THE EFFECT OF HAY TYPE AND SOAKING ON GLYCEMIC RESPONSE IN HORSES

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ABSTRACT

Dietary management of equine metabolic syndrome includes minimizing glycemic response. Soaking hay prior to feeding reduces nonstructural carbohydrate content, but little research indicates if soaking hay reduces glycemic response. Glycemic response of four hay diets were evaluated: dry or soaked prairiegrass hay, and dry or soaked alfalfa hay. Twelve healthy horses were randomly assigned into two groups and fed the hay diets at 0.5% BW in a 2x2 factorial design. Blood samples were collected at 0, 30, 60, 90, 120, 180, 240, and 300 min after feeding. Plasma glucose was analyzed using a colorimetric assay, and incremental area under the curve (AUC) of glucose response calculated. Data were analyzed using a mixed model with repeated measures. Plasma glucose and the AUC was higher *($P = 0.0001$)* in healthy horses fed alfalfa compared to grass hay, with no differences identified due to soaking *($P = 0.82$).* Additional research is needed to determine if soaking hay has physiological merit in horses with metabolic issues.

Keywords

Glycemic response, Hay soaking, Horses

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CHAPTER I: LITERATURE REVIEW

Introduction

Horses are hind gut fermenting herbivores, making forage the greatest proportion of the horse's diet. Forage provides a great fiber source that is fermented slowly. The slow fermentation process allows the survival and multiplication of beneficial bacterial that provide volatile fatty acids, which are then used by the horse as a major energy source. In addition to providing fuel for bacterial fermentation, fiber in forages also maintains gut health by providing scratch factor (Warner, 1991). Scratch factor pulls away dead cells along the gut lining and allows for better secretion of gut enzymes. Lastly fiber provides structural support for the gut. Horses that have continuous access to forage maintain proper gut structure, which helps in preventing twists and kinks.

In addition to fiber, forage contains storage carbohydrates including fructans, starch, and simple sugars; which are nonstructural carbohydrates (NSC). Fructans and simple sugars act as an energy source for cool-season grasses, while starches are stored in legumes and warm-season grasses. Starch and simple sugars can be hydrolyzed in the foregut, but fructans are rapidly fermented in the hindgut (Hoffman et al., 2001). Furthermore if starch is consumed in excess of 400 g per 100 kg BW per meal (Potter et al., 1992), it overwhelms the capacity of the foregut and is fermented rapidly in the hindgut. The rapid fermentation of these carbohydrates in large quantities has been demonstrated to cause colic and laminitis (Cohen et al., 1999; Longland et al., 1999). The NSC content in pasture forages undertake circadian and seasonal variation, directly affecting the horses metabolism and perhaps exacerbating metabolic disease (McIntosh, 2006).

Researchers evaluating metabolic disorders recommend reducing rapidly fermentable nonstructural carbohydrates (Frank, 2009). Although simply reducing the amount fed is a possibility, the large amount of fiber is important for gut health. A management practice that is becoming commonly recommended is soaking hay.

Martinson et al. (2011) studied the effect of soaking on carbohydrate removal of different hays and found a significant loss in nonstructural carbohydrates with 30 minutes of soaking in warm water or 60 minutes in cold water. This management practice has been proven as a viable way to reduce NSC in hay. The next matter at hand would be whether the soaked hay has a lowered effect on blood glucose and insulin. The large spike in blood glucose and insulin exacerbates metabolic conditions in the horse.

Carbohydrate Digestion in the Horse

Although forage is the largest portion of their diet, performance horses are commonly supplemented with grain concentrates. Dietary carbohydrate composition in grain range from simple sugars and starch to slowly fermentable and indigestible fiber. The horse’s digestive system is organized to hydrolyze disaccharides and starches to yield simple sugars, which are absorbed in the small intestine. Fermentation of fibrous carbohydrates in the hindgut yields volatile fatty acids. The determination of where carbohydrates are digested depends on the linkage of its sugar molecules. Alpha 1, 4 linkages predispose the carbohydrate to hydrolysis by enzymes in the small intestine, while β 1, 4 linkages are subject to microbial fermentation in the hindgut (NRC, 2007).

Hydrolytic digestion is achieved using enzymes produced by the horse. The enzymes include alpha amylase, alpha glucosidase (sucrase, glucoamylase, and maltase) and beta galactosidase (lactase). Alpha amylase is secreted from the pancreas and the other enzymes are brush border enzymes. Disaccharides and starches are hydrolyzed to an extent by the low acidity of the stomach however, the majority of hydrolysis occurs in the small intestine by alpha amylase. Amylopectinase cleaves α 1, 4 linkages leaving disaccharides and oligosaccharides. The brush border enzymes then complete hydrolysis yielding free sugars glucose, galactose, and fructose.

Microorganisms prosper in the large intestine of horses because of a pH greater than five and sufficient retention time. The pH is optimally greater than six, because lower pH favors lactic acid producing bacteria (Radicke et al., 1991). Small amounts of fermentation are thought to occur in the fundic region of the stomach; these bacteria are also lactic acid producing. Some fermentation occurs in the distal region of the small intestine, but it is uncertain if this occurs independently of the horse's hind gut or if this is due to reflux of the hindgut contents. The large majority of fermentation happens in the cecum and large intestine. Nonstructural carbohydrates that pass through the small intestine unhydrolyzed are rapidly fermented in the hindgut yielding lactate. This occurs typically when the concentration of starch exceeds 400 g per 100 kg of body weight per meal (Potter et al., 1992). Carbohydrates that can be digested in the foregut yield greater energy than those fermented in the hindgut (Blaxter, 1989; Kronfeld et al., 1996).

Fermentation of fiber produces desired volatile fatty acids acetate, propionate, butyrate, and usually smaller amounts of lactate and valerate (Argenzio et al., 1974). Lactate is an undesired end product of rapid fermentation because it is poorly absorbed, and accumulated amounts of lactic acid lower cecal pH below six and further favors the production of bacteria that yield lactate. The decrease in pH leads to death of desired fiber fermenting microbes creates microdamage to the intestinal epithelium (Clarke et al., 1990). The dying microbes release endotoxins that are absorbed through the damaged epithelium, leading to endotoxemia and laminitis (Pollitt and Visser, 2010).

Diseases and Conditions Related to Sugar Sensitivity

Several equine disorders are characterized by sensitivities to sugar and starch intake. These sensitivities are most commonly related insulin resistance or, in some cases, extreme sensitivity to insulin. These metabolic conditions include Insulin resistance, Equine Metabolic Syndrome, Polyssacharide Storage Myopathy, and Laminitis.

Insulin resistance is defined as a state when normal concentrations of insulin fail to obtain a normal biological response (Kahn, 1978). Normal concentrations of insulin do not lower blood glucose effectively. In response, the pancreas then produces increasing amounts of insulin. Dietary recommendations for horses with insulin resistance suggest for low nonstructural carbohydrate content. Consuming diets with large amounts of simple sugars can worsen symptoms of obesity, hyperinsulinemia, and laminitis. An optimal diet for insulin resistant horses contains low nonstructural carbohydrate content and high fiber (Johnson et al., 2012).

The major clinical signs of Equine Metabolic Syndrome include obesity and or regional adiposity, prior or current laminitis, and insulin resistance (Frank, 2009). The definite causes of Equine Metabolic Syndrome are unknown, but there seem to be genetic correlations (Brosnahan et al., 2010). Common factors include genetic predisposition, low activity, diets with excess energy and high glycemic indices. Horses with Equine Metabolic Syndrome have recommended dietary restrictions due to symptoms of Insulin resistance.

Polysaccharide Storage Myopathy is characterized with clinical signs of muscle cramping and pain with exercise (Frishman, 2005). Horses diagnosed with Polysaccharide Storage Myopathy have muscle damage and are deficient in energy generation. Studies show when feeding Polysaccharide Storage Myopathy afflicted horses grain based concentrated feeds containing large amounts of simple sugars and starch, muscle pain and exercise intolerance is worsened (Ribeiro, 2004). Polysaccharide Storage Myopathy horses secrete less insulin to a glucose load (De la Corte, 1999), exhibiting insulin sensitivity and enhanced blood glucose uptake (Annandale et al., 2004). Feeding a ration restricting starch and sugar content (total ration < 8% DE from starch and sugar) and adding fat (> 10% of total DE) results in affected horses improving clinically (Frishman, 2005; Riberio, 2004). Recommendations for hay fed to Polysaccharide Storage Myopathy horses include feeding hay containing 11% nonstructural carbohydrates or less (Borgia et al., 2011).

Laminitis is a disabling disease in horses and ponies, which is a failure of the attachment between the dero-epidermal junction, resulting in pain and lameness in affected horses (Pollitt et al., 2003). Of the cases reported to the USDA (2000), lush pasture consumption is the number one cause of laminitis at 46%, followed by grain overload at 7%. Laminitis has been induced experimentally by administering starch and fructans at a dosage between 7.5 to 12.5 g per kg BW (French and Pollitt, 2004). Rapid fermentation causes the proliferation of lactic acid producing saccharolytic and amylolytic bacteria which decreases the hindgut (Garner, 1977). King and Mansmann (2004) recommended hay containing less than 15% nonstructural carbohydrates for healthy horses, preventing the onset of laminitis. Maintenance of laminitic horses also includes prevention of excess weight, increasing the importance of limiting excess carbohydrates.

Grain intake has been well documented as the culprit in the onset and exacerbating these disorders (Clarke et al., 1990; Kronfeld and Harris 2003; Frank, 2009). Controlling nonstructural carbohydrate intake and the glycemic response to feeds is paramount to managing equine metabolic disorders.

Glycemic Response

Glycemic response occurs as the body's goal is to maintain a safe level of blood glucose. The horses' normal level is between 72 to 114 mg/dl of blood. After a meal is consumed, the gut breaks down the carbohydrate and starch down into simple sugars. The simple sugars are then absorbed into the blood. Blood glucose increases while absorption rate exceeds clearance. Pancreatic β cells detect a rise in blood glucose and produce insulin in response, causing a pronounced rise in blood insulin. Insulin then facilitates the uptake of glucose into muscle and liver cells where it is converted into glycogen and stored. The uptake of glucose causes serum levels of glucose to decrease and insulin follows this pattern. The amount blood glucose and insulin rises varies dramatically and depends on the food consumed.

Nonstructural Carbohydrate Affects the Glycemic Index

The glycemic index was created in the 1980s for the use in human nutrition. It was a method to classify different foods by their potential to raise blood glucose levels, which cannot be predicted by chemical analysis of the food. As defined by the Food and Agriculture Organization and World Health Organization, the glycemic index of a food is the incremental area under the blood glucose response curve when administered a 50 g carbohydrate portion of a test food. The glycemic index of the test food is then expressed as a percentage of the blood glucose response curve of a standard food (FAO/WHO, 1998).

This description entails that foods with high glycemic indices produce higher peaks and overall blood glucose response than foods with a low glycemic index (Jenkins, 1981). Foods with a low glycemic index elevate blood glucose less or at a slower rate. The glycemic index is calculated as a percent of a response when compared to a standard food, which is assigned a glycemic index of 100. The standard food used to evaluate the glycemic response in human studies is most commonly white bread (FAO/WHO, 1998). Horse research has frequently used oats or corn as a standard food of comparison (Jose-Cunilleras et al., 2004; Rodiek and Stull, 2005). Like human glycemic index, equine research has divided horse feed glycemic indices into three groups; high (> 70), medium (55 to 69), and low (< 55) (Kronfeld et al., 2004).

Compared to glycemic index, the glycemic load has been defined as the glycemic index multiplied the amount of carbohydrate available in the portion of the food. The glycemic load is used to quantify the overall glycemic effect using quantity and quality of carbohydrate. A low glycemic load can be obtained through decreasing the total carbohydrates consumed by feeding smaller meals, or by reducing glycemic index of the carbohydrates consumed by choosing feeds with a low glycemic index (Vervuert, 2006).

There are three major factors where glycemic response is influenced differently humans when compared to horses. First there are carbohydrates that are fermentable and accessible to equines but not humans, and these fermentable carbohydrates may affect glycemic response. Secondly when horses consume meals with large amounts of nonstructural carbohydrate, digestive disturbance can occur. These disturbances are cause by the capacity of the small intestine being overwhelmed, thus pushing the excess carbohydrate load to the hindgut where it is rapidly fermented. Rapid fermentation of nonstructural carbohydrates yield primarily lactic acid, which in turn lowers the pH of the colon causing disturbance of the bacterial population, potentially leading to colic, osmotic diarrhea or laminitis. Lastly, humans consuming fructose have smaller serum insulin concentrations as compared to consuming glucose, a response not noted in horses (Bullimore et al, 2000; Vervuert et al. 2004).

Variability in horse glycemic index research has created difficulties when comparing published results. Researchers may compare feeds to different standardized feeds (oats or corn) or different amounts of feeds (Jose-Cunilleras et al., 2004; Rodiek and Stull, 2005; Pagan, 1999a). The amount of starch consumed, the feed source, and the processing of the feed source alters the breadth and scope of the postprandial blood glucose response to a meal.

Source of Starch

Rodiek et al. (2005) evaluated ten common horse feeds and their glycemic response. During this study oats were set as the standard feed and assigned a glycemic index of 100. All ten samples were fed in isocaloric amounts, at 4.0 MCal DE. The results showed sweet feed, corn, jockey oats, and oats were all considered high on the glycemic index, while beet pulp, alfalfa, rice bran, and soy bean hulls were all considered low glycemic index. Both barley and wheat bran were in the middle, but not significantly different than feeds in the high and low groups (Rodiek, 2005). In order for the feeds to be evaluated at the same DE, the weights of the meals varied from 1.18 to 2.36 kg. In other equine glycemic index research, glucose concentration time to peak increased by 45 minutes when increasing the amount fed from 0.75 to 1.5 kg (Pagan et al., 1999b). The large variation in meal size may alter the glycemic response however, in order to evaluate the same quantity of starch among samples the weights had to differ. Another likely cause in variation is the time of feed consumption, which ranged from 15 to 300 minutes for individual horses. Furthermore there was feed refusal for low glycemic responsive feeds. The values of feed refusal rates were 14% soy hulls, 31% rice bran, and 37% beet pulp. Regardless of the variation these results were similar to glycemic index values compiled by Kronfeld et. al., (2004). The difference is Kronfeld et al.,'s interpretation of the high, medium, and low values of the glycemic response; that is, feeds with high glycemic index are greater than 70; feeds with medium glycemic indexs are in the range of 50 to70; and feeds with glycemic indexes less than 50 were considered low (Kronfeld et. al., 2004).

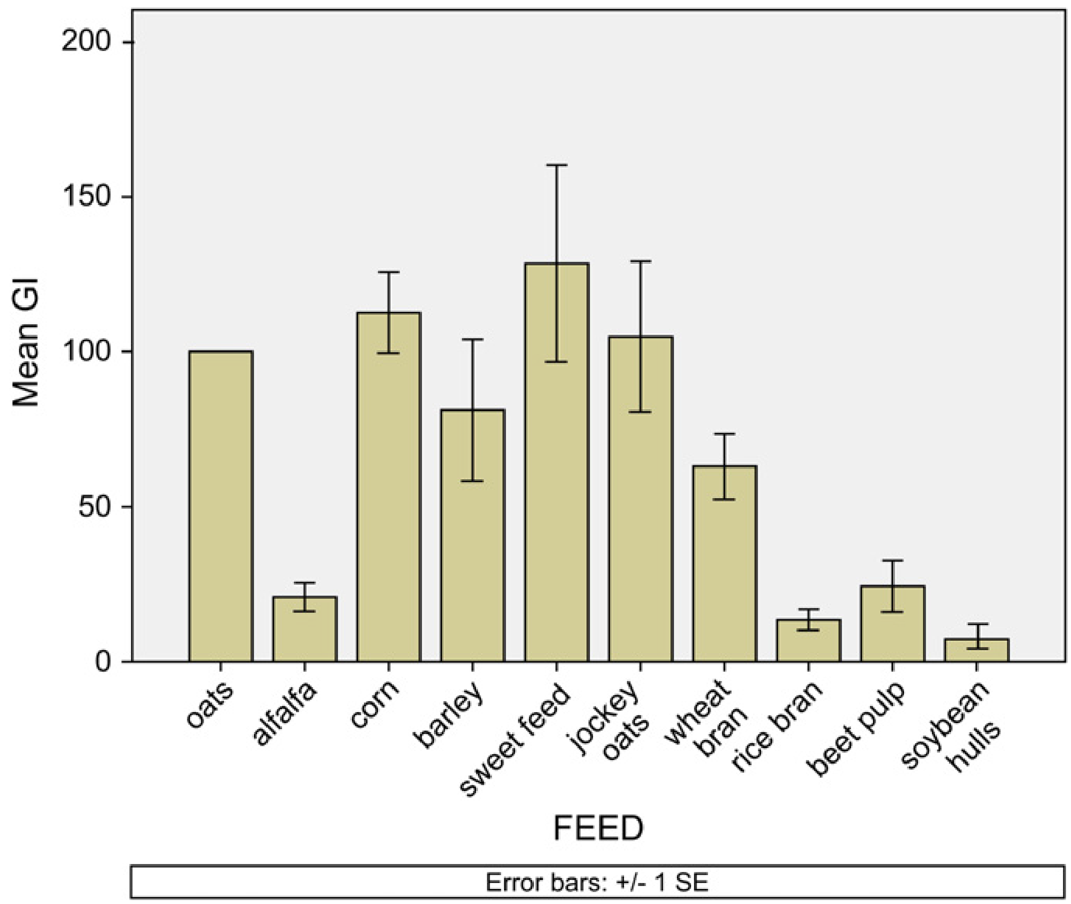

Figure 1. (Rodiek and Stull, 2005) mean glycemic indices of 10 horse feeds.

Processing of Starch

Horse's apparent prececal digestibility of starch is high (Brand et al, 1985). Processing of starches can increase the digestibility and the glycemic response (Meyer et al., 1993). The amount of processing has a great influence on the availability of starch. Starch that is contained within a waxy seed coat creates a greater difficulty for pancreatic amylase to make contact with it. Milling and grinding increases the ability of hydrolysis, as evidenced by a. study investigated by Meyer et al. (1993) that evaluated the preileal digestion on oats, corn, and barley starch in relation to processing. Five ponies were fitted with fistulas at the end of the jejunum. Whole oats were compared to rolled oats; whole corn compared to ground corn and popped corn; and rolled barley was evaluated. There was no difference in hydrolysis as evidenced by jejunal chyme between whole and rolled oats, and no difference was demonstrated between crushed and whole corn. Grinding corn improved its digestibility by 16.7% and popping by 61.2%.

Hoekstra et al. (1999) had studied the effects of processing corn on starch digestibility, using glycemic index as an indirect measure of pre-caecal starch digestibility in response to cracking, grinding, or steam processing. Steam-flaked corn had the highest glycemic response. Vervuert et al. (2003, 2004) evaluated the effects of mechanical and thermal processing on oats, barley, and corn. No difference in glycemic response was found due to processing when the starch amount was moderate (1.2 to 1.5g starch/kg BW). Processing affected blood glucose response in rolled, steam flaked, and extruded barley when the starch meal was increased to 2g starch/kg BW. In these studies, thermal processing was the primary factor in increased starch digestibility.

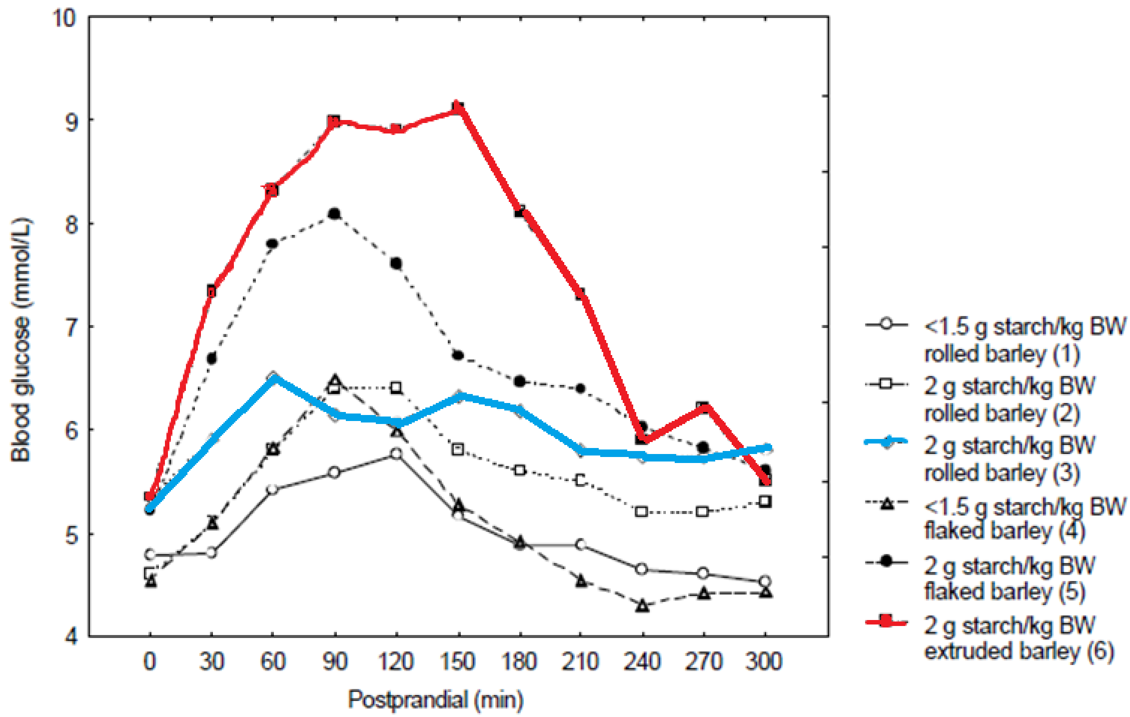

Figure 2. Effect of processing on the Glycemic Index of barley. Adapted from Vervuert et al. (2003).

Amount of Starch

The next factor that influences the glycemic response and starch digestibility is the amount of starch consumed. Vervuert et al. (2009) studied the effect of feeding increasing quantities of starch on the glycemic and insulinaemic responses. During this study six quantities of starch were evaluated including 0.3, 0.6, 0.8, 1.1, 1.4, and 2gstarch/ kg body weight. The feed used was a commercial compounded feed containing oats, wheat, corn, and barley as starch sources. There was a significant increase in serum glucose when starch intake exceeded 1.1g /kg body weight. The glycemic index for starch intakes between 1.1 and 2.0 g starch/kg body weight were not different, while there was a difference in meal sizes varying from 0.45 to 3.1 kg dry matter, suggesting that glycemic index may reach a maximal response, as meal sizes larger than 1.1 g starch/kg body weight did not appear to continue influence the glycemic index (Vervuert et al., 2009).

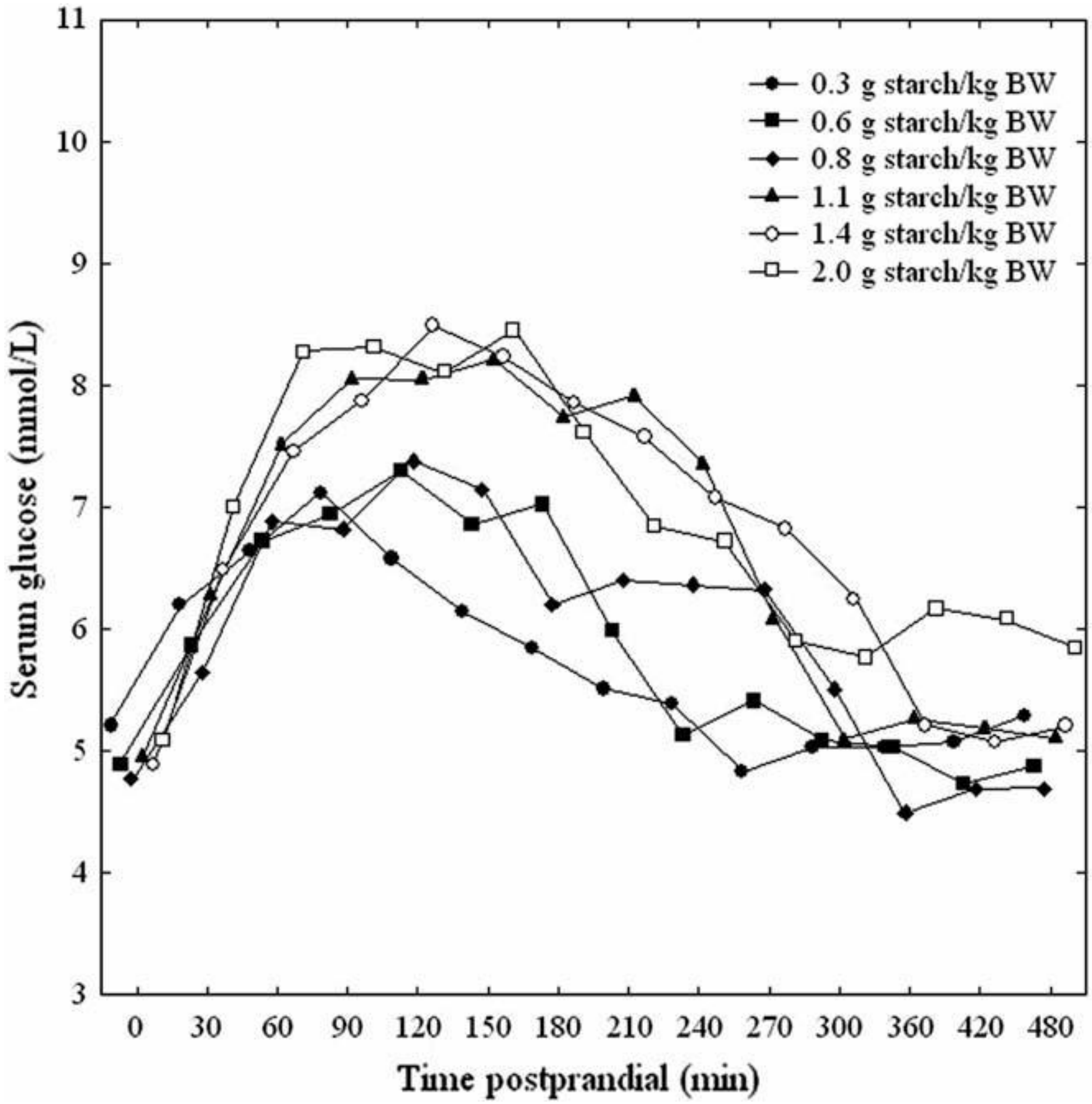

Figure 3. Postprandial serum glucose concentrations increase with increasing amounts of starch (Vervuert, 2009).

Mixed Diets

Generally meals are not made of one food in isolation but a complex mixture of feeds. The glycemic index of mixed meals is difficult to predict, as the glycemic indices of mixed meals are not linear. Forage has a large effect the digestibility and absorption of carbohydrates in the foregut. Research investigated by Pagan et al. (1999a) studied the effect of time of hay feeding, using glycemic response as an indicator of prececal starch digestibility. The treatments included feeding horses hay four hours after grain intake, feeding hay two hours before grain intake, and feeding hay and grain at the same time. Feeding hay at the same time or two hours before the grain significantly decreased the glycemic response.

Rate of gastric empting plays a huge role in understanding why meal size and the addition of fat and fiber affect the glycemic index. Larger meal size and higher starch content have been linked to slower gastric empting in horses (Métayer et al. 2004). Human research has demonstrated dietary fat to slow gastric empting, and with the release of gut hormones, glucose clearance increases (Thorne et al., 1983). Similar effects of added dietary fat on the lowering of glycemic index have been demonstrated in horses (Stull and Rodiek, 1988), attributed to an increased insulin response and more rapid glucose clearance (Veruert, 2006).

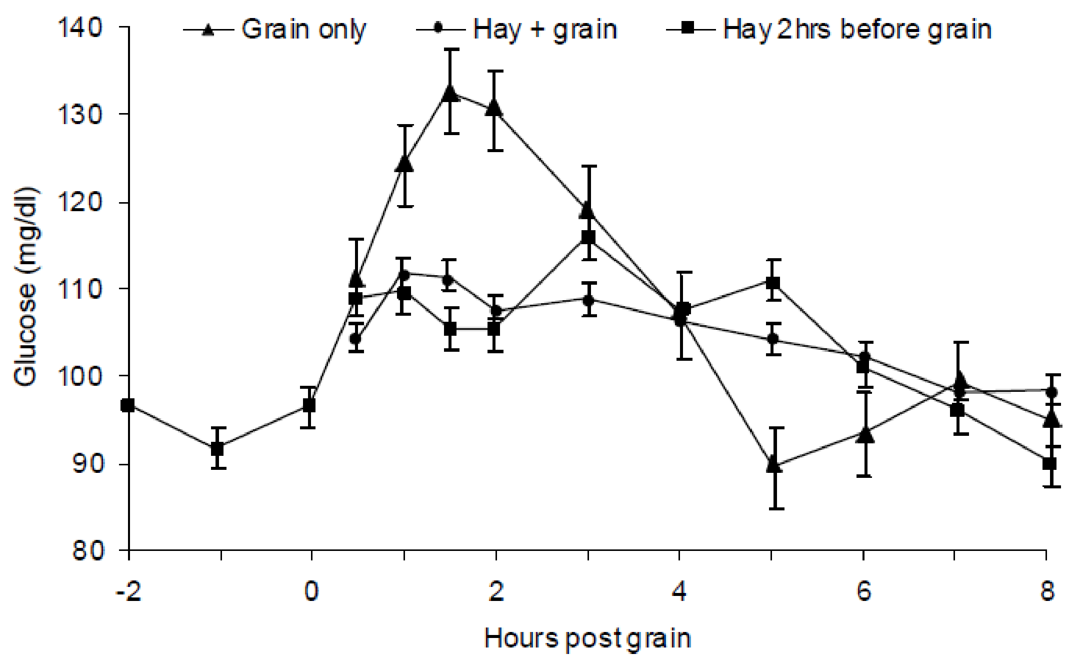

Figure 4. Effect of time of hay feeding on glycemic response (Pagan, 1999a).

Challenges with High Glycemic Index Feeds in Horses

Many adverse equine conditions are exaggerated with a diet containing a high glycemic load. Previously explained in this review, the conditions caused may include offsetting bacterial populations in the hind gut. These populations are extremely important to maintain fiber fermentation. Furthermore large amounts of starch digestion and a changed bacterial population can also lead to hind gut acidosis. Like previously mentioned fiber fermenting bacteria prefer a pH of greater than six and bacterial populations in general prefer a pH greater than five. Low pH in the hind gut irritates the colon lining and inhibits efficient absorption (Clarke et al., 1990). Simple sugar rich diets in horses have been associated as a risk factor insulin resistance. Horses with metabolic syndrome and insulin resistance demonstrate a greater glycemic response when compared to horses that do not have the condition. Common management practices work to lower the glycemic load, which can be accomplished by lowering the glycemic index of the foods consumed or lowering the quantity of hydrolysable carbohydrates.

Grass Hay vs. Legume Hay and the Glycemic Response

Forage is a main staple in the horse's diet, and horse owners provide it though pasture or hay. Common legumes used as forage are alfalfa and clover. Many grass species are used in horse diets including orchardgrass, timothy, Kentucky bluegrass, and tall fescue. Forages all contain a nonstructural carbohydrate including simple sugars, starches, and fructans; and a structural carbohydrate including cell wall components and lignin. Both the nonstructural carbohydrates and the strutural carbohydrates provide the energy base of forages utilized by horses. The simple sugars and starch can be digested by endogenous enzymes in the small intestine; however the cell wall carbohydrates and fructans cannot be digested in the foregut. The carbohydrates that undergo enzymatic hydrolysis contain a α-1,4 or α-1,6 linkages. These carbohydrates include disaccharides, starch, and some oligosaccharides. Horse enzymes cannot cleave carbohydrates containing β-1,4 linkages. Carbohydrates including these β-1,4 linkages include cellulose, hemicelluloses, lignocelluloses, fructans, galactans, and soluble fibers. Due to β-1,4 linkages not being hydrolyzed, they bypass the small intestine and are broken down by microbial fermentation in the hindgut (NRC,2007). Digestion of hydrolysable carbohydrates can produce a large spike in blood glucose and insulin response. The carbohydrates that are fermented do not produce a pronounced blood glucose spike (Harris, 2009) The major storage carbohydrate of grass and legume seeds, in addition to vegetative tissues of legumes and warm season grasses, is starch (Chatterton et al., 1989). However cool season grass vegetative tissues store their carbohydrates as fructans (Ojima, K., and T. Isawa. 1968). Cool season grasses contain up to 50 to 60% cellulose, 30 to 50% hemicellulose, and 2 to 4% pectin (Longland et al.,1995). Legumes contain up to 30 to 50% cellulose, 25 to 30% hemicellulose, and up to 30% pectin (Nordkvist and Åman, 1986). The differences in pectin makes legumes such as alfalfa a highly soluble fiber source. In addition to these differences, mid-maturity cool season grass hays contain 13.3% protein and mid-maturity legume hays contain 20.8% protein. Legumes are generally higher in calcium, potassium, magnesium, copper, zinc, iron, and cobalt than grasses (NRC, 2007). The differences between legumes and grasses cause horses to digest them differently and create different levels of glycemic response. Horses consuming alfalfa produce a more pronounced glycemic index than those consuming grass due to the higher nonstructural carbohydrates and less cellulose and hemicelluloses (Rodiek and Stull, 2005).

Plant carbohydrate varies greatly between plant type (variety and species) stage of growth, growth conditions, temperature, rainfall sunlight hours and intensity, and any stressors put on the plant. Typically the more immature the plant, the higher the fructan and starch content. The growth stage provides the plant with lots of stored energy, and the time of the year the plants are immature also plays a huge role. The spring and early summer months provide an abundance of sunlight, rain, and ideal temperatures. Plant stressors such as frost or bright cold days cause the plant to produce excess energy. While grass or legumes undergoing flowering or seeding may be high in fructans and starch, plants that have already undergone these processes tend to be lower in fructan and starch. The more mature the plant, the less excess energy it stores, and the more indigestible fiber it contains (King et al., 2004). These environmental factors create a great variation in plant carbohydrate.

The circadian and seasonal variability of cool season grass (Max-Q tall fescue) was evaluated by McIntosh (2006) during the months of April, May, August, October, and January of the following year. Large variation in nonstructural carbohydrates in the pasture occurred within hours, days and seasons. Nonstructural carbohydrate in cool season grass pasture was lowest (17.6 ± 0.3%) during the morning and highest by late afternoon (22.2 ±0.3%). April provided the pasture with the highest nonstructural carbohydrate content due to its ambient temperature, amount of sunlight, and humidity. Grazing horses demonstrated higher plasma lactate levels and lower fecal pH when compared to control horses being fed mix grass hay. Furthermore, McIntosh (2006) found a link between forage nonstructural carbohydrate content and alterations in glucose and insulin concentrations (Figure 5). These carbohydrates are stable in hay, making environmental factors that alter nonstructural carbohydrates and the timing of making hay important to feeding horses with sugar sensitivities

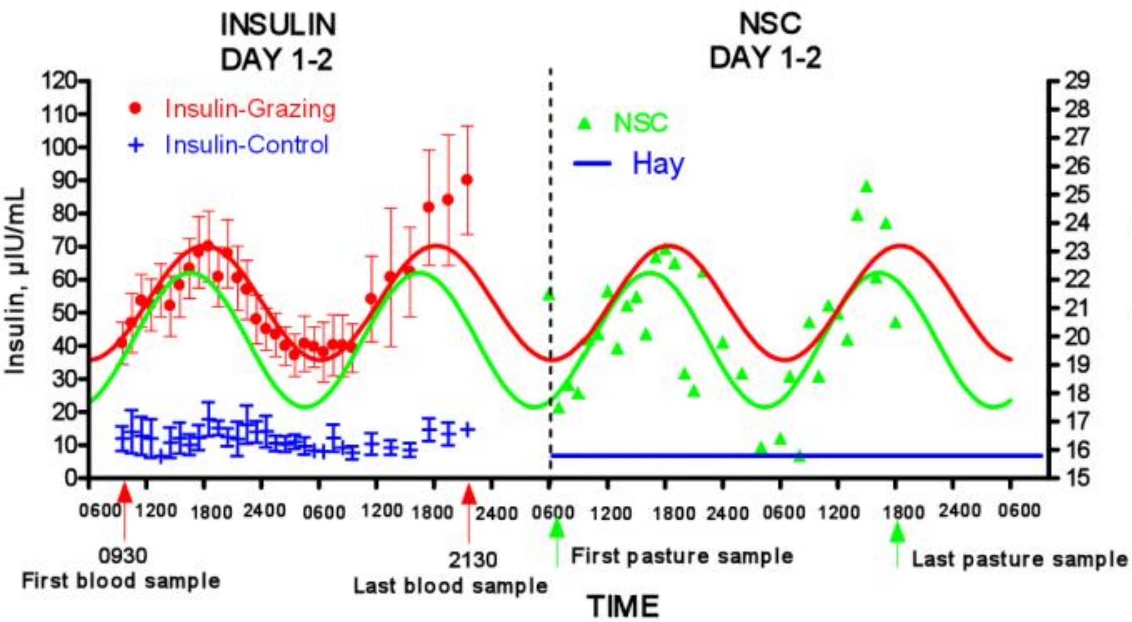

Figure 5. Sinusoidal circadian patterns in nonstructural carbohydrate and insulin in grazing horses (McIntosh et. al, 2006). Graph on the right shows NSC variation in growing pasture over two day period, with high NSC during the day and low NSC during the night. The graph on the left shows insulin in grazing horses (red) vs horses eating hay (blue). Insulin concentrations in grazing horses follow circadian variation of NSC in pasture.

Hay Soaking

Soaking hay affects dry matter loss and fructan removal in immature orchard grass and alfalfa hay, based on water temperature and soaking time (Martinson et al., 2011). Hays were soaked for 15, 30, 60 min or 12 hour time intervals and in warm or cold water. Water temperature did not have a significant effect on soaking times except during the 15 and 30 minute soaks. Dry matter loss was not significant until 12 hours of soaking all hays except for immature orchard grass, which had significant DM loss within at 1 hour of soaking in warm water. All time intervals greater than 15 minutes for all hay except the mature alfalfa hay exhibited greater loss in nonstructural carbohydrates than the control unsoaked hay. Both alfalfa hays were below the recommended 10% nonstructural carbohydrate recommendation (Borgia et al., 2011) but also had a significant loss of nonstructural carbohydrate after a 15 min soak in warm and cold water. Before the trials the mature orchard grass contained 14.3% nonstructural carbohydrates and the immature orchard grass contained 13.8% nonstructural carbohydrates. Mature orchard grass was below the recommended 10% nonstructural carbohydrates within 15 minute of soaking in warm water (9.2%) and 30 minutes of soaking in cold water (9.5%). Soaking the mature alfalfa and orchard grass and immature orchard grass for 12 hours produced the largest loss of nonstructural carbohydrate, but recommended levels of nonstructural carbohydrate for metabolically diseased horses was met within a hour of soaking (Martinson et al., 2011).

A study by Longland et al. (2011) also evaluated time of soaking on nonstructural carbohydrates loss. Nine different meadow hays varying in water soluble carbohydrate

(WSC) were soaked in cold water 20 min, 40 min, three hours, and 16 hours. A 2 to 9% loss of WSC was noted within 20 minutes of soaking. For a majority of the hays, WSC increasingly leached out as time increased. After 16 hours of soaking in water, 9 to 54% of the WSC had diffused out. The results of the study give the impression of hay soaking to be a viable practice in lowering nonstructural carbohydrates for horses with sugar sensitivities. It was noted, however, that soaking for longer periods of time may leach enough nutrients out excessively, to the point that the horse's nutrient requirements may not be met (Longland et al., 2011).

Cottrell et al. (2005) evaluated the glycemic and insulin responses to horses being fed soaked hay with high NSC. The study used 12 Belgian X Quarter horse weanlings, aging between four to five months old. Horses were paired according to body size then randomly assigned to one of two treatments. The research design was a simple cross over design. The two trials included a single 0.91kg meal of 12% NSC hay or a single meal of 22% NSC hay fed at 0.3% body weight. Both trails were fed dry and soaked for 30 minutes. Findings included fecal pH decrease after 36 hours during the 12% NSC trial but not during the 22% NSC trial. Plasma glucose AUC decreased during the trials. Peak glucose was lower during the 22% NSC trial. Lastly peak insulin and AUC were both decreased during both trials. Further research is needed to evaluate the glycemic and insulin responses in mature horses consuming long stem hay with a moderate NSC content.

CHAPTER II: THE EFFECT OF DRY VERSUS SOAKED HAY ON GLYCEMIC RESPONSE IN HORSES

Low glycemic feeds and forages are recommended in the management of horses with equine metabolic diseases. Research has demonstrated the exacerbating effects of high nonstructural carbohydrates loads have on the horse and how they can further worsen the symptoms of a metabolically disabled horse. There is limited research that has evaluated reducing chemical composition of nonstructural carbohydrate (starches, sugars, fructans) in feeds and forages, rather than determining the actual glycemic impact of dietary components. Veterinarians and horse nutritionists recommend reducing nonstructural carbohydrate content of the forage portion of the equine diet by soaking hay prior to feeding. This recommendation arose from previous reports of reducing nonstructural carbohydrate content in soaked hay compared to dry hay from Martinson et. al.(2011) and Longland et al. (2011). Although soaked hay, compared to dry hay, has lower nonstructural carbohydrate as measured chemically in the laboratory, to date, research has not been found that examines the glycemic impact of soaked versus dry hay. The goal of this study is to examine the glycemic response in horses fed soaked versus dry hay in order to determine if the common recommendation of soaking hay has a physiological effect in horses.

Materials and Methods

Twelve healthy horses aged 17 ± 4 yr, weighing 549 ± 51 kg, with BCS 5 to 6 were randomly assigned into two groups and one of four hay diets in a 2x2 factorial design. Four hay diets were evaluated for effect on glycemic response: dry prairiegrass hay, soaked prairiegrass hay , dry alfalfa hay , and soaked alfalfa hay .

For the hay glycemic response test, horses were placed in 9.3 m^2 stalls and offered water but no hay or grain for 10 h prior to the onset of feeding hay for the study. Remaining hay and grain was cleaned out of the stalls at 2200 h the night before the test. Horses were weighed using an electronic scale, and the hay diet allotment for each horse was measured at 0.5 % of BW on a dry basis.

Hay to be soaked was placed in a hay net and then soaked in 10 to 12 gallons of cold water for 60 min prior to the beginning of each feeding, following procedures recommended by Martinson et al. (2011). After 60 min of soaking, each wet hay diet was raised from the soak water in the hay net and drained until water stopped running out of the hay. The wet hay was removed from the hay net, and hay for each horse (wet or dry) was placed in an elevated corner hay rack, 1.4 m from the floor. Early completion of meals or cessation of eating bouts were recorded, as well as renewed periods of eating. Nutrient profiles of the hay are shown in Table 1 and moisture contents are shown in Table 2.

Hay diets were offered to the horses beginning at 0800 h on the morning of the glycemic response trials in order to avoid any potential effect of diurnal variation on glucose response. Blood samples were collected prior to offering hay, and at 30, 60, 90, 120, 180, 240, and 300 min after hay feeding began. Samples were collected into heparinized tubes, placed immediately in ice, centrifuged, and plasma aliquots frozen at -4°C pending analysis. Plasma glucose concentrations were analyzed using a colorimetric assay (Wako Diagnostics Auto Glucose kit, Wako Chemicals USA Inc., Richmond, VA). The magnitude of the incremental area under the curve of postprandial glucose response to the meal in each horse was calculated using graphical approximation (Slide Write Plus Ver. 7, Advanced Graphics Software, Inc., Rancho Santa Fe, CA).

Data were tested for normality and analyzed using a mixed model with repeated measures (SAS Inst. Ver 9.2, Inc., Cary, NC) with hay type and treatment (dry vs wet) as main effects, horse as the subject, and sample time as the repeated effect.

Table 1. Hay analysis based on percent dry matter (Equi-Analytical, Ithaca, NY).

%	Dry Grass	Soaked Grass	Dry Alfalfa	Soaked Alfalfa
Crude Protein	6.0	7.2	16.9	23.8
Acid Detergent Fiber (ADF)	39.1	39.5	39.0	38.0
Neutral Detergent Fiber (aNDF)	65.7	69.1	49.3	45.1
Water Soluble Carbohydrates (WSC)	9.5	6.8	8.8	6.6
Ethanol Soluble Carbohydrates (Simple Sugars)	6.4	4.4	6.7	5.3
Non- Fiber Carbohydrates (NFC)	19.2	14.6	21.7	19.1
Starch	1.2	0.6	0.9	1.6
Calcium	0.60	0.50	1.24	1.44
Phosphorus	0.08	0.08	0.19	0.19
Magnesium	0.21	0.13	0.21	0.18
Potassium	0.92	0.59	1.57	0.81
Sodium	0.061	0.002	0.081	0.031

Table 2. Hay moisture analysis on an as fed basis (Equi-Analytical, Ithaca, NY).

%	Dry Grass	Soaked Grass	Dry Alfalfa	Soaked Alfalfa
Dry Matter	93.6	34.2	91.1	25.0
Moisture	6.4	65.8	8.9	75.0

Results

Plasma glucose concentrations over time were higher *(P = 0.001)* in horses fed alfalfa compared to grass hay, while no difference was identified in horses fed dry vssoaked grass *(P = 0.99)* or dry vs soaked alfalfa *(P = 0.82)*. The AUC of glucose response was higher *(P =0.0004)* in horses fed alfalfa compared to grass hay. There was no difference in AUC of glucose response in horses fed dry grass hay as compared to soaked grass hay (2780 ± 563 vs 1271 ± 465 ug*dL^{-1}*min^{-1}, respectively; *P = 0.26*). There was also no difference in AUC of glucose response in horses fed dry alfalfa as compared to soaked alfalfa hay (5156 ± 905 vs 3347 ± 473 ug*dL^{-1}*min^{-1}, respectively; *P = 0.13*). While type of hay fed influenced glucose response and glucose AUC, no difference in physiological glucose response or glucose AUC was observed in healthy horses fed dry vs soaked hay. Additional research is needed to determine if soaking hay has physiological merit in horses with metabolic issues sensitive to nonstructural carbohydrate.

Discussion

Type of hay fed influenced glucose response and glucose area under the curve. No difference in physiological glucose response or glucose area under the curve was observed in healthy horses fed dry vs. soaked hay. However, results may vary with diseased horses. Previous research (Rodiek, 2004) evaluating the effect of cool season grasses, warm season grasses, and alfalfa on the glycemic index in the horse were similar to results from this study regarding the type of hay. The current study found no significant difference between soaked and unsoaked hay in either the prairiegrass or alfalfa hay treatments, these results differ from Cottrell et al., (2005). Variation in methods and materials used between Cottrell et al., (2005) may be the cause for different results. Cottrell et al. (2005) used chopped hay as compared to this study using long stem hays. The differences in processing changes exposed surface area. The smaller surface area in chopped hay may have caused differences in digestibility and the leaching of nonstructural carbohydrates during the soaking period. Furthermore, Cottrell et al., 2005 used 4 to 5 month old draft light breed horse cross weanlings, as compared to our study using mature horses. Differences in age of horses may cause a difference in digestibility and insulin sensitivities to feedstuffs consumed. Lastly, Cottrell et al., 2005 used hays containing high levels of nonstructural carbohydrates. The hays they used contained 12 and 22% nonstructural carbohydrates and the hays we used had moderate levels of nonstructural carbohydrates. These differences in nonstructural carbohydrate content may have caused glycemic index differences.

Conclusion

Owners of healthy horses do not need to soak hay. It is unknown if soaking hay holds merit for diseased horses. Type of hay fed influenced glucose response and glucose AUC. No difference in physiological glucose response or glucose AUC was observed in healthy horses fed dry vs. soaked hay.

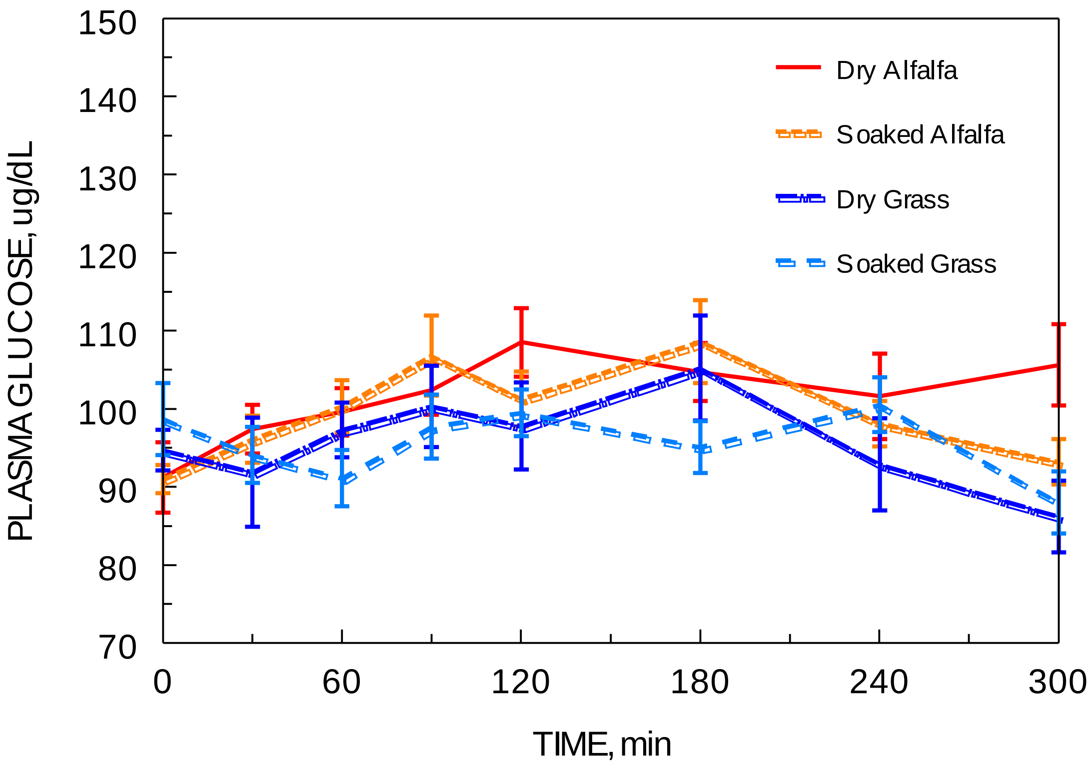

Figure 6. Postprandial plasma glucose response after feeding different hays. There was no effect of soaking but glucose response to alfalfa hay was higher than grass hay *(P = 0.041).*

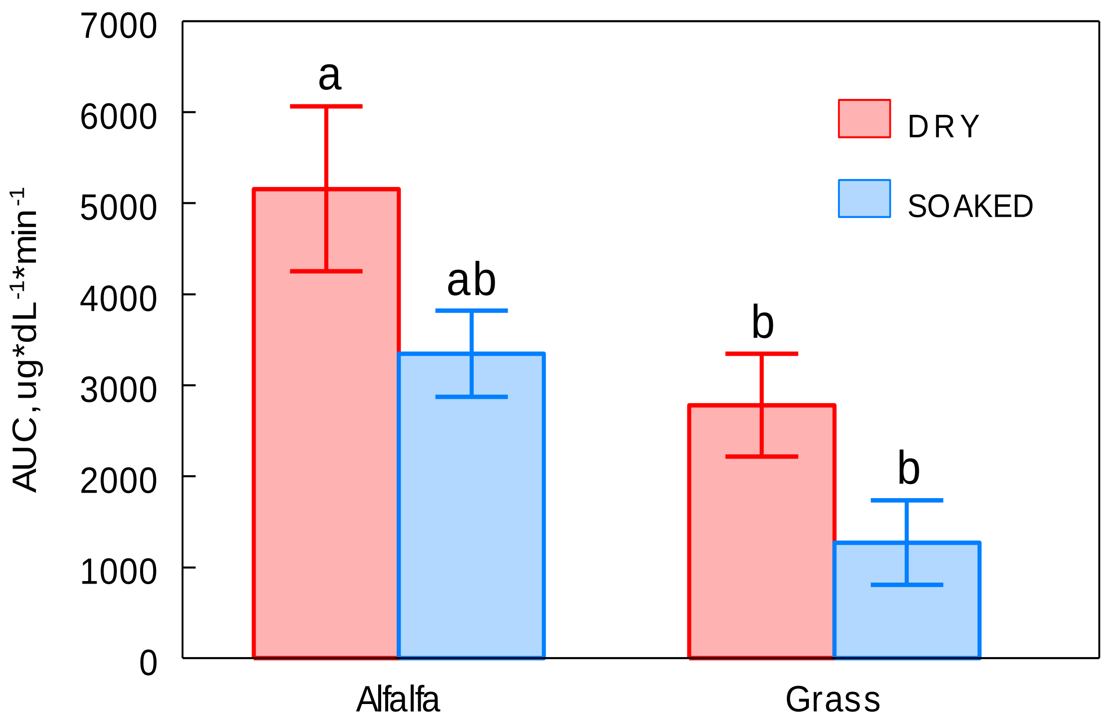

Figure 7. Incremental area under the curve (AUC) of plasma glucose response to feeding dry vs soaked alfalfa or grass hay. There was no effect of soaking but AUC for alfalfa hay was higher than grass hay *(P = 0.0004)*.

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APPENDICES

APPENDIX A: IACUC Approval

7/10/2014

Investigator(s) Name: Rhonda M. Hoffman, Josie Collins, John Haffner, Holly Spooner
Investigator(s) Email: Rhonda.Hoffman@mtsu.edu, jc5w@mtmail.mtsu.edu, John.Haffner@mtsu.edu, Holly.Spooner@mtsu.edu

Protocol Title: "The Effect of Dry versus Soaked Hay on Glycemic Response in Horses"
Protocol Number: 14-013

Dear Investigator,

The MTSU Institutional Animal Use and Care Committee has reviewed your research proposal identified above and has approved your research in accordance with PHS policy. Approval is granted for three (3) years. Your study expires 7/10/2017. Please note you will need to file a Progress Report annually regarding the status of your study and submit an end-of-project report.

According to MTSU Policy, an investigator is defined as anyone who has contact with animals for research purposes. Anyone meeting this definition needs to be listed on the protocol and needs to complete the IACUC training through citiprogram. If you add investigators to an approved project, please forward an updated list of investigators to the Office of Compliance before they begin to work on the project.

Any change to the protocol must be submitted to the IACUC before implementing this change. Any unanticipated harms to subjects or adverse events must be reported to the Office of Compliance at (615) 494-8918.

Also, all research materials must be retained by the PI or faculty advisor (if the PI is a student) for at least three (3) years after study completion. Should you have any questions or need additional information, please do not hesitate to contact me.

Sincerely,

Kellie Hilker
Compliance Officer
615-494-8918
kellie.hilker@mtsu.edu