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The Effect of Grooming and Therapeutic Riding by Adolescents with Autism Spectrum Disorder on Equine Salivary Cortisol Concentrations

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THE EFFECT OF GROOMING AND THERAPEUTIC RIDING BY ADOLESCENTS WITH AUTISM SPECTRUM DISORDER ON EQUINE SALIVARY CORTISOL CONCENTRATIONS

by

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ii. Abstract

The use of horses in therapeutic riding programs, especially for adolescents with autism spectrum disorder (ASD), has grown, yet the impact of grooming and riding on equine stress levels remains unknown. The aim of this study was to compare the effects of grooming and therapeutic riding on salivary cortisol concentrations of 10 horses in a therapeutic riding program. Samples were taken pre and post over two control and grooming days and over two series of nine therapeutic riding lessons for adolescents with ASD. On grooming days, each horse was groomed using a set protocol. On riding days, horses were ridden following a set lesson plan. On control days, horses remained in their stalls. The samples were analyzed using an enzyme-linked immunosorbent assay (ELISA) to determine cortisol concentrations. The mean change in cortisol concentrations over time for control, grooming, and riding samples were compared using one-way repeated measures ANOVA. Paired t-tests were used to compare the change over time for individual lessons and controls. No significant differences were found for the change over time during therapeutic riding, grooming, and control. A significant post-riding increase in salivary cortisol concentrations was found during lesson four when compared with controls ($P=0.02$). This was the first lesson focusing on walk-trot transitions, and the riders' posture may have impacted the horses. Overall, the data indicate that therapeutic riding and grooming had no major effect on equine cortisol concentrations, suggesting that these human-animal interactions did not compromise the well-being of the horses.

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1. Introduction:

In recent years, the therapeutic use of horses has become increasingly popular, and their use in therapeutic riding programs has grown. Therapeutic riding is a type of animal assisted therapy with the goal to help treat populations with a wide range of disabilities. Therapeutic riding is defined as utilizing the act of horseback riding to help cognitively, socially, and psychologically benefit populations with physical or mental disabilities (Bass et al., 2009). This study investigated the impact of therapeutic riding programs on the horses used in order to determine any effects these programs may have on equine welfare.

Impact of Therapeutic Riding Programs on the Participants

Currently, therapeutic riding programs have been shown to be beneficial for veterans (Johnson et al., 2018), children with developmental delays (Winchester et al., 2002), and children with autism spectrum disorder (ASD) (Bass et al., 2009). In programs designed to benefit veterans with post-traumatic stress disorder (PTSD), it was found that 6 weeks of therapeutic riding resulted in both clinically and statistically lower PTSD scores (Johnson et al., 2018). Therapeutic riding has also been shown to result in significant improvements in gross motor function in children with developmental disorders (Winchester et al., 2002). For adolescents with ASD, therapeutic riding has been demonstrated to result in improved sensory integration, directed attention, social motivation, and decreased sedentary behaviors (Bass et al., 2009), along with decreased irritability and hyperactivity (Gabriels et al. 2015). Additionally, a beneficial therapeutic bond may develop between the horses and the participants as a result of these programs (Bass et al., 2009). Therapeutic riding programs are therefore beneficial for the participating riders; however, the exact impact of these programs on the therapeutic riding horses requires additional research.

Salivary Cortisol as an Indicator of Stress

One way to assess the impact of therapeutic riding on the horses used in such programs is by examining the cortisol levels of each animal. Cortisol is a hormone produced by the adrenal glands as part of the hypothalamic pituitary axis (HPA). Cortisol is released into the bloodstream by the adrenal glands in response to increased levels of corticotrophin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), which are released as part of an animal's natural response to stress. In horses, an increased stress response, and therefore increased levels of cortisol, is associated with the body's attempt to reestablish homeostasis following intense exercise, injury or discomfort, or psychological strain (Wagner, 2010). As this is the case, an increase in cortisol can be used as in indication of discomfort, pain, and psychological stress in the horse, therefore suggesting compromised animal welfare (Wagner, 2010). In the bloodstream, cortisol is typically bound to plasma proteins, although non-protein bound cortisol is found in other bodily fluids such as saliva. Plasma cortisol levels are directly correlated to salivary cortisol levels and, as a result of this, salivary cortisol concentrations can be successfully used to determine cortisol release in equines (Kędzierski et al., 2013). Therefore, cortisol concentrations found in both blood and saliva can be used to determine how stressed an animal is (Möstl & Palme, 2002).

Other Variables Affecting Equine Salivary Cortisol

In addition to stress; the time of day, exercise intensity, and the act of feeding have also been shown to effect cortisol levels. It is known that in equines, salivary cortisol levels fluctuate during the day in a diurnal pattern due to a natural circadian rhythm, and salivary cortisol concentrations are typically highest around 10:00am and lowest in the evenings (Bohák et al., 2013). The highest salivary cortisol levels tend to differ from the lowest levels by roughly 0.2

ng/mL (Bohák et al., 2013). Equine salivary cortisol levels also fluctuate seasonally, with the highest cortisol concentrations occurring in December (Aurich et al., 2015). Moderate to intense exercise also has been found to increase blood cortisol levels in horses (Kędzierski et al., 2013); however, further study is needed to understand more about how therapeutic riding, which typically involves low intensity exercise, affects salivary cortisol. Whether a horse has recently eaten a meal can also effect cortisol levels. In a study comparing ACTH of both fasted and fed horses, ACTH levels were significantly higher in horses that had been fed within two hours as opposed to those which had not (Castro et al., 2014).

Impacts of Riding on Equine Salivary Cortisol

The impacts of both equestrian competitions and recreational riding on equine salivary cortisol levels have also been studied. Salivary cortisol levels have been shown to increase in sport horses in response to competitions (Becker-Birck et al., 2013). In this study it was found that equine salivary cortisol levels tend to peak 20 minutes after the conclusion of a show-jumping competition. It has also been found that horses with higher increases in salivary cortisol levels tend to perform better in the competition overall (Peeters et al., 2013). Additionally, while the increase in equine salivary cortisol is greater in competition horses, salivary cortisol levels have also been shown to increase in response to recreational riding and training (von Lewinski et al., 2013). The experience level of the rider, at least in sport horses, has not been shown to affect the salivary cortisol levels of the horse (Ille et al., 2013). In a study comparing the cortisol levels of 16 horses ridden over the same show-jumping course, cortisol and heart rate were shown to increase in response to the exercise (Ille et al., 2013). However, when the post-riding cortisol increases in the 8 horses ridden by experienced professionals were compared to the 8 horses ridden by less-experienced riders, no significant differences were found.

Impacts of Therapeutic Riding on the Horse

The effects of different types of therapeutic riding programs and stress responses in therapeutic riding horses has also been studied. In a therapeutic riding program designed to benefit veterans with PTSD, baseline salivary cortisol levels were collected on rest days for each of the five horses used in the program and compared to the levels after each lesson, in addition to before and after the tacking session preceding that lesson. No significant differences were found in the cortisol level post lesson when compared to the baselines for each horse, indicating that the horses carrying the veterans were under no undue stress (Johnson et al., 2017). This study also analyzed the cortisol levels of the horses after they were ridden by experienced riders following the same program as the veterans and found salivary cortisol levels to be significantly higher in horses ridden by veterans as compared to experienced riders (Johnson et al., 2017). This result is contradictory to both the study's previous findings and also the finding that rider experience has not been shown to increase equine salivary cortisol (Ille et al., 2013). It is therefore suggested by the researchers of this study that perhaps the horses experienced more stress before the therapeutic lesson, during the tacking process with the veterans, than they did with the experienced riders. Fazio and colleagues (2013) found that blood cortisol concentrations were lower in horses following participation in therapeutic riding lessons for riders with varying psychomotor disabilities when compared with recreational lessons for riders without disabilities. The same routine and exercise intensity were followed for riders in both of those groups. In another study, the frequency of equine stress-related behaviors in response to different types of therapeutic riding targeting different groups of individuals was analyzed. These behaviors included ear pinning, head tossing, head raising, and defecation. The study found the frequency of these equine stress-related behaviors were significantly higher in the horses ridden by at-risk

children, whereas there were no differences found in the horses ridden by recreational riders without disabilities, riders with physical disabilities, or riders with developmental delay or mental health diagnosis (Kaiser et al. 2006).

Impacts of Various Activities on Equine Stress Levels

In addition to riding, horses are exposed to a variety of other activities all with the potential to illicit stress responses. Horses are often transported and increases in salivary cortisol have been found in response to transport and trailering (Schmidt et al., 2010). Additionally, the coats of domestic horses are often clipped during the winter to ease their use in riding. Clipping is typically done supplementary to grooming and only occurs every couple weeks depending on the preference of the horse owner. Many horses, however, seem stressed by this procedure. In a study done investigating the impact of clipping on equine salivary cortisol, a significant increase was found post clipping session for horses during the procedure (Yarnell et al., 2013). The horses used in therapeutic lessons, and domestic horses in general, are often groomed daily, and this is a procedure typically conducted before riding. Grooming is assumed to be a fairly calming activity that must be done in order to ensure the welfare of the animal. However, a search of the literature did not reveal any studies conducted to investigate the impact of this common daily activity on the stress levels of the horse.

Impacts of Petting/Grooming on Stress Responses of Other Species

In dogs housed in animal shelters, 25-minute human contact/exercise sessions were shown to reduce salivary cortisol (Menor-Campos et al. 2011), while grooming, petting, and practice of obedience commands for 45 minutes resulted in either decreased salivary cortisol or no change, depending upon the number of days after the dog's arrival at the shelter. The study

found that the decreases in salivary cortisol only occurred on day 3 after the dog's arrival at the shelter and no change in cortisol for any of the other days (Coppola et al. 2006). It has also been found that shelter dogs participating in a human interaction program of four sessions of walking, grooming, and petting had significantly lower salivary cortisol levels post session when compared to baseline levels and exhibited significant improvements in temperament (Bergamasco et al., 2010). These studies overall suggest that the act of grooming have a positive impact on the stress levels of the animals groomed.

Objective of the Study

As therapeutic riding programs have been shown to be effective in the management of several different human physical disabilities or mental health conditions, the popularity of these programs will only increase. Additionally, grooming is an integral part of the daily welfare of the horse, both in therapeutic riding programs and traditional riding programs. Knowledge of how grooming and therapeutic riding affect HPA activity and cortisol production, and therefore stress levels in the horse, will allow directors of therapeutic riding programs and horse owners in general to be able to make the best decisions concerning the welfare of their animals. The aim of this study was to compare the effects of both grooming and therapeutic riding on salivary cortisol concentrations, and therefore determine the impact on the welfare of, 10 horses used in a university therapeutic riding program designed for adolescents with ASD. The results of this study may prove beneficial for both directors of therapeutic riding programs designed to help children with ASD and general horse owners alike.

2. Methodology

Saliva samples were collected from horses for analysis of salivary cortisol immediately before and after a therapeutic riding, grooming, and a stalled control period. Each of the therapeutic riding lessons started at 4:00 pm and ended at 4:30 pm, therefore on each collection date for grooming and control samples, the samples were collected at 4:00 pm (designated "pre") and 4:30 pm (designated "post") to account for any fluctuations due to the horses' natural circadian rhythms.

Horses

Saliva samples were collected from 10 horses who actively participate in a therapeutic riding program at Slippery Rock University (SRU) in Pennsylvania. Out of the 10 horses, 6 were geldings and 4 were mares. The horses were of several different breeds, including Haflinger, Quarter Horse, and Paso Fino. All horses used in the study were housed at Storm Harbor Equestrian Center in Slippery Rock, Pennsylvania. The horses did not have access to hay or feed within 60 minutes prior to sampling to avoid affecting baseline cortisol levels.

Saliva Collection

During each saliva collection, a swab (Salimetrics) was placed by a volunteer under the tongue of each horse for 60 seconds. The swab was then deposited into an empty sample tube and frozen at -20˚C. The samples were then transported on dry ice to the Equine Science Lab at Otterbein University in Westerville, Ohio. The samples were stored at -20ºC until analysis.

Control and Grooming Samples

Control and grooming samples were taken over a one week period in December 2018 with the control samples taken over two days (Days 1 and 2) and grooming samples taken

consecutively after over four days (Days 3, 4, 5, and 6) to ensure no horses had been ridden during the sampling. Control samples were taken on the two control days (Days 1 and 2) when horses were not used for any other activities. During these control dates, horses remained in their stalls between the pre and post sampling times. Grooming samples were taken from each horse on two days when the horse was not used for riding or other activities. For each horse's two days of grooming, the samples were collected over a period of four days (Days 3, 4, 5, and 6) total to avoid sampling on any dates when a horse was needed for lessons, but again had each individual horse only sampled on two dates. On grooming sampling days, each horse was groomed in a cross-tie area for a total of 30 minutes using a set grooming protocol by experienced volunteers or student workers. The same grooming protocol was followed for all horses (Table 1). Volunteers started by collecting a pre-grooming sample at 4:00 pm following standard sampling protocol. Each horse was then groomed according to protocol, which was timed and supervised by a research assistant, before collecting a post-grooming sample at 4:30 pm.

Table 1: Standard grooming protocol used for each of the horses.

TIME (PM)	**PROTOCOL**
4:00 – 4:01	Saliva collection (Pre)
4:01 – 4:05	Hoof picking
4:05 – 4:15	Curry with rubber curry comb
4:15 – 4:20	Brush with hard bristled brush
4:20 – 4:25	Brush with soft bristled brush
4:25 – 4:30	Gently comb out mane and tail
4:30 – 4:31	Saliva collection (Post)

Riding Samples

The riding samples were taken over the course of two nine week lesson periods during a period of time prior to when the control and grooming samples were collected. This portion of the study was completed in April 2018 and were compared to the grooming and control samples taken in December 2018.

A total of 18 riding lessons were sampled. One riding lesson and thus one pre and post riding sample occurred each week. On riding sampling days, horses were ridden by adolescents with ASD and the instructor followed a set 30-minute lesson plan. A series of nine different

riding lesson plans, including a progression of riding skills, was used (Table 2). The riding lesson plans were repeated in the second period, but the horses were ridden by a different group of riders with ASD. Over the course of each nine week riding lesson program, the content of each riding lesson progressed from focusing on posture and walk-halt-walk transitions (week 1) to independent riding, collecting/extending the trot (week 8), and a mock horse show (week 9).

Table 2: Therapeutic riding lesson plans for each of the two nine week-long lesson periods.

LESSON #	**LESSON FOCUS**
1	Balance and position, learning how to halt
2	Basic steering at the walk
3	Trotting, basic steering
4	Walk-trot transitions, advanced steering
5	2-point at trot, trotting through corners
6	2-point at trot, independent steering
7	No-stirrup riding, trotting 20 m circles
8	Independence at trot, learning leg yields
9	Independent riding, collect and extend trot

Salivary Cortisol Analysis

The samples were analyzed using an ELISA kit (Salimetrics) designed to estimate salivary cortisol concentrations. To analyze cortisol concentrations, the tubes containing the saliva samples were first thawed for 30 minutes and centrifuged for 15 minutes at 1500 xg. Once samples had been thawed and centrifuged, 25 µL of each sample was pipetted into each well in a 96 well microtitre assay plate. The standards added to each assay were 25 µL of cortisol standards in a saliva-like matrix. The same amount of high and low controls were also added to each assay. Additionally, a zero control consisting of assay diluent with no sample was also run with each assay. A 1:1600 dilution of 15 µL of enzyme conjugate and 24 mL of assay diluent was created and 200 µL was added to each well using a multichannel pipette. Each plate was then mixed for 5 minutes at 500 rpm on a plate rotator and incubated at room temperature for an hour. Each plate was washed using 1X wash buffer solution by pipetting 300 µL into each well and then discarding the excess liquid over a sink. This was repeated four times before each plate had 200 µL of TMB substrate solution added. The plates were then mixed for 5 minutes on a plate rotator at 500 rpm and incubated in the dark for 25 minutes before 50 µL of stop solution was added. Each plate was briefly passed over with a hairdryer on low before being run to ensure no bubbles were present in any of the wells. Each plate was then read using an absorbance microplate reader at 450 nm with a secondary filter correction at 492 nm (eMax Plus, Molecular Devices). Each assay contained standards, high, and low controls, and a zero control. A standard curve was established for each plate and R values were recorded. The samples themselves were run in duplicate and analyzed for any internal deviations.

Statistics

The mean change in salivary cortisol concentrations over time (pre-post) for control, grooming, and riding samples were compared using one-way repeated measures ANOVA. The one-way repeated measures ANOVA was run using SPSS Version 25 Software. Paired t-tests were used to compare the change over time, from 4:00-4:30 pm, for individual lessons and controls. The paired t-tests were calculated using Microsoft Excel. Statistical significance was set at $P < 0.05$. The change in mean salivary cortisol (pre-post) of all 10 horses in response to grooming and therapeutic riding were compared to control levels as was also the change (pre-post) in mean salivary cortisol in response to individual riding lessons each week.

3. Results

Mean Equine Salivary Cortisol Concentrations

The mean equine salivary cortisol concentration pre-grooming was 0.071 ± 0.009 µg/dL and 0.058 ± 0.007 µg/dL post-grooming (Fig. 1). The mean salivary cortisol concentration pre-therapeutic riding was 0.096 ± 0.007 µg/dL and post-therapeutic riding was 0.114 ± 0.008 µg/dL. The mean control salivary cortisol concentration at 4:00 pm (pre) was 0.085 ± 0.020 µg/dL and 0.096 ± 0.029 µg/dL at 4:30 pm (post). No significant differences were found for the change in salivary cortisol concentration over time when comparing therapeutic riding to the control (Fig 1). Additionally, no significant differences were found when comparing the change in mean salivary cortisol concentrations over time for pre grooming to post grooming and pre riding to post riding (Fig 1). Salivary cortisol concentrations pre and post grooming were also not significantly different from the control levels (Fig 1).

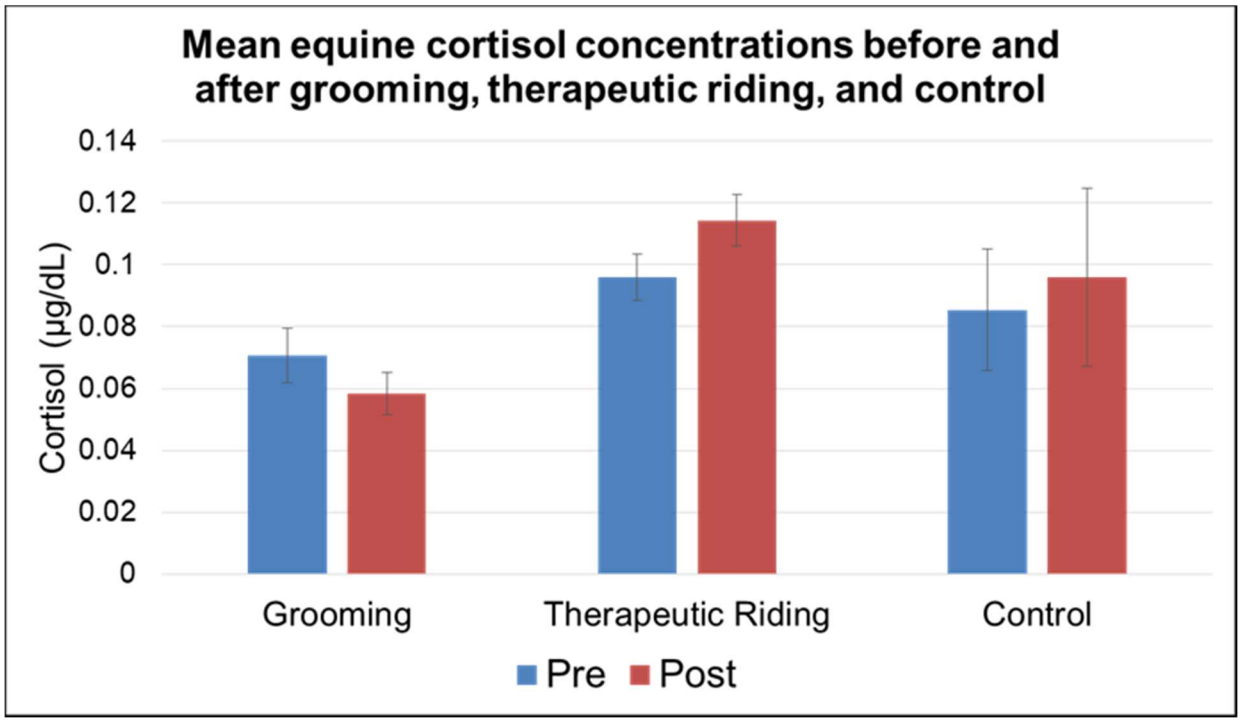

Fig. 1: The mean equine cortisol concentrations with standard error bars are shown before (pre) and after (post) control, grooming, and therapeutic riding.

Change in Mean Equine Salivary Cortisol in Response to Therapeutic Lessons

The change (pre-post) in mean equine salivary cortisol levels in response to each lesson was as follows (Table 3). The mean change in control cortisol concentration was -0.011 ± 0.011 µg/dL. When analyzing the individual lessons (Fig. 2), a significant post-riding increase in salivary cortisol concentrations was found during lesson four when compared with the control (P=0.02).

Table 3: The mean change in equine salivary cortisol concentration (pre-post) in response to individual lessons over the course of a nine week therapeutic riding program.

Lesson #	Change in Mean Salivary Cortisol (Pre-Post)
Lesson 1	0.018 ± 0.011 µg/dL
Lesson 2	0.021 ± 0.034 µg/dL
Lesson 3	-0.038 ± 0.054 µg/dL
Lesson 4	-0.134 ± 0.043 µg/dL
Lesson 5	0.017 ± 0.014 µg/dL
Lesson 6	-0.00035 ± 0.018 µg/dL
Lesson 7	0.020 ± 0.009 µg/dL
Lesson 8	-0.0042 ± 0.012 µg/dL
Lesson 9	-0.030 ± 0.013 µg/dL

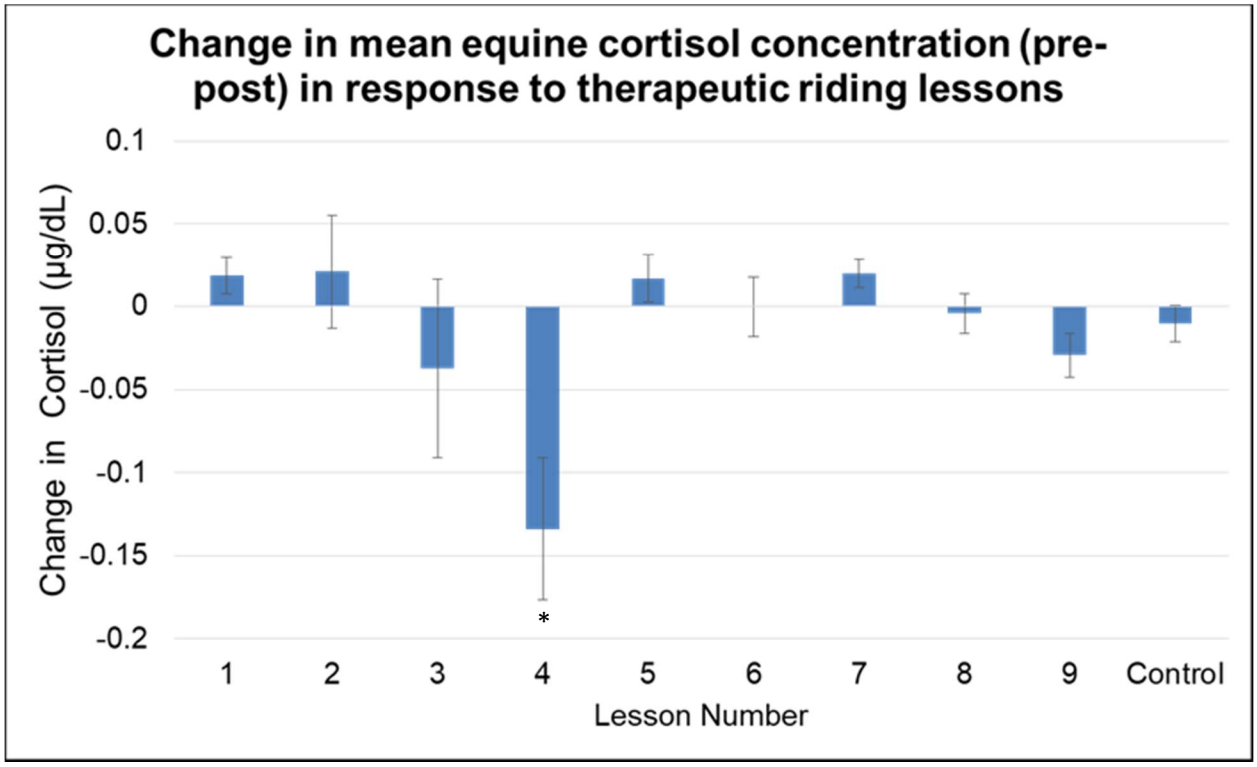

Fig. 2: The change in mean equine salivary cortisol concentration (pre-post) over nine lessons is shown with standard error bars. *Indicates $P<0.05$ when compared with control.

4. Discussion:

The analysis of the mean change in salivary cortisol in response to therapeutic riding and grooming when compared to the control showed no significant differences. The change in mean equine salivary cortisol levels in response to just therapeutic riding lessons for adolescents with ASD showed no statistically significant differences, when compared to the control. This is in agreement with the study done by Kaiser et al. (2006) which found no differences in equine stress related behaviors in response to riders with physical disabilities, or riders with developmental delay or mental health diagnoses. The results of this study, which suggest that grooming and therapeutic riding do not put undue stress onto the animal, are also in agreement with a study done investigating the effects of a therapeutic riding program designed for veterans with PTSD. In the study done by Johnson and colleagues (2017), it was also concluded that the therapeutic riding did not result in undue stress to the horse. However, in the results of this project, there is disagreement with the study done by Fazio et al. (2013), which found therapeutic lessons designed for riders with varying psychomotor disabilities to result in lower equine blood cortisol levels. This discrepancy may be due to the fact that the horses in this study were ridden according to a different lesson plan designed to benefit riders with ASD as opposed to individuals with motor disabilities.

When analyzing the change in mean equine salivary cortisol compared to the control for each of the individual riding lessons, a significant increase in salivary cortisol concentration was found during lesson four. Lesson three was the first lesson the students were introduced to trotting and lesson four was the first lesson that focused on walk-trot transitions. In the study done by Johnson et al., it was thought that interaction with the less experienced participants of the therapeutic programs could have contributed to the increases in salivary cortisol that they saw

(Johnson et al., 2017). Considering this, it is possible that compromised rider balance and position, as a result of the lower experience levels of the riders in this study, during the walk-trot transitions focused on in lesson four, led to the increased equine salivary cortisol concentrations. Although moderate to intense exercise is known to increase salivary cortisol concentrations (Kędzierski et al., 2013), no significant increases in salivary cortisol were found during subsequent trot lessons, so it is not likely that the increase in cortisol concentration was solely due to the introduction of trot work. That said, the effect exercise has on equine salivary cortisol during these programs is certainly less than if the horse were to undergo a normal moderate to intense workload as investigated by Kędzierski et al.

Future Directions

Since the results of this study indicate that grooming did not have a statistically significant effect on salivary cortisol, it would be worth investigating other activities such as mane pulling to determine the effect these activities have on equine salivary cortisol levels. Due to the relatively small sample size of this study, it could be possible that grooming may have a positive impact on equine welfare well worth future investigation. Certain equine management activities such as clipping have already been found to result in increased equine salivary cortisol levels and therefore further studies should be undertaken in order to assess the impact of these activities on the welfare of the horse (Yarnell et al., 2013). As there seem to be differences in the results of the effects of different types of therapeutic riding on horses, this study provides important information for the directors of therapeutic riding programs designed specifically for adolescents with ASD. In particular, this study shows that the current curriculum followed by Slippery Rock University's therapeutic riding program does not compromise the welfare of the horses used in their program and therefore is a good model for the design of future therapeutic

riding programs for individuals with ASD. In order to provide more information for directors of different programs, additional studies exploring the effects other types of therapeutic riding programs, either designed for children with varying levels of ASD symptoms or other similar disabilities, on equine salivary cortisol should be established. Additionally, since in this study the participants in the program did not tack their horses before the lesson, it would be worth future investigation to see if any interactions pre-lesson have an effect on equine stress levels during the lesson. The effects of different riders on equine stress levels, adolescents with ASD versus experienced riders riding at the same exercise intensity, should be compared in the future in order to provide additional information on the effects of therapeutic riding. A limitation of this study is the fact that the riding samples were collected during a different time of year than the grooming and control. Another limitation is the fact that the sample size was small, and it would be worth the effort in the future to conduct a study with a larger sample size, perhaps encompassing several therapeutic riding programs for individuals with ASD. Although the effects of circadian rhythm were not taken into account since all samples were collected at the same time of day, salivary cortisol does tend to fluctuate seasonally.

5. Conclusion:

Overall there was an increase in salivary cortisol concentration following lesson four, indicating the horses may have been stressed during this beginner trot lesson. Therapeutic riding instructors should ensure adequate rider skill competency and readiness prior to early trot lessons. Otherwise, the data indicate that therapeutic riding and grooming did not have a statistically significant effect on equine cortisol levels, suggesting that these human-animal interactions did not compromise the well-being of the horses.

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