ABSTRACT

Title of Thesis: EFFECTS OF GRAZING MUZZLES ON GRAZING MINIATURE HORSE BEHAVIOR AND PHYSIOLOGICAL STRESS

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Grazing muzzles are highly effective at reducing forage intake in horses and are a popular tool to control horse weight. However, grazing muzzle design may cause horses stress. The objective of these studies was to determine how grazing muzzles impact behavior and physiological stress in grazing horses. Two groups of 6 miniature horses, housed individually or in a herd, wore grazing muzzles for 0, 10, and 24 h/d. Over 9 weeks, body weight, heart rate parameters, salivary cortisol concentrations, and observations of behavior were collected. Results indicate muzzling did not seem to cause physiological stress as measured by cardiac and salivary cortisol parameters but did alter grazing and locomotive patterns. Muzzling for 24 h/d was necessary for weight loss and was associated with lower heart rate and higher heart rate variability. These findings suggest that muzzles do not cause stress in horses, even if left on for 24 h/d.

EFFECTS OF GRAZING MUZZLES ON GRAZING MINIATURE HORSE BEHAVIOR AND PHYSIOLOGICAL STRESS

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Masters of Science
2019

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Acknowledgements

Thank you to my advisor, Dr. Amy Burk, for her countless hours of guidance, staunch support, and innumerable edits. For their advice and support, I'd like to express gratitude to my committee members, Dr. Rachel Dennis and Dr. Richard Erdman.

Thanks also to my graduate peers, the crew at CMREC, and to the small army of undergraduate volunteers who helped facilitate our research. Special thanks in particular to graduate student Marie Iwaniuk for her advice in planning and statistical analysis, and to undergraduate Anna Schilling for her unwavering assistance.

Sincerest thanks and much love to my family, who were patient and encouraging throughout the entire graduate school process.

Finally, thank you to all the miniature horse owners who allowed us to borrow their horses for these studies.

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List of Abbreviations

AC – Abdominal circumference

ACh – Acetylcholine

ANS – Autonomic nervous system

BW – Body weight

CNS – Cresty neck score

GC – Girth circumference

GPS – Global positioning satellite

HR – Heart rate

HRV – Heart rate variability

M0 – Unmuzzled

M10 – Muzzled for 10 hours

M24 – Muzzled for 24 hours

NE – Norepinephrine

PNS – Parasympathetic nervous system

SNS – Sympathetic nervous system

CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

There exists within the domestic equine population a problem with obesity. A survey by the University of Maryland estimated over 40% of horses in Maryland are overconditioned or obese (Jaqueth et al., 2017); the rate within the rest of the United States is between 20-51% (Pratt-Phillips et al., 2010; Thatcher et al., 2012). Obesity in horses is associated with an increased risk of diseases such as insulin resistance (Hoffman et al., 2003) and laminitis (Geor, 2008). Many horse owners are concerned about the weight and condition of their horses and use a variety of management strategies to reduce weight gain. Among Maryland horse owners, the most-used strategies were confinement to dry lots, restricted grazing time, and application of grazing muzzles (Jaqueth et al., 2017). One breed frequently in need of weight management is the Miniature horse. Some Miniature horses may be described as "thrifty", or easily able to gain weight with few extra calories, possibly due to being descended from ancestors who adapted to surviving on low quality forage (Geor, 2008). Due to this "thriftiness", Miniature horses are particularly susceptible to obesity and its associated risks (Jeffcott et al.,1968; Geor, 2008).

Equine Weight Management Strategies

Confinement to a dry lot allows a horse owner to completely control the horse's diet. Dry lots are relatively small turn-out areas that are bare of any vegetation and require feeding of hay to meet horse nutritional needs (Guthrie, 2011). While a dry lot allows for a horse owner to maintain exact control of what and how much their horse eats, it does so at the expense of inhibiting some natural horse behaviors. Dry lots are usually much smaller than pastures, and smaller turn-out areas have been shown to reduce the amount of horse voluntary exercise to the point of sedentariness (Hampson et al., 2010). Horses that are managed on dry lots for weight control may not receive turn-out time with horses that don't require management for weight loss. This can reduce or eliminate socialization opportunities for the dry lot-managed horse. Additionally, dry lots can be expensive to construct and maintain and require the constant purchase of hay.

Instead of restricting intake via a dry-lot, some horse owners elect to restrict grazing time on pasture and will turn a horse out for a few hours each day to graze. However, this is counterproductive to natural equine grazing patterns. Horses tend to graze an average of 13 hours a day, sometimes up to 17 hours a day (Longland et al., 2016). Restricting available grazing hours has been shown to cause a dramatic increase in dry matter intake during the few hours that horses are on pasture (Glunk and Siciliano, 2011). Horses have the ability to consume more than 1% of their body weight in only 3 hours of focused grazing (Longland et al., 2016). Most weight loss plans for horses involve daily dry matter intake of only 1-1.25% of their body weight. Thus, restricting grazing to 3 hours or more could give a horse enough time to exceed their daily dry matter intake requirement. Muzzling is likely necessary for weight loss if a horse is to be left on pasture for more than 3 hours daily.

Grazing Muzzles

Most grazing muzzles follow the basic design of a webbed basket with a small hole in the base. The basket is fitted over a horse's mouth and nose and held in placed via a halter or series of halter-like straps. The design prevents the horse from eating at a normal pace, reducing their intake by forcing them to take small bites of grass. Grazing muzzles are very effective at reducing horse dry matter intake while on pasture. Dry matter intake has been documented to be reduced by anywhere from 30% up to 80% when horses are muzzled (Longland et al., 2011;

Glunk et al., 2014). With pasture intake reduced, horse body weight may be maintained or reduced.

Grazing muzzles hold a distinct advantage over dry-lot and restricted grazing management strategies. With a grazing muzzle, a horse may have forage intake restricted, yet may still graze pasture with herdmates and engage in voluntary exercise. Pasture access is beneficial to horse physical and psychological health. Horses allowed to graze pasture show fewer stereotypical behaviors than horses confined to stalls (Pell and McGreevy, 1999). Pastured horses are at lower risk for gastric ulcers (Murray, 1994) and colic (Hudson et al., 2001) than horses without pasture access. Additionally, pastured horses have the opportunity to participate in voluntary exercise, which is an important factor in weight management (de Laat et al., 2016).

Grazing muzzles are very popular with horse owners (Jaqueth et al., 2017) for both their ease of use and proven effectiveness as a weight loss tool (Longland et al., 2011; Longland et al., 2016). A survey estimated that 60% of owners of overweight horses in Maryland use grazing muzzles as their main weight loss tool (Jaqueth et al., 2017). Though popular, muzzles have the potential to be a welfare issue as they inhibit some horse behaviors. The design of a muzzle is such that it encompasses the entire nose and mouth of a horse, rendering a horse unable to use its mouth to bite, groom itself or others, or communicate with facial expressions. By depriving a horse of the full use of its mouth and nose, it is possible that muzzling constitutes a welfare issue. Some research studies report that horses seemed to become fractious or depressed after muzzle application (Longland et al., 2016). There are differing opinions on how long to leave a grazing muzzle on a horse to mitigate these welfare issues. Some groups such as the British Horse Society recommend removing a muzzle after 12 hours (British Horse Society, NEWC, 2015). The ASPCA, Humane Society of the United States, and American Horse Council do not offer any recommendations for grazing muzzle use. Anecdotally, some horse owners will leave grazing muzzles on for 24 hours a day and report no behavioral differences in the horse.

HORSE BEHAVIOR

Grazing Behavior

Horses have evolved to consume small, frequent meals of forage (Clarke et al., 1990). They are selective grazers and tend to follow a step-bite-step-bite grazing pattern (Archer, 1973; Marinier and Alexander, 1991; Olson-Rutz et al., 1996). Horses will engage in periodic grazing and ingest forage throughout the day and night, with the highest grazing activity occurring shortly after dawn and before dusk (Mayes and Duncan, 1986). On average, horses spend between 63% and 75% of the day grazing, and between 49% and 50% of the night, with 15-20 distinct grazing sessions (Bott et al., 2013). That horses spend the majority of their time on grazing demonstrates the importance of allowing pasture access.

Grazing muzzles have been shown to alter the foraging behavior of horses, particularly in the area of preference according to pasture height. Unmuzzled horses tend to prefer taller sward (~17cm) over shorter grasses (~6cm) (Scherer-Hoock, et al., 2011). Short, upright grasses are preferred by muzzled horses for ease of consumption through the muzzle aperture, as longer grasses tend to buckle under the muzzle and become inaccessible for grasping with teeth (Longland et al., 2016). When attempting to ingest longer grasses, muzzled horses show signs of frustration including pawing and slamming the muzzle against the ground. (Longland et al., 2016). When longer grasses are grasped for ingestion, horses often uproot the entire sward of grass rather than crop it (Longland et al., 2016). Therefore, muzzled horses may selectively graze areas of the pasture with shorter grass. The height of the grass seems to be the determining factor in horse selection, as muzzling has not been found to affect horse preference for specific grass species (Glunk et al., 2014). This may have implications for pasture management, as muzzled horses may cause damage to pastures by uprooting plants or increasing grazing pressure on specific areas.

Social Behavior

Houpt (1990) found that when two horses are pastured together, if one starts to graze the other will follow and begin grazing as well. This is reflective of the highly social nature of horses; horses are herd animals that prefer to live in groups. Within these groups they naturally form dominance hierarchies and close relationships with conspecifics (Houpt, 1978).

Dominance hierarchies in horse herds are non-linear and are established and maintained through agonistic behaviors (Waring, 2003). Agonistic behaviors are behaviors related to social conflict and may include aggression, threats of aggression, submission, and acts of conciliation. During initial formation of a herd hierarchy there are increased instances of aggression and physical contact between horses (Waring, 2003). However, once the hierarchy has been established, instances of aggression decrease. The hierarchy is maintained mainly through threats from dominant horses and retreats from submissive horses (Waring, 2003, McDonnel, 2013). Montgomery (1957) investigated agonistic behaviors within an established herd of horses (composed of geldings and mares). Over 14 hours of observation, 488 agonistic displays were catalogued. Of these displays, 47% were bite threats and 27% were completed full-contact bites, as compared to kick actions which comprised only 6% of the displays. Over 70% of the aggressive actions observed by Montgomery (1957) were conducted via the mouth. As grazing muzzles prevent biting it is possible that muzzling may reduce the effectiveness of such threats from a dominant horse to a subordinate, thus disturbing the herd hierarchy.

To establish and strengthen close relationships, horses will engage in two main affiliative behaviors: play and allogrooming (Waring, 2003; McDonnel, 2013). As play is mostly performed by younger animals, allogrooming is the behavior necessary to form social bonds and friendships in mature adults (Dunbar, 1991; Seyfarth and Cheney, 2012). Allogrooming usually involves two horses standing antiparallel to one another and simultaneously nipping, rubbing, or nuzzling each other gently with their teeth. Typical allogrooming sessions occur on the favored areas of the neck, withers, and back, and horses will mirror each other's grooming location (Waring, 2003, McDonnel, 2013). Allogrooming is reported to lower heart rate and blood pressure in horses (Keiper, 1998; Feh and Maziéres, 1993), as well as to reduce tension after social conflict (Aureli et al., 2000). Grazing muzzles may inhibit allogrooming by preventing the full use of teeth during grooming sessions. Rubbing of the grazing muzzle on another horse may substitute for the grooming sensation but it is unknown if the horse grooming or horse being groomed may shorten or reject the session due to the different sensation.

To communicate with each other, horses make use of a wide range of facial expressions. There are at least 14 discrete facial movements in the lower face alone, the motions of which are complex and often change in rapid sequences and combinations (Wathan et al., 2015). Raising and lowering of the upper lip, pulling of the lip corners, lifting of nostrils, and lower lip flattening or relaxation are just a few ways horses augment their facial expressions (Wathan et al., 2015). Horses are predominantly visual animals and use these subtle changes in lip and nostril position to convey various emotional states. Masking the lower face with a grazing muzzle would likely inhibit communication between horses.

Coping Behavior

Horses may adopt coping behaviors in response to stress. Generally, stress may be defined as any threat to the psychological or physiological homeostasis of an individual (McEwen, 1999). A stressor will disrupt homeostasis and the body will attempt to restore balance (Seyle, 1936). Grazing muzzles may qualify as a stressor if they prevent the horse from performing natural behaviors. When natural behaviors are stymied, a horse may rely instead on coping behaviors to counter the stressor (Herd, 1991). These coping behaviors may develop into stereotypies, or repetitive behaviors that seem to serve no discernible function other than an attempt to cope (Mason, 1991; Mason and Rushen, 2006). Stereotypies may be oral or locomotive in nature; examples include cribbing, wood chewing, pawing, head-tossing, pacing, and weaving (Houpt and McDonnell, 1993). Nagy et al. (2009) reported that stress from food placed just out of reach triggered stereotypic activity in horses. The horses mainly engaged in cribbing, pawing, and head-tossing to combat the frustration of being unable to reach the food. Horses may experience a similar sense of frustration while wearing grazing muzzles. The restriction of forage intake imposed by grazing muzzles may cause the horse to react with stereotypic behaviors.

PHYSIOLOGICAL RESPONSE TO STRESS

In addition to behavioral changes, stress induces a series of physiological responses in the horse. These responses can be measured via changes in heart rate, heart rate variability, and salivary cortisol concentrations.

Heart Rate

Normal horse heart rate fluctuates between 20-45 bpm (Mlyneková et al., 2016) throughout the day. Under acute stress conditions during transport, heart rate has been observed to rise to 66 ± 13 bpm (Schmidt et al., 2010). Heart rate response to acute stress in horses is controlled by the autonomic nervous system (ANS).

The ANS is comprised of two main branches: the sympathetic and parasympathetic nervous systems (SNS and PNS, respectively). The SNS is responsible for preparing the body to respond to emergency or stressful situation, i.e. “fight or flight”. Sympathetic nervous system stimulation increases heart rate and strength of heart contractions. The PNS has the opposite function, and is more active under calm, restful situations, i.e. “rest and digest”. After a stressful situation the PNS is responsible for restoring the body’s homeostasis by decreasing heart rate (Acharya et al., 2006; Gordon et al., 2015). The slowing and acceleration of heart rate is initiated via hormones. The SNS causes release of norepinephrine (NE) while the PNS causes release of acetylcholine (ACh) (Gordon et al., 2015).

Norepinephrine is released into the bloodstream from the adrenal glands in response to increased SNS activity. After NE is transported to cardiac cells, it binds to plasma proteins within the cell membrane called beta-adrenergic receptors. This activates G proteins, composed of 3 subunits, that reside inside the cell. The G-proteins are bound to guanosine diphosphate when inactive; when activated, guanosine diphosphate is replaced by guanosine triphosphate and one of the G-protein subunits will uncouple from the main protein. The G-protein subunit diffuses through the cell cytoplasm until it encounters the membrane enzyme adenylyl cyclase and activates it. Adenylyl cyclase catalyzes the formation of cyclic adenosine monophosphate from adenosine triphosphate. Cyclic adenosine monophosphate diffuses through the cytoplasm until it binds to and activates protein kinase-A. Protein kinase-A phosphorylates and activates the protein Phospholamban. Phospholamban regulates the calcium pump in cardiac smooth muscle cells, and when activated causes sequestration of Ca^{2+} ions in the sarcoplasmic reticulum. The influx of calcium ions results in faster and stronger smooth muscle contractions, increasing the heart rate and contractile strength (Gordan et al., 2015).

The opposite effect occurs with the release of ACh hormone in response to increased PNS activity. Acetylcholine binds to muscarinic receptors within the cardiac cell membrane. Once activated, muscarinic receptors in turn activate G inhibitory proteins, which inhibit production of cyclic adenosine monophosphate. Reduced amounts of circulating cyclic adenosine monophosphate means reduced activation of protein kinase-A. Without activation of protein kinase-A, conductance of Ca^{2+} is reduced. Additionally, when the G inhibitory protein is activated a subunit (G beta-gamma) uncouples from the main protein. G beta-gamma subunits open potassium ion channels in cardiac smooth muscle cells and allow for outward flow of K^+ ions, slowing the heart rate (Gordan et al., 2015).

However, there is evidence that regulation of heart rate is not controlled solely by the SNS and PNS systems. Hays and Webster (1971) conducted a series of experiments involving administration of propranolol to sheep. Propranolol is a beta-adrenergic blocking agent that is used to treat high blood pressure and tachychardia by suppressing SNS activity. After propranolol administration, sheep were subjected to cold stress conditions and were either fed or had feed withheld. Hays and Webster (1971) demonstrated that propranolol successfully inhibited acceleration of heart rate during cold stress and withholding of feed, but only slightly reduced the heart rate acceleration observed during cold stress coupled with feeding. This observation indicates that consumption of feed may provide a pathway to increase heart rate independent of the sympathetic nervous system. Many other studies have demonstrated the same increase in heart rate during feeding, both in sheep and in cattle (Ingram and Whittow, 1962;

Young, 1966; Webster, 1967; Webster and Hays, 1968; Berzins, 1969; Christopherson and Webster; 1972).

One theory as to why heart rate increases in response to feeding is to do with maintaining oxygen levels. Christopherson and Webster (1972) found that blood pH decreased during feeding due to increases in blood partial pressure carbon dioxide. As carbon dioxide levels increased, blood pH fell, causing oxygen saturation of venous blood to fall. Oxygen consumption rate increased to compensate for the lowered oxygen saturation, inducing increased respiratory rate and heart rate. In humans, Ueda et al. (2002) demonstrated that food intake induced a significant increase in muscle oxygen consumption, thus increasing the body's oxygen demands and respiratory rate. This in turn increases heart rate as the action of the heart pumping is necessary for oxygen distribution to tissue.

Regulation of heart rate is also affected by body weight. Weight gain is linked to a higher resting heart rate; the larger a body, the harder the heart must work to supply it with blood. The opposite is also true. In humans, both resting heart rate and heart rate during activity was shown to be lower after weight loss (Hunter et al., 2012). Body weight may also affect how stress influences heart rate. Wan et al. (2003) reported that intermittent fasting in five-month-old rats was associated with lower resting heart rate and with a lower increase in heart rate during stress situations as compared to rats that were fed ad libitum.

Heart Rate Variability

Heart rate variability (HRV) has a close, inverse relationship with heart rate, increasing as the other decreases and vice versa (Kazmi et al., 2016). It's best described as the beat-to-beat fluctuations that occur within the heart rate, or as the variation in timing between each heart beat (Steward, Moser, & Ryan-Wenger, 2001; De Jong and Randall, 2005; Yasuma and Hayano, 2004). Heart rate variability reflects the changes in influence of the SNS and PNS branches on the heart (von Borrel et al., 2007). During times of stress, the SNS increases heart rate and reduces variability in the timing between heart beats to produce a more metronome-like rhythm. This ensures a consistent blood supply to the body as it prepares to deal with the stressor. During times of rest and recovery, the PNS decreases heart rate and allows for an increase in the variability between heart beats. Therefore, measurement of the variability of timing between consecutive heart beats can be used to quantify SNS and PNS activity (Rietmann, 2004).

In humans, HRV has been extensively used to assess shifts in SNS and PNS cardiac control of individuals suffering from different diseases associated with psychological disorders and stress (Thayer et al., 1996, Sloan et al., 1997, Bernasconi et al., 1998, Sato et al., 1998, Bernardi et al., 2000, Hanson et al., 2001, Papousek et al., 2002). In livestock, von Borell et al. (2007) suggests HRV may be used as assessment of stress and welfare. Greater HRV values while under stress is reflective of less SNS and greater PNS activity, indicating an individual's increased ability to respond to the stressor's demand. Lower HRV indicates the opposite, highlighting an individual's relative inability to respond to a stressor (Porges, 1995; Bazhenova, Plonskaia,and Porges, 2001).

Recently HRV has been used in horses to assess ANS response to acute stress. Schmidt et al. (2010 A) reported decreased HRV in three-year old warmbloods undergoing initial training. Lowest HRV values were found when horses were mounted by riders for the first time, indicating that in young horses learning to be mounted by a rider induces stress. Similar results were reported from a follow-up study trailering transport-naïve horses for a series of four hour trips. Low HRV values during the initial trip indicated that horses experienced a stress event. HRV values remained low overall but slightly increased during subsequent trips, suggesting that though horses were still stressed they became acclimatized to the stressor (Schmidt et al., 2010b). The same result was observed after exposing yearling horses to novel objects. During initial exposure to the novel object horses experienced a marked decrease in HRV values, indicating a shift of the ANS towards SNS influence. Horses that received further training did not show as drastic a decrease in HRV when exposed to novel objects at a later time (Visser, 2002). Werhahn (2012) examined the HRV measurements of horses not allowed turnout as compared to horses regularly turned out. When no turnout was allowed, horses exhibited lower HRV values than horses allowed free exercise, suggesting they experienced a higher degree of stress. The similar responses in HRV evoked by a wide range of stressors indicate the suitability of HRV as an assessment of horse physiological response to stress.

Similar to HR, changes in HRV are not solely driven by the presence or absence of stress. As mentioned previously, HR and HRV have an inverse relationship; HRV is dependent on HR and will change according to acceleration or deceleration in HR. Kazmi et al., 2016 demonstrated that in humans, rabbits, and rats, HRV was inversely correlated with HR. The nature of the correlation was very strong, suggesting that HRV may be entirely dependent on HR. Heart rate variability may not be able to stand alone as evidence that an individual is under stress, and HR should be taken into consideration during analysis. As an example, several studies have indicated an increase in HR during feeding. When HR increases during feeding, HRV would consequently decrease. In that situation looking at HRV alone may give the impression that an individual is experiencing stress when in fact they are not.

As body weight (BW) effects changes in heart rate, so too does it effect changes in HRV. Loss of BW is correlated with lower resting heart rate; as heart rate decreases, HRV will increase. Mager et al. (2006) demonstrated that rats maintained on intermittent fasting or caloric restriction diets had higher HRV values than rats that were fed ad libitum, suggesting that dieting produced decreased SNS activity and increased PNS activity. Dieting rats reached maximal HRV values after one month, and HRV decreased to baseline values after dieting rats were switched to ad libitum feeding. Even short-term feed restriction can alter HRV. Ohmura et al., 2010 found increased HRV measurements in horses that were deprived of feed for 24 hours, while horses on a typical feeding schedule had lower HRV measurements. Decreased HRV values are a risk factor for the development of heart disease in humans (Liao et al., 1997). If the opposite holds true and increased HRV values indicate decreased risk, then there is likely a cardiovascular benefit of dietary restriction. (Mager et al, 2006). When taken in context with heart rate and the mechanisms through which it is driven, HRV may be used to help interpret horse physiological response to being muzzled.

Salivary Cortisol

In times of acute stress, the increase in SNS activity also increases activity of the hypothalamic-pituitary-adrenal axis, which leads to increased cortisol release (Nader et al., 2010). Cortisol is a major glucocorticosteroid hormone and is produced in the adrenal cortex. Cortisol disperses to all areas of water within the body and can be detected in blood serum or saliva. Serum levels of cortisol are frequently used in research settings, but venipuncture during collection may increase stress, skewing results (Gozansky et al., 2005; Vollenhoven, 2016). Additionally, venipuncture is not always an appropriate collection technique, especially when attempting to measure stress during daily activities. On the other hand, salivary samples are easily collected without causing additional stress. Salivary cortisol is also thought to represent only the bioactive fraction of total cortisol, i.e. cortisol that remains unbound to protein. Only about 1 to 15% of serum cortisol is bioactive (Robin et al., 1977), while in saliva all cortisol is bioactive (Vining et al., 1987). Salivary cortisol is highly correlated with serum cortisol and saliva sampling has been validated as a non-invasive technique for cortisol assessment (Aardal-Erikkson, 1998; Perogamvros et al., 2010). Salivary cortisol sampling has been used successfully in horses to assess their response to stressful situations (Schmidt et al., 2010; Peeters et al., 2011; von Lewinski et al., 2013).

Cortisol production has been demonstrated to increase during exposure to aversive situations. For example, cortisol increased not only when livestock were subjected to acute stress from procedures such as castration, but also when they were separated from conspecifics, mixed with unfamiliar animals, restrained, transported, or frustrated from the inability to perform a behavior or obtain a reward (Kent and Ewbank, 1983; Boissy et al., 1997; Sylvester et al., 1998; Mason et al., 2001; Mormede et al., 2007). The opposite effect seems to occur when an individual is subjected to chronic stress. In humans, suffering from chronic back pain or psychological 'burnout' is associated with lower cortisol levels (Pruessner et al., 1999; Muhtz et al., 2013). The same effect has been observed in chronically lame sheep (Ley et al., 1991) and in calves that are kept in long term social isolation (Van Reenen et al., 2000). Houp et al. (2001) reported that pregnant mares housed in tie stalls that received only 30 minutes of exercise every 14 days had lower cortisol levels than mares that were exercised 30 minutes every day. Similar results were reported by Visser et al. (2008) after examining how horses responded to long term individual or paired housing. Horses that were stabled individually for 12 weeks had lower cortisol levels than horses that were stabled in pairs. When compared to baseline levels, salivary cortisol sampling will provide a useful tool to assess both acute and chronic stress of horses wearing grazing muzzles.

OBJECTIVES

Very little research has been conducted that focuses specifically on how horses are behaviorally and physiologically impacted by wearing a grazing muzzle. Similarly, there has been little research that indicates or supports a minimum or maximum amount of time for muzzle wear to mitigate welfare issues that may arise. We hope to fill these gaps in the literature by conducting two studies, each exploring how wearing grazing muzzles for different lengths of time affects the behavior and physiology of individual or group-housed horses. We hypothesize that horses wearing muzzles for longer periods of time will exhibit more signs of behavioral and physiological stress than horses that are unmuzzled or muzzled for shorter periods.

CHAPTER 2: EFFECTS OF GRAZING MUZZLES ON BEHAVIOR AND PHYSIOLOGICAL STRESS OF INDIVIDUALLY HOUSED MINIATURE HORSES

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ABSTRACT

Grazing muzzles are used to control weight in horses by limiting dry matter intake through a small hole. Muzzles cover the mouth and nose and can impede self-grooming and biting. Therefore, use of grazing muzzles may negatively affect horse welfare. The objective of this study was to assess the effects of muzzling for different lengths of time on horse behavior and physiological stress. Six mature miniature horses (initial BW of 104.6 ± 8.9 kg; BCS of 6.0 ± 0.4) were studied during fall 2017. The study was a 3x3 Latin Square with horses receiving one of three treatments over three 21 d periods. Treatments were unmuzzled (M0), muzzled for 10hr (M10, 0830-1830 h), or muzzled for 24hr (M24). After a 10d acclimation, horses were housed in adjacent individual 0.11 ha grass paddocks for 24hr/d. Daily at 0800h, horses were groomed and fed 0.5 oz vitamin and mineral supplement; muzzles were re-applied at 0830h. Body morphometrics were assessed at the start and end of each period. Each horse's willingness to accept muzzle application was measured daily using a 1 (strongly accepts) to 5 (strongly rejects) scale. Horse behavior was monitored twice weekly with video recordings for 1h each in the morning and afternoon. Physiological stress was assessed weekly using salivary cortisol concentrations, heart rate (HR), and heart rate variability (HRV). Data were analyzed as repeated measures with treatment and week as main effects. M24 horses lost approximately 0.5kg BW while M0 and M10 horses gained 1.5kg BW ($P = 0.01$). M24 horses spent more time grazing than M10 horses ($P = 0.04$). M0 horses spent more time walking than M10 or M24 horses ($P = 0.02$). M10 horses spent more time resting than M0 or M24 horses ($P < 0.01$), and a trend was observed where M10 horses spent more time lying down than M24 horses ($P = 0.06$). There was no effect of treatment on behaviors associated with frustration including pawing and head shaking. There was no effect of treatment on muzzle acceptability score; however, all horses were less accepting of muzzle application by the third week of each experimental period (P = 0.01). Salivary cortisol concentrations were not different between treatments. HR was lower and HRV was higher in M24 horses than in M0 horses (P = 0.02, 0.04 respectively). Muzzling horses while on pasture for 24hrs a day resulted in a loss of BW and altered physiologic response of the autonomic nervous system but did not appear to negatively impact behavior.

KEY WORDS behavior, grazing muzzles, miniature horse, physiological stress

INTRODUCTION

It has been estimated that 1.7% of horses in the United States are overconditioned with a body condition score ≥7 (USDA, 2015), but this is likely an underestimate. Based on other reported surveys, the prevalence of overconditioned horses was at 51% in Virginia (Thatcher et al. 2012), 48% in North Carolina (Pratt-Phillips et al., 2010), and 40% in Maryland (Jaqueth et al., 2017). To control or reduce horse weight, horse owners may resort to diet restriction by limiting grazing time, confinement to a bare paddock, or application of a grazing muzzle. Limitation of grazing time has been shown to have minimal effect on intake restriction. Horses with restricted grazing time have been shown to double their intake rate and can consume approximately 55% of their daily digestible energy requirements within the first 4 hours of a restricted grazing period (Glunk et al., 2011). Management via horse confinement to bare paddocks, or dry lots, provides owners the ability to strictly control diet through hay rationing. However, dry lots don't allow use of available pasture and increase hay costs (Jaqueth et al., 2017). Denying the ability to graze may cause horses to develop behavioral stereotypies (Roberts et al., 2017) and the relatively small size of dry lots may decrease horse voluntary exercise (Hampson et al., 2010B). Grazing muzzles have gained in popularity as a management tool due to both their documented success at limiting horse forage intake (Longland et al., 2011) and their relatively low expense to the owner. Horses wearing grazing muzzles may socialize with herdmates and more closely mirror natural grazing patterns on pasture as compared with horses that are managed with restricted grazing or dry lots. While popular for diet restriction, muzzles may present an animal welfare issue. Muzzle design is such that the mouth and nose are usually entirely encompassed in a basket; this can prevent self-grooming and self-defensive behaviors. Longland et al. (2016) reported observations that muzzled animals appeared fractious or despondent. Anecdotally, owners report horses running from muzzle application or damaging, destroying, or losing muzzles while on pasture. Longland et al. (2016) reported that it is necessary to leave grazing muzzles on for at least 10 hours to reduce the rate of weight gain, while longer periods may be required to effect weight loss. It is unclear whether muzzling for shorter intervals may mitigate potential animal welfare issues. While many studies have been conducted on grazing muzzle efficacy (Glunk et al., 2013; Glunk et al., 2014; Longland et al., 2011; Longland et al., 2016; Venable et al., 2016), few studies have been conducted on how muzzling affects horse behavior. Therefore, the objective of this study was to assess changes in horse physiological stress and behavior under continuous muzzling or muzzling for the minimum recommended time.

MATERIALS AND METHODS

This study was conducted at the University of Maryland's Central Maryland Research and Education Center in Ellicott City, MD from October 11 to December 13, 2017 and was approved by the Institutional Animal Care and Use Committee at the University of Maryland (R-SEP-17-37).

Animals and Design

Six Miniature Horses (three geldings and three mares), aged 9.3 ± 1.8yr, BW of 230 ± 19.6 kg, and BCS 6 ± 0.4 (Henneke, 1985) were used in the study. Horses were free-leased to the University from private owners, contingent upon proof of negative Coggins, prior vaccination of Rabies, Tetanus, and Rhinopneumonitis, and overall good health. Horses were required to have at least six months of prior experience wearing muzzles. After all horses arrived, they received a 10 d acclimation to the facility prior to the start of the study where they were allowed access to a 0.03 ha fenced dry lot that had a water source and run-in shed, and an adjacent 0.08 grass paddock. On d 1 of the acclimation period, all horses were dewormed based on BW (Ivermectin, Bimeda Inc., Oakbrook Terrace, IL) and received hoof trims and dental floating from contracted professionals.

The study was designed as a 3 x 3 Latin Square in which the six horses were blocked by period (21 days each) and horse within paddock. During each period, horses were individually housed in adjacent 0.11 ha grass pastures with an individual run-in shelter and had free access to water and a white salt block (Roto Salt Company, Penn Yan, NY). Prior to the start of each period, pastures were mowed to a height of 12.7 cm to allow for normal grazing behavior and to provide enough biomass for each horse to meet or exceed its daily digestible energy requirements (NRC, 2007). Horses remained in their individual grazing cells for the duration of each period, after which, horses were moved to a rested 0.11 ha individual grazing cell to begin a new period (Figure 2.1). Grazing cells were divided using fiberglass push-in posts with 2 strands of electrobraid with a top height of 76.2 cm. At 0800 h each day, horses were groomed and fed ~ 14 g of a supplement (VitaPlus Complete Vitamin and Mineral Supplement, Farnam Companies Inc., Phoenix, AZ). The supplement was given to meet or exceed the vitamin and mineral requirements of horses fed forage only diets (NRC, 2007). At 0800 h and 1600 h each day, horses were visually assessed for illness or injury.

Treatments

Treatment consisted of continuous grazing with either no grazing muzzle (**M0**), a grazing muzzle applied for 10 h from 0830 to 1830 h (**M10**) or a grazing muzzle applied for 24 h (**M24**). Muzzles (Tough 1 Grazing Muzzle, JT International, Indianapolis, IN) were fit such that: 1) two fingers were able to fit between the noseband and nose of the horse, and 2) there was a one-inch gap between the base of the muzzle and the horse's lips. Muzzles were removed once daily at

0800 h to ensure proper fit as well as for inspection of the muzzle for damage and horse for injury and then re-applied.

Body Morphometrics

Body weight (**BW**) was determined at the start of each week during each period using a platform scale (Preifert, Mt. Pleasant, TX). Additionally, body condition score (**BCS**, Henneke et al., 1983), cresty neck score (**CNS**, Carter et al., 2009), girth circumference (Carter et al, 2009), and abdominal circumference (Carter et al., 2009) was assigned at the beginning (d1) and end (d21) of each period by a trained investigator.

Behavior

An ethogram was adapted from Ransom et al. (2009) and McDonnell (2003) to quantify frequency and duration of certain behaviors (Table 2.1). On days 3, 5, 10, 12, 17, and 19 of each period, horses were continuously observed using a DVR and video cameras (1080p Security System, Annke, Rowland Heights, CA) for a period of 180 minutes at 2 h after sunrise and 3 h before sunset (9:08 ± 4m and 14:15 ± 10m). Six cameras, one positioned in a corner of each grazing cell, were set at an angle to capture the entire area of the respective grazing cells. Visual observations of the weather were conducted prior to recording sessions; if rain was present, recording was rescheduled for the next clear day. Behavioral scoring was conducted using BORIS software (v. 5.1.3, Friard and Gamba, University of Turin, Turin, Italy) on the first 60 minutes of each video observation. Behaviors listed in the ethogram were classed as state and point behaviors and logged by three separate observers for both frequency and duration. Analysis of video recordings was randomized to avoid observer bias. Inter-observer and intra-observer reliability, as measured with Cohen's kappa coefficient, were high ($K \geq 0.91$). Behavioral data were calculated as hourly means for behaviors that occurred throughout the duration of video recording.

Grazing Muzzle Acceptance

Willingness to accept the grazing muzzle being re-applied was scored each morning by the horse's handler. A five-point system was developed, with "1" indicating the highest degree of willingness and "5" indicating the lowest degree of willingness (Table 2.2).

Physiological Stress

On days 6, 13 and 20 of each period, heart rate (**HR**), heart rate variability (**HRV**), and salivary cortisol levels were assessed. Heart rate variability was monitored using equine heart rate monitors (HRMs; S10; Polar Electro Inc, Lake Success, NY). Prior to application, a small portion of the hair coat was shaved on the horses' girth area to ensure better contact of the heart rate monitor with the horse. On assessment days, heart rate monitors were secured to each horse with a girth strap at 1400 h ± 30 min and removed after 5 min. All horses performed the same grazing activity for the 5 min duration of heart rate monitoring. HR and HRV was analyzed with Kubios software (Kubios Oy, Kuopio, Finland) Artefacts were corrected by setting the custom filter of the program at 0.3 (Schmidt et al., 2010). Data were detrended according to the procedure described by Tarvainen et al., 2002. Salivary cortisol was measured via saliva samples collected at 1000 h on days 6, 13 and 20 of each period using Salivette tubes (Sarstedt, Numbrecht, Germany) with cotton wool. The cotton was inserted and rubbed into the horse's mouth and then placed under the tongue of the horse for 60 s. All saliva samples were centrifuged for 15 minutes and frozen at −20°C immediately after collection. Samples were assayed in triplicate using the Salimetrics Salivary Cortisol Enzyme Immuno-Assay Kit (Diagnostic System Laboratories Inc., Webster, TX, USA) according to the manufacturer's protocols, previously described in detail by Schwinn et al. (2016). The minimal detectable concentration was 0.007 μg/dL, and the intra- and inter-assay coefficient of variation were 14.3% and 15.6% respectively.

Forage Availability and Nutrient Composition

Vegetative cover was determined at the beginning of each period by step point method (Evans and Merton, 1957) to assess pasture density. Biomass (kg/ha) available for grazing was determined at the beginning and end of each period by randomly placing a $0.25m^2$ quadrat in the field thrice and then hand harvesting grass with clippers within the quadrat at a height of 7.62 cm. At the beginning of each period, forage samples (~ 500 g), were randomly collected at a height of 7.62 cm by hand clipping and shipped immediately on dry ice for determination of nutrient composition by a commercial laboratory (Equi-analytical Laboratories, Ithaca, NY) utilizing traditional analytical methods to determine moisture, dry matter, digestible energy, protein, fiber, carbohydrates and minerals.

Statistical Analysis

Prior to the study, a power analysis was conducted using the GLMPOWER procedure of SAS (SAS, v. 9.4, SAS Institute Inc. Cary, NC) with the following parameters: 1) three experimental treatments, 2) an alpha set at 0.05 to declare significance, 3) a power of 80% to detect potential differences in treatment means, 4) a projected difference of 0.09 ug/dL in salivary cortisol concentration between treatment means, and 5) an average standard deviation value of 0.07 which was calculated based on standard deviations published in the literature for similar studies (Kedzierski et al., 2013; Peeters et al., 2013; Kang and Lee, 2016). The results of the power analysis indicated that five horses were required per treatment. Due to resource limitations, a 3 x 3 Latin square design was implemented which resulted in six horses per treatment, yielding 86.4% power. Data were analyzed using repeated measures Analysis of Variance (ANOVA) in PROC MIXED (SAS 9.4.) with treatment and week serving as fixed effects and horse and period serving as random effects. Response variables analyzed were muzzle acceptance score, gain in bodyweight (**BW**), girth circumference (**GC**), abdominal circumference (**AC**), HR, HRV, and salivary cortisol concentrations. Data for frequency and duration of behaviors were analyzed using repeated measures ANOVA with treatment serving as a fixed effect and horse and period as random effects. Data are reported as LSMEANS ± SE. BCS and CNS were not analyzed as they remained constant for each horse throughout the study. Normality and equality of variance of the sampling mean distribution of sampling means were tested using the Shapiro-Wilk test and Levene test, respectively (Sachs, 1984). The null hypothesis for each test was not rejected; therefore, it was concluded that the normality and equality of variance assumptions had been met to perform to the repeated measures ANOVA. Results were considered significant at the $P < 0.05$ level.

RESULTS

Body Morphometrics

An overall effect of treatment on BW was observed such that horses on M24 weighed less overall than M10 horses ($P = 0.02$, Table 2.3). Additionally, M24 horses exhibited loss of BW while M0 and M10 horses gained a similar amount of BW ($P < 0.01$, Table 2.3). An interaction between treatment and week was also observed whereby M0 horses and M10 horses gained the most BW during Wk 1, while M24 horses lost the most ($P = 0.05$, Table 2.3). There was no effect of treatment, week, or their interaction on GC or AC. Average AC and GC for all horses was 134.5 ± 2.0 cm and 117.2 ± 1.8 cm, respectively ($P > 0.05$).

Behavior

Application of grazing muzzles altered the amount of time horses spent resting, walking, grazing, autogrooming, and laying down but it varied with muzzle treatment ($P < 0.05$; Table 2.5). Compared to when horses were not muzzled, muzzling for 10 h/d increased resting behavior while muzzling for 24 h/d decreased resting behavior ($P < 0.01$). There was also a trend for muzzling 24 h/d to reduce time spent laying down ($P = 0.07$). Muzzling for either 10 or 24 h/day decreased the amount of time spent walking compared to when horses were not muzzled ($P = 0.01$). Horses muzzled for 24 h/d spent more time grazing than when muzzles were left on for 10 h/d ($P = 0.04$), but both muzzle treatments spent about the same amount of time grazing compared to when muzzles were not worn. Muzzling horses for 24 h/d reduced the amount of time horses spent autogrooming with their mouths ($P = 0.04$). Some behaviors were not performed and thus data was not presented. There was no effect of treatment on time spent performing other behaviors and no week or treatment by week interaction was observed.

Muzzle Acceptance

Muzzle acceptance scores did not differ between the M10 and M24 treatments ($P > 0.05$; Table 2.4). However, there was an effect of week with muzzle acceptance scores increasing for both M10 and M24 by wk 3. Acceptance scores were 2.7 ± 0.1 and 2.6 ± 0.1 for wk 1 and 2, respectively, and increased to 3.0 ± 0.1 in wk 3 ($P = 0.01$). There was no interaction of treatment by wk observed ($P > 0.05$).

Physiological Stress

The effects of muzzle treatment and wk on HR, HRV, and salivary cortisol concentrations in miniature horses are summarized in Table 2.6. There was an effect of treatment such that M0 horses had higher average HR than M24 horses ($P = 0.02$). There was an effect of week on HR where average HR in wk 1 for all horses was higher than average HR in wk 3 *($P = 0.05$)*. There was no effect of treatment by week interaction on HR ($P > 0.05$). There was an effect of treatment such that M0 horses had lower average HRV than M24 horses ($P = 0.04$). There was no effect of week or treatment by week interaction on HRV *($P > 0.05$)*. There was no effect of week, treatment, or their interaction on salivary cortisol concentrations in horses ($P > 0.05$).

Forage Availability and Nutrient Composition

Mean pasture cover was 90.0 ± 2.0% grass and legume with 10.0 ± 2.0% weed and bare soil. Mean biomass before grazing was 464.9 ± 36.4 kg/ha and 366.3 ± 25.0 kg/ha after grazing. Mean pasture height before grazing was 28.4 ± 3.8 cm and 18.2 ± 3.1 cm after grazing. Nutrient composition is summarized in Table 2.7. Pasture cover, biomass, and height values indicate sufficient grazing material available to sustain animals throughout the study

DISCUSSION

The objective of this study was to assess how behavior and welfare of grazing horses were affected by muzzling for 10 hours a day and 24 hours a day. There were two main findings from this study. The first was that wearing muzzles for 10 hours a day was not effective at controlling weight gain; muzzles had to be left on for 24 hours to induce weight loss. The second finding was that muzzling horses did not appear to increase physiological stress when assessed by behavior, heart rate, heart rate variability, and salivary cortisol.

Due to the relatively short length of treatment time (three weeks), it’s possible that our BW observations are more reflective of changes in gut fill rather than significant weight loss. Horses on the M10 treatment gained a similar amount of BW as unmuzzled horses, suggesting they engaged in compensatory grazing after muzzles were removed at 1830h. Glunk and

Siciliano (2011) found that horses on restricted grazing time had higher dry matter intake rates per hour than free grazing horses. Rather than restricting time allowed for grazing, muzzles limit intake by restricting the ability to graze. When a grazing muzzle is removed after being worn for a period of time, a horse may increase intake rate in response to the prior restriction. Dowler and Siciliano (2009) reported that horses are able to consume approximately 55% of their daily maintenance DE requirements within the first 4 hours of a grazing period. It's likely that 12 hours overnight unmuzzled was sufficient for M10 horses to consume excess of their daily digestible energy requirements, resulting in weight gain. Our results contrast with those of Longland et al. (2016), who reported that ponies muzzled for 10 h/d exhibited reduced rate of weight gain as compared to ponies allowed to freegraze. This may be explained by experimental design, as ponies used in the Longland et al. (2016) study were larger than miniature horses and were also lightly exercised three times a week. The treatment by week interaction observed during Wk 1 may be due to the novelty of new treatment assignments. Horses on M0 and M10 treatments possibly gained more BW during Wk 1 because they were newly afforded the ability to free graze (in M10's case, after muzzle removal at 1830) and took full advantage. The increase in BW experienced by M24 during Wk 3 may support the findings of Longland et al. (2016), who observed muzzled ponies lose weight during the first week of a trial and gain weight in subsequent weeks, suggesting that ponies became more adept at grazing with muzzles over time. The lack of change in horse BCS, CNS, AC, or GC indicates that the three week period was not enough time to effect changes in these parameters.

Video recordings revealed particularly interesting behavior patterns in horses on the M10 treatment. We speculate that M10 horses spent less time grazing and more time resting than M24 because M10 horses were allowed increased access to forage after muzzle removal at 1830h.

Afternoon video recordings ended before muzzles were removed. While not directly captured on video, it's likely that M10 horses were more active and grazed after being unmuzzled. The finding of increased time resting coupled with weight gain by M10 horses supports the conjecture they engaged in compensatory grazing after muzzles were removed. Increased time spent walking by M0 horses as compared to M10 and M24 may have resulted from increased steps taken while grazing. Horses are selective grazers and tend to follow a step-bite-step-bite grazing pattern (Archer, 1973; Marinier and Alexander, 1991; Olson-Rutz et al., 1996). It's possible the intake restriction imposed by a muzzle dampens a horse's inclination to be selective, as priority shifts from selection to meeting daily digestible energy requirements. As a result, muzzling may have the unintended side effect of reducing voluntary exercise. Voluntary exercise is an important aspect of horse weight control and further study on the effects of muzzling on amount of horse voluntary exercise is needed. Though no horse spent much time on autogrooming, horses on M0 used their mouths to groom significantly more than horses on M24. Muzzled horses were observed briefly swinging the muzzle towards an area needing grooming on multiple occasions. M0 also performed this action, but frequently coupled it with sustained contact of teeth to skin. This finding suggests muzzling hampers a horse's ability to groom itself with its mouth. Pawing, head tossing, and head shaking have been associated with frustration in horses (Houpt and McDonnell, 1993). Very few instances of head shaking were observed; these tended to happen on days of high insect activity. No pawing behavior was observed during the video observation period. One horse in particular engaged in a substantial amount of head tossing while on M24 treatment; however, no other horses were observed performing this behavior. It is likely that frustration responses due to wearing a muzzle are determined on an individual basis, according to each horse's personality, and that some horses are more accepting of grazing while muzzled than others.

Overall low muzzle acceptance scores indicate horses did not object to muzzle application. The slight increase in acceptability score for Wk 3 implies horses became less accepting of muzzle application as time went on. However, Wk 3 average muzzle acceptance score was a '3', indicating that horses stood still for muzzling with only minor signs of avoidance. Stronger reactions such as rearing, running from the handler, and bucking after application were not observed, suggesting that horses accustomed to wearing muzzles aren't distressed by application.

The higher HR observed in M0 horses as compared to M24 horses may be due to effects of feeding. Early studies demonstrate that HR increases during feeding in sheep and cattle (Ingram and Whittow, 1962, Young, 1966; Webster, 1967; Webster and Hays, 1968; Christopherson and Webster; 1972). Christopherson & Webster, 1972 showed that feeding in sheep caused a decrease in blood pH due to increased carbon dioxide saturation. This results in an increase in oxygen intake to increase oxygen saturation. In humans, Ueda et al., 2002 found that food intake caused an increase in muscle oxygen consumption, which increased the body's oxygen demand and respiratory rate. To meet the increased demand for oxygen distribution to the body HR would need increase. In this study HR and HRV were measured at 1400 h when horses were unrestrained and grazing. M0 horses had unrestricted intake and therefore ingested more during grazing than M24 horses, possibly inducing the higher HR.

Heart rate variability has been used extensively in humans to assess the shift in balance of the autonomic nervous system in response to stress (Rechlin et al., 1994, Sloan et al., 1994, McCraty et al., 1995, Thayer et al., 1996, Laio et al., 1997, Bernardi et al., 2000) and in recent years has shown use as a barometer for the stress response in horses (Schmidt, 2010; Schmidt et al, 2010; Visser, 2002; Werhahn, 2012; Mareiki, 2013, von Borell et al, 2007). Lower HRV values reflect a shift toward more sympathetic influence, while higher HRV values indicate a shift toward parasympathetic influence. Observed higher HRV in M24 horses may indicate they were less stressed than M0 horses. However, the lower HRV observed in M0 horses is likely due to the increase in HR from feeding. Heart rate variability is highly dependent on HR and the two have an inverse relationship, with HRV declining as HR rises and vice versa (Kazmi et al., 2016). Higher HRV in M24 horses may simply be an indicator of lower HR during grazing rather than less stress when compared to M0 horses. In addition, some forms of dietary restriction have been shown to induce higher HRV values. Mager et al. (2006) found that rats on caloric restriction exhibited higher HRV values than rats that were fed ad libitum. Ohmura et al., 2010 reported that 24 hours of withholding feed in Thoroughbred horses caused lower HR and higher HRV than in horses not feed restricted, suggesting that feed restriction reduces sympathetic nerve activity and engages energy conservation in response to caloric deficit. While horses in the present study were allowed to graze and not deprived of feed entirely, muzzling for 24 hours resulted in horse weight loss indicative of caloric restriction. It's possible this form of caloric restriction effected similar changes in horse HR and HRV as found in 24 hour feed restriction. The overall lower mean HR in Wk 3 compared to Wk 1 suggests horses became less stressed towards the end of each period; it's likely by the third week of each period horses had adjusted to their new treatment and paddock assignments and were more relaxed in their environment.

Salivary cortisol sampling has been validated as non-invasive cortisol assessment technique in horses (Schmidt et al., 2010) and has been used as a reliable indicator of horse stress

(Peeters et al., 2011; von Lewinski et al, 2013). Mean salivary cortisol values found in this study are within the ranges of normal values reported by Bohák et al. (2013) and Aurich et al. (2015). The lack of effect on salivary cortisol suggests that muzzling for either 10 or 24 hours a day had no ill effects on stress.

CONCLUSIONS

This study assessed the behavioral and physiological response of grazing horses to being muzzled for different amounts of time. Muzzled horses did have altered behavioral responses, but an increase in incidence of stereotypic behaviors was not observed. Additional, salivary cortisol data suggest a lack of stress in response to being muzzled. This suggests that even though grazing muzzles may need to be left on 24hrs to achieve weight control, they do not seem to induce stress.

Table 2.1. Ethogram developed to quantify behaviors in miniature horses with or without grazing muzzles applied.[1]

Behavior	**Description**
Rest	Standing with weight resting on 3 or 4 legs for longer than 2 minutes
Walk	Slow four beat gait of forward movement wherein at least one foot has contact with the ground
Graze	Using teeth to shear and chew grass
Autogroom (mouth)	Using teeth, mouth, or muzzle to scratch part of own body
Lay	Lying with sternum in contact with ground surface, legs folded under body or stretched out on side with legs stretched out
Stand	Standing upright with all 4 feet simultaneously in contact with the ground.
Drink	Placing mouth in water trough
Roll	Drop to knees, roll onto side, then back and perhaps all the way over, then return to feet
Head rub	Rubbing any part of head on ground or any part of fence, shelter, or water trough
Head shake	Rapid rhythmic rotation of head only
Shake	Rapid rhythmic rotation of head, neck and upper body along axis while standing with feet planted
Trot	Two beat diagonal gait of forward movement
Canter	Three beat gait of forward movement
Gallop	Fast four beat gait of forward movement wherein all four feet leave ground at some point
Autogroom (foot)	Using hind foot to scratch part of own body
Scratch	Rubbing any part of body against part of the fence, shelter, or water trough or using hind foot to scratch part of own body
Head toss	Head rapidly and repetitively moved up and down
Paw	Striking a vertical or horizontal surface or the air with forelimb

[1]Adapted from Ransom, J.I., Cade, B.S. (2009) Quantifying equid behavior: A research ethogram for free-roaming feral horses. U.S. Geological Survey Techniques and Methods Report 2-A9; and from McDonnell, S. (2003) The Equid Ethogram. Eclipse Press, Lexington KY.

Table 2.2. Scoring system used to quantify miniature horse willingness to accept muzzle application[1].

Score	Corresponding Behaviors
1	Proactively places nose in muzzle; ears forward
2	Stands quietly during and after muzzle application
3	Stands for muzzling but indicates signs of avoidance (looking away, leaning away from handler, shifting weight, ears to side)
4	Actively avoids muzzle (tosses head, dances around, paws at ground, flicks tail, rubs muzzle on object after application)
5	Exhibits defensive behaviors (biting, kicking, striking, ears pinned, flees from handler, bucking or slamming muzzle into ground after application)

[1]'1' indicates highest degree of willingness and '5' indicates lowest degree of willingness.

Table 2.3. Effect of muzzle treatment and week on miniature horse body weight (kg).

	Bodyweight, kg				Gain in Bodyweight, kg			
Treatment[1]	M0	M10	M24	Weekly Average	M0	M10	M24	Weekly Average
Week								
1	108.2	110.2	106.8	108.4	2.6[a]	3.8[a]	-3.6[b]	0.9
2	108.7	110.9	104.3	108.0	0.5	0.8	-2.4	-0.3
3	109.8	112.0	105.8	109.2	1.1	1.1	1.4	1.2
Treatment Average	108.9[ab]	111.0[a]	105.6[b]		1.4[a]	1.9[a]	-1.5[b]	
Treatment	*P* = 0.0243, SE ± 1.6				*P* = 0.0084, SE ± 0.6			
Week	NS, SE ± 0.8				NS, SE ± 0.7			
Treatment*Week	NS, SE ± 1.4				P = 0.0528, SE ± 1.2			

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.
[a,b,c]Means in rows with different superscripts differ ($P < 0.05$).

Table 2.4. Effect of muzzle treatment and week on average daily muzzle acceptance score in miniature horses.

Muzzle Acceptance Score			
Treatment[1]	M10	M24	
Week			SE
1	2.7	2.7	0.1
2	2.5	2.7	
3	3.0	3.0	

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.

Table 2.5. Effect of muzzle treatment on mean percentage of time miniature horses spent performing different behaviors within a 2 hr daily time period[1].

	Treatment[2]			
	M0	M10	M24	
Behavior				SE
Rest	11.0[a]	27.6[b]	3.8[c]	1.6
Walk	8.3[a]	5.7[b]	5.5[b]	0.8
Graze	66.3[ab]	49.7[a]	78.0[b]	8.9
Autogroom (mouth)	0.1[a]	0.1[ab]	0.03[b]	0.03
Lay	4.7[x]	13.5[x]	1.8[y]	3.1
Stand	70.3	66.7	79.5	6.4
Drink	0.1	0.1	0.1	0.02
Roll	0.2	0.1	0.1	0.1
Head rub	0.2	0.2	0.02	0.1
Head shake[3]	22.3	26.8	17.7	7.7
Shake[3]	2.0	3.5	1.5	1.0
Trot[4]	-	-	-	-
Canter[4]	-	-	-	-
Gallop[4]	-	-	-	-
Autogroom (foot) [4]	-	-	-	-
Scratch[4]	-	-	-	-
Head toss[4]	-	-	-	-
Paw[4]	-	-	-	-

[1] Behavior was assessed 6 times during each period for 2hr each day for a total of n = 12 for each horse. Behaviors were not scored to be mutually exclusive; i.e. 'walking' may have occurred concurrently with 'grazing'.
[2] M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.
[3]Behaviors are point events of extremely short duration; reported as frequency of occurrence over 12 hours.
[4]Behaviors were not performed often enough to provide sufficient data.
[a,b,c]Means within a row with unlike superscripts differ ($P < 0.05$).
[x,y,z]Means within a row with unlike superscripts differ ($P = 0.0666$).

Table 2.6. Effect of muzzle treatment and week on Least Square Means of heart rate (HR), heart rate variability (HRV), and salivary cortisol concentrations (SC) in grazing miniature horses.

	HR, bpm				HRV, ms				SC, ug/dL			
Treatment[1]	M0	M10	M24	Weekly LSMeans	M0	M10	M24	Weekly LSMeans	M0	M10	M24	Weekly LSMeans
Week												
1	70.4	62.6	59.3	64.1^{x}	851.9	970.7	1017.3	946.6	0.1	0.17	0.11	0.13
2	65.3	61.1	56.3	60.9^{xy}	922.2	1014.3	1073.9	1003.5	0.13	0.13	0.10	0.12
3	63.7	58.8	56	59.5^{y}	972.5	1043.7	1089.4	1035.2	0.13	0.13	0.12	0.12
Treatment LSMeans	66.5^{a}	60.9^{ab}	57.2^{b}		915.5^{a}	1009.6^{ab}	1060.2^{b}		0.12	0.14	0.11	
Treatment	P = 0.0204, SE ± 1.8				P = 0.0405, SE ± 33.2				NS, SE ± 0.02			
Week	P = 0.0561, SE ± 1.7				NS, SE ± 27.0				NS, SE ± 0.01			
Treatment*Week	NS, SE ± 2.8				NS, SE ± 46.5				NS, SE ± 0.02			

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs
[a,b,c]Means within a row with unlike superscripts differ (*P < 0.05*).
[x,y,z]Means within a column with unlike superscripts differ (*P < 0.05*).

Table 2.7. Summary of nutrient composition of pasture at the beginning of each period.

Nutrients	Period 1	Period 2	Period 3
DE, Mcal/kg	2.19	2.43	2.62
CP, %	23.2	21.8	21.6
ADF, %	31.0	25.2	22.3
NDF, %	50.7	45.3	38.5
WSC, %	9.9	20.5	29.1
ESC, %	5.6	15.2	21.9
Starch, %	0.9	0.8	1.1
Ca, %	0.43	0.40	0.39
P, %	0.40	0.48	0.32
Mg, %	0.25	0.24	0.24
Na, %	0.011	0.011	0.012

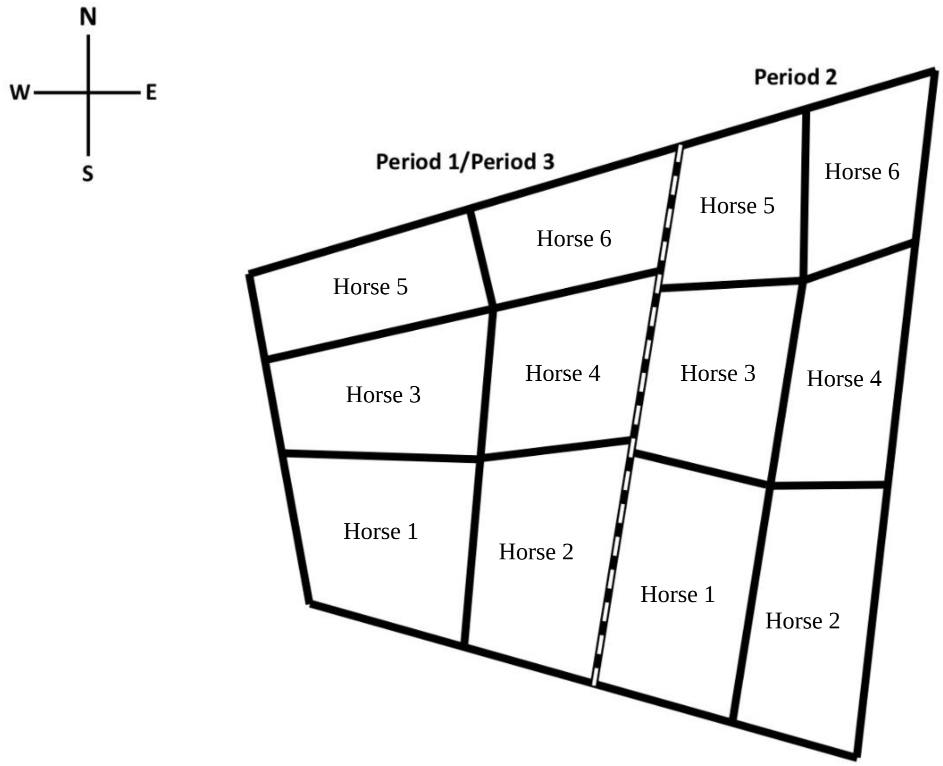

Figure 2.1. Diagram of individual 0.11 ha grass grazing cells separated by electric fencing used for grazing miniature horses with or without muzzles applied. The same grazing cells were used in period 1 and 3 after a three-week pasture rest and regrowth period.

CHAPTER 3: EFFECTS OF GRAZING MUZZLES ON VOLUNTARY EXERCISE AND PHYSIOLOGICAL STRESS OF MINIATURE HORSES IN A HERD

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ABSTRACT

Grazing muzzles are a popular and effective management tool used to prevent weight gain in obese-prone equids. Concerns have been raised over the possible negative impacts they may have on horse welfare as muzzles interfere with social activities. The objective of this study was to assess the effects of grazing muzzles used for different lengths of time on behavior and physiological stress of horses housed in a herd. Six mature miniature horses (initial BW of 114.9 ± 11.4 kg; BCS of 6.0 ± 0.8) were studied during fall 2018. The study was a 3x3 Latin Square with horses receiving one of three treatments over three 21 d periods. Treatments were unmuzzled (M0), muzzled for 10 h (M10, 0830-1830 h), or muzzled for 24 h (M24). After a 10 d acclimation period, horses were housed as a herd on a 0.6 ha grass pasture for 24 h/d. Body weight (BW) was assessed weekly. Reaction to muzzle application was scored daily using a 1-5 point scale. Physiological response to muzzling was assessed weekly using salivary cortisol concentrations and changes in heart rate (HR) and heart rate variability (HRV). Voluntary exercise was measured for 24 h twice during each period using a GPS device. A dominance rank was assigned to horses on d 21 of each period. Data were analyzed as repeated measures with treatment and week as main effects. M24 horses lost BW while M0 and M10 horses gained BW ($P = 0.01$). M24 horses spent more time grazing ($P = 0.04$) and less time resting ($P = 0.03$) than M10 horses. M0 horses spent more time walking than M24 horses ($P < 0.01$), more time cantering than M10 ($P = 0.02$), and more time trotting than M10 and M24 ($P = 0.04$ and 0.01 respectively). M10 and M24 spent less time grooming with their mouths than M0 horses ($P < 0.01$). There was no effect of treatment on stereotypic behaviors. M24 horses had lower HR than M0 horses ($P = 0.01$) and higher HRV than M0 and M10 horses ($P = 0.01$ and 0.03 respectively) during grazing. There were no effects of treatment on muzzle acceptability score, salivary cortisol concentration, amount of voluntary exercise, or change in dominance rank. Muzzling for 24 h was necessary for weight control. Changes in HR and HRV in M24 while grazing demonstrated an altered response of the autonomic nervous system likely related to the effects of feed restriction and BW loss. Results indicate that muzzling did not seem to cause stress as measured by cardiac and salivary cortisol parameters.

KEY WORDS behavior, grazing muzzles, miniature horse, physiological stress

INTRODUCTION

It's estimated that 40-50% of the horse population on the East Coast of the United States is overconditioned (Pratt-Phillips et al., 2010; Thatcher et al., 2012; Jaqueth et al., 2017). Overconditioned horses (those with a body score greater than 7 out 9) are at risk for metabolic diseases such as insulin resistance and laminitis (Kronfeld et al., 2005; Geor, 2008). To manage that risk and to prevent weight gain, horse owners may use grazing muzzles to reduce forage intake of horses on pastue. Grazing muzzles have proven to be highly effective at reducing intake in grazing horses; some studies report that intake may be reduced by up to 80% (Longland et al, 2011; Glunk et al, 2013; Glunk et al, 2014; Longland et al, 2016; Venable et al, 2016). While much research has been conducted on their effectiveness, little has been conducted on how muzzling effects horse behavior and physiological stress. Most muzzles are designed as a basket which encompasses the nose and mouth of horse, reducing the ability of the horse to use its lips, teeth, and nose. Consequently, muzzling may mask horse facial expressions (Wathan et al, 2015), prevent horses from allogrooming (Waring, 2003), and disturb establishment and maintenance of horse hierarchy (Montgomery, 1957; Waring, 2003). Additionally, muzzling may reduce the horse's voluntary exercise (Davis, 2019). Exercise is an important factor in horse weight loss (de Laat et al., 2016); if muzzling reduces voluntary exercise it may contravene the goal of weight loss. Overall, the inhibition of social behaviors and impact on voluntary exercise may have negative consequences for horse welfare, rendering the grazing muzzle an effective yet inhumane weight loss tool. Therefore, the objective of this study was to assess how muzzling horses housed in a herd affects physiological stress and behavior while grazing.

MATERIALS AND METHODS

This study was conducted at the University of Maryland's Central Maryland Research and Education Center in Ellicott City, MD from September 1 to November 2, 2018 and was approved by the Institutional Animal Care and Use Committee at the University of Maryland (R-SEP-17-37).

Animals and Design

Six Miniature Horses (two geldings and four mares), aged 5.8 ± 1.4yr, BW of 114.9 ± 11.4 kg, and BCS 6 ± 0.8 (Henneke, 1985) were free-leased to the University from private owners for use in the study. The lease was contingent upon proof of negative Coggins, prior vaccination of Rabies, Tetanus, and Rhinopneumonitis, a BCS between 5-7 out of 9 (Henneke, 1983), and overall good health. Horses were required to have at least six months of experience wearing muzzles. Horses received a 10 d acclimation period to the facility prior to the start of the study. During acclimation, horses were housed in a 0.03 ha fenced non-vegetative lot that had a water source and run-in shed. Horses were introduced to the research pasture by being turned out to graze for 1 h/d starting on d 2 of acclimation and increasing by an additional 1 h every d thereafter. On d 1 of the acclimation period, all horses were dewormed based on BW (Ivermectin, Bimeda Inc., Oakbrook Terrace, IL) and received hoof trims and dental floating.

The study was designed as a 3 x 3 Latin Square in which horses were blocked by period. There were a total of three periods, each consisting of 21 days. During each period, horses were housed on half of the 1.16 ha pasture with free access to a run-in shelter, water, and a salt block (Roto Salt Company, Penn Yan, NY). Horses remained on half of the research pasture until the 21d period was complete, whereupon they were moved to the other half of the pasture for the next 21d period. The pasture was divided using fiberglass push-in posts with 3 strands of electrobraid with a top strand height of 76.2 cm. Prior to the start of each period, pasture height was mown to 12.7cm to provide for a consistent grazing height that would promote normal grazing behavior yet still provide enough biomass for horses to meet digestible energy requirements (NRC, 2007). At 0800 h each day, horses were fed 14 g of a supplement (VitaPlus Complete Vitamin and Mineral Supplement, Farnam Companies Inc., Phoenix, AZ) to meet the vitamin and mineral requirements of horses fed forage only diets (NRC, 2007). Horses were groomed and assessed for illness and injury concurrently with supplement feeding.

Treatments consisted of continuous grazing with either no grazing muzzle (**M0**), a grazing muzzle applied for 10 h from 0830 to 1830 h (**M10**) or a grazing muzzle applied for 24 h (**M24**). Muzzles (Tough 1 Grazing Muzzle, JT International, Indianapolis, IN) were fit according to criteria described previously (Davis, 2019). Muzzles were removed once daily at 0800 h for feeding of supplement. After removal muzzles were inspected for damage and then re-applied.

Body Morphometrics

Body weight was determined on d 1, 8, and 15 of each period using a platform scale (Preifert, Mt. Pleasant, TX). Girth circumference (GC) and abdominal circumference (AC) (Carter et al., 2009) were assigned at the beginning (d1) and end (d21) of each period by a trained investigator.

Behavior

An ethogram was adapted from McDonnell (2003) and Ransom et al. (2009) to quantify frequency and duration of certain behaviors (Table 3.1 and Table 3.2). Horses were continuously observed using a digital video recorder and video cameras (1080p Security System, Annke, Rowland Heights, CA) for 60 m on d 3, 5, 10, 12, 17, and 19 of each period. Recordings began 2 h after sunrise and 3 h before sunset (9:04am ± 2m and 15:51 ± 3m) to capture times when horses were most active. Eight cameras, one positioned in each corner of the research pasture and four positioned in the center, were set at angles to capture the entire area of the research pasture (Figure 3.1). Weather was noted prior to recording sessions. If inclement weather was present, recording was rescheduled for the next clear day. Behavioral scoring was conducted using BORIS v. 5.1.3 (Friard and Gamba, University of Turin, Turin, Italy). Behaviors were classed as state and point behaviors and logged for frequency and duration. Two observers analyzed the videos in a random order to avoid observer bias. Inter-observer and intra-observer reliability were measured with Cohen's kappa coefficient ($K \geq 0.89$). Behavioral data were calculated as hourly means for behaviors that occurred throughout the duration of video recording.

Dominance ranks among the horses were determined by adding the number of observed bite and kick threats, bites, kicks, and chases and subtracting the number of retreats (McDonnell, 2003; Kreuger and Heinze, 2008). A rank was assigned to each horse with '1' indicating the horse with the highest score as the most dominant and '6' indicating the horse with the lowest score as least dominant.

Grazing Muzzle Acceptance

A horse's response to having the grazing muzzle re-applied was scored each morning by the horse's handler using a scoring system where "1" indicated the highest degree of acceptance and "5" indicated the lowest degree of acceptance (Davis, 2019, Table 2.2).

Physiological Stress

On d 13 and 20 of each period, heart rate (**HR**) and heart rate variability (**HRV**) were assessed. All horses performed the same grazing activity during assessment. Heart rate variability was monitored using equine HR monitors (HRMs; S10; Polar Electro Inc, Lake Success, NY). To ensure best contact of HR monitor to horse, a small portion of the hair coat was shaved on the girth area prior to monitor application. During assessment, HR monitors were attached to each horse with a girth strap at 1000 h ± 30 min and removed after 5 min. Heart rate and HRV was analyzed with Kubios software (Kubios Oy, Kuopio, Finland). Artefacts were corrected by setting the custom filter of the program at 0.3 (Schmidt et al., 2010) and data were detrended according to the procedure described by Tarvainen et al, 2002.

On d 6, 13, and 20 of each period, salivary cortisol was assessed via saliva samples collected using Salivette tubes (Sarstedt, Numbrecht, Germany). At 1100 h on assessment days, cotton wool was inserted and rubbed into the horse's mouth and then placed under the tongue of the horse for 60 s. All saliva samples were centrifuged for 15 min and frozen at −20°C immediately after collection. Samples were assayed in triplicate using the Salimetrics Salivary Cortisol Enzyme Immuno-Assay Kit (Diagnostic System Laboratories Inc., Webster, TX, USA) according to the manufacturer's protocols, previously described in detail by Schwinn et al. (2016). The minimal detectable concentration was 0.007 μg/dL, and the intra- and inter-assay coefficient of variation were 19.1% and 7.3% respectively.

Voluntary Exercise

Voluntary exercise was assessed using GPS devices (i-gotU GT-120 GPS logger, Mobile Action Technology, Inc., Taipei, Taiwan) on d 6 and 20 of each period. Thirty min prior to recording, devices were wrapped in plastic wrap, placed on a small square (6.4 cm^2) of foam padding, and secured to the front left cannon of each horse with vet wrap (Figure 3.2). Starting at 0800 h, devices recorded for 24 consecutive h and were set to record at 5 min intervals. Data points were screened for accuracy by removing any points with a horizontal position error of greater than 15 m (Friar et al., 2010; Blake et al., 2013). Devices were removed the next morning at 0830 h after recording was completed.

Forage Availability and Nutrient Composition

Forage characteristics were assessed at the beginning of the study. Vegetative cover was determined at the by step point method (Evans and Merton, 1957) to assess pasture density. Biomass (kg/ha) available for grazing was determined by randomly placing a 0.25m^2 quadrat in the field thrice and then hand harvesting grass with clippers within the quadrat at a height of 7.62 cm. Forage samples (~ 500 g), were randomly collected at a height of 7.62 cm by hand clipping and shipped immediately on dry ice for determination of nutrient composition by a commercial laboratory (Equi-analytical Laboratories, Ithaca, NY) utilizing traditional analytical methods to determine moisture, dry matter, digestible energy, protein, fiber, carbohydrates and minerals.

Statistical Analysis

Data were analyzed using repeated measures Analysis of Variance (ANOVA) in PROC MIXED (SAS 9.4.) with treatment and week serving as fixed effects and horse and period serving as random effects. Response variables analyzed were gain in BW, AC, GC, HR, HRV, salivary cortisol concentrations, muzzle acceptance, and total distance travelled. Frequency and duration of behaviors and change in dominance hierarchy rank were analyzed using multi-way ANOVA with treatment serving as a fixed effect and horse and period as random effects. Data are reported as LSMEANS ± SE. Normality and equality of variance of the sampling mean distribution of sampling means were tested using the Shapiro-Wilk test and Levene test, respectively (Sachs, 1984). The null hypothesis for each test was not rejected; therefore, it was concluded that the normality and equality of variance assumptions had been met to perform to the repeated measures ANOVA. Results were considered significant at the $P < 0.05$ level.

RESULTS

Body Morphometrics

A weekly effect on BW was observed such that all horses weighed less in Wk 1 than horses in Wk 2 or 3 ($P < 0.01$, Table 3.3). An overall effect of treatment was observed for change in BW such that on average, M24 horses lost 0.5kg BW per week overall while M0 and M10 horses gained 1.5kg BW ($P < 0.01$, Table 3.3). A treatment by week interaction was observed during Wk 1 whereby M0 and M10 horses gained weight while M24 lost weight ($P = 0.05$; Table 3.3). There was no effect of treatment, week, or interaction on AC or GC ($P > 0.05$). Average AC and GC for all horses were 139.7 ± 1.4 cm and 116.9 ± 1.1 cm, respectively.

Behavior

Length of time a grazing muzzle was worn altered the amount of time horses spent resting, moving, grazing, autogrooming with the mouth, and standing ($P < 0.05$; Table 3.5). Horses muzzled for 24 h/d spent less time resting than when muzzles were left on for 10 h/d ($P = 0.03$), but both muzzle treatments spent similar amounts of time resting as unmuzzled horses. Unmuzzled horses spent more time walking than M10 horses ($P < 0.01$), more time cantering than M24 horses ($P = 0.02$), and more time trotting than M10 and M24 horses ($P = 0.04$ and $P = 0.01$ respectively). Muzzling horses for 10 h/d reduced the amount of time spent grazing ($P = 0.02$) and increased the amount of time spent standing ($P = 0.04$) and resting ($P = 0.03$) as compared to M24 horses. Both muzzle treatments reduced the amount of time horses spent autogrooming with their mouths as compared to unmuzzled horses ($P < 0.01$ and $P = 0.02$ respectively). Some behaviors were not performed and thus data was not presented. There was no effect of treatment on time spent performing other behaviors and no week or treatment by week interaction was observed ($P > 0.05$). A summary of frequency of agonistic behaviors performed by horses is provided in Table 3.6. While muzzle treatment may have changed dominance rank in some individual horses, that was no significant difference in treatment groups (Table 3.7).

Muzzle Acceptance

There was no effect of treatment, week, or their interaction on muzzle acceptance ($P > 0.05$; Table 3.4). Mean muzzle acceptance for horses throughout the study was 2.9 ± 0.1.

Physiological Stress

The effects of muzzling on horse HR and HRV are summarized in Table 3.8. There was no effect of week or treatment by week interaction ($P > 0.05$), but there was an effect of treatment such that M24 horses had lower HR and higher HRV than M0 and M10 horses ($P < 0.01$). There was no effect of treatment, week, or their interaction on salivary cortisol concentrations ($P > 0.05$; Table 3.9). Average salivary cortisol concentrations for all horses were 0.07 ± 0.02 ug/dL.

Voluntary Exercise

There was no effect of treatment, week, or their interaction on mean distance travelled by horses in a 24 h period ($P > 0.05$; Table 3.10). On average, horses traveled 6.4 ± 0.65 km over a 24 h period**.**

Forage Availability and Nutrient Composition

Mean pasture cover was 71.0 ± 0.1% grass and legume with 24.0 ± 0.1% weed and bare soil. Mean biomass before study beginning was 5760.6 ± 442.5 kg/ha. Mean pasture height was 11.8 ± 0.7cm. Nutrient composition is summarized in Table 3.11. Pasture cover, biomass, and height values indicate sufficient grazing material available to sustain animals throughout the study.

DISCUSSION

The overall objective of this study was to assess whether muzzling horses for different lengths of time affected the behavior and physiological stress of grazing horses. There were two main findings from this study. The first was that muzzles had to be left on for 24 hours for them to be effective at controlling weight. The second finding was that muzzling horses for either 10 or 24 hours a day did alter behavior but did not appear to increase physiological stress as measured by the variables in this study or alter herd dynamics as related to hierarchy.

Due to the relatively short length of treatment time (three wks), it's possible that our BW observations are more reflective of changes in gut fill rather than significant weight loss, especially given the observed loss of BW in M24 horses during Wk 1. A similar amount of BW gain between M0 and M10 horses support the findings of a previous study (Davis, 2019) that found muzzles needed to stay on for 24 hours to promote weight loss. Horses muzzled for 10 h/d gained as much bodyweight as M0 horses likely due to compensatory grazing after muzzle removal. Glunk and Siciliano (2011) reported that horses on a restricted grazing schedule increased hourly dry matter intake rate compared to horses allowed to free graze. Restricting intake via a muzzle may produce the same compensatory effect, with horses increasing intake once the muzzle is removed. M10 horses were unmuzzled overnight for 14 hours. Horses have the ability to consume 55% of daily maintenance digestible energy requirements within the first four hours of a grazing period (Dowler and Siciliano, 2009). Applying those findings here, fourteen hours unmuzzled would provide ample time for M10 horses to consume excess of their daily digestible energy requirements, which would result in weight gain.

The treatment by week interaction observed during Wk 1 whereby change in weight gain was most apparent may be due to the novelty of new treatment assignments. If horses were previously assigned a treatment that imposed greater intake restriction than that imposed by their new treatment, they may have increased their intake during Wk 1. For example, horses transitioned from M24 to M10 or M0 treatments would abruptly be allowed to free graze for at least some portion of the day. Conversely, horses newly assigned to M24 had to adjust to being restricted full time by a muzzle during grazing; this may have caused significant weight loss during the first week of wear. Horses seemed to adjust to treatments by Wks 2 and 3 by slightly reducing intake during free graze periods if on M0 or M10 treatments or increasing intake by becoming more adept at grazing while muzzled if on M24 treatment.

Video observations of behavior support the assertion that M10 horses engaged in compensatory grazing. It's likely M10 horses spent more time resting during the day and instead actively grazed at night after muzzles were removed. These results closely align with a previous study (Davis, 2019) investigating the effects of muzzling on individual horse behavior. Unmuzzled horses appeared to have exhibited increased activity compared to muzzled horses, with more time spent on walking, trotting, and cantering. Reduced walking and trotting activity on behalf of M10 horses may be explained by the horses resting during the day and becoming active at night after muzzle removal. Reduced trotting and cantering activity on behalf of M24 horses may be explained by the need for energy conservation under feed restriction. However, M24 horses also walked about as much as M0 horses so the total distance travelled during the day may not have been impacted.

Tracking devices using GPS have been validated as a method for estimating travelling distance in both wildlife and domestic animals (Chivers et al, 2012; Stevenson et al, 2013, McClune et al, 2015, Morris and Connor, 2017). The lack of effect of muzzling on total distance travelled suggests muzzling does not significantly impact voluntary exercise. Overall, horses in this study travelled less than reported values for wild horses (average 17.9km per day; Hampson et al., 2010a) and domestic horses (average 7.5km per day; Hampson et al., 2010b). Hampson et al., 2010b found that horse daily travel distance decreased as pasture size decreased. Taking into account the size of the research pasture and the size of the subjects (approximately ¼ the size of a normal horse), the authors feel represents a normal amount of travel for miniature horses in a small pasture.

Horses spent relatively little time grooming, but unmuzzled horses spent more time grooming themselves with their mouths than muzzled horses. Muzzled horses often attempted to groom with their mouths via a brief swinging motion toward the area needing grooming. Unmuzzled horses, in addition to performing the same action, frequently used teeth and lingered on the area needing grooming. The design of the muzzle likely hinders the ability of a horse to use its mouth for grooming. Muzzled horses did not seem to compensate for this difficulty by increasing time spent grooming with the hindfoot or by scratching themselves on objects. Behaviors associated with frustration in horses, such as pawing, head shaking, and head tossing (Houpt and McDonnell, 1993) were rarely observed. Overall, it seems that horses in this study were not frustrated by muzzling for either 10 or 24 h/d. However, a period length greater than 3 wks may have produced different results.

Horses established a stable dominance hierarchy during the acclimation period and tended to maintain their rank in the hierarchy throughout the study. Only one horse (Horse 3) ascended two ranks after switching from a muzzled to unmuzzled treatment. Horse 3 transitioned from Rank 6 on M10 to Rank 4 with no muzzle, mainly due to increased instances of chasing and biting the other gelding (Horse 4) during Period 3. Period 3 was the only instance in which Horse 3 was unmuzzled while Horse 4 was muzzled during the day. Increased chasing and biting on Horse 3's behalf arose mainly from behavior observed after play bouts. After play bouts, Horse 4 tended to disengage and retreat while Horse 3 pursued. It's possible that there may be a gender difference in response to muzzling as play bouts were not observed among mares. Applying muzzle treatments to an all-gelding herd may produce different results. While the change in rank was not statistically significant in this study, it is likely that any effect muzzling may have on herd hierarchy is dependent on the individual horse and its personality. Future studies with larger sample sizes are necessary to make this determination. Due to our relatively small herd size, the dominance hierarchy was expected to be linear (Houpt et al., 1978); therefore, the linear nature of the ranking system used was appropriate for our study. However, the system used is fairly rudimentary and did not take into account self-defensive behaviors from subordinate horses. For example, a subordinate horse may retreat from a charging dominant horse and kick out during flight; in our ranking system that kick would be scored as aggressive behavior. A more sophisticated ranking system might be employed in future studies to differentiate between true aggression and self-defensive subordinate behavior, thus rendering a more accurate portrayal of herd hierarchy.

Physiological response to feeding may account for the differences seen in HR between M0 and M10 horses. Consumption of feed has been shown to increase HR in sheep and cattle (Ingram and Whittow, 1962, Young, 1966; Webster, 1967; Webster and Hays, 1968; Christopherson and Webster; 1972.). This increase in HR is likely induced by increased oxygen demand; when food is consumed, blood pH drops due to low oxygen saturation, and muscle oxygen consumption increases (Christopherson and Webster, 1972; Ueda et al., 2002). An increase in oxygen demand triggers a faster respiratory rate, which in turn increases HR to meet the demand for oxygen distribution. We collected HR and HRV data at 1000 h when horses were grazing. M0 horses were unmuzzled and likely ingested more forage during grazing than M24 horses, thus effecting a higher HR.

Heart rate is very closely linked to HRV and the two have an inverse relationship (Kazmi, et al., 2016). As HR increases, HRV decreases and vice versa. In humans, HRV has been successfully used to monitor the shift in balance of the autonomic nervous system (ANS) in response to stress (Rechlin et al., 1994, Sloan et al., 1994, McCraty et al., 1995, Thayer et al., 1996, Laio et al., 1997, Bernardi et al., 2000) and in recent years has proven viable for monitoring the stress response in horses (Schmidt, 2010; Schmidt et al, 2010; Visser, 2002; Werhahn, 2012; Mareiki, 2013, von Borell et al, 2007). In times of stress, a shift towards increased sympathetic activity of the ANS manifests as lower HRV values, while higher HRV values indicate a shift toward parasympathetic activity. Higher HRV values for M24 horses than M0 horses may indicate M24 horses were less stressed while grazing. It is possible that muzzling for 24 h/d simulates a more natural grazing pattern by reducing the rate of forage intake and requiring the horse to spend more time grazing. In the wild, available forage is often poor and horses generally spend about 75% of their day grazing in order to meet nutritional demands (Bott et al., 2013). In contrast, domestic horses are often provided improved pasture as forage, and therefore don't require as much grazing time to meet nutritional demands. Slowing intake rate with a grazing muzzle may reduce stress levels in horses by mimicking a more natural grazing environment. Additional research is necessary to determine how reduction of intake rate affects HR and HRV.

It is also possible that M0 HRV values are a direct result of the increased HR from feed ingestion. Heart rate variability is dependent on HR and will decrease as HR increases (Kazmi et al., 2016). Higher HRV in M24 horses may simply be an indicator of lower HR during grazing rather than less stress when compared to M0 horses. Additionally, dietary restriction has been shown to induce greater HRV values. Rats on a caloric restriction diet have been shown to have higher HRV values than rats fed ad libitum (Mager et al., 2006). This finding has also been reported in horses. Ohmura et al., 2010 found that overnight feed restriction caused lower HR and higher HRV in Thoroughbred horses than in horses fed ad libitum. Ohmura et al., 2010 suggested that witholding feed for 24 h reduced sympathetic nerve activity and engaged energy conservation in response to caloric deficit. Horses in this study that were muzzled for 24 hours experienced weight loss indicative of caloric restriction. Caloric restriction from muzzling may induce similar patterns in horse HR and HRV as found from withholding feed entirely. Additionally, HR and HRV findings from this study closely mirror those found in the previous study (Davis, 2019).

Muzzle acceptance scores remained neutral throughout the study, indicating that horses did not object to muzzle application. Only one instance of ‘5’ behavior was recorded; this coincided with severe weather. Horses were competing for position within the run-in shelter and the ‘5’ score was likely more reflective of social conflict than of response to muzzling. The lack of effect on muzzle score suggests that horses acclimatized to muzzles aren’t unduly effected by application.

Salivary cortisol sampling is a non-invasive cortisol assessment technique (Schmidt et al., 2010) and has been used as a reliable indicator of horse stress (Peeters et al., 2011; von Lewinski et al., 2013). Values found in this study are within a range of normal values reported by Bohák et al. (2013) and Aurich et al. (2015). The lack of effect on salivary cortisol suggests that muzzling horses had no ill effects on stress and substantiate similar findings from the previous study (Davis, 2019). Interestingly, it appears that salivary cortisol levels were lower overall in group-housed horses than in individually housed horses (Davis, 2019). While individually housed horses were still able to see and hear one another, they were unable to make physical contact. It's possible that salivary cortisol levels may lower by allowing horses physical contact in a group-housed.

CONCLUSIONS

This study assessed the effects of muzzling grazing horses for different lengths of time on behavior and physiological stress. While grazing muzzles did alter behavior, they did not appear to induce physiological stress and needed to be left on for 24 h/d for weight control.

Table 3.1. Ethogram developed to quantify individual and social behaviors in miniature horses with or without grazing muzzles applied.[1]

Behavior	Description
Rest	Standing with weight resting on 3 or 4 legs for longer than 2 minutes
Walk	Slow four beat gait of forward movement wherein at least one foot has contact with the ground
Trot	Two beat diagonal gait of forward movement
Canter	Three beat gait of forward movement
Graze	Using teeth to shear and chew grass
Autogroom (mouth)	Using teeth, mouth, or muzzle to scratch part of own body
Stand	Standing upright with all 4 feet simultaneously in contact with the ground.
Lay	Lying with sternum in contact with ground surface, legs folded under body or stretched out on side with legs stretched out
Play	Frolicking either alone or with another; nipping of legs, neck wrestling, rearing.
Drink	Placing mouth in water trough
Roll	Drop to knees, roll onto side, then back and perhaps all the way over, then return to feet
Head rub	Rubbing any part of head on ground or any part of fence, shelter, or water trough
Head shake	Rapid rhythmic rotation of head only
Shake	Rapid rhythmic rotation of head, neck and upper body along axis while standing with feet planted
Head toss	Head rapidly and repetitively moved up and down
Mutual Grooming	Two horses stand beside one another grooming each other's neck, withers, back or rump by gently nipping, nuzzling, or rubbing.
Gallop	Fast four beat gait of forward movement wherein all four feet leave ground at some point
Autogroom (foot)	Using hind foot to scratch part of own body
Scratch	Rubbing any part of body against part of the fence, shelter, or water trough or using hind foot to scratch part of own body
Paw	Striking a vertical or horizontal surface or the air with forelimb

[1]Adapted from Ransom, J.I., Cade, B.S. (2009) Quantifying equid behavior: A research ethogram for free-roaming feral horses. U.S. Geological Survey Techniques and Methods Report 2-A9; and from McDonnell, S. (2003) The Equid Ethogram. Eclipse Press, Lexington KY.

Table 3.2. Ethogram developed to quantify agonistic behaviors in miniature horses[1].

Behavior	**Description**
Bite	Opening and rapid closing of the jaws with teeth grasping the flesh of another horse.
Bite Threat	Similar to bite but no contact is made.
Kick	One or both hind legs lifted off the ground and rapidly extended towards another horse.
Kick threat	Similar to a kick but without sufficient extension or force to make contact.
Chase/Charge	Direct, quick, aggressive movement toward another horse with intent to make other horse move
Retreat	Movement that maintains or increases an individual's distance from an approaching horse

[1]Adapted from Ransom, J.I., Cade, B.S. (2009) Quantifying equid behavior: A research ethogram for free-roaming feral horses. U.S. Geological Survey Techniques and Methods Report 2-A9; and from McDonnell, S. (2003) The Equid Ethogram. Eclipse Press, Lexington KY.

Table 3.3. Effect of muzzle treatment and week on change in miniature horse body weight (kg).

	Bodyweight, kg				Gain in Bodyweight, kg			
Treatment1	M0	M10	M24	Weekly Average	M0	M10	M24	Weekly Average
Week								
1	120.1	119.5	117.3	119.0[x]	2.5[a]	2.3[a]	-2.3[b]	0.09
2	121.0	120.4	118.8	120.0[y]	0.9	0.9	1.4	0.04
3	121.9	121.6	118.1	120.5[y]	0.9	1.2	-0.7	0.07
Treatment Average	121.0	120.5	118.0		1.5[a]	1.5[a]	-0.5[b]	
Treatment	NS, SE ± 0.9				P = 0.0074, SE ± 0.4			
Week	P = 0.0026, SE ± 0.6				NS, SE ± 0.5			
Treatment*Week	NS, SE ± 1.4				P = 0.0529, SE ± 0.9			

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.
[a,b,c]Means in rows with different superscripts differ (*P < 0.05*).
[x,y,z]Means in rows with different superscripts differ (*P < 0.05*).

3.4. Effect of muzzle treatment and week on average daily muzzle acceptance score in miniature horses.

Muzzle Acceptance Score			
Treatment[1]	M10	M24	
Week			SE
1	3.1	2.7	0.1
2	3.0	2.9	
3	2.9	2.7	

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.

Table 3.5. Effect of muzzle treatment on mean percentage of time miniature horses spent performing different behaviors within a 2 hr daily time period[1].

	Treatment[2]			
	M0	M10	M24	
Behavior				SE
Rest	18.34[ab]	25.51[a]	13.89[b]	2.45
Walk	10.65[a]	7.78[b]	9.36[ab]	0.44
Trot	0.16[a]	0.08[b]	0.06[b]	0.02
Canter	0.05[a]	0.03[ab]	0.01[b]	0.01
Graze	58.31[ab]	46.51[a]	64.96[b]	3.63
Autogroom (mouth)	0.22[a]	0.05[b]	0.11[b]	0.02
Stand	58.28[ab]	54.46[a]	68.45[b]	1.51
Lay	2.39	3.24	0.96	0.76
Play	0.39	0.47	0.02	0.19
Drink	0.31	0.44	0.57	0.15
Roll	0.22	0.13	0.1	0.09
Head rub[3]	0.21	0.24	0.02	0.14
Head shake[3]	22.33	26.83	17.71	7.66
Shake[3]	2	3.54	1.53	0.96
Head toss[3]	2.1	2.8	1.2	0.7
Mutual Groom[4]	-	-	-	-
Gallop[4]	-	-	-	-
Autogroom (foot)[4]	-	-	-	-
Scratch[4]	-	-	-	-
Paw[4]	-	-	-	-

[1] Behavior was assessed 6 times during each period for 2hr each day for a total of n = 12 for each horse. Behaviors were not scored to be mutually exclusive; i.e. ‘walking’ may have occurred concurrently with ‘grazing’.
[2] M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.
[3]Behaviors are point events of extremely short duration; reported as frequency of occurrence over 12 hours.
[4]Behaviors were not performed often enough to provide sufficient data.
[a,b,c]Means within a row with unlike superscripts differ ($P < 0.05$).

Table 3.6. Summary of muzzle treatment on frequency of miniature horse agonistic behaviors within a 2 hr daily time period[1].

	Miniature Horse Frequency of Agonistic Behavior		
Treatment[1]	M0	M10	M24
Behavior			
Chase/Charge	5.8 ± 2.5	4.0 ± 2.0	5.8 ± 2.8
Bite	0.3 ± 0.2	0	0
Bite Threat	4.0 ± 1.8	3.5 ± 1.8	3.0 ± 1.0
Kick	1.2 ± 0.4	0.8 ± 0.8	0.3 ± 0.2
Kick Threat	1.0 ± 0.4	1.3 ± 0.4	0.3 ± 0.2
Retreat	16.0 ± 5.9	27.0 ± 6.6	23.4 ± 9.3

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.

Table 3.7. The effect of muzzle treatment on dominance rank of miniature horses[1]

	Horse					
	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6
Treatment[2]						
M0	3	1	4	4	6	2
M10	3	1	6	5	5	1
M24	3	2	5	4	6	2

[1]Dominance rank was assessed by adding the number of observed bite threats, kick threats, bites, kicks, and chases and subtracting the number of retreats (McDonnell, 2003; Kreuger and Heinze, 2008). A rank was assigned to each horse with '1' indicating the horse with the highest score as most dominant and '6' indicating the horse with the lowest score as least dominant.
[2]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.

Table 3.8. Effect of muzzle treatment and week on Least Square Means of heart rate (HR) and heart rate variability (HRV) in grazing miniature horses.

	HR, bpm				HRV, ms			
Treatment[1]	M0	M10	M24	Weekly LS Means	M0	M10	M24	Weekly LS Means
Week								
2	59.2	53.3	47.7	53.4	1035.2	1149.8	1311.1	1165.4
3	54.5	51.5	45.8	50.6	1112.4	1197.5	1331.7	1213.9
Treatment LS Means	56.8[a]	52.4[ab]	46.8[b]		1073.8[a]	1173.7[a]	1321.4[b]	
Treatment	P = 0.0061, SE ± 1.6				P = 0.0023, SE ± 33.0			
Week	NS, SE ± 1.2				NS, SE ± 26.1			
Treatment*Week	NS, SE ± 2.1				NS, SE ± 45.2			

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs

[a,b,c]Means within a row with unlike superscripts differ ($P < 0.05$)

Table 3.9. Effect of muzzle treatment and week on Least Square Means of salivary cortisol (SC) in grazing miniature horses.

	SC, ug/dL			
Treatment[1]	M0	M10	M24	Weekly Average
Week				
1	0.07	0.12	0.06	0.09
2	0.05	0.03	0.03	0.04
3	0.07	0.07	0.05	0.07
Treatment Average	0.07	0.08	0.05	
Treatment	NS, SE ± 0.02			
Week	NS, SE ± 0.02			
Treatment*Week	NS, SE ± 0.03			

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.

Table 3.10. Effect of muzzle treatment and week on mean distance traveled (km) by miniature horses in 24 hours.

	Treatment[1]			
Week	M0	M10	M24	SE
1	6.4	6.6	7.6	0.65
3	4.9	5.9	6.9	

[1]M0 = no muzzle, M10 = muzzled for 10hrs, M24 = muzzled for 24hrs.

Table 3.11. Summary of nutrient composition of research pasture.

Nutrients	
DE, Mcal/kg	2.27
CP, %	20.2
ADF, %	30.0
NDF, %	53.5
WSC, %	10.8
ESC, %	8.1
Starch, %	2.1
Ca, %	0.33
P, %	0.35
Mg, %	0.23
Na, %	0.015

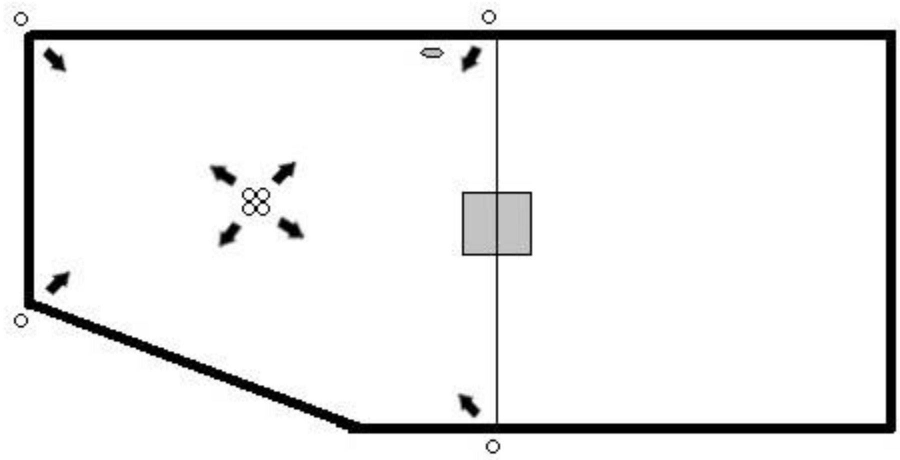

Figure 3.1. Diagram of 1.16 ha research pasture separated by electric fencing used for grazing miniature horses with or without muzzles applied. Small circles represent locations of cameras with arrows indicating direction of lens. The gray square represents the run-in shelter while the grey oval indicates position of water source. The right half of the pasture was used in period 1 and 3 after a three-week pasture rest and regrowth period, while the left half was used in period 2.

Figure 3.2. Photographs of i-gotU GPS unit just prior to application (left) and after securing with vet wrap to miniature horse (right).

CHAPTER 4: SUMMARY AND CONCLUSIONS

Grazing muzzles are highly effective, popular tools for controlling horse weight. The findings presented within in these studies suggest that grazing muzzles do not cause stress even though their design inhibits some horse behaviors. Our goal was to assess the effects of muzzling for 0, 10, and 24 hours a day on horse behavior and physiological stress in grazing miniature horses. We found no evidence that muzzles induced stereotypic behavior or stress in individually housed horses. We observed similar results for group housed horses, with the additional finding that muzzling did not alter the amount of voluntary exercise or statistically affect herd dynamics regarding dominance hierarchy. Muzzling for 24 hours a day was necessary for weight control in horses housed on pasture while muzzling for 10 hours a day allowed for weight gain similar to that observed in unmuzzled horses. Horses altered their behavior according to how long muzzles were left on. Horses muzzled for 10 hours a day tended to spend more time resting during the day and likely conducted the majority of their grazing activity overnight, after muzzle removal. Horses muzzled for 24 hours a day tended to spend more time grazing and exhibited lower HR and higher HRV than unmuzzled horses. Low resting HR and high HRV are markers of low mental and physical stress but may also be observed during feed restriction and weight loss conditions. It is likely that the cardiac trends observed in M24 horses reflect the latter. Future studies conducted with a longer length of treatment applications would be useful for assessing effects of muzzle wear on horse weight loss. Applying muzzle treatments for longer than 3 weeks at time may help mitigate the uncertainty of whether observed weight changes are due to changes in gut fill rather than overall weight gain or loss.

These findings will allow us to make recommendations for grazing muzzle use to owners of overweight horses. In order to control weight gain, muzzles should be left on while a horse is on pasture. If the muzzle needs to be removed or the owner wishes to give the horse a reprieve from the muzzle, the horse should be removed from pasture to prevent compensatory grazing. As muzzling for 24 hours a day did not appear to influence stress markers used in this study, owners may consider leaving a grazing muzzle on for longer than the maximum recommended time of 10 hours a day.

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