GLYCEMIC RESPONSE TO MEAL LENGTH IN HORSES

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Jinger Bland

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GLYCEMIC RESPONSE TO MEAL LENGTH IN HORSES

Jinger Bland

A Thesis

Submitted to

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GLYCEMIC RESPONSE TO MEAL LENGTH IN HORSES

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Signature of Author

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Date of Graduation

VITA

Jinger Bland, daughter of Dr. John Bland and Lesley Slaton, was born on January 18, 1985 in Shreveport, LA. She lived in Woodworth, LA until the age of 12, at which time she moved to Niceville, FL with her parents. After residing in Florida for one year, she moved to Pineville, LA with her mother before moving to Texarkana, TX in 1999. As a youth, Jinger was extremely active and competitive in swimming as well as barrel racing and pole bending. Upon graduation from Pleasant Grove High School in 2003, she attended Louisiana State University where she earned a Bachelor of Science in Animal, Dairy, and Poultry Sciences in December of 2006. She then began graduate school at Auburn University working towards a Master of Science in Animal Sciences, concentrating on equine nutrition.

THESIS ABSTRACT

GLYCEMIC RESPONSE TO MEAL LENGTH IN HORSES

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Cereal grains are an ideal feedstuff for horses with high energy demands because of their high soluble carbohydrate content. However, consumption of a single meal high in soluble carbohydrates causes an immediate increase in blood glucose. This increase could be problematic for horses with certain health conditions such as insulin resistance or laminitis. Because some horses require additional energy for growth, performance, pregnancy or lactation, it becomes difficult to completely remove cereal grains from their diet. Therefore, new feeding management strategies are needed to attenuate the blood glucose response to meals high in soluble carbohydrates. The objective of this study was to assess the glycemic response to a concentrate meal based on time required to consume

that meal as measured by peak plasma glucose and insulin concentrations, glucose and insulin areas under the curve, and time to peak plasma glucose and insulin. Eight mature, idle horses were used in an experiment consisting of eight 7 d periods in which treatment combinations were systematically arranged in a 2 × 4 factorial of feeds and meal portions. Horses were offered approximately 4 Mcal of oats or textured sweet feed twice daily at 0600 and 1800 h. Horses were offered their respective concentrate in 1, 2, 3, or 4 equal portions in 15 min intervals, thereby restricting rate of intake of the concentrate. Blood was collected via indwelling jugular catheters at the evening feeding on d 7 of each period, with a baseline sample collected 30 min prior to feeding (1750 h), then every 30 min post-feeding until 0000 h. Plasma glucose and insulin concentrations were determined, and resulting data were analyzed by the GLM procedure of SAS. Time to peak plasma insulin was longer ($P < 0.05$) for horses consuming oats, but there were no other significant differences for concentrate type, portions, or treatment interactions for glucose and insulin. A period effect ($P < 0.05$) was noted for peak plasma glucose and time to peak plasma glucose. There was a trend ($P < 0.10$) toward a period effect for glucose area under the curve, but no period effects were observed for measures of insulin. A horse effect ($P < 0.05$) was also noted for peak plasma glucose and insulin as well as glucose and insulin area under the curve (AUC). Glucose and insulin responses to a concentrate meal were not altered by time to consume the meal in the present study.

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The author also wishes to express her gratitude for those who participated in the blood collection period of this project: Whitney Brown, Teresa Fenn, Judson “Kyle” Grubbs, Lindsay Henderson, Erica Hobbs, Mike Huggins, Grace Owen, Shayna Penn, William “Cobie” Rutherford, Jordan Towns, and Patty Tyler. Without their sacrifice, this project never could have been completed. She also wishes to thanks Ashley Bruce for her assistance with data input.

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Style manual or journal used: _____Journal of Animal Science_____

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REVIEW OF LITERATURE

Horses have evolved to be continuous grazers. Feral horses generally spend 65% of their time grazing low quality, high fiber forage (Laut et al., 1984). In the wild they have adapted to a diet consisting of forages with relatively large amounts of water, soluble proteins, lipids, sugars and structural carbohydrates, but very little starch (Frape, 2004). Domesticated horses, however, are often managed differently. Meal feedings generally are restricted for various reasons (most often being the caretaker's schedule), and increased energy requirements for production and/or performance often result in the feeding of high-concentrate rations of grains or grain by-products to horses (Massey et al., 1985), with pasture turnout restricted in many cases.

Common diets fed to horses seldom contain more than 5% fat and 7-12% protein (Frape, 2004), so these represent minor sources of energy relative to carbohydrates. Recently, however, some manufacturers have begun to develop higher-fat and fiber, lower-starch feeds that with time and further research could become the new trend for horse diets. Most of the energy in grains is typically found as starch, and a diet containing 30% starch (3.4 g starch/ kg BW/ meal) is generally considered to be a high-starch diet (NRC, 2007). Typical grain-based mixes can contain as much as 30-50% starch (NRC, 2007). In contrast to ruminants, horses are able to hydrolytically digest

and absorb digestive end-products prior to post-gastric fermentation (Argenzio and Hintz, 1970). Carbohydrates digested and absorbed as monosaccharides in the small intestine yield more energy for metabolism than those digested by microbial fermentation, with the amount of starch ingested in a single meal possibly affecting the percentage of starch that disappears before reaching the large intestine (NRC, 2007). Potter et al. (1992) reported that about 80% of the starch was digested and absorbed before it reached the terminal ileum when a small amount of oats was fed to horses. However, when a large amount of oats was fed, only around 58% of the starch was digested before reaching the ileum. If levels of dietary carbohydrates exceed the capacity of the equine small intestine to digest and absorb them, they will spill over into the hindgut. Potter et al. (1992) suggested that a starch intake of 3.5 to 4.0 g/kg BW is the upper limit for the small intestine for starch digestion. Starch spill-over can alter the microbial populations and the metabolic products of the cecum, and can predispose the horse to gastrointestinal disorders (Dyer et al., 2002). Any gut dysfunction in a horse is threatening because it can precede colic, which is the main cause of equine mortality (Hintz and Cymbaluk, 1994). A lower limit of starch intake has been suggested for feeding horses prone to developing laminitis (Frape, 2004).

Carbohydrate digestion and fermentation yield predominantly glucose and acetic, propionic and butyric volatile fatty acids (VFA), all of which are readily absorbed into the equine bloodstream. It has been suggested that the proportion of digestive end-products absorbed as glucose or VFA and lactic acid may be influenced by the extent of prececal, or maybe even preileal, digestion (Frape, 2004). Glinsky et al. (1976) indicated that VFA from the cecum may be sufficient to supply up to 30% of a horse's energy

needs at maintenance, while Vermorel et al. (1997) proposed that consuming a diet composed primarily of hay might meet more than 80% of the energy needs from VFA. However, it is important to note that not all carbohydrates are fermented at the same rate or produce the same proportions of VFA (NRC, 2007).

Glucose is the principle form of carbohydrate used to produce ATP. Cells can acquire glucose from the circulation or from intracellular glycogen stores. Because of this, healthy horses and ponies typically sustain a blood plasma glucose concentration within certain limits. Ralston (2002) indicated that fasting blood plasma glucose concentrations in horses are usually between 60 and 90 $mg \cdot dL^{-1}$, while fasting blood plasma insulin concentrations are typically between <5 and 20 µIU. This blood glucose concentration is the expression of a balance of glycogen breakdown (glycogenolysis) and glycogen synthesis (glycogenesis), and the production of glucose from other sources such as amino acids, lactic acid and propionate through gluconeogenesis. Red blood cells lack mitochondria, so they are totally dependent on substrate-level phosphorylation. As a result, these cells depend almost entirely on glucose as a source of energy producing lactate as an end-product of glycolysis. The clearance of glucose from blood results mainly from uptake by the liver and muscle cells, where it is then converted to glycogen and fat (Frape, 2004). The muscle is conservative with its glycogen reserves such that glycogen is only available to the muscle as a fuel source, not to other tissues. However, glycogen stored in the liver may be broken down to allow glucose utilization by other tissues (NRC, 2007).

The process of converting blood glucose into glycogen in various body tissues is stimulated by the anabolic hormone insulin, which activates enzymes directed towards

storage in response to a rise in blood glucose. Inversely, insulin inhibits glucose breakdown. By stimulating uptake in tissues, insulin prevents glucose from being excreted in urine, thus lowering blood glucose concentration. Insulin also enhances fat metabolism, or lipolysis, by stimulating lipoprotein lipase in the adipose tissue. The effects of insulin are counterbalanced by other hormones such as glucagon, glucocorticoids, catecholamines, epinephrine and norepinephrine (Frape, 2004). In this manner, the system is maintained in a state of dynamic equilibrium. The type of diet consumed can influence fluctuations between resting and peak levels of glucose (and insulin). Equine diets containing more grain and less roughage tend to lead to higher peaks and lower troughs. Gordon and McKeever (2005) suggested that the concurrent ingestion of high-fiber hay might actually slow down the absorption of glucose in the gastrointestinal tract, as illustrated by the lack of a substantial postprandial increase in both glucose and insulin.

There are two procedures commonly used to evaluate glucose response in horses. The first is known as the glucose tolerance test, which measures plasma glucose and insulin responses to a glucose challenge (oral or intravenous). This technique provides information about the animal's glucose metabolism. Another process, known as the glycemic index, measures plasma glucose and insulin responses to a meal. This method provides information about the feed, but not necessarily the animal.

The glycemic index has been widely used in human nutrition, especially for diabetics, to formulate diets that have a low glycemic impact (Wolever et al., 1991). In horses, it has been used to describe meal-related responses of blood glucose and insulin to different diets (Stull and Rodiek, 1988; Williams et al., 2001). The glycemic index has

been defined as the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of test food, expressed as a percentage of the blood glucose response curve to the same amount of carbohydrate from a standard food consumed by the same individual (FAO/WHO, 1998). For humans, the 50 g carbohydrate portion should contain 50 g of available carbohydrate, with the standard food being either white bread or glucose (FAO/WHO, 1998). The methods used in equine studies have been extremely variable, making it difficult to interpret results across the board (NRC, 2007). Factors affecting glycemic index include meal size, concentrations of hydrolyzable carbohydrates, fat and fiber, processing, intake time, gastric emptying, digestibility and rate of absorption (Hoffman et al., 2003b). A glycemic index developed for horses and ponies needs to account for feed differences when they are mixed with other ingredients (i.e., not fed alone) because this method is routine in the equine industry.

Plasma glucose response to a meal is generally measured as the area under the curve (AUC). The more rapidly glucose is cleared (the greater the tolerance), the smaller the area. Horses and ponies tend to have a lower glucose tolerance than humans or pigs, but a slightly greater one than ruminants. However, there are variations in tolerance among equines. For example, some hot-blooded horses such as Thoroughbreds generally have a higher glucose tolerance than ponies because ponies tend to secrete less insulin and their tissues may be less sensitive (more resistant) to insulin (Frape, 2004), although there can be considerable adaptation to diets.

Glucose conservation has been shown to be favored when animals are deprived of glucogenic precursors, such as with feed restriction (Waghorn et al., 1987). The horse may change its pattern of glucose metabolism so that it partially relies on the metabolism

of other substrates (Evans, 1971). When they are well fed, less adaptation to glucose conservation may occur (Powell et al., 2000). Therefore, a more rapid decline in plasma glucose concentrations may occur when adequate intakes are provided (McNiven, 1984). For example, when subjected to a glucose infusion, fasted ponies were less sensitive to the action of insulin than normally fed ponies. A larger insulin response was necessary to affect glucose uptake (Argenzio and Hintz, 1970; 1971). Powell et al. (2000) also suggested that diet adaptation may play a role. These researchers indicated that, during short-term feed restriction, glucose uptake could be slowed following feeding in working horses adapted to a high-roughage diet. Sticker et al. (1995) showed that mares on long-term dietary energy and protein restriction were able to gradually adjust to that restriction by increasing plasma glucose concentrations, regardless of intake. Jacobs and Bolton (1992) indicated that horses adapted to pasture had a higher response to an oral glucose dose and 1.8 times as much glucose AUC than horses fed a typical stable diet of hay and commercial feed. The horses fed the stable diet consumed about 3.5 times as much hydrolyzable carbohydrate as the pasture horses, resulting in a higher glycemic index that influenced their response to the oral glucose challenge. An adaptation to meals with a higher glycemic index may have enhanced the ability of the stabled horses to clear glucose at a much faster rate than the horses accustomed to a natural pasture grazing pattern. Also, Ralston (2002) hypothesized that the time between feedings, and possibly the fasting that occurs before a feeding, can affect glucose and insulin responses.

Despite appearing healthy, horses adapted to high-glycemic feeds may exhibit changes in altered insulin sensitivity and compensation. Diets rich in simple sugars have been associated with insulin resistance in several animal and human studies (Bessesen,

2001; Storlien et al., 2000), so the common management practice of feeding starch-rich cereal grains in two meals per day may actually promote insulin resistance in some horses.

While horses very rarely develop insulin-dependent diabetes, noninsulin-dependent diabetes (insulin resistance) does occur. Insulin resistance has been generally defined as a state in which normal concentrations of insulin fail to elicit a normal physiological response (Kahn, 1978), and is fundamental in the pathology of type II diabetes in humans. Resistance can refer to inefficient insulin signaling at the cell surface (low insulin sensitivity) or disruption of insulin signaling pathways within the cell (insulin ineffectiveness) (Kronfeld, et al., 2005). Insulin resistance has been associated with a variety of equine disorders, such as obesity and laminitis (Jeffcott et al., 1986; Pass et al., 1998), and may play a role in colic (Hudson et al., 2001), exertional rhabdomyolysis (Valentine et al., 2001), and osteochondrosis dissecans (Ralston, 1996).

Frost et al. (1996) demonstrated that consumption of a diet with a low glycemic index appeared to elevate insulin sensitivity in human heart-disease patients. Ingestion of another low-glycemic-index diet resulted in a higher disposition index (an index that describes ß-cell responsiveness and accounts for the influence of both endogenous insulin secretion and insulin sensitivity, Hoffman et al., 2003a) and tended to improve insulin sensitivity in humans with insulin resistance (Wolever and Mehling, 2002).

Adaptation to a high-glycemic diet is associated with increased insulin resistance and a compensatory increase in insulin secretion (Treiber et al., 2005). A study by Hoffman et al. (2003a) used the minimal model, a mathematical tool that provides a quantitative measure of insulin sensitivity, to examine glucose-insulin dynamics in obese

vs. non-obese geldings. Results showed that obese geldings were insulin resistant, indicating that they seemed to rely primarily on glucose-mediated glucose disposal. The researchers also noted that feeding a diet rich in sugar and starch decreased the insulin sensitivity of all horses (obese and non-obese), with that sensitivity approximately 80% lower in obese horses. These results are similar to a reported 76% reduction in insulin sensitivity in obese vs. normal-weight humans (Lee et al., 1992). The horses also had lower acute insulin responses to glucose (endogenous insulin secretion in response to a glucose dose) and lower disposition indices when fed a high sugar-starch diet. The lower disposition index suggests less ß-cell responsiveness. This study ultimately demonstrated that the maintenance of body condition and avoidance of grain-based meals rich in sugar and starch should be beneficial in decreasing the risk of developing insulin resistance and associated metabolic disorders in horses, especially for horses at risk for these conditions.

Because of the innate differences in grains commonly fed to horses, the availability of glucose after ingestion of a grain meal may vary. Previous studies performed in horses have compared the effect of ingestion of varying amounts of grains or concentrates on plasma glucose and serum insulin concentrations (Pagan et al., 1999; Ralston, 1992; Stull and Rodiek, 1988; Williams et al., 2001). These studies showed that ingestion of grain or concentrate meals results in moderate to marked hyperglycemia and hyperinsulinemia between 1 and 3 hours after eating. However, these studies compared ingestion of different grains or concentrates as equal-weight or isocaloric meals, which did not result in ingestion of equal amounts of starch and sugar.

Several studies using horses or ponies with ileal or cecal fistulae have determined that small-intestinal starch digestibility depends on its botanical origin and prior physical

or thermal treatment (Healy et al., 1995; Meyer et al., 1993). De Fombelle et al. (2004) indicated that prececal starch disappearance was most affected by the botanical source of starch. Still, there is conflicting evidence for the difference in small-intestinal starch digestibility among various cereal grains such as corn, oats, and barley (De Fombelle et al., 2001; Meyer et al., 1993; Potter et al., 1992; Radicke et al., 1991). Despite possible differences in starch digestibility among different grains observed in previous studies, the glycemic response assessed as glucose AUC did not differ among corn, oat groats, or barley in comparison to an intragastric glucose infusion based on equal amounts of hydrolyzable carbohydrates in a study by Jose-Cunilleras et al. (2004). Plasma glucose concentration peaked in all 4 treatments by 1.5 to 2 hours after feeding, and remained higher than baseline throughout 8 hours for oat groats and barley. For corn-fed and glucose-administered horses, plasma glucose returned to baseline by 5 to 6 hours post-feeding. The shape of the glycemic responses after glucose administration and corn ingestion were similar and had larger fluctuations in plasma glucose compared with oats and barley. It is important to note that the rate of ingestion was unequal among the grains. A slower meal consumption in oat groats-fed horses than corn-fed horses may explain the lower glycemic peak and extended clearance time for oat groats compared with corn.

The normal feeding pattern of horses and ponies is one of small meals at frequent intervals. In today's equine industry, however, many horses consume grain meals once or twice daily. Steelman et al. (2006) performed a study in which horses received the same amount of concentrate per day, allocated into meals fed 2, 3, or 4 times per day. Results showed that peak glucose concentration, mean concentrations of glucose, as well as glucose AUC were all greater in horses fed twice daily. No differences were observed

between the 3× and 4× daily schedules, which indicates that the larger amounts of concentrate fed per meal twice daily resulted in a larger plasma glucose response than the smaller amounts fed either three or four times per day. Smaller, more frequent meals allow the small intestine to continually, and maximally, digest and absorb carbohydrates resulting in a more constant glycemic response. Large, infrequent meals can overload the capacity of the small intestine, causing an excess of undigested carbohydrate to enter the cecum where the microflora convert it to VFAs and lactic acid. This meal situation results in a more dynamic glycemic response with rapid, high peaks and broad, low troughs.

Considering the negative consequences and disadvantageous economic costs of overfeeding starch, it is valuable to establish what levels can be safely fed in a meal-feeding situation. Trials conducted with different sizes of horses and ponies ingesting various levels of starch suggest that single-meal starch intakes exceeding 0.2 to 0.4% of body weight vastly increase the amount of starch presented to the cecum and large intestine (Potter et al., 1992; Meyer et al., 1993; Kienzle, 1994). High-quality forage diets and diets containing significant amounts of digestible fiber and added fat reduce the need for starch as an energy source (NRC, 2007). Results from a study by Treiber et al. (2005) showed a tendency for a lower glucose response to fat-added feed vs. a traditional starch concentrate over 30 days.

The glycemic responses to the ingestion of different diets in horses are complex. Blood glucose and insulin levels appear to be alterable through dietary manipulation, but individual horse responses to meal-fed concentrates will vary. Additional influences, such as the form and processing of feedstuffs, will alter the suggested recommendations for

limiting the starch levels of meals, as well as the amounts fed per meal and the meal frequency. Because some horses require additional energy for growth, performance, pregnancy or lactation, it becomes difficult to completely remove cereal grains from their diet. Therefore, new feeding management strategies are needed to attenuate the blood glucose response to a soluble carbohydrate-rich meal.

If daily schedules of horse owners are highly restricted, it is oftentimes most feasible to meal-feed horses once or twice per day. These meals are generally large, concentrate meals. As previously stated, these large, infrequent meals are not the best feeding protocol for horses in regard to glycemic and insulinemic responses. The objective of this study was to use concentrate feeds that are commonly fed to horses to assess the glycemic and insulinemic responses to a meal based on the time allowed to consume that particular meal. Perhaps by reducing the rate of intake of a large concentrate meal, the caretaker could easily prevent extremely dynamic glycemic and insulinemic responses.

MATERIALS AND METHODS

Eight mature, idle horses were used in the present study. Ages ranged from 4 to 14 yr, with an average of 6.25 yr. Five were American Quarter Horses (four mares and one gelding), one mare was an Arabian, one gelding was an Appaloosa, and one gelding was an Arabian x Appaloosa cross. Body weights (BW) averaged 504 ± 82 kg. The animals were maintained at the Auburn University Horse Unit according to a protocol that was approved by the Institutional Animal Care and Use Committee. Horses had access to pasture containing predominantly Coastal bermudagrass (*Cynodon dactylon)*, and were allowed *ad libitum* access to clean, fresh water with meal feedings twice daily.

Initially, horses were randomly allocated to different treatments for the first week of the project, and then systematically assigned to a 2 × 4 factorial arrangement of feeds and meal portions consisting of eight 7 d periods (Appendix 1). Approximately 4 Mcal DE of Nutrena® Racehorse Oats or Nutrena® Triumph® textured sweet feed (Appendix 2) was offered twice daily at 0600 and 1800 h. The approximate 8 Mcal DE fed per day represents roughly half of the daily Mcal DE required by a 500 kg adult horse at maintenance (NRC, 2007). The amount of feed offered was calculated from DE content based on vendor information for the sweet feed and tabular values for oats (NRC, 2007). The textured sweet feed contained approximately 10% CP, 4% crude fat, and 8% crude

fiber according to the manufacturer's label. Horses were placed in individual stalls and offered their respective meal in 1, 2, 3, or 4 equal portions in 15 min intervals.

On d 7 of each period, horses were fasted in individual stalls for 6 h beginning at 1200 h prior to feeding the test diet. They were allowed ad libitum access to fresh water. Jugular-vein catheters were inserted 2 h prior to the initial baseline blood collection. Prior to insertion, the hair on the collection area of the neck was shaved with standard grooming clippers, and the insertion site was disinfected using a 3 × 3 surgical scrub procedure with Betadine® and isopropyl alcohol. One and one-half cc of Lidocaine® was administered subcutaneously at the catheter-insertion site. A 14GA × 140mm jugular-vein catheter (Abbocath®-T Radiopaque FEP I.V. Catheter) was inserted, and an extension set (Hospira® 7in extension set with Option-Lok®) attached. Super glue® was used to anchor the catheter port to the animal's neck. Gauze squares were placed over the insertion site, and Elasticon® elastic tape was wrapped around the horse's neck to ensure that the catheter and extension set remained clean and in place.

A baseline blood sample was collected at 1730 h, 30 min prior to the initial evening feeding at 1800 h, with sequential samples collected every 30 min after the initial feeding for 6 h (1830, 1900, 1930, 2000, 2030, 2100, 2130, 2200, 2230, 2300, 2330, and 0000 h). Five cc of blood was collected and discarded, followed by collection of 20 cc. The extension set and catheter were then flushed with 7 cc of a heparinzed saline solution (0.9 % NaCl) to prevent the formation of blood clots. Each blood sample was immediately transferred into 2 tubes containing a glycolytic inhibitor (sodium fluoride) and 2 tubes with sodium heparin for analysis of glucose and insulin concentrations, respectively.

Blood samples were cooled in ice water for 15 min prior to centrifugation. An IEC® Centra CL2 (Thermo Electron Corp., Milford, MA) centrifuge was used to spin samples for 10 min at 1560 × g. Plasma was separated into microcentrifuge tubes and frozen at -20°C prior to laboratory analyses of glucose and insulin concentrations.

Plasma samples were thawed at room temperature. Glucose was analyzed using an automated glucose and L-lactate analyzer (YSI 2300 Stat Plus Analyzer, Yellow Springs, OH), while insulin samples were analyzed using radioimmunoassay (Coat-A-Count Insulin, Dept. of Anatomy and Physiology and Pharmacology, Auburn University College of Veterinary Medicine). Duplicates were run of each sample for each assay.

On each blood collection day, samples of each feed were collected for subsequent laboratory analysis. DM was determined using the procedure of the Association of Official Analytical Chemists (1995) in which samples were dried in an oven at 65°C for 72 h. Samples were ground using a Wiley Mill with a screen size of 1.0 mm. Concentrations of NDF and ADF were determined by sequential fractionation using a heat-stable amylase according to procedures of Van Soest et al. (1991). Megacalories of DE were then calculated for each concentrate feed using the following formula (NRC, 2007).

$$\text{DE (Mcal/kg)} = 4.07 - 0.055 \times (\%\text{ADF})$$

Data for blood plasma values were analyzed using the Proc GLM procedure of SAS with classes of horse, period, feed, and portion, using a $P < 0.05$ level of significance to determine differences in the peak concentration, time to peak

concentration, and area under the curve (AUC) for both glucose and insulin. AUC was calculated using the trapezoidal method. A simple one-way ANOVA was used to analyze data from the feed samples with a $P < 0.05$ level of significance.

RESULTS AND DISCUSSION

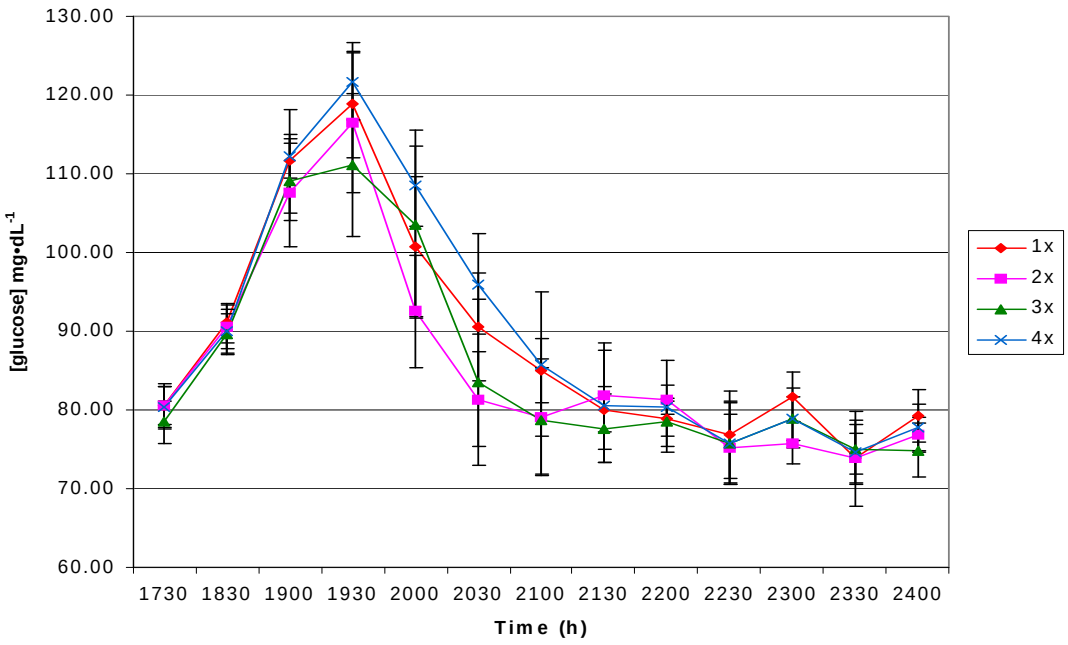
One horse did not consume its experimental diet during the first wk of the project, so its treatment was repeated for that particular mare on wk 9 of the project. In the statistical analysis, data from wk 9 was treated as if from wk 1 for this particular horse. Another horse had to be sedated with xylazine for catheterization throughout the study. This horse was catheterized first (approximately 5 h before the baseline sample) to allow sufficient time for effects of the xylazine to dissipate prior to blood collection. All horses consumed their meals in fewer than 10 min for all feedings throughout the study. Except for the one horse during wk 1, there were no feed refusals during the course of the study.

Laboratory analysis of experimental feeds revealed that estimated DE consumed per meal differed ($P < 0.05$) between oats and sweet feed (Appendix 3). Horses fed oats consumed 4.1 Mcal DE per meal, whereas those fed sweet feed consumed 4.5 Mcal DE. Although these values are dissimilar, they only differ from their mean by less than 5%.

No difference ($P < 0.05$) was found for peak glucose concentration based on the number of portions fed or concentrate type (Figures 1 and 2). For time to peak glucose, there was no difference among the number of portions or type of concentrate consumed. Glucose AUC ($mg \cdot dL^{-1} \cdot h^{-1}$) also was not different among meal portion offered or type of concentrate fed.

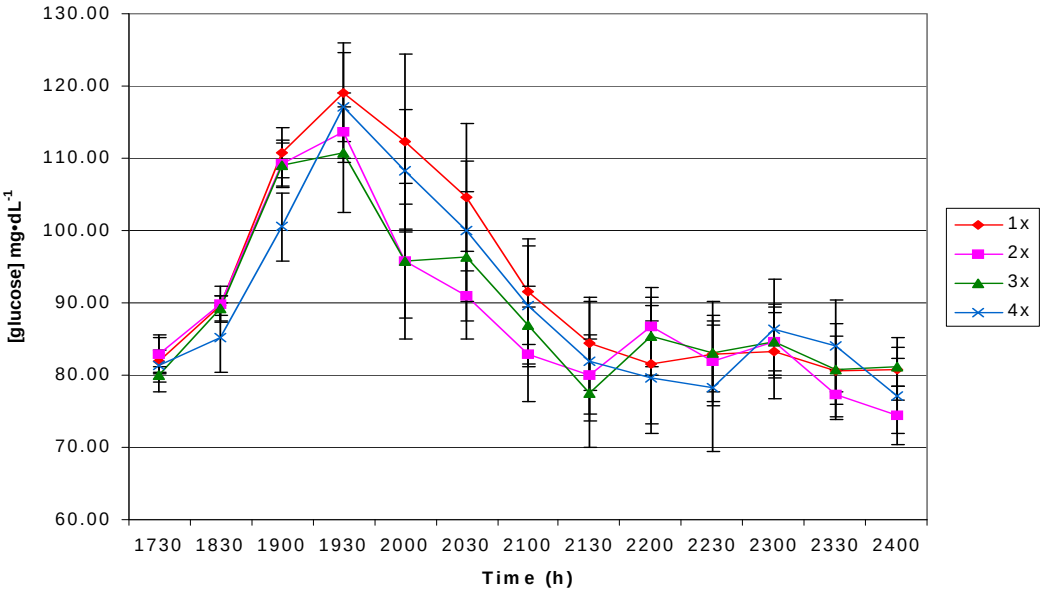
There was no difference for peak insulin concentration ($\mu U \cdot mL^{-1}$) throughout the study based on the number of portions offered or type of concentrate fed (Figures 3 and 4). For time to peak insulin, there were no differences among portions fed. However, there was a difference ($P < 0.05$) between oats and sweet feed for time to peak insulin concentration, with oats having a longer time to peak. Portions and concentrate type did not differ for insulin AUC ($\mu U \cdot mL^{-1} \cdot h$).

Figure 1. Mean plasma glucose concentrations for horses consuming a meal* of sweet feed offered in 1, 2, 3, or 4 equal portions.



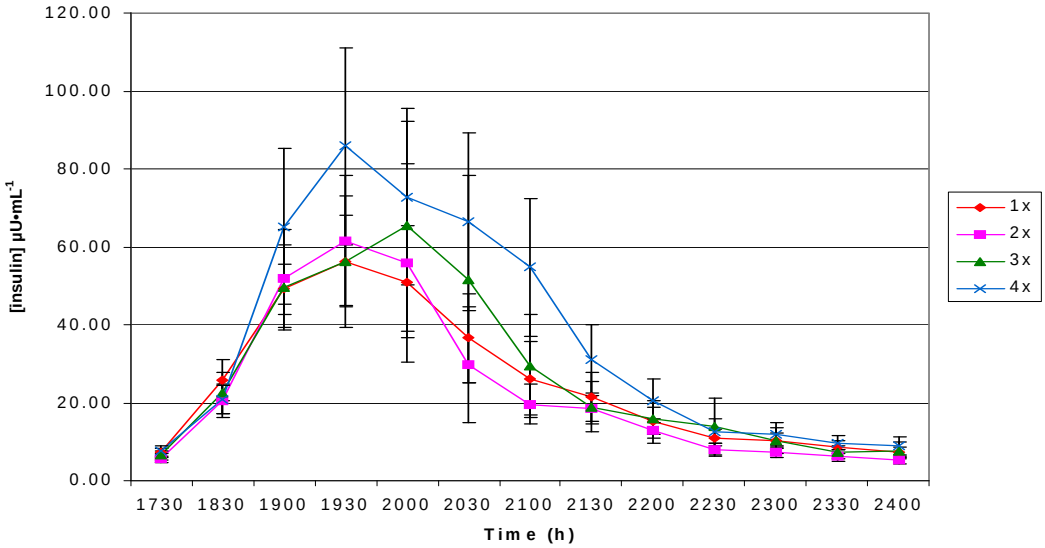
*The initial offering of concentrate occurred at 1800 hrs.

Figure 2. Mean plasma glucose concentrations for horses consuming a meal* of oats offered in 1, 2, 3, or 4 equal portions.



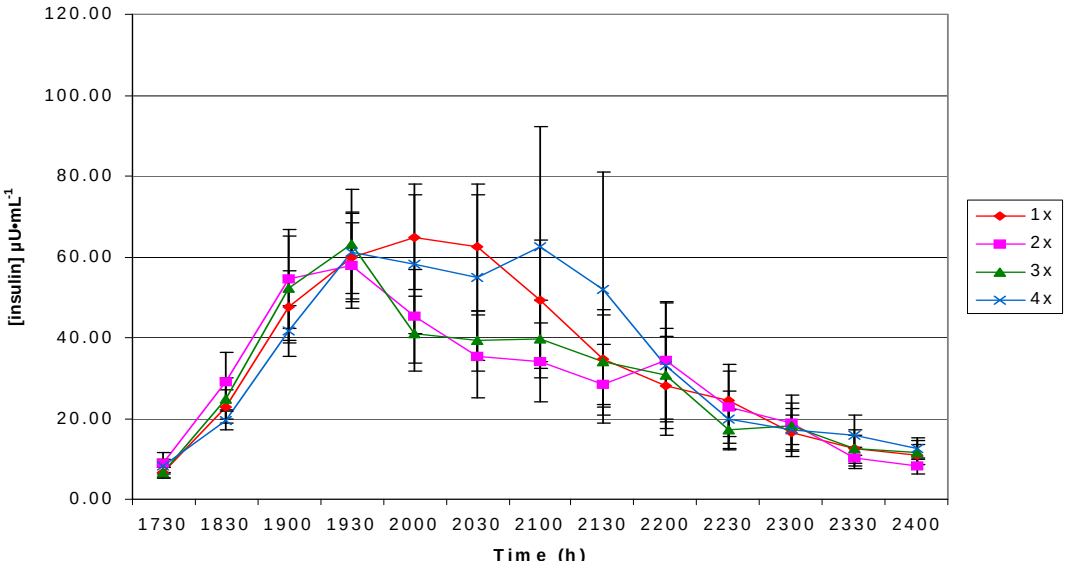
*The initial offering of concentrate occurred at 1800 hrs.

Figure 3. Mean plasma insulin concentrations for horses consuming a meal* of sweet feed offered in 1, 2, 3, or 4 equal portions.



*The initial offering of concentrate occurred at 1800 hrs.

Figure 4. Mean plasma insulin concentrations for horses consuming a meal* of oats offered in 1, 2, 3, or 4 equal portions.



*The initial offering of concentrate occurred at 1800 hrs.

Peak concentrations of glucose and insulin were not altered by time allowed to consume a concentrate meal in the present study (Tables 1 and 2). Times to peak concentrations of glucose and insulin were not affected by time allowed to consume a concentrate meal (Tables 3 and 4). However, time to peak plasma insulin was longer ($P < 0.05$) for horses consuming oats than sweet feed (Table 4). The area under the time × concentration curve for glucose and insulin was not affected by time to consume a concentrate meal (Tables 5 and 6). These results indicate that extending concentrate intake up to 1 h was not sufficient to alter measures of glycemic response in the present study.

Table 1. Mean peak glucose concentrations ($mg \cdot dL^{-1}$) for horses consuming a meal of oats or sweet feed offered in 1, 2, 3, or 4 equal portions.

# Portions	Oats		Sweet Feed		Mean[1]	
	LSMean	SE	LSMean	SE	LSMean	SE
1	126.13	± 4.01	122.14	± 4.01	124.13	± 2.83
2	115.75	± 4.01	120.55	± 4.01	118.15	± 2.83
3	118.25	± 4.01	116.23	± 4.01	117.24	± 2.83
4	124.76	± 4.01	122.88	± 4.01	123.82	± 2.83
Mean[2]	121.22	± 2.00	120.45	± 2.00		

[1] Mean of oats and sweet feed based on portion.
[2] Mean of portions based on concentrate type.

Table 2. Mean peak insulin concentrations ($\mu U \cdot mL^{-1}$) for horses consuming a meal of oats or sweet feed offered in 1, 2, 3, or 4 equal portions.

# Portions	Oats		Sweet Feed		Mean[1]	
	LSMean	SE	LSMean	SE	LSMean	SE
1	77.60	± 12.28	62.20	± 12.28	69.90	± 8.68
2	65.49	± 12.28	78.63	± 12.28	72.06	± 8.68
3	66.99	± 12.28	74.16	± 12.28	70.58	± 8.68
4	82.78	± 12.28	89.03	± 12.28	85.90	± 8.68
Mean[2]	73.21	± 6.14	76.00	± 6.14		

[1] Mean of oats and sweet feed based on portion.
[2] Mean of portions based on concentrate type.

Table 3. Mean time to peak glucose concentrations (h) for horses consuming a meal of oats or sweet feed offered in 1, 2, 3, or 4 equal portions.

# Portions	Oats		Sweet Feed		Mean[1]	
	LSMean	SE	LSMean	SE	LSMean	SE
1	1.94	± 0.23	1.94	± 0.23	1.94	± 0.17
2	2.00	± 0.23	2.19	± 0.23	2.09	± 0.17
3	1.94	± 0.23	2.31	± 0.23	2.13	± 0.17
4	2.81	± 0.23	2.13	± 0.23	2.47	± 0.17
Mean[2]	2.17	± 0.12	2.14	± 0.12		

[1] Mean of oats and sweet feed based on portion.
[2] Mean of portions based on concentrate type.

Table 4. Mean time to peak insulin concentrations (h) for horses consuming a meal of oats or sweet feed offered in 1, 2, 3, or 4 equal portions.

# Portions	Oats		Sweet Feed		Mean[1]	
	LSMean	SE	LSMean	SE	LSMean	SE
1	2.44	± 0.30	1.81	± 0.30	2.13	± 0.21
2	2.69	± 0.30	2.00	± 0.30	2.34	± 0.21
3	2.38	± 0.30	2.06	± 0.30	2.22	± 0.21
4	2.63	± 0.30	2.44	± 0.30	2.53	± 0.21
Mean*[2]	2.53	± 0.15	2.08	± 0.15		

* Means in row differ ($P < 0.05$)
[1] Mean of oats and sweet feed based on portion.
[2] Mean of portions based on concentrate type.

Table 5. Glucose AUC ($mg \cdot dL^{-1} \cdot h^{-1}$) for horses consuming a meal of oats or sweet feed offered in 1, 2, 3, or 4 equal portions.

# Portions	Oats		Sweet Feed		Mean[1]	
	LSMean	SE	LSMean	SE	LSMean	SE
1	598.86	± 14.07	576.03	± 14.07	587.44	± 9.95
2	575.59	± 14.07	557.94	± 14.07	566.77	± 9.95
3	579.04	± 14.07	558.59	± 14.07	568.81	± 9.95
4	585.29	± 14.07	580.65	± 14.07	582.97	± 9.95
Mean[2]	584.70	± 7.03	568.30	± 7.03		

[1] Mean of oats and sweet feed based on portion.
[2] Mean of portions based on concentrate type.

Table 6. Insulin AUC ($\mu U \cdot mL^{-1} \cdot h^{-1}$) for horses consuming a meal of oats or sweet feed offered in 1, 2, 3, or 4 equal portions.

# Portions	Oats		Sweet Feed		Mean[1]	
	LSMean	SE	LSMean	SE	LSMean	SE
1	220.06	± 27.69	165.12	± 27.69	192.54	± 19.58
2	195.92	± 27.69	149.33	± 27.69	172.62	± 19.58
3	195.07	± 27.69	176.75	± 27.69	185.91	± 19.58
4	227.03	± 27.69	229.38	± 27.69	228.20	± 19.58
Mean[2]	209.52	± 13.85	180.12	± 13.85		

[1] Mean of oats and sweet feed based on portion.
[2] Mean of portions based on concentrate type.

A period effect ($P < 0.05$) was found for peak glucose concentration and time to peak glucose concentration. There was also a trend toward a period effect ($P < 0.10$) for glucose AUC. No period effects were observed for any measures of insulin. The observed period effect for glucose was not surprising given the study took place over the duration of eight weeks. While environmental factors were controlled as best as possible, they could have affected glucose responses by increasing cortisol levels as a result of stress from daily activity around the barns or inclement weather.

A horse effect ($P < 0.05$) was found for peak glucose concentration and glucose AUC. There was also a horse effect for peak insulin concentration and insulin AUC. The observed horse effect was expected, as there is always individual variation among subjects. However, it is interesting to note that there was no subject variation ($P < 0.05$) for times to peak glucose or insulin.

One factor that may have affected the results of this study is cortisol. Research has shown that cortisol displays a normal diurnal rhythm with concentrations highest in early morning and lowest in the evening (Irvine and Alexander, 1994; Cartmill et al., 2003; Gordon and McKeever, 2005, 2006; Storer et al., 2007). This study utilized evening blood collections, and it would be expected to see declining cortisol concentrations during this time of day. However, cortisol is known as the “stress hormone” because its concentrations increase during stressful circumstances. Activity around the barn or catheterization could have stressed the horses, causing an increase in cortisol concentrations. Ralston (2002) indicated that the glycemic response to a meal is dramatically influenced by circulating cortisol concentrations in horses. Because cortisol indirectly leads to an increase in circulating blood glucose, this could have affected the

results. Perhaps utilizing morning blood draws would have somewhat countered this effect. If cortisol concentrations are already peaking, stress may not influence blood glucose concentrations as much. It might have also been beneficial to insert catheters the night prior to a morning feeding and blood collection. This would not only allow horses to adapt to their stalled environment, but also allow their stress from catheterization to decrease overnight.

It has been suggested that time of day is a factor when evaluating glycemic and insulinemic responses (Williamson et al., 2008). Ralston (2002) indicated that horses had lower ($P < 0.005$) insulin responses in the afternoon than in the morning, even though glucose concentrations did not differ. Follow-up studies found that the length of fast and plasma cortisol concentrations were more highly correlated with the associated glucose and insulin responses to a meal of grain than the actual time of day. It has also been suggested that horses are much more stressed during a daytime fast verses a nighttime fast. An attempt should be made to avoid any stimuli that could arouse behavioral or physiological effects that could cause a defensive endocrine response (Gordon and McKeever, 2006).

CONCLUSIONS

The hypothesis that time to consume a concentrate meal would alter the glycemic response to that meal was not validated in the present study. Neither the number of portions fed, or concentrate type had any significant effects on peak glucose concentration ($mg \cdot dL^{-1}$), time to peak glucose concentration (h), or glucose AUC ($mg \cdot dL^{-1} \cdot h^{-1}$). There were also no significant differences for peak insulin concentration ($\mu U \cdot mL^{-1}$) or insulin AUC ($\mu U \cdot mL^{-1} \cdot h^{-1}$) based on portions fed or type of concentrate. However, there was a significant difference ($P < 0.05$) for time to peak insulin (h) between concentrate type, with oats having a longer time to peak that sweet feed.

These results indicate that extending concentrate intake up to 1 h was not sufficient to alter measures of glycemic response in the present study. It is possible that the amount of concentrate fed per meal was not sufficient to overload the capacity of the equine small intestine for starch digestion and absorption. Perhaps by increasing the amount of concentrate fed per meal or extending the amount of time allowed to consume a given meal, the glycemic response would be alterable.

Low-glycemic diets based on the glycemic index (GI) have been recommended for the avoidance and management of a variety of diseases in humans that involve insulin resistance, such as diabetes mellitus type II and coronary heart disease. Further research is needed to examine the GI of feedstuffs commonly fed to horses. This will aid in the

control of a marked postprandial hyperglycemia and hyperinsulinemia, which can be devastating to horses with certain chronic metabolic problems associated with insulin resistance, as well as digestive disturbances related to rapid fermentation of starch in the hindgut.

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APPENDICES

APPENDIX 1

Feeding rotations throughout the duration of the study.

Week	Oats				Textured Sweet Feed			
	1×	2×	3×	4×	1×	2×	3×	4×
1	Rose	Flaxen	Cricket	Roan*	Ellie	Casper	Moon	Blue
2	Blue	Rose	Flaxen	Cricket	Roan	Ellie	Casper	Moon
3	Moon	Blue	Rose	Flaxen	Cricket	Roan	Ellie	Casper
4	Casper	Moon	Blue	Rose	Flaxen	Cricket	Roan	Ellie
5	Ellie	Casper	Moon	Blue	Rose	Flaxen	Cricket	Roan
6	Roan	Ellie	Casper	Moon	Blue	Rose	Flaxen	Cricket
7	Cricket	Roan	Ellie	Casper	Moon	Blue	Rose	Flaxen
8	Flaxen	Cricket	Roan	Ellie	Casper	Moon	Blue	Rose
9				Roan*				

*This horse did not consume the treatment diet during week 1, so this horse × diet was repeated on week 9.

APPENDIX 2

Ingredient composition of Nutrena® Triumph® textured sweet feed.

Ingredient
Whole oats*
Wheat midds*
Winterlass (molasses)
Corn chops
Corn germ
Crimped oats
Peanut hulls
$CaCO_3$
Salt
Cottonseed meal
Calcium propionate
Trace mineral premix
Vitamin A

*Together, whole oats and wheat midds comprise over half of the total feed composition.

APPENDIX 3

Laboratory analyses for oats and sweet feed.

Feed	As-fed intake (kg)	DM (%)	ADF (% DM)	DE/kg (% DM)	DE (Mcal/kg)
Oats	1.37	89.79	13.67	3.32	4.1*
Sweet feed	1.36	87.67	5.38	3.77	4.5*

*$P < 0.05$