EFFECT OF LOW-DOSE ELECTROLYTE SUPPLEMENTATION TO UNIVERSITY RIDING HORSES DURING HOT AND HUMID WEATHER

By

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To my friends and family for their love and support, and to my professors for believing in me and always pushing me to be my best.

ABSTRACT

Electrolyte supplementation is a frequent practice in human and equine athletes, as exercise in hot and humid weather can exacerbate electrolyte and fluid losses. Previous equine electrolyte research has focused on supplementation to horses in heavy workloads with limited research in horses in light to moderate workloads. This study covered 5 consecutive weekdays in hot and humid conditions. Five treatment horses received a low-dose oral electrolyte supplement daily comprised of 0.07g NaCl/kg BW and 0.02g KCl/kg BW, and six control horses received 0.09g/kg BW granulated sugar as a placebo. Measurements included water intake, blood glucose, and plasma electrolyte levels. Treatment did not affect water consumption (P=0.94), or serum levels of sodium (P=0.18), potassium (P=0.92), and chloride (P=0.26). Average blood glucose tended to be higher in control animals (P=0.06). These results suggest that horses in light to moderate work receiving a commercial concentrate may not require additional low-dose electrolyte supplementation.

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CHAPTER I: LITERATURE REVIEW

Introduction

Thermoregulation of sweating animals during hot and humid weather is directly related to body fluids and electrolytes, such as sodium (Na), potassium (K), and chloride (Cl). Physical exercise or stress involves various metabolic pathways and induces physiological changes in the horse, including the use of glucose as fuel and loss of electrolytes. During intense exercise, a large amount of water and electrolytes are lost through sweating, as horses may sweat at rates up to 10-15 L/h during prolonged exercise (Sampieri et al., 2006). Many international competitions such as the Olympics and high-stake races are held in hot and humid weather conditions, such as the Tokyo 2020 Olympic games (Munsters et al., 2022). Losses of fluids and electrolytes are exacerbated when weather is hot and humid, as sweating rate increases and efficiency of heat loss by evaporation is reduced (Hyyppa et.al, 1996). Despite having a large reserve of fluids in the gastrointestinal tract, losses in water and electrolytes from exercise can cause an imbalance in electrolytes (hypovolemia), dehydration, and a decrease in performance (Assenza et al., 2014). Previous studies have focused on these biological markers in horses completing endurance rides or 3-day-event show jumping competitions. While previous studies may apply to endurance horses and horses in heavy or very heavy exercise, there is little insight to if electrolyte supplementation would be beneficial to horses in light to moderate exercise.

Electrolytes

Electrolytes are minerals which are vital for basic life functions. Their functions include maintaining electrical neutrality in cells, regulating thirst, along with the transmission of action potentials in nerves and muscles (Shrimanker and Bhattarai, 2019). Electrolytes must be kept at appropriate levels to allow proper muscle contraction and encourage optimal performance, as an imbalance could result in life-threatening complications (Shrimanker and Bhattarai, 2019). Rather significant, the heart is a muscle and could not beat or undergo contraction without electrolytes. In addition, without proper electrolyte levels, the body is not capable of maintaining the proper fluid in and around cells. Sodium, potassium, and chloride are pertinent when discussing electrolyte balance related to loss in sweat during exercise. As seen in **Table 1**, sodium, potassium, and chloride are the major constituents in equine sweat.

Sodium

Sodium is one of the major cations found in extracellular fluid and is impermeable, therefore unable to cross cell membranes. Sodium induces movement of water across cell membranes, balancing extracellular fluid volume and regulating membrane potential of cells. Being an osmotically active cation, it is a great contributor to plasma tonicity and osmolality (Borer and Corley, 2006a). Sodium is exchanged along with potassium across cell membranes as a part of active transport, a part of the sodium-potassium adenosine triphosphatase (ATPase) pump (Shrimanker and Bhattarai, 2019). The ATPase pump is an enzyme embedded in cell membranes that determines cellular membrane potential and regulates proper electrolyte equilibrium. When activated, the sodium-potassium ATPase pump exchanges two extracellular potassium ions (K^+) for three intracellular sodium ions (Na^+), based on physiological excitation or inhibition (Stone et al., 2016).

Dehydration or overloading with water can interfere with water homeostasis and can result in a deviation of cellular sodium levels. (Borer and Corley, 2006a). *Hypernatremia,* or a high concentration of sodium in the blood, usually occurs due to water loss or a net gain of sodium. Hypernatremia and water loss are necessary for normal metabolic function, as these cues are responsible for rehydration and triggering the horse's thirst signal. Hypernatremia is of concern when discussing exercise as it is a result of dehydration. Dehydration and hypernatremia can present themselves as a central nervous system dysfunction, commonly as muscle weakness and lethargy. If hypernatremia persists, it can elicit permanent neurological damage (Borer and Corley, 2006a). On the other hand, *Hyponatremia,* or low concentration of sodium in the blood, is typically a result of overhydration. Furthermore, hyponatremia can be encountered in human athletes or marathon runners that drink too much water, diluting sodium concentrations and potentially leading to collapse and death if untreated (McKeever 2008).

Chloride

Chloride is the major anion in extracellular fluid, and the electrolyte is known for maintaining acid-base balance, renal tubular function, and production of gastric acid in the stomach. Plasma chloride concentration is correlated with sodium concentrations and highly dependent on water balance, therefore it is crucial that chloride levels are interpreted with acknowledgement to sodium ion concentrations (Borer and Corley, 2006a). The kidneys primarily regulate serum chloride levels and filter chloride, which is then reabsorbed by the proximal and distal tubules by active and passive transport (Shrimanker and Bhattarai, 2019). Renal reabsorption is influenced by plasma sodium concentrations and acid-base balance, as well as endocrine components such as parathyroid hormone, calcitonin, anti-diuretic hormone, and angiotensin II to regulate fluids in the horse (Borer and Corley, 2006a). Chloride disorders are not as frequent as sodium disorders; however, in humans *hyperchloremia* and *hypochloremia* present themselves in cases of gastrointestinal bicarbonate losses such as vomiting, diarrhea, or excess water gain. An extreme loss of chloride ions can lead to a serious metabolic alkalosis, or too much bicarbonate in the blood. This can be seen predominantly in horses diagnosed with colic and post-operative colic patients due to a large loss of gastric fluid in reflux (Borer and Corley, 2006a).

Potassium

In contrast to sodium and chloride, potassium is the most abundant cation in intracellular fluid, predominantly the intracellular space of skeletal muscle. The uptake of potassium into cells is stimulated by increased circulating insulin concentration and B-adrenergic stimulation (Borer and Corley, 2006b). Potassium plays a key role in maintaining cell function and ion exchange, with notable influence on transmembrane electro-chemical gradients (Stone et al., 2016). This gradient of potassium across the cell membrane in the ATPase pump governs membrane potential, with the ratio of intracellular to extracellular potassium ions typically generating -90mV (Stone et al., 2016). Whether it is resting or active potential, the electro-chemical difference stimulated by these electrolytes is compulsory for nervous tissue and muscle contraction during movement.

Hypokalemia, or low potassium concentration, affects electoral conduction across the cell membrane, resulting in a greater negative resting potential; therefore, lowering chances of action potential occurring. Symptoms of hypokalemia are dependent on the speed of potassium decrease, but typically present as muscle weakness and reduced gastrointestinal motility (Borer and Corley, 2006b). On the other hand, *hyperkalemia* appears as muscle weakness, or in extreme cases ventricular fibrillation or cardiac arrest. Hyperkalemia can be associated with metabolic acidosis, or in cases of colic, lactic acid accumulation (Borer and Corley, 2006b). Hyperkalemic periodic paralysis is a codominant autosomal genetic disorder occurring in American Quarter Horses and related breeds, affecting the sodium-potassium ATPase pump. If affected, this channel mutation results in extreme sensitivity to potassium intake, and may result in symptoms such as muscle fasciculations, weakness, increased respiratory rate, and possible death. Potassium intake with horses diagnosed with hyperkalemic periodic paralysis (HYPP) should be restricted to <1% of total diet to prevent symptoms of hyperkalemia (Naylor, 1997).

Table 1: Electrolyte Sweat Composition in the Horse (Coenen, 2005)

Electrolyte	Concentration
Major Constituents (g/L)	
Na	2.8
K	1.4
Cl	5.3
Minor constituents (g/L)	
Ca	0.12
Mg	0.05
P	<0.01
Trace constituents (mg/L)	
Fe	4.3
Cu	0.3
Zn	11.4
Mn	0.16
Se	<0.005

Glucose

Glucose and glycogen as energy sources

Glucose serves as another factor to consider when discussing metabolites that may be affected by performance. Carbohydrates stored as muscle glycogen are the primary energy source for anaerobic glycolysis, specifically during intense exercise in humans and horses. However, repeated bouts of maximal intensity exertion or a combination of prolonged running and repeated sprints results in a decline of up to 50% in muscle glycogen concentration of horses (Lacombe et al., 2001). As supplementing carbohydrates has been proven to increase time to fatigue, competitive endurance horses are often fed meals high in carbohydrates (such as sugar beet or bran) at rest stops to provide glucose as fuel and delay fatigue (Bullimore et al., 2000). The inclusion of hydrolysable carbohydrates such as starch or sugar in the regular diet of a performance horse is crucial for replenishing glycogen reserves and availability of the substrates needed to fuel muscular work (McKeever 2008). Resynthesis of muscle glycogen stores requires 48-72 hours in horses and requires adequate intracellular water and K^+ (Waller et al., 2009).

Glucose and electrolytes

Glucose is not an electrolyte, though is of the essence relating to electrolyte absorption and rehydration. Within muscle and liver, glycogen is stored in a hydrated form, tightly associated with K^+. As muscle contraction occurs and glycogen is utilized, water and K^+ is simultaneously released from the glycogen store (Waller et. al, 2008). This may lead to post-exercise dehydration, and slower glycogen replenishment. To examine the effects of electrolytes on post-exercise rehydration and glycogen replenishment, a study by Waller and colleagues (2009) examined the efficacy of a commercial electrolyte solution (Perform' N Win, Buckeye Nutrition, consisting of 44 g dextrose along with 12 g Na, 24 g Cl, 9 g K, 1 g Ca, and 1 g Mg in 8 liters of water). This solution was administered to Standardbreds in moderate-intensity exercise following a competitive exercise test (CET), in comparison to hay and grain alone. Muscle glycogen content decreased by 21.9% in the electrolyte and 22.6% in the control treatment following the CET, much lower compared to other studies measuring depletions from intense exercise (Waller et. al 2008). Muscle biopsies in addition to venous blood samples were collected at rest and following the CET to analyze muscle glycogen, blood glucose, and muscle electrolytes. There were no differences in muscle electrolyte contents with electrolyte treatment, however, there was greater glycogen content following exercise compared to the control. Plasma Na^+ and Cl^- increased in horses receiving the electrolyte solution, with a 1% decrease in total body K^+. Furthermore, plasma osmolality in horses receiving the electrolyte administration increased up to 4 hours following administration, further eliciting the thirst response (Waller et. al 2008). Results from this study conclude that this electrolyte solution generated faster restoration of plasma hydration status compared to the control and enhanced the rate of muscle glycogen resynthesis.

Based on human literature, it has been recognized that the inclusion of a carbohydrate in conjunction with electrolytes can enhance the uptake of sodium ions and water from the intestinal epithelial cells (Monreal et al., 1999). Not only can glucose increase electrolyte absorption but enhance palatability for equine utilization. Following carbohydrate consumption, D-glucose transport takes place across the equine intestinal brush-border membrane by a high affinity, low capacity, Na+ glucose cotransporter (SGLT1), meaning glucose will only be transported across the membrane when it is in high concentration inside the cell (Dyer et al., 2002). Through this intestinal absorption, glucose is absorbed to provide ATP for aerobic and cell metabolism and stored as glycogen in muscle.

Fluids, Rehydration, and Sweat in the Horse

Fluids

The body of the horse is primarily composed of water and electrolytes, compartmentalized within and outside of cells. The combination of intracellular and extracellular fluid comprises the total body water (TBW), accounting for 50-70% of total body weight (McKeever, 2008). Two-thirds of water is contained within cells as intracellular fluid, with the other one-third found in extracellular fluid space. Extracellular fluid can include areas such as the vascular space, interstitial fluid space, lymphatics, transcellular fluids, and most importantly, the gastrointestinal tract (McKeever 2008). The gastrointestinal tract serves as a temporary reservoir for fluids, in addition to stored minerals consumed through diet. It has been found that increased roughage consumption may increase water and electrolyte storage in the gastrointestinal tract of the horse (Coenen, 2005).

Horses lose water and electrolytes in a variety of ways, not just through exercise-induced sweat. The intake of water is counteracted through excretion by the kidneys via urine and feces to keep balance in a neutral range (Coenen, 2005). Excretion through sweat depends on the duration and intensity of exercise and environmental conditions and is not reliant on water intake. As seen in **Figure 1**, the renal excretion of water and electrolytes is the only dependent variable which reflects differences in intake and additional output via sweat (Coenen, 2005).

Sweat

The equine sweat gland is unique in its simplicity compared to other species. In contrast to human sweat that is hypotonic, horse sweat is hypertonic, or has a high concentration of sodium, potassium, and chloride. The horse has a less favorable surface area to volume ratio when compared to humans, further eliciting fluid and electrolyte deficits (McKeever 2008). Equine sweat contains the protein latherin, that gives the sweat a white and foamy appearance. This protein aids in cooling and evaporation by moving moisture from skin to the surface of the coat. Furthermore, equine sweat glands are not responsive to aldosterone, the hormone responsible for sodium conservation and potassium excretion. This results in extra salt in the sweat and can contribute to a higher loss of sodium. Nonetheless, this feature can alter the evaporation point and possibly enhance evaporative cooling (McKeever 2008). Evaporative cooling can be defined as heat loss by evaporation of sweat and is several times more effective than other routes of heat exchange (Geor et al., 1995). Increased evaporative cooling of the upper respiratory tract is enhanced by an increased respiratory rate and decrease in tidal volume, which can be seen as panting in dogs. In horses, respiratory rates can increase up to 200 breaths per minute following exercise to experience these same cooling effects (Geor et al., 1995).

Environmental influence on sweat and water uptake

Contrary to other weather conditions, hot and humid weather provides the greatest opportunity for dehydration from greater heat stress and reduced cooling ability. As seen in **Figure 2**, in comparison to exercising in cool and dry or hot and dry conditions, hot and humid conditions add thermoregulatory strain due to higher heart rates during exercise, and longer recovery times (Geor et al., 1995). Under hot and humid weather conditions, evaporative cooling becomes ineffective because sweat will not evaporate. Previous studies have reported that under conditions of high heat and humidity, horses can experience sweat losses of 12L per hour. Even properly fueled and hydrated horses can experience hyperthermia in these weather conditions, and can experience fatigue, cramps, heat stroke, and other thermal injuries (McKeever 2008).

Specifically for elite sport horses that travel frequently, it is not possible to eliminate adverse effects of various environmental conditions on performance. However, numerous studies in both humans and horses have found that appropriate exercise programs along with proper time for acclimation can help mitigate the effects of climate (McCutcheon and Geor, 2008). In addition, maintenance of fluid and electrolyte balance is crucial for adapting to a hot environment. Horses used in the 1996 Olympic games in Atlanta, Georgia were recommended to arrive three weeks ahead of time, based on previous research studies stating that 14 days is the ideal period for heat acclimatization (McCutcheon and Geor, 2008). A recent study examined the possibility of using heated indoor arenas to acclimate horses to hot climates prior to the 2020 Tokyo Olympics (Munsters et al., 2022). This study discovered that 14 days of normal training in an indoor arena resulted in a reduction in cardiovascular and thermal strain in hot environments, giving insight into future training for international competitions.

Rehydration and water intake

Electrolyte and fluid losses are inevitable and are necessary to support thermoregulatory cooling. An equine athlete can typically recover from acute fluid and electrolyte losses from exercise, with the implementation of proper nutrition, water uptake, and an exercise routine. Repeated exercise and disturbances in electrolyte balance evokes an adaptive response that better prepares the horse for these acute losses. Adequate water, a normal diet, and a salt and mineral supplement are all inclusions that can help maintain hydration in equine athletes (McKeever 2008). Water intake will vary between horses due to different workloads, climates, and unique thirst signals; however, the National Research Council recommends 4-6 liters of water per 100 kg of body weight daily (NRC, 2007). Horse water intake in warm climates may increase by 15-30%, and in combination with heavy work it may increase by 200-300% (Geor 2008).

The physiological stimuli of thirst are increased plasma osmolality and or hypovolemia. Since sweat is hyper- or isotonic compared to plasma, an increase in plasma sodium or plasma osmolality is not always experienced, therefore thirst may not be stimulated until hypovolemia reaches a certain extent (Nyman et al., 2002). Watering routines and management may have an impact on fluid balance, and transportation to different competitions or training could reduce voluntary water intake. The degree of workload (light, moderate, heavy) could also influence rehydration and thirst stimulation. Previous research in high-intensity exercise has found a decrease in plasma volume, while low-intensity exercise findings are contradictory in that there may be no change, decrease, or an increase in plasma volume (Nyman et al., 2002). Exercise and the associated hypervolemic response can enhance cardiovascular and thermoregulatory stability as seen in **Figure 3**, as the increase in TBW provides extra volume to maintain venous return and cardiac output (McKeever 2008).

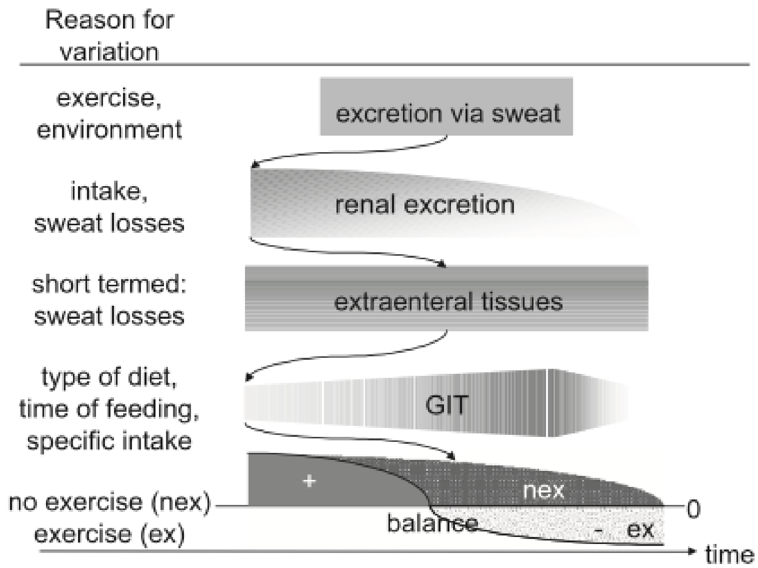

Figure 1: Variation in fluids due to water and electrolyte exchange between compartments (Coenen, 2005)

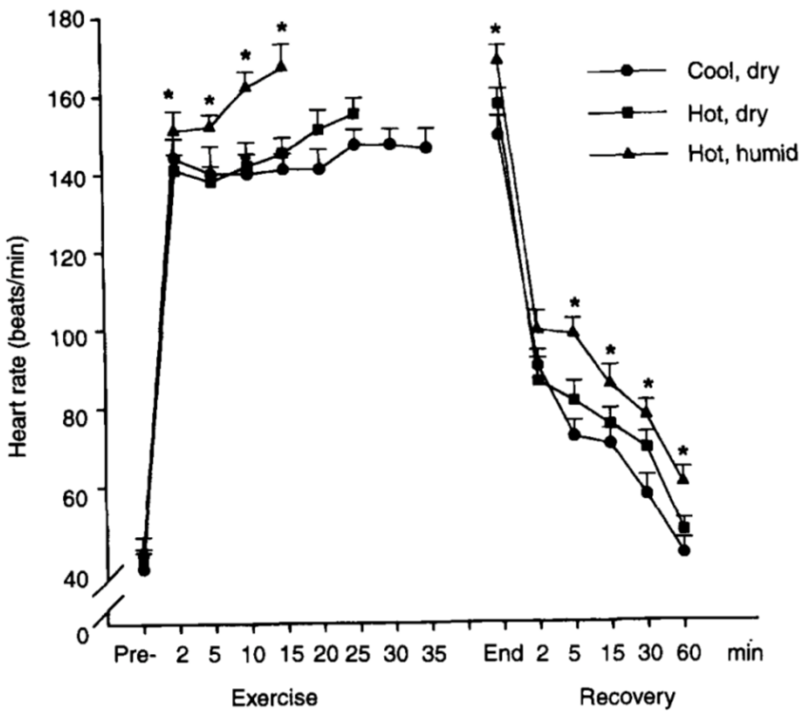

Figure 2: Mean heart rate in 5 horses during exercise at 50% of maximal O_2 consumption and for 60 min after exercise in cool, dry, hot, dry, and hot, humid ambient conditions (Geor et al., 1995).

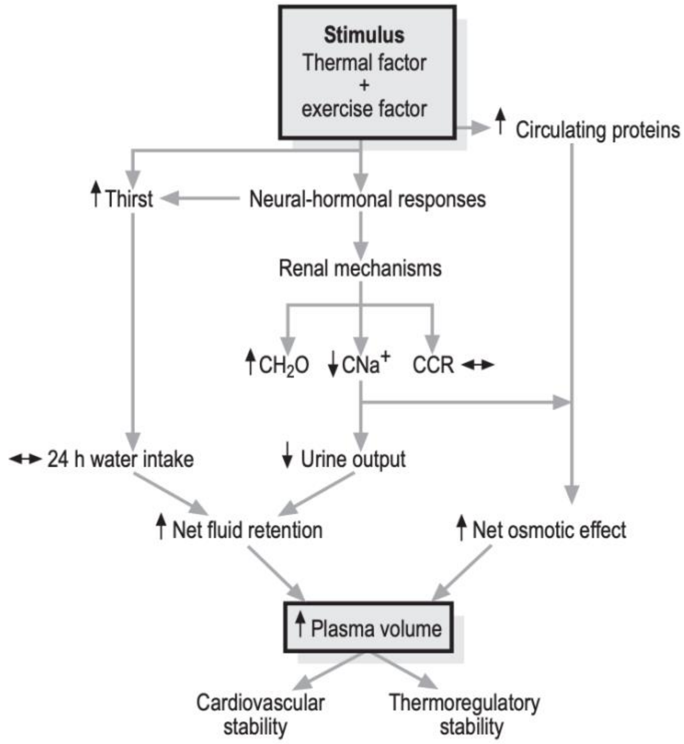

Figure 3: Mechanisms for exercise training-induced hypervolemia in horses (McKeever 2008)

Exercise influence on sweat

Previous electrolyte research tends to focus on effects of supplementation to endurance horses or horses in heavy workloads. The National Research Council defines heavy exercise in horses to be 4-5 hours a week, while very heavy to be a variable number of hours, but may include racing, endurance, or elite three-day event horses (NRC, 2007). Research concentration in heavy workloads can be attributed to the clinical disorders encountered during prolonged exercise from fluid and sweat loss, heat accumulation, and intramuscular glycogen depletion. Bodyweight losses of 3-7% routinely develop following intense exercise and may take longer than 24 hours to be recovered (Sampieri et al., 2006). Moreover, many athletic horses with who have diets higher in starch and energy may increase their likelihood for electrolyte deficiency as forage serves as an electrolyte and water reservoir (Walker and Collins, 2017).

Electrolyte pastes are often given by endurance riders at rest stops to support their athletic condition and delay the onset of fatigue. Although, the actual quantity of electrolytes in these commercial pastes is contentious if they provide the adequate amount of electrolytes these hard-working horses need. One endurance study investigated if varying doses of an oral electrolyte would attenuate bodyweight losses by increasing water intake or have possible effects on speed and performance (Sampieri et al., 2006). Horses either received a high-dose electrolyte (0.2 grams (g) NaCl/kg bodyweight (BW) and 0.07 KCl/kg BW) or a low-dose electrolyte (0.07 g NaCl/kg BW and 0.02 g KCl/kg BW) 30 minutes before an 80 km ride. Results from this study found that the horses who received the high dose had enhanced water intake, but there was no competitive advantage or change in performance for either dose in 80 km rides.

Likewise, another study relating to moderate-intensity exercise aimed to evaluate training-induced hydration and electrolyte balances in horses competing in a longer 120 km endurance race (Robert et al., 2010). Blood and urine samples were taken before, during, and after the rides to calculate fractional excretion of electrolytes. These measurements contribute to the understanding of renal control mechanism of endurance exercise, as tubular conservation of electrolytes would be of essence to conserving water and sodium, and possible electrolyte disorders. Roberts and colleagues (2010) found that during the endurance rides, fluid losses appeared balanced by water intake, while renal reabsorption seems insufficient to compensate for losses of Na+ and Cl- through sweat. These results support the possible benefit of including an electrolyte in a supplement regimen during training or competition.

Walker and Collins (2017) performed a study to examine the influence of varying intensities of exercise and electrolyte supplementation on heart rate and plasma electrolyte concentrations. Eleven standardbreds in varying workloads (rest, 20 min of jogging, 2 min race) received or had the lack of an electrolyte supplement. The electrolyte supplement consisted of 18 g sodium chloride (60%), 6 g potassium chloride (2%), 4.8 g dextrose (16%), 0.9 g inactive ingredients (silicon dioxide and flavor (3%); 0.240 g magnesium oxide (0.8%), and 0.060 g calcium carbonate (0.2%). The powder supplement was diluted in water and administered via oral syringe 2 hours prior to exercise. Immediately following exercise, heart rate measurements and blood samples were taken for analysis. Results from this study found oral administration of the electrolyte supplement affected heart rate, blood potassium and sodium concentrations, but not blood chloride concentrations (Walker and Collins, 2017). As expected, horses who received the oral supplement at rest had significantly higher plasma sodium concentrations compared to rest horses that did not receive the supplement. Furthermore, different intensities of exercise had a significant effect on plasma potassium and sodium concentrations, but no significant changes in chloride concentrations. Regardless of treatment, potassium concentrations were higher in jogging horses in comparison to rest and racehorses, yet sodium levels increased significantly following horses who raced in comparison to resting or jogging horses (Walker and Collins, 2017). This study gave clarity into the effects of different intensities of exercise on plasma electrolyte levels. heart rate, and the impact of an additional electrolyte supplement.

Conclusion

Electrolytes are minerals that support basic life functions, including action potentials, thirst regulation, and maintaining neutrality in cells. Sodium and chloride are the main electrolytes found in extracellular fluid, while potassium is the main cation found in intracellular fluid. Like human athletes, horses experience fluid and electrolyte losses through sweat and exercise. Continuous losses in water and electrolytes from exercise can cause an imbalance in electrolytes, dehydration, or a decrease in performance (Assenza et al., 2014). Equine sweat is hypertonic to plasma, meaning it has a higher concentration of sodium, potassium, and chloride. In combination with their less favorable surface area to volume ratio, equine athletes may experience greater fluid and electrolyte deficits than humans. Research in endurance horses has found that including an electrolyte to support recovery and replenish what is lost in sweat may increase water intake but may not have an impact on performance (Sampieri et al., 2006). Furthermore, fluid losses from exercise may be balanced by water intake, but Robert and colleagues

(2010) found sodium and chloride losses are not balanced by renal absorption and endurance horses may benefit from including supplemental electrolytes in the diet.

While there has been prevalent research in electrolyte supplementation to horses in heavy workloads, the equine industry could benefit from studies regarding supplementing to horses in light to moderate workloads. Horses in a light to moderate workload may include lesson horses, horses in a university program, therapeutic riding horses, or some show horses (NRC, 2007). Typical horse owners could benefit from education and knowledge of these different NRC exercise categories (light, moderate, heavy, very heavy) to decide what category their horse may fall in and if they need an electrolyte supplement. Furthermore, the average horse owner wouldn't have the knowledge to balance an appropriate ration compared to an educated animal nutritionist. With research knowledge in this subject, the average owner could either: continue to administer electrolytes to support their horse's health or remove electrolytes from their supplement regimen. If removal is recommended, this could alleviate financial and environmental waste. Further research could give clarity into the necessity of electrolyte supplementation to horses in light or moderate exercise.

CHAPTER II: EFFECT OF LOW-DOSE ELECTROLYTE SUPPLEMENTATION TO UNIVERSITY HORSES DURING HOT AND HUMID WEATHER

Introduction

During the late summer and early fall months, horses used in collegiate riding programs are placed in light to moderate exercise as the school year begins. Horses exercised in hot and humid conditions may experience exacerbated fluid and electrolyte losses, as sweating rate increases and efficiency of heat loss by evaporation is reduced (Hyyppa et.al, 1996). Endurance horses and horses in prolonged bouts of exercise are often supplemented with electrolytes to counteract these fluid losses, however there is little research if these rehydration practices are needed in horses in light to moderate exercise. Several endurance studies conclude that supplementing electrolytes increases plasma concentrations of sodium and chloride, with decreasing or varying results of potassium concentrations (Coenen et al., 1995; Dusterdieck et al., 1999; Sampieri et al., 2006). Sampieri et al., 2006 evaluated the efficacy of varying electrolyte doses in endurance horses, concluding that a high-dose supplementation exhibited a greater effect on water intake than a low-dose, with no competitive advantage. Further, Dusterdieck et al., 1999 examined if administering glycerol in combination with an electrolyte to endurance horses provided any additional benefit for rehydration rather than electrolytes alone. Like Sampieri and colleagues (2006), this study found oral electrolyte pastes to enhance water intake, however concurrent glycerol administration had no additional benefit to plasma electrolyte levels or water consumption.

In this current study, university riding horses were administered an oral supplement to determine if a low-dose electrolyte would affect serum concentrations of electrolytes and glucose, water intake, and willingness to work. Based on previous research, it was hypothesized that horses receiving the low-dose oral electrolyte would have increased plasma electrolyte levels, water intake, and perceived horse willingness compared to control horses. Should the electrolyte supplement improve these measurements, recommendations can be made to horses with similar workloads in similar climates. Conversely, these results could give clarity that horses in this level of work in a hot and humid climate may not require a low-dose electrolyte supplement.

Materials and Methods

Horses

Eleven mature horses of mixed breed and age (19±5 yr) were used to test low-dose electrolyte supplementation to university riding horses during one week of hot and humid weather. The study horses were comprised of four mares and seven geldings of stock breeds consisting of nine American Quarter Horses and two American Paint Horses. Horses were selected from the teaching and research herd at Middle Tennessee State University (MTSU). The horses were utilized for riding classes, Intercollegiate Horse Show Association (IHSA) practices and shows, and research. The study took place over 5 consecutive days in Murfreesboro, Tennessee during the third week of September, experiencing record high temperatures and hot and humid weather as seen in **Table 2**. This study was approved by the MTSU Institutional Animal Care and Use Committee (Protocol # 23-2001, Appendix) and exempt from Institutional Review Board status (Protocol #23-1012).

Table 2: Weather in Murfreesboro Tennessee, September 19-23, 2022 (National Weather Service, 2022)

	Monday 9/19	Tuesday 9/20	Wednesday 9/21	Thursday 9/22	Friday 9/23
High Temperature	33°C	36°C	37°C	28°C	26°C
Low Temperature	19°C	23°C	22°C	17°C	13°C
Average Humidity %	59.0%	55.1%	54.6%	53.1%	43.6%

Study Protocol

Horses remained on their regular schedule of low to moderate work during five weekdays, as this is the typical workload for university riding horses. Horses are typically ridden once per day, with a few horses ridden twice per day in lighter works. For example, a horse used in an English lesson in the morning for beginner riders performing foundational walk and trot maneuvers, may also be used in a Western lesson for beginner riders in the afternoon. One week prior to the beginning of the study, horses were randomly assigned to treatments and then striated by weight and sex. To determine how many animals should be used, alpha = 0.05 and power (1-B) = 0.80 were used. A two-sided test was assumed, as biomarkers may increase or decrease in relation to exercise, dosage, and supplementation. Based on these assumptions, a minimum of 4 horses are needed for each treatment group in this study. Six horses were proposed for each treatment group in order to account for potential variation in biomarkers measured outside of glucose. Five horses were assigned to the treatment electrolyte group (one horse was removed from the treatment group for unrelated health reasons prior to beginning the study) and six horses were assigned to the control group. Body condition score (BCS) was monitored throughout the research period and if a horse reached a BCS of less than 4, it was removed from the study. No horse reached a BCS lower than 4 during the study period.

Horses remained on their usual diets while stalled throughout the study, consisting of a commercial pelleted concentrate (Purina Strategy), prairie grass hay, and *ad libitum* access to water. Treatment group horses received a low-dose electrolyte oral supplement daily for five days that was comprised of 0.07g NaCl/kg BW and 0.02g KCl/kg BW (Sampieri et al., 2006) and control horses received 0.09g granulated sugar per kg BW as an equivalent. During the study period, the calculated oral supplementation based on BW was mixed in study horses pelleted concentrate each morning. All study horses were weighed each day after their second feeding to calculate accurate supplement dosage for the following day. Horses were monitored during feeding and there were no reports of horses refusing supplementation.

Sample Collection

Venous blood samples were collected via jugular venipuncture into heparinized tubes each morning fasted; prior to feeding the appropriate oral supplementation. Before centrifuging, blood glucose was analyzed via the AlphaTrak™ Glucometer, a glucometer tailored to veterinary use and specifically equines (Hackett and McCue, 2010). After recording blood glucose, blood samples were centrifuged at 3000 rpm for 10 minutes, then plasma was pipetted into individual microcentrifuge tubes. Plasma was stored in a -

80°C freezer until shipped to Cornell University Animal Health Diagnostic Center to conduct a mineral and electrolyte panel.

Water intake was a constituent of interest for this study, specifically by reason of combining an electrolyte supplement with hot and humid weather. Water intake for study horses was monitored for 24 hours throughout the 5-day period, with specific water buckets labelled with liter marks in order to accurately measure water consumed. Only researchers were permitted to refill and take measurements of water during the research period. On the last day of the study, water intake was only measured until 12 o'clock as this was the university horse's regular work schedule before weekend turnout.

Survey

To retrieve information on perceived horse performance and willingness to work, riders for each horse were given a survey following each ride via Qualtrics (Appendix). Topics of the survey encompassed the perception of the horse's responsiveness to rider aids, horse's perceived sweat, level of fatigue, and the amount of time for the horse to cool down. All horses in the study were ridden at least once each day, either being a riding class or equestrian team lesson. Horses could be ridden for a one-hour or two-hour duration, varying by day and riding assignment. Riders given the survey were recognized as having an intermediate to advanced level of experience, ensuring the survey questions were comprehended and answered appropriately. Riders, coaches, and instructors were blind to the horse's treatment and were aware of the horse receiving an oral supplement, but not specifically an electrolyte.

Statistical Analysis

Data were analyzed using SAS Ver. 9.4 (SAS Stat. Inc., Cary, NC). After verifying that data were normally distributed using the Shapiro-Wilk statistic, a mixed model with repeated measures was used to compare the effects of electrolyte treatment on water intake, body weight, and plasma glucose, using horse as the individual subject, and day as the repeated effect, with a *post hoc* Tukey's adjustment to account for an unequal number of horses per electrolyte treatment.

Similarly, a mixed model with repeated measures was used to compare the effects of electrolyte treatment and exercise duration on riders' assessment of horse performance variables, using horse as the individual subject, day and time (morning, mid-day, afternoon) as repeated effects, and a *post hoc* Tukey's adjustment to account for an unequal number of horses per electrolyte treatment. Because not all horses were used at every possible lesson time or exercise duration, the interactions of day*time, exercise duration*time, and exercise duration*day did not provide enough statistical power to estimate interaction effects, so these interactions were dropped from the model.

The effects of diet on plasma sodium, potassium, chloride, bicarbonate, anion gap, calcium, phosphorus, magnesium, and lipemia were analyzed using pooled post-supplementation samples from each electrolyte treatment group and a General Linear Models procedure.

Pearson's correlation coefficients were used to examine relationships between body weight, water intake, plasma glucose, sodium, potassium, chloride, bicarbonate, anion gap, calcium, phosphorus, magnesium, and lipemia.

A P-value of P<0.05 was considered significant, while 0.05<P<0.10 was considered a trend. Correlation coefficients were interpreted as follows: r>0.90 were considered very strong; r between 0.70 to 0.90 as strong; r between 0.50 to 0.70 as moderate; r between 0.30 to 0.50 as weak, and r <0.30 were negligible correlations.

Results

Plasma Electrolyte Levels

Treatment did not affect plasma concentration of sodium (P=0.18), potassium (P=0.92), or chloride (P=0.26), but did affect magnesium (P=0.011) and bicarbonate (P=0.034, Table 3). There were strong correlations between sodium and potassium (r=0.71,P<0.0001), sodium and calcium (r=0.96,P<0.0001), sodium and chloride (r=0.99,P<0.0001). In addition, strong correlations were present between chloride and potassium (r=0.76, P<0.0001), as well as chloride and calcium (r=0.94,P<0.0001). There was no interaction of day by treatment, which was dropped from the model.

Blood Glucose

There was no day effect (P=0.55), however a treatment trend existed, with the control treatment tending to have greater average blood glucose (P=0.06). There was no day by treatment interaction (P=0.84).

Body Weight

Treatment did not alter horse weight (P=0.66), however there was a day effect (P<0.0001), and a day by treatment interaction (P=0.0007, Figure 4). There was a weak correlation (r=0.44,P=0.0027) between body weight and water intake.

Water intake

Day had an effect on water consumption ($P<0.01$, Figure 5), with water consumption peaking on day 3 with the highest ambient temperature. Treatment did not affect water consumption ($P=0.94$), and there was no day by treatment interaction ($P=0.93$).

Survey

Participation

There was no day ($P=0.41$), time of day (morning, mid-day, afternoon) ($P=0.86$), or treatment effect ($P=0.48$). Regardless of treatment, there was a time duration trend ($P=0.06$), horses ridden for two hours rather than one hour tended to have a lower participation score.

Sweat

There was a day effect ($P=0.0004$, Figure 6), with perceived sweat highest on day 3. There was no treatment ($P=0.86$) or time of day effect ($P=0.60$), however there was a time of day (morning, mid-day, afternoon) and treatment interaction ($P=0.017$, Figure 7). Control horses had a higher perceived sweat in morning rides ($P=0.009$), with electrolyte supplemented horses having higher perceived sweat in mid-day rides than morning rides ($P=0.029$).

Fatigue

Results from the survey concluded riders perceived horse fatigue was not different between treatments ($P=0.23$). There was no day ($P=0.49$) or time of day ($P=0.94$) effect, however a duration trend ($P=0.10$) found that horses ridden for two hours tended to fatigue quicker than those ridden for one hour. There was a time of day by treatment interaction (P=0.043, Figure 8), results found electrolyte horses were perceived to fatigue quicker in the afternoon (P=0.025) compared to control horses.

Rider Aids

Survey results concluded that perceived horse responsiveness to rider aids had no day effect (P=0.66), treatment (P=0.38), or day by treatment interaction (P=0.50).

Cool Down

Results regarding horse cool down reported a day effect (P=0.005, Figure 9) with the longest cool down time on day 3, corresponding to the highest ambient temperature of the study period. There was no treatment effect (P=0.68) or day by treatment interaction (P=0.88).

Table 3. Plasma Electrolyte Results via Mineral and Electrolyte Panel from Cornell Animal Health Diagnostic Center

Variable	**CONTROL**	**ELECTROLYTE**	**P-Value**
Sodium, mEq/L	136 ± 1.8	133 ± 2.0	0.18
Potassium, mEq/L	3.79 ± 0.08	3.78 ± 0.09	0.92
Magnesium, mEq/L	1.50 ± 0.03	1.38 ± 0.03	0.011
Phosphate, mEq/L	3.45 ± 0.16	3.50 ± 0.18	0.85
Chloride, mEq/L	99.5 ± 1.56	96.8 ± 1.74	0.26
Calcium, mEq/L	12.0 ± 0.21	11.5 ± 0.24	0.16
Lipemia, mEq/L	5.80 ± 0.47	5.17 ± 0.53	0.38
Anion Gap, mEq/L	12.5 ± 0.31	12.9. ± 0.35	0.35
Bicarbonate, mEq/L	28.4 ± 0.44	26.9 ± 0.49	0.034

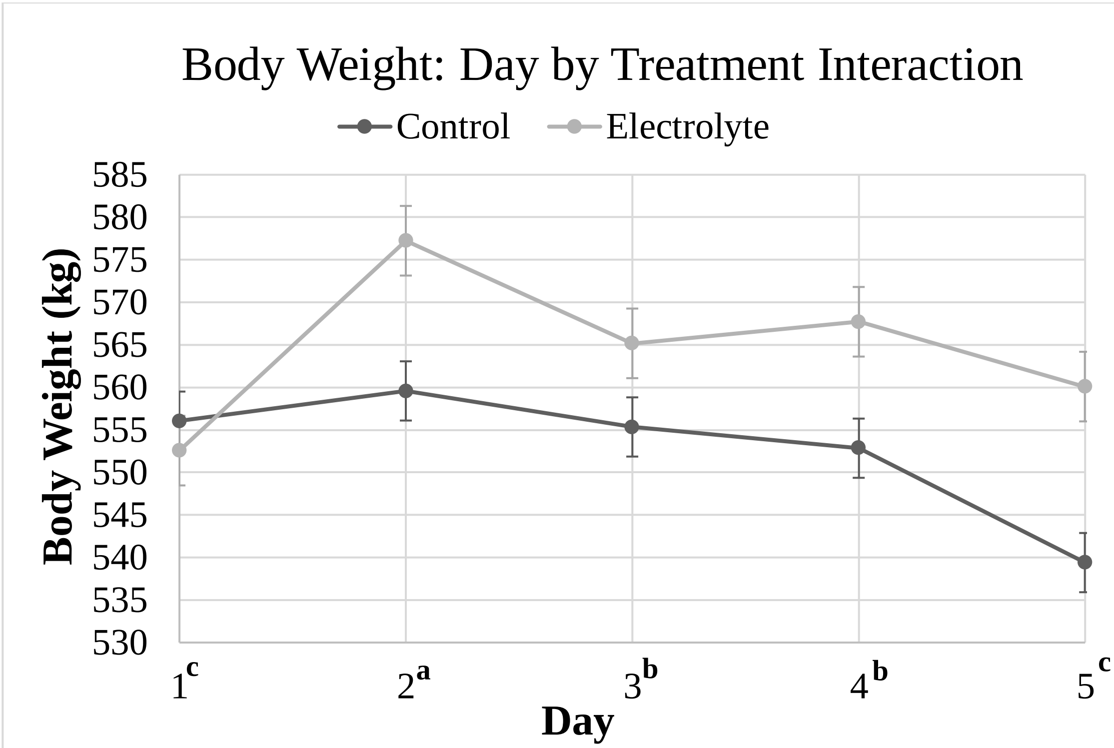

Figure 4. Average treatment group body weight (kg) by day of study, day treatment effect (P=0.0007Body weight measurements were taken each day of the study following the second feeding.

[a,b,c] Days lacking common superscript differ (P<0.001)

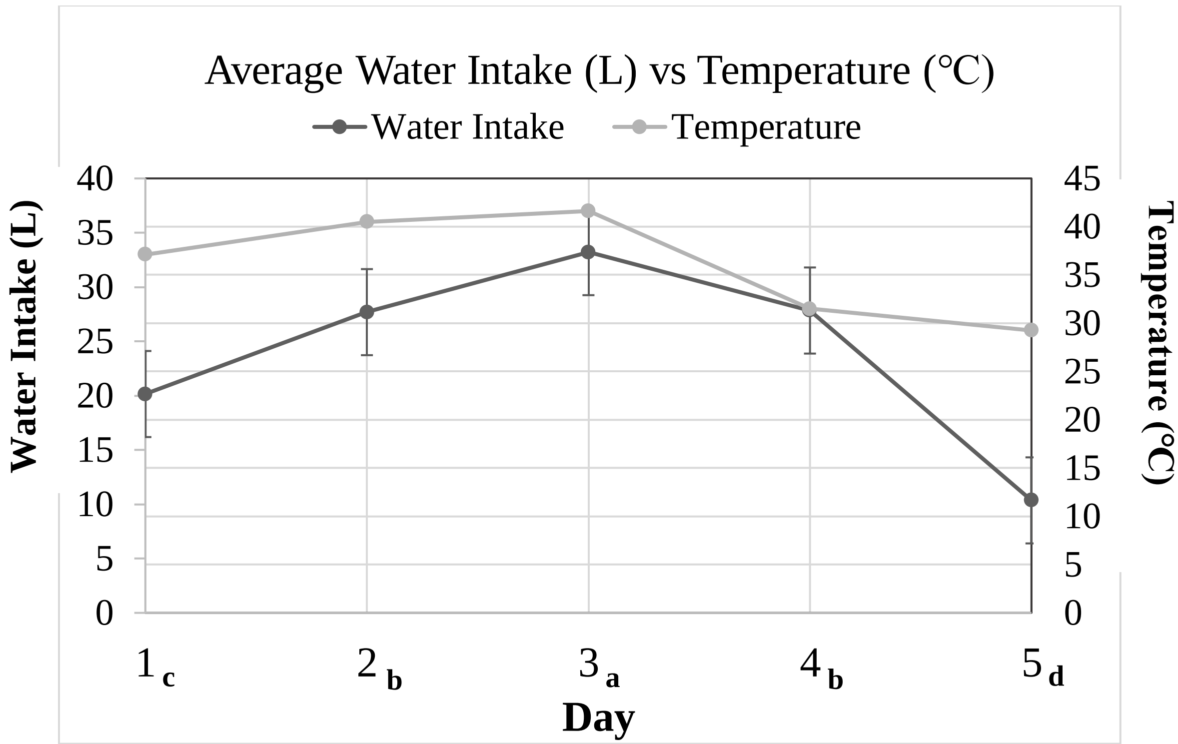

Figure 5. Water intake in liters compared to daily high temperature throughout study. Day 5 water intake was only measured until 12 o'clock due to lesson horse turnout schedule.

[a,b] Days lacking common superscript have means for water intake that differ ($P<0.0001$)

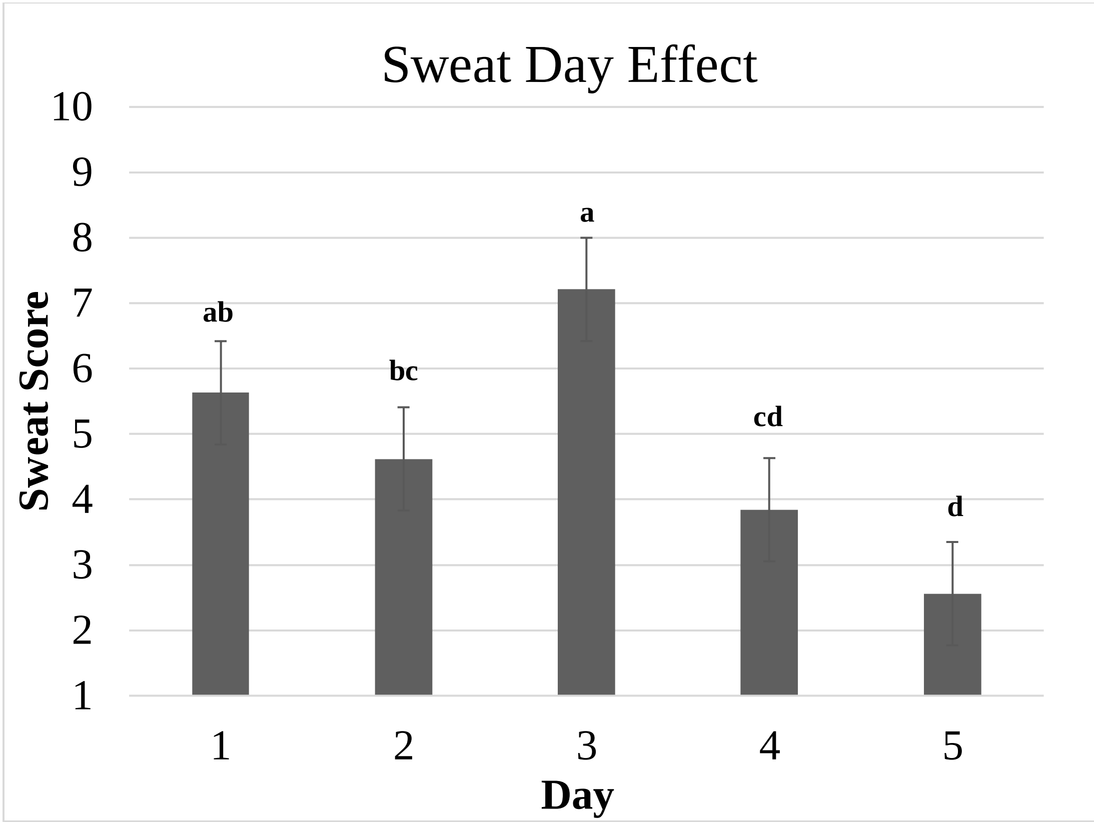

Figure 6: Survey perceived sweat score by day of study. Day 3 exhibited the highest perceived sweat in conjunction with the highest perceived temperature.

[a,b,c,d] values lacking common superscript differ (P=0.0004)

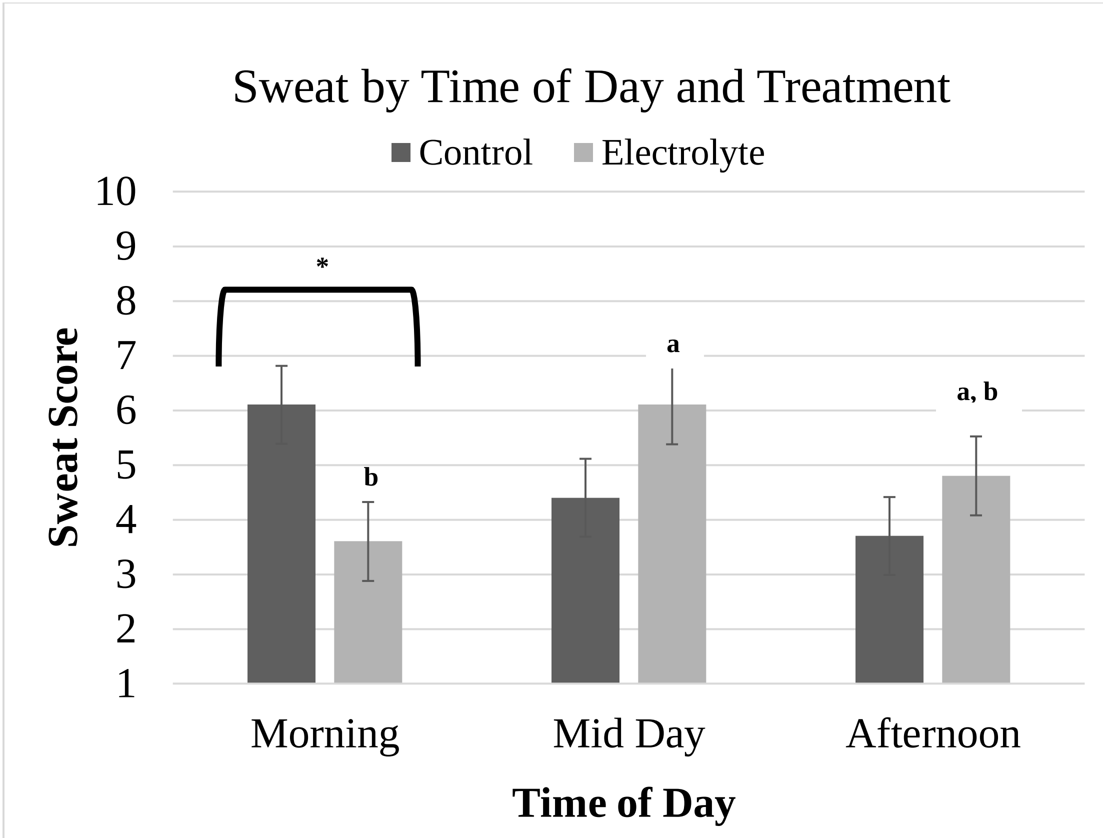

Figure 7: Survey perceived sweat score by time of day ridden.

[a,b] Values lacking common superscript indicate different sweat score among times of day within a treatment (P=0.029).
*Indicates different sweat score between treatments within a time-of-day (P=0.0086).

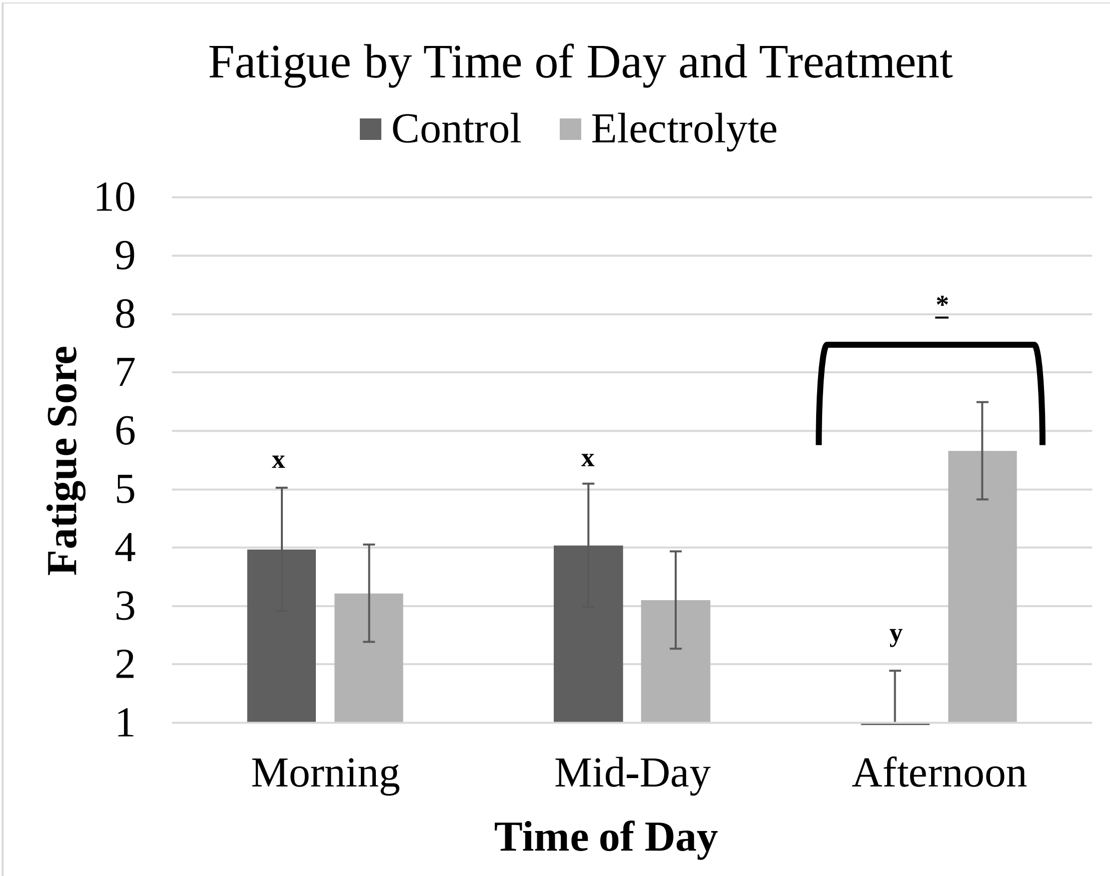

Figure 8: Survey perceived fatigue score by time of day ridden.

^{x,y} Values lacking common superscript indicate trending different fatigue score among times of day within a treatment (P=0.06).

*Indicates different fatigue score between treatments within a time-of-day (P=0.025).

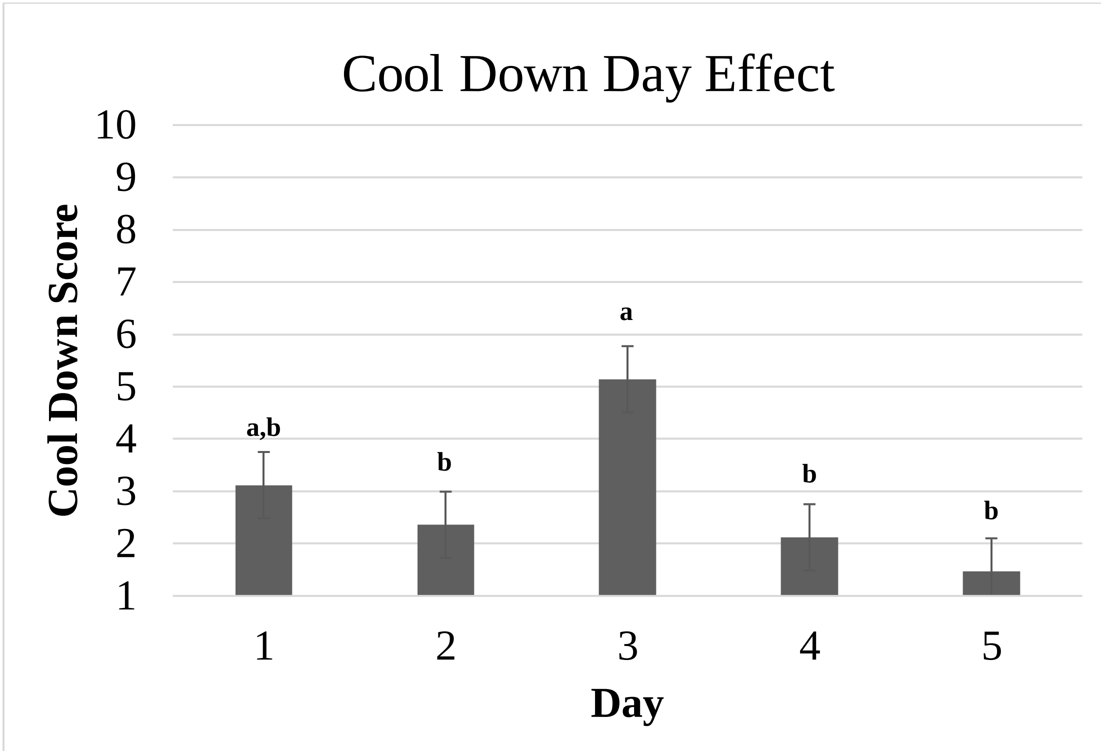

Figure 9: Survey perceived cool down score by day of study.
[a,b] Different letters indicate cool down score difference (P=0.005).

Discussion

Sample Collection

Several studies conclude that supplementing electrolytes increases plasma concentrations of sodium and chloride, with decreasing or varying results of potassium concentrations (Coenen et al., 1995; Dusterdieck et al., 1999; Sampieri et al., 2006). The current study found that the presence of an electrolyte supplement did not change plasma concentrations of sodium, potassium, or chloride. This could be attributed to the dose of the electrolyte, as the current study administered a low-dose electrolyte like Sampieri and colleagues (2006). Sampieri and colleagues (2006) displayed a slight increase in sodium and chloride and a decrease in potassium plasma concentrations with a low-dose electrolyte, however not as significant as those given a high-dose. Similar studies administering a higher dose of these electrolytes (0.4 g/kg BW NaCl and 0.2 g/kg BW KCl) to endurance horses saw a greater increase in plasma concentrations of these electrolytes (Dusterdieck et al., 1999). Coenen and colleagues (1995) concluded that the time the electrolyte supplement is administered prior to exercise impacts plasma concentrations and water intake as well, with supplementary salt intake being more effective 4 hours prior to exercise rather than 1 hour. In the current study, the electrolyte or placebo was given with morning concentrate. Horses were ridden daily at various times of the day, some in the afternoon past the 4-hour mark, or some within an hour of receiving their supplementation.

Strong positive correlations between plasma electrolytes were expected, especially between sodium and potassium and sodium and chloride as these electrolytes were supplemented together. Control horses had higher concentrations of magnesium and bicarbonate. There is normally an inverse relationship between chloride and bicarbonate levels in the blood (Feldman and Dickson, 2018), and higher bicarbonate in the control correlates with this understanding due to the lack of additional chloride in the control group. Lower levels of magnesium in the treatment group correlates to findings with rats conducted by Lee et al. 2013, as administering a high salt substrate was found to increase magnesium excretion. Future successful studies would control exercise in relation to the timing after supplementation, and also may evaluate excretion of electrolytes in sweat and urine, which was not done in this study.

Further, it is important to consider that horses in this study remained on their normal diet and received a pelleted concentrate twice daily (Strategy Professional Formula GX, Purina Mills). Purina Strategy contains 1.25% dry-matter potassium and 0.3% dry-matter sodium, with no notable concentrations of chloride (Purina Mills, 2023). For an average horse receiving 2.7 kg daily of this feed, this is 30g K and 7.21g Na; which may be meeting their electrolyte requirements alone, along with forage. These results could imply that a commercial concentrate is sufficient for meeting electrolyte requirements in horses in similar workloads, thus may not require additional supplementation.

For the present study, blood glucose levels did not vary by day between treatments. Control horses tended to have higher blood glucose levels; however, they were receiving granulated sugar as a placebo. Horses that received the electrolyte supplement had no significant changes in blood glucose levels; yet this was predicted due to blood glucose levels being tightly regulated by regular metabolic processes (Roder et al., 2016). Furthermore, the school horses used for the study are in light to moderate exercise, while previous studies have looked at blood glucose and muscle glycogen levels in conjunction with electrolytes in heavy working horses. Waller and colleagues (2009) found similar results regarding blood glucose levels of an electrolyte solution given nasogastrically in standardbred horses. There was no difference in blood glucose between horses given an electrolyte solution and the control, as in both treatments' glucose increased following 3 to 4 hours following exercise and then returned to normal levels (Waller et al., 2009). One limitation to the current study was the inability to perform muscle biopsies to measure muscle glycogen, the primary indicator of energy available for exercise.

The presence or lack of an electrolyte supplement did not alter horse weight; however, body weight did vary by day during the 5-day period. This variation could be attributed to increased water consumption and retention, as there was a weak correlation between body weight and water intake. Further, there was a day by treatment interaction. At the start of the study the body weight was similar between the two treatments, then body weight peaked on day 2 of the study and tended to decrease in the following days with decreasing temperatures. Horses receiving the electrolyte had greater BW on day 2 along with greater water consumption, which may be attributed to the consumption of supplementary salt. Dusterdieck et al. 1999 demonstrated that horses that received water alone experienced lower water intake; therefore, greater weight loss following exercise in comparison to horses receiving electrolytes or a glycerol electrolyte combination. Horses that received an electrolyte or electrolyte glycerol combination had a much higher voluntary water intake, hence greater body weight and weight retention. Thus, electrolytes supported greater drinking rather than a difference in sweating rate between treatments (Dusterdieck et al., 1999).

Regardless of treatment, all horses consumed more water throughout the study in hot and humid conditions. Due to the record high temperatures (33 to 37°C) in mid-September, overall increased water intake was anticipated. Average water consumption peaked on day 3 at 37L, in conjunction with the highest ambient temperature of the study period (37 °C). In contrast to the lack of treatment effect from this study, previous studies in endurance horses have found that electrolyte supplements do increase overall water consumption compared to horses without electrolyte supplementation (Coenen et al., 1995; Dusterdieck et al., 1999; Sampieri et al., 2006). University horses in light to moderate work may not experience the same fluid and bodyweight losses (sweat, urine, feces) as horses in prolonged exercise, or require as intense of rehydration practices.

Survey

Like Sampieri et. al 2006, this study demonstrated that supplementing an electrolyte did not enhance perceived horse performance. Treatment did not affect perceived horse participation or responsiveness to rider aids during the horse’s daily ride, however horses ridden for two hours rather than one hour tended to have a lower perceived participation score. This is expected as lesson horses ridden for longer periods may become less willing to participate with student riders in hot and humid conditions. Student riders may also become more tired themselves, and this may impact their perception of horse participation.

Perceived sweat did differ by day with the higher sweat scores on day 3, also in alignment with the highest ambient temperature. Perceived sweat scores decreased in the following days as the average temperature decreased. Horses receiving electrolytes were not perceived to sweat more, and horses did not sweat more at different times of day. There was a time of day and treatment interaction; with control horses having higher perceived sweat in morning rides, and electrolyte supplemented horses having higher perceived sweat in mid-day rides and afternoon rides. These results could make us believe horses that received the electrolyte may sweat more efficiently; however, quantity and of sweat and composition were not analyzed to confidently make that conclusion.

The presence or lack of an electrolyte did not change how quickly horses were perceived to fatigue during rides. Control horses tended to be perceived to fatigue quicker in the morning and mid-day rides, yet; horses that received the electrolytes were perceived to fatigue quicker in the afternoon. These results vary from other studies as it is often found electrolytes may prolong time until fatigue and increase the duration of submaximal exercise (Lindinger and Ecker, 2013). In addition, there was no change in perceived fatigue between days of study or the time of day the horse was ridden. Horses ridden for two hours tended to be perceived to fatigue relatively quicker than those ridden for one hour. This was expected as it was a longer bout of exercise in hot and humid conditions.

Day 3 of the study exhibited the longest cool down time out of the study period, but there was no treatment difference or day by treatment interaction. Skin and rectal temperatures were not taken to determine precise internal and external temperature, yet after exercising in hot and humid conditions two days prior, a longer cool down time was expected on day 3. Day 3 was the hottest day of the study, and as exercising in hot and humid environments can limit evaporation and convection, this can prolong heat dissipation (Takahashi and Takahashi, 2020).

Conclusion

Collectively, the results of this study suggest that horses in a light to moderate workload receiving a commercial concentrate may not require additional low-dose electrolyte supplementation based on the parameters evaluated. Supplementation of a low-dose electrolyte did not alter plasma electrolyte levels of the electrolytes administered, horse weight, or blood glucose levels. One of the primary reasons horse owners may include electrolytes in their horse's diet is to drink more water, and contrary to other studies; horses that received the electrolyte did not drink more water on average over the week than control horses. However, it is important to note that electrolyte horses did have higher water intake on specific days compared to the control. This study took place in record high temperatures and all horses consumed more water throughout the study period. Further, the presence or lack of an electrolyte did not affect perceived willingness to work or horse performance. Horses had higher sweat scores, cool down times, and water intake on day 3 of the study, which all aligned with the highest recorded temperature (37°C).

These results are beneficial as there is little research in electrolyte supplementation to horses in light to moderate work. It is possible that administering a higher dose of these electrolytes can exhibit more drastic effects to horses in similar workloads. However, it is important to consider that university horses may not exhibit the same sweat and electrolyte losses as horses in more intense work and may not require as intense rehydration practices. Further research in this topic is needed to evaluate if additional supplementation beyond hay and commercial grain is needed.

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APPENDIX

IACUC APPROVAL

IACUC
INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

MIDDLE TENNESSEE STATE UNIVERSITY

IACUCN006: DMR PROTOCOL APPROVAL NOTICE

Date of original notice: September 8, 2022

Senior Investigator	**Alyssa Logan** (ROLE: Principal Investigator)
Co-Investigators	Rhonda Hoffman, John Haffner
Investigator Email(s)	Alyssa.logan@mtsu.edu, Rhonda.Hoffman@mtsu.edu, John.Haffner@mtsu.edu
Department	School of Agriculture-Horse Science Program
Protocol Title	*Effect of low dosage electrolyte supplementation to university riding horses during hot and humid weather*
Protocol ID	**23-2001**
Funding	None

Dear Dr. Logan,

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the ***Designated Member Review (DMR) mechanism***. The IACUC has determined that your animal use protocol meets the guidelines for approval in accordance with PHS policy. In view of the current COVID- 19 restrictions, the IACUC also introduced a few policies to protect students and junior staff. A summary of the IACUC action(s) and other particulars of this this protocol are tabulated below:

IACUC Action	**APPROVED for one year**	
Date of Expiration	9/8/2023	
Number of Animals	Twelve (12)	
Approved Species	***Equine***	
Category	☐ **Teaching**	☒ **Research**
Subclassifications	☐ Classroom ☐ Laboratory	☐ Laboratory ☒ Field Research ☐ Field Study ☒ Handling/Manipulation ☐ Observation
	Comment: NONE	
Approved Site(s)	MTSU Horse Science Center	
Restrictions	**1. Must comply with all IACUC requirements;** **2. Mandatory compliance with CDC guidelines during COVID-19; Social distancing guidelines are made by the Dean of CBAS.** **3. The PI must make alternative plans to ensure proper animal care, including euthanasia if needed, in the event the research team is quarantined due to COVID19**	
Comments	NONE	

IACUCN006 Version 1.3 Revision Date 05.03.2016

This protocol expires on 9/8/2023 and it can be extended for THREE years until **9/8/2025** by requesting a continuing review by submitting annual progress reports. The investigator(s) MUST file a Progress Report annually updating the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be **approved** by the IACUC prior to 9/8/2023 for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	9/8/2023	NONE
Second year report	9/8/2024	NONE
Final report	9/8/2025	NONE

Post-approval Amendments:

Date	***Amendment***	***IACUC Notes***
NONE	NONE	NONE

Post-approval Actions:

Date	***Amendment***	***IACUC Notes***
NONE	NONE	NONE

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. In addition, refer to MTSU Policy 129: Records retention & Disposal (https://www.mtsu.edu/policies/general/129.php) for Tennessee State requirements for data retention. Please be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,
Matthew Klukowski, Ph.D. (Chair IACUC, acting on behalf of IACUC Office)
Compliance Office
Middle Tennessee State University
Tel: 615 494 8918
Email: iacuc_information@mtsu.edu (for questions) and
Iacuc_submissions@mtsu.edu (for sending documents)

QUALTRICS SURVEY

Survey Questions - Electrolyte Study

Start of Block: Block 1

Informed consent
The following information is provided to inform you about the research project in which you have been invited to participate. Please read this disclosure and feel free to ask any questions. The investigators must answer all of your questions and please save this page as a PDF for future reference. • Your participation in this research study is voluntary. • You are also free to withdraw from this study at any time without loss of any benefits. For additional information on your rights as a participant in this study, please contact the Middle Tennessee State University (MTSU) Office of Compliance (Tel 615-494-8918 or send your emails to irb_information@mtsu.edu. (URL: http://www.mtsu.edu/irb). Please read the following and respond to the consent questions in the bottom if you wish to enroll in this study.

1. Purpose: The purpose of this study is to determine the effects of an oral supplement given to horses in university lesson programs.

2. Description: You will be asked questions relating to your horse's performance, such as willingness, presence of fatigue, or sweating. There is no identifying information recorded in this survey.

3. IRB Approval Details
Protocol title: Effect of low dosage electrolyte supplementation to university riding horses during hot and humid weather
Primary investigator: Dr. Alyssa Logan
PI Department and college: Agriculture, CBAS
Protocol ID: IACUC ID (horse use) 23-2001 IRB ID (rider survey) 23-1012
Approval date:
Expiration date:

4. Duration: This is a 5-day study. You will participate once after each ride on a dedicated study horse. The riding will be your normal riding time for a class at the MTSU Horse Science Center. The post-ride survey will take 5-10 minutes.

5. Here are your rights as a participant:

• Your participation in this research is voluntary.
• You may skip any item that you don't want to answer, and you may stop the experiment at any time (but see the note below)
• If you leave an item blank by either not clicking or entering a response, you may be

warned that you missed one, just in case it was an accident. But you can continue the study without entering a response if you didn't want to answer any questions.

- Some items may require a response to accurately present the survey.

6. Risks and discomforts:
There are no expected discomforts or risks as a result of your participation in this survey.

7. Benefits:
Benefits to you: There are no direct benefits to you from this study
Benefits to the field of science of the community: This survey impacts the current knowledge on equine welfare and exercise. This information will be part of a Master's Thesis and have the potential to be presented at the 2023 Equine Science Society Symposium as well we be published in a journal article.

8. Identifiable Information: You will NOT be asked to provide identifiable personal information

9. Compensation: There is no compensation for participating in this study

10. Confidentiality. All efforts, within reason, will be made to keep your personal information private but total privacy cannot be promised. Your information may be shared with MTSU or the government, such as the Middle Tennessee State University Institutional Review Board, Federal Government Office for Human Research Protections, if you or someone else is in danger or if we are required to do so by law.

11. 11. Contact Information. If you should have any questions about this research study or possibly injury, please feel free to contact Dr. Alyssa Logan by telephone (615-494- 8849) or by email (alyssa.logan@mtsu.edu)/ You can also contact the MTSU Office of compliance via telephone (615 494 8918) or by email (compliance@mtsu.edu). This contact information will be presented again at the end of the experiment.

You are not required to do anything further if you decide not to enroll in this study. Just quit your browser. Please complete the response section below if you wish to learn more or you wish to part take in this study.

Q1 I have read this informed consent document pertaining to the above identified research

○ Yes (1)

○ No (2)

Q2 The research procedures to be conducted are clear to me

○ Yes (1)

○ No (2)

Q3 I confirm I am 18 years or older

○ Yes (1)

○ No (2)

Q4 I am aware of the potential risks of the study

○ Yes (1)

○ No (2)

Q5 By clicking below, I affirm that I freely and voluntarily choose to participate in this study. I understand I can withdraw from this study at any time without facing any consequences.

○ Yes I consent (1)

○ NO I do not consent (2)

End of Block: Block 1

Start of Block: Default Question Block

Q1 On a scale of 1-10 (1 being not willing, 10 being very willing), how willing was your horse to participate today?

▼ 1 (1) ... 10 (10)

Q2 On a scale of 1-10 (1 being not responsive at all, 10 being maximum responsiveness), how responsive was your horse to rider aids today?

▼ 1 (1) ... 10 (10)

Q3 Did your horse defecate or urinate during your ride?

◯ YES (1)

◯ NO (2)

Display This Question:

If Did your horse defecate or urinate during your ride? = YES

Q3 If YES, what was the consistency?

◯ Diarrhea (1)

◯ Loose and Unformed (2)

◯ Firm and Formed (3)

◯ Hard and Dry (4)

Q4 On a scale of 1-10, (1 being not at all, 10 being an excessive amount) to what extent did your horse sweat today?

▼ 1 (1) ... 10 (10)

Q5 On a scale of 1-10 (1 being no fatigue at all, 10 being fatigued quickly) how quickly did your horse fatigue today?

(Signs of fatigue may include decreased responsiveness, need to take frequent breaks, inability to increase speed)

▼ 1 (1) ... 10 (10)

Q6 On a scale of 1-10 (1 being a short amount of time, 10 being a long amount of time) how long did it take for your horse to cool down after riding?

▼ 1 (1) ... 10 (10)

Q8 Which horse did you ride today?

____

Q9 What was the date and time of your riding session?

____

End of Block: Default Question Block