DIGESTIBILITY OF A DIET WITH OR WITHOUT REED SEDGE PEAT IN EXERCISED HORSES

by

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ABSTRACT

An experiment was conducted to determine the effect of a diet containing Reed Sedge Peat (RSP) on nutrient digestibility, and serum glucose and insulin concentrations in mature exercised horses. Six stock-type geldings, ranging from 8 to 19 yr of age, were randomly assigned in a replicated 3 × 3 Latin Square design, and fed a diet consisting coastal Bermudagrass hay and a concentrate with or without RSP. Three dietary treatments consisted of: control with no RSP (CON); 10 lb/ton (4960 mg/kg) RSP (RSP10; Kent Nutrition Group, Muscatine, IA); and 20 lb/ton (9920 mg/kg) RSP (RSP20). The 12-wk study consisted of three 21-d dietary adjustment periods followed by a 3-d fecal collection period. Blood samples were collected via I.V. pre- and post-prandial, and before and immediately following a Standard Exercise Test (SET) on d 21 of each period. Horses were fed hay at a minimum 2% of body weight each day and 2.2 kg/d of each dietary treatment. Feed and feces were analyzed for DM, CP, ether extract (EE), ADF, NDF, ash, and calcium (Ca) and phosphorous (P) concentrations. Serum was analyzed for glucose and insulin concentrations. There was an effect of period on apparent DM ($P = 0.02$), NDF ($P = 0.04$), ADF ($P = 0.01$), Ca ($P = 0.03$), and P ($P = 0.01$) digestibility. Horses consuming RSP20 had greater ($P = 0.03$) overall mean serum glucose concentrations compared to horses consuming RSP10 and CON. Overall mean serum insulin concentrations increased ($P < 0.01$) after horses consumed the grain and hay diet, then decreased significantly 8 h post-prandial. Analysis of the data recorded during the SET indicated mean serum glucose and insulin concentrations decreased ($P < 0.01$) after submaximal exercise. Data reported in this study suggests no benefit was

observed in nutrient digestibility when RSP was added to horses' diets. However, greater glucose concentrations after RSP20 diet consumption suggests feeding RSP20 alters glucose metabolism and increases energy availability for performance horses, but the mode of action is not clearly understood. Therefore, further research may be warranted to investigate effects of RSP on glucose and insulin dynamics in horses.
Key words: horse, glucose, Reed Sedge Peat

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CHAPTER I INTRODUCTION

Numerous research trials have been performed in an attempt to identify dietary additives that improve nutrient digestibility in horse diets. Feed additives have a broad range of purposes, but in horses, much interest has been focused in promoting exercise performance via improved nutrient digestibility and metabolism (Church and Kellems, 1998). Furthermore, addition of novel ingredients to the diet could improve feeding value while decreasing the costs associated with equine feed (Geor and Pagan, 2001). Reed sedge peat (RSP) is a type of humate derived from the breakdown of organic plant material by bacteria (Visser, 1973). The composition of humates include humus, humic acid, fulvic acid, ulmic acid, and trace minerals (Stevenson, 1994). Humic and fulvic acids are the major extractable components of soil humates and are predominantly used to improve soil fertility and enhance nutrient uptake of plants by chelating nutrients with trace minerals to enable uptake of nutrients by plant cells (Rajendiran and Purakayastha, 2016). However, use of humates as a nutrient source for livestock has a relatively short history. Lenk and Benda (1989) and Griban et al. (1991) first studied the effects of humic acid on the immune system of calves and demonstrated improved growth performance compared to calves fed a control diet with no humic acid. Kuhnert et al. (1989, 1991) studied the effects of humic acid on the mucosal lining of the intestinal tract of dogs and cats as a method to prevent non-infectious digestive disorders, and reported humic acids decreased the incidence of enteritis. Humic acids are also thought to benefit the intestinal

flora by increasing villus length and subsequently increasing nutrient absorption (Islam et al., 2005).

In previous research with horses, replacing traditional cereal grains with non-starch carbohydrate feeds reduces the dietary starch content and may alter the glycemic effect of the diet (Stull and Rodiek, 1988). For example, higher blood glucose concentrations caused by increased dietary starch would facilitate a greater insulin response to aid in the uptake of glucose by the working muscles (Hiney and Potter, 1996). This nutritional approach may benefit the exercising horse because maintaining high blood glucose concentrations during exercise could reduce the onset of fatigue (Frape, 1988; Hiney and Potter, 1996). Reed sedge peat has been used in horse feed as a minor source of trace minerals, for absorbing excess moisture, and as a binder for toxins (Crandell, 2016). However, to this author's knowledge, no data is available in the literature that investigates the metabolic effects of RSP on mature exercising horses. Therefore, this study was designed to determine the effect of a diet containing RSP at two different concentrations on nutrient digestibility, and serum glucose and insulin concentrations in mature exercised geldings.

CHATPTER II LITERATURE REVIEW

Equine Digestive Function

Equine nutrient requirements are based on assumptions about how well horses digest and absorb nutrients (Pagan and Geor, 2005). Anatomically, horses are classified as non-ruminant herbivores, or hindgut fermenters (Stevens, 1988). Horses use endogenous enzymes to digest carbohydrates, protein, and fat in the stomach and small intestine, while microflora ferment organic matter in the hindgut. The stomach is relatively small and comprises less than 7% of the empty weight of the digestive system, while the small intestine makes up approximately 27% (Duren, 1998). The hindgut (cecum and colon) is the largest component of the gastrointestinal tract of the horse and accounts for approximately 64% of the empty weight of the digestive system (McMeniman et al., 1987). The large intestine of the horse is a fermentative vat of bacteria and protozoa that synthesizes the enzymes needed to digest structural carbohydrates including cellulose and hemicellulose (Pagan, 1998). Fermentation in the equine hindgut results in the production of carbon dioxide, methane, and VFA. (Pond et al., 2005). Volatile fatty acids can supply a horse with a substantial portion of their digestible energy (DE) requirements. Fermentation in the cecum alone can provide up to 30% of the horses DE at maintenance (NRC, 2007). Therefore, forage should be the primary component of equine diets (Raymond et al., 2003). The NRC (2007) suggests a minimum of 1% BW of long-stemmed forage be fed for a horse at maintenance.

Diet composition, feed processing, and level of intake will affect the rate of passage between the small intestine and the hindgut (Potter et al., 1992). The relative importance of hindgut digestion will increase with greater concentrations of forage in the diet. Digestion and metabolism of structural carbohydrates in the large intestine may meet the energy needs of many horses at maintenance, but is unable to meet the needs of horses with higher requirements when used at the sole feed source (NRC, 2007). Traditionally, dietary energy density has been increased by adding grains (oats, barely, corn, or others) or grain by-products to the horse's diet. Most of the energy in grains is present as starch (NRC, 2007).

Feeding a "high" starch diet (≥ 30% starch) to horses has been reported to decrease the concentration of cellulolytic bacteria and increase the concentration of amylolytic bacteria in the hindgut (Medina et al., 2002). On the contrary, Moore and Dehority (1993) reported minimal differences in the abundance of cecal cellulolytic bacteria in ponies receiving diets consisting of 90% hay and 10% concentrate compared to ponies receiving 60% hay and 40% concentrate. However, the higher concentrate diet was associated with increased total bacteria and protozoa in the colon (NRC, 2007). In mature Thoroughbred horses, Willard et al. (1977) reported both cecal pH and the molar proportion of acetate were reduced when horses were switched from an all-hay diet to an all-concentrate diet, but the molar proportion of propionate increased. Shifts in bacteria population in the hindgut of horses could facilitate differences in diet digestibility and nutrient utilization.

Digestibility

Digestibility can be defined as a measure of the degree of digestion of a particular nutrient in the digestive tract (Pagan, 1998). Digestibility is not always a constant value as feed intake and physiological state (e.g. age, gender, activity level) can influence digestibility (Aiken et al., 1989; Pagan et al., 1998). Nutrient digestibility can be expressed in two different ways: apparent digestibility and true digestibility. Apparent digestibility is calculated by determining the amount of a specific nutrient recovered in the feces subtracted from the total intake of that nutrient. The amount that disappeared (intake minus feces) is then divided by the total daily intake of the nutrient to express digestibility as a percentage of nutrients consumed (Pagan, 1998). This method is not considered the most exact (or "true") measure of digestibility of some nutrients because not all the material in feces is residue from the diet (Geor and Pagan, 2001). The non-dietary fraction is called the "endogenous fraction" (Schryver et al., 1970). Regression equations and removal of endogenous losses from feces using neutral detergent solution are two of the ways to account for endogenous losses in true digestibility studies (Mertens, 2002). However, because of the relatively low cost involved and difficulty in measuring endogenous nutrients, apparent digestibility is more common than true digestibility studies (Mertens, 2002).

Digestibility Trials

Digestibility trials often require maintaining a constant or consistent daily feed intake, so that day to day variation in fecal excretion is minimized (Pond et al., 2005). From 1877 to 1895, Wolff and co-authors conducted some of the earliest digestion trials in horses, although they were performed with few experimental units (Martin-Rosset and

Dulphy, 1987). Patterson (1902) recognized the need for more in-depth information on nutritive value and digestibility of varying feeds in horses and multiple trials were performed to determine the digestibility of oats, corn, "new corn product," timothy hay, and other feeds. The total collection methods used in Patterson's experiments were adapted from experiments with steers, which included a 9-d adaption period followed by a 5-d digestion trial. Compared to other collection methods, equine total collection studies are considered to be accurate because digestibility in different metabolic conditions can be studied and are the basis of the French Horse Net Energy System (Miraglia et al., 1999). Kentucky Equine Research, Inc., in Versailles KY, uses a 3-wk dietary adjustment period followed by a 5-d collection period (Pagan and Geor, 2005). Although there are differing accounts of the necessary length and time for total collection trials, the NRC (2007) suggests that at least a 7-d dietary adjustment period is necessary, and a total fecal collection period greater than 2 d is needed to ensure the passage of nutrients (NRC, 2007).

Carbohydrate Digestion

Most of the carbohydrates in equine diets originate from forages, grains, and grain by-products. Traditionally, dietary energy density in the horse's diet is increased by the addition of starch from grains (NRC, 2007). Irrespective the starch source, total tract apparent digestibility is usually very high (Cuddeford, 2001). Arnold et al. (1981) reported apparent starch digestibility values for corn, oats, and sorghum as 97.0, 96.7, and 97.0%, respectively. However, within the study, considerable differences were observed in pre-cecal starch digestibility. In monogastric species, dietary starch is digested in the small intestine (NRC, 2007). The starch that reaches the large intestine

where it will be fermented by microbes into VFA is categorized as resistant starch in other monogastric species (Englyst and Hudson, 1996; Englyst and Englyst, 2005). Resistant starch is defined as starch that is not broken down by digestive enzymes because of its structure (Cummings et al., 1996; Tharanathan, 2002). Therefore, in horses, pre-cecal starch digestion increases the proportion of carbohydrates absorbed as glucose in the small intestine (NRC, 2007).

Glucose Metabolism and Storage

After starch consumption, most glucose absorption appears to occur in the proximal small intestine in monogastric species before entering the blood stream (Dyer et al., 2002). Blood glucose concentration rises when a meal is consumed. Likewise, insulin levels will increase, which enhances glucose uptake by muscle and adipose tissues. Absorbed glucose may be immediately used or stored for later use (NRC, 2007). The monosaccharide glucose is the primary form of energy used for ATP production (Lewis et al, 2020). Glycolysis is the metabolic pathway that converts glucose into pyruvate and a hydrogen ion. The energy released during this process is used to form high-energy molecules, such as ATP and NAD. The storage carbohydrate in animals is glycogen, and the main storage sites include the muscle and liver. Glycogen stored in the muscle is available primarily to the muscle as a fuel source. However, glycogen stored in the liver may also be broken down to augment blood/plasma glucose, which is available to other tissues. Glucose that is not used for immediate energy or for glycogen synthesis may also be used for fat synthesis (NRC, 2007).

Insulin

In response to glucose metabolism, the release of insulin is governed by a complex signaling network linking plasma glucose to pancreatic ß-cell function (Leibiger et al., 2008). Insulin is characterized as a classic pleiotropic hormone, which influences multiple organ systems and metabolic pathways (Pollak et al., 2004). In response to elevated glucose levels, insulin is released from β-cells within the pancreatic islet. Insulin receptors regulate metabolism in the canonical ‘‘big three’’ tissues: liver, adipose tissue, and skeletal muscle (Schmidt and Hadley, 2009). However, insulin also affects the central nervous system, vascular bed, kidneys, heart, pancreas, and gastrointestinal tract; (Pollak et al., 2004; Biddinger and Kahn, 2006; Bertrand et al., 2008). In each case, the role of insulin is to enhance the uptake of glucose via receptors on target cells where long chains of glucose are metabolized, linked, and stored as glycogen, or used as an energy source for the synthesis of proteins or fats (Hadley and Levine, 2006). Regulation of the cellular actions of insulin is essential to maintenance of macronutrient metabolism, body weight regulation, and a diverse range of integrative physiologic functions (Schmidt and Hadley, 2009).

Within the liver, insulin also activates glycogen synthetase, which pushes glucose towards glycogen formation (Hadley and Levine, 2006). Glycogen is readily broken back down to glucose for use as an energy source when needed (Sell et al., 2012). Insulin stimulates the active transport of glucose and amino acids into muscle cells, and subsequently, enhances protein synthesis. Glycolysis and oxidative phosphorylation of glucose derivatives provide the energy required for such metabolic activity (Hadley and Levine, 2006).

Influence of Grain Source on the Glycemic and Insulinemic Responses

Blood glucose and subsequently insulin concentrations rise when a meal high in starch is consumed (NRC, 2007). Research investigating the glycemic effects of different feeds in resting and exercising horses indicates that blood glucose levels are highly influenced by the diet (Rodiek et al., 1991). Stull and Rodiek (1995) reported greater blood glucose concentrations in 2-yr-old Quarter Horse geldings fed a combined diet of 50% corn and 50% alfalfa compared to a 100% alfalfa diet. Insulin concentrations closely followed the glucose concentrations in both diets (Stull and Rodiek, 1995). The authors stated that greater blood glucose and insulin concentrations are likely due to a higher starch content in the combined diet.

Increased starch availability in the small intestine may alter the metabolic response as more substrates are absorbed (Geor and Pagan, 2001). A study by Hansen et al. (2020) suggested high-soluble fiber diets may reduce and delay post-prandial glucose and insulin responses in Quarter Horse geldings fed diets containing Orchardgrass hay, beet pulp, or almond hulls. Blood was serially sampled for 6 h after feeding to evaluate glycemic and insulinemic responses. Horses consuming Orchardgrass hay had lower glucose and insulin concentrations compared to horses consuming beet pulp. Foods that elicit low post-prandial glycemic responses are considered beneficial in horses with metabolic diseases (Hansen et al., 2020). In horses with impaired glucose metabolism, plasma glucose concentrations remain higher for longer periods of time, and horses were less sensitive to insulin (Geor and Pagan, 2001). Therefore, reducing carbohydrate intake may be used for prevention and management of glucose intolerance in horses.

Glycemic and Insulinemic Response Before Exercise

In addition to carbohydrate availability affecting the metabolic response in horses during digestion, the contribution of carbohydrates to energy production increases with increasing work intensity (NRC, 2007). Carbohydrates are an important source of energy for contracting skeletal muscle in the exercising horse. The carbohydrate used during exercise may arise from muscle glycogen storage, blood glucose derived from the diet, hepatic gluconeogenesis, or hepatic glycogenolysis (NRC, 2007). In response to exercise, insulin stimulates active transport of glucose and amino acids into muscle cells. Both exercise and insulin dependently mediate the translocation of the glucose transporter isoform glucose transporter-4 (GLUT4) to the cell membrane. The exercise response can upregulate the expression of GLUT4 so that more total transport capacity is present (Goodyear and Kahn, 1998). Glycolysis and oxidative phosphorylation of glucose derivatives provides the energy required for such metabolic activity (Hadley and Levine, 2006).

Muscle and liver glucose storage can decrease dramatically when strenuous exercise continues for an extended period (NRC, 2007). Nimmo and Snow (1983) studied changes in muscle glycogen concentrations of Thoroughbred horses following various maximal exercises. The authors observed 13% depletion of glycogen concentrations during a 506-m sprint. When distance lengthened to a 1,025-m sprint, glycogen concentrations depleted to approximately 35%. However, when average speed decreased, glycogen concentrations decreased to approximately 20% when distance lengthened to 3,620 m during exercise. The authors suggested the extent of muscle glycogen depletion may be affected by exercise intensity and duration. High intensity exercise has lower

muscle glycogen utilization in comparison to long term and moderate exercise with a higher rate of glycogen use (NRC, 2007).

Maintaining adequate carbohydrate availability is important for performance and offsetting fatigue (NRC, 2007). However, composition of the diet can affect glycemic availability in the blood during exercise (Stull and Rodiek, 1995; Pagan and Harris, 1999). Dietary management before exercise may affect performance because of altered energy metabolism during exercise. In several studies, corn fed 1, 2, 3, or 4 h before exercise decreased plasma glucose and insulin concentrations during exercise to below pre-feeding levels (Rodiek et al., 1991; Lawrence et al., 1995; Stull and Rodiek 1995; Pagan and Harris,1999). Horses that began exercise with high blood glucose and insulin (e.g., after corn feeding), experienced transient hypoglycemia during exercise. During exercise, a drop in blood glucose concentration may indicate a lack of glucose availability for the muscle or brain and might have a deleterious effect on performance (Pagan and Harris 1999). Additionally, Lawrence et al. (1993) reported higher pre-exercise glucose levels when horses consumed corn while the size of the meal (1, 2, or 3 kg/d) did not affect the glucose response. In contrast, horses with lower pre-exercise blood glucose and insulin concentrations (e.g., after alfalfa feeding) maintained steady glucose and insulin levels throughout exercise (Lawrence et al., 1993; Pagan and Harris, 1999). In the performance horse, developing feeding strategies that include feedstuffs with a high carbohydrate content and low glycemic index is the ideal diet when horses have higher energy demands (Geor and Pagan, 2001).

Previous research indicates that blood glucose concentrations are influenced by diets in both resting and exercising horses. A study by Stull and Rodiek (1995) evaluated

the metabolic and hormonal responses in horses after consuming corn, alfalfa, or fasting at differing intervals before moderate aerobic exercise. A group of 6 mature Thoroughbreds were used to determine the effects of 3 feeding regimes and 2 feeding intervals on the availability of energy metabolites during exercise. Blood was sampled every 15 min for 8.5 h except during the 55-min SET, in which 6 samples were drawn during the SET. The authors reported the blood glucose levels in corn-fed horses fell below pre-feeding concentrations during the exercise test, but rebounded at the termination of cantering in tests performed by horses exercising 1 and 4 h after feeding. In contrast, blood glucose concentrations in alfalfa-fed or fasted horses gradually rose throughout the exercise test. The authors concluded the composition and timing of a meal before exercise may be manipulated to influence glycemic metabolites available in the blood.

Feed Additives

Feed additives for animals can be defined as non-nutritive ingredients that stimulate growth or other types of production, improve the efficiency of feed utilization, or benefit health and metabolism (Church and Kellems, 1998). Much research interest surrounding feed additive use has been focused on the potential role in promoting animal health and well-being, performance, and disease mitigation or prevention. Further, feed additives not currently recognized as an essential nutrient may have beneficial role(s) in metabolism to facilitate and enhance body function (NRC, 2007).

In human health, fitness, and nutrition, inclusion of dietary additives for the purpose of improving health and performance has expanded the market for such products and has generated new terminology. There is similar interest within horse nutrition (NRC,

2007). However, some concern has been raised relative to the safety and efficacy of such products, as well as the role of regulatory oversight (Boothe, 1998). In feeding horses, as with any species, emphasis should be placed on feeding a complete and balanced diet (NRC, 2007).

Reed Sedge Peat

Reed Sedge Peat (RSP) moss is a type of humate that is derived from the breakdown of organic plant material, mainly by bacteria (Visser, 1973). Peatlands are characteristically waterlogged locations found on relatively flat landscapes. These landscapes often produce cold and anaerobic conditions slightly beneath the waterlogged surfaces, which cause organic residues to accumulate to deeper depths, often up to several feet below the surface (Gorham, 1991). A series of complex decomposition and accumulation processes in this kind of environment lead to the formation of peat. Peat bogs form where there is a presence of excess water for most or all the year. This condition prevents the complete degradation of organic substances, leading to an accumulation of sediments that form peat (Bozkurt et al., 2001).

The composition of humates include humus, humic acid, fulvic acid, ulmic acid, and trace minerals (Stevenson, 1994). Humic and fulvic acids are the major extractable components of soil humates and are predominantly used to improve soil fertility (Rajendiran and Purakayastha, 2016). Most humic substances are chemically bonded to inorganic components (clay and oxides). Therefore, humic substances can easily combine with inorganic attachments such as metal ions, oxides, and clay minerals to form water soluble or insoluble complexes, which allows interaction with organic substances including alkenes, fatty acids, capillary-active substances, and pesticides (Islam et al.,

2005). Humic acids increase permeability of cell membranes and thus facilitate transport of minerals from blood into cells (Visser, 1973). Figure 1 illustrates an oxidized humic acid. This organic molecule is naturally oxidized and therefore negatively charged, thus, positive ions, such as micronutrients and microflora will be attracted at the site of oxidation, allowing for absorption to occur (Islam et al., 2005). Some common examples of micronutrients are vitamins (including riboflavin, folate, and biotin), minerals, and trace elements needed for energy metabolism, organ function, and cellular growth (Ishiguro et al., 2018).

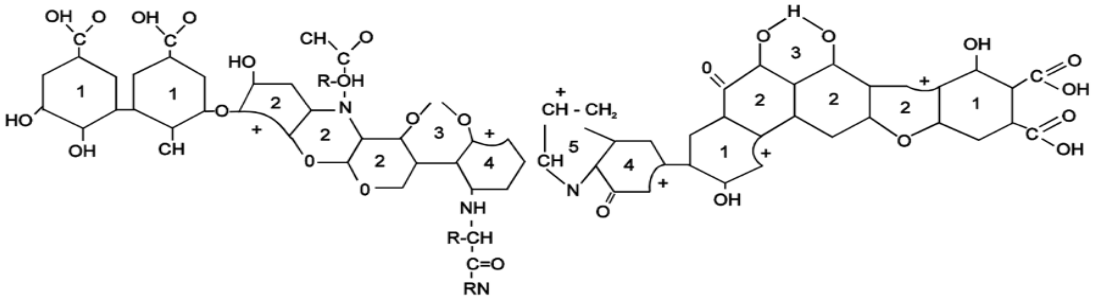

Figure 1. Oxidized Humic Acid Molecule

The application of humates in animal nutrition has a very short history (Kocabagli et al., 2002). Livestock producers and owners have used peat as a non-traditional supplement in animal nutrition. Positive effects of humic substances on the performance and health status of animals have been documented in various studies (Stepchenko et al., 1991; Zhorina and Stepchenko, 1991; Fuchs et al., 1995; Bailey et al., 1996; Kocabagli et al., 2002; Yoruk et al., 2004). Lenk and Benda (1989) first studied the effects of humic acid on non-infectious enteritis in calves. After humic acid administration, reports recorded the frequency of enteritis was reduced by more than 30%. The lower frequency of enteritis may be from humic acids in peat covering the open nerve endings in mucosa causing a reduction in excitability, which prevents lesions of intestinal mucosa (Slavik,

1999). Additionally, authors observed an increased growth performance in calves after humic acid administration.

Research suggests humic substances can also act as natural antibiotics, resulting in increased growth performance in cattle and goats (Cusack, 2008; Degirmencioglu, 2014). Humic substances have been observed to have antimicrobial (Váradyová et al., 2009), binding, and detoxifying properties, which in soils have been described to promote microbial growth (Islam et. al, 2005). Fuchs et al. (1995) studied peat preparation fed as a supplement to piglets and observed improved growth and general health status. These authors concluded that piglets that received peat preparation or antibiotics had increased growth from d 59 of life compared to a negative control.

Peat contains a large number of water-soluble components, which have stimulatory effects on the spontaneous contractile activity of smooth muscle tissue (Beer and Lukanov, 1998; Beer et al., 2000; Zagorchev et al., 2000). The excitatory effect occurred via activation of the *αlpha 2*-adreno and D2-dopamine receptors. However, inactivation of the same receptors after prolonged impact will inhibit these effects. (Beer et al., 2000). The effect of peat extracts on the spontaneous contractile activity of smooth muscles may be due to the organic compound's molecular weight (Zagorchev et al., 2000).

Existing studies have been inconclusive when evaluating the effects of humic substances in ruminant diet. A study by Terry et al. (2020) suggested humic substances as a feed additive can provide increased milk production in dairy cows due to their ability to modify rumen fermentation patterns, however, the mechanism of action of humic acids

remain unclear. The author also observed humic substances increased nitrogen intake and CP digestibility. A similar response was observed in pigs supplemented with humic substances, as apparent total nitrogen digestion increased (Ponce et al., 2016). Shi et al. (2001) also observed the nitrogen binding capacity of humic substances in feedlot cattle. Humic and fulvic proportions of humic substances can act as strong chelators (Mackawaik et al., 2001) and may be responsible for binding minerals, and possibly converting them to a chemical state that is more readily absorbed by cells and organisms. Trace elements within humic substances may also act as cofactors in nitrogen digestion by increasing proteolytic enzyme activity (Hayirli et al., 2005).

Previous studies have reported that the addition of RSP to the diet can improve feed efficiency and health in livestock. Supplementation of nutritional additives to the diet in horses may be beneficial to increase nutrient digestibility and alter metabolism of the horse. To this author's knowledge, no research has been conducted between RSP and the effect of glucose and insulin metabolism on the performance of livestock. Peat has been used as a feed additive in horses to supply a minor source of trace minerals, absorbing excess moisture, and binding toxins. Moreover, the addition of RSP to the concentrate portion of the diet may benefit horse owners by decreasing the cost of feed. Therefore, the objective of the current study was to determine the effect of three levels of RSP concentrate on nutrient digestibility, and serum glucose and insulin concentrations in mature exercised horses.

CHAPTER III MATERIALS AND METHODS

All procedures using animals in the study were approved by the West Texas A&M University (WTAMU) Institutional Animal Care and Use Committee (Protocol # 2020.02.005). Diets were formulated to meet or exceed the "Nutrient Requirements of Horses: Sixth Revised Edition" published in 2007 by the National Research Council (NRC, 2007) for moderately exercised horses.

Experimental Design

The 12-wk study consisted of three 24-d periods, each consisting of a 21-d adaption period followed by a 3-d sample collection period. Six stock-type geldings, 8 to 19 yr of age (n = 6) were used in a replicated 3 × 3 Latin Square design experiment to determine the effect of 3 levels of RSP on nutrient digestibility, serum glucose and insulin concentrations in exercised horses.

Diets and Treatments

The treatment diet consisted of a 1:4 grain to hay diet with concentrate pellets fed with coastal Bermudagrass hay. The experiment used 3 treatments (Table 1): a control (CON) that contained no added RSP; RSP10 concentrate pellet that contained approximately 10 lb/ton (4960 mg/kg) of RSP (Kent Nutrition Group); and RSP20 pellet concentrate that contained approximately 20 lb/ton (9920 mg/kg) of RSP (Kent Nutrition Group). Two cuttings of coastal Bermudagrass hay were fed in the trial due to Hay 1 was

from a cutting fed in period 1 (P1) and 2 (P2), while hay 2 was from a cutting fed in period 3 (P3).

Horse Care and Management

Horses were housed in 3 groups of 2 in 6 × 20 m dry-lot pens at the WTAMU Horse Center in Canyon, TX. Horses were fed at 0700 and 1600 individually in 2 × 5 m pens. Horses were fed hay in concrete bunks initially at 2% body weight (BW). The amount of hay offered to each horse was then adjusted to a minimum of 2% BW in an attempt to maintain body weight while reducing orts. Horses were fed 2.2 kg/d of a proprietary pellet concentrate from Kent Nutrition Group (Muscatine, IA) with or without added RSP with 20-L buckets used to supply the concentrate pellets. Horses were allowed 3 h to consume diets before being turned out into 6 × 20 m group housing pens where no feed was available. Water was available ad-libitum throughout the trial. Additionally, horses were turned out in a 40 × 60 m dry-lot exercise paddock between morning and evening feedings 3 times per wk.

Horses were weighed at 0700 before morning feeding on d 0 and weekly thereafter. Routine farrier work, vaccinations, and veterinary care were administered consistent with facility SOP's. During the trial, all horses were used in beginner horsemanship classes or exercised 4 times per wk at the WTAMU Horse Center, which would classify them under "moderate work" according to the NRC (2007) weekly workloads of horses in light, moderate, heavy, and very heavy exercise categories.

Exercise Regimens

Horses were exercised 4 d/wk for the duration of the trial using 2 exercise regimens. The ridden exercise regimen was used 3 d/wk and consisted of horses ridden at a walk, trot, and lope for 1 h. The longe exercise regimen was used 1 d/wk and consisted of horses being individually longed in a circle (5 or 7 m radius) for 5 min on both left and right rein at a walk, trot, and lope. When there was no scheduled exercise, horses were turned out in 40 × 60 m dry-lot exercise paddock.

Standard Exercise Test

On d 21 of each period, a standard exercise test (SET) was conducted at the WTAMU Horse Center outdoor arena (150 × 250 m; 225 m radius). Horses were ridden by 6 riders with similar riding experience. The SET began with a warmup of 10 min at a walk and trot. After warm up, the SET (all on the left rein and lead) consisted of: walk 450 m, trot 675 m, lope 675 m, and extended lope 900 m (Harris et al., 2007). Horses were then walked for a 10-min recovery period.

Collection Procedures

Hay Collection. A Penn State Forage Sampler (Scientific Systems, Atkinson, WI) was used to collect random core samples from 10% of the hay at delivery. Hay refusals were weighed daily 3 h after feeding. There were no refusals for the concentrate pellets. Hay and orts were weighed using an industrial floor scale (M3315, Escali Products, NM). Hay samples were stored in 3.8 L Ziploc bags at room temperature until later analysis.

Fecal Collection. Horses were fitted with fecal collection harnesses (Bun Bag™; Sagle, ID) and moved into individual pens over the last 3-d of each experimental period. Total feces voided was collected every 4 h, or when fecal bags were full. After bags were emptied into a weigh boat, total fecal weights were recorded using a bench scale (Ranger 7000, OHAUS Corporation, Parsippany, NJ). Fecal samples were mixed and 10% samples were taken from the total weight and stored separately in sealed plastic bags at -10°C.

Blood Collection. On d 21 of each period, blood samples were collected via jugular venipuncture using a 20-ga needle attached to a Vacutainer hub. Blood was collected into a 4 mL BD vacutainer EDTA collection tube (Franklin Lakes, NJ) for plasma and 10 mL SST BD vacutainer tube (Franklin Lakes, NJ) for serum. Venous blood was collected 30 min pre-feeding (0630), 30 min post-feeding (0730), 8 h post-feeding**,** 30 min pre-SET (1430); then 30 sec, and 10, 30, 60, and 120 min post-SET. Plasma tubes were immediately inverted 5 to 6 times to ensure mixing of anticoagulants to prevent blood clotting. The serum blood tubes were allowed to clot and samples were stored in a refrigerator at 4°C for up to 3 h.

Blood Sample Preparation. Blood samples were taken to the WTAMU Ruminant Health and Immunology Laboratory (Canyon, TX) where plasma and serum samples were centrifuged (Beckman Coulter Allegra Model 6R, Indianapolis, IN) for 25 min at 2,058 x g. Both serum and plasma samples were transferred using plastic pipettes to a 2 mL polypropylene tube in duplicate, and frozen at -20 °C for later analysis.

Laboratory Analysis

Sample Preparation and Dry Matter Determination. The DM procedure was adapted from the methods outlined by Van Soest (1967) to allow for subsequent analysis of lignin and ADF. Fecal samples were thawed for 24 h at room temperature and placed in 9 × 11 cm aluminum drying pans, and initial and final weights were recorded using an electronic bench top scale (Ranger 4000, OHAUS Corp., Parsippany, NJ). After drying in an oven (SMO28HP model, Sheldon Manufacturing Inc., Cornelius, OR) at 55°C for 72 h, dry samples were then composited by an equal weight basis. The resulting composite was a representation of each horse for each time period. Dried fecal samples were ground through a 2-mm screen (Wiley-Mill Model 4, Thomas-Wiley Mill, Philadelphia, PA). Ground samples were placed in a Whirl-Pak storage bag (Nasco, Fort Atkinson, WI) and stored at room temperature for later analysis.

Nutrient Analysis. Feed and fecal analysis was performed at ServiTech Laboratory (Amarillo, TX). Nitrogen (N) was analyzed using the LECO FP928 (LECO Corporations, St. Joseph, MI), which quantitatively analyzes the combustion products of the sample by evaluating changes in the thermal conductivity of the reference gas. The CP concentration was then calculated by multiplying N by a coefficient of 6.25 into CP. Ether extract analysis was performed using the AOAC Method 920.39. Neutral and acid detergent fiber analysis was performed with the “filter bag technique” (ANKOM Technology, 2011) using the AOAC Method 2002-04. Ash content of feed and fecal samples were determined by heating in a muffle furnace at 600 °C for 24 h (Servi-Tech; Amarillo, TX).

Blood Glucose and Insulin Analyses. Blood serum was analyzed for fasting in all insulin and glucose concentrations at Michigan State University Veterinary Diagnostic Laboratory. Serum insulin concentrations were analyzed using RIA. Serum glucose concentrations were measured with 340 nm absorbance spectrophotometrically using the Hexokinase (glucose-6-phosphate dehydrogenase) reaction method.

Statistical Analyses

Data for nutrient digestibilities, and serum glucose and insulin were analyzed using the Proc Mixed Procedure of SAS v. 9.4 (SAS Institute, Inc., 2014) with period, treatment, and time as fixed effects as a replicated 3 × 3 Latin Square. The interactions of each independent variable (treatment × time) were also analyzed. Horse was considered as a random effect. The covariance structures tested used the lowest AIC criterion, which included compound symmetry and variance components. Means were separated by least significant difference. Linear and quadratic effects were analyzed. Significant differences between means were declared at $P \leq 0.05$. Differences between means were declared as trends $P \leq 0.10$.

CHAPTER IV RESULTS AND DISCUSSION

Horse mean BW (Table A-1) was 546 kg throughout the study. Nutrient analyses of RSP pellets and coastal Bermudagrass hay analyses on a DM basis are shown in Tables 1 and 2, respectively. Effect of period on nutrient intake in exercised horses are shown in Table 3. Effect of treatment and period on overall mean apparent digestibility of DM, NDF, ADF, CP, Ca, and P for horses consuming 3 levels of RSP treatment and coastal Bermudagrass hay are shown in Table 4. Effect of dietary treatments on overall and nutrient intake and output for a 500 kg horse at moderate exercise are shown in the Appendix (Table A-3).

Nutrient Digestibility

There was no effect of treatment ($P = 0.59$) on mean apparent DM digestibility (Table 4). Intake of DM decreased ($P < 0.01$) in P3 (Period 3; 8381 g/d) compared to P1 (Period 1; 9618 g/d) and P2 (Period 2; 8593 g/d; Table 3). Overall DM digestibility was greater ($P = 0.02$) during P1 (53.03%) and P2 (50.02%) compared to P3 (47.26%; Table 4). Apparent DM digestibility values observed in this study for P1 and P2 agree with those observed by Pagan and Harris (1998) who reported a summary of data collected from various experiments in exercised Thoroughbred geldings consuming a similar grain and hay diet. While there was a statistical difference observed for period in the current study, the difference can be attributed to the change in hay and nutrient content of hay in P3.

There was no effect of treatment ($P = 0.51$) on ADF digestibility (Table 4). Overall ADF digestibility in the current study were within previously reported ranges of 31.6 to 37.9% (NRC, 2007). However, intake of ADF decreased ($P < 0.01$) in P3 (2437 g/d) because of lower ADF concentrations in hay fed in P3 compared to P1 (3232 g/d) and P2 (2835 g/d; Table 3). In the current study, fiber intake contrasts that reported by Son and Kim (2015), where fiber intake increased with increased fiber concentrations. Horses in the current study increased fiber intake in response to higher fiber concentrations in hay fed in P1 and P2. In the current study, overall ADF digestibility in P1 (36.60%) and P2 (30.85%) were greater ($P = 0.01$) than P3 (26.34%; Table 4). While there was a statistical difference observed for period in the current study because of the change in hay and fiber quality in P3 hay, all ADF digestibility values were within previously reported ranges for horses consuming similar diets (Pagan and Harris, 1998; Bowen et al., 2014).

There was no effect of treatment ($P = 0.38$) on NDF digestibility (Table 4). Intake of NDF decreased ($P < 0.01$) in P3 (5062 g/d) because of lower NDF concentrations in the hay fed in P3 compared to P1 (6132 g/d) and P2 (5397 g/d; Table 3). Overall mean NDF digestibility was greater ($P = 0.04$) during P2 (38.50%) and P3 (36.58%) compared to P1 (43.09%; Table 4). Overall NDF digestibilities in the current study are lower than those observed by Bowen et al. (2014) who reported an apparent NDF digestibility of 53.97% in mature horses consuming orchardgrass hay. However, the digestibility values observed in the current study are within the digestibility ranges reported by Aiken et al. (1989), who observed NDF digestibility of 39.5% in horses consuming a nutritionally similar diet of coastal Bermudagrass hay.

Table 1. Three Levels of Reed Sedge Peat Treatment Analysis.

Ingredient[1], units	CON[2]	RSP10[3]	RSP20[4]
Moisture, %	8.1	8.5	7.8
DM, %	91.9	91.5	92.2
CP, %	18.2	18.3	18.3
ADF, %	14.2	14.6	15.6
CFat, %	6.6	6.9	6.4
CF %	10.6	11.2	11.3
NDF, %	34.3	35.0	35.6
Ash, %	10.0	9.8	10.2
Ca, %	1.5	1.4	1.3
P, %	1.1	1.1	1.2
Calculated DE, Kcal/L	1,585.3	1,575.3	1,565.4

[1]All values are expressed on a dry matter basis.
[2]No supplemental Reed Sedge Peat (RSP).
[3]10lb/ton (4960 mg/kg) of RSP (Kent Nutrition Group, Muscatine, Iowa).
[4]20lb/ton (9920 mg/kg) of RSP (Kent Nutrition Group).

Table 2. Coastal Bermudagrass Hay Analysis.

Ingredient[1], units	Hay A[2]	Hay B[3]
Moisture, %	7.2	7.3
DM, %	92.8	92.7
CP, %	6.5	12.3
ADF, %	38.8	33.8
CFat, %	0.8	1.2
CF, %	33.0	29.1
NDF, %	71.7	68.8
Ash, %	8.2	6.5
Ca, %	0.39	0.31
P, %	0.16	0.19
TDN, %	58.8	65.1
Calculated DE, Mcal/L	215.5	238.5

[1]All values are expressed on a dry matter basis.
[2]Hay was fed in Period 1 and Period 2.
[3]Hay was fed in Period 3.

Table 3. Effect of Period on Nutrient Intake in Exercised Horses (n = 6) Consuming CON, RSP10, and RSP20.

	PERIOD				
Item[1]	1	2	3	SEM	*P* - value
DM, g/d	9618	8593	8381	7.03	< 0.01
NDF, g/d	6132	5397	5062	18.13	< 0.01
ADF, g/d	3232	2835	2437	30.72	< 0.01
CP, g/d	869	803	1154	0.95	< 0.01
Ca, g/d	58	54	48	44.96	< 0.01
P, g/d	34	32	34	12.31	< 0.01

[1]All values are expressed on a dry matter basis.

Table 4. Effect of Treatment and Period on Overall Mean Apparent Digestibility in Exercised Horses (n = 6) Consuming CON, RSP10, and RSP20.

	Treatment				Period				*P*-value	
Item, units	CON[1]	RSP10[2]	RSP20[3]	SEM	1	2	3	SEM	Trt	Pd
DM, %	50.92	49.21	49.83	1.30	53.03[a]	50.02[a]	47.26[b]	7.03	0.59	0.02
NDF, %	41.08	37.98	39.12	1.10	43.09[b]	38.50[a]	36.58[a]	5.03	0.38	0.04
ADF, %	32.79	30.03	30.97	1.40	36.60[a]	30.85[a]	26.34[b]	9.94	0.51	0.01
CP, %	67.09	66.38	65.89	3.92	67.39	65.43	66.54	1.14	0.71	0.43
Ca, %	50.84	50.93	50.48	0.24	54.43[a]	53.77[a]	44.03[b]	13.85	0.99	< 0.01
P, %	17.09	16.75	20.46	2.05	26.66[a]	19.59[a]	8.05[b]	7.58	0.71	0.01

[a, b]Means in rows with differing superscripts differ, $P < 0.05$.
[x, y]Means in rows with differing superscripts tend to differ, $P < 0.10$.
[1]No supplemental Reed Sedge Peat (RSP).
[3]10lb/ton (4960 mg/kg) of RSP (Kent Nutrition Group, Muscatine, Iowa).
[4]20lb/ton *9920 mg/kg) of RSP (Kent Nutrition Group).

There was no effect of treatment ($P = 0.71$) or period ($P = 0.43$) on apparent CP digestibility (Table 4). However, intake of CP increased ($P < 0.01$) in P3 (1154 g/d) because of higher CP concentrations in hay fed in P3 (12.3%) compared to P1 and P2 (6.5%, Table 2). The difference in the CP content in P3 hay was likely influenced by increased CP intake in P3 (1154 g/d) compared to P1 (869 g/d) and P2 (803 g/d; Table 3). La Casha et al. (1999) observed similar CP digestibilities (67.8%) in Quarter Horse yearlings consuming coastal Bermudagrass hay. Additionally, apparent CP digestibility for P1 (67.39 %) and P2 (65.43%; Table 4) in the current study were within previously reported ranges of 57 to 64% (NRC, 2007) for horses consuming coastal Bermudagrass hay.

There was no effect of treatment ($P = 0.99$) on apparent Ca digestibility (Table 4). Overall Ca intake decreased ($P < 0.01$) in P3 (48 g/d) compared to P1 (58 g/d) and P2 (54 g/d; Table 3). In the current study, Ca intake were within the previously reported reference of 35 g/d (NRC, 2007) by all horses in all periods. Calcium apparent digestibility values observed in this study for P1 (54.43%) and P2 (53.77%) were greater ($P < 0.01$) compared to P3 (44.03%; Table 4). However, the Ca digestibility values observed in this study agreed with those observed by Pagan and Harris (1998) who reported Ca digestibility of 48.3%. Overall Ca digestibilities observed in this study are mostly within the reported range of 50% in the NRC (2007) for horses consuming coastal Bermudagrass hay.

There was no effect of treatment ($P = 0.71$) on P digestibility (Table 4). Intake of P increased ($P < 0.01$) in P1 (34 g/d) and P3 (34 g/d) compared to P2 (32 g/d; Table 3). While the intake of P is different compared to previous effects, intakes were within the

previously reported reference range of 21 g/d (NRC, 2007) and provided a 2:1 ratio of Ca to P. Furthermore, P digestibility was greater (P = 0.01) in P1 (26.66%) and P2 (19.59%) compared to P3 (8.05%; Table 4). Overall P digestibilities from the current study are lower than those observed by Pagan and Harris (1998), who observed a P digestibility of 34.6% in Thoroughbreds consuming a mixed hay and grain diet. Overall digestibilities of P observed in this study are also lower than previously reported range of 30 to 55% (NRC, 2007). Low P digestibility values in the current study may be due to the apparent total tract digestibility method, which may underestimate the available P due to the existence of endogenous losses in the feces (She, 2017).

Serum Glucose and Insulin Prandial Response

Before the P1 SET, one horse exhibited lameness and was removed from the SET. However, pre- and post-prandial blood collections were included from this horse.

The effect of treatment and the interaction between treatment by time on pre- and post-serum glucose concentrations are shown in Fig. 2 and Fig. 3 over an 8-h sampling period, respectively. All serum glucose and insulin concentrations reported were within normal ranges (insulin = 4.9 to 20.0 ulU/mL; glucose = 77 to 105 mg/dL) according to the Michigan State University Veterinary Diagnostic Endocrinology Department. Serum glucose concentrations were greater (P = 0.03) for horses consuming RSP20 (95.5 mg/dL) compared to horses consuming RSP10 (87.2 mg/dL) and CON (86.6 mg/dL; Fig. 2). This observation may suggest an area of further research due to increased blood glucose in horses consuming RSP20, which may increase energy availability for performance horses. However, pre- and both post-prandial serum glucose concentrations were not different (P = 0.64; Table A-5). Serum glucose concentrations reported in this

study were similar to the range (82.0 to 89.6 mg/dL) reported in previous studies by Harris and Pagan (1998) and Stull and Rodiek (1995), who observed that post-prandial glucose and insulin concentrations returned to pre-feeding concentrations approximately 8 h after a meal and before exercise. There was no interaction between treatment and time ($P = 0.85$) on pre- and post-prandial glucose concentrations. Changes in serum glucose concentrations 30 min before consumption, 30 min post, and 8 h after a meal (Fig. 3). In the current study, post-prandial serum glucose concentrations decreased after horses consumed CON (84.8 to 88.5 mg/dL) and RSP10 (88.50 to 86.5 mg/dL) compared to increased serum glucose concentration in horses consuming RSP20 (95.0 to 100.0 mg/dL; Fig. 3). Post-prandial serum glucose concentrations returned to pre-prandial serum glucose concentrations within 8 h (Fig. 3). Further, glucose concentrations were within ranges described by Stull and Rodiek (1987).

There was no effect of treatment ($P = 0.41$) or the interaction of time × treatment ($P = 0.87$; Fig. B-1) on overall pre-and post-prandial mean serum insulin concentrations (Table A-5). However, post-prandial serum insulin concentrations (17.81 uIU/mL) were greater ($P < 0.01$) compared to both pre-prandial (7.1 uIU/mL) and 8 h post-prandial (6.52 uIU/mL; Fig. 4) suggesting an insulinemic metabolic response in horses after consumption of a meal. Mean serum insulin concentrations in the current study are similar to those reported by Hansen et al. (2020) who reported post-prandial insulin concentrations were greater than pre-prandial concentrations 30 min after mealtime in horses consuming an almond hulls diet.

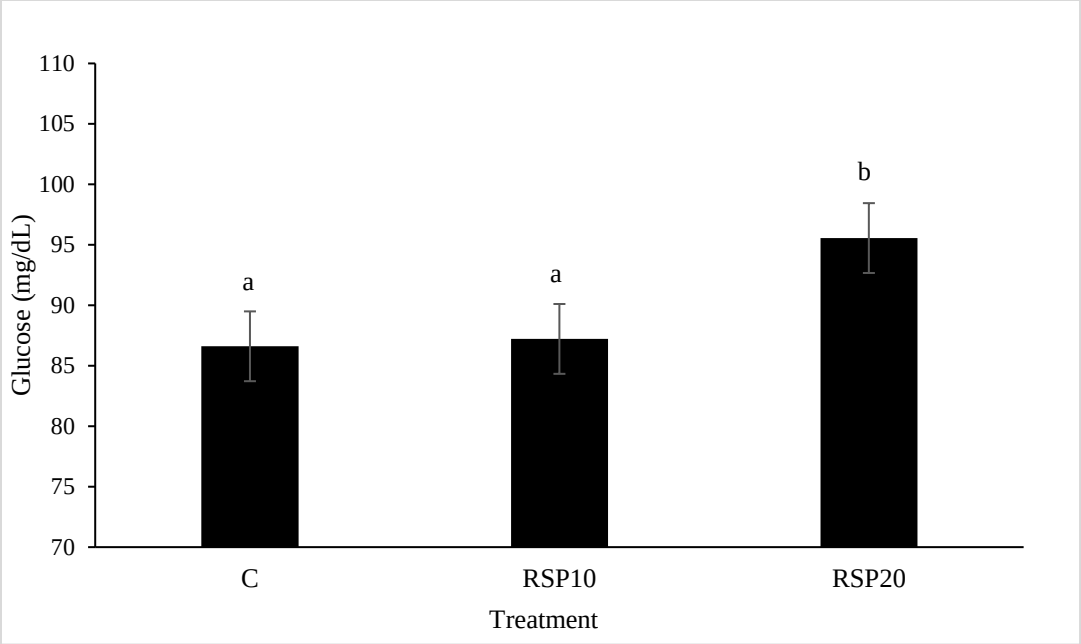

Figure 2. The Effect of Treatment on Pre- and Post-Prandial Mean Serum Glucose in Exercised Horses Consuming CON, RSP10, and RSP20 ($P = 0.03$).

[a,b]Means in with unlike superscripts differ, ($P < 0.05$).
Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

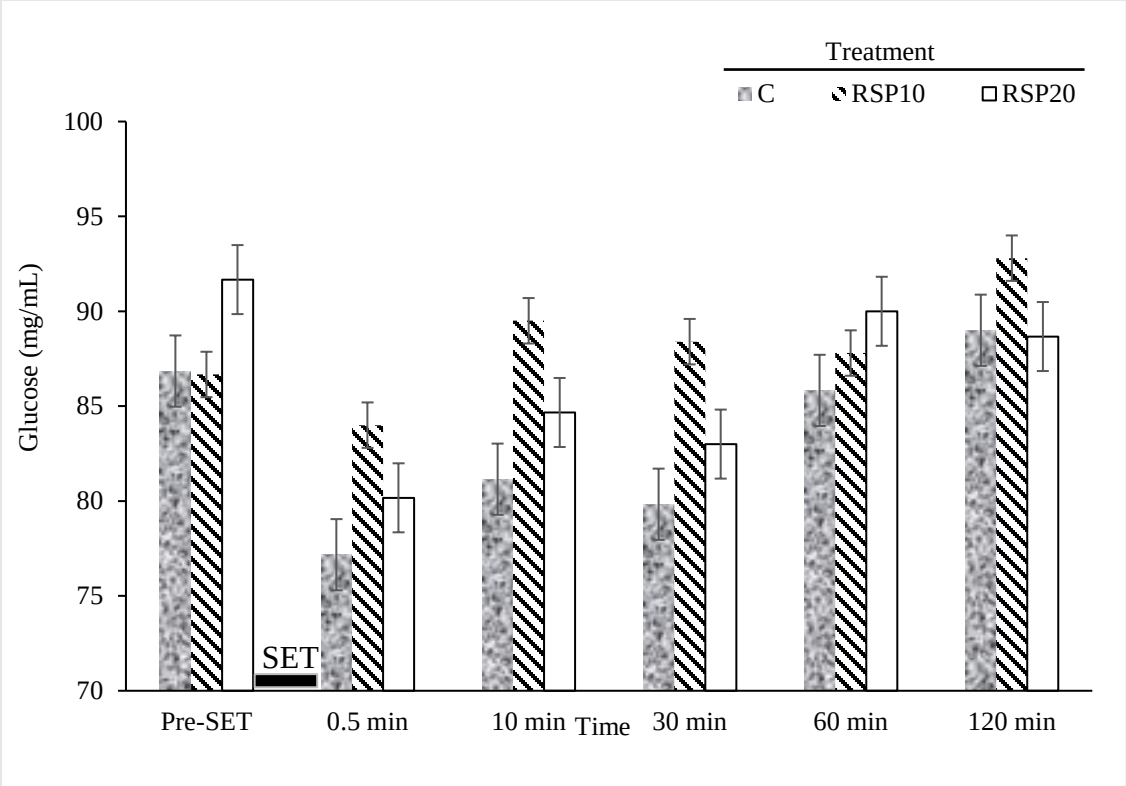

Figure 3. The Interaction of Treatment (Trt) by Time on Pre- and Post-Prandial Mean Serum Glucose in Exercised Horses Consuming CON, RSP10, and RSP20 (P = 0.85).

Blood samples were collected prior to morning feeding (fasted), 30 min after feeding, and 8 h after feeding.
[1]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

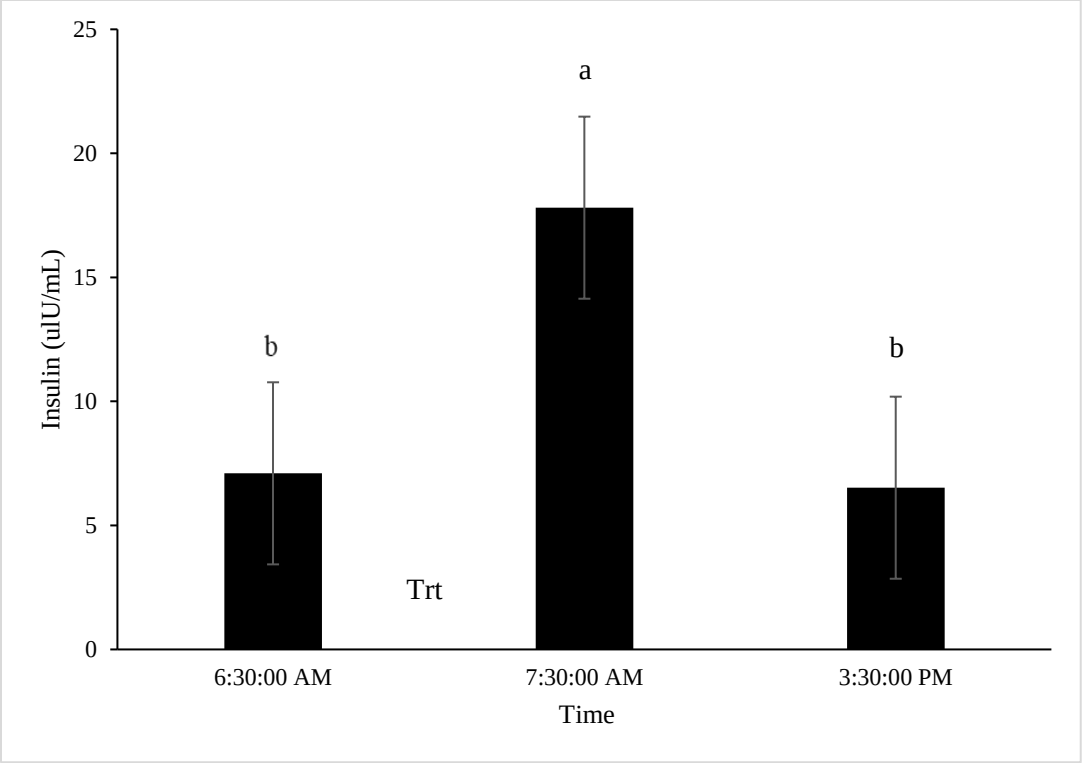

Figure 4. Effect of Time on Pre- and Post-Prandial Mean Serum Insulin in Exercised Horses Consuming CON, RSP10, and RSP20 ($P < 0.01$).

[a,b]Means in with unlike superscripts differ, $P < 0.05$.
Blood samples were collected prior to morning feeding (fasted), 30 min after feeding, and 8 h after feeding. Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

Serum Glucose and Insulin SET Response

There was no effect of treatment ($P = 0.91$; Table A-6) or interaction of treatment × time ($P = 0.99$; Fig. B-2) on serum mean glucose concentrations 30 min pre, 0.5, 10, 30, 60, and 120 min post-SET. However, serum glucose concentrations decreased ($P < 0.01$) from 86.43 mg/mL pre-SET to 80.13 mg/dL immediately after exercise (Fig. 5). The decline in glucose concentration after the SET in this study may be due to an uptake of glucose by the muscles with the continued action of insulin following submaximal exercise (Stull and Rodiek, 1995). Serum glucose concentrations returned to basal concentrations (91.8 mg/dL) within 120 min post-exercise (Table A-6). Serum glucose concentrations in the current study are similar to Rodiek et al. (1991), who reported glucose concentrations of 79.4 mg/dL immediately after exercise in horses consuming alfalfa hay. In the same study, Rodiek et al. 1991 reported that glucose concentrations recovered to 90.0 mg/dL 70 min after exercise.

There was no effect of treatment ($P = 0.98$) on serum insulin concentrations 30 min pre , 0.5, 10, 30, 60, and 120 min post-SET (Table A-6). However, during the SET, serum insulin concentrations over time followed serum glucose concentrations, as insulin decreased ($P < 0.01$) from 5.56 ulU/mL pre-SET to 3.05 ulU/mL post-SET, then recovered to basal concentrations of 7.07 ulU/mL at 120 min post-SET; suggesting an increased energy availability for the performance horse after exercise. To foresee further changes, additional sampling-time points would allow a more accurate illustration of blood glucose and insulin concentrations. The serum insulin concentrations in the current study were within normal ranges, and were similar to those reported by Crandell et al. 1999; Rodiek et al., 1991; and Stull and Rodiek, 1995.

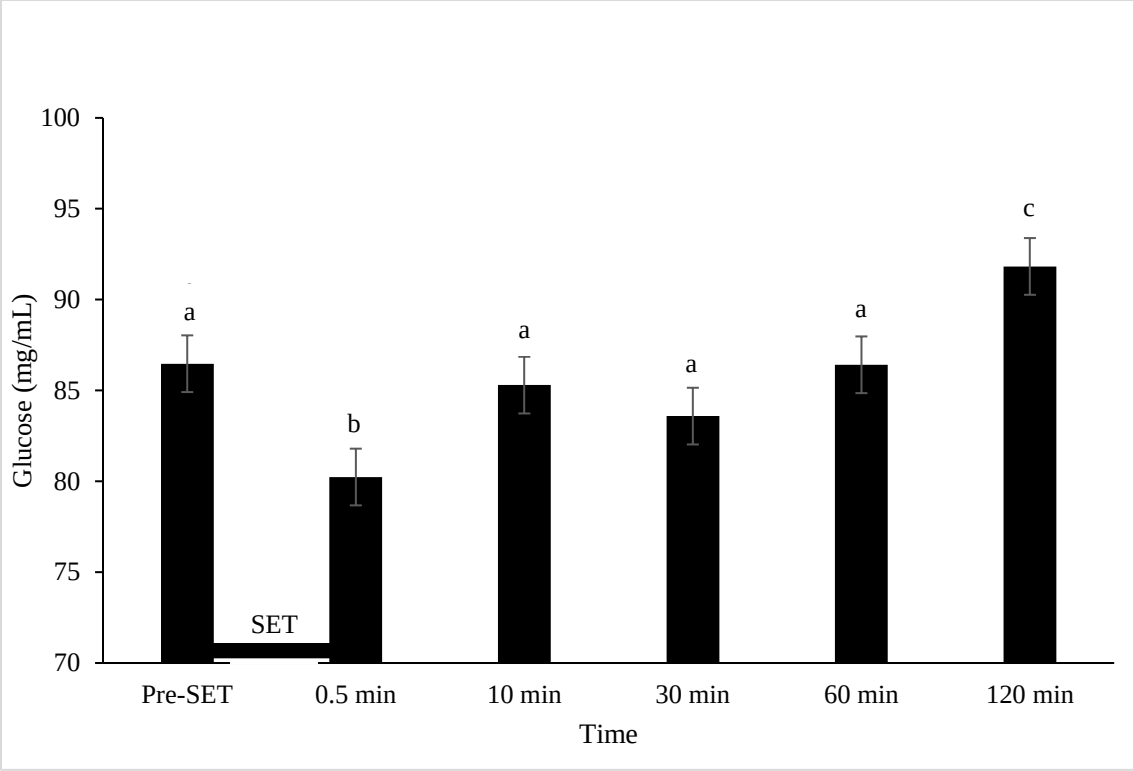

Figure 5. The Effect of Time on Pre- and Post-SET Mean Serum Glucose in Exercised Horses Consuming CON, RSP10, and RSP20 ($P < 0.01$).

[a, b] Means in with unlike superscripts differ, $P < 0.05$.
Blood samples collected prior to the morning feeding (fasted), 30 min after feeding, followed by 8 h after feeding.
Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

CHAPTER V CONCLUSIONS AND IMPLICATIONS

The data reported in this study suggests no benefits were observed in nutrient digestibility when RSP was added to horses' diets. Results from this experiment indicate that mature horses consuming RSP20 at 20 lb/ton (9920 mg/kg) had greater post-prandial blood glucose concentrations compared to horses consuming CON and RSP10. Horses consuming added RSP exhibited no negative effects in overall health such as loss of BW, appetite or fitness.

Greater glucose concentrations after RSP20 diet consumption suggest RSP20 may alter the metabolic response as more substrates are absorbed. These observations may be due to enhanced glucose uptake by the muscles and adipose tissue leading to a higher contribution of glucose for energy production in response to increasing work intensity. The carbohydrate via glucose used during exercise may have arisen from the blood glucose derived from the RSP20 diet. However, the data observed for nutrient digestibility did not differ in horses consuming added RSP.

To this author's knowledge, there is limited research regarding the metabolic response of livestock to humic substances. Therefore, further research is needed to investigate the effects of added RSP on nutrient metabolism and possible effects on glucose metabolism during exercise. The present study did not measure serum concentrations of glucose or insulin before the trial. Having a baseline concentration of blood glucose and insulin levels may add value in comparing potential differences in

glucose levels before and during the trial. Furthermore, measuring more variables such as blood lactate to estimate how much glucose is being used during various stages of exercise may be needed. While previous reports have observed positive effects of RSP on the performance and health status of animals, further research may be warranted to investigate the effects on glucose and insulin dynamics in horses.

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APPENDIX

Table A-1. Overall Mean Body Weights by Period

		Period 1				Period Two				Period Three			
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Horse	Weight, kg												
A	573	560	559	560	565	559	553	555	556	555	552	559	558
B	507	493	494	497	504	497	491	488	495	491	491	500	499
C	583	575	576	580	585	579	578	570	580	575	575	582	577
D	624	614	612	612	614	606	602	585	585	587	585	587	579
E	575	572	572	574	562	562	557	553	550	557	547	552	555
F	495	485	486	485	491	485	481	484	483	484	481	482	484

[1]Horse's were weighed and recorded once weekly pre-feeding.

Table A-2. Mean Intake and Fecal Output by Period in Exercised Horses Consuming CON, RSP10, and RSP20 on a Dry Matter Basis.

Period[1]	Horse	Treatment[2]	Hay DMI, kg/d	Grain DMI kg/d	Fecal Output kg/d
1	A	C	7.21	2.07	4.080
	B	C	7.23	2.07	4.325
	C	RSP10	8.69	2.08	5.601
	D	RSP10	7.93	2.08	4.964
	E	RSP20	7.22	2.10	4.027
	F	RSP20	6.93	2.10	4.257
2	A	RSP10	6.05	2.08	4.044
	B	RSP10	5.40	2.08	4.031
	C	RSP20	7.97	2.10	4.963
	D	RSP20	6.10	2.10	4.283
	E	C	6.68	2.07	4.083
	F	C	6.88	2.07	4.354
3	A	RSP20	6.13	2.10	4.484
	B	RSP20	5.81	2.10	4.364
	C	C	6.99	2.07	4.487
	D	C	6.39	2.07	5.062
	E	RSP10	6.18	2.08	4.023
	F	RSP10	6.28	2.08	4.302

Data presented as true means (n = 6).
[1]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.
[2]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

Table A-3. Effect of Treatment on Overall Mean Apparent Digestibility and Nutrient Intake and Output for a 500 kg Horse at Moderate Exercise.

	Control			RSP10			RSP20		
Item, units	Input	Output	Digestibility%	Input	Output	Digestibility%	Input	Output	Digestibility%
DM, g/d	8968	4389	50.92	8835	4484	49.21	8789	4387	49.83
NDF, g/d	5591	3280	41.08	5511	3412	37.98	5488	3315	39.12
ADF, g/d	2858	1902	33.79	2820	1971	30.03	2824	1931	30.97
CP, g/d	955	315	67.09	939	314	66.38	933	318	65.88
Ca, g/d	56	27	50.84	53	26	50.92	51	25	50.48
P, g/d	33	28	17.09	33	28	16.75	33	26	20.46

Data presented as true means (n = 6).
[1]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.
[2]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

Table A-4. Apparent Dry Matter Digestibility Type-3 Test of Fixed Effects.

Effect	Num *DF*	Den *DF*	*F* Value	*Pr* > *F*[3]
Trt[1]	2	8	0.57	0.587
Pd[2]	2	8	7.03	0.017

Least significant difference was used to determine the probability of treatment effect within period (SAS Institute, Inc., 2014).

[1]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

[2]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.

[3]Significant differences between treatment means and time was declared at $P \leq 0.05$.

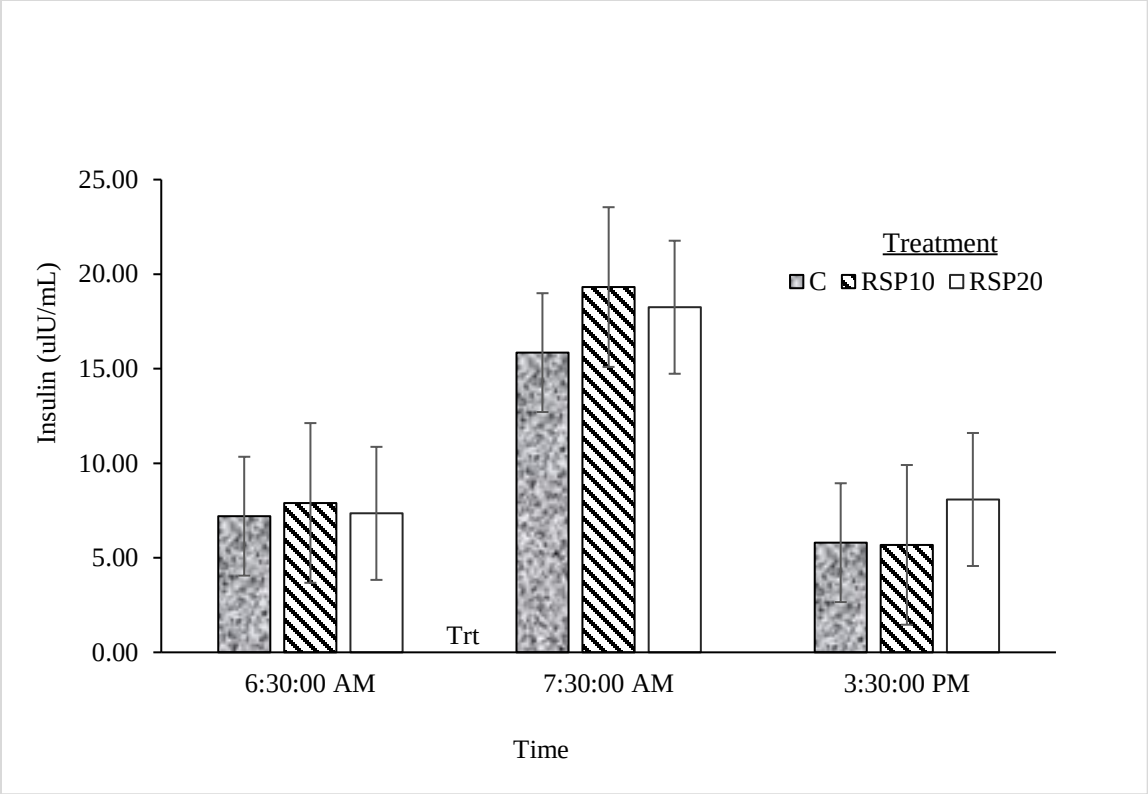

Figure B-1. The Interaction of Treatment (Trt) by Time on Pre- and Post-Prandial Mean Serum Insulin in Exercised Horses Consuming CON, RSP10, and RSP20 (P = 0.87).

Blood samples collected prior to the morning feeding (fasted), 30 min after feeding, followed by 8 h after feeding.
Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

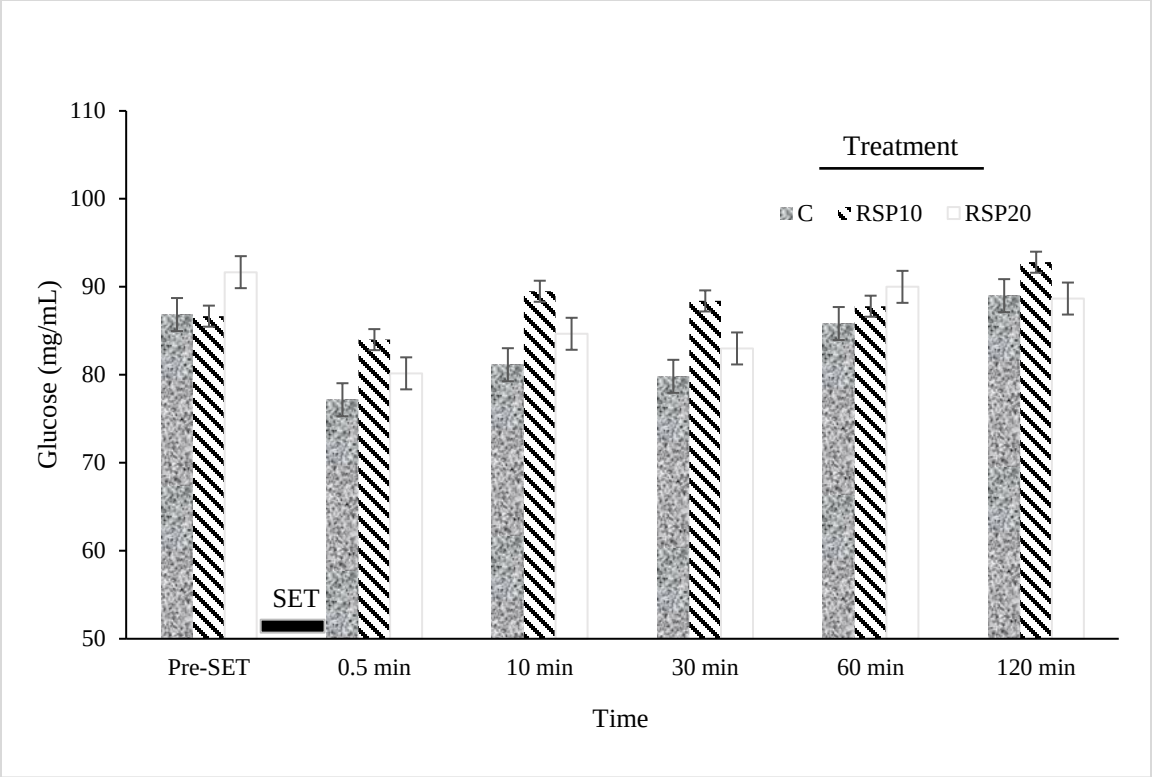

Figure B-2. The Interaction of Treatment (Trt) by Time on Pre- and Post-SET Mean Serum Glucose in Exercised Horses Consuming CON, RSP10, and RSP20 (P = 0.94).

Blood samples were collected before and after protocols in which horses underwent a SET 8 h after feeding.
Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

Table A-5. Effect of Treatment, Period and Time on Pre- and Post-Prandial Mean Serum Glucose and Insulin in Exercised Horses (n = 6) Consuming CON, RSP10, and RSP20.

Item, units				
Treatment[1]	CON	RSP10	RSP20	SEM
Glucose, mg/dL	86.61[a]	87.22[a]	95.56[b]	3.78
Insulin, ulU/mL	9.23	10.96	11.22	0.92
Period[2]	1	2	3	SEM
Glucose, mg/dL	98.17	90.37	80.94	4.62
Insulin, ulU/mL	11.67	10.34	9.42	0.38
Time (min)[3]	Pre	Post	Post8hr	SEM
Glucose, mg/dL	89.44	91.67	88.27	0.45
Insulin, ulU/mL	7.10[b]	17.81[a]	6.52[b]	31.60
P-value	Trt	Pd	Time	Time × Trt
Glucose, mg/dL	0.03	< 0.01	0.64	0.85
Insulin, ulU/mL	0.41	0.38	< 0.01	0.87

[a, b, c]Means in rows with differing superscripts differ, $P < 0.05$.
[1]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).
[2]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.
[3]Blood samples were collected before and after protocols in which horses underwent a SET 8 h after feeding.

Table A-6. Effect of Treatment, Period and Time on Pre- and Post-SET Mean Serum Glucose and Insulin in Exercised Horses (n = 6) Consuming CON, RSP10, and RSP20.

Item, units							
Treatment[1]	CON	RSP10	RSP20	SEM			
Glucose, mg/dL	85.44	85.94	85.43	0.09			
Insulin, ulU/mL	5.11	5.06	5.12	0.02			
Period[2]	1	2	3	SEM			
Glucose, mg/dL	82.73[b]	90.01[a]	83.61[b]	15.08			
Insulin, ulU/mL	5.01	5.07	5.25	0.41			
Time (min)[3]	Pre	0.5	10	30	60	120	SEM
Glucose, mg/dL	86.47	80.24	85.23	83.59	86.44	91.80	7.37
Insulin, ulU/mL	5.56	3.05	4.44	4.82	5.64	7.03	19.00
P-value	Trt	Pd	Time	Time × Trt			
Glucose, mg/dL	0.91	< 0.01	< 0.01	0.99			
Insulin, ulU/mL	0.98	0.67	< 0.01	0.94			

[a, b, c]Means in rows with differing superscripts differ, $P < 0.05$.
[1]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).
[2]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.
[3]Blood samples were collected before and after protocols in which horses underwent a SET 8 h after feeding.

Table A-7. Insulin Pre- and Post-Prandial Type-3 Tests of Fixed Effects

Effect	Num *DF*	Den *DF*	*F* Value	*Pr* > *F*[4]
Time[1]	2	38	31.60	< 0.01
Trt[2]	2	38	0.92	0.41
Time*trt	4	38	0.31	0.88
Pd[3]	2	38	1.00	0.37

Least significant difference was used to determine the probability of treatment effect within period (SAS Institute, Inc., 2014).
[1]Blood samples were drawn prior to the morning feeding (fasted), 30 min after feeding, followed by 8 h after feeding.
[2]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).
[3]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.
[4]Significant differences between treatment means and time was declared at $P \leq 0.05$.

Table A-8. Glucose Pre- and Post-Prandial Type-3 Tests of Fixed Effects

Effect	Num *DF*	Den *DF*	*F* Value	Pr > *F*[4]
Time[1]	2	38	0.34	0.64
Trt[2]	2	38	3.78	0.03
Time*trt	4	38	0.34	0.85
Pd[3]	2	38	11.25	< 0.01

Least significant difference was used to determine the probability of treatment effect within period (SAS Institute, Inc., 2014).
[1]Blood samples were drawn prior to the morning feeding (fasted), 30 min after feeding, followed by 8 h after feeding.
[2]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).
[3]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.
[4]Significant differences between treatment means and time was declared at $P \leq 0.05$.

Table A-9. Insulin Pre- and Post-SET Type-3 Tests of Fixed Effects.

Effect	Num *DF*	Den *DF*	*F* Value	Pr > *F*[4]
Time[1]	5	77	19.00	< 0.01
Trt[2]	2	77	0.02	0.98
Time*trt	10	77	0.41	0.94
Pd[3]	2	77	0.41	0.67

Least significant difference was used to determine the probability of treatment effect within period (SAS Institute, Inc., 2014).
[1]Blood samples before and after protocols in which horses underwent a SET 8 h after feeding.
[2]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).
[3]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.
[4]Significant differences between treatment means and time was declared at $P \leq 0.05$.

Table A-10. Glucose Pre- and Post-SET Type-3 Tests of Fixed Effects.

Effect	Num *DF*	Den *DF*	*F* Value	Pr > *F*[4]
Time[1]	5	77	7.37	< 0.01
Trt[2]	2	77	0.09	0.92
Time*trt	10	77	0.09	0.99
Pd[3]	2	77	15.08	< 0.01

Least significant difference was used to determine the probability of treatment effect within period (SAS Institute, Inc., 2014).
[1]Blood samples before and after protocols in which horses underwent a SET 8 h after feeding.
[2]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).
[3]Study consisted of three 24-d periods, each consisting of a 21-d feeding period followed by a 3-d sample collection period.
[4]Significant differences between treatment means and time was declared at $P \leq 0.05$.

Table A-1. Laboratory Analysis of Blood Serum Glucose and Insulin Concentrations.

Horse	Time	Date	Period	Treatment[1]	Insulin, (ulU/ML)	Glucose, (mg/DL)
A	6:30	5/2/20	1	CON	6.8	95
A	7:30	5/2/20	1	CON	17.6	86
A	14:30	5/2/20	1	CON	6	102
A	30 Sec	5/2/20	1	CON	1.9	87
A	10 Min	5/2/20	1	CON	2.3	87
A	30 Min	5/2/20	1	CON	5.2	92
A	60 Min	5/2/20	1	CON	7.2	78
A	120 Min	5/2/20	1	CON	9.1	103
A	6:30	5/29/20	2	RSP10	5.8	90
A	7:30	5/29/20	2	RSP10	33	91
A	14:30	5/29/20	2	RSP10	6.4	101
A	30 Sec	5/29/20	2	RSP10	1.8	96
A	10 Min	5/29/20	2	RSP10	2.4	97
A	30 Min	5/29/20	2	RSP10	5.3	92
A	60 Min	5/29/20	2	RSP10	5.6	93
A	120 Min	5/29/20	2	RSP10	6.5	102
A	6:30	6/26/20	3	RSP20	9.8	89
A	7:30	6/26/20	3	RSP20	14.9	82
A	14:30	6/26/20	3	RSP20	3.6	84
A	30 Sec	6/26/20	3	RSP20	2	95
A	10 Min	6/26/20	3	RSP20	2.1	92
A	30 Min	6/26/20	3	RSP20	4.3	91
A	60 Min	6/26/20	3	RSP20	7.3	98
A	120 Min	6/26/20	3	RSP20	6.6	94
B	6:30	5/2/20	1	CON	7.2	91
B	7:30	5/2/20	1	CON	19.5	90
B	14:30	5/2/20	1	CON	4	92
B	30 Sec	5/2/20	1	CON	1.6	73
B	10 Min	5/2/20	1	CON	2.6	83
B	30 Min	5/2/20	1	CON	3.5	79
B	60 Min	5/2/20	1	CON	3.4	85
B	120 Min	5/2/20	1	CON	5	82
B	6:30	5/29/20	2	RSP10	6.3	92
B	7:30	5/29/20	2	RSP10	19.4	85
B	14:30	5/29/20	2	RSP10	4.3	95
B	30 Sec	5/29/20	2	RSP10	1.4	91
B	10 Min	5/29/20	2	RSP10	2.6	87
B	30 Min	5/29/20	2	RSP10	4.2	85
B	60 Min	5/29/20	2	RSP10	5.3	88
B	120 Min	5/29/20	2	RSP10	6.7	92

B	6:30	6/26/20	3	RSP20	6.2	82
B	7:30	6/26/20	3	RSP20	18.3	77
B	14:30	6/26/20	3	RSP20	3.8	78
B	30 Sec	6/26/20	3	RSP20	1.7	77
B	10 Min	6/26/20	3	RSP20	3.6	84
B	30 Min	6/26/20	3	RSP20	6.4	84
B	60 Min	6/26/20	3	RSP20	6.5	83
B	120 Min	6/26/20	3	RSP20	6.3	83
CON	6:30	5/2/20	1	RSP10	3.8	75
CON	7:30	5/2/20	1	RSP10	24.5	86
CON	14:30	5/2/20	1	RSP10	4.2	88
CON	30 Sec	5/2/20	1	RSP10	5	68
CON	10 Min	5/2/20	1	RSP10	4	73
CON	30 Min	5/2/20	1	RSP10	3.2	73
CON	60 Min	5/2/20	1	RSP10	4.1	80
CON	120 Min	5/2/20	1	RSP10	4.7	85
CON	6:30	5/29/20	2	RSP20	6.8	80
CON	7:30	5/29/20	2	RSP20	19.9	88
CON	14:30	5/29/20	2	RSP20	6.6	87
CON	30 Sec	5/29/20	2	RSP20	2.7	79
CON	10 Min	5/29/20	2	RSP20	3.2	83
CON	30 Min	5/29/20	2	RSP20	5.2	80
CON	60 Min	5/29/20	2	RSP20	4.3	85
CON	120 Min	5/29/20	2	RSP20	7.9	89
CON	6:30	6/26/20	3	CON	6.5	79
CON	7:30	6/26/20	3	CON	16.5	78
CON	14:30	6/26/20	3	CON	7.5	72
CON	30 Sec	6/26/20	3	CON	3.1	74
CON	10 Min	6/26/20	3	CON	3.6	81
CON	30 Min	6/26/20	3	CON	4.6	78
CON	60 Min	6/26/20	3	CON	3.5	86
CON	120 Min	6/26/20	3	CON	9.5	85
D	6:30	5/2/20	1	RSP10	15	106
D	7:30	5/2/20	1	RSP10	3.4	92
D	14:30	5/2/20	1	RSP10	4.2	92
D	30 Sec	5/2/20	1	RSP10	2.1	73
D	10 Min	5/2/20	1	RSP10	2.2	89
D	30 Min	5/2/20	1	RSP10	2.6	81
D	60 Min	5/2/20	1	RSP10	2.9	90
D	120 Min	5/2/20	1	RSP10	5.3	99
D	6:30	5/29/20	2	RSP20	4	98
D	7:30	5/29/20	2	RSP20	11.2	106
D	14:30	5/29/20	2	RSP20	3.2	97

D	30 Sec	5/29/20	2	RSP20	1.9	89
D	10 Min	5/29/20	2	RSP20	2.8	91
D	30 Min	5/29/20	2	RSP20	2.8	93
D	60 Min	5/29/20	2	RSP20	2.5	93
D	120 Min	5/29/20	2	RSP20	4.4	108
D	6:30	6/26/20	3	CON	3.4	82
D	7:30	6/26/20	3	CON	9.2	97
D	14:30	6/26/20	3	CON	2.8	80
D	30 Sec	6/26/20	3	CON	2.3	83
D	10 Min	6/26/20	3	CON	2.3	82
D	30 Min	6/26/20	3	CON	2.3	82
D	60 Min	6/26/20	3	CON	3.3	88
D	120 Min	6/26/20	3	CON	4.9	92
E	6:30	5/2/20	1	RSP20	9.5	141
E	7:30	5/2/20	1	RSP20	28	153
E	14:30	5/2/20	1	RSP20	22.9	119
E	6:30	5/29/20	2	CON	4.7	85
E	7:30	5/29/20	2	CON	19.8	95
E	14:30	5/29/20	2	CON	5.5	89
E	30 Sec	5/29/20	2	CON	4.2	96
E	10 Min	5/29/20	2	CON	5.4	96
E	30 Min	5/29/20	2	CON	5.1	90
E	60 Min	5/29/20	2	CON	6.6	92
E	120 Min	5/29/20	2	CON	6	94
E	6:30	6/26/20	3	RSP10	7.3	87
E	7:30	6/26/20	3	RSP10	16.5	84
E	14:30	6/26/20	3	RSP10	5.4	72
E	30 Sec	6/26/20	3	RSP10	4	86
E	10 Min	6/26/20	3	RSP10	6.7	92
E	30 Min	6/26/20	3	RSP10	5.8	89
E	60 Min	6/26/20	3	RSP10	6.5	88
E	120 Min	6/26/20	3	RSP10	8.4	87
F	6:30	5/2/20	1	RSP20	7.8	80
F	7:30	5/2/20	1	RSP20	17.2	94
F	14:30	5/2/20	1	RSP20	8.4	85
F	30 Sec	5/2/20	1	RSP20	5.2	54
F	10 Min	5/2/20	1	RSP20	11.8	73
F	30 Min	5/2/20	1	RSP20	6.3	72
F	60 Min	5/2/20	1	RSP20	8.4	78
F	120 Min	5/2/20	1	RSP20	8.8	86
F	6:30	5/29/20	2	CON	7.7	77
F	7:30	5/29/20	2	CON	12.5	85
F	14:30	5/29/20	2	CON	9	84

F	30 Sec	5/29/20	2	CON	4.6	69
F	10 Min	5/29/20	2	CON	8.7	83
F	30 Min	5/29/20	2	CON	7.5	77
F	60 Min	5/29/20	2	CON	9.3	84
F	120 Min	5/29/20	2	CON	10.9	96
F	6:30	6/26/20	3	RSP10	9.2	81
F	7:30	6/26/20	3	RSP10	19.1	81
F	14:30	6/26/20	3	RSP10	9.6	72
F	30 Sec	6/26/20	3	RSP10	6.2	74
F	10 Min	6/26/20	3	RSP10	8.4	77
F	30 Min	6/26/20	3	RSP10	6.8	83
F	60 Min	6/26/20	3	RSP10	8.5	80
F	120 Min	6/26/20	3	RSP10	8.9	84

Data presented as true means (n = 6).

[1]Horses were fed 3 supplement treatments (Kent Nutrition Group; Muscatine, Iowa): Control (CON) with no added Reed Sedge Peat (RSP); RSP10 (10lb/ton added RSP); RSP20 (20lb/ton added RSP).

FEED AND FECAL LABORATORY ANALYSIS

Sample Preparation and Dry Matter Determination. All feed and feedstuff were calculated and expressed on a dry matter basis The DM procedure was adapted from the methods outlined by Van Soest (1967). The process is designed to allow for subsequent analysis for CF, lignin, and ADF.

For DM determination, fecal and hay samples were thawed for 24 h at room temperature for analysis. Wet fecal samples were placed in 9 × 11 cm diameter aluminum drying pans and all weights were determined using an electronic bench top scale (OHAUS Corp., Parsippany, NJ). The weight of the weigh boat was recorded (W_1), then the fecal or hay sample was placed in the weigh boat and the weight recorded (W_2). All samples were placed in a forced-air oven (SMO28HP model, Sheldon Manufacturing Inc., Cornelius, OR) at 55°CON for 72 h. Samples were removed from the oven and the weight was recorded (W_3). The percent DM was then calculated as:

$$\% \text{ Total DM} = [((W_3-W_1)/(W_2-W_1))*100]$$

The dry samples were composited by percentage of each sample weight. The resulting composite was a representation of each horse for each time period. Dried fecal samples were ground through a 2 mm screen (Wiley-Mill Model 4, Thomas-Wiley Mill, Philadelphia, PA). The ground sample was placed in a Whirl-Pak storage bag (Nasco, Fort Atkinson, WI) and stored at room temperature for later compositing.

Crude Protein. Feedstuff and fecal samples CP analysis was performed at ServiTech Laboratory (Amarillo, TX). Samples were analyzed using the LECO FP928 (LECO Corporations, St. Joseph, MI), which quantitatively analyzes the combustion products by the changes in the thermal conductivity of the reference gas. Analysis provides an estimate of the total proteins in a sample by using a general coefficient of

6.25 to transform total nitrogen into CP. The thermal conductivity is used to for the detection of nitrogen in the combustion gas.

Ether Extract. Ether extract analysis was performed at ServiTech Laboratory. This method indirectly determines CF by extraction with petroleum ether or other common fat solvents. The process used the Soxhlet principle in a closed stainless-steel extraction vessel allowing solvent temperatures to exceed solvent boiling points for the fat extraction procedure. The analysis was achieved by measuring the loss of mass due to the extraction of fat from the sample encapsulated in a filter bag.

Ash. The inorganic component of the feed and fecal samples was determined by ashing at the ServiTech Laboratory. The weight of an empty tin foil crucible was weight and recorded (W_1). Two-gram sample were placed into a dried crucible (W_2) and heated at 600 °CON for 2 h. After heating, samples were transformed to desiccator and allowed to cool to room temperature. Cooled samples were then weighed, and weight was recorded. Ash content was determined by the following calculation:

Crude Fiber. Methods used to determine fiber content have been developed mainly for ruminants or humans. Consequently, neither bovine or human digestive systems translated well with the digestive system and intermediary metabolism of the horse (NRC, 2007). Crude Fiber represented the less readily digestible carbohydrates, mostly cellulose and some lignin (Van Soest, 1997). The CF fraction was measured by boiling the sample in in weak acid and weak alkali. However, the weak alkali solution dissolves hemicellulose and lignin, resulting in incorporation of some dietary fibers (Pond et al., 2005). However, ADF and neutral detergent fiber (NDF) are more useful

measures of feeding value and should be used to evaluate forages and formula rations (Pond, 2005). Van Soest (1997) published a more reliable way to evaluate the dietary fiber fraction and further partition of the cell wall's major subcomponents known as the NDF, the insoluble fiber, and ADF, the indigestible, fractions. Crude fiber analysis was performed in sight of the Van Soest (1997) methods using the "filter bag technique" (ANKOM Technology, 2011) at ServiTech Laboratory.

Neutral Detergent Fiber. The CF fraction of the proximate analyses has further divided into the NDF fraction, which contained hemicellulose, cellulose, and lignin. Neutral detergent fiber analysis was performed at ServiTech Laboratory using the "filter bag technique" (ANKOM Technology, 2011). The percent NDF fraction (as-received basis) was calculated by the following equation:

$$\% \text{ NDF} = \frac{(W3 - (W1 \times C1))}{W2} \times 100$$

Acid Detergent Fiber. Acid Detergent Fiber analysis process performed at ServiTech Laboratory extracted hemicellulose from the NDF fraction, leaving only cellulose and lignin in the residue. Acid detergent fiber analysis were performed using the "filter bag technique" (ANKOM Technology, 2011). Percent ADF (as received basis) was calculated after extraction as:

$$\% \text{ ADF} = \frac{(W3 - (W1 \times C1))}{W2} \times 100$$

Percent Hemicellulose was calculated as: same

$$\% \text{ Hemicellulose} = \text{NDF-ADF}$$