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The Effect of Slow-Feeding Hay Nets on Post-Prandial Equine Salivary Cortisol Levels

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THE EFFECT OF SLOW-FEEDING HAY NETS ON POST-PRANDIAL EQUINE SALIVARY CORTISOL LEVELS

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Abstract

Throughout its evolution, the *Equus caballus*, or horse, developed a physiological response to environmental alterations involving the synthesis and release of cortisol from the hypothalamic-pituitary-adrenal axis, to regulate blood glucose levels, vascular tone and hormone release throughout the body (Ambrojo et al., 2018). With a hypothesis that changing a horse's feeding method can alter salivary cortisol levels, the aim of this research project was to determine if feeding forage to horses in slow-feeding haynets would significantly reduce acute cortisol salivary level fluctuations, which would be valuable to minimize horses' stress and increase overall welfare. Two feeding methods were tested with 20 clinically healthy horses randomly assigned to two equal groups at Otterbein University's Austin E. Knowlton Center for Equine Science: treatment N with daily forage presented in a slow-feeding hay net and treatment G with forage presented on the ground. Group 1 received treatment N on days 0-7 and treatment G on days 8-14 and Group 2 received treatment G on days 0-7 and treatment N on days 8-14, with forage removed 2.75 hours after presentation. One salivary sample of 200-1000 µl was collected per horse on testing periods (days 6,7,13 and 14) and a competitive enzyme-linked immunosorbent assay (ELISA) was used to quantify the concentration of salivary cortisol within each sample (Salimetrics LLC, State College, PA). The mean individual values of each horse between treatments were compared, with a mean difference of -0.075 ± 0.290 µl/dl. These values were non-significant at $p<0.05$, although 65% of the horses displayed lower salivary cortisol concentrations with treatment N compared to treatment G. The high percentage of horses displaying lower cortisol levels with treatment N indicated that longer duration of foraging time mimics the horse's natural consummatory behavior and thus decreases the stress experienced by the horse during feeding.

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1. Introduction

1.1. Function of cortisol as a stress hormone

Cortisol, also widely known as a stress hormone, is one of the most vital hormones in physiological pathways found in the equine body. Cortisol is responsible for maintenance of cardiovascular homeostasis, immune and inflammatory responses, electrolyte and fluid balance, protein and lipid metabolism, and is susceptible to modification through stressful or painful stimuli. According to Leah et al. (2011), elevated cortisol levels ensure the delivery of adequate nutrients to the brain and other areas of the body susceptible to compromise during stressful events or injury, resulting in acute immunosuppression. These acute alterations to equine cortisol levels can be measured in saliva, plasma and feces, with salivary tests accurately measuring small and transient changes in cortisol release as a response to stress triggers (Ambrojo et al., 2018, Peeters et al., 2011).

The synthesis and secretion of cortisol begins when physiological, environmental or pathophysiological signals are received from the equine's peripheral or central nervous system. Transmission of these signals to the hypothalamus activates the release of corticotrophin-releasing hormone, which then acts upon the anterior pituitary gland. Once the anterior pituitary gland has been acted upon by corticotrophin-releasing hormone, receptors on the surface of corticotropic cells release adrenocorticotropic hormone into the body's systemic circulation (Hart et al., 2011). Adrenocorticotropic hormone stimulates the adrenal glands located on each kidney, which in turn synthesize and release cortisol directly into systemic circulation (Ambrojo et al., 2018). Since cortisol is a lipophilic hormone, it is primarily transported by binding to plasma proteins, such as globulin and albumin, but it is also found in a non-protein bound and

biologically active form (Hart et al., 2011). This smaller, free form experiences a significant increase in salivary concentration when the horse experiences acute stress (Ambrojo et al., 2018).

Saliva is produced by three salivary glands, the parotid, submandibular and sublingual pairs, and is a complex fluid that contains variable amounts of hormones, plasma exudates and microorganisms (Bayazit, 2009). Hormones, such as free cortisol, can diffuse through acinar cells, mucosal surface abrasions, or capillary cell walls to enter saliva, and salivary cortisol concentrations are a direct reflection of plasma concentrations (Bayazit, 2009). With a non-invasive method of collection, saliva is a useful biological fluid to detect such biomarkers (Bayazit, 2009). As Hart et al. found in a 2011 research study with neonatal foals, increases in free salivary cortisol levels are consistent with stress induced activation of the hypothalamic-pituitary-adrenal axis, which influences the horse's adaptability to external and internal changes.

In horses, cortisol concentrations in both plasma and saliva follow a circadian rhythm without external cues from humans or the environment, with peaks occurring between the 0600 and 0900 hour and a trough between the 1900 and 2300 hour (Irvine, 1994). This naturally occurring rhythm can be altered by external stimuli, especially with stimuli resulting from stressful conditions such as exercise and transportation or restricted access to forage and presentation of large amounts of concentrate in limited meal times (Ambrojo et al., 2018). Observations of such alterations include higher serum cortisol concentrations present thirty minutes prior to AM feedings compared to thirty minutes after feed intake, as well as significant postprandial increase in endogenous adrenocorticotropic hormone (Ambrojo et al., 2018). These fluctuations in cortisol concentration indicate that the equine body responds to such external stimuli by activating the hypothalamic-pituitary-adrenal axis and sending the body into a stress response. A research study done by Irvine and Alexander, found that the secretion of cortisol is

episodic, with a novel environment causing a mean peak frequency of 0.56 ± 0.03 peaks per hour, without causing a difference from baseline day and night frequencies (1994). Since modern management of horses limits feeding frequency and duration, their cortisol release in response to the novelty of food is subject to such episodic secretions, in response to intermittent fasting and meal times. Chronic fluctuations in cortisol concentrations can lead to loss of adrenal sensitivity, tissue atrophy, and increased immunosuppression, which impairs the equine's ability to fight disease and maintain physical homeostasis (Leal et al., 2011).

1.2 Effects of domestication upon the horse's natural foraging behaviors

Throughout the horse's domestication, the natural roaming and grazing lifestyle of the equine species has been replaced by human-made, artificial feeding systems. Equines are natural herbivores designed to graze during the main portion of the day, with a digestive tract dependent upon continuous forage intake. The primary foundation of the equine diet should be forage fed fresh or preserved, in order to allow their digestive tract to continuously digest herbage that is low energy (Ellis, 2010). This continuous feeding of forage presents itself as a challenge to horse owners, as horses consume hay at a rapid rate, and it is difficult for owners to fed or afford *ad libitum* forage. Glunk et al. (2013), found that horses have an intake rate of loose hay that is 25% higher than hay presented in a medium sized hay net, which limits the horse's ability to consume forage over an extended period of time. Haynets are commonly recommended as a tool to help increase the overall time spent foraging without having to increase the amount of forage presented, in an effort to preserve the horse's natural intake behavior (Ellis et al., 2015).

Boyd et al. (1988) examined Przewalski horses kept in a zoo over the course of two summers and found that they spent approximately 46.4 ± 5.9% of their time foraging. Feral

horses have been observed to display foraging behaviors for greater than 70% of the day, while stabled horses forage for less than 40% of the day, which alters the body's overall hormone concentrations and rhythms (Ellis et al., 2015). An absence of the opportunity to forage has been seen to result in behaviors such as coprophagy, bed-eating and wood-chewing, which may be a reflection of motivation to consume fiber outside of domestic-determined mealtimes (Hothersal & Nicol, 2009). These actions may also result from an inability to express foraging behavior, or lack of stimulation, and develop into the repetitive and functionless behavioral expressions of boredom, known as stereotypies (Hothersal & Nicol, 2009).

A study performed by Winskill et al. (1996), examined the effect of a foraging device upon the behavior and overall time budget of stabled horses. The foraging device was comprised of a cylinder with an internal food store and a food dispensing hole and horses were observed through video recordings. Five Standardbred horses were subjected to three consecutive treatment test periods during stabling; baseline treatment included observing the horses under their normal management for three consecutive days, while device treatment involved exposure to the device containing 4 kg of high fiber pelleted feed for five consecutive days, and the post-enrichment treatment consisted of three consecutive days after the device was removed from the stable. The device was associated with a significant decrease in the baseline stereotypic behaviors of moving, ingesting concentrates and nose bedding. Winskill et al. (1996) concluded that the overall time budget change reflected the time budget allocations of free-ranging horses, which indicated an increase in animal welfare. The use of slow-feeding haynets is hypothesized to have similar effects upon the overall time budget allocations of the stabled horse, by increasing horse-specific foraging behavior. A study done by Glunk et al. (2014), found that as hay net opening size decreases, the time spent foraging increases in adult horses. Horses feeding

from hay nets with medium and small openings exhibited a longer duration of foraging behavior, which resulted in a reduced dry matter intake rate compared to horses feeding from a large-opening hay net and the ground (Glunk et al., 2014). This decrease in hay net opening size limits the horse's access to hay, allowing foraging behavior to continue over a longer duration of time, with controlled amounts of forage presented.

1.3. Evaluating the horse's overall welfare

The primary goal behind researching how a specific feeding method impacts the horse's cortisol levels is to determine a feeding method that can decrease stress and increase overall welfare. A study by Bayazit in 2009 found that assessing salivary cortisol levels is a useful method of assessing an animal's stress and well-being, as an evaluation of the animal's housing conditions, physiological status and feeding practices. Common physiological indicators of stress include weight gain or loss, changes in blood pressure, enzymatic shifts, altered heart rate or abnormal sleep patterns, with only the most extreme forms of suffering or stress causing visible effects (Bayazit, 2009). Absence of chronic stress is considered to be an indicator of animal welfare, but a standard method of measuring animal stress has not yet been developed (Möstl and Palme, 2002). Measuring the effects of an animal's feeding practice upon their cortisol concentrations, could determine which feeding methods decrease the animal's cortisol levels and thus increase their physiological welfare.

The present study describes the effect of altering an equine's feeding method through presentation of forage in a slow-feeding haynet upon salivary cortisol levels. As the primary corticosteroid in equine plasma and saliva has been identified as cortisol, examining salivary samples for free cortisol concentration changes can provide insight into the impact of the given

feeding method upon equine stress (Leal et al., 2011). This study will analyze salivary cortisol concentrations in equines following afternoon feeding, while taking into account the equine circadian rhythm and individual equine's variations in concentration. Observing an alteration in cortisol levels following the experimental treatment of feeding forage in a slow-feeding haynet, could provide insight into the improvement of feeding practices in the equine industry. With increased knowledge, the industry can begin developing feeding methods to prevent acute increases in cortisol concentrations, and potentially improve overall equine health and welfare through advanced equine management.

2. Methodology

2.1 Animals used

This research project used 20 clinically healthy horses (Table 1) at Otterbein University's Austin E. Knowlton Center for Equine Science. Out of the 20 horses used, four were mares and 16 were geldings, with varying breeds including five Thoroughbreds, three Quarter Horses, two American Paint Horses and ten warmbloods. The horses weighed 559.3 ± 49.1 kg and were 14.1 ± 3.7 years of age. The horses were individually housed in loose boxes (minimum size 3.05m x 3.66m), with 15 turned out daily in paddocks between 08:00h-15:00h, four turned out in fields between 08:00-15:00 and one turned out in a field between 21:00h-06:00h. All 20 horses were bedded on wood shavings, with the shavings cleaned once daily in the morning. The horses were all fed morning between 06:30h and 07:00h, and evening between 18:00h and 18:30h, with 3.60 ±0.57 kg of hay fed and were accustomed to their hay being presented from the ground during both their AM and PM feeding.

The 20 horses used were lesson horses for a collegiate riding program, and all horses were ridden one hour per day on days 1-5 and days 8-12. During the testing periods (days 6,7,13 and 14), the horses were not ridden or exposed to any external treatments that had the possibility of influencing the horses' salivary cortisol levels.

2.2. Study Design

This project was approved in January 2019, according to the animal use and welfare standards set by the Otterbein University Animal Care and Use Committee. This study was performed at Otterbein University's Austin E. Knowlton Center for Equine Science, with a testing period of 14 days. The 20 horses were randomly assigned two equal treatment groups,

consisting of ten horses in each group. Group 1 received treatment N on days 0-7 and treatment G on days 8-14, and Group 2 received treatment G on days 0-7 and treatment N on days 8-14. Prior to receiving treatment G, each group of horses were given a five-day acclimation period to the slow-feeding hay nets, with the amount of hay presented to each horse remaining consistent throughout the study duration. During the acclimation periods, the horses were fed by the barn management staff at the center and were fed by the research student during the testing days. The haynets were hung approximately 1.219 m above the ground, and the hay presented and left-over was weighed by the researcher prior to feeding on testing days. Each horse's forage was removed 2.75 hours after presentation, with the amount of leftover hay measured and recorded per horse. One salivary sample was collected per horse on each testing day, one hour after the horses' forage was removed. The salivary samples were collected with SalivaBio Children's Swabs, which were inserted into the corner of the horse's mouth for 60 seconds, with a sample recovery volume of the swabs designed to fall between 200-1000 µl. A total of four salivary samples were collected per horse over the duration of the experiment. The salivary samples were immediately placed in swab storage tubes and stored at -20^{o}C.

2.3. ELISA Measurements

A competitive enzyme-linked immunosorbent assay (ELISA) was used to quantify the concentration of salivary cortisol within each sample (Salimetrics LLC, State College, PA). On the day the ELISA was performed, the salivary samples and reagents were brought to room temperature for 1.5 hours until all materials were thawed completely. The saliva samples were then vortexed and centrifuged at 1500 x g for 15 minutes, in order to remove mucins and other particulate matter that could interfere with antibody binding sites. The standards, high and low

controls and salivary samples were pipetted in 25 µl quantities into the appropriate wells of the ELISA plate, with 25 µl of Assay Diluent serving as the zero in two wells. All standards, controls, and samples were run in duplicate. Diluted enzyme conjugate was prepared by adding 15 µl of the conjugate to 25 ml of assay diluent. The diluted enzyme conjugate mixture was then vortexed and 200 µl were immediately added to each well using a multichannel pipette. The addition of the enzyme caused the binding of the antibodies at the bottom of each well to bind to the free-floating cortisol found in the salivary samples. The plate was mixed on a plate rotator for 5 minutes at 500 rpm and incubated at room temperature for 1 hour.

After incubation, the plate was washed four times with 300 µl of wash buffer in each well. After completion of the washes, 200 µl of tetramethylbenzidine substrate solution was added to each well and the plate was placed on a plate rotator for 5 minutes at 500 rpm. The addition of substrate caused a reaction between the cortisol-bound enzymes to form a blue-colored solution within the wells. The plate was then incubated in the dark at room temperature for 25 minutes before 50 µl of an acidic stop solution was added to each well. Following addition of the stop solution, the plate was mixed on a plate rotator for 3 minutes at 500 rpm and a color change was observed as the wells turned varying degrees of yellow depending on the cortisol concentration in each individual well.

The plate was then read in a plate reader (EMax Pro, Molecular Devices, San Jose, CA) at 450 nm within 10 minutes of adding the stop solution. The plate reader calculated the mean optical density for each individual well, with the amount of cortisol enzyme conjugate detected being inversely proportional to the amount of cortisol present in the sample. Concentrations were determined using a 4-parameter non-linear regression curve fit.

2.4. Statistics Analysis

All statistical analyses were performed using SPSS (v.24) with a level of significance set to $p<0.05$. A Shapiro Wilks test was performed to examine the data for normal distribution. A Wilcoxon signed ranks test was used to compare mean cortisol levels observed during each treatment.

Table 1: Characteristics of animals used in the present study

Sample Size	Sex	Age (years)	Age Range (years)	Breed
n=20	Mare (n=4) Gelding (n=16)	14.1 ± 3.7	5.0-20.0	Thoroughbred (n=5) Quarter Horse (n=3) American Paint (n=2) Warmblood (n=10)

Weight (kg)	Weight Range (kg)	Forage Fed (kg)	Forage Fed Range (kg)
559.3 ± 49.1	471.7- 671.3	3.6 ±0.57	2.7-5.4

3. Results

3.1. Statistical Analysis Results

Once salivary samples were collected from all the individual horses used in the study (n=20), with four samples collected per horse over the duration of the experiment (n=80), mean cortisol levels were calculated per treatment. The mean and standard deviation of the salivary cortisol levels observed in treatment N, with forage presented in a slow feeding haynet, was 0.194 ± 0.136 µl/dl. In treatment G, where forage was presented on the ground, the mean salivary cortisol level was 0.269 ± 0.222 µl/dl.

The individual mean values of each horse between treatments were compared, to find the difference between the two treatments. The mean difference between treatment N and treatment G was -0.075 ± 0.290 µl/dl, with 65% of the horses displaying lower salivary cortisol concentrations with treatment N compared to treatment G.

The Shapiro-Wilks test was used to determine if the salivary cortisol concentrations followed a normal distribution, with the test being appropriate for the small sample size of n=20. The test produced a test statistic of 0.830 and a p-value of 0.002. Since the statistical significance value was less than 0.05, the data is not normally distributed. Due to the small sample size within the experiment, and a lack of normal distribution, a Wilcoxon signed rank test was run to compare the mean salivary cortisol levels between treatment N and treatment G. The result of this test was p=0.332, which was determined to be non-significant when compared to the significance value of $p< 0.05$. This indicates that there was no statistical difference between the two treatments.

3.2. Percentage of Forage Consumed

The forage presented to the horses during each treatment was weighed on a scale prior to feeding and upon removal 2.75 hours after initial presentation. The percentage of forage consumed in treatment N was 80.1 ±12.6%, with a range of 58.3-100.0%. Comparatively, the percentage of forage consumed in treatment G was 92.0 ±10.7%, with a range of 69.4-100.0%. Upon comparison of the percentage of forage consumed between the two treatments, 75.0% of the horses consumed more forage with treatment G than treatment N, with 60.0% of the horses completely finishing their forage in treatment G and 5.0% completely finishing forage in treatment N. After the salivary samples were collected, the horses were allowed to finish the remainder of the initially presented forage.

Table 2: Salivary cortisol data results in µg/dl

Treatment N (slow-feeding haynet)	Treatment N (range)	Treatment G (ground)	Treatment G (range)
0.194 ± 0.136	0.050-0.567	0.269 ± 0.222	0.071-0.859

Treatment N-Treatment G	Treatment N-Treatment G (range)
-0.075 ± 0.290	-0.685-0.424

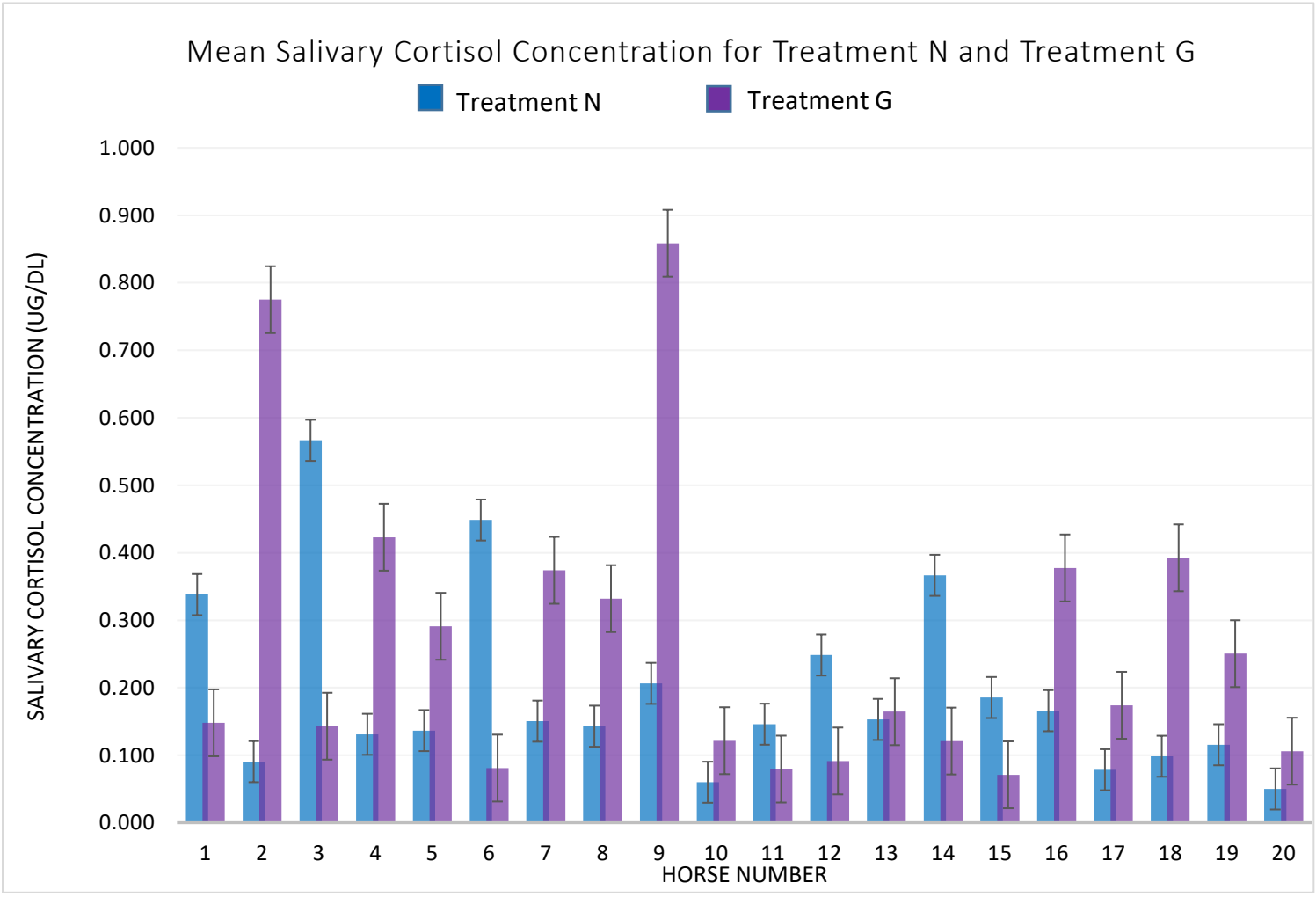

Figure 1. Mean (µg/dl) salivary cortisol concentrations per horse for treatments N and G

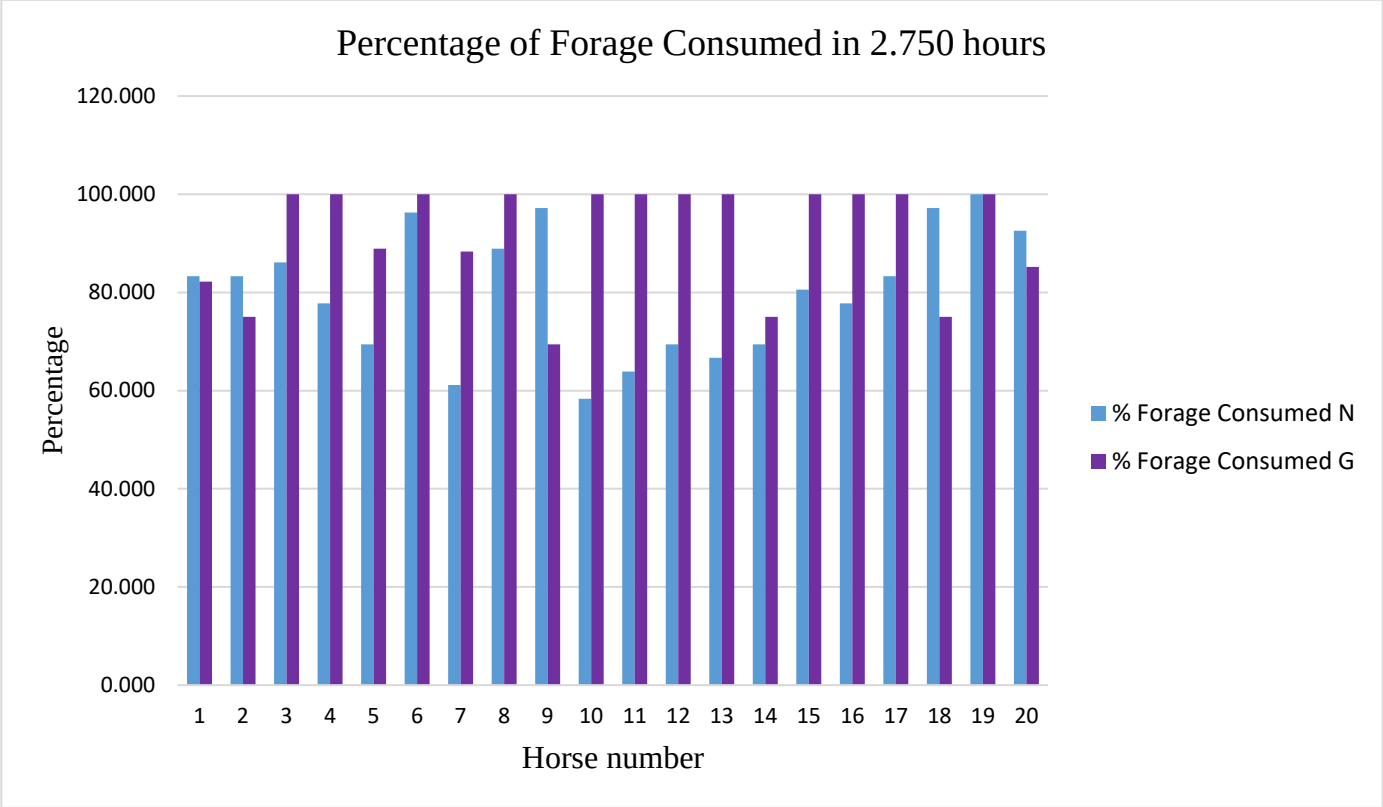

Figure 2. Percentage of forage consumed per horse for treatments N and G

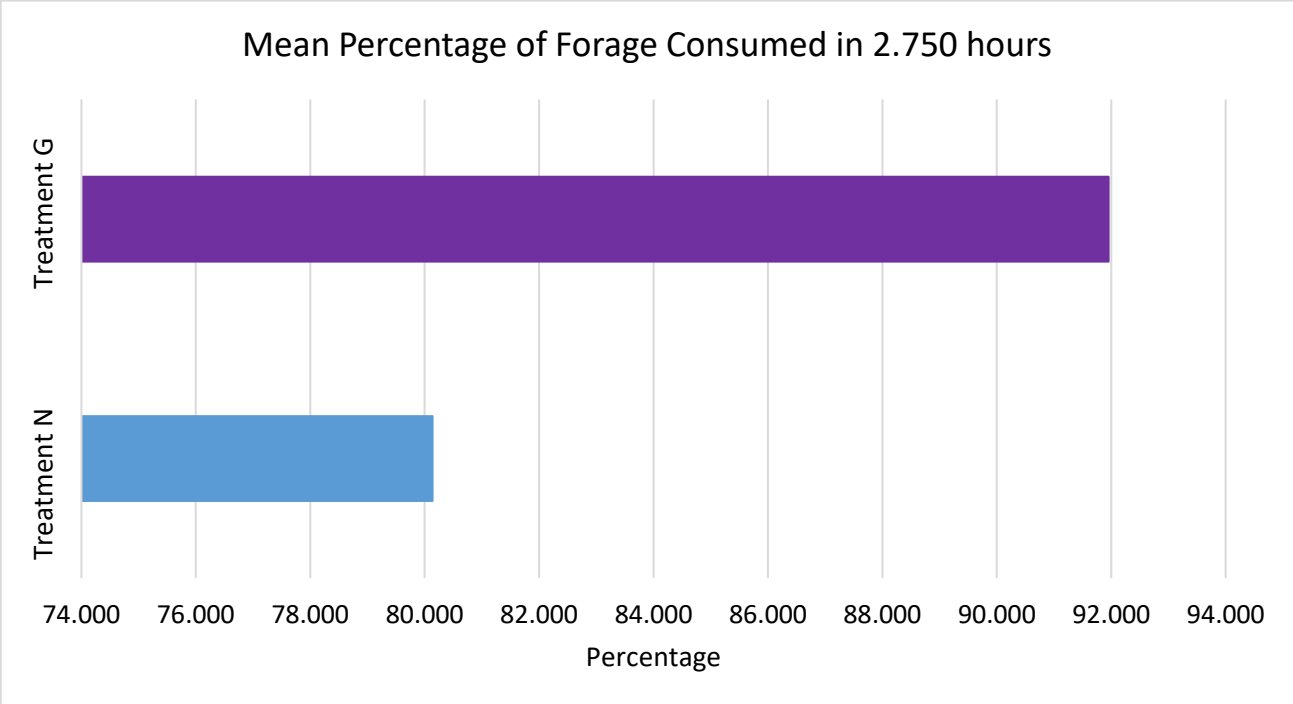

Figure 3. Mean percentage of forage consumed in 2.750 hours for treatments N and G

4. Discussion

While the above findings determined that the difference between salivary cortisol levels between treatment N and treatment G was non-significant, the study did find that 65% of the horses used in the experiment displayed lower salivary cortisol levels with treatment N. This high percentage indicates that with a larger sample size and resources for a longer experiment duration, the difference between salivary cortisol levels between treatments could become significant.

The presence of lower salivary cortisol levels after altering the medium through which forage is presented is important to consider, as the cortisol found in saliva represents the cortisol that is biologically active. As Ambrojo et al., state, cortisol found in plasma composes 90% of the cortisol in the horse's body, which is bound to albumin and CBG, and thus is unable to interfere with the body's receptors in response to acute stimuli (2018). The final 10% of cortisol is found in unbound in saliva and feces, giving it the ability to diffuse readily through capillaries to form bonds with steroid receptors and induce systemic effects during an acute stress response (Ambrojo et al., 2018). Since feeding of the modern domesticated horse is an event that causes acute stress, rapid alterations in cortisol concentrations will take place in cortisol's unbound form, indicating the underlying chronic changes in cortisol concentration due to the continuous, daily acute stress of feeding. With cortisol concentrations found to be lower in 65% of the horses used in this study, it is possible that those 13 horses may experience long-term benefits from having their daily forage presented in slow-feeding haynets. Avoiding an acute stress response to the external, psychological stimulus of feeding, allows these horses to maintain internal homeostasis, as their bodies will not be experiencing the systemic effects of cortisol concentration elevation.

Such systemic effects include inhibition of the immune system, as nutrient delivery is directed to the brain and nervous system in order to improve the body's chances of survival in the midst of a stressful stimuli (Ambrojo 2018). Glucocorticoids have the ability to inhibit the synthesis of cytokines or other cells that control the body's inflammatory and immune reactions, as they inhibit antigen presentation, proliferation of T cells, B cells and macrophages, and atrophy of the thymus (Sapolsky, 2000). It has also been found that one of the primary physical coping responses due to a stressor, is rapid cardiovascular activation (Sapolsky, 2000). This is due to the corticotrophin-releasing hormone's ability to not only regulate adrenocorticotropic hormone secretion, but also serve the role of a neurotransmitter that initiates the sympathetic nervous system (Sapolsky et al., 2000). Once the sympathetic nervous system is activated, through cortisol concentration rising above the basal level, the horse loses the ability to maintain homeostasis. As Sapoksly et al. (2000) found, once glucocorticoid levels rise above the basal level, the cognitive response is decreased, reproductive ability is suppressed, and the body is more susceptible to disease.

Considering the overall effects of elevated cortisol concentration upon the horse's homeostasis is critical, as the daily acute stress of feeding can result in chronic alterations to the bound plasma corticosteroids (Alexander, 1998). Since the body's response to any stressor, internal or external, follows the same pathophysiology, chronic elevations in cortisol concentration from feeding can have the same impact upon the body as cortisol alterations due to chronic predation. A study done in 1998 by Alexander and Irvine examined the effects of social stress upon both bound and unbound cortisol concentrations in horses. Results indicated that disruption of social patterns caused acute activation of the hypothalamic-pituitary-adrenal axis, with prolonged stress elevating basal free cortisol levels overall. After exposure to continued

stress, 18% of the horses used in the study developed respiratory infections, which was attributed to the inhibitory effect of corticosteroids upon immune activity. The control horses not exposed to an extended period of stress did not develop any clinical signs of disease, with basal free cortisol concentrations decreasing throughout the day. The study concluded that the horses experiencing prolonged social stress had a lowered binding capacity of plasma cortisol and increased basal concentrations of free cortisol.

An increase in overall basal concentration of free cortisol not only causes the sympathetic nervous system to be initiated, but has the potential to cause exhaustion of the hypothalamic-pituitary-adrenal axis. This exhaustion can result from experiencing prolonged stress, since the horse's body is not designed with enough resources to continuously synthesize glucocorticoids above its natural basal level. Avoiding such an increase in basal free cortisol concentration through proper equine feeding management, can help prevent such exhaustion.

Mimicking the natural feeding environment of the horse is critical to allowing basal free cortisol concentration to follow its natural circadian rhythm. This study found that the mean amount of forage consumed with the slow-feeding haynets within the duration of 2.75 hours was lower than the amount of forage consumed with ground presentation, indicating that feeding forage through a slow-feeding haynet causes forage to be consumed over a longer period of time. Continuous foraging is considered to be a horse's primary consummatory behavior, which artificial feeding environments restrict, by limiting the amount of time the horse spends foraging (Ninomiya, 2004). When a horse eats forage from a slow-feeding hay net, the lengthened time spent eating allows the horse's digestive system to experience the same intake as natural roaming and grazing.

In her book studying animal behavioral responses to stimuli, Temple Grandin describes the horse as "a prey species herbivorous grazing animal" (105). The natural instinct found within prey species causes novel stimuli to trigger the "fear system" within the amygdala and sub-cortex to be initiated, making the horse hypersensitive to environmental changes (Grandin 8). In order to eradicate such hypersensitivity to specific novel stimuli, the process of habituation is required to give the horse time to adjust and normalize the amount of cortisol released in response to the stimuli. In order to address such marked release, the horses in this present study were given 5 days to habituate to the slow-feeding hay nets used during treatment N. The time given to habituate to the slow-feeding haynets provides the horses used to overcome the immediate fear response to the novel stimuli initiated by the haynets, and turn on their "seeking system", which encourages "the basic impulse to search, investigate and make sense of the environment" (Grandin 6). As Grandin found, an initiation of the seeking response overpowers the effects of the fear response, as the two systems are "opposed in the brain" (130). Giving horses enough time to investigate and habituate to the slow-feeding haynets permitted the horses to no longer view the nets as a novel stimulus, thus allowing the salivary cortisol levels sampled in this study to be unaffected by any fear response to the nets themselves.

It is also important to consider that restricting an animal's natural consummatory behavior not only influences their cortisol release, but also their observable behavior and stress (Ninomiya et al., 2004). In a 2004 study done by Ninomiya et al., eight horses were exposed to five treatments using different methods of hay feeding, including cutting timothy hay into 5 cm lengths, delaying feeding for 1 hour, giving portions of hay over an extended period of time, feeding with hay in three different locations and mixing species of hay. It was found that when the hay-eating time was decreased significantly, through the treatment of cutting hay, stereotypic

behaviors such as bed-eating increased significantly. The treatments of increasing the frequency of feeding, feeding locations and hay varieties both extended the duration of hay-eating and encouraged the natural consummatory behaviors of bedding investigation. This encouragement of natural behaviors around feeding not only leads to increased feeding satisfaction in the horse, but also allows the horse to behave naturally. The increased duration of feed consumption with slow-feeding hay nets thus can improve the horse's welfare by allowing normal behavior to be exhibited.

5. Conclusion

As the body's primary stress hormone is cortisol, analyzing how salivary cortisol concentrations fluctuate in response to the horse's environment can provide valuable information about the horse's overall welfare and level of stress. The salivary cortisol concentration data results of this study were not statistically significant, but the 65% of horses used in the study who had lower cortisol concentration levels with treatment N can individually benefit from having their daily forage presented in a slow-feeding hay net. This study also found that the longer duration of foraging time when eating from hay nets mimics the horse's natural consummatory behavior. Further research into the effects of slow-feeding hay nets with varying hole sizes, presentation of forage at different heights or presentation of mixed forage within slow-feeding hay nets, may provide additional information to better mimic the horse's natural feeding environment.

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