GLYCEMIC RESPONSES TO CARBOHYDRATE SOURCES IN THE HORSE

by

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Abstract

There is increasing interest in the use of point-of-care glucometers to monitor glucose concentrations in horses with metabolic disorders. The first study reported herein compared equine glucose concentrations obtained by a handheld glucometer using whole blood or plasma, a YSI 2300 bench top glucose analyzer using whole blood or plasma, and a SEVEN continuous glucose monitoring (CGM) device that measured glucose in interstitial fluid to readings obtained by a standard laboratory glucose analyzer utilizing plasma. In addition, glucose concentrations obtained by the CGM were compared to those obtained by the handheld glucometer using whole blood or plasma. Post-prandial increases and decreases in glucose concentrations were detected utilizing all glucometers tested. When glucose measurements obtained with the CGM in interstitial fluid were compared to glucose measured using the handheld glucometer in plasma or whole blood, glucose measurements from plasma had better reproducibility. Although the CGM could be a useful instrument for collecting nearly continuous data for the researcher and clinician, there are technical difficulties related to the CGM that must first be overcome. The second study was designed to compare the effects of consuming a twice-daily meal of sweet feed (SF) to *ad libitum* access to a molasses-based block (BL) supplement on patterns of interstitial glucose concentrations in horses. A novelty effect of the BL was observed, in which horses consumed increased quantities in the first 12 h. Treatments had no effect on intake of forage in this study. The range and means of glucose values were similar between treatments, and significant glucose responses to treatments had lag times that were indirectly similar, even though molasses intake was greater for horses on BL. Variability between horses was noted in quantity of BL consumed as well as timing and magnitude of glucose responses. Based on the results of this experiment, there does not appear to be a clear advantage to either treatment, SF or

BL, in attenuating post-prandial glucose increases or in minimizing glucose fluctuations in the

horse.

Table of Contents

List of Figures

List of Tables

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Dedication

This thesis is dedicated to all equine enthusiasts that seek further knowledge of these magnificent animals.

Chapter 1 - Literature Review: A Nutritional Overview of Carbohydrates and the Impacts of High-Starch Diets in the Equine

Abstract

Carbohydrates constitute the primary source of energy in the equine diet and can be divided into nonstructural (NSC), which are high in free sugars, starch, and fructan, and structural (SC) fractions, which are high in fiber. Structural carbohydrates make up the cell wall tissue of plants, while NSCs contain the sugars found within the plant cell. The mechanisms by which carbohydrates are digested, absorbed and utilized depend on the type of carbohydrate consumed. Structural carbohydrates are fermented by bacteria in the stomach and hindgut, while NSC are digested by enzymes in the small intestine. Like SC, NSC can also be fermented by bacteria in the stomach and hindgut. One important exception is that, the NSC fructan is unavailable for enzymatic digestion. The end products of microbial fermentation are primarily volatile fatty acids (VFA) and lactate. Fermentation of excessive NSC can result in an abundance of VFA and lactic acid generated in the cecum, which can cause a decline in pH. A reduction in pH can cause acidosis, which can be detrimental to the gastric and hindgut mucosa, resulting in acute digestive disorders. As well, starch absorbed in the small intestine can elicit systemic glycemic and insulinemic responses. Exaggerated glycemic responses have been associated with metabolic disorders in the horse. Pastures rich in storage carbohydrates, such as starch and fructan, have been linked to a variety of metabolic disorders, such as laminitis,

obesity, and insulin resistance in the horse. There are management techniques that can be utilized to aid in the prevention of overconsumption of storage carbohydrates from pastures, hay, and grain. Some of these include limiting (or regulating) frequency and time of day horses graze, soaking hay to lessen NSC content, and providing feeds that have low to moderate glycemic and insulinemic effects. The challenge is to provide a diet that avoids rapid microbial fermentation of NSC in the hindgut while also minimizing post-prandial glycemic and insulinemic effects. This is challenging because, while increased absorption of NSC in the small intestine lessens the substrate available for rapid microbial fermentation in the hindgut, it simultaneously increases the glycemic and insulinemic response. Providing low- to moderate-glycemic feeds in small, frequent meals may be an effective regimen for maintaining the balance between stability of the hindgut microbial environment and the metabolic health of horses.

Introduction

Horses are classified as non-ruminant herbivores and subsist on a diet that consists primarily of plant material (Janis, 1976). Carbohydrates constitute the primary source of energy for the horse and are obtained primarily through the consumption of forages, grain, and grain byproducts (NRC, 2007). A thorough understanding of how horses digest and utilize different types of carbohydrates is fundamental to developing effective management strategies. Inappropriate consumption of carbohydrates can result in severe gastrointestinal and metabolic disorders that can be fatal to the horse (Kronfeld and Harris, 2003). This review examines the carbohydrate fractions in the equine diet and their effects on the gastrointestinal tract,

metabolism, and ultimately, the health of the horse. As well, management strategies that facilitate the safe feeding of carbohydrates to horses are reviewed.

Carbohydrates in Equine Diets

Types of Carbohydrates

Dietary carbohydrates consist of nonstructural (NSC) and structural (SC) fractions (Dey and Harborne, 1997).   The NSC fraction is found within the cellular contents of plants and includes simple sugars, such as glucose and fructose, and storage carbohydrates, such as sucrose, starch, and fructan (Dey and Harborne, 1997). The SC fraction is composed of fibrous components of the plant that give it physical structure. These include cellulose, hemicellulose, and pectin (Dey and Harborne, 1997).

Pasture grasses, a major dietary component for most equines, can be grouped into cool-season and warm-season grasses (Bender and Smith, 1973). Cool-season grasses, such as brome (*Bromus sp.*), fescue (*Festuca sp.*), timothy (*Phleum sp.*), and small grain hays (oat, wheat, rye, triticale), thrive in temperate conditions (Watts, 2004; Chatterton et al., 1989).   Fructans (polymers of fructose and glucose) are the major storage carbohydrates in these grasses (Ojima and Isawa 1968; Smith, 1968), and they accumulate in reserve tissues, such as roots, tubers, bulbs, leaves, and seeds (Dey and Harborne, 1997; Chatteron et al., 1989). Warm-season grasses, such as Bermuda (*Cynodon dactylon*), bahiagrass (*Paspalum sp.*), bluestem (*Andropogon sp.*), and crabgrass (*Digitaria sp.*), prefer subtropical or tropical conditions (Watts,

2004; Chatterton et al., 1989). Starch is the major storage carbohydrate in these grasses (Ojima and Isawa, 1968, Smith, 1968) and is deposited in the chloroplasts of leaves and the amyloplasts in non-photosynthetic reserve tissues such as roots, tubers, and seeds (Dey and Harborne, 1997). Like warm-season grasses, the main storage carbohydrate in legumes is starch, which accumulates in the vegetative tissues and seeds (NRC, 2007). Typically, cool-season grasses have higher NSC content compared to warm-season grasses (Table 1.1; Watts, 2004; Chatteron et al., 1989). Cool-season grasses can accumulate large concentrations of fructan because its accumulation is not self-limiting, as opposed to starch production which ceases once the chloroplasts in the leaf become saturated (Watts, 2004; Longland and Byrd, 2006; Chatterton et al., 1989).

Many factors can induce changes in NSC concentrations in both cool-season and warm-season forage (Longland and Byrd, 2006; Watts and Chatterton, 2004; Watts, 2004). For example, diurnal variation can affect NSC concentrations in forage, with greater NSC content occurring during daylight hours (Shewmaker et al., 2006; Ciavarella et al., 2000). Cold stress in cool-season grasses, such as an abrupt decrease in temperature or frost, is a major cause of increased NSC concentrations (Moriyama et al., 2003; Vagujfalvi et al., 1999; Pollock et al., 1983). Furthermore, NSC in cool-season grasses can be increased by many other environmental factors, such as drought (Volaire and Lelievre, 1997; Spollon and Nelson, 1994; Busso et al. 1990) or nitrogen deficiency (Batten et al., 1993; McGrath et al., 1997; Watts, 2005).

Carbohydrate Digestion

Digestion of NSC

The processes used to digest NSC material in the equine largely depend on the type of carbohydrates consumed. Starch and simple sugars in the NSC fraction have α1-4 and α1-6 linkages that can be hydrolyzed by pancreatic amylase (Roberts, 1974; Alexander and Chowdhury, 1958) and brush border enzymes, such as sucrase, maltase and lactase, in the small intestine (Dyer et al., 2002; Roberts et al., 1974; Alexander and Chowdhury, 1958). However, there is less amylase secreted in horses compared to carnivores or omnivores (Roberts, 1974). In addition to enzymatic digestion, α linkages can be broken through microbial fermentation occuring in the stomach (Varloud et al., 2007; Varloud et al., 2004) and the hindgut (de Fombelle et al., 2003) of the horse. Like starch, fructan are NSC polysaccharides, however, they are resistant to digestion by mammalian enzymes because they contain β linkages. These β2-1 and β2-6 linkages, however, are readily fermented by microorganisms within the gastrointestinal tract (Milinovich et al., 2008; Crawford et al., 2007; Bailey et al., 2002).

Starch is reported to have nearly complete total tract digestibility (Varloud et al., 2004; Potter et al., 1992). The upper limit to starch digestion in the foregut of a meal-fed horse has been estimated to be 0.35 to 0.4% body weight (BW) per feeding (Potter et al., 1992). However, prececal digestibility of starch varies widely and can be affected by amount consumed (Meyer et al., 1995; Potter et al., 1992), processing (Kienzle et al., 1997; Meyer et al., 1995; Potter et al., 1992) and individual animal idiosyncrasies, such as chewing behavior (Kienzle et al., 1997; Meyer et al., 1995) and amylase activity (Kienzle et al. 1997; Kienzle et al., 1994; Kienzle, 1994). Also, botanical structure has been reported to be an important factor in influencing

prececal starch digestibility (Kiensle et al., 1997; Kienzle et al., 1998; Meyer et al., 1995). For instance, Meyer et al. (1995) reported 83.5 ± 9.0% pre-ileal digestibility of whole oats compared to 28.9 ± 20% pre-ileal digestion of whole corn. This corresponds to data from a related study by Kienzle et al. (1998) that showed the outer layer of oat starch granuals can be digested easily by exocorrosion (the breakdown of starch all around granules) while the outer layer of corn starch granules are digested more slowly by endocorrosion (amylase only has access through pin holes in the starch granule). In addition, the effect of processing, in which starch digestibility increases with destruction of the original starch structure, is another important factor affecting prececal starch digestibility (Kienzle et al., 1997; Potter et al., 1992). For example, Meyer et al. (1995) reported 47.3 ± 12% pre-ileal digestibility of starch from ground corn compared to 28.9 ± 20% pre-ileal digestibility of whole corn.

Digestion of SC

The SC fraction contains β1-4 linkages that can only be digested through microbial fermentation because horses lack the necessary enzymes needed to break these structures down (Theander et al., 1989). Volatile fatty acids (VFA) and lactic acid are the primary products of microbial fermentation of both NSC and SC (Argenzio et al., 1974). The predominant VFAs produced are acetate, propionate, and butyrate (Bergman, 1990). Fermentation of SC will increase the acetate: propionate ratio while fermentation of NSC will decrease the acetate: propionate ratio (Bergman, 1990; Hintz et al., 1971b).

Carbohydrate Uptake and Utilization

Uptake and Utilization of NSC

Once the NSC with α linkages are enzymatically hydrolyzed into free sugars, they can be absorbed across the small intestine (Merediz et al., 2004; Dyer et al., 2002; Roberts, 1975) and used to provide an immediate energy source via glycolosis and the citric acid cycle (Horton et al., 2006). Alternatively, these sugars may be stored as glycogen or converted to fat for later use (Pond et al., 2005). Glycogen is stored in muscle and liver tissue until needed, at which time the enzyme glycogen phosphorylase catabolizes it in a process called glycogenolysis. The product of glycogenolysis, glucose 1-phosphate, is converted to glucose 6-phosphate, which can enter a number of energy generating pathways (Horton et al., 2006; Ellis and Hill, 2005). Fat is stored as triacylglycerols (esters of glycerol and fatty acids) in muscle and adipose tissue and can supply energy when hydrolyzed into glycerol and free fatty acids (Pond et al., 2005). Glycerol is used by the liver for gluconeogenesis, which is the formation of new glucose. Free fatty acids are oxidized to release energy in tissues, such as skeletal muscle and liver (Horton et al. 2006).

Although many hormones contribute to carbohydrate metabolism (Storer et al., 2007; Buff et al., 2006; DePew et al., 1994), insulin and glucagon are the two primary regulators (Pond et al., 2005). An increase in blood glucose following carbohydrate intake stimulates the release of insulin from the β cells of the pancreas. In turn, insulin stimulates glucose uptake by liver, muscle, and adipose tissues, thus removing glucose from blood (Pond et al., 2005). Insulin also inhibits the hydrolysis and oxidation of stored triglycerols for energy (Horton et al., 2006). Conversely, low blood glucose concentrations stimulate glucagon secretion from pancreatic α

cells to signal gluconegogenesis and glycogenolysis (Pond et al., 2005). Thus, insulin and glucagon contribute to maintaining a homeostatic balance between glucose uptake and metabolism to aid in sustaining euglycemia.

Fructans are digested and absorbed more like SC because of their β linkages. Volatile fatty acids that are produced from microbial fermentation of fructans are absorbed and transported across the large intestinal mucosa (Argenzio et al., 1974) and are metabolized to produce adenosine triphosphate (ATP) for energy.

Uptake and Utilization of the SC

Like fructans, VFAs that are produced from microbial fermentation of SC are absorbed and transported across the equine large intestinal mucosa (Argenzio et al., 1974) and are metabolized to produce ATP. Horses can utilize VFAs to meet 60 to 70% of their energy requirements (Argenzio, 1975; Argenzio et al., 1974). Acetate and butyrate energy sources for tissues and are utilized for de novo synthesis of long-chain fatty acids in the horse (Ellis and Hill, 2005). They can be utilized for energy production for muscles (Pratt et al., 2005). Propionate can be used for hepatic glucose synthesis by means of gluconeogenesis and (Simmons and Ford, 1991; Ford and Simmons, 1985).

Impacts of High-Starch Diets

Glycemic and Insulinemic Effects

Glycemic Indices

A diet composed primarily of SC may meet the energy requirements of horses at maintenance but often will not meet the needs of horses with higher energy requirements, such as working or lactating horses (NRC, 2007). Thus, concentrates can be included to increase dietary energy (NRC, 2007; Hintz, 1978). These types of supplements, primarily grains and grain byproducts, contain large amounts of starch and sugar and have been reported to elicit high glycemic responses (Kronfeld et al., 2004; Table 1.2).

Glycemic and insulinemic responses observed in horses to various dietary components have been attributed to differences between horses and differences among horse feeds (Tables 1.3 and 1.4). Some work has been done in an attempt to create a standardized glycemic index (GI) of common horse feeds to facilitate understanding of carbohydrate digestion and metabolism in horses and to provide knowledge that might aid in diet formulation (Rodiek and Stull, 2007).

The GI was originally developed to provide information about post-prandial blood glucose responses elicited in humans by various foods (Jenkins, 1981). The GI of a food is calculated and expressed as a percentage of glucose response elicited by a particular food compared to the glucose response elicited by a standard food, such as white bread (Jenkins et al., 2002). Glycemic response has been broken into 3 categories for human foods: high (GI > 70),

medium (GI = 55 to 69) or low (GI < 55; Atkinson et al., 2008). Rodiek and Stull (2007)

quantified the glycemic responses in horses fed 10 common horse feeds on an isocaloric basis

using whole oats as a standard feed for comparison, calculated a GI for the feeds, ranked the

feeds by GI, and categorized feeds into groups based on their GI. These researchers reported a

significant difference in GI values between a high group (GI > 70) that included grains and a low

group (GI < 55) that included roughage and byproduct feeds, with an intermediate group (GI =

55 to 69) of barley and wheat bran that was not significantly different from either the high or the

low groups (P > 0.05). Kronfeld et al. (2004) grouped horse feeds into similar categories, in

which the high group consisted mainly of grains, while forages and byproduct feeds were

included in the low GI group. Both Rodiek and Stull (2007) and Kronfeld et al. (2004)

concluded that, unlike human foods, there may only be a small number of equine feeds that

should be classified into an intermediate group (GI = 55 to 69).

There are some inconsistencies among reported studies with regard to glycemic and

insulinemic indices reported for equine feeds (Nielson et al., 2010; Vervuert and Coenen, 2005;

Kronfeld et al., 2004). First, equine studies designed to develop glycemic and insulinemic

indices do not all share a common standard. Standards that have been used include oats (Rodiek

and Stull, 2007), dextrose (Nielson et al., 2010), and cracked corn (Hoekstra et al., 1999).

Nielson et al. (2010) illustrate limitations to using feed ingredients, such as oats or corn, as a

standard for calculating GI; they can vary in quantity and availability of starch and glycemic

response may be affected by rate of consumption (Ralston, 2005). Nielson et al. (2010)

recommend using dextrose as a standard in an attempt to minimize possible variation. Secondly,

studies designed to develop equine glycemic and insulinemic indices differ in the amount of

feedstuff administered. Researchers have administered treatments on the basis of available

carbohydrate (Vervuert and Coenen, 2005), feed weight (Nielsen et al., 2010) or feeding an amount calculated to provide a specified amount of digestible energy (Rodiek and Stull, 2007). These differences between studies can also be seen in the Kronfeld et al. (2004) compilation of GI values of horse feeds from six studies that differ in the method that treatments were administered.

In humans, large amounts of variation can occur in the calculated GI of a food due to differences such as volume and structure, cooking methods, or processing (Wolever, 2006; Wheeler and Pi-Sunyer, 2008; Riccardi et al., 2008). Variation in GI also can be attributed to differences within individuals, such as age, sex, and ethnicity (Wolever, 2009). Inconsistency is common in determining the GI of human foods; therefore, variations of 10 to 15% are considered to be within the error associated with the measurement of GI (Foster-Powell and Miller, 1995). As well, glycemic indices for horse feeds have large errors (Kronfled et al., 2004). Glycemic indices and loads are based on linear calculations, yet the glycemic response of a subject depends on non-linearly regulated processes such as gastric emptying and rates of glucose removal from blood (Kronfeld et al., 2005). Therefore, Kronfeld et al. (2005) suggest, "The simple arithmetic of adding glycemic indices and loads, and finding weighted averages, must yield inexact results."

Although it has been reported that GI values have a high positive correlation to the NSC% (adjusted $r^2 = 0.829$) and starch% (adjusted $r^2 = 0.833$) in equine feedstuffs (Rodiek and Stull, 2007), proximate analysis of carbohydrates does not allow for differences in digestion and metabolism among substances and thus is inadequate for the precise prediction of glycemic effects (Kronfeld et al., 2004). However, Englyst et al. (1999) successfully accounted for the glycemic response of dietary carbohydrates in an *in vitro* analysis by classifying them into rapidly available glucose and slowly available glucose by measuring the rate of hydrolysis.

11

Rapidly available glucose is significantly and positively correlated to glycemic effect while slowly available glucose is significantly and negatively related to glycemic effects (Englyst et al., 1999; Englyst et al., 2003). Kronfeld (2005a) suggests that once validated, this technique may provide a superior method of predicting the glycemic effect in horse feeds compared to the glycemic index. Kronfeld (2005a) also suggested the technique may need to be altered for forages compared to concentrates due to different effects each has on gastric emptying and intestinal transit.

Excess Carbohydrate Consumption

Excess consumption is the primary causative factor in triggering equine grain-associated disorders (EGAD), a group of acute digestive disturbances attributed to rapid fermentation and to metabolic disorders involving insulin resistance (Kronfeld and Harris, 2003). As well, excess consumption of lush pasture containing high starch or fructan content can lead to acute digestive disturbances.

Acute Digestive Disturbances

If horses overgraze lush pastures that are high in fructan or consume too much NSC, it can be problematic. An abrupt accumulation of lactic acid and VFAs in the stomach can cause the pH to drop below 4.5 in the non-glandular region, which has been associated with gastric ulceration in horses (Nadeau et al., 2003a; Nadeau et al., 2003b). Additionally, if the NSC content of a meal exceeds the digestive or absorptive capacity of the small intestine, the unabsorbed digesta passes to the large intestine where it will be rapidly fermented by starch

utilizing bacteria, such as lactobacilli and streptococci, which produce lactic acid and VFAs (Sprouse and Garner, 1982; Garner et al., 1978). The buildup of lactic acid and VFAs can cause the pH to drop below the normal range of 6.0 to 6.8 in the large intestine (de Fombelle et al., 2003; Argenzio et al., 1974; Kern et al., 1973), thus leading to hindgut acidosis (Krueger et al., 1986; Sprouse and Garner, 1982; Garner et al., 1978). The deleterious consequences of rapid microbial digestion of NSC include diarrhea (Durham, 2010; Argenzio, 1975), colic (Durham, 2010; Tinker et al., 1997; Clarke et al., 1990), and laminitis (Garner et al., 1977; van Eps and Pollit, 2006).

Laminitis has been estimated to account for 15% of lameness problems in horses in the United States (USDA, 2002). Carbohydrate overload was the most often cited cause of laminitis, with overgrazing lush pasture accounting for 45.6% of cases and grain overload accounting for 7.4% of horses with laminitis (USDA, 2000). Although the specific pathway is unknown, it is believed that acidodic conditions created by the accumulation of acidic end products of lactate producing bacteria lead to the destruction of the luminal wall in the cecum which contributes to the onset of laminitis (Krueger et al., 1986; Garner et al., 1978). A rapid decline in pH alters cecal conditions so that it is inhospitable to some native species of bacteria, such as *Enterobacteriae*, and a significant number of these bacteria die (Garner et al., 1978). When the luminal mucosal layer is damaged, lactic acid and endotoxins produced by dying bacteria can be absorbed into portal circulation (Milinovich et al., 2008; Krueger et al., 1986; Garner et al., 1978). Researchers have also suggested that amines formed by starch utilizing bacteria after decarboxylation of amino acids may be a contributing factor in the development of laminitis, causing digital vasoconstriction if released into circulation after a carbohydrate overload (Bailey

et al., 2004). These starch-utilizing bacteria rapidly proliferate during a carbohydrate overload, leading to increased production of vasoactive amines (Bailey et al., 2003; Bailey et al., 2002).

Metabolic Disorders

Equine grain-associated disorders encompass many metabolic conditions associated with the consumption of large grain meals, leading to exaggerated glycemic and insulinemic responses (Kronfeld and Harris, 2003). Laminitis, some forms of exertional rhabdomyolysis, osteochondritis dissecans (OCD), growth fluctuations, and hyperlipidemia are among the metabolic disorders that have been linked to excessive feeding of grain (Kronfeld and Harris, 2003). Insulin resistance (IR) is an underlying factor in many metabolic disorders associated with excess grain feeding. Insulin resistance has been defined as "a less than normal biological response" to normal concentrations of the hormone insulin (Kahn, 1978). Insulin resistant horses may demonstrate decreased sensitivity to insulin at the cell surface, which disrupts glucose metabolism inside cells, particularly in insulin-sensitive cells such as muscle, adipose, and liver (Kronfeld, 2005b; Kronfeld et al., 2005). Regular consumption of meals with high starch and sugar content has been shown to reduce insulin sensitivity in Thoroughbred geldings fed a concentrate at 0.30 to 0.98% of BW twice daily (NSC = 56.7 ± 1.4 %; Quinn et al., 2008), in those fed approximately 0.51% BW twice daily (NSC = 62.4 ± 0.8% of meal on dry matter basis; Hoffman et al., 2003b), and weanlings fed approximately 0.58% BW twice daily (NSC = 40.0 ± 1.7% of meal on dry matter basis; GI = 129 %; Treiber et al., 2005a).

Nomenclatures assigned to metabolic conditions are often based on common risk factors, some of which have been derived from human medicine (Kronfeld, 2003). Equine Syndrome X was derived from Syndrome X in humans in which a series of related variables, such as IR, hyperglycemia, and hypertension, are related to coronary artery disease and type 2 diabetes

mellitus (Reaven, 2000; Kronfeld, 2003). Kronfeld (2003) used the term Equine Syndrome X to describe a group of metabolic disorders associated with long-term consumption of excess grain and molasses and that were also hypothesized to be linked to IR. Some of these metabolic disorders include hyperlipidemia, pituary adenoma, OCD, and laminitis (Kronfeld, 2003; Kronfeld and Harris 2003).

The term Equine Metabolic Syndrome (EMS) was derived from the human metabolic syndrome, or "MetS", which represents a compilation of risk factors used to predict the occurrence of coronary artery disease and type 2 diabetes mellitus (Frank et al., 2010, Fulop et al., 2006). In a recent consensus statement, the American College of Veterinary Internal Medicine (ACVIM) applied the term Equine Metabolic Syndrome to horses that have increased regional adiposity, IR, and that are predisposed to laminitis (Frank et al., 2010). The consensus statement pointed out that pituitary pars intermedia dysfunction (PPID), equine Cushing's disease, and EMS may be difficult to differentiate between because they share clinical symptoms, such as regional adiposity and laminitis (Frank et al., 2010). Although IR has been detected in horses with PPID, the ACVIM panel determined that it is more common for horses with PPID have normal insulin sensitivity (Frank et al., 2010). However, EMS and PPID can occur concurrently because some horses with EMS subsequently develop PPID (Frank et al., 2010). Horses with EMS require special dietary consideration that avoids feeds that exacerbates IR such as pasture grass that can fluctuate in fructan content and feeds high in sugar (Frank, 2007).

An association between high-glycemic feeds and OCD has been reported in young horses (Ralston, 1996; Pagan et al., 2001) and therefore high-glycemic diets should be avoided for young horses at risk for OCD (Kronfeld et al., 2005). Kronfeld et al. (2005) point out that

although an association between high-glycemic feeds and OCD exists, there is no evidentiary support that a low-glycemic diet will prevent the disease.

Dietetics

Avoidance of Excess Energy Intake

Dietary energy should be provided according to the mass, production class, and work load of a horse (NRC, 2007). Excess energy intake can cause hyperglycemia which, over time, can result in IR and increased adiposity.   Increased adiposity, if significant, can lead to obesity, which has been shown to decrease insulin sensitivity in the horse (Hoffman et al., 2003b). Energy intake of obese horses should be reduced to encourage weight loss by restricting total number of calories consumed while also increasing the level of physical activity (Frank et al., 2010).   The benefits of exercise were confirmed by Powell et al. (2002) who reported insulin sensitivity was improved by 60% and increased glucose utilization was apparent after 7 d of low-intensity exercise in obese mares compared to sedentary obese mares.

Meal Composition, Size, and Frequency

High-starch feeds have been defined as having NSC > 40% (Quinn et al., 2008; Hoffman et al., 2003b; Treiber et al., 2005a or having a starch content < 0.35% BW (Potter et al., 1992). Although high-starch feeds carry a risk for EGAD, if managed properly they can be beneficial to horses that require increased dietary energy. Complete pre-cecal starch absorption allows the horse to avoid acute digestive disturbances associated with rapid microbial fermentation in the hindgut, such as colic, laminitis, and diarrhea (Kronfeld and Harris, 2003). The upper limit of

pre-cecal absorption of starch is reported to be 0.35 to 0.4% BW per meal feeding (Potter et al. 1992).   However, increased pre-cecal starch absorption elicits greater glycemic responses (Nielsen et al., 2010; Kronfeld and Harris, 2003). Therefore, diets that elicit a low- to moderate-glycemic response are preferable when coupled with nearly complete pre-cecal digestibility of starch to avoid overflow into the hindgut, simultaneously averting metabolic disorders associated with high-glycemic responses and those associated with hindgut acidosis (Nielsen et al., 2010; Kronfeld and Harris, 2003).

Increased meal size has been associated with increased glycemic responses (Nielsen et al., 2010; Veruvert et al., 2009a; Pagan et al., 1999). Thus, Vervuert et al. (2009a) recommended meals with a starch intake of < 1.1 g/kg/BW or a meal size of 0.3 kg/100 kg BW with a starch content of 30 to 40% to avoid risk of digestive disturbances and metabolic disorders associated with EGAD. As well, Meyer et al. (1995) suggest that a meal should not contain < 2 g of starch/ kg BW. Some advocate increasing the number of meals rather than the size of meals when feeding high intakes of grain (Kronfeld and Harris, 2003; Frank, 2007).

Pasture Starches and Fructans

Overconsumption of fresh vegetation with elevated carbohydrate content has been linked to pasture laminitis, obesity, and the development of IR (Longland and Byrd, 2006; van Eps and Pollitt, 2006; Milinovich et al., 2006). Overconsumption of NSC can be avoided using appropriate management strategies (Longland and Byrd, 2006; Watts and Chatterton, 2004). Limiting grazing time to early morning (0300 h to 1000 h), when carbohydrates are often lowest, while restricting pasture access during the day, when storage carbohydrates are increased, can aid in avoiding overconsumption (Longland and Byrd, 2006; Watts and Chatterton, 2004). Nonstructural carbohydrate content in plants has been shown to increase concurrently with

sudden decreases in temperature (Pollock et al., 1983), so limiting grazing time under these conditions may be a prudent management tool for regulating intake of NSC (Watts and Chatterton, 2004). Also, given that the effect of shading has been shown to reduce NSC content (Watts, 2009; Ciavarella et al., 2000), selecting grazing areas that are shaded may reduce the level of NSC intake (Watts, 2009; Longland and Byrd, 2006; Watts and Chatterton, 2004).

Another option for controlling excessive intakes of NSC from pasture is to physically impede intake with a grazing muzzle (Longland and Byrd, 2006). Grazing muzzles reduce the total quantity of grass consumed and limits consumption to the tops of leaves where NSC concentrations tend to be lowest (Longland and Byrd, 2006; Watts, 2004, Watts, 2010). Strip grazing with portable fence can also limit NSC intakes by reducing access to grass (Watts, 2004, Watts 2010).

Because starch-utilizing bacteria rapidly increase in the presence of increased quantities of NSC (Sprouse and Garner, 1982; Garner et al., 1978), it may be prudent to gradually increase the amount of time a horse is allowed to graze per day. This gradual adaptation should facilitate an increase in the population of lactate-utilizing bacteria in the horse's hindgut, which can attenuate the drop in pH that accompanies high NSC intake through increased production of lactic acid by starch utilizing bacteria. Though, due to constantly changing and highly variable storage carbohydrate concentrations in some pastures, it may be difficult for the hindgut microflora to reach and maintain a balanced, steady environment (Longland and Byrd, 2006). Additionally, horses predisposed to laminitis or that have a chronically perturbed hindgut environment may be more sensitive to acute ingestion of elevated NSC than other horses (Longland and Byrd, 2006).

Initiation of meals is influenced by many internal and external cues, such as the smell of a feedstuff and the sight of other horses grazing (Ralston, 1984), factors which may be virtually impossible to control. Size and duration of meals are regulated by the degree of hunger the horse experiences before starting to eat (Ralston, 1984). Therefore, providing horses with feedstuffs low in NSC prior to a short-term turnout may help initiate or increase duration of satiety, decreasing the desire to graze.

In addition, pasture management techniques can be implemented to reduce NSC consumption (Longland and Byrd, 2006; Watts and Chatterton, 2004). The application of nitrogen fertilizers has been shown to increase growth, thus increasing the utilization of NSC by the plants themselves, therefore reducing NSC reserves available to the horse (Watts, 2010). By maintaining appropriate soil fertility and providing adequate moisture levels for optimal growth, storage of NSC in the plant may be abated (Longland and Byrd, 2006; Watts and Chatterton, 2004). Also, Watts and Chatterton (2004) advise against overgrazing pastures, as storage carbohydrates accumulate at the base of plant stems. Instead they recommend rotational grazing to maintain a grass height of 10.2 to 15.2 cm. As well, Longland and Byrd (2006) caution that pastures in the seed-bearing stage or stubble from recent harvesting will be high in NSC and should be avoided. Additionally, some plant varieties tend to be lower in NSC (Table 1.1), such as warm season grasses and species native to an area. Pasturing horses on these varieties may aid in lowering the NSC content of pastures (Watts, 2010). Watts (2010) suggests that varieties of grass that are foreign to a region are more likely to be stressed by the environment and are likely to produce more fructans as a protective mechanism than species native to the region. Watts (2007b) reported that most native grasses that feral horses in the Intermountian West region of the United States consumed were lower in NSC (NSC < 12%) than introduced,

improved grasses (NSC = 15 to 18%) commonly used for pasture and hay in the same region under the same environmental conditions. Although planting or maintaining native varieties may be beneficial to the horse, these varieties are generally less competitive, more expensive, and take longer to establish than invasive species (Watts, 2010).

Cultivated Hay

Premium quality hay is characterized as leafy, high in protein, has lesser SC content, and often contains greater concentrations of NSC (NRC, 2007; Watts, 2004). While premium quality hay may be well-suited for performance horses, low-quality hay containing less NSC and greater SC concentrations is recommended for EMS-affected horses (Johnson et al., 2010). Similarly, Watts (2004) suggested that laminitic horses should not consume premium quality forages, as they are often associated with large quantities of hydrolyzable carbohydrates. Frank (2007) suggests feeding hay with a NSC content of < 12% to horses with endocrine disorders, such as IR and EMS.

The NSC content in cultivated hay varies and reflects the NSC content of the pasture that it was harvested from (Watts and Chatterton, 2004; Watts, 2004). Cut forage continues to utilize sugars for respiration until the moisture content is below approximately 40%. Thus, the faster hay is dried, the greater the NSC content will be (Trevino et al., 1995). As hay matures, the amount of stem material increases in proportion to leaves, and protein content declines (NRC, 2007). With senescence, SC content increases while NSC content decreases, and these concentrations are reflected in the hay (Jafari and Rezaeifard, 2010). Caution is advised, however, that mature plants in the seed-bearing stage can contain great amounts of NSC, as the seed is a storage organ (Longland and Byrd, 2006; Waite and Boyd, 1953).

Environmental factors, such as cold stress, can influence carbohydrate content (Watts and Chatterton 2004; Watts, 2004). For example, oat hay cut at a mature stage but exposed to cool temperatures was reported to be as high in NSC as oat hay cut in a pre-heading stage, which would typically contain greater concentrations of NSC (Watts and Chatterton, 2004). Watts (2007a) reported a correlation of fiber to NSC of -0.66 and concluded that NSC concentration cannot be accurately inferred from fiber content. Therefore, hay analyses must be performed to determine an accurate NSC assessment (Longland and Byrd, 2006).

Measuring NSC in cultivated hay provides an estimate of the concentration of carbohydrates stored in the hay and can be a useful tool in feeding management (Longland and Byrd, 2006). However, Kronfeld et al. (2004) warns the word "sugar" is being used in two ways to describe carbohydrate content in feed analyses. Forage laboratories in the United States were originally developed to aid the dairy industry. For dairy producers, the distinction of carbohydrates by how they are digested (enzymatically vs. microbially) is not a primary concern as both are fermented in the rumen of cattle (Longland and Byrd, 2006; Kronfeld et al., 2004). Some laboratories extract only mono- and disaccharides (true sugar), while others extract water-soluble carbohydrates (WSC; true sugars plus fructan). Other laboratories report NSC which could either represent the sum of all starch, true sugar, fructans, pectins, gums, and mucilages, or may simply reflect the sum of starch, true sugar, and fructans (Longland and Byrd, 2006). Thus, it is important that the user knows which fractions are being measured in order to correctly implement a management plan for the horse (Longland and Byrd, 2006; Kronfeld et al., 2004). To estimate fructan content, Kronfeld et al. (2004) suggests calculating the difference between the WSC fraction and the true sugar fraction which can be extracted using 80% or 90% ethanol.

Soaking

Soaking beet pulp in water over night and then rinsing (until glucose in water was < 1 mg/dL) reduces the glycemic response in Thoroughbred horses and may be beneficial for horses that require diets that elicit a low glycemic response (Groff et al., 2001). In addition, Conttrell et al. (2005) reported that soaking chopped orchard grass hay containing 12% or 22% WSC for 30 min in cold water reduced the percent of sugar from 12 to 5.6% and 22 to 13.4%, respectively. Soaking also reduced the area under the curve (AUC) for glucose and insulin response compared to unsoaked hay ($P < 0.01$; Conttrell et al., 2005). As well, fecal pH was higher in horses fed 12% WSC soaked hay, suggesting that less NSC were fermented in the large intestine compared to unsoaked hay ($P < 0.05$; Conttrell et al., 2005). Furthermore, Watts and Sirois (2003) reported that soaking hay for at least 60 min in clean, cold water (27.8°C), or 30 min in hot water (50.3°C) and then draining the water before feeding significantly reduced sugar concentrations in multiple varieties of grass and legume hays by 31 and 29%, respectively. Longland et al. (2011) reported a mean loss of 27% WSC in hay soaked for up to 16 h in water at a mean temperature of 8°C. Longland et al. (2010) reported average proportional losses of total WSC from 3 meadow hays after soaking hay for 1, 3, and 16 h at 8°C, 16°C, and 49°C. Longland et al. (2010) concluded that soaking hays at cold temperatures may result in more moderate losses of WSC compared to soaking in warmer conditions. Warr and Petch (1992) reported a 2 to 4% loss of WSC after soaking hay for a 12 h period and observed that WSC decreased as the duration of the soaking period increased. Conversely, Blackman and Moore-Colyer (1998) found no difference in WSC content in medow hay that had been soaked for 0, 10, or 30 min or steamed at 100°C for 80 min.

Fat and Oils

Fat is digested and absorbed in the small intestine (Hintz and Schryver, 1978; Frape, 2010). Fats consumed can delay or decrease the peak glycemic response in humans by slowing gastric empting (Wolever, 1990; Frape, 2010). Although dietary fat and oils provide twice the energy of grain, some suggest they may be a safer and more efficient energy source than grain. Fat-and-fiber feeds, in which grain and molasses have been replaced with corn oil and various fiberous sources, have been shown to lower the glycemic response in horses by 40% and the insulinemic response by 85% (Williams et al., 2001; Kronfeld et al., 2005). Horses fed these fat-and-fiber feeds maintained greater insulin sensitivity compared to pasture-fed horses (Hoffman et al., 2003a) and horses adapted to high-glycemic meals (Treiber et al., 2005a; Hoffman et al., 2003b).

The type of fat included in the diet also may affect insulin sensitivity. Research performed in rodent species has indirectly and directly linked saturated fat to IR but has indicated that polyunsaturated fatty acids (PUFAs) may be beneficial by increasing insulin sensitivity (Storlien et al., 2000). Correspondingly, research performed in humans has shown that excessive intake of saturated fat worsens insulin sensitivity while consumption of omega-6 type PUFAs improve it (Rivellese and Lilli, 2003). Dietary inclusion of omega-3 type PUFAs have been shown to improve insulin sensitivity in rodents but not humans (Rivellese and Lilli, 2003).

Neeley and Herthel (1997) reported that feeding 237 mL (1 cup) of essential fatty acid supplementation twice daily for 1 month prior to and during a carbohydrate overload prevented laminitis, compared to control horses that received no supplementation. These researchers proposed that decreased inflammation, decreased vasoconstriction, control of hypertension, and

effects on coagulation may have been mechanisms by which essential fatty acid supplementation prevented clinical manifestation of laminitis in the study (Neeley and Herthel, 1997).

Pro- and Prebiotics

Pro- and prebiotics are feed additives that confer benefits upon the wellbeing and health of the host when given in appropriate and regular quantities (Julliand, 2006). These additives may play a role in managing some of the health problems associated with excess carbohydrate consumption. Probiotics are live organisms, such as bacteria or yeast, that are thought to impact animal health by colonizing the gut. Prebiotics are non-living organisms or compounds that can be fermented by microbial populations of the host, which also impact animal health by enhancing microbial populations in the gut (Chaucheyras-Durand and Durand, 2010; Julliand, 2006).

Yeast probiotics have been used in ruminant, monogastric, and equine species (Chaucheyras-Durand and Durand, 2010; Hill et al., 2001). Feeding 20 g of yeast culture (*Saccharomyces cerevisiae* strain Yea- Sacc 1026) to ponies for 2 weeks was reported to increase post-prandial cecal pH for 8 h compared to ponies not supplemented with yeast (More and Newman, 1994). Likewise, feeding 10 g of yeast culture (*Saccharomyces cerevisiae* strain CBS 493.94) for 21 d has been shown to attenuate undesirable changes in the large intestinal microbial population, reducing the decrease in pH, and reducing the accumulation of lactic acid following starch overload in the horse (Medina et al., 2002). Conversely, an *in vitro* experiment in which yeast (*Aspergillus oryzae*) was added to cecal fluid from a horse fed a more moderate amount of starch at 0.07 g/ L resulted in little change in pH after 24 or 48 h, but the pH increased when 0.7 g/L was added (McDaniel et al., 1993).

In addition to changes in cecal pH, changes in bacterial biomass have been observed in horses after being fed yeast (Jouany et al., 2009). Jouany et al.(2009) reported lactobacilli (P = 0.099) and lactic acid utilizing bacteria (P = 0.067) were greater in the cecum but remained similar in the colon of horses fed 10 g of yeast culture (*Saccharomyces cerevisiae* strain CBS 493.94) for 25 days. Although the yeast had an effect on the microbial population, it was concluded that the main positive effect was the increases in enzymatic activities of bacteria involved in the digestion of cellulosic material (Jouany et al., 2009).

Bacterial probiotics have been used with the goal of maintaining an optimal microbial balance in the rumen of cattle (Chaucheyras-Durand and Durand, 2010). Some researchers theorize that maintaining sufficient ruminal populations of lactate utilizing bacteria in cattle by supplementing lactate-producing bacteria may help prevent ruminal acidosis (Krehbiel et al., 2003).In both *in vitro* and *in vivo* studies, administration of the lactate utilizing bacteria *Megasphaera elsidenii* has been shown to prevent lactate accumulation during the transition from low- to high-carbohydrate diets in cattle (Greening et al., 1991; Kung and Hession, 1995; Krehbiel et al., 2003). Swyers et al. (2008) supplemented a target dosage of 10^8 cfu/50 kg of BW daily of lactic acid-producing bacteria in horses for 26 d and reported a limited reduction in risk of acidosis in horses after an abrupt increase in dietary starch (Swyers et al., 2008).

Prebiotics of short chain fructo-oligosaccharides (scFOS) have been reported to affect composition and activity in the hindgut microflora in horses (Respondek, 2006, Respondek, 2007; Berg et al., 2005). This type of prebiotic was shown to be effective in curbing disruptions of the microbial population in the hindgut of the horse after an abrupt increase in starch. The authors suggested that supplementing with 0.05 to 0.07 g/ kg of BW daily may be beneficial in preventing digestive disorders associated with starch intake (Respondek et al., 2008).

In addition, supplementation of scFOS has been shown to improve insulin sensitivity in obese horses (Respondek et al., 2011). After 6 weeks of scFOS supplementation at 45 g daily in obese, insulin resistant horses, a modest improvement on insulin sensitivity was observed (Respondek et al., 2011). Although the mechanisms by which insulin sensitivity was improved after supplementation of scFOS in horses have not been elucidated, one hypothesis is that short chain fatty acids, a fermentation product of scFOS, can have direct effects on insulin signaling mechanisms and indirect effects on the hormones that contribute to regulation of insulin-mediated glucose disposal (Respondek et al., 2011). It has also been speculated that scFOS have anti-inflammatory effects that may contribute to improved insulin sensitivity (Respondek et al., 2011).

Summary

The type and amount of carbohydrate consumed affects how it will be digested, absorbed, and utilized by the horse. Excess consumption of NSC is often a causative factor in the development of acute digestive and metabolic disorders. Complete pre-cecal absorption of NSC prevents rapid microbial fermentation in the hindgut that is often associated with acute digestive disturbances. However, increased pre-cecal absorption is associated with increased glycemic and insulinemic responses, which are, in turn, associated with metabolic disorders. Therefore, the challenge is providing a diet that aids in moderating the glycemic effect that accompanies increased pre-cecal starch absorption while limiting the amount of NSC fermented in the hindgut. Management strategies that prevent overconsumption of NSC should be implemented to avoid the development of EGAD and other metabolic concerns in the horse.

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Table 1.1 Percent nonstructural carbohydrate (NSC) in forage and feedstuff on a dry matter basis.[1]

Item	Samples	Mean	Normal Range	Standard Deviation
			% NSC[2]	
MMG pasture[3]	2,213	12.81	7.85 - 17.77	4.96
Grass pasture[4]	1,888	13.07	6.07 - 20.08	7.01
Grass hay[4]	17,590	12.85	8.04 - 17.66	4.81
Legume hay	39,412	11.00	8.76 - 13.25	2.25
Warm-Season Grass				
Bermuda hay	3,418	13.18	9.05 - 17.31	4.13
Cool-Season Grass				
Oat hay	2,703	22.11	14.89 - 29.34	7.23
Triticale hay	157	21.77	14.87 - 28.68	6.91
Barley hay	91	19.16	12.06 - 26.26	7.10
Rye hay	381	18.23	11.68 - 24.78	6.55
Wheat hay	500	17.63	10.48 - 24.79	7.15
Grain and Byproducts				
Steam flaked corn	85	73.85	70.39 - 77.30	3.46
Molasses	43	59.71	46.85 - 72.57	12.86
Barley	76	59.47	51.91 - 67.02	7.56
Oats	63	48.68	37.24 - 60.13	11.45
Rice bran	60	25.10	16.40 - 33.79	8.70
Beet pulp	192	11.81	3.94 - 19.68	7.87

[1]Data reported from 2011 Feed Composition Library at Dairy One Forage Lab, Ithaca, NY; from accumulated crop years: 05/01/2000 through 04/30/2011; Complete listing at http://www.dairyone.com/Forage/FeedComp/MainLibrary.asp

[2] Nonstructural carbohydrate (NSC) values represent the sum of sugars and fructan.

[3]Mixed mainly grass (MMG) pasture has a mixture of grass and legume.

[4]Grass pasture and grass hay includes random mixtures of grass species.

Table 1.2 Compilation of glycemic and insulinemic responses observed in common horse feeds.[1]

Description of feed item	Number of horses measured	Reference Feed	Base[2]	Glycemic Responses			Insulinemic Responses			Reference
				AUC	SD[3] of AUC	GI	AUC	SD[3] of AUC	II	
Alfalfa	6	Oats (untreated)	DE	4	2.8	15				Rodiek and Stull, 2007
Alfalfa/Oats (untreated)[5]	4	Oats (untreated)	CF;SC	446	248	164	16,888	7,855	158	Vervuert et al., 2009b
Alfalfa + Oats (untreated)[5]	4	Oats (untreated)	CF;SC	407	70	150	12,961	4,874	121	Vervuert et al., 2009b
Barley	6	Oats (untreated)	DE	16	8.3	60				Rodiek and Stull, 2007
Barley (finley ground)	6	Barley (whole)	SC	127	58.5	79	2,923	1,532	143	Vervuert et al., 2007
Barley (popped)	6	Barley (whole)	SC	242	53.5	150	2,173	1,194	106	Vervuert et al., 2007
Barley (rolled)	7	Glucose solution	AC	505	403	62!	3,274	513[4]	42	Jose-Cunilleras et al., 2004
Barley (steamed)	6	Barley (whole)	SC	103	61	64	2,440	1,338	120	Vervuert et al., 2007
Barley (steam-flaked)	6	Barley (whole)	SC	205	62.9	127	3,837	1,440	188	Vervuert et al., 2007
Barley (whole)	6	*	SC	161	87.2	100	2,041	614	100	Vervuert et al., 2007
Beet pulp	6	Oats (untreated)	DE	6	7	24				Rodiek and Stull, 2007
Beet pulp shreds (hydrated)	4	Oats (whole)		3,017	406[4]	72				Groff et al., 2001
Beet pulp shreds + Molasses	4	Oats (whole)		3,834	406[4]	92				Groff et al., 2001
Beet pulp shreds (rinsed)	4	Oats (whole)		1,422	406[4]	34				Groff et al., 2001
Corn	6	Oats (untreated)	DE	25	8.8	92				Rodiek and Stull, 2007

Corn (cracked)	6	*	FW	1,734	191[4]	100				Hoekstra et al., 1999
Corn (cracked)	7	Glucose solution	AC	529	108[4]	65	5,747	1315[4]	73	Jose-Cunilleras et al., 2004
Corn (finely ground)	6	Corn (untreated)	SC	1,527	175	94	4,539	2,456	105	Vervuert et al., 2004
Corn (ground)	6	Corn (cracked)	FW	1,887	191[4]	109				Hoekstra et al., 1999
Corn (micronized)	6	Corn (untreated)	SC	1,505	101	92	5,129	2,415	118	Vervuert et al., 2004
Corn (popped)	3	Corn (untreated)	SC	1,691	283	104	3,511	1,929	81	Vervuert et al., 2004
Corn (steamed)	6	Corn (untreated)	SC	1,480	111	91	4,291	2,271	99	Vervuert et al., 2004
Corn (steam-flaked)	4	Corn (untreated)	SC	1,513	48	93	4,373	1,796	101	Vervuert et al., 2004
Corn (steam-flaked)	6	Corn (cracked)	FW	2,500	191[4]	144				Hoekstra et al., 1999
Corn (untreated)	6	*	SC	1,630	170	100	4,334	2,129	100	Vervuert et al., 2004
Glucose solution	7	*	AC	818	170[4]	100	7,860	2342[4]	100	Jose-Cunilleras et al., 2004
Oats (crimped)	6	Oats (untreated)	DE	20	11.9	74				Rodiek and Stull, 2007
Oats (finely ground)	6	Oats (untreated)	SC	1,697	318	102	9,946	11,415	164	Vervuert et al., 2003
Oat groats	7	Glucose solution	AC	510	38[4]	62	5,275	1526[4]	67	Jose-Cunilleras et al., 2004
Oats (popped)	3	Oats (untreated)	SC	1,576	186	95	4,998	3,166	83	Vervuert et al., 2003
Oats (steamed)	6	Oats (untreated)	SC	1,638	253	99	7,641	5,930	126	Vervuert et al., 2003
Oats (steam-flaked)	6	Oats (untreated)	SC	1,549	67	93	4,662	1,351	77	Vervuert et al., 2003
Oats (untreated)	6	*	SC	1,659	254	100	6,052	4,623	100	Vervuert et al., 2003
Oats (untreated)	6	*	DE	27	18	100				Rodiek and Stull, 2007

Feed	n	Reference feed	Base	AUC	SD	GI	AUC	SD	II	Citation
Oats (untreated)[5]	4	*	SC	272	76	100	10,686	3,506	100	Vervuert et al., 2009b
Oats (untreated)/Alfalfa[5]	4	Oats (untreated)	SC; CF	431	38	158	17,140	11,641	160	Vervuert et al., 2009b
Oats (whole)	4	*		4,175	406[4]	100				Groff et al., 2001
Rice bran	6	Oats (untreated)	DE	4	4.3	16				Rodiek and Stull, 2007
Soybean hulls	6	Oats (untreated)	DE	2	2.1	6				Rodiek and Stull, 2007
Sweet feed	6	Oats (untreated)	DE	29	15.7	106				Rodiek and Stull, 2007
Wheat bran	6	Oats (untreated)	DE	15	7.8	57				Rodiek and Stull, 2007

[1]Glycemic index (GI) values were calculated from glucose area under the curve (AUC) from test feed of /glucose AUC from reference feed (*) that was set to 100%. Insulinemic index (II) values were calculated from insulin AUC from test feed of /insulin AUC from reference feed (*).

[2]Base refers to the basis on which the amount of feed offered was calculated: starch content (SC), feed weight (FW), available carbohydrate (AC), or crude fiber (CE).

[3]Standard devation (SD) of the AUC mean.

[4]Values represent standard error of the AUC mean.

[5]Feeds were offered in 3 different ways: alfalfa followed by oats (A/O), oats followed by alfalfa (O/A), or a mixture of alfalfa and oats (A + O).

Table 1.3 Variables affecting glycemic and insulinemic responses observed in the horse.

	Variable		Research cited that reported:			
			Effects on glycemic response		Effects on insulinemic response	
			Increase[1]	Decrease[2]	Increase[3]	Decrease[4]
Physiological state	Early lactation			Hoffman et al., 2003a		Hoffman et al., 2003a
	Late Gestation		Hoffman et al., 2003a; George et al., 2011		Hoffman et al., 2003a; George et al., 2011	
	Disease	OCD	Ralston, 1996; Pagan et al., 2001; Ralston et al., 1988;		Ralston et al., 1988; Ralston, 1996; Pagan et al., 2001	
		PSSM		Benders et al., 2005; De La Corte et al., 1999a; De La Corte et al., 1999b		De La Corte et al., 1999a; De La Corte et al., 1999b
		Acute abdominal disorder	Hassel et al., 2009; Hollis et al., 2007			
	Previously exposed to laminitis		Jeffcot et al., 1986		Treiber et al., 2005b; Jeffcot et al., 1986; Bailey et al., 2007	
	Obesity		Hoffman et al., 2003b; Jeffcot et al., 1986; Powell et al., 2002; Vick et al., 2007		Jeffcot et al., 1986; Hoffman et al., 2003b; Vick et al., 2007; Powell et al., 2002	
	Age			Murphy et al., 1997	Nielsen et al., 2010	
Breed	Pony		Jeffcot et al., 1986		Jeffcot et al., 1986	Rijnen et al., 2003?
Exercise			De La Corte et al., 1999b	Treiber et al., 2010; Powell et al., 2002; Quinn et al., 2008; Pagan and Harris, 1999; Lawrence et al., 1993	De La Corte et al., 1999b	Powell et al., 2002; Quinn et al., 2008; Pagan and Harris, 1999; Lawrence et al., 1993
Seasonal influence	Spring		Treiber et al., 2008		Borer et al., 2010; Treiber et al., 2008	
	Summer				Borer et al., 2010	

[1]Represents an increase or a greater glycemic response observed in relation to variable.
[2]Represents a decrease in glycemic response or greater blood glucose disappearance observed in relation to variable.
[3]Represents an increase or greater insulenimic response or a decrease in insulin sensitivity in relation to variable.
[4]Represents a decrease in insulenimic response or an increase in insulin sensitivity in relation to variable.

Table 1.4 Dietary variables that impact glycemic and insulinemic responses observed in the horse.

Dietary Variable	Increase[1]	Decrease[2]	Increase[3]	Decrease[4]
		Research cited that reported:		
Thermal processing	Hoekstra et al., 1999; Nielsen et al., 2010; Vervuert et al., 2007		Vervuert et al., 2007	
Pelleted form of concentrate	Healy et al., 1995; Harbour et al., 2003; Gordon et al., 2008		Gordan et al., 2008	
Addition of fat		Pagan et al., 1999		
Increase of fructan content			Bailey et al., 2007	
Increase of starch and sugar content	George et al., 2011; George et al., 2009; Staniar et al., 2007; Vervuert et al., 2009a	Hoffman et al., 2003a; Zeyner et al., 2002; Pagan and Harris, 1999; Lawrence et al., 1993	Quinn et al., 2008; Hoffman et al., 2003b; Trieber et al., 2005a; George et al., 2011; George et al., 2009; Staniar et al., 2007; Vervuert et al., 2009a; Ralston, 1992	Pagan and Harris, 1999;
Increase of fat and Fiber content	Zeyner et al., 2002	George et al., 2011		Quinn et al., 2008; Hoffman et al., 2003b; Trieber et al., 2005a; George et al., 2011
Short chain fructo-oligosaccharides				Respondek et al., 2011
Preparation method of rinsing with water		Groff et al., 2001		
Preparation method of soaking with water		Contrell et al., 2005		Contrell et al., 2005
Acclimation to meal feeding of starch and sugar		Hoffman et al., 2003a	Treiber et al., 2005a	
Fasting or restriction of feed	Powell et al., 2000	Jose-Cunilleras et al., 2002; Alexander, 1955; Ralston et al., 1979; Pagan and Harris, 1999	Powell et al., 2000	Ralston et al., 1979; Jose-Cunilleras et al., 2002; Pagan and Harris, 1999
Meal size	Pagan et al.,1999; Vervuert et al., 2009a		Vervuert et al., 2009a	
Addition of molasses	Groff et al., 2001			

[1]Represents an increase or a greater glycemic response observed in relation to dietary variable.
[2]Represents a decrease in glycemic response or greater blood glucose disappearance observed in relation to dietary variable.
[3]Represents an increase or greater insulenimic response or a decrease in insulin sensitivity in relation to dietary variable.
[4]Represents a decrease in insulenimic response or an increase in insulin sensitivity in relation to dietary variable.

Chapter 2 - Assessment of Different Methodologies for Measuring Glucose Concentrations in the Horse

Abstract

This study compared equine glucose concentrations obtained by a One Touch® Ultra® (OTU; Life Scan, Milpitas, CA) handheld glucometer using whole blood or plasma, a YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI; YSI Inc., Yellow Springs, OH) benchtop glucometer using whole blood or plasma, and a SEVEN® (SEVEN; Dexcom, San Diego, CA) continuous glucose monitor (CGM) that measured glucose in interstitial fluid to readings obtained by a standard laboratory Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire, UK) glucose analyzer utilizing plasma. In addition, glucose concentrations obtained by the CGM were compared to those obtained by the handheld glucometer using whole blood or plasma. Six 4-year-old American Quarter horse geldings with a mean body weight of 488 ± 20 kg were used. For each blood collection interval, glucose was measured by the OTU and the YSI in whole blood; the OTU, YSI, and AA in plasma; and the SEVEN in interstitial fluid. Ten mL of whole blood was collected in vacuum tubes via jugular catheter on 2 consecutive days, every 30 minutes, for 4 hours. The first 2 collections were 30 min apart, taken pre-prandially, and considered baseline measurements. Then horses were fed 1.13 kg of sweet feed concentrate (Sweet Stuff 12, Cargill Animal Nutrition, Minneapolis, MN). Immediately following each collection, whole blood was utilized to obtain glucose readings using both the OTU and the YSI. After centrifugation ($876 \times$ g for 10 min) of remaining blood, 10 µL of plasma was used to

measure glucose concentrations with the OTU. Remaining plasma was separated, frozen at -21°C, and later thawed for glucose assays with the YSI and AA. The SEVEN glucose values were manually recorded from the SEVEN receivers at the time of each blood collection. The YSI and the SEVEN presented technical challenges during the experiment. These difficulties resulted in missing data points, but these did not effect statistical analysis. Of the methods tested, glucose concentrations derived from plasma using the YSI generated the most readings within 20% of those generated by AA. All methods detected a postprandial increase and subsequent decrease in glucose concentrations. Of the fixed random regression models with an autoregressive parameter that were fitted to all predictor methods, only the YSI used with plasma was unbiased in that the intercept was not different from 0 (P = 0.0824) and the slope was not different from 1 (P = 0.0805). With AA as the response variable, the YSI used with plasma had the strongest concordance coefficient (0.77) while the SEVEN had the weakest (0.39). The SEVEN had a stronger concordance coefficient with the OTU used with plasma (0.59) compared to the OTU used with whole blood (0.47). The OTU used with whole blood and the YSI used with plasma had diffmeans closest to 0 (0.021 and -0.096, respectively) when the response variable was the AA. When the response variable was OTU, the diffmean of the SEVEN was closer to 0 when plasma was used in the OTU (0.934) rather than whole blood (3.25). The glucometers studied here may provide convenient and methods of detecting post-prandial increases and decreases in glucose concentrations, but if exact glucose values are required, the YSI used with plasma will likely provide the most reliable data. Technical difficulties of the SEVEN need to be overcome in order for it to be a reliable source for continuous glucose monitoring in the horse.

Key Words: equine, continuous glucose monitor, glucometer, glucose

Introduction

Interest in using point-of-care glucometers in the horse has increased because of the need to closely monitor glucose for those in critical care or for those affected by metabolic disorders (Hollis et al., 2008; Hollis et al., 2007; Russell et al., 2007). Handheld glucometers are convenient because they are portable, use a minimal amount of blood, provide a quick glucose reading, and often have an option to download data onto computers. Continuous glucose monitors (CGM) provide a minimally invasive way to carry out nearly-continuous monitoring of glucose for use in the horse by using a sensor inserted in adipose tissue that measures glucose in interstitial fluid (Johnson et al., 2011; Wiedmeyer et al., 2003).

This study compared equine glucose concentrations obtained by a handheld glucometer using whole blood or plasma, a benchtop glucometer using whole blood or plasma, and a CGM that measured glucose in interstitial fluid to readings obtained by a standard laboratory glucose analyzer utilizing plasma. In addition, glucose concentrations obtained by the CGM were compared to those obtained by the handheld glucometer using whole blood or plasma. The goal of the study was to quantifiably characterize system performance, assess potential for error, and identify method-to-method differences.

Materials and Methods

Horses

All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University. Six 4-year-old American Quarter horse geldings with a mean body weight of 488 ± 20 kg were used. Throughout the experiment, horses were housed in individual 3.05 m X 3.66 m stalls. Native prairie hay was provided *ad libitum* along with 1.13 kg sweet feed concentrate (Sweet Stuff 12, Cargill Animal Nutrition, Minneapolis, MN; Table 2.1) twice daily.

Instrumentation

Handheld Glucometer

The One Touch[®] Ultra[®] (OTU; Life Scan, Milpitas, CA) handheld glucometer was used in the current experiment. It was stored and maintained according to the manufacturer's specifications. To obtain a glucose measurement, approximately 10 µl (1 µl minimum) of blood was applied to a channel at the edge of a test strip where the blood or plasma diffused through a membrane and glucose was then measured through an electrochemical reaction that utilized the enzyme glucose oxidase. The OTU had a glucose measurement range of 1.11 to 33.33 mM according to the manufacturer. The OTU was calibrated with the control solution provided by the manufacturer before using it for the first time each day and each time a new vial of test strips was utilized.

Continuous Glucose Monitor

Each horse was equipped with a SEVEN® CGM (SEVEN; Dexcom, San Diego, CA). The SEVEN sensor was inserted into subcutaneous tissue overlying the posterior dorsum lateral to the dock of the horse after the area was shaved and cleaned with rubbing alcohol. The sensor was adhered to the skin using the adhesive pad that was pre-attached to the sensor and provided by the manufacturer. Additionally, a cyanoacrylate adhesive was applied to the pad as reinforcement. The SEVEN sensor obtained an interstitial glucose reading every 5 min through an electrochemical reaction that used the enzyme glucose oxidase and transmitted this information to a receiver secured onto the tail of the horse. According to the manufacturer, the SEVEN had an effective glucose measurement range of 2.22 to 22.20 mM. Sensors were calibrated according to the manufacturer's specifications utilizing an OTU handheld glucometer with whole blood. The SEVEN receiver was calibrated every 12 h and each time new sensors were installed.

The SEVEN system was designed for use in humans, and, to the authors' knowledge, had not previously been used in horses. Thus, 2 preliminary experiments were conducted to evaluate the performance of the SEVEN in the equine. In the first preliminary experiment, sensors were inserted into the adipose tissue overlying the latissimus dorsi, slightly posterior to the anterior end of the scapula. Receivers were attached to halters on the horses' heads to ensure they were kept within the manufacturer's suggested range (1.8 m) of the sensors. In this experiment, there were several hours worth of missing data points and 1 of the sensors ceased proper function. Dexcom technical support representatives suggested the metal buckles on the halters may have been interfering with proper function of the SEVEN. Because of the difficulties experienced, a

second preliminary experiment was conducted in which 1 horse was equipped with sensors placed subcutaneously in the adipose tissue overlying the crest of the neck, poll, olecranon, and the posterior dorsum lateral to the dock of the horse. Receivers were braided into the mane and tail or secured to the girth of the horse. The SEVEN had the fewest errors when located on the posterior dorsum lateral to the dock of the horse and when the receiver was braided into the tail. Therefore, this placement of the sensor and receiver was chosen for the current experiment.

Benchtop Glucometer

The YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI; YSI Inc., Yellow Springs, OH) was used to measure glucose in whole blood and in plasma. Approximately 25 µL of whole blood or plasma was aspirated, automatically diluted with 475 µL of buffer solution, and dialyzed before the total glucose in the aliquot was measured through an electrochemical reaction using glucose oxidase. The YSI linearity was 0 to 55.5 mM and the machine self calibrated every 15 min or every 5 measurements. The YSI produced an intrassay CV of 0.12% for whole blood and 0.03% for plasma samples.

Auto Analyzer

The Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire, UK) was used as the reference method for this experiment. Approximately 6.7 µL of plasma was aspirated and automatically diluted in saline (0.9% NaCl), dialyzed into the glucose oxidase/phosphate buffer reagent, and total glucose in the aliquot was measured through a colorimetric reaction using glucose oxidase and peroxidase. The AA linearity was 0 to 12 mM and it self-calibrated using 5 standards before measuring the group of plasma samples (120 samples). The AA produced an intrassay CV of 0.35% for plasma samples.

Blood Collection and Analysis

For each blood collection interval, glucose was measured by the OTU and the YSI in whole blood; the OTU, YSI, and AA in plasma; and the SEVEN in interstitial fluid. An indwelling 14 gauge × 13 cm jugular catheter was placed in all horses on the day prior to the experiment. During the collection periods, 10 mL of whole blood was collected in vacuum tubes via jugular catheter on 2 consecutive days, every 30 minutes, for 4 hours. This regimen produced a total of 120 observations. The first 2 collections on each day were taken pre-prandially and considered baseline measurements. Then horses were fed 1.13 kg of sweet feed concentrate, thus enabling sampling over a range of glucose concentrations. Immediately following each collection, approximately 10 µL and 25 µL of blood were utilized to obtain glucose readings in whole blood with both the OTU and the YSI, respectively. Remaining blood was centrifuged at 876 × g for 10 min. Promptly after centrifugation, 10 µL of plasma was used to measure glucose concentrations with the OTU. Remaining plasma was separated, frozen at -21°C, and later thawed for glucose assays with the YSI and AA. The SEVEN glucose values were manually recorded from the SEVEN receivers at the time of each blood collection interval.

Statistics

Three techniques were performed using SAS® version 9.2.1 software (SAS Institute, 2007) to evaluate how accurately each method of glucose measurement statistically reproduced the glucose values produced by the AA (considered the reference technique in this study). Because the SEVEN system was calibrated by the OTU, the OTU also was assessed for

reproducibility of glucose values from both plasma and whole blood in which the SEVEN system was the response variable (X) using the same 3 techniques.

First, a random coefficients regression model was fitted (Littell et al., 1996) to account for inherent random variability from different days and different horses, in which AA was the response variable (Y) and all other methods were the predictor variable (X). The parameters estimated for each model included overall (fixed) intercept and slope, variance components (for random intercept, random slope, residual, and covariance component between random intercept and random slope), and an autoregressive parameter which accounted for serial correlation within each horse-day combination. All models were fitted using the SAS® MIXED procedure with REML estimation, and normality of residuals was checked using the SAS® UNIVARIATE procedure (SAS Institute, 2007). For reproducibility assessment, t-tests were performed to make sure the model was unbiased (that the intercept was not different from 0 and the slope was not different from 1 for each model). Also, 95% confidence intervals were calculated for the regression parameters and are reported.

To further compare the regression models, the Akaike's Information Criterion (AIC) was used for overall comparison of all predictor methods by comparing unbiased regression models with complex variance-covariance structures (Burnham and Anderson, 1998; Littell et al., 1996). The method with the smallest AIC value best predicts the AA values. Predictors with AIC values ± 2 of the minimum AIC are considered comparable to the predictor with the smallest AIC. Predictor methods with AIC differences ≥ 7 from the smallest AIC are considered different in their ability to predict glucose values obtained with the AA (Burnham and Anderson, 1998).

The second statistical technique used was Lin's Concordance Coefficient (Lin, 1989) which evaluates both the strength of the linear association between 2 variables (X and Y) and the

degree of departure from the 45° line through the origin. This technique is reported with a 95%

confidence interval for each predictor method and results of this confidence interval should

contain 1 if there is reproducibility between 2 methods. Concordance coefficients were

performed on both original observations and on residuals + estimated means from the SAS®

MIXED procedure to account for variability due to horse-day combinations. There were some

differences between concordance coefficients based on raw data versus those based on residuals,

so concordance coefficients based on residuals are reported.

Lastly, the third statistical technique used was Bland-Altman calculations (Bland and

Altman, 1986). These calculations provided the mean difference between measurements of 2

methods, the number and percent of observed differences outside the 95% prediction interval

based on a t-distribution, and the standard deviation of differences. The mean difference in this

study is defined as the response variables glucose mean minus the mean glucose of a predictor

method. Positive mean differences signify that, on average, the predictor method had lower

glucose concentrations than the response variable, whereas negative mean differences indicate

the predictor method generated greater glucose concentrations than the response varible. The

acceptability criteria for the number and percent of observed differences outside the 95%

prediction interval requires differences to be approximately normal with only 5% of values

outside the prediction interval. Bland-Altman calculations were performed on both original

observations and on residuals plus estimated means from the SAS® MIXED procedure (SAS

Institute, 2007) to account for variability due to horse-day combinations. Because results

between the original and residuals plus estimated means were not different (the horse-day

variance component was typically very small relative to residual variability), Bland-Altman

calculations reported here are from the original data.

There were some missing measurements from the SEVEN and the YSI using whole blood. Although these 2 predictor methods had smaller sample sizes as a result, removing these data points had no impact on the validity of the statistical analyses.

Results

Technical Challenges

The YSI and the SEVEN presented technical challenges during the experiment. During the glucose analysis in whole blood, air bubbles in samples caused the YSI to generate unrealistic values. These obviously inaccurate values were removed from the data set and resulted in 33 missing data points. The SEVEN had 12 missing data points reported that corresponded with blood collections. However, it should be noted that the SEVEN is designed to collect glucose information every 5 min, thus there were far more missing data points that occurred between blood collections.

Reproducibility

Descriptive Statistics

Of the methods tested, plasma glucose concentrations derived from the YSI generated the most readings within 20% of those generated by AA. The OTU used with whole blood was the only other method that had greater than 40% of values generated that fell within 20% of those

obtained with the AA (Table 2.2). However, all methods detected a postprandial increase in glucose concentrations, which was eventually followed by a decrease (Figure 2.1).

Random Regression Models

Of the fixed random regression models with an autoregressive parameter that were fitted to all predictor methods, only the YSI used with plasma was unbiased in that the intercept was not different from 0 ($P = 0.0824$) and the slope was not different from 1 ($P = 0.0805$; Table 2.3). This can be visually confirmed in Figure 2.2, which provides visual comparison of the raw glucose concentrations obtained via each method compared to those obtained with the AA. Glucose measurements obtained by the OTU using plasma or whole blood, when compared to the interstitial glucose values obtained with the SEVEN, were both biased. This can be visually confirmed in Figure 2.3. Although AIC values are reported, the AIC only works if the random regression model is unbiased, so comparison of all predictor methods could not be performed using the AIC.

Lin's Concordance Coefficient

With AA as the response variable, the YSI used with plasma had the strongest concordance coefficient (0.77) while the SEVEN had the weakest (0.39). The SEVEN had a stronger concordance coefficient with the OTU used with plasma (0.59) compared to the OTU used with whole blood (0.47). None of the predictor methods contained 1 in the 95% confidence intervals, although the YSI used with plasma was closest (Table 2.4).

Bland-Altman Calculations

In the Bland-Altman calculations, no predictor method had greater than 4.5% of the diffmean (response variable mean - the mean glucose of a predictor method) outside the prediction interval, thus all diffmeans were approximately normal. The OTU used with whole

blood and the YSI used with plasma had diffmeans closest to 0 (0.021 and -0.096, respectively) when the response variable was glucose measurements generated by the AA. All methods using glucose from whole blood and interstitial fluid had diffmeans that were positive, meaning they generated lower glucose concentrations than the AA (Table 2.5; Figure 2.4). In contrast, all methods using glucose from plasma had negative diffmeans which indicate the mean glucose concentrations from these methods were, on average, higher than those obtained with the AA. When the response variable was glucose measurements obtained with the OTU, the diffmean of the SEVEN used with interstitial fluid was closer to 0 when plasma was used in the OTU (0.934) rather than whole blood (3.25; Table 2.5; Figure 2.5).

Bland-Altman analysis showed the SEVEN produced glucose concentrations that were, on average, greater than of the OTU when used with blood or plasma. The glucose measured from the OTU using whole blood had the smallest diffmeans and thus had the better reproducibility according to Bland Altman calculations.

Discussion

Comparisons of Measurement Methods

The OTU, when used with plasma, had the highest mean glucose measurements of all methods tested, and was greater than the AA (diffmean = -2.19). Similarly, Hollis et al. (2008) reported glucose measured in equine plasma with a handheld glucometer yielded greater values than glucose measurements obtained from a plasma reference technique (diffmean = -0.60 ± 0.73). Mean glucose values measured with the OTU using whole blood in this study was slightly less than the plasma glucose measurements from the AA (diffmean of 0.02 ± 1.18 mM). This

agrees with Hollis et al. (2008) who reported glucose measured from equine whole blood with a handheld glucometer was lesser than their plasma reference technique (diffmean = 2.48 ± 1.91 mM). However, Russell et al. (2007) used a handheld glucometer designed for humans and measured glucose from whole blood on neonatal foals in an intensive care unit reported less than ideal agreement in plasma glucose values generated with a laboratory standard, with glucometer readings greater than the reference technique which utilized plasma (diffmean = 1.11 ± 0.89 mM). It has been shown that glucose concentrations generated from plasma are typically greater than glucose values measured in whole blood (Holtkamp et al., 1975). Dilution effect due to size and number of erythrocytes (Kilpatrick et al., 1993) or the fact that the rate of glucose consumption by red blood cells in whole blood samples has been reported to be 5% to 10% per hour (Stockham, 1995) may explain why glucose measured in plasma is often greater than glucose measured in whole blood, which was also observed in this experiment.

Glucose measurements obtained with the OTU in plasma had better reproducibility with readings obtained in plasma by the AA than were readings obtained with the OTU when using whole blood. This agrees with Hollis et al. (2008) who observed that glucose measurements obtained with a handheld glucometer when used with plasma were more highly correlated to a laboratory standard than when used with whole blood. Using repeated measures analysis with a mixed model, researchers have reported a highly significant correlation between glucose measurements obtained by the OTU using whole blood and glucose measurements obtained by the AA using plasma ($R^2 = 0.48$, $P < 0.0001$; Gunkel et al., 2009). A later analysis of the same data using the random coefficients model used in the present study was conducted and resulted in a bias intercept estimate of 2.93 and a slope estimate of 0.52 ($P < 0.05$). Ideal reproducibility would require an intercept not different than 0 and a slope not different than 1. Although the

earlier study had intercept estimates slightly closer to 0 and slope estimates slightly closer to 1

than the present study, the data collected in both studies agree that readings obtained from AA

are not reproducible using the OTU.

Hematocrit levels can affect glucose values obtained by glucometers (Tang et al., 2000a;

Kilpatrick et al., 1993; Wiener, 1991). Hematocrit levels may influence glucometer readings by

changing the viscosity of the blood sample, thus impeding plasma from reaching the reaction

surface of the test strip, and therefore affecting the diffusion kinetics or displacing plasma

volume (Louie et al., 2000; Dacombe et al., 1981). The manufacturer of the OTU states that a

hematocrit level above 55% or below 30% can cause inaccurate results. Because the normal

resting hematocrit range is reported to be 32 to 55% and 24 to 44% for healthy light horses and

heavy horses, respectively (Schalm, 1961), a glucometer used in horses should ideally be

accurate at 24 to 55% hematocrit. The OTU hematocrit range is similar to the normal

hematocrit range in horses. Hackett and McCue (2010) reported that glucose measurements

were underestimated with hematocrit greater than 65% in horses admitted to a hospital or

reproductive laboratory when using a veterinary handheld glucometer. However, Hollis et al.

(2008) did not experience any hematocrit effect on equine glucose measurements using a

glucometer designed for human use. Although hematocrit levels were not monitored in this

study, it is likely that hematocrit levels were not increased by stress because animals appeared

calm during the study.

In addition to hematocrit levels, blood oxygen tension levels (Tang et al., 2001; Louie et

al., 2000; Kilpatrick et al., 1994), blood pH (Tang et al., 2000b; Kilpatrick et al., 1994), and

variation between lots of glucometer test strips (Kimberly et al., 2006; Kristensen et al., 2005)

have been reported to influence glucometer measurements in glucose oxidase-based devices.

Although these factors were not monitored in the current study, these are all possible sources of error that could have affected the glucose values obtained. With the exception of blood pH and variation between lots of glucometer test strips, these factors are most likely to affect methods measuring whole blood rather than plasma due to erythrocytes.

Time differences for the equilibrium of glucose between blood and interstitial fluid have been reported in which glucose concentrations in interstitial fluid tends to lag behind glucose concentrations in blood from 4 to 10 min in humans (Boyne et al., 2003, Kulcu et al, 2003; Regittnig et al., 2003). Additionally, others have reported interstitial fluid time lags due CGM sensor processing time as a substantial source of error (Kovatchev et al., 2009; Boyne et al., 2003). Johnson et al. (2011) reported a 15-min lag time of glucose measured from a CGM compared to glucose measured from a handheld glucometer in horses and discovered that fitting a 15 min lag time into a regression line made the results unbiased. However, Kamath et al. (2009) found that the SEVEN had an average sensor lag time of 5.7 ± 0.5 min and reported that accuracy was maintained during periods of rapidly changing glucose concentrations in humans. Although not measured here, it is possible that there was a delay between interstitial glucose values and blood glucose concentrations in the current study.

Technical Challenges

The technical challenges experienced with the YSI glucose analysis in whole blood easily could have been avoided had the air bubbles been removed prior to analyzing samples. The technical difficulties that were encountered with the SEVEN, however, are not so easily addressed, and will need to be overcome in order for it to be a reliable source for continuous glucose monitoring in the horse. When the sensors were removed (due to sensor failure or at the completion of the experiment), some were bent at an acute angle which may have impeded

sensor function. Although this occurred in both the current and preliminary experiments, it occurred most often in the preliminary experiment in which the sensor was placed into the adipose tissue overlying the latissimus dorsi, slightly posterior to the anterior end of the scapula. Because the latissimus dorsi aids in movement of the humerus, contraction of this large muscle may have applied enough mechanical force to physically bend the sensor probe. Although the SEVEN sensor was tested in numerous locations, perhaps another sensor location would enhance the performance of the SEVEN. Wiedmeyer et al. (2003) reported a CGM device with a sensor located in subcutaneous tissue overlying the masseter muscle worked well for a 24 h period. This location was not tested in the current study and would perhaps be another option, although it would have had to function well for 2 d to be sufficient.

Missing data from the SEVEN in the present study may be attributed to tissue reactions of biomaterial-mediated inflammation, which have an effect on the function of sensors (Bridges and García, 2008; Dungel et al., 2008; Onuki et al., 2008). As well, the SEVEN has a working range of 2.22 to 22.20 mM glucose. Therefore readings of 2.16 mM, which were frequently obtained, likely represent the minimum glucose value the SEVEN will provide, despite the fact that actual glucose concentrations may have been even lower. Even so, the lowest glucose concentration observed using the AA (3.6 mM) was greater than the minimum glucose concentration using the SEVEN. As well, the SEVEN had a lesser glucose mean than the AA (4.89 mM and 5.82 mM, respectively) and a positive Bland-Altman difference mean (0.957), which would indicate it consistently measured glucose at a lesser concentration than the AA.

Furthermore, the ability of a device to measure a broader working range of glucose concentrations would be better suited for use in the horse. The SEVEN's working range of 2.22 to 22.20 mM is insufficient for monitoring and diagnosing a hypoglycemic horse in a clinical or

70

experimental setting because the point at which prompt clinical attention is needed is 0.66 to 1.12 mM for hypoglycemia (Lyle and Keen, 2010; Mayhew, 2009) in horses. Although 22.20 mM will capture most measurements of hyperglycemia ($\geq$ 7.3 mM) and extreme hyperglycemia (> 10 mM; Hollis et al., 2007), glucose values greater than 22.20 have been reported in the horse (Hassel et al., 2009; Hollis et al., 2007).

The International Organization for Standardization established criteria for the accuracy of glucose monitoring systems that requires 95% of glucose results to fall within $\pm$ 0.83 mM of the reference technique when glucose values are < 4.2 mM and within $\pm$ 20% at glucose concentrations $\geq$ 4.2 mM (IOS, 2003). Although the YSI used with plasma and the OTU used with whole blood were the closest to reference values obtained by the AA, the ISO criteria was not met by any of the methods in this study. However, these criteria were made in reference to human glucose concentrations. This analysis was also performed with raw data that does not account for possible autocorrelation between concentrations, which must be taken into consideration.

Conclusion

If a precise and accurate glucose measurement is needed in horses, laboratory reference techniques will provide the most reliable data. However, if the goal is to examine and investigate patterns of glucose appearance and disappearance, the glucometers studied here may provide convenient and adequate methods of measuring glucose, as all were able to detect post-prandial increases and decreases in glucose concentrations. When glucose measured using the SEVEN in interstitial fluid was compared to glucose measured using the OTU in plasma or

whole blood, glucose measurements from plasma rather than whole blood had better

reproducibility when analyzed by Lin's Concordance Coefficient but whole blood rather than

plasma had better reproducibility when analyzed by Bland Altman calculations. Because

measuring glucose using plasma requires more steps and introduces a greater potential for error

than when using whole blood, it may be prudent to use whole blood to calibrate the SEVEN.

Although the SEVEN could be a resourceful instrument for collecting nearly continuous data for

the researcher and clinician, the technical difficulties of the SEVEN need to be overcome in

order for it to be a reliable source for continuous glucose monitoring in the horse.

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8:172-175.

Table 2.1 Nutrient Analysis of Sweet Feed.[1]

Item (%)	
Crude protein, minimum	12.0
Crude fat, minimum	3.0
Crude fiber, maximum	15.0
Ca, minimum	1.25
Ca, maximum	1.75
P, minimum	0.45
Molasses	9.0

[1]Sweet Stuff 12, Cargill Animal Nutrition, Minneapolis, MN.

Table 2.2 Descriptive statistics of glucose data collected from each measurement method tested.[1,2]

	AA	OTU		YSI		SEVEN
	Plasma	**Blood**	**Plasma**	**Blood**	**Plasma**	**Interstitial Fluid**
Mean (mM)	5.82	5.8	8.02	4.15	5.92	4.89
Range (mM)	3.8 - 8.8	1.39 - 9.11	4.0 - 12.89	1.47 - 6.87	2.63 - 9.22	2.17 - 11.11
Percent of values within 20 % of AA	N/A	70.8	21.7	21.7	84.2	37.5
Missing data points (out of 120)	0	0	0	33	0	12

[1]Calculations based on raw data and therefore do not account for any autocorrelation.

[2]Instrumentation: Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire, UK), One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA), YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI; YSI Inc, Yellow Springs, OH), and SEVEN® continuous glucose monitoring system (SEVEN; Dexcom, San Diego, CA).

Table 2.3 Random coefficients regression models comparing glucose measurements from each method tested.[1,2]

		AA[3]					OTU[4]	
		Plasma					Blood	Plasma
		OTU		YSI		SEVEN	SEVEN	
		Blood	Plasma	Blood	Plasma	Interstitial Fluid	Interstitial Fluid	
Intercept	**β**	3.71	2.83	4.35	1.41	4.6	3.97	5.7
	SE	0.45	0.43	0.65	0.72	0.38	0.45	0.49
	P-value (Ho: β = 0)	$P < 0.0001$	$P = 0.0001$	$P = 0.0002$	$P = 0.0824$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	95% Confidence Interval	(2.74, 4.68)	(1.81, 3.85)	(2.81, 5.89)	(-0.25, 3.07)	(3.72, 5.48)	(3.07, 4.87)	(4.68, 6.72)
Slope	**β**	0.35	0.37	0.32	0.74	0.25	0.37	0.43
	SE	0.08	0.06	0.16	0.13	0.1	0.09	0.1
	P-value (Ho: β = 1)	$P < 0.0001$	$P < 0.0001$	$P = 0.0001$	$P = 0.0805$	$P = 0.0001$	$P < 0.0001$	$P = 0.0003$
	95% Confidence Interval	(0.17, 0.53)	(0.23, 0.51)	(-0.05, 0.69)	(0.44, 1.04)	(0.01, 0.49)	(0.19, 0.55)	(0.20, 0.66)
	AIC	295.9	265.0	251.7	251.2	284.9	367.5	347.9

[1]Regression coefficients with standard error (SE), P-values, and 95% confidence interval for slope and intercept. In addition, Akaike's Information Criterion (AIC) for random-coefficients regression models were generated for each method.

[2]Instrumentation: Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire, UK), One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA), YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI; YSI Inc, Yellow Springs, OH), and SEVEN® continuous glucose monitoring system (SEVEN; Dexcom, San Diego, CA).

[3]Glucose measured with the OTU, YSI, and SEVEN (predictor variables) from blood, plasma, or interstitial fluids were compared to glucose values obtained with the AA (response variable) from plasma.

[4]Glucose measured with the SEVEN (predictor variable) in interstitial fluid was compared to values obtained with the OTU (response variable) using plasma and whole blood.

Table 2.4 Lin's Concordance Coefficient comparisons of glucose measurements for each method tested.[1,2]

	AA[3] Plasma					OTU[4] Blood Plasma	
	OTU		YSI		SEVEN	SEVEN	
	Blood	Plasma	Blood	Plasma	Interstitial Fluid	Interstitial Fluid	
Observations (n)	120	120	87	120	108	108	108
Lower CL	0.46	0.55	0.22	0.69	0.23	0.32	0.45
Concordance coefficient	0.58	0.64	0.41	0.77	0.39	0.47	0.59
Upper CL	0.68	0.72	0.57	0.84	0.52	0.6	0.7

[1] Lin's Concordance Coefficient is reported with a 95% confidence interval for each predictor method and results of this confidence interval should contain 1 if there is reproducibility between methods.

[2] Instrumentation: Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire, UK), One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA), YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI; YSI Inc, Yellow Springs, OH), and SEVEN® continuous glucose monitoring system (SEVEN; Dexcom, San Diego, CA).

[3] Glucose measured with the OTU, YSI, and SEVEN (predictor variables) from blood, plasma, or interstitial fluids were compared to glucose values obtained with the AA (response variable) from plasma.

[4] Glucose measured with the SEVEN (predictor variable) in interstitial fluid was compared to values obtained with the OTU (response variable) using plasma and whole blood.

Table 2.5 Bland-Altman calculations of glucose measurements for each method tested.[1,2]

	AA[3] Plasma					OTU[4] Blood Plasma	
	OTU		YSI		SEVEN Interstitial Fluid	SEVEN Interstitial Fluid	
	Blood	Plasma	Blood	Plasma			
Observations (n)	120	120	87	120	108	108	108
Diffmean[5]	0.021	-2.194	1.668	-0.096	0.957	0.934	3.25
SD	1.18	1.31	1.19	0.73	1.6	1.63	1.64
No. outside diffmean ± 2 SD	5	4	4	4	2	4	4
% outside diffmean ± 2 SD	4.2	3.3	4.5	3.3	1.9	3.7	3.7
95% confidence interval of diffmean	[-2.34, 2.38]	[-4.81, 0.43]	[-0.71, 4.05]	[-1.56, 1.36]	[-2.24, 4.16]	[-2.33, 4.19]	[-0.03, 6.53]

[1]Bland-Altman calculations provided the mean difference (diffmean) between measurements of 2 methods, the number and percent of observed differences outside the 95% prediction interval based on t-distribution, and the standard deviation (SD) of differences.
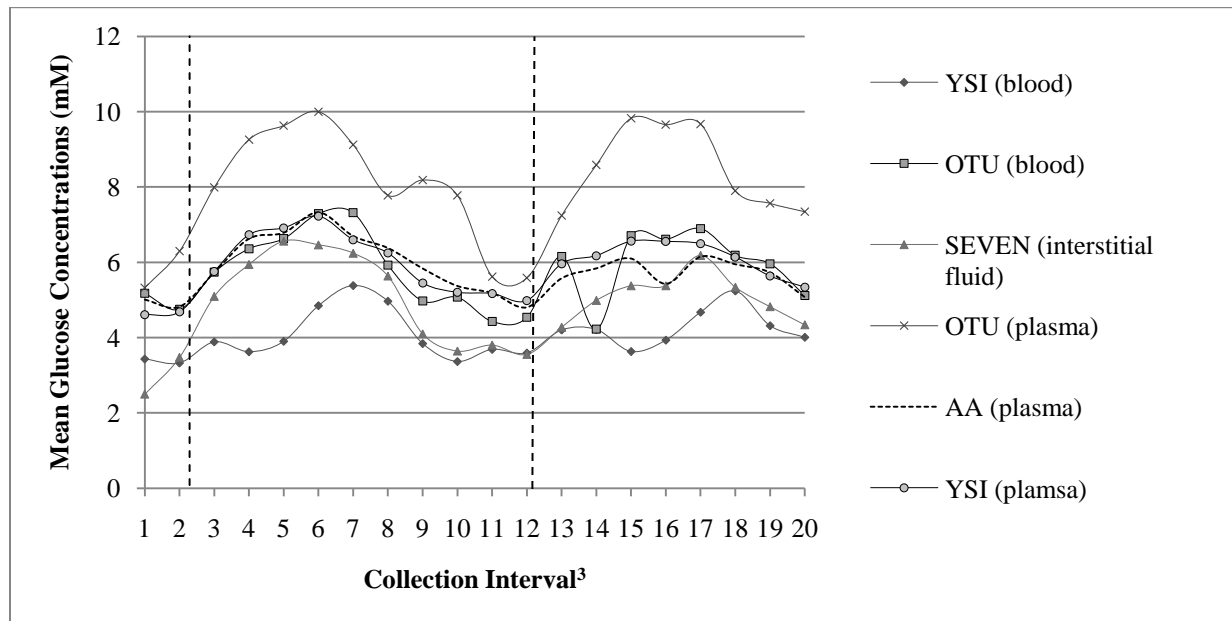
[2]Intsrumentation: Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire, UK), One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA), YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI; YSI Inc, Yellow Springs, OH), and SEVEN® continuous glucose monitoring system (SEVEN; Dexcom, San Diego, CA).

[3]Glucose measured with the OTU, YSI, and SEVEN (predictor variables) from blood, plasma, or interstitial fluids were compared to glucose values obtained with the AA (response variable) from plasma.

[4]Glucose measured with the SEVEN (predictor variable) in interstitial fluid was compared to values obtained with the OTU (response variable) using plasma and whole blood.

[5]The (diffmean) is defined as the response variables glucose mean minus the mean glucose of a predictor method.

Figure 2.1 Glucose measurements from all methods across all collection intervals.[1, 2]

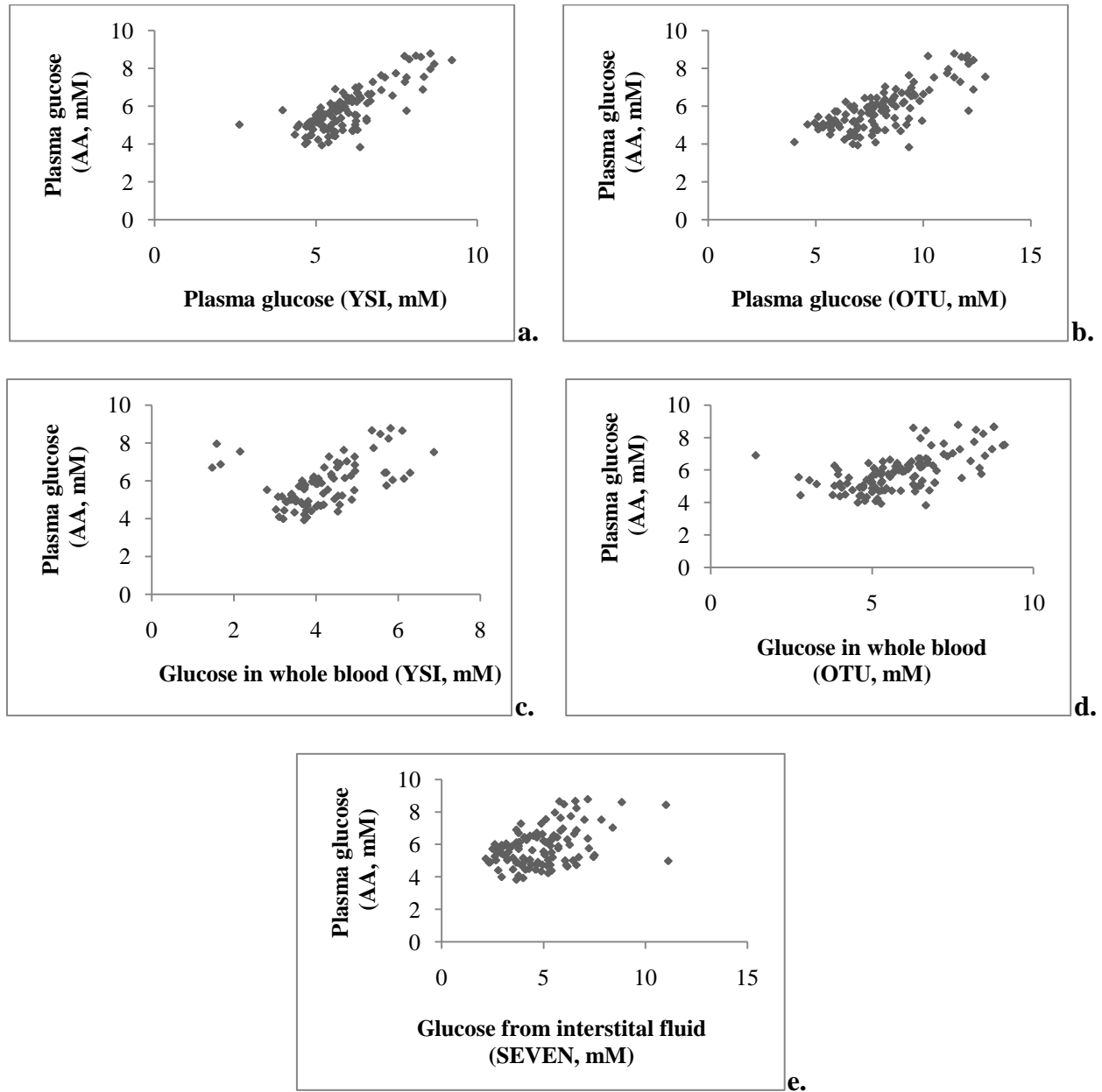[1]Instrumentation: Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire,

UK), One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA), YSI 2300 Stat

Plus Glucose and Lactate Analyzer (YSI; YSI Inc, Yellow Springs, OH), and SEVEN®

continuous glucose monitoring system (SEVEN; Dexcom, San Diego, CA).

[2]Glucose measurements were performed using the AA with plasma, OTU and YSI using plasma

or whole blood, and the SEVEN using interstitial fluid on 6 horses.

[3]Collection interval 1 to 10 and 11 to 20 were performed on 1 and 2, respectively. Collection

interval 1, 2, 11, and 12 represent pre-prandial baseline measurements. Feed administration is

1.13 kg sweet feed concentrate (Sweet Stuff 12, Cargill Animal Nutrition, Minneapolis, MN)

indicated by vertical dashed lines.

Figure 2.2 Scatter plots of raw data comparing the association of glucose measurements from all methods to those obtained with the Auto Analyzer 3 Digital Colorimeter.[1,2]

[1]Intramentation: Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire, UK), One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA), YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI; YSI Inc, Yellow Springs, OH), and SEVEN® continuous glucose monitoring system (SEVEN; Dexcom, San Diego, CA).

[2] The Y-axis represents glucose measurements obtained by the AA from plasma. The X-axis

represents glucose measurements from one of the following predictor methods: a) YSI using

plasma, b) OTU using plasma, c) YSI using whole blood, d) OTU using whole blood, or e)

SEVEN using interstitial fluid.

Figure 2.3 Scatter plots of raw data comparing association of glucose measurements obtained by the SEVEN to those obtained by the One Touch® Ultra®.[1,2]
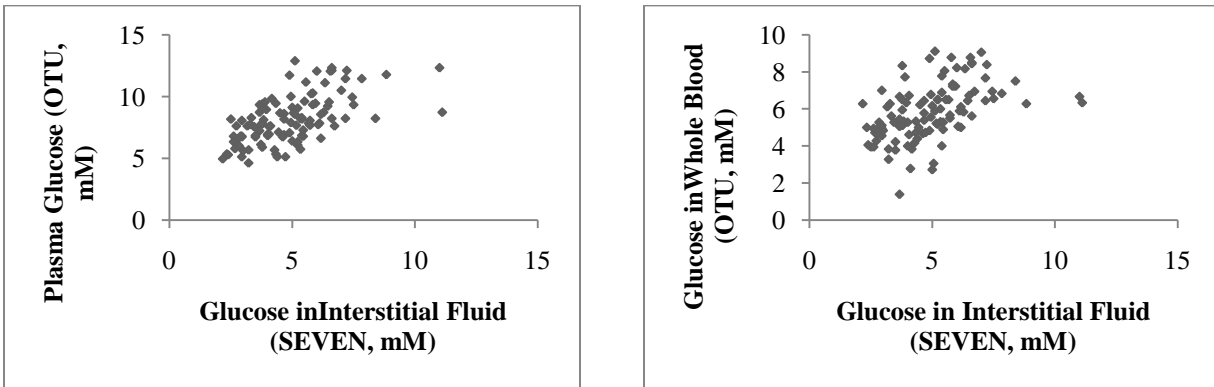
[1]Instrumentation: One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA) and SEVEN® continuous glucose monitor system (SEVEN; Dexcom, San Diego, CA).

[2]The Y axis represents glucose measurements obtained by the OTU from either a) plasma or b) whole blood. The X-axis represents glucose measurements from the SEVEN using interstitial fluid which is the predictor varible.

Figure 2.4 Bland-Altman plots comparing glucose measurements of all methods to the Auto Analyzer 3 Digital Colorimeter.[1,3]
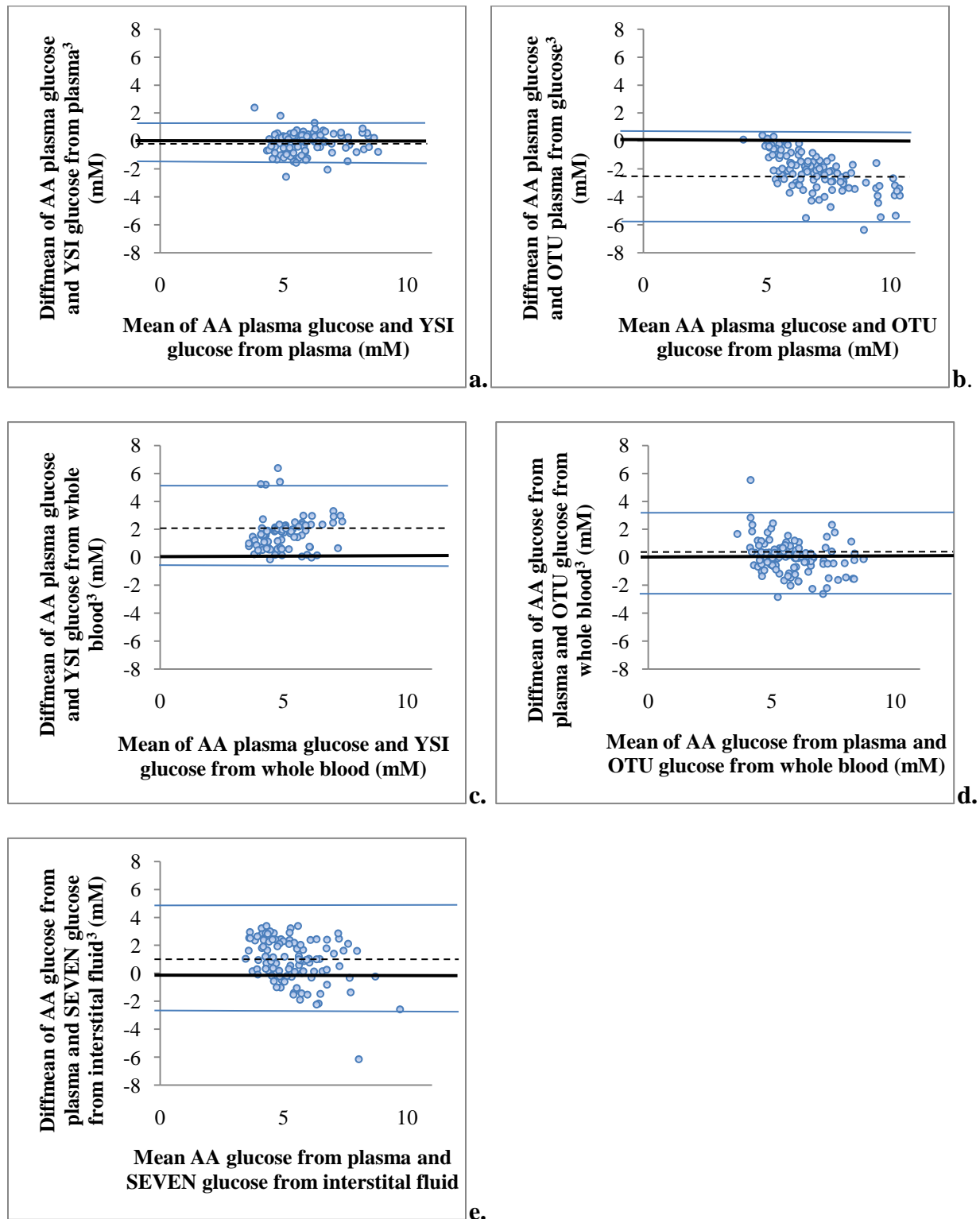
[1]Instrumentation: Auto Analyzer 3 Digital Colorimeter (AA; Seal Analytical Ltd, Hampshire, UK), One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA), YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI; YSI Inc, Yellow Springs, OH), and SEVEN® continuous glucose monitoring system (SEVEN; Dexcom, San Diego, CA).

[2]Bland-Altman calculations provided the mean difference (diffmean) between measurements of 2 methods, the number and percent of observed differences outside the 95% prediction interval based on t-distribution, and the standard deviation of differences. The diffmean is represented with a dashed line, the limits of the 95% prediction interval are represented by thin solid lines and the heavy solid line represents 0 on the Y-axis which is the point at which there would be no differences between means.

[3]The diffmean is defined as the response variable's glucose mean minus the mean glucose of a predictor method. The Y-axis represents the diffmeans of glucose measurements of the AA from plasma and glucose measurements obtained from one of the following predictor methods: a) YSI from plasma, b) OTU from plasma, c) YSI from whole blood, d) OTU from whole blood or e) SEVEN from interstitial fluid. The X-axis represents the glucose measurements obtained from the AA using plasma averaged with glucose measurements obtained from one of the following predictor methods: a) YSI from plasma, b) OTU from plasma, c) YSI from whole blood, d) OTU from whole blood or e) SEVEN from interstitial fluid.

Figure 2.5 Bland-Altman plots comparing glucose values of SEVEN to the One Touch® Ultra®.[1-3]
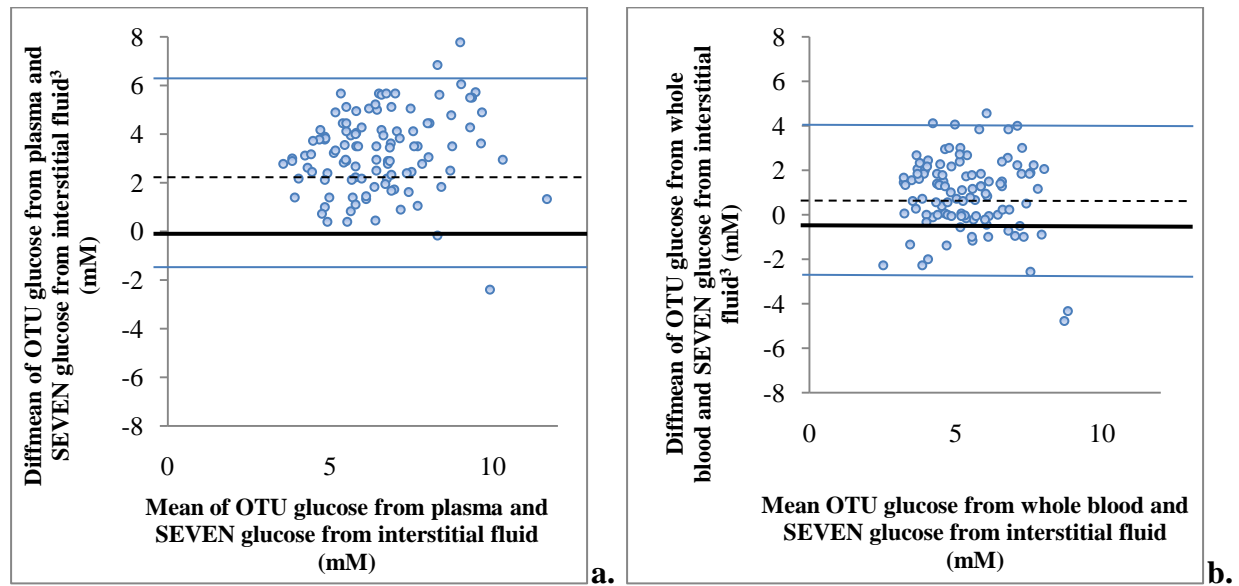
a. **b.**

[1]Instrumentation: One Touch® Ultra® handheld glucometer (OTU; Life Scan, Milpitas, CA) and SEVEN® continuous glucose monitoring system (SEVEN; Dexcom, San Diego, CA).

[2]Bland-Altman calculations provided the mean difference (diffmean) between measurements of 2 methods, the number and percent of observed differences outside the 95% prediction interval based on t-distribution, and the standard deviation of differences. The diffmean is represented with the dashed line, the limits of the 95% prediction interval are represented by thin solid lines and the heavy solid line represents 0 on the Y-axis which is the point at which there would be no differences between means.

[3]The diffmean is defined as the response variable's glucose mean minus the mean glucose of a predictor method. The Y-axis represents the diffmeans of glucose measurements of the a) OTU from plasma or b) OTU from whole blood minus the mean of glucose measurements from the SEVEN. The X-axis represents the glucose measurements obtained from the a) OTU from

91

plasma or b) OTU from whole blood averaged with glucose measurements obtained from the

SEVEN.

Chapter 3 - Blood Glucose Concentrations over Time in Horses Fed Sweet Feed or a Low Moisture Molasses-Based Block Supplement

Abstract

This study compares the effects of consuming a twice-daily meal of a sweet feed (SF) concentrate to an *ad libitum* molasses-based block (BL) supplement on interstitial glucose parameters and patterns over time in the horse.   Six 4-year-old geldings (mean BW = 468 ± 26 kg) were used in a switchback design over two 6-d periods. Each horse was equipped with a continuous glucose monitoring (CGM) system that recorded glucose concentrations every 5 min. Diets consisted of *ad libitum* prairie hay with either a treatment of SF concentrate fed twice daily or *ad libitum* access to a BL supplement. Consumption rates of hay were also measured. A motion-sensing camera was placed above each BL feeder to document time and duration of BL usage. Total intake for the 6-d period of treatments (SF or BL, not including hay) for SF was 16.49 ± 0.90 g DM/ kg BW for SF and 15.60 ± 3.37 g DM/ kg BW for BL (P = 0.6132). Quantity of non structural carbohydrates (NSC) consumed was significantly higher for horses in the BL group than those in the SF group (P = 0.0052).   A novelty effect of the BL was observed, in which horses consumed more BL in the first 12 h they were exposed to the BL than any other time during the 6 days. Total forage intake over the 6-d period was similar between treatments with a Least Squares Mean (LSM) of 137 g ± 2 g DM/ kg BW for horses on BL and 142 ± 1.3 g DM/ kg BW for horses on SF (P = 0.3333). Mean interstitial glucose concentration was 4.16 ± 1.13 mM for horses consuming BL and 2.96 ± 0.23 mM for horses consuming SF (P > 0.05).

Horses consuming SF had an initial glucose response that ranged from instantaneous to 2 in all time periods analyzed and a second significant glucose response in 1 to 2 horses per time period that ranged from 2 h to 4 h after feeding. Glucose patterns varied for the horses on BL: no significant correlation between amount of BL consumed and glucose response was detected in Horse 1, while 3 horses showed significant correlation ($P \leq 0.05$). Post-prandial glucose response patterns varied from an instantaneous effect, to a delay of 15 min, and to a delay of 4.5 h ($P \leq 0.05$). Glucose responses were similar between treatments, although variability between horses was noted in quantity of BL consumed as well as timing and magnitude of glucose responses.

Key Words: continuous glucose monitor, glucose, glycemic response, horse, molasses-based blocks, sweet feed

Introduction

Various supplements, such as minerals, omega-3 fatty acids, prebiotics, and non-protein nitrogen (for ruminants), can be incorporated into molasses blocks, which represent one form of free-access supplementation. Molasses is palatable to the horse, which thus provides an incentive to consume the lick (Goodwin et al., 2005; Randall et al., 1978), and it provides 17.0 MJ/ kg digestible energy (NRC, 2007). However, because molasses is a sugar byproduct, usually made from sugarcane or sugarbeets (NRC, 2007), its dietary inclusion generally raises the glycemic indices of horse feeds (Rodiek and Stull, 2007; Kronfeld et al., 2004). Excessive consumption of

94

high-glycemic feeds has been associated with metabolic diseases in the horse (Treiber et al., 2005; Kronfeld, 2005; Kronfeld and Harris, 2003).

Because a block-type supplement (BL) is assumed to be consumed more slowly and over a longer period of time than a meal of sweet feed (SF), one might hypothesize that the glycemic effects of a BL may be less than that of SF, despite the fact they both contain ingredients, such as molasses, that are high in nonstructural carbohydrates (NSC). This study was designed to compare the effects of consuming a twice-daily meal of SF to an *ad libitum* molasses-based BL supplement on glucose parameters and patterns in the horse.

Materials and Methods

Horses

All procedures were approved by Institutional Animal Care and Use Committee at Kansas State University. Six 4-year-old American Quarter horse geldings with a mean body weight of 468 ± 26 kg were used. Throughout the experiment, horses were housed in individual stalls (3.05 m X 3.66 m). Horses received daily turnout into a dry lot (15.24 m X 36.58 m) during the 4-d washout period between treatments. Long-stemmed prairie hay (Table 3.1), salt blocks, and water were offered to all horses *ad libitum.*

Treatments

A crossover design was employed over two 6-d periods. Treatments consisted of a SF concentrate (9% molasses, 22% NSC, Sweet Stuff 12, Cargill Animal Nutrition, Minneapolis, MN) provided twice daily or *ad libitum* access to a 5-kg molasses-based BL supplement (67.8% molasses, 43% NSC, HorseLic®, New Generation Feeds, Belle Fourche, SD) presented in fence-mounted feeders (Table 3.2). Sweet feed was fed at 0700 and 1400 h daily, gradually increasing meal size over the first 3 feedings, which brought concentrate consumption to a rate of 0.82 kg per meal. Consumption of the BL and hay were measured every 12 h for the first 3 d and then each was weighed on a staggered schedule every 6 h for the final 3 d of the treatment period.

Equipment

Each horse was equipped with a SEVEN® continuous glucose monitor (CGM) system (Dexcom, San Diego, CA). A sensor was subcutaneously inserted into the adipose tissue on the posterior dorsum lateral to the dock of each horse. Sensors obtained an interstitial glucose reading every 5 min which was transmitted to a wireless receiver attached to the tail of the horse. Sensors were calibrated every 12 h and each time new sensors were installed according to the manufacturer's specifications. To calibrate, about 0.50 ml of blood was obtained via jugular venipuncture and approximately 10 µl was placed into a channel on a test strip inserted into the One Touch® Ultra® hand-held glucometer (OTU; Life Scan, Milpitas, CA). The blood glucose concentration that appeared on the screen of the OTU was manually entered into the SEVEN® receiver. The OTU was stored and maintained according to the manufacturers' specifications.

A motion-sensing camera with infrared technology, either a Digital Game Camera DV-5 (Vibrashine Inc, Taylorsville, MS) or a STC-1540IR (Stealth Cam, LLC, Grand Prairie, TX), was placed above each BL feeder to generate time-stamped photographs when a horse approached the BL and thus documented the amount of time each horse spent at the BL. The cameras took a maximum of 1 photograph per min each time movement of a horse's head broke the vertical infrared beam. This enabled both the monitoring of when the BL was consumed and the duration of each consumption episode.

Statistics

Intake of forage were analyzed as a crossover design using the MIXED procedure of SAS (SAS Institute, 2007). Type 3 F-tests and dietary treatment means and standard errors were calculated for treatment and period main effects (SAS Institute, 2007), and an alpha level of 0.05 was used. Total 6-d intake of dietary treatments, total 6-d intake of NSC, minimum and maximum glucose measurements, range of glucose measurements, and treatment means were compared by a paired t-test using the TTEST procedure of SAS (SAS Institute, 2007).

There were significant technical difficulties with the CGM that resulted in large blocks of missing glucose data for 2 animals and intermittently scattered missing values for the other 4 animals. Consequently, glucose was modeled for only 4 horses. Glucose values were averaged to obtain 15-min means for modeling purposes. Linear interpolation was used to estimate the few remaining missing values. Time-series models were necessary to account for serial correlation in glucose measurements, and were performed using the ARIMA procedure of SAS (SAS Institute, 2007) for periods where data was complete. First, for each horse modeled, Box-Jenkins Auto-

97

Regressive Integrated Moving Average models (Box et al., 1994) were obtained using the AIRMA procedure of SAS and were used to estimate the mean glucose, the standard error of the mean (SEM), and 95% confidence intervals for the mean.

Additional analyses examined patterns of glucose over time for each treatment. First, cross-correlations (Box et al., 1994) were used to examine the strength of the linear association between min spent at the BL and mean glucose per 15-min period. Next, Box-Jenkins intervention models were used to examine glucose patterns generated after horses were fed SF relative to the pre-prandial glucose mean over 2 consecutive days, broken up into feeding times (AM and PM). The Box-Jenkins intervention models had an intervention and noise component. The intervention component modeled the magnitude and timing of glucose concentration effected by the event of feeding SF relative to the pre-prandial glucose mean, while the noise component modeled residual variability. The pre-prandial glucose mean was determined using data over a period of approximately 6 h prior to feeding.

Treatments can be compared directly for response: intake of forage, NSC, and dietary treatment; and minimum, maximum, and range of glucose measurements. However, it should be noted that due to the contrasting nature of the treatments (i.e. limited access to SF compared to self-regulated access to BL), treatments cannot be compared directly for continuously-monitored glucose, except for when comparing mean concentration of glucose. For glucose means, the Box-Jenkins time series models are qualitatively different, and therefore are not directly comparable statistically. However, patterns of glucose responses produced by the treatments can be indirectly compared descriptively.

Results

Technical Challenges with Equipment

The primary challenges encountered during this study were due to technical difficulties with the CGM and infrared cameras. While the CGM sensors were reported to last greater than 5 d in humans, they functioned properly, on average, 3.7 d in horses during this study. Sensors that failed were either reinitialized or replaced. Because of the 2 h initialization period, there was loss of data during each re-initialization. Most horses in this study required 1 to 3 sensors to complete the 6-d treatment period. The CGM monitors had problems as well. Due to high humidity inside the barn, condensation collected on the inside of the receiver screen. The text and graphics appeared faded or nonexistent until the humidity lessened. The manufacturer states that the SEVEN® receiver should be stored within a range of 10 to 85% humidity. While relative humidity was not monitored during this experiment, it is possible that relative humidity levels were greater than 85%. The moisture damage necessitated the purchase of new monitors.

The infrared cameras also presented technical challenges. As a result of the large number of pictures being taken, the cameras required frequent changes of batteries, which ranged from once to twice daily. Although the cameras were checked for battery function periodically throughout each day, there were periods of missing data due to low batteries. The Digital Game Camera DV-5 used more batteries, while the STC-1540IR ran more efficiently and consistently.

Intake of Treatments, NSC, and Forage

Horses typically consumed each SF meal within a 5-min period. Sweet feed and BL were not significantly different for total intake over the 6-d period (P = 0.6132; Table 3.3). Block total intake over the 6-d period was variable between horses, with 6-d consumption rates ranging from 11 to 20 g DM/kg BW. Total intake of NSC over the 6-d period was 3.08 ± 1.5 g/kg BW greater for horses consuming BL compared to horses receiving SF (P = 0.0052; Table 3.3). There appeared to be a novelty effect for all horses, as the amount of BL consumed in the first 12-h period ranged from 1.6 to 3.2 times greater than the average daily consumption rates for succeeding 12-h periods. Two of the 6 horses consumed more BL in the first 12 h than during any other 12-h period.

Total 6-d forage intake between treatments was similar (Table 3.4). While there was a period effect (P = 0.0001), there was not a treatment effect (P = 0.3333) on forage consumption. Total forage intake was greater in period 1 compared to period 2.

Glucose Parameters

The mean, minimum, and maximum glucose values and standard error of the mean (SEM) were highly variable among horses (Table 3.5). This was attributed to the idiosyncrasies of individual horses (i.e. physiological differences and individual eating patterns, as some horses were observed to bite off sections of BL while others simply licked the BL, etc.). Mean glucose concentrations, peak glucose values and glucose ranges for treatments were not statistically different between treatments (P > 0.05). Horses on BL treatment had a mean glucose

concentration of 4.18 ± 2.3 mM, while horses on SF treatment had a mean glucose concentration of 2.96 ± 0.23 mM (P > 0.05). In the BL group, glucose concentration varied within horse with an average range of 4.11 mM, while horses in the SF group had an average range of 4.81 mM but these ranges were not significantly different (P = 0.7297). The average maximum glucose concentrations were 7.15 mM for horses on BL and 7.03 mM for horses on SF treatment (P > 0.05).

Sweet Feed Intervention Models

Intervention models were created for the SF treatment to analyze patterns created by the event of feeding SF at either 0700 or 1400 h relative to the pre-prandial glucose mean (data over a period of approximately 6 h prior to feeding). Details of intervention models are given in Appendix A. The intervention component started with glucose data collected prior to feeding and then for a duration of approximately 11 h following feeding. Adequate intervention models were not found for the first AM feeding on Horse 2 and the first PM feeding on Horse 4, so, for these horses, 3 of the 4 time periods were analyzed. Glucose values at all feeding times fluctuated considerably in Horse 2 and 4, while Horse 1 and 3 had more stable glucose values (Figure 3.1). Horse 1 had an initial significant glucose increase in 3 of the 4 time periods ranging from 15 to 45 min following feeding and had an immediate (within 15 min) significant glucose decrease for one of the time periods (P ≤ 0.05). These initial increases in glucose ranged from about 0.23 to 0.45 mM. In 2 time periods, Horse 1 had a second increase that occurred 2.5 to 2.75 h after feeding and in 1 time period a third occurred 4 h after feeding. Horses 2 and 3 had initial significant glucose increases in all time periods analyzed (P ≤ 0.05). Initial significant glucose increases

were observed from 30 min to 2 h after eating for Horse 2 and 30 to 45 min following feeding for

Horse 3 (P ≤ 0.05). These initial increases in glucose ranged from 0.92 to 1.48 mM and 0.14 to

0.54 mM in horses 2 and 3, respectively. Horse 3 had a second increase that occurred 2.75 to

3.75 h after feeding in 3 time periods. Horse 4 had an initial glucose increase of 1.24 mM in 1

time period that occurred at 45 min with a second increase that followed 3.75 h after feeding.

Horse 4 had initial glucose decreases for 2 time periods that occurred at 45 min and 1 h after

feeding and ranged 0.08 to 1.99 mM (P ≤ 0.05). All horses that were modeled had an initial

significant glucose response that ranged from instantaneous to 2 h after feeding in all time periods

analyzed (P ≤ 0.05). These responses ranged in magnitude from 0.14 to 1.48 mM for initial

increases and 0.08 to 1.99 mM for initial decreases. A second significant glucose increase was

observed for all horses in at least 1 to 2 time periods that ranged from 2 h to 4 h after feeding (P ≤

0.05). These responses ranged in magnitude from 0.19 to 1.77 mM. Only Horse 1 had a third

significant glucose response that increased 0.39 mM at 4 h after feeding.

Cross Correlations for Block Consumption

Cross correlations between min spent at the BL and mean glucose concentrations per 15-

min period provided information on the delay, or lag, between BL intake and glucose appearance

in the interstitial fluid (Figure 3.2). Approximate 2 standard errors for correlation are: 0.15 for

Horse 1 (n = 244) and Horse 3 (n = 208) and 0.10 for Horse 2 (n = 489) and Horse 4 (n = 281).

Once again, there was a large amount of variation between horses. No significant correlation was

detected in Horse 1 (P > 0.05), while significant correlation was detected in 3 horses (P ≤ 0.05).

Lag patterns varied from an immediate effect, to a delay of 15 min, and to a delay of 4.5 h. Horse

2 had a steady correlation that was detected almost immediately, peaked at 30 min, and then quickly declined. The correlation built back to a second peak at 4.25 h and significant correlation was sustained for about 5.75 h ($P \leq 0.05$). Horse 3 had a greater lag period initially with a correlation that peaked for a shorter duration of time. Significance was detected at a lag of 4.25 h and had a 2.25 h duration ($P \leq 0.05$). Horse 4 had an immediate response ($\leq$ 15 min) that was more intensely correlated and which completely diminished at an earlier time (5.25 h lag time) than the other horses ($P \leq 0.05$).

Discussion

The average daily BL consumption in this study (1.26 ± 0.33 kg/d) was greater than daily intake values (0.9 kg/d) reported by De and Singh (2002) in cattle fed molasses-based blocks *ad libitum*. Aside from the obvious differences of specie studied, blocks utilized in the bovine study contained monensin and urea which may have affected intake in cattle, but perhaps the boredom of stall confinement contributed to the high intake values reported in horses here. Similarly, Titgemeyer et al. (2004) observed that heifers housed in pens and fed a cooked molasses blocks *ad libitum* consumed a much greater amount of the supplement than that typical of free-range cattle. Also, the novelty effect of the BL may have been a factor in this study, whereas the cattle studies were conducted over several weeks.

Effects of supplementation with a molasses based BL on forage intake have been studied extensively in cattle. Greenwood et al. (1998) observed greater total forage organic matter (OM) intake in steers consuming a cooked molasses BL compared to steers without BL supplementation. As well, steers supplemented with cooked molasses blocks had greater forage

intake of lower quality forages (Greenwood et al., 2000). Similar treatment effects were not noted in the current experiment, but it should be noted that differences in consumption reported in bovine studies may be attributed to added protein in the blocks, which has been shown to be the most limiting nutrient in cattle fed low-quality forages (Koster et al., 1996; Hannah et al., 1991). As well, the daily intake of digestible nutrients in ruminants may be reduced on low-quality feeds because of the relatively slow rate of passage through the rumen (Fisher, 2002). Compared to horses, ruminants can consume smaller amounts of low-quality forage and can derive more energy and protein content from it through ruminal fermentations (Houpt, 2006). Thus, they may intake less than horses and this may be an influence when comparing forage intake between these species. Furthermore, the above ruminant studies were carried out for several weeks so differences in intake may be magnified. Perhaps if the current study was carried out longer, differences would be easier to detect.

Although the range of glucose values in this study (2.16 to 9.49 mM) does not fall within reported normal ranges in the horse (3.44 to7.44 mM; Hollis et al., 2007; Lumsden et al., 1980), the average glucose values detected in horses on both treatments do fall within the reported normal ranges. Biorhythm can influence biochemical values, physiological processes, and eating patterns in the horse (Gordon and McKeever, 2005; Yashiki et al., 1995; Evans et al., 1974; Kern and Bond, 1972). Consequently, these averages, although generated by an auto regressive procedure, are likely not taking all possible periodicity into account and should be looked at as a rough estimation.

It is important to note that the SEVEN® has a working a range of 2.22 to 22.20 mM and therefore readings of 2.16 mM likely represent the minimum glucose value the CGM will provide despite the fact that actual glucose concentrations may have been even lower. The point at which

prompt clinical attention is needed is lower in horses (0.66 to 1.12 mM; Lyle and Keen, 2010; Mayhew, 2009) than in humans (< 2.22 mM; Wallach, 1996), so a broader glucometer working range would be more advantageous for use in the horse.

Variable peak glucose concentrations have been reported in equines after consuming a meal of SF. In Thoroughbred horses, peak glucose values of 5.99 mM (Pagan et al., 1999 in Williams et al., 2001) and approximately 7.21 mM (Williams et al., 2001) have been reported, while glucose concentrations in ponies peaked at 5.89 mM (Healy et al., 1995) after a meal of a textured feed with molasses. Differences in peak glucose concentrations between these studies could be attributed to differences in number of horses used, meal sizes, horse sizes, individual variability, insulin sensitivity, and breed to some extent. With the exception of Williams et al. (2001), the values for average glucose peaks reported in the above studies are lesser than those in the present study (7.03 mM for horses on SF and 7.15 mM for horses on BL), but they still fall within the range of maximum glucose values observed in the current study (5.46 to 9.49 mM for SF and 5.60 to 9.16 mM for BL). Glucose peaks have been observed at 1.5 to 3.5 h after meal feeding in horses (Williams et al., 2001; De La Corte et al., 1999; Stull and Rodiek, 1988) and in ponies (Healy et al., 1995; Ralston and Baile., 1982; Hintz et al., 1971) which is similar to the time frames detected in this study (instantaneous to 2 h for BL and 2 to 4 h time frames for SF) in which significant glucose responses were observed.

Although other equine studies have not reported using molasses-based BL, studies in buffalo calves (Singh et al., 2010b; Tiwari et al., 1990) and goats (Singh et al., 2010a) have reported significant increases in glucose concentrations after animals were fed molasses-based blocks. Singh et al. (2010b) reported a significant increase in glucose concentrations in buffalo calves after consuming 400 to 600 g daily of 35% molasses BL supplementation for 30 d resulting

in a mean glucose concentration of 3.28 mM. Similarly, Tiwari et al. (1990) observed a glucose

mean of 2.78 ± 0.04 mM in buffalo calves after being fed a 38% molasses-based BL *ad libitum*

for 130 d, which was a significant increase over the control (no BL). Singh et al. (2010a) reported

a significant increase in glucose concentrations in goats fed 200 g daily of 35% molasses BL for

30 days which resulted in a mean glucose concentration of 3.80 mM. Horses on BL treatment in

this study had a greater mean glucose concentration (4.18 ± 2.3 mM) than the ruminant species

reported. This dissimilarity may be attributed to the differences associated with the mechanism

by which starch is digested in ruminants compared to non-ruminants. Before glucose reaches the

small intestine, microbes ferment starch in the rumen and ruminants rely on the end products of

microbial fermentation, such as acetate and lactate, which are utilized to synthesize glucose in the

liver by means of gluconeogenesis (Pond et al., 2005). Thus glucose is generally considered an

indirect product, unless it reaches the small intestine intact where it can be directly absorbed. In

contrast, non-ruminants absorb starch directly (Pond et al., 2005).   In addition, the BL in the

cited studies had lesser amounts of molasses (35 to 38%) than the current study (67.8%) and all

but one of the cited studies limited intake of BL treatment, which was not the case in the current

equine experiment.

In the current study, the mean, minimum, maximum, and range of glucose concentrations

were similar between treatments ($P > 0.05$) despite the fact that the total intake of NSC was

greater for horses on the BL ($P = 0.0052$). While increasing or decreasing the quantity of SF

offered at each meal would likely have a significant impact on glucose data collected, which may

lead to significant treatment differences, this study was designed to mimic a typical management

scenario and thus to compare the effects of consuming a "typical" twice-daily meal of SF to an *ad*

libitum molasses-based BL supplement on glucose parameters and patterns in the horse.

Minimum crude fat was higher in the BL (6%) compared to the SF (3%) treatment. In humans, it has been reported that fat delays gastric emptying which can decrease the glycemic response to a meal (Wolever, 1990). Effect on glycemic response depends on the amount of fat added (Wolever, 1990). While decreases in equine glycemic responses have been reported due to the addition of fat (Pagan et al., 1999) or diets higher in fat and fiber (14% fat and 7% fiber) compared to higher starch diets (39% starch; George et al., 2011), the quantity or type of fat that is needed to affect glycemic response is not known in horses. It is possible, but one cannot state with certainty that the differences in fat content between the diets may have influenced glycemic responses in this study with the higher fat content in the BL attenuating glycemic response despite increased NSC consumption compared to horses receiving SF.

In further comparison, patterns of glucose responses were analyzed over time with an intervention analysis for SF and a cross-correlation analysis for BL. The BL cross-correlations showed significant responses at greater lag times of up to 5.75 h compared to the SF intervention analysis which had a maximum lag time of 4 h. However, for both treatments, significant responses were seen, initially, in a range from instantaneous to 2 h and, secondly, in a range from 2 to at least 4 h. Even so, lag times of glucose responses from the treatments can only be indirectly compared due to the differences the delivery of the treatments (i.e. controlled intake of SF compared to self-regulated intake of the BL). Also, one must remember, lag times observed in the cross-correlation analysis (BL) were averaged over the entire run of data where as those of the intervention analysis (SF) were done in separate time periods.

As Figures 1 and 2 depict, a great amount of variability between horses was observed in relation to magnitude and timing of glucose responses. In order to better characterize and more fully assess individual horse variability, further experiments with greater numbers of horses

107

would be warranted to gain further understanding of both pre-prandial and post-prandial glucose patterns associated with meal-fed SF consumption and *ad libitum* access to BL.

Conclusion

The present study design, in which the BL was fed *ad libitum*, provided insight into the amount of molasses-based BL supplement horses will consume. A novelty effect of the BL was observed, in which horses consumed increased quantities in the first 12 h. Treatments did not have an effect on intake of forage in this study. The glucose range and means were similar between treatments and significant glucose responses from the treatments had lag times indirectly comparable even though NSC intake was greater for horses on BL. This evidence suggests that glucose responses were similar between treatments; however, variability between horses was noted in quantity of BL consumed as well as time and magnitude of glucose responses. This variability needs to be further assessed to gain an improved understanding of both pre-prandial and post-prandial glucose patterns. Based on the results of this experiment, there does not appear to be a clear advantage to either treatment, SF or BL, in attenuating post-prandial glucose increases or in minimizing glucose fluctuations in the horse.

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Table 3.1 Hay analysis.[1]

Item (%)	
Dry matter	92.62
Crude protein[2]	5.49
NDF	69.69
ADF	41.15

[1]Results are reported on a 100% dry matter basis.

[2]Crude protein was calculated using a 6.25 conversion factor.

Table 3.2 Proximate analysis of treatments.

Item (%)	Diet	
	SF	BL
Crude protein, minimum	12.0	12.0
Crude fat, minimum	3.0	6.0
Crude fiber, maximum	15.0	2.0
Ca, minimum	1.25	1.5
Ca, maximum	1.75	2.0
P, minimum	0.45	1.0
Molasses[1]	9.0	67.8
Non structural carbohydrates	22.0	43.0

[1]Sweet feed (SF) contained cane molasses while block (BL) contained beet molasses.

Table 3.3 Mean total 6-d intake of treatments (BL or SF, no hay) and molasses across all horses.

Item	Mean Total Intake (g DM/kg BW)[1]	
	SF	BL
Treatment	16.49 ± 0.90	15.60 ± 3.37
NSC	3.6 ± 0.2^a	6.7 ± 1.4^b

[1]Mean $\pm$ standard error of the mean of total intake of treatments and molasses as g of dry matter/kg of body weight (g DM/kg BW) for horses on block (BL) or sweet feed (SF) treatments.

[a,b]Values with different superscripts are different (P = 0.0052).

Table 3.4 Mean total 6-d intake of forage across all horses.

		Total Intake (g DM/ kg BW)[1]
Treatment	BL	137 ± 2
	SF	142 ± 1.3
Period	1	147 ± 2.9^{a}
	2	132 ± 1.3^{b}

[1]Least Squares Means ± standard error of the mean of total intake of forage as kg of dry matter/kg of body weight (g DM/kg BW) for horses on block (BL) or sweet feed (SF) treatments.

[a,b]Values with different superscripts are different (P = 0.0001).

**Table 3.5 Summary of Box-Jenkins model of glucose parameters.**

Horse ID	Treatment	Mean Estimate[1] (û; mM)	SEM Estimate[1] Se (û)	95% CI[1] (mM)	Minimum Glucose[2] (mM)	Maximum Glucose[2] (mM)
1	SF	2.73	0.11	[2.51, 2.90]	2.16[3]	5.60
	BL	4.47	0.22	[4.03, 4.90]	3.16	8.05
2	SF	4.02	0.25	[3.53, 4.51]	2.39	6.48
	BL	3.60	0.15	[3.30, 3.90]	2.16[3]	9.49
3	SF	2.82	0.12	[2.59, 3.06]	2.16[3]	6.88
	BL	4.80	0.05	[4.70, 4.90]	3.75	5.60
4	SF	4.61	0.10	[1.39, 3.13]	2.16[3]	9.16
	BL	3.86	0.08	[3.70, 4.03]	3.05	5.46

[1]Box-Jenkins models that accounted for serial correlation (non-independence) among glucose readings over time were used to calculate the glucose mean, standard error of the mean (SEM) and 95% confidence interval (CI).

[2]The minimum and maximum reported are the least and greatest glucose concentrations observed for each horse respectively.

[3]The SEVEN® has a working range of 2.22 to 22.20 mM and therefore readings of 2.16 mM indicate glucose values that were below the CGM's minimum threshold and may not have been accurately measured by the CGM.

**Figure 3.1 Sweet feed intervention analysis plots for each horse.[1]**
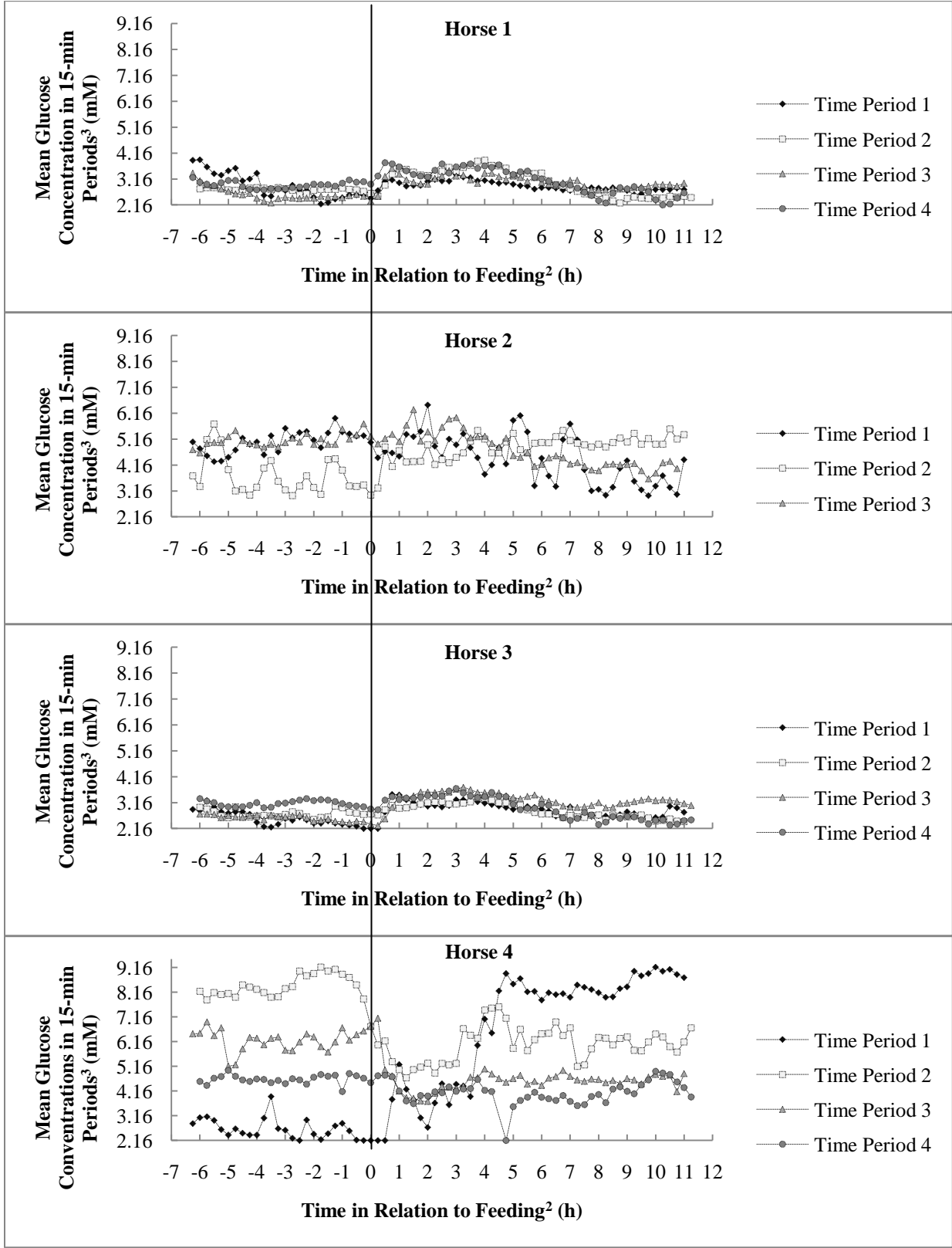
[1]The ARIMA procedure (SAS Version 9.2) was used to fit Box-Jenkins intervention models to evaluate the SF treatment effect on glucose values over 2 consecutive days broken up into 4 time periods. The time periods consisted of a 6-h period of pre-prandial glucose concentrations and an 11-h period of post-prandial glucose concentrations.

[2]The horizontal axis corresponds to time in relation to when SF meal was fed. The numbers -1 to -7 represent time (h) before the SF meal, 0 represents time of feeding, and numbers 1 to 12 represent hours after the SF meal. The solid vertical line illustrates time of feeding.

[3]The SEVEN® CGM had a working range of 2.22 to 22.20 mM. Glucose readings of 2.16 mM likely represent the minimum glucose value the CGM would provide, despite the fact that actual glucose may have been even lower.

Figure 3.2 Cross-correlations for each horse on block treatment.[1]
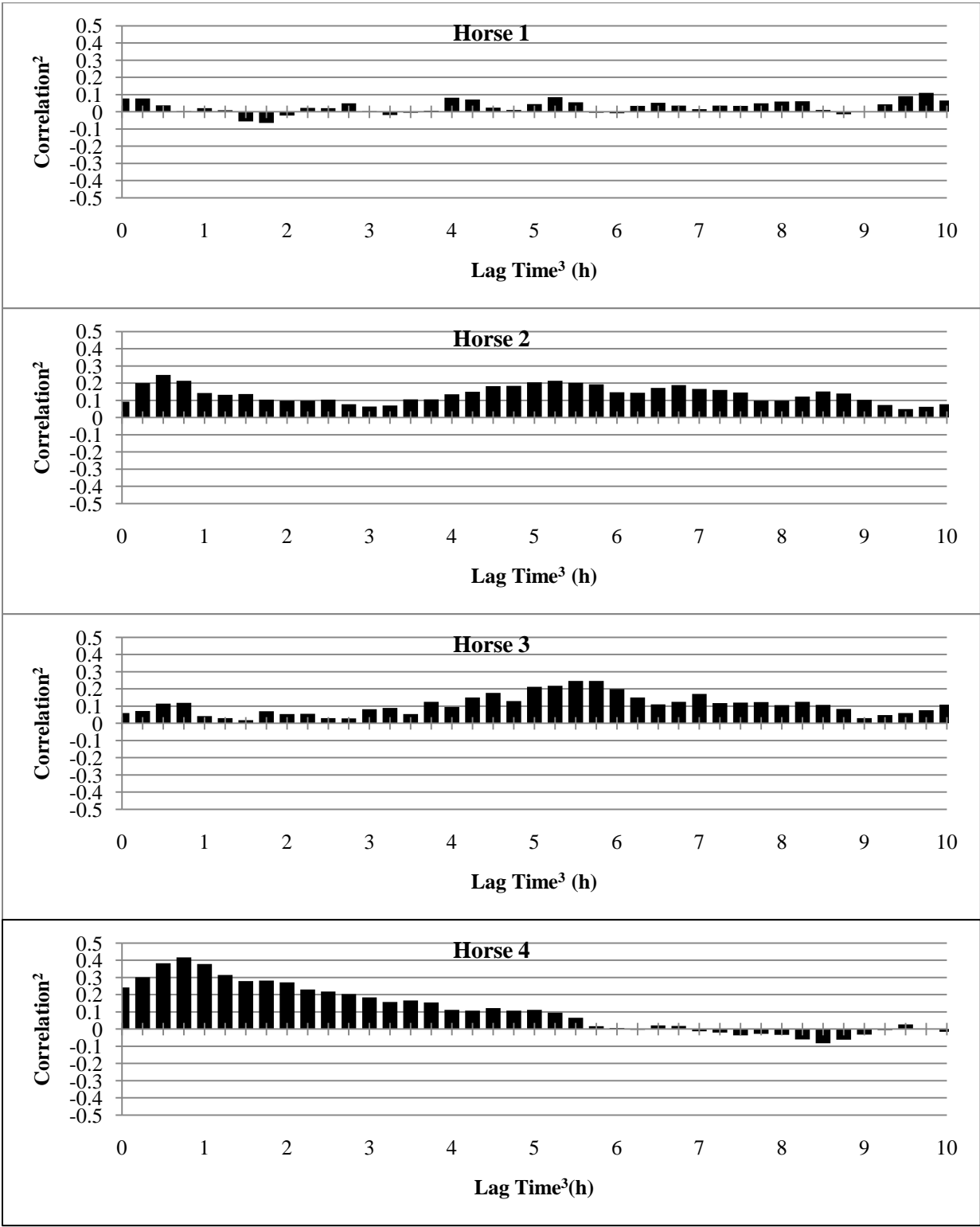
[1]Cross correlations between min spent at the block (BL) and the mean glucose per 15-min period provided information on the delay between BL intake and glucose appearance.

[2]Correlation refers to the strength of linear association between min spent at BL and mean glucose concentration per 15-min period.

[3]Lag time corresponds to the delay between BL intake and glucose appearance. For example, if significance is reached at lag 0, it signifies the min spent at the BL had an immediate (within 15 min) effect on blood glucose, and if significance is reached at lag time 10, it signifies an effect on blood glucose occurred 10 h after spending time at the BL.

Appendix A - Appendix

Table A.1 Predominant patterns of glucose increases/decreases after being fed SF across all horses.[1]

Horse ID	Feeding Time	PPM[2]; (SEM)[3]	Pattern[4]
	AM	2.60; (0.05)	Increase of 0.45 mM over the PPM at 45 min; Second increase of 0.33 mM at 3.25h; Damping of 0.05 mM beginning at 3.5 h.
	PM	2.73; (0.10)	Increase of 0.45 mM over the PPM at 30 min; Two more increases of 0.44 mM at 45 min, 0.26 mM at 1 h; Decrease of 0.19 mM at 4.25 h.
1	AM	2.43; (0.10)	[a]Decrease of 0.23 mM over the PPM instantaneously ($\leq$ 15 min); Three increases of 0.06 mM at 15 min, 0.67 mM at 30 min, and 0.48 mM at 45 min; Damping of 0.04 mM beginning at 1 h. [b]Increase of 0.21 mM over the PPM at 2 .75 h. [c]Increase of 0.39 mM over the PPM at 4 h; Damping of 0.05 mM beginning at 4.25 h.
	PM	2.75; (0.13)	[a]Increase of 0.33.0 mM over the PPM at 15 min; Second increase of 0.55 mM at 30 min; Damping of 0.06 mM beginning at [b]Increase of 0.30 mM over the PPM at 2.5 h.
	PM	4.63; (0.27)	Increase of 1.48 mM over the PPM at 2 h.
2[5]	AM	3.40; (0.36)	Increase of 1.17 mM over the PPM at 30 min; Second increase of 0.70 mM at 2 h; Decrease of 0.38 mM at 3.75 h.
	PM	5.30; (0.21)	Increase of 0.92 mM at 1.5 h.
	AM	2.38; (0.06)	[a]Increase of 0.54 mM over the PPM at 30 min; Second increase of 0.65 mM at 45 min; Damping beginning at 1.5 h to return to baseline. [b]Increase of 0.33 mM over the PPM at 3 h; Damping beginning at 3.25 h.
	PM	2.53; (0.10)	[a]Increase of 0.14 mM over the PPM at 45 min. [b]Increase of 0.19 mM over the PPM at 3.75 h; Damping beginning at 4 h.
3	AM	2.66; (0.07)	Increase of 0.46 mM at 45 min; Second increase of 0.50 mM at 1 h; Damping of 0.06 mM beginning at 1.25 h.
	PM	3.15; (0.11)	[a]Increase of 0.26 mM over the PPM at 30 min; Second increase of 0.23 mM at 45 min. [b]Increase of 0.22 mM over the PPM at 2.75 h; Second increase of 0.17 mM at 3 h; Damping of 0.04 mM beginning at 3.25 h.
	AM	N/A[6]	[a]Increase of 1.24 mM over the PPM at 45 min; Second increase of 1.62 mM at 1 h; Damping of 0.04 mM beginning at 1.25 h. [b]Increase of 1.77 mM over the PPM at 3.75 h; Second increase of 1.57 mM at 4 h.
4[5]	AM	6.26; (0.13)	Decrease of 1.99 mM over the PPM at 45 min; Damping of 0.06 mM beginning at 1 h.
	PM	4.32; (0.15)	Decrease of 0.08 mM over the PPM at 1 h; Second decrease of 0.81 mM at 4.5 h; Final decrease of 1.60 mM at 4.75 h.

[1]The ARIMA procedure (SAS Version 9.2) was used to fit Box-Jenkins intervention models to evaluate the sweet feed (SF) treatment effect on glucose values relative to the pre-prandial glucose means over 2 consecutive days broken up into 4 time periods according to time in which feed was given.

[2] The pre-prandial glucose mean (PPM; mM) was determined using data over a period of approximately 6 h prior to feeding time using an ARIMA procedure (SAS Version 9.2).

[3]Standard error of the mean (SEM).

[4]For feeding times in which more than one pattern is observed, [a,b,c] represent the first, second, and third patterns respectively.

[5]Adequate intervention models were not found for the first AM feeding on Horse 2 and the first PM feeding on Horse 4.

[6]Pre-prandial glucose mean (PPM; mM) of differenced glucose value was 0.03. Differencing of data was necessary for Box-Jenkins modeling due to non-stationarity of original glucose data.