overview of the facial expressions featured in the ethogram in Mullard et. al. (2017).

Table 2
Overview of Facial Expressions Graded in the Ethogram

Round versus almond-shaped eyes with tension of musculus levator anguli oculi medialis
Eyes open or semi closed
Eyes normal expression or intense stare or cannot tell
Sclera exposed, yes/no
Ears forward; erect and parallel with pinnae facing forward; erect and to side, with pinnae facing outward (divergent); one forward and one erect and to side (divergent); one erect and to side (divergent) and one back; one ear forward and one back; or both ears back

Mouth closed; tongue out but otherwise lips closed; lips separated but cannot see teeth; lips open showing teeth and no gum; lips open showing teeth and gum and teeth apposed; mouth open, that is, teeth slightly separated, but cannot see tongue; mouth open, that is, teeth widely separated, but cannot see tongue; mouth open and teeth slightly separated, exposing tongue; mouth open, teeth exposed and separated, and tongue outside oral cavity
Jaw crossed, that is, upper and lower teeth not aligned, yes/no/cannot tell
Salivation, yes/no
Nostrils, tear drop or oval shape or rounded and angular; wrinkle between nostrils, yes/no; nostrils drawn to one side, yes/no/cannot tell
Upper muzzle in line with lower muzzle; upper muzzle extended and angled.
Lower muzzle, relaxed with curved contour; muzzle tense and angled
Tongue in; tip of tongue protruding; large part of tongue out but cannot see teeth; large part of tongue out and teeth exposed
Head erect, straight; head tipped to one side, nose to left or right; head turned
Front of head vertical; front of head behind vertical up to 10°; front of head behind vertical >10-30°; front of head behind vertical >30°; front of head in front of/above vertical up to 10°; front of head in front of vertical >10-30°; front of head >30° in front of/above vertical.

Note: Adapted from Development of an ethogram to describe facial expressions in ridden horses (FEReq) by Mullard, Berger, J. M., Ellis, A. D., & Dyson, S. (2017). *Journal of Veterinary Behavior*, *18*, 7–12. https://doi.org/10.1016/j.jveb.2016.11.005

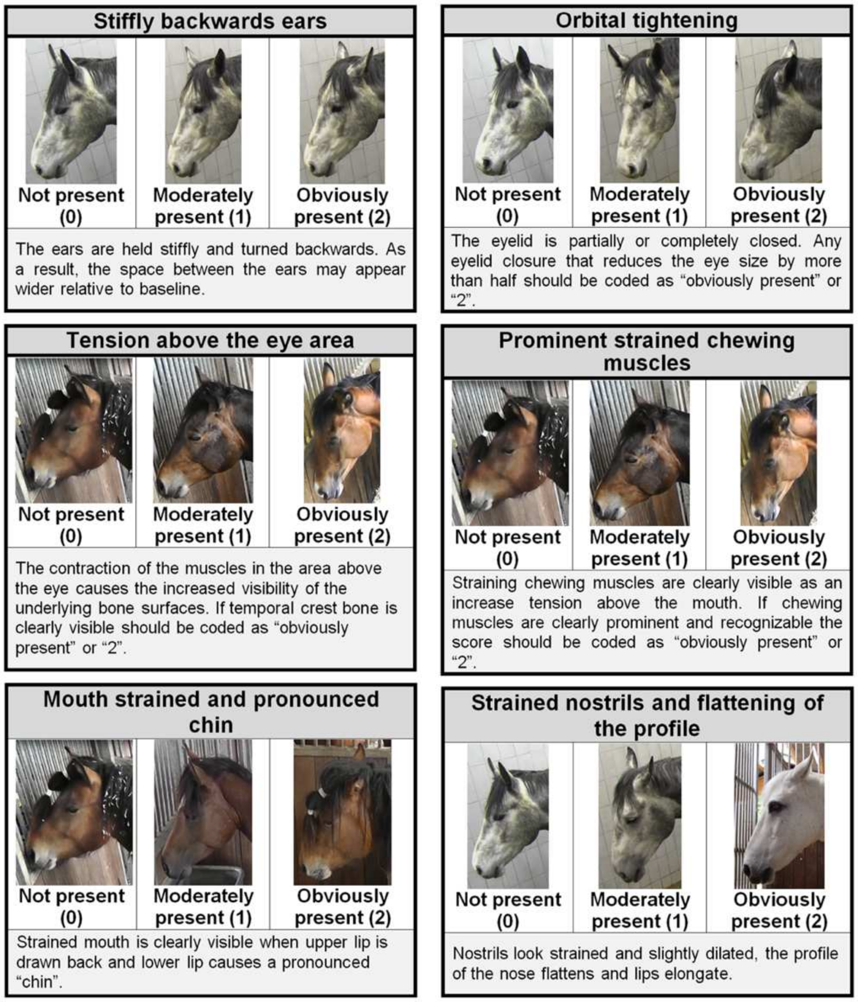
All research observers were blinded to the horse group, and they were unaware of the number of horses in each group. The authors' results stated that the overall agreement between the researchers grading the same photos was 80%. The authors' stated that the results could differ between ridden horses and non-ridden horses as well as horses could be influenced with bits in their mouth. The study conclusion also states that the ethogram for facial expressions in ridden horses has been reliably developed and should be utilized by people in different professional equine related backgrounds to code behavior of horses as a measurement of pain or stress (Mullard et. al., 2017).

Equine Discomfort Ethogram

A recent study by Torcivia & McDonnell (2021) illustrated a catalog of equine behaviors associated with discomfort and stress in an ethogram labeled Equine Discomfort Ethogram. The objective of the study was to improve the unambiguous recognition of physical discomfort with the use of ethograms. Hundreds of horses over the course of 35 years were evaluated with a minimum of 24-hour continuous video recording. Healthy horses, breeding program horses, hospitalized horses with various medical issues were observed resting in a stall. The results included a total of 64 specific equine behaviors in the ethogram grouped into 8 categories: "posture and weight bearing; limb and body movements; head, neck, mouth, and lip movements, attention to area; ear and tail movements; overall demeanor; altered eating or drinking; and vocalizations/ audible sounds" (p.3). The authors propose their findings, which also validates previous literature on other equine related ethograms, be used in helping recognize stress, pain, and discomfort in horses in all stages of work (Torcivia & McDonnell, 2021).

Horse Grimace Scale (HGS)

The Horse Grimace Scale (HGS) was designed by Dalla Costa et. al. (2014) which includes photographs and detailed descriptions to illustrate various components of equine discomfort. The HGS was developed to validate and standardize the pain scale based on facial expressions in horses (Dalla Costa, et. al., 2014). The figure below was developed by Dalla Costa et. al. (2014) which shows The Horse Grimace Pain Scale with images and explanations for each of the 6 facial action units.



Note: **Figure 1.** Figure above is called the Horse Grimace Scale which is adapted from "Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration," by Dalla Costa, Minero, M., Lebelt, D., Stucke, D., Canali, E., & Leach, M. C. (2014). *PloS One*, *9*(3), e92281–e92281. https://doi.org/10.1371/journal.pone.0092281

There are many strengths to using behavioral measures to measure stress in horses. Ethograms are noninvasive and require little expenses to obtain data. Ethograms can be used to reliably code equine behaviors associated with stress, particularly when interrater reliability is used. Coding behaviors by using applied ethograms has the potential to compliment the physiological indicators of stress. For this study, the researchers used an applied ethogram to code the behavior of the horses. To ensure more accurate results the authors used interrater reliability to code behaviors.

There are a few limitations of applied ethograms. Applied ethograms require interrater reliability to ensure the reliability of the ethogram results. Potential bias could occur if interrater reliability is not used when coding with an ethogram. Certain behaviors in the ethogram have had more research than others supporting that they are indicative of equine stress—generally, "author-created ethograms" have less data supporting their validity. In this thesis study, the researchers included equine behaviors that have already been defined in previous research and interrater reliability was used when coding with the applied ethogram.

After reviewing all the pertinent literature, I decided on how to best measure equine stress during a study of EAL for youth with social- emotional concerns by looking at both physiological and behavioral measures of equines in EAL, including salivary cortisol, eye temperature using IRT, and an applied ethogram.

CHAPTER 2: BEHAVIORAL AND PHYSIOLOGICAL MARKERS OF STRESS IN HORSES DURING EQUINE-ASSISTED LEARNING FOR YOUTH WITH SOCIAL-EMOTIONAL CONCERNS

The current research sought to observe signs of stress in horses participating in equine-assisted learning for youth with social- emotional concerns. Equine assisted services (EASs) refer to multiple services in which professionals incorporate horses and other equines to benefit people (Wood, 2021). There are several different types of EASs, the focus of this paper is one service called equine-assisted learning (EAL). EAL is an educational service that engages people of all ages in learning processes that focus on academic skills, character development, or the promotion of relevant life skills such as problem solving or critical thinking skills. Horses are integrated into EAL to help participants achieve the designated educational outcomes (Wood, 2021). The focus of this study is EAL for youth with social- emotional concerns.

Youth with social- emotional concerns have difficulties with social-emotional competencies, including: self-awareness, self-management, responsible decision making, social awareness, and relationship skills (Casel, n.d). In the past, these youth have been referred to as "youth at-risk" which describes youths who are at risk for experiencing negative life outcomes as they transition to adulthood such as higher probability of negative developmental outcomes, poor academic success, and reduced mental health when they engage in or are exposed to adverse risk (Wilkie et. al., 2016). Among the many factors that increase youth's risk of experiencing negative life outcomes are emotional and behavioral difficulties, family conflicts, maltreatment, low social competence, and/or low socioeconomic status (Kaiser et. al., 2006; Wilkie, 2016). EAL can focus on teaching social-emotional skills to youth who have trauma, which may serve as a protective factor to decrease the risk of youth experiencing negative life outcomes. The current paper uses the term "youth with social- emotional concerns" to be in line with most up-to-date terminology recommendations. This study focuses on examining stress in horses that are integrated into EAL focused on teaching social-emotional skills to youth with social- emotional concerns in the community.

A growing body of research has examined equine welfare in horses during EASs. Most studies have demonstrated that there are not higher levels of stress in horses participating in EASs when compared to recreational riding (Arrazola & Merkies, 2020; Kaiser et. al., 2006; Nobbe, 2016). However, a few studies have demonstrated that EASs with humans with history of trauma or characterized as "at-risk" could cause behavioral and physiological changes in horses (Kaiser et. al., 2006; Merkies et. al., 2018). After reviewing the pertinent literature, I was only able to locate two research studies that observe equine stress during EASs with youth with social- emotional concerns, referred to by the authors as "youth at-risk" (Arrazola & Merkies, 2020; Kaiser et. al., 2006).

In the research study by Arrazola & Merkies, (2020), they studied the effects of human attachment style of "at- risk youth" on horses participating with EASs. The conclusion stated that therapy horses did not exhibit physiological or behavioral distress during EASs programs, and the human attachment style if the "at-risk humans" can influence the horse response during the horse-human interaction (Arrazola & Merkies, 2020). Kaiser et. al., (2006) found that the horses did exhibit higher levels of stress in EASs compared to other populations offered in EASs, and concluded horses should be limited in how often they work with the population of "youth at-risk" (Kasier et. al., 2006).

The studies above are preliminary and more rigorous research is needed to help determine if horses experience increased when working with youth with social- emotional concerns. Furthermore, if there is acute stress, does it change over time as the horse interacts with the youths? Lastly, does equine stress differ by the nature of the interaction with youth with social- emotional concerns (i.e., during grooming, tacking, riding, etc.) Therefore, there is a current need for more research investigating equine welfare during EASs for youth with social-emotional concerns.

Purpose and Research Questions

To address this need for additional research, the purpose of this study is to examine physiological and behavioral indicators of stress in horses involved in EAL for youth with social-emotional concerns. To achieve this purpose, we asked the following research questions:

1. Do horses integrated into EAL for youth with social- emotional concerns demonstrate increased stress in comparison to horses integrated into EAL for a population without trauma?
2. Does equine stress differ during 3 different phases of EAL for youth with social-emotional concerns in comparison to the control condition:
    a. Baseline before interaction with participants
    b. Tie rail with participants
    c. Mounted/ unmounted work in the arena with participants
3. Does equine stress during EAL for youth with social- emotional concerns differ over the course of 5-weeks?

Methods

Design

This study implemented a prospective within subjects design that compared indicators of equine stress during EAL for two different populations of human participants: EAL for youths with trauma (experimental condition), and EAL for young adults with developmental disabilities (control condition). Each horse served as its own control. The Institutional Animal Care and Use Committee and Institutional Review Board approved all study procedures.

Setting

The study occurred at Hearts and Horses, a nonprofit PATH Intl. Premier Accredited Center located in Northern Colorado. Hearts and Horses provides EASs to an array of individuals who have physical, emotional, and psychological disabilities.

Horses

Eleven horses that were integrated into EAL at Hearts and Horses were included in this study.

Experimental Condition: "Changing Leads" EAL Program for Youth with Social-Emotional Concerns (EAL-CL)

For the experimental condition, the horses were integrated into a program called "Changing Leads," an EAL program delivered to youth with social- emotional concerns from a local school district (EAL-CL). This three-and-a-half-hour program took place once a week for eight weeks in the Fall of 2022. Hearts and Horses provides EAL to help facilitate positive social and emotional learning, and self-discovery for youths with trauma. By providing the youths with tools to be functional members in their society, EAL-CL hopes to encourage human growth and development. The program staff describe this population of human participants as having a high incidence of previous trauma and/or current social-emotional difficulties. The general structure of these sessions included classroom time followed by unmounted and mounted activities with the horses. Youth participants were at least 12 years of age, in the 7th or 8th grade, and referred by their school counselors for participation in the program if they demonstrated a need for social-emotional instruction.

Control Condition: EAL for Participants with Developmental Disabilities (EAL-DD)

This control condition was a convenience control condition of an EAL program provided at Hearts and Horses to young adults who have developmental disabilities (EAL-DD). This group is described by Hearts and Horses as a population that is not generally stressful for the horses to work with, which is why they were selected as the control condition. Similarly, to the experimental condition, this group interacted for three and a half hours once a week for 8 weeks on a different day than the experimental condition. This population has low incidence of extensive physical impairments, low incidence of behaviors that would be considered stressful for the horses (i.e., screaming, body rocking), and low incidence of trauma, psychological diagnoses, or trauma compared to the EAL-CL group. Hence, this group represented a group of participants who traditionally participate in EAL, but do not have trauma.

Data Collection

Pilot Day

Researchers went to Hearts and Horses one month before the study started to introduce saliva swabs to the eleven horses. This was done in hopes to reduce any potential stress due to the novelty of the swabs. The week before data collection, the researchers conducted a pilot day during week 2 of EAL-DD (non-data collection day). No riding was done and only the 7 horses in the EAL-DD control condition were piloted on. Researchers piloted their data collection methods, including taking pictures with the IRT camera and recording behaviors; saliva was not collected on this pilot day.

During the 8-week EAL-CL and EAL-DD programs, data on the horses were collected on weeks three, five, and seven. This allowed time for the horses to accommodate to the program routines in weeks one and two so as not to capture possible stress due to novelty of participating in the program. In addition, weeks three, five and seven were all similar in structure, including both unmounted and mounted activities. Data were collected from the horses in an indoor arena during each of the three phases of the EAL programs: Phase 1, baseline (1-B; the tie rail before interacting with EAL participants), Phase 2, tie rail with participants (2-TR; in the tie rail with the EAL participants), and Phase 3, arena work (3-AW; participants with horses in unmounted or mounted work at the stop or walk)

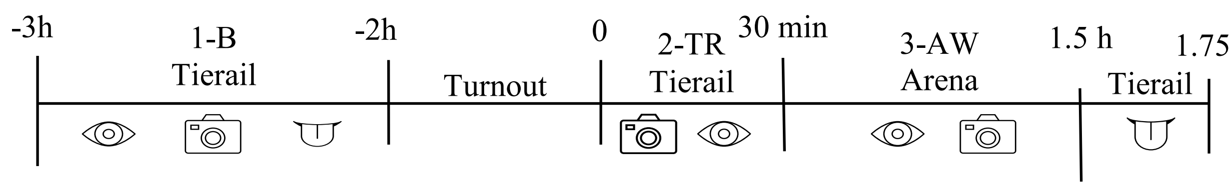


Figure 2
Day structure timeline of sessions

Behavior Ethogram

Photos with Infrared Thermography Camera

Saliva samples

Infrared Thermography (IRT)

A trained research assistant took pictures of the right eye of each horse using a FLIR E6-XT Handheld IRT camera during each of the three phases. This camera has range features 160 x 120 thermal resolution and a -20-to-550-degree Celsius temperature range. To ensure proper use of the IRT camera, the research assistant was trained by a research team member with Infrared thermography certification (ITC). All photos were captured at a 90-degree angle about a meter away or until the camera was in focus. In Phase 1, a photo was captured from each horse alone in the tie rail, before any interaction with the program participants. In Phase 2, the camera operator captured a photo while the horses were being groomed and tacked up in the tie rail by a participant. Lastly in phase 3, the camera operator captured a photo of each horse in the arena while the youths were mounted. An undergraduate research assistant recorded the current temperature and humidity while each photo was captured.

Cortisol

Saliva samples were collected from each horse using the Salimetrics SalivaBio Children's Swab (SCS) Kit. Saliva was collected from each horse in the morning at 9:00am in the cross ties at least one-hour after the horses had their breakfast and before the youths interacted with the horse (1-B). Saliva samples were also collected immediately at the end of the session day at 1:00 pm, when the youths stopped their interaction with the horses. As an exploratory process, saliva was also collected in the same three horses after Phase 2-TR in both conditions, to allow for exploratory analyses into the effect of Phases 1 and 2 on salivary cortisol. To collect saliva, the swab was inserted into the side of the horse's mouth and was held under the tongue or in the cheek for a total of 35 seconds to ensure at least 1mL of saliva was collected. After collection, samples were immediately stored in a freezer and then transferred to a temperature-controlled ice box during transportation back to the university. Samples were then stored in a freezer below 20-degrees Celsius until they were sent to Salimetrics overnight on dry ice for analysis.

Upon arrival to Salimetrics, samples were thawed to room temperature, vortexed, and then centrifuged for 15 minutes at approximately 3,000 RPM (1,500 x g) immediately before the assay was performed. Samples were tested for salivary cortisol using a high sensitivity enzyme immunoassay (Cat. No. 1-3002). Sample test volume was 25 µl of saliva per determination. The assay had a lower limit of sensitivity of 0.007 µg/dL, a standard curve range from 0.012-3.0 µg/dL, an average intra-assay coefficient of variation of 4.60%, and an average inter-assay coefficient of variation 6.00%,. This met the manufacturers' criteria for accuracy and repeatability in Salivary Bioscience and exceeds the applicable NIH guidelines for Enhancing Reproducibility through Rigor and Transparency (Salimetrics).

Behavior Ethogram

To measure behavioral indicators of equine stress, this study used an applied ethogram from behaviors that were drawn from previous literature. Common behaviors associated with autonomic responses or releases of tension and common behaviors associated with avoidance and conflicts, have been included. All of the categories of these behaviors were included because I wanted to look for low-level indicators of stress in horses in EASs which has not been done in previous literature, to my knowledge. The applied ethogram included the following 15 behaviors which were consistently observed as low levels signs of stress and which was drawn from previous literature.

Table 3:
Applied Ethogram of Behaviors Indicative of Stress During EASs

Behavior	Definition
Licking and Chewing	Rotating movement of the lower jaw, possibly also producing sound, with tongue pushing straight forward, out of the mouth and retracted again (Draaisma, & Strous, S., 2018).
Jaw and/or Body Stretch	Opened mouth with or without uncovering the teeth nor protruding tongue (Draaisma, & Strous, S., 2018). Ridged extension of the limbs, arching of the neck and back (McDonnell, 2003).
Neck and/ or Body Shake	Rapid, rhythmic rotation of the whole body or just the head and neck along the long axis. (Torcivia et al., 2021)
Lowering the Head and Neck	Head/Neck is lowered downward (Eisersio et al., 2021)
Itching (Rubbing) Head and Neck	Rubbing face against forelimb (autogrooming) (Torcivia et al., 2021)
Head Raise	Head/Neck is raised upward (Eisersio et al., 2021)
Pawing	Reaching a forelimb cranially and dragging the hoof along or above the substrate while sweeping caudally, often in rhythmic series (Torcivia et. al., 2021)
Step Forward/ Back	One step forward or one step back with either front or hind limb while stationary (McDonnell, 2003).
Head Turn with Ears Pinned	Head turned horizontally to handler or object, with ears pinned

Ears Pinned	Ears pressed caudally against the head and neck (McDonnell, 2003)
Tail Swish	Moving tail suddenly from side to side (Torcivia et al., 2021)
Head Toss	Quick rotational toss of the head (Torcivia et al., 2021)
Kick Threat	Intent to kick, swinging rump, backing up, or stamping hind leg toward stimulus (Seamen et. al., 2002).
Bite Threat	Bite intention movement with ears back and neck extended, with no actual contact (Seamen et. al., 2002).
Lip Quiver	Involuntary movement (twitching) of the lips, often with relaxation (drooping) of the lower lip. (Torcivia et al., 2021)

(*Note:* Behaviors adapted from: McDonnell, 2003; Draaisma & Strous, 2018; Seamen et. al., 2002; Eisersio et al., 2021; Torcivia et al., 2021).

Two researchers coded equine behaviors simultaneously during all three phases. In Phases 1-B each horse was observed for two 1-minute intervals. In Phase 2-TR each horse was observed for one 1-minute interval. Lastly, in Phase 3-AW, each horse was observed for three 1-minute intervals.

The researchers established interrater reliability on their use of the applied ethogram by obtaining 94% agreement on observed behaviors. If there were any discrepancies within the two observers with the behaviors, they were due to obstructed view or simply missed behavior. Researchers discussed the few discrepancies and came to a joint consensus erring on the side of recording more behaviors rather than fewer.

Human Participant Characteristics

Hearts & Horses collects demographic data from all participants in their program using an initial enrollment form that includes the following information: age, race, ethnicity, gender, medications, family household size, living with relatives, previous medical diagnosis, physical disabilities, and if they have history of trauma, emotional or behavioral concerns. Hearts & Horses provided researchers access to this de-identified information in order to characterize the humans participating in EAL-CL and EAL-DD.

Data Analysis

Analysis was done using SAS 9.4. Summary statistics were calculated for each variable, condition, week and phase. A mixed model was fit for each response variable separately. Specifically, fixed effects included condition (experimental or control), week (3, 5 or 7) and phase (1, 2 or 3) and all two-way interactions. Horse was included as a random effect to account for repeat observations on horses. F-tests for fixed effects were used to determine pairwise comparisons of interest. Tukey adjusted p-values were used when comparing factors with more than two levels. Residual diagnostic plots were used to evaluate model assumptions of normality and equal variance.

To answer our research question 1, we examined main effects of condition, to determine if equine stress differed during EAL-CL compared to EAL-DD. To answer research question 2, we examined condition x phase interaction effects, to determine if equine stress differed during different phases (baseline, tie-rail, or arena work) during EAL-CL compared to the EAL-DD control condition. And finally, to answer research question 3, we examined condition x week interaction effects, to see if equine stress during EAL-CL differed over the course of 5 weeks in comparison to the EAL-DD control condition.

Results

Table 4 provides equine characteristics for horses included in this study. The eleven horses ranged from six years of age to twenty-five years of age, averaging sixteen years of age. All horses were housed in similar conditions living in stalls with runs at night and turned out to pasture during the day. The geldings were housed on one side of the property and the mares lived on the other. The horses were fed morning and night in their individual pens and fed again once they were out to pasture during the day. All horses were on a 6–8-week farrier cycle, up to date on veterinary care, received acupuncture, massage and chiropractic when needed and all horses had been professionally fitted to saddles.

Table 4
Equine Characteristics

Horse Name	Sex	Age	Breed	Years in EASs	Medications that could affect cortisol	Current medical history
Rocket	Gelding	25	Quarter Horse	4	Daily Previcox	N/A
Nugget	Gelding	9	Quarter Horse	1	N/A	N/A
Varsity	Gelding	10	Gypsy Vanner	7	N/A	N/A
Niko	Gelding	15	Quarter Horse	<1 year	N/A	History of gastrointestinal issues
Mohica	Mare	17	Quarter Horse	2	N/A	History of gastrointestinal issues
Wrangler	Gelding	20	Paint	<1 year	Daily Previcox	N/A
Ben	Gelding	21	Connemara	6	N/A	N/A
Apollo	Gelding	18	Percheron	<1 year	Daily Previcox	Metabolic, history of foundering
Hope	Mare	6	Haflinger	2	N/A	N/A
Leroy	Gelding	15	Warmblood	<1 year	Daily Previcox	N/A
Ray	Gelding	21	Quarter Horse	<1 year	N/A	N/A

Table 5 provides characteristics and demographics for the humans who participated in EAL-CL and EAL-DD. Consistent with provider reports at Hearts & Horses, Table 5 demonstrates that youth in the EAL-CL condition had higher incidence of trauma compared to young adults in the EAL-DD condition.

Table 5

Demographics and Characteristics of EAL-CL and EAL-DD participants

Participant Characteristic	Youths with Social-Emotional Concerns (N=11)	Control Condition (N= 7)
Gender: m/f/non-binary/trans	4/5/1/1	2/5/0/0
Age: mean(range)	13(12-14)	20(19-21)
Race: White/not reported	10/1	5/2
Hispanic/Latino: yes/no	3/8	4/3
Previous Riding Experience: yes/no/not reported	4/6/1	6/1/0
On current Medications: yes/no/not reported	6/4/1	6/1/0
Physical Disabilities: yes/no/not reported	0/9/2	2/5/0
Diagnosis:		
ADHD	5	
Autism Spectrum Disorder	2	2
Anxiety	5	2
Depression	2	
Dyslexia	1	
Developmental Delays		1
Down Syndrome		1
Intellectual Disability		2
Mood/ Personality Disorder	2	
PTSD	1	
Smith Magenis Syndrome		1
Living with relatives: yes/no	11/0	6/1
Household size: median (range)	5 (2-6)	3(3-6)
History of: (yes/no/not reported)		
Trauma	3/8/0	0/6/1
Behavioral Concerns	6/5/0	2/4/1
Emotional/Mental Health Concerns	9/2/0	1/6/1

Equine Eye Temperature

To answer research question 1, there was no evidence of a main effect for condition (EAL-CL verse EAL-DD), F=0.73(1,136), p=0.39, indicating that on average horses did not demonstrate increased eye temperature during EAL-CL in comparison to EAL-DD (Figure 3).

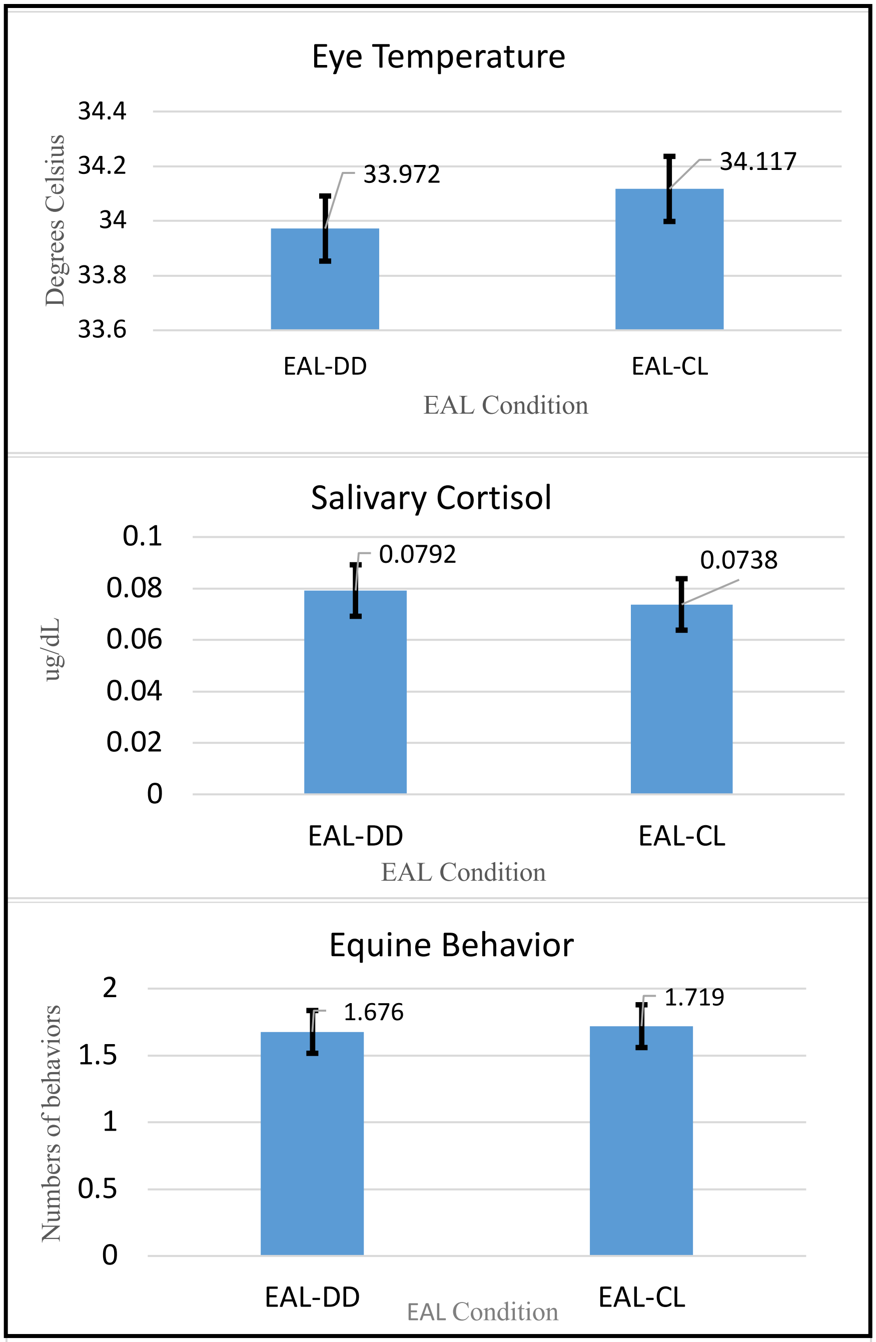
To answer research question 2, there was no significant condition x phase interaction effect (F= 1.11(2,136), p= 0.33), indicating horses did not demonstrate increased stress at the tie rail or in the arena during EAL-CL compared to EAL-DD (Figure 4). To answer research question 3, there was a significant interaction effect of condition x week, F=6.47 (2,136), p=0.002 (Figure 5). Equine eye temperature was higher in the EAL-CL condition compared to the EAL-DD condition in week 3, t=-3.22(136), p=0.002. However, there were no differences between the EAL-CL and EAL-DD in weeks 5 and 7 (week 5 t=0.26(136), p=0.79; week 7 t=1.69(136), p=0.09). Although not directly related to our research questions, there was a significant phase effect (F=4.47 (2,136), p=0.01) such that eye temperature was significantly higher in phase 3-AW compared to phase 1-B, t=-2.91(136), p=0.01. Eye temperature did not differ between phase 1-B and 2-TR, or between phase 2-TR and phase 3-AW (t=-1.00(136), p= 0.12.; t=-0.91(136), p=0.64). There were no significant week x phase interaction effects (F=1.80(4,136), p=0.13).

Equine Salivary Cortisol

To answer research question 1, equine cortisol did not significantly differ between experimental conditions (EAL-CL vs EAL-DD; F=0.36(1,85.8), p=0.55) indicating that on average horses did not demonstrate increased levels of cortisol during EAL-CL in comparison to EAL-DD (Figure 3). In answering research question 2 there were no significant interaction effects of condition x phase (RQ2) indicating that horses did not demonstrate increased stress at the tie rail or in the arena in EAL-CL compared to EAL-DD (Figure 4) F=0.00(1,81.7), p>0.99. In answering research question 3, there was also no significant interaction effects of condition x week indicating that salivary cortisol did not differ by week during EAL-CL compared to EAL-DD (Figure 5;F=1.18(2,81.7), p=0.31. Although not directly related to our research questions there was evidence of a significant main effect for week (F=7.77(2,82.20) p=0. 001), such that equine cortisol was significantly higher in week 3 compared to week 5 (*t*=3.94(82.4), *p*=0.001). There was also evidence of a significant phase effect (*F*=4.24(1,81.7), *p*=0.04) such that cortisol was significantly greater in phase 3-AW compared to phase 1-B,*t*=-2.06(81.7), *p*=0.04. There were no significant effects in week x phase *F*=1.72(2,81.7), *p*=0.19). As an exploratory process, saliva was also analyzed in the three horses after Phase 2-TR in both conditions, to allow for the exploratory analyses into the effect of Phases 1 and 2 on salivary cortisol, which was found to have no significant effects.

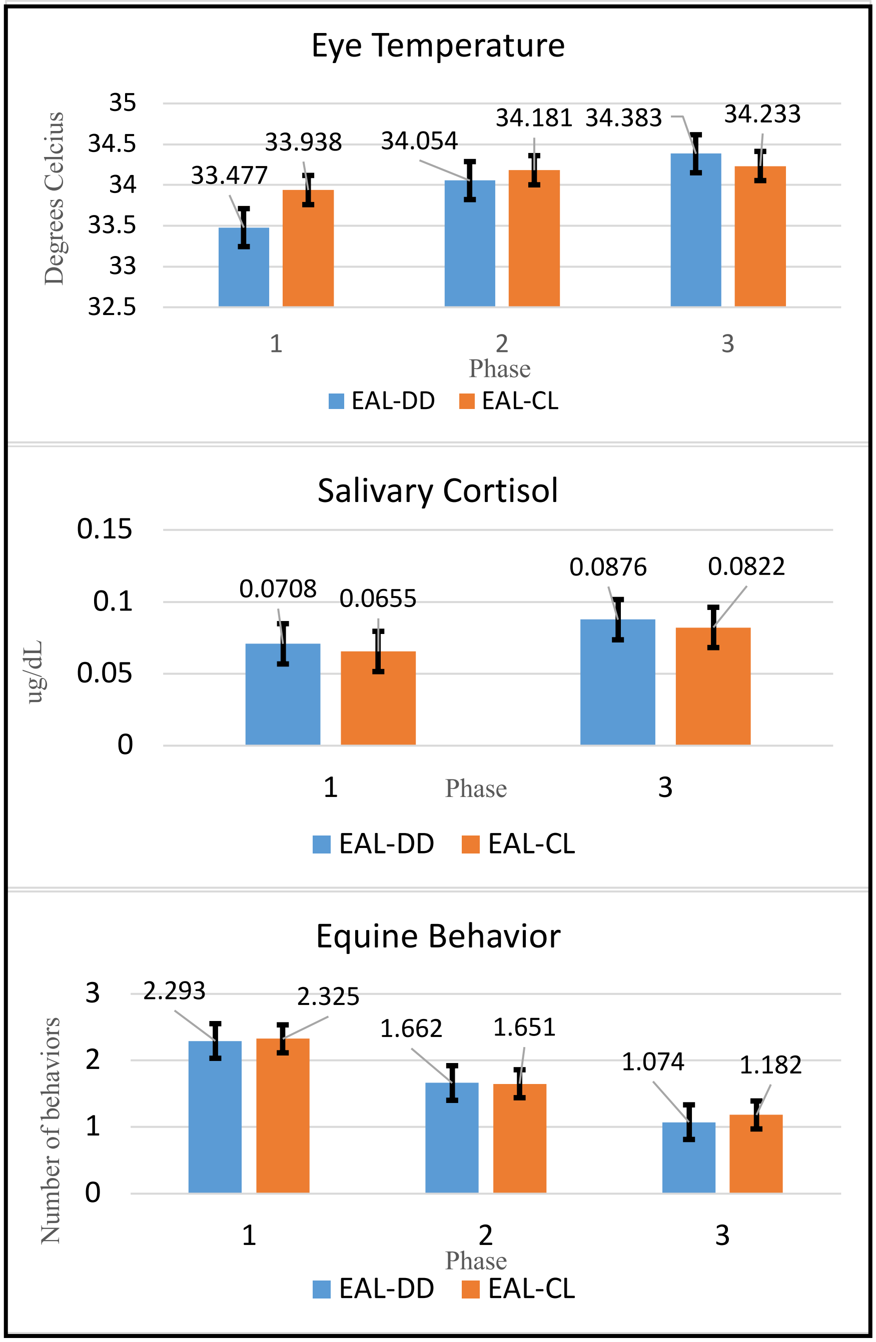
Equine Behavior

In answering research question 1, there was no evidence of a main effect for condition (EAL-CL verse EAL-DD), F=0.06(1,135), p=0.81 indicating that on average horses did not demonstrate increased behaviors during EAL-CL in comparison to EAL-DD (Figure 3). In answering research questions 2, there were no significant interaction effects of condition x phase (indicating horses did not demonstrate increased stress at the tie rail or in the arena during EAL-CL compared to EAL-DD (Figure 4) F=0.04(2,126). Lastly in answering research question 3, there were also no significant interaction effects of condition x week indicating that horses did not demonstrate increased stress in the EAL-CL compared to EAL-DD over the weeks (Figure 5) (F=0.70(2,127), p=0.5. Although not directly related to the research questions, there were no main effects for week, F=0.27(2,128), p=0.77 and no week x phase interaction effect p= 0.99; F=0.38(4,126), p=0.82).There was a significant phase effect, F=16.83(2,126), p=<0.0001 such that there were significantly more equine behaviors in phase 1-B than 2-TR, t=3.18(126), p=0.01. There were also significantly more equine behaviors in phase 1-B than 3-AW (t=5.79(126), p=<0.0001) and significantly more equine behaviors in phase 2-TR than 3-AW (t=2.54(126), p=0.03).

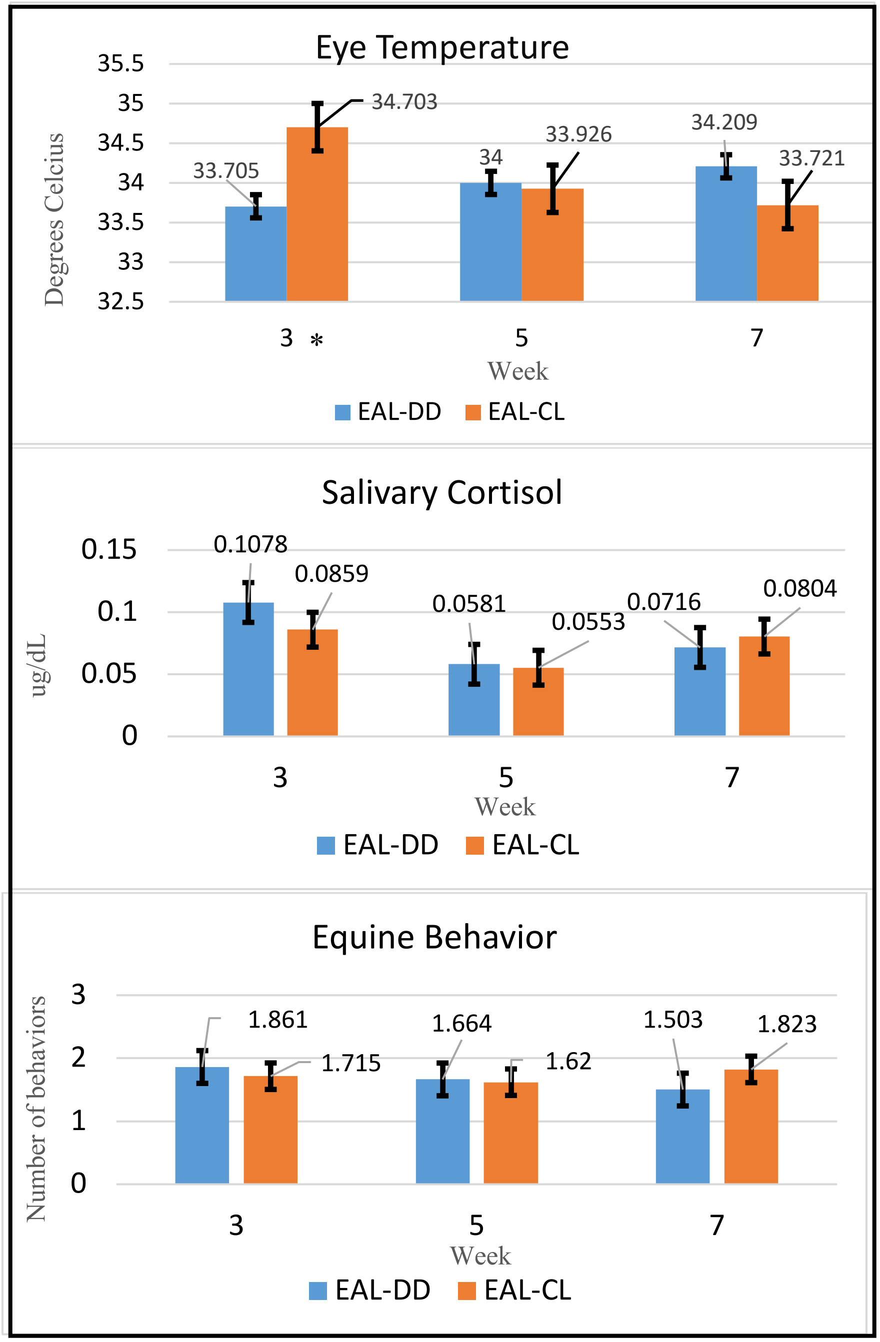


Note: **Figure 3**. *Research Question 1: Indicators of equine stress during different EAL Conditions*

Temperature is measured in degrees Celsius. Cortisol concentrations measured in ug/dL. Behavior is reported as number of behaviors in a 1-minutes observation period. There were no statistically significant effects.



Note: **Figure 4**. *Research Question 2: Indicators of equine stress during different phases of EAL* Temperature is measured in degrees Celsius. Cortisol concentrations measured in ug/dL. Behavior is reported as number of behaviors in a 1-minute observation period. There were no statistically significant effects.



Note: **Figures 5.** *Research Question 3: Indicators of equine stress across 5 weeks of EAL* Temperature is measured in degrees Celsius. Cortisol concentrations measured in ug/dL. Behavior is reported as number of behaviors in a 1-minutes observation period. There is a statistically significant effect in eye temperature p value < 0.01

Discussion

The purpose of this study was to examine physiological and behavioral indicators of stress in horses involved in EAL for youth with social- emotional concerns. To achieve this purpose, we examined equine stress during EAL for youth with social- emotional concerns compared to an EAL control condition. We examined three phases of EAL (baseline, tie-rail, and arena work) and collected data across 5 weeks.

The researchers' findings indicated that there was not an increase in stress in horses integrated into EAL for youth with social- emotional concerns compared to the horses integrated into EAL for a population without social- emotional- behavioral concerns. This finding is opposite from Kaiser et. al. (2006) findings, which demonstrated an increase of equine behavioral indicators of stress during EASs for "youth at-risk" compared to the other populations participating in EASs. It is important to note that Kaiser's population with "youth at-risk" is not well characterized. Kaiser also write in the discussion, "subtle signs of impatience, aggressive disposition, or displeasure towards the horse was *seen* in their population of "youth at risk" (p. 43), yet this was not seen in the youth with social- emotional concerns in my study. My findings showed that horses that are integrated into an EASs population with youth with social- emotional concerns are not more stressed than being integrated into any other population in EASs; which is also consistent with the previous research by Arrazola & Merkies (2020).

For research question 2, no differences were found in equine stress during interactions with youth with social- emotional concerns compared to the control condition among the three phases of EAL. Thus, in the current study, getting groomed and tacked at the tie rail by youth with social- emotional concerns was no more stressful for horses than the same activities performed by young adults with developmental disabilities. Similarly, arena work was no more stressful for horses during EAL for youth with social- emotional concerns compared to young adults with developmental disabilities. This is the first study to examine differences in equine stress during EASs for youth with social- emotional concerns during different phases (tie rail vs arena work) of the service.

Lastly for research question 3, there were no differences in salivary cortisol or behavior during EAL-CL compared to EAL-DD across the 3 different weeks that data were collected. However, eye temperature was higher in week 3 in EAL-CL compared to EAL-DD; these differences between conditions no longer existed in Weeks 5 and 7. Week 3 of EAL-CL was the first day of data collection for the study and was also the first day that EAL participants began riding (as opposed to groundwork only). Only 7 of the 11 horses in the EAL-CL condition were present for the data collection pilot day, so data collection (i.e. IRT camera, equine behavior observers) was novel to 4 of the horses that day. Therefore, we believe increased eye temperature in Week 3 of EAL-CL may be attributed to these changes in routine, rather than increased stress caused by interacting with youth with social- emotional concerns.

Although not directly related to our research question, the data analysis also demonstrated significant differences across different phases of EAL, regardless of the participant population. Specifically, there was an increase of behavioral indicators of stress during phase 1-B compared to the other two phases, indicating that the horses demonstrated more behavioral signs of stress while alone in the tie rail compared to EAL sessions for either population. This is consistent with the research by Merkies et. al. (2018), where horses demonstrated increased behavioral indicators of stress while alone in a round pen, than during interactions with EASs populations. We also found an increase of eye temperature and cortisol in phase 3-AW compared to the other two phases. This increase can likely be attributed to the increase in physical activity during arena work compared to the tie rail; however, more research is needed to understand if equine eye temperature increases with such light physical activity that occurs with EAL, as current outside research only examines higher-intensity exercise (Jannsson et. al., 2021; Bartolomé et. al., 2013; Stewart et. al., 2007).

Limitations

The researchers collected baseline data in the tie rails, to ensure all IRT photographs were captured in the same indoor arena, so any differences in temperature were not due to dust, temperature, humidity, or direct sun exposure. However, standing alone in a tie-rail may be stressful for horses, and therefore is not an ideal baseline condition. Future studies may consider a different baseline condition. Our study was also limited by the use of convenience samples of human participants already enrolled in EAL programming at Hearts & Horses; while youth participating in EAL-SE had higher incidence of previous trauma and social-emotional-behavioral concerns, some participants in the EAL-DD group were characterized as having behavioral concerns (n=2) or emotional concerns (n=1). Future research not reliant on convenience samples could assign human participants to EAL conditions based on inclusion/exclusion criteria, so the control condition has no presence of social-emotional-behavioral concerns. Finally, our study is limited by the small sample size of horses (n=11).

Conclusion

Researchers found that there was not increased stress in horses integrated into EAL with youth with social- emotional concerns compared to a different population that commonly partakes in EASs. This study adds to the growing body of literature on equine welfare during EASs. While our study is congruent with previous research that interacting with participants during EASs is less stressful than no-human control conditions, it also contradicts previous research that concluded interacting with youth with social- emotional concerns is more stressful than other common EASs populations. Further research with larger sample sizes and a better baseline condition is needed.

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APPENDICES

Study Timeline

- October 3rd, 2022: First Day of Data collection for (EAL-CL)
- October 7th, 2022: First day of Data collection for (EAL-DD)
- October 24th, 2022: Data collection for (EAL-CL)
- October 28th, 2022: Data Collection for (EAL-DD)
- November 4th, 2022: Last day of Data Collection for (EAL-DD)
- November 7th, 2022: Last day of Data Collection (EAL-CL)

Table 6
Equine Applied Ethogram Used for this Study

Phase 1, 2 or 3 (circle)	**Group: experimental or control (circle)**			
Horse Name:				
Date:				
Behavior Coding Unit		**Time 1**	**Time 2**	**Time 3**
Bite Threat				
Ears Pin				
Head Raise				
Head Turn w/ Ears Pin				
Head Toss				
Itching Head/Neck				
Jaw Stretch/ Body Stretch				
Kick Threat				
Licking/Chewing				
Lowering Head/Neck				
Neck/Body Shake				
Pawing				
Step Forward/Step Back				
Tail Swoosh				
Lip Wiggle				
Total Behaviors				
Notes:				
Unmounted or mounted?				
Stationary or moving?				
Gate Transition?				