

ABSTRACT

KUTZNER-MULLIGAN, JENNA MARIE GILCHRIST. The Effect of Different Feed Delivery Methods on Time to Consume Feed and the Resulting Changes in Post-prandial Metabolite Concentrations in Horses. (Under the direction of Dr. Shannon Pratt-Phillips.)

Management techniques that reduce the insulin response to feeding in horses have application in preventing insulin resistance (IR) and potential associations (e. g., laminitis). Eight mature idle horses of BCS between 5 and 6.5 and with no previous indication of IR were fed a meal of concentrate feed under 4 feed delivery treatments in a repeated Latin Square design. Treatments were all based on a bucket of equal dimensions. The treatments included a control (CON) and 3 treatments hypothesized to increase time to consume feed (TIME_{feed}): mobile obstacles above the feed (BALL), stationary obstacles below the feed (WAFF), and feed with water added (WTR). Jugular venous blood samples were taken at feed delivery, every 10 min for the first hour, and then every 30 min until 300 min post feed delivery. TIME_{feed} was greater ($P = 0.004$) for BALL and WAFF when compared to CON and WTR. Compared to CON and WTR, average glucose and insulin concentrations tended to decrease due to BALL ($P = 0.059$) and WAFF ($P = 0.072$) and the peak glucose concentration was decreased ($P = 0.049$). Compared to all other treatments, peak insulin concentrations ($P = 0.030$) and area under the curve of insulin ($P = 0.051$) were decreased and time to peak insulin was increased ($P = 0.031$) due to BALL. Therefore, feed delivery methods that include obstacles effectively increase TIME_{feed} and attenuate post-prandial glucose and insulin parameters. A second experiment was designed to determine if the TIME_{feed} changes associated with BALL and WAFF in Exp. 1 remain effective over 4-d periods. Four horses with no recent or regular history of consuming meals were fed

concentrate meals for 4 consecutive days using the same treatments described in Exp. 1 under a Latin Square design. Horses were subject to a 4-d adaptation period (ADP) and were randomly assigned to 4-d treatment periods using the 4 previously described treatments. During ADP, TIMEfeed decreased ($P = 0.018$). However, following adaptation, TIMEfeed did not decrease significantly over 4 days of any treatment feed delivery method, but BALL and WAFF had higher TIMEfeed when compared to CON and WTR ($P < 0.001$) and maintained prolonged TIMEfeed after 4 d of use ($P = 0.006$). Utilizing obstacles to increase TIMEfeed on a daily basis may be an effective method to reduce post-prandial glucose and insulin concentrations, thereby decreasing the risk of insulin resistance development in horses

The Effect of Different Feed Delivery Methods on Time to Consume Feed and the Resulting
Changes in Post-prandial Metabolite Concentrations in Horses

by
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DEDICATION

This thesis is dedicated to my husband Ryan, who has quietly listened to more about the deep recesses of digestion... and statistics... than he ever wanted to know; my mom Susan, who believes in what I'm doing just because I'm doing it; my dad Morgan, who gladly humors my physio-pathological theories; my brother Jarrod, who tells people I'm a scientist now; my pug Jazzira, who has patiently "helped" me study for countless hours; and my horse Silverado, who has remained my stead-fast inspiration to use my life to make a difference in the lives of animals.

"Life has a way of making the foreseeable that which never happens...
and the unforeseeable that which your life becomes."

BIOGRAPHY

Jenna Kutzner-Mulligan was born in Greenville, South Carolina. Her love for animals and wonder at the possibilities of science began early in her life, as she fondly remembers the first time she found out there were actually whole careers where you could study how animals live and interact with their environment. She graduated from Travelers Rest High School with highest honors and initially pursued a Bachelor's Degree in Zoology at Mars Hill College. However, after two years at Mars Hill and a change of academic heart, she decided to instead pursue her (now) husband Ryan, who had just become a Supply Core Officer in the US Navy. He was stationed in Pascagoula, MS, so Jenna left school to move to Mississippi. After a year of living on the coast and working in veterinary clinics, Hurricane Katrina devastated the Mississippi coast, destroying the apartment Jenna and her husband lived in. Jenna was required to evacuate herself and her animals back to Greenville, SC, but Ryan remained stationed along the coast to help deliver supplies to the community and assist in clean-up efforts.

A short time later, Ryan was medically discharge from the Navy and Jenna and Ryan bought a house near Clemson, SC. Jenna finally completed her Bachelor's degree at Clemson University, but this time in Animal and Veterinary Science and with a concentration in Equine Business Management. She graduated Summa Cum Laude from Clemson and was ranked first in her class and first in her major. Jenna took a position as the property and animal manager at a private equestrian estate in Fletcher, NC. After a year of back-breaking manual labor, her first large-scale attack of poison ivy, and over 60 in of snow that trapped

Jenna and Ryan in their home multiple times, Jenna decided to pursue her Master's degree in Animal Science, with an emphasis in Equine Nutrition. She has been a Teaching and Research Assistant in the department of Animal Science and has been working on her Master's degree at North Carolina State University for almost 2 years now. In those 2 years, she has been involved in 8 research projects, has been the teaching assistant in 2 laboratory courses, and has maintained a 4.0 GPA. She hopes to receive a lecturer position in a land-grant institution or become an extension agent in the either North or South Carolina upon her graduation in Summer 2012.

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I would like to thank Dr. Pratt-Phillips for her artful, simultaneous combination of guidance and lee-way in the past 2 years of my life. She has stepped in for me when I needed it but has allowed me the freedom to explore my interests and ideas whenever possible. I've never felt alone in these endeavors, but I also never felt controlled. That combination has given me the opportunity to develop skills I never knew I had. I'll never forget the day she called me "organized". No one had ever called me that, but I realized (after telling every member of my family that I had been referred to as "organized") that it had become true. My growth as an academic, researcher, and employee has only been possible through her mentoring, and I deeply thank her for that.

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My team of graduate student accomplices has often been the only reason I've been able to cope with hectic schedule, pressing demands, and numerous deadlines. When my family couldn't understand what I was doing with my time, my advisors seemed to enjoy my tortures because "we've all been there" and "at least you get to use a computer for that now", and my professors thought I lived to study their class, I could always find a fellow graduate student with the same complaints (or something even worse) that put my problems in perspective. We have all stepped in for each other when it counted and let each other off the

hook when it was deserved in the same way brothers and sisters are “your best link to your past and the people most likely to stick with you in the future”. I thank you all for helping me feel normal.

Finally, my friends and family have been both my inspiration to commit to my education and my escape from the realities of it. You’ve all helped me push through when times were tough through your support and words of wisdom, but you’ve also helped me get out of my own head when I’ve been dreaming about blood pulls and editing papers. Thanks for reminding me why I’ve been working so hard. I hope I’ve made you all proud.

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber
ADP	Adaptation of Meal Feeding Period (Using Control Treatment Bucket)
AIRg	Acute Insulin Response to Glucose
AUC	Area Under the Curve
AUCglu	Area Under the Curve of Post-Prandial Glucose Concentrations
AUCins	Area Under the Curve of Post-Prandial Insulin Concentrations
BALL	Ball Containing Treatment Bucket (Mobile Obstacles Above Feed)
BW	Body Weight
BCS	Body Condition Score
CGIT	Combined Glucose-Insulin Test
CON	Unaltered Treatment Bucket, Unaltered Feed (Control)
DI	Disposition Index
DM	Dry Matter
DOT	Day of Treatment (Within Period)
EHC	Euglycemic-Hyperinsulinemic Clamp
EMS	Equine Metabolic Syndrome
FSIGT	Frequently Sampled Intravenous Glucose Tolerance
IR	Insulin Resistance
JM	Jaw Movements
LSM	Least Squares Mean

NDF	Neutral Detergent Fiber
NSC	Non-Structural Carbohydrate
OMD	Organic Matter Digestibility
PEAK	Highest Post-Prandial Metabolite Concentration Detected within 300 min Post-Feeding
PEAKglu	Highest Post-Prandial Glucose Concentration Detected within 300 min Post-Feeding
PEAKins	Peak Post-Prandial Insulin Concentration Detected within 300 min Post- Feeding
SI	Sensitivity to Insulin
Sg	Glucose Effectiveness
TIMEPEAK	Time Until Highest Post-Prandial Metabolite Concentration (in minutes)
TIMEPEAKglu	Time Until Highest Post-Prandial Glucose Concentration (in minutes)
TIMEPEAKins	Time Until Highest Post-Prandial Insulin Concentration (in minutes)
VFA	Volatile Fatty Acid
WAFF	Waffle Insert Containing Treatment Bucket (Stationary Obstacles Below Feed)
WTR	Water Mixed with Feed but Unaltered Bucket Treatment Bucket

CHAPTER ONE

Literature Review

INTRODUCTION

Horses evolved as grazing animals and therefore have a digestive tract developed for consumption of small amounts of forage at a nearly continuous rate throughout the day. However, substantial pasture area is needed to produce adequate forage to solely support equine populations, so it is often necessary to supplement the equine diet with concentrated feeds to meet the energy requirements of horses. Additionally, horses with dental afflictions and high performing equine athletes, growing equine athletes, and pregnant and lactating mares with heightened daily nutritional needs may not meet nutrient requirements by grazing alone, even when plentiful, high quality pasture is available (National Research Council, 2007). Therefore, daily inclusion of concentrate in the equine diet has become widespread and is regularly included in feeding and farm management practices (Hoffman et al., 2009; Richards et al., 2006; United States Department of Agriculture, 1998).

Despite general acceptance for inclusion of concentrate in the equine diet, potential risks associated with concentrate meal consumption are considerable, including metabolic disorders like insulin resistance (Hoffman et al., 2003; Quinn et al., 2008), laminitis (Garner et al., 1977), and risk of choke (Chiavaccini and Hassel, 2010; Feige et al., 2000). Current methods to reduce insulin resistance involve changing the energy sources of the diet (Hoffman et al., 2003; Stull and Rodiek, 1987; Williams et al., 2001) or increasing the number of meals per day and reducing the size of each meal (Gordon et al., 2007). Both of these suggestions can increase feed or labor costs, but a novel concept to reduce risk of insulin resistance is to prolong the time horses take to consume a concentrate meal.

Some techniques shown to increase time to consume feed include leaving grains unprocessed or lightly processed (Bergero and Nardi, 1996; Brökner et al., 2008) or adding forages to the concentrate meal (Brökner et al., 2008; Meyer et al., 1975). However, both of these methods have negative impacts on digestibility of feeds (Bailoni et al., 2006; Drougal et al., 2001; Rosenfeld and Austbø., 2009) and unprocessed grains can increase the amount of resistant starch that reaches the hind-gut (Bailoni et al., 2006) which can alter the microbial environment of the hind-gut (McLean et al., 2000). An alternative way to prolong time to consume feed is therefore needed in order to assess the impact of increasing time to consume feed on the risk factors of metabolic disorders. Therefore, this literature review and subsequent research investigates common equine feed components, the carbohydrate digestive physiology of horses, the metabolic effects of feed consumption, the effects of meal feeding horses, and means to reduce the risks associated with concentrate meal feeding in horses.

COMMON EQUINE CONCENTRATE FEEDS AND CARBOHYDRATE COMPONENTS OF FEED

Common Concentrate Feeds and Various Ways to Process Feeds

Concentrated feedstuffs for horses may include oats, corn, barley, sorghum, wheat, or soybeans or by-products of these grains, and grains can be fed whole or processed in a variety ways (Richards et al., 2006). Processing can impact the digestibility of feeds (Bailoni

et al., 2006; Rosenfeld and Austbø, 2009) and can influence the microbial population of the equine hind-gut (Drougal et al., 2001; Julliand et al., 2001; McLean et al, 2000), so it is of interest to define common processing techniques in a discussion of the impact of concentrate meal feeding horses.

Common grain processing can be classified as simple physical processing, such as cracking, rolling, crimping, and grinding, or methods which also alter the chemical or nutritive qualities of the feed due to the inclusion of water and/or heat during the processing methods, such as micronizing, steam flaking, and extruding (Richards et al., 2006; Rosenfeld and Austbø, 2009). Concentrated feeds are also commonly given as pellets, cubes, or as a combination of grains and pelleted feeds (United States Department of Agriculture, 1998). Sweet feeds have molasses added as a binding agent and as a flavor enhancer and may comprise whole grains, processed grains, pellets, or a combination of these components (United States Department of Agriculture, 1998). Pelleted grains are often included in performance, complete, and senior feeds. Complete and senior feeds are marketed as products that can be fed alone without additional forages due to their high fiber content, which is achieved by the inclusion of fibrous ingredients such as beet pulp, peanut hulls, rice bran, soybean hulls, and/or wheat middlings (Purina Mills, LLC, St. Louis, MO; Southern States Cooperative, Inc, Richmond, VA; Triple Crown Nutrition, Inc, Waysata, MN).

Different Carbohydrate Components of Feed

Carbohydrates within feedstuffs for horses are first broadly divided into structural carbohydrates, as in the cell walls of forages or the fibrous coating of grains, and non-structural carbohydrates (NSC), which represent the carbohydrates within cell contents of forages or the interior of grains (Van Soest, 1963). The structural carbohydrate content is described by the neutral detergent fiber (NDF) portion, which comprises the total cell wall and consists of insoluble fiber (Hoffman et al., 2001). Further, NDF includes hemicelluloses and an acid detergent fiber (ADF) portion, which comprises the celluloses, lingo-cellulose, and lignin within the cell wall (Hoffman et al., 2001). The NSC portion of equine feeds includes hydrolyzable carbohydrates (hexoses, disaccharides, and some oligosaccharides) and non-hydrolyzable, rapidly fermentable carbohydrates (resistant starches, galacto-oligosaccharides, fructo-oligosaccharides, and soluble fibers) (Hoffman et al., 2001). Resistant starch is defined as the percent of starch unlikely to be hydrolyzed by mammalian digestive enzymes (Bailoni et al., 2006), so resistant starch would be undigested within the small intestine and could reach the cecum and large intestine as by-pass starch. Microbes of the hind-gut would then rapidly ferment the starch, resulting in changes in hind-gut pH, volatile fatty acids, lactic acid production, and bacterial population profiles (Julliand et al., 2001).

EQUINE DIGESTIVE PHYSIOLOGY WITH A FOCUS ON CARBOHYDRATES

Feed Type Effects on Chewing, Saliva Components, and the Effects of Saliva on the Gastric Environment

Horses at pasture may spend around 70% of their time eating (Crowell-Davis et al., 1985), but horses housed in stalls often are fed a few large meals followed by periods of time without access to feed (Hoffman et al., 2009; Richards et al., 2006). Horses not maintained by grazing pasture are commonly fed a combination of roughages, which are higher fiber feeds like hay and hay products, and concentrated feeds, which are higher energy feeds like grains and grain products (United States Department of Agriculture, 1998). The feed type a horse consumes dictates how much chewing is required, as measured by jaw movements/g dry matter (DM), with roughages requiring more chewing than concentrate feeds (Brökner, et al., 2008). Within roughages, the stage of maturity and level of dry matter (DM) affects the amount of chewing needed, with fresh green hay requiring less chewing than fully dried hays and hays requiring less chewing than straw (Brökner, et al., 2008). Within concentrate feeds, pelleted feeds require less chewing than whole grains and whole grains require less chewing than a mix of grains and pellets (Brökner, et al., 2008).

Saliva is produced in glands located around the mouth of the horse, including the parotid, mandibular, and sublingual glands, and saliva contains a variety of electrolytes, such as sodium, potassium, calcium, chloride, and bicarbonate (Alexander, 1966), as well as trace amounts of lipases (Moreau et al., 1988). Chewing stimulates saliva production from the

parotid salivary gland, and saliva ceases to flow from the parotid salivary gland once the horse stops chewing (Alexander, 1966). Therefore, feeds which require more chewing, like roughages, result in increased parotid saliva production when compared to feeds that require less chewing, like concentrate feeds.

Saliva mixes with feed as the horse chews, and the mixture travels down the esophagus to the rest of the gastrointestinal tract. Because the pH of parotid saliva is around 7.5, regular saliva production has the ability to buffer against the acidic environment of the stomach (Alexander, 1966). As a result, low roughage diets and meal feeding concentrates may lead to decreased pH in the stomach and increases the risk of gastric ulcers in the non-glandular portion of the stomach (Nadeau et al., 2003). The non-glandular portion of the stomach does not secrete substantial mucus and is therefore less protected from gastric acids, so if gastric pH decreases as a result of decreased saliva flow to the stomach, the occurrence of gastric ulcers becomes more likely (Nadeau et al., 2003).

Microbes as a Component of the Equine Digestive Tract

Different feedstuffs have unique combinations of carbohydrate components, which are subject to fermentation by microbes to various extents. The degree of potential microbial contribution to digestion in the hind-gut is also impacted by the extent of feed processing (Rosenfeld and Austbø, 2009). The equine digestive tract involves a variety of microbial populations which vary along the different sites of the tract, and microbes begin contributing to digestion starting in the stomach (de Fombelle et al, 2003). The stability and health of

microbial populations increases the bioavailability of feeds because microbes are capable of hydrolyzing of bonds of fibrous plant material, freeing cell contents for use by the horses and improving total digestion (Koller et al., 1978). Specifically, common microbes of the equine digestive tract include lactobacilli, streptococci, cellulolytic bacteria, and lactate using bacteria (de Fombelle et al., 2003). Lactobacilli and streptococci are starch using bacteria, and cellulolytic bacteria are capable of hydrolyzing beta bonds found in plant material which would otherwise remain unbroken by the equine digestive tract (Narisawa et al., 2007). Maintaining healthy microbial populations is important because bacteria produce volatile fatty acids (VFAs) through fermentation, including acetate, propionate, and butyrate, which can contribute to the energy intake and metabolic pathways of horses (Argenzio et al., 1974).

Digestion and Absorption in the Stomach

Limited carbohydrate digestion takes place in the stomach, but the stomach contains substantial microbial populations, including the highest concentrations of lactobacilli, lactate using bacteria, and total anaerobic bacteria along the digestive tract (de Fombelle et al., 2003). While the stomach contains among the lowest values of cellulolytic bacteria and VFA concentrations, it is capable of absorbing some of the VFAs produced (Argenzio et al., 1974). The stomach has the lowest pH of the entire tract (de Fombelle et al., 2003) because horses continuously release hydrochloric acid in the stomach, regardless of the presence of feed material, making the pH of the gastric environment decrease substantially after prolonged

absence of feed (Murray and Schusser, 1993). As a result, prolonged time in between meals leads to decreased stomach pH and increases the risk of ulcers (Nadeau et al., 2003).

Gastric lipases are present in the stomach, but they are in amounts that contribute little to total lipid digestion when compared to levels associated with the small intestine (Moreau et al., 1988). However, gastric lipases and the gastric environment may be important as a means to begin lipid digestion, increasing the effectiveness of pancreatic lipases and bile salts (Moreau et al., 1988). Little protein digestion takes place in the stomach of the horse, but there is an increase in water soluble nitrogen and a decrease in total nitrogen concentrations as a result of pepsin secreted in the stomach (Glade, 1983). Therefore, some lipid and protein digestion occurs in the stomach, and nitrogen within the digesta is becoming more available as it transitions to water soluble nitrogen.

Digestion and Absorption in the Small Intestine

As digesta enters the small intestine, the pancreas contributes amylase capable of hydrolyzing alpha bonded carbohydrates, and the brush border region of the small intestine is lined with disaccharidases, leading to the greatest amount of carbohydrate digestion occurring within the small intestine (Hintz et al., 1971a). The greatest absorption of carbohydrates also occurs within the small intestine (Hintz et al., 1971a). Some of the highest values of streptococci and the lowest values of cellulolytic bacteria and lactobacilli are found across regions of the small intestine (de Fombelle et al., 2003). Further, the ileum has the highest pH and some of the lowest counts of total anaerobic bacteria of the entire

tract (de Fombelle et al., 2003). In relation to these characteristics, the lowest concentrations of VFAs are found within the small intestine (de Fombelle et al., 2003).

Lipid digestion in the small intestine is accomplished through pancreatic lipases and micelle formation with the addition of bile salts so that the greatest lipid digestion occurs within the small intestine (Meyer et al., 1997). Some forms of nitrogen are greatly reduced within the small intestine, but the addition of digestive juices and normal loss of intestinal cells makes it difficult to differentiate between exogenous and endogenous protein values of digesta (Glade, 1983). Even so, it has been calculated that over 50% of protein disappearance from digesta occurs within the small intestine (Hintz et al., 1971a).

Digestion and Absorption in the Cecum and Large Intestine

The hind-gut is the site of greatest water absorption and fiber digestion throughout the tract of the horse (Hintz et al., 1971a). As the horse is a hind-gut fermenter, the cecum is large, well developed, and houses a substantial microbial population capable of digesting fibrous material (Koller et al., 1978). Within the cecum, anaerobic bacteria, lactobacilli, streptococci, and lactate using bacteria are in the lowest concentrations of the tract but cellulolytic bacteria are in highest (de Fombelle et al., 2003). As a result, VFAs are found in the second highest concentration of the tract within the cecum, and, compared to the entire tract, the cecum contains the highest concentration of propionate (de Fombelle et al., 2003). Within the cecum, VFAs are also absorbed at one of the highest amounts, providing energy and substrates for metabolism (Argenzio et al., 1974)

The lowest numbers of lactate utilizing bacteria and some of the largest numbers of total anaerobic bacteria and cellulolytic bacteria are found in the colon (de Fombelle et al., 2003). The highest concentrations of VFAs are found within the right ventral portion of the colon, which directly follows the cecum, and this area specifically contains the highest concentration of acetate and butyrate found within the tract (de Fombelle et al., 2003). The ventral portion of the large intestine also has the highest capacity for VFA absorption (Argenzio et al., 1974).

Extra lipids in the diet that are not digested and absorbed within the small intestine can inhibit microbial activity within the cecum and large intestine, decreasing fiber digestion within these regions (Jansen et al., 2007). The cecum and large intestine contributes large amounts of endogenous protein to digesta from the microbial population, making it difficult to assess true protein digestion within these regions without some sort of labeled markers. Even so, apparent nitrogen digestibility increases within the cecum and large intestine and water soluble nitrogen decreases, indicating both protein digestion and absorption are occurring (Glade, 1983).

THE EFFECTS OF FEED TYPE AND FEED PROCESSING ON DIGESTIBILITY

The Effects of Feed Processing on Digestibility

Generally, processing feeds increases digestibility, and more extensive processing leads to greater digestibility (Bailoni et al., 2006; Rosenfeld and Austbø, 2009). Bailoni et

al. reported in 2006 regarding the differences in resistant starch and organic matter digestibility (OMD) for the most common grains fed to horses. Thirty samples of corn, barley, and oats that were whole, flaked, rolled or extruded were subject to nutritional analysis, liquid chromatography, and in vitro digestibility techniques. Resistant starch of corn decreased through processing, as whole corn, extruded, and steam flaked contained 10.6%, 5.1%, and 0.7% resistant starch, respectively. Processing did not substantially increase resistant starch in barley or oats, but the average resistant starch across all barley and oat processing techniques was only 2.0% of total starch (Bailoni et al., 2006). Extrusion slightly increased OMD of oats, but steam flaking decreased OMD when both were compared to the OMD of whole oats. However, both steam flaking and extrusion increased OMD of barley and corn when compared to the whole grain OMD of each (Bailoni et al., 2006). Therefore, processing grains decreases the resistant starch content of corn and generally increases OMD for horses.

Rosenfeld and Austbø (2009) conducted digestibility trials on cecally cannulated horses using the mobile bag procedure. The horses had been previously fitted with a permanent cecal cannula located near the ileo-cecal junction. Oats, barley, and corn were either ground, pelleted, extruded or micronized and, after treatment to mimic the physical changes due to mastication, were placed in a mobile bag and intubated through a nasogastric tube. Mobile bags were either collected from the cecal cannula or from the feces, so the original nutritional contents, the pre-cecal digestibility, and total digestibility of the feeds could be compared. Micronizing resulted in the highest pre-cecal (84.8%) and total tract (97.6%) starch digestibility of the grains (Rosenfeld and Austbø, 2009). Extrusion led to the

lowest pre-cecal starch digestibility (69.7%), with grinding (72.3%) and pelleting (82.2%) resulting in intermediate pre-cecal digestibility; but, for total tract digestibility of starch, pelleting (95.8%) and extrusion (97.8%) were similar to micronizing (97.6%), and all 3 methods were more effective than the total tract starch digestibility of grinding (91.5%) (Rosenfeld and Austbø, 2009).

The Effects of Diet Type on Digestibility and Microbial Populations

The inclusion of concentrates in a diet generally increases digestibility of the organic matter (Drougal et al., 2001), but concentrate inclusion in diets can cause alterations in microbial populations (Julliand et al., 2001). However, different concentrate processing methods have varying impacts on microbial populations (McLean et al., 2000). Drougal et al. (2001) fed horses diets of varying proportions of hay and grain, including a 100% chopped hay diet, 70% chopped hay with 30% rolled barley, and 50% chopped hay with 50% rolled barley, and determined the apparent digestibility and transit rate of each diet type. Diets were fed to be roughly equal in organic matter percent, and thus as the ratio of hay to barley decreased (100:1 to 7:3 to 1:1) the energy content increased (from 0.40 to 0.63 to 0.78 UFC/kg dry matter (DM), respectively) and DM content decreased (102.7 to 70.1 to 58.4 g DM/kg BW, respectively). The addition of grain to the diet created a linear increase in OMD and a linear decrease in acid detergent fiber (ADF) digestibility with each additional increase in percent grain (Drougal et al., 2001). The hay only diet had greater digestibility of neutral detergent fiber (NDF) than either diet that included grain, and thus any addition of grain

reduced fiber digestibility (Drougal et al., 2001). Finally, as the percent of grain increased and the DM content of the diet decreased, transit rate through the tract also decreased (Drougal et al., 2001).

Julliand et al. (2001) reported on the alterations in the microbial profile and activities of horses under the 3 diets described previously by Drougal et al. (2001), including 100% chopped hay, 70% chopped hay with 30% rolled barley, and 50% chopped hay with 50% rolled barley. In both the cecum and the large intestine, the inclusion of barley in the diet decreased cellulolytic bacteria and increased anaerobic bacteria, including lactate utilizing bacteria, lactobacilli, and streptococci (Julliand et al., 2001). Increasing the proportion of barley decreased the pH of both the cecum and large intestine linearly, and the inclusion of barley in the diet in any amount decreased acetate and increased propionate production (Julliand et al., 2001).

McLean et al. (2000) utilized 3 cecally cannulated horses in a 3x4 incomplete Latin Square changeover design to determine the differences in pH, lactate, and volatile fatty acid (VFA) profiles of the cecum when horses consumed meals of only hay or a 50:50 ratio of hay to either rolled, micronized, or extruded barley. Rolled barley reduced cecal pH (6.26) when compared to the hay diet (6.5), while micronizing (6.33) and extrusion (6.36) of barley resulted in intermediate pH levels which were not statistically different from either hay or rolled barley (McLean et al., 2000). Lactate concentration was increased due to rolled barley (0.97 mmol/L) when compared to the hay diet (0.11 mmol/L), micronized barley (0.18 mmol/L), and extruded barley (0.26 mmol/L), but only rolled barley and hay only were statistically different from each other due to a large standard error of 0.411 mmol/L (McLean

et al., 2000). The acetate molar proportions under the hay and micronized barley diets (767 and 716 mmol/mol, respectively) were greater than acetate under rolled or extruded barley (630 and 680 mmol/mol, respectively) while propionate molar proportions under hay and micronized barley diets (127 and 220 mmol/mol, respectively) were less than propionate under rolled and extruded barley (302 and 254 mmol/mol, respectively) (McLean et al., 2000). Therefore, the extent of processing barley effects the cecal environment, only physical processing, such as rolling barley, has the greatest undesirable impact on the cecal environment, and micronizing barley has the least impact on pH, lactic acid, and VFA profiles.

GLUCOSE AND INSULIN RESPONSES TO FEED CONSUMPTION

Glucose

Glucose is the major end product of carbohydrate digestion within the digestive tract, and glucose is predominantly absorbed from the digestive tract by GLUT-2 and SGLUT-1 transporters within the small intestine. Liver uptake of glucose is accomplished by GLUT-2 transporters, and muscle and adipose tissue uptake of glucose is facilitated by GLUT-4 transporters. Glucose is the primary substrate for the first step of glycolysis, which is the metabolic pathway that generates usable products for physiological energy and replenishment of substrates for use in other pathways of metabolism. When glucose is not present in sufficient amounts to support glucose-requiring tissues, such as the brain and red blood cells,

gluconeogenesis takes place, which is the reverse of glycolysis in order to create glucose for use by tissues. When glucose is present in sufficient quantities, glycogen is created as a storage form of glucose and is stored within the liver and the skeletal tissues.

Insulin

Insulin is the primary hormone involved in stimulating tissue uptake of glucose when glucose levels increase following a meal. Insulin is produced from the beta cells of the pancreas and facilitates skeletal and cardiac muscle uptake of glucose by stimulating the migration of GLUT-4 transporters (which are considered insulin dependent glucose transporters) to the surface of cells to facilitate glucose uptake. Insulin also directs the body toward an anabolic state, as it signals that glucose is present in sufficient quantities, and thus stimulates glycogen synthesis from glucose, fatty acid synthesis, and adipose tissue creation, and insulin decreases gluconeogenesis, glycogenolysis, and lipolysis (Froesch et al, 1965). As a result of insulin's association with adiposity, obesity and heightened or aberrant insulin concentrations in horses are often related (Frank et al., 2006; Hoffman et al., 2003).

Glucose and Insulin Dynamics

Blood glucose concentrations increase following intake of a meal, but because glucose is tightly regulated by homeostatic mechanisms, differences in meal size and feed type may not significantly alter the glucose response to feeding (Hintz et al., 1971b; Stull and

Rodiek, 1987). If the percent non-structural carbohydrate (NSC) of 2 treatment meals is the same, it is possible for no differences to be seen among the average glucose, area under the curve (AUC) of glucose, and peak glucose of the 2 diets, even when 1 of the treatment meals is double the size of the other (Gordon et al., 2007). However, when meals of the same size are fed but the percent of NSC is doubled then average glucose, AUC of glucose, and peak glucose are all higher under the high NSC diet (Gordon et al., 2007). Diets with drastically different energy sources (starch versus fat) may generate different average glucose responses to feeding, AUC of glucose concentrations, peaks in glucose, and/or time to peak glucose (Williams et al., 2001). Different feed types have different glycemic indexes, which is a comparison of the AUC of glucose following consumption of a feed when compared to AUC of glucose of a pre-determined standard feed, such as oats in horses (Rodiek and Stull, 2007).

Insulin concentrations increase shortly after glucose concentrations increase following a meal, and therefore diets which are capable of increasing glucose following feeding generally lead to heightened insulin concentrations since the insulin response is closely related to the glucose response to feeding (Gordon et al., 2007; Stull and Rodiek, 1987; Williams et al., 2001). However, the insulin response to a meal is often much more exaggerated than the glucose response, and additional factors besides the glucose response and the NSC content of feed likely influence insulin levels, including the primary energy source of the diet (Williams et al., 2001), meal size (Gordon et al., 2007), and diet components (Stull and Rodiek, 1987). Relatively small difference in average glucose concentrations may be found due to treatment meals high in either starch and sugar or high in fat and fiber, such as an approximately 50% higher average glucose concentration due to the

starch and sugar diet (Williams et al., 2001), but the high starch and sugar diet may entice an approximately 500% higher average insulin concentration (Williams et al., 2001). When 2 treatment meals contain the same percent NSC but one meal is double the size of the other, the meals may not elicit sizeable differences in the glucose parameters following feeding (Gordon et al., 2007), but the larger meal can still lead to increased average insulin and AUC insulin concentration (Gordon et al., 2007). Diets of different compositions and a wide range in fiber, fat, and starch containing ingredients may not lead to any marked difference in average or AUC glucose concentrations (Stull and Rodiek, 1987), but they can still lead to substantial differences in average and AUC insulin concentrations (Stull and Rodiek, 1987). Clearly, glucose and insulin dynamics in horses are multi-factorial and are not as of yet fully understood.

INSULIN RESISTANCE AND OTHER METABOLIC PROBLEMS ASSOCIATED WITH FEED CONSUMPTION IN HORSES

Equine Metabolic Syndrome

Equine metabolic syndrome (EMS) was first officially termed by Johnson in 2002 to refer to horses with a combination of obesity, insulin resistance (IR), and history of laminitis without heightened adrenocorticotropin levels associated with Cushing's disease (Johnson, 2002). Dietary and managerial factors can all be associated with development of obesity, IR, and laminitis or risk factors for these conditions (Hoffman et al., 2003; Julliand et al., 2001;

McLean et al., 2000; Pratt et al., 2006; Quinn et al., 2008), and often these disease states are related to and coincide with each other (Frank et al., 2006; Hoffman et al., 2003; Laat et al., 2010; Walsh et al., 2009). It is uncertain the specific prevalence of EMS risk factors among horses, but one survey and sample collection of 366 horses in North Carolina found that 7.8% of horses were considered hyperinsulinemic (resting insulin > 30 uU/mL), 20% were considered obese (body condition score (BCS) > 7.0), an additional 28% were considered moderately fleshy (BCS of 6.0 to 7.0), and 4.6% had been diagnosed with laminitis (Pratt et al., 2010). Further, BCS was positively correlated with resting insulin concentrations and negatively correlated with glucose to insulin ratios (Pratt et al., 2010), indicating that nearly half of the horses represented in this survey were at least moderately overweight, and this corresponds to risk factors for metabolic abnormalities and higher risk for EMS development.

Insulin Resistance

Insulin resistance is a disease state in which skeletal muscle, cardiac muscle, adipose tissue, and the liver become under-responsive to insulin, and because insulin is less able to stimulate uptake of glucose by tissues, blood glucose levels remained elevated for prolonged periods of time (Frank et al., 2006). When glucose levels are elevated due to consumption of a high starch diet, additional insulin is released from the pancreas as the body attempts to attenuate glucose concentrations, leading to increased circulating concentrations of insulin (Gordon et al., 2007; Stull and Rodiek, 1987; Williams et al., 2001). Initially, increasing

insulin concentrations will correct glucose levels, but repeated increases in insulin further decrease tissue sensitivity to insulin until eventually even resting glucose and insulin levels remain excessively elevated in horses with reduced tissue sensitivity to insulin (Treiber et al., 2005). Therefore, primary indicators of reduced tissue sensitivity to insulin are increased or seemingly uncontrolled blood glucose levels despite increased blood insulin levels following a meal, overall elevated insulin concentrations following a meal, and/or increased basal blood glucose and insulin levels (Frank et al., 2006; Hoffman et al., 2003; Kaske et al., 2001; Pratt et al., 2006; Pratt et al., 2009). Because IR is a risk factor for and is correlated with obesity (Frank et al., 2006; Hoffman et al., 2003) and laminitis (Laat et al., 2010; Walsh et al., 2009), it is of value to investigate the pathology of IR and management decisions that could reduce the risk of IR development.

Diagnosis of IR can be accomplished through a variety of methods which range in effectiveness and accuracy. Resting glucose and insulin concentrations can be used to estimate insulin sensitivity, but single samples are highly subject to environmental and physiological variations in horses (Pratt et al., 2009). Jugular blood sampling at pre-determined intervals following feeding a meal can be used to establish glucose and/or insulin concentrations at specific post-prandial time points (Gordon et al., 2007; Rodiek and Stull, 2007; Stull and Rodiek, 1987; Williams et al., 2001). The concentrations following feeding can then be used to calculate the area under the curve (AUC) of the glucose or insulin response, which quantifies the total metabolite response to feeding, as well as the peak concentration and time to reach the peak concentration of the metabolites (Gordon et al., 2007; Rodiek and Stull, 2007; Stull and Rodiek, 1987; Williams et al., 2001). The peak

value shows the maximum concentration measured that tissues are exposed to, and the time to peak value demonstrates how long it took following feeding until the concentration began to decrease. Using paired glucose and insulin concentrations, the glucose to insulin ratio can be calculated, which quantifies how rising glucose levels are matched by rising insulin levels (Pratt et al., 2009). Low glucose to insulin ratios demonstrate that when glucose increases only a small amount, insulin increases accordingly. High glucose to insulin ratios therefore mathematically demonstrate that glucose has increased without an equal insulin response, indicating insulin may not be effectively responding to and/or controlling glucose levels. However, total glucose and insulin concentration should be considered before using the glucose to insulin ratio to diagnosis IR, and the consistency of the glucose to insulin ratio may have more value in understanding if a horse has IR. Modified insulin to glucose ratios estimate pancreatic responsiveness to increasing glucose levels since it mathematically depicts how much insulin is present given the concentration of glucose detected (Pratt et al., 2009).

Similar to feeding a meal and assessing post-prandial blood glucose and insulin concentrations, an oral glucose tolerance test involves oral consumption of a specific amount of glucose, followed by blood sample collection to quantify changes in glucose and insulin dynamics (Pratt et al., 2006). The benefit of administration of glucose alone is there are no confounding factors associated with digestion of various feeds. Bioavailability of glucose from a diet is related to the type of diet the horse is previously adapted to, the maturity of forage based feedstuffs, and the percent of structural carbohydrate of the feed, so

administration of a glucose bolus ensures a specific of glucose is administered to the horse to elicit a metabolic response (Pratt et al., 2006).

A variety of tests involve administration of exogenous glucose and insulin via jugular catheters to assess glucose and insulin effectiveness and tolerance. The euglycemic-hyperinsulinemic clamp (EHC) involves administering a primer dose of insulin followed by a continuous infusion of insulin for a set amount of time, plus infusion of glucose calculated to maintain basal blood glucose levels to avoid hypoglycemia (Kaske et al., 2001). Blood samples are regularly drawn in order to determine glucose and insulin levels and variations in insulin-mediated tissue uptake of glucose (Kaske et al., 2001). The combined glucose-insulin test (CGIT) involves administration of a bolus of glucose and insulin simultaneously, followed by blood sample collection and analysis for glucose and insulin (Frank et al., 2006). Diagnosis of IR using a CGIT may be determined by an arbitrary time frame in which it is deemed acceptable that glucose levels return to baseline, such as 45 min post-glucose administration (Frank et al., 2006). The frequently sampled intravenous glucose tolerance test (FSIGT) determines how quickly and efficiently tissues take up an amount of glucose delivered intravenously before a bolus of insulin is later administered, as blood samples are taken following glucose administration and continue throughout insulin bolus administration and beyond (Hoffman et al., 2003; Quinn et al., 2008). Results from blood sample analysis can be used to fit a minimal model of glucose and insulin dynamics, which is a non-linear model that is used to separately calculate insulin independent and insulin dependent glucose uptake by tissues, quantifying tissue sensitivity to insulin and the ability of the pancreas to respond to reduced sensitivity to insulin (Hoffman et al., 2003; Quinn et al., 2008). The

combination of FSIGT and the minimal model of glucose and insulin provide the greatest understanding of post-prandial metabolite dynamics, but the FSIGT may have greater variability than similar methods such as the EHC (Pratt et al., 2008).

Quinn et al. (2008) utilized a FSIGT to assess changes in glucose and insulin dynamics in horses that were fed either high starch or high fat feeds, first at maintenance levels and then at levels designed to encourage weight and body condition score increases. Horses were fitted with jugular catheters, an intravenous bolus of glucose was administered, and blood sampling began. Twenty minutes after the glucose bolus was given, an intravenous bolus of insulin was administered, and blood samples continued until 240 minutes post-glucose bolus administration. A minimal model of glucose and insulin dynamics was developed to quantify insulin sensitivity (SI), glucose effectiveness (Sg), the acute insulin response to glucose (AIRg), and the disposition index (DI). The SI depicts how well insulin facilitates tissue uptake of glucose, Sg depicts glucose uptake regardless of a change in insulin or how well glucose mediates its own uptake, AIRg depicts the insulin response to the glucose bolus in the first 10 min of sampling (prior to exogenous insulin bolus administration), and DI reflects the ability of the pancreas to increase insulin concentrations in response to reduced SI. The high starch diet decreased tissue SI by the end of the maintenance period and increased AIRg by the end of the entire trial (Quinn et al., 2008). Under both diets, Sg decreased during the high calorie intake period when horses gained weight (Quinn et al., 2008). Therefore, high starch diets decrease tissue responsiveness to insulin directed uptake of glucose and reduce the endogenous insulin

response to increasing blood glucose levels, and excessive calorie intake reduces the effectiveness of glucose to mediate its own uptake.

Obesity and Insulin Resistance

Since insulin is a key hormone that signals increased fatty acid synthesis and adipose tissue formation and reduces lipolysis, heightened insulin levels in IR horses also is associated with a state of obesity (Frank et al., 2006; Froesch et al., 1965). Frank et al. investigated glucose and insulin concentration differences among non-obese and obese horses with IR using a CGIT. Obese horses were considered a BCS of 7 or greater (Henneke et al., 1983), and diagnosis of IR was based on the horses having heightened glucose concentrations that were not controlled for greater than 45 min during a CGIT (Frank et al., 2006). The median AUC glucose, the median AUC of insulin, and resting insulin was higher in the obese-IR horses group, and these 3 parameters were all positively correlated with increasing BCS (Frank et al., 2006).

Hoffman et al. (2003) investigated the effects of both obesity and diet on SI, Sg, AIRg, and DI using a FSIGT test and minimal model of glucose and insulin dynamics. Horses were classified as non-obese (BCS of 5.0 to 5.9), moderately obese (BSC of 6.0 to 6.9), or obese (7.0 to 9.0) (Henneke et al., 1983) prior to the start of treatments, which included either a diet high in starch and sugar or a diet high in fat and fiber. Insulin concentrations were steadily higher and insulin sensitivity was lower in obese horses than non-obese or moderately obese horses (Hoffman et al., 2003). Further, the peak insulin

concentration was higher in obese horses than non-obese horses (Hoffman et al., 2003). However, Sg and AIRg was higher in obese horses, and the authors attribute this to the obese horses relying on glucose-mediated glucose uptake and the increased production of insulin as a result of the reduced SI status (Hoffman et al., 2003). Analysis of the effect of diet showed that the diet high in starch and sugar reduced SI, AIRg, and DI for all BCS groups, and within non-obese horses the high starch and sugar diet reduced SI (Hoffman et al., 2003).

Laminitis

Laminitis is marked by separation of the hoof lamellar-distal phalangeal attachment and is associated with unwillingness to maintain weight on all hooves, stride normally, and, at the greatest severity, to walk or move at all (Garner et al., 1977). Glucose availability and normal glucose metabolism may be necessary for hoof health, as the absence or sudden removal of glucose from in vitro solutions of hoof tissue results in separation of the lamellae of the hoof (Pass et al., 1998). Heightened insulin concentrations may also be a factor in laminitis development, as prolonged infusion of insulin by an EHC induces laminitis within 48 hr (Laat et al., 2010). Therefore, horses with IR or EMS may be at higher risk of developing laminitis (Walsh et al., 2009).

Diet is another risk factor for laminitis. By-pass or resistant starches from high starch feeds may reach the cecum or large intestine and be rapidly fermented, causing alterations in pH, microbial populations, and volatile fatty acid production within the cecum and large intestine (Julliand et al., 2001; McLean et al., 2000). These changes due to high starch intake

can lead to lactic acidosis, a state marked by substantial increases in plasma L-lactate, which is a risk factor for development of laminitis (Garner et al., 1977).

CONCENTRATE MEAL FEEDING HORSES

Occurrence of Concentrate Meal Feeding

Meal feeding and concentrated feed use for horses have become a common practice across the world. The United States Department of Agriculture published in a 1998 report that 87.4% of equine operations supplied concentrates during winter months, 86.8% of equine operations that feed concentrates purchased from retail sources, and the reported types of concentrates horses received included 57.2% unpelleted sweet feed mixes, 42.9% unpelleted whole or rolled grains, 21.9% grain mix with pellets, and 18.7% complete feed pellets or cubes (feed types not mutually exclusive as some operations use a mix of multiple feed types) (United States Department of Agriculture, 1998).

Richards et al. (2006) published the results of an 18 question survey regarding equine feeding practices representing 3% of thoroughbred trainers in Australia. The survey found that 98.7% of trainers surveyed fed their horses meals of grains or concentrates each day, with 82% feeding their horses in only 2 meals per day. Oats, corn, and commercial pre-mixed feeds were the most common grains the horses received, at 80.6%, 73.6%, and 73.6%, respectively, and the average amount of grain fed per day was reported as 7.3 ± 0.24 kg per horse (Richards et al., 2006).

Hoffman et al. found in a survey that represented the feeding practices for 337 horses in New England that 96% of the horse owners fed at least one concentrate to their horses with the average number of meals per day as 2.1 ± 0.7 meals with the most common concentrate being pelleted feed (Hoffman et al., 2009). Further, this study reported that the median number of hours of turn-out to fresh pasture each horse received was only 2 hr, with a range of 0.5 to 24 hr/day.

Purposes to Meal Feed Horses

Common reasons to include concentrate meal feeding in an equine management plan are wide-ranging and vary in validity. Necessary reasons to include concentrate meals are based on either the energy demands of the horse or the limited energy supplying capacity of the pasture available or both. Daily feed intake rates of horses are generally considered to range between 1.5% and 3.0% of body weight (BW) per day on a dry matter (DM) basis (National Research Council, 2007), so low energy density forages may not be able to meet the heightened energy needs of breeding mares and high performance horses, particularly for horses who do not consume pasture at the higher end of the intake rates. Even if pasture is of average quality or supplemental forages, such as free-choice hay, are provided, the nutritive demands of some horses would be greater than forages alone could provide.

The National Research Council estimates that 500 kg lactating mares require between 27.23 and 31.73 Mcal/day (depending on month of lactation), and horses considered in heavy work or training require 26.64 Mcal/day (National Research Council, 2007). If a 500 kg

horse consumes 2% of its BW per day (10 kg on a DM basis), cool season grass pasture (at 2.39 Mcal/kg and 20.1% DM) would supply only 24.02 Mcal, and a legume forage pasture (at 2.71 Mcal/kg and 21.4% DM) would supply only 27.26 Mcal (National Research Council, 2007). Therefore horses in lactation and heavy work would be unable to meet their energy needs from pasture alone at this feed intake rate. Even at an intake rate of 2.5% of BW (12.5 kg on a DM basis), mares in the first 3 months of lactation would not meet their energy needs from cool season pasture grasses, which would supply only 29.78 Mcal, but legume forage pasture would meet the energy demands of a lactating mare at this intake rate. However, it should be noted the lactating mares would have to consume approximately 60 kg of the legume pasture on an as-fed basis for her energy needs to be met (National Research Council, 2007) and gut fill could limit the horse's willingness to reach this level of intake. Further, the horse would be more than doubling her crude protein requirements at this level of legume pasture intake which is excessively straining on a horse's kidney function (National Research Council, 2007).

Horse owners may not be able to maintain their horses on pasture when stocking rates are too high to maintain the health of the pasture. Also, owners may choose to keep their horses off pasture for cosmetic reasons, such as maintaining coat quality for show horses, or for behavioral reasons, such as aggressive herds of horses increasing the risk of injury while at pasture. In these instances, concentrate meals may be necessary to supplement the horse's energy and nutritive needs. Additional reasons horse owners include meal feeding in their management plans include tradition, perceived quality of grain, nutritional advice from a variety of sources, or as a means to administer supplements, medications, nutraceuticals, or

behavior modifying compounds (Hoffman et al., 2009; Richards et al., 2006). While some of these purposes have validity, many are unfounded and meal feeding could compromise the physiological status of horses if implemented without consideration for the nutritional needs of the horse.

Problems Associated with Meal Feeding

The components of common equine concentrate feeds can generate digestive or metabolic problems for horses. Common concentrated feeds are relatively high in non-structural carbohydrate (NSC) content when compared to forages and other high fiber feeds (Hoffman et al., 2001; Rodiek and Stull, 2007), and increasing the NSC content of the equine diet can lead to rapid or exaggerated increases in glucose and insulin responses following feeding (Rodiek and Stull, 2007; Stull and Rodeik, 1987; Williams et al., 2001). High NCS diets are a risk factor for insulin resistance (IR) development because long-term (≥ 8 weeks) administration of high NSC diets can lead to decreased tissue sensitivity to insulin in horses (Hoffman et al., 2003; Quinn et al., 2008). Increasing levels of NSC in feeds also increase the likelihood that some starch will be undigested and unabsorbed within the small intestine, leading to by-pass starches reaching the cecum or large intestine (Chapman et al., 1985). By-pass starches due to high NSC diets are rapidly fermented within the microbial environment of the equine cecum and large intestine and lead to altered bacterial population profiles and volatile fatty acid (VFA) production, decreased pH of the hindgut, and increased plasma lactate concentrations (Garner, et al., 1977; Julliand et al., 2001; McLean et al., 2001;

Richards et al., 2006), making high NSC diets a risk factor laminitis (Garner, et al., 1977).

High NSC diets are also implemented as a risk factor for colic, likely also due to disturbances in microbial populations (Hudson et al., 2001)

A further deleterious effect of meal feeding is the time in between meals when no feed is entering the digestive track. Stomach pH averaging < 2.0 has been measured in fasted horses (Murray and Schusser, 1993), and particularly low stomach pH is a risk factor for gastric ulcers (Nadeau et al., 2003). Further, mobilization of glycogen (Froesch et al., 1965), heightened circulating non-esterified fatty acid concentrations (Lawrence et al., 1995), and destructive behavioral patterns (Cooper et al., 2005) are all undesirable effects of withholding feed for 8 hr or more in horses. Upon feed re-introduction, horses are likely to consume feed at a more rapid pace than if feed were available in constant supply, and rapid feed intake is associated with choke in horses (Chiavaccini and Hassel, 2010).

Meal Associated Choke

Choke, or esophageal obstruction, is defined as impaired passage of feedstuffs in the esophagus (Feige et al., 2000) and common non-physiological causes include excessively rapid ingestion of dry fibrous, pelleted, or cubed feedstuff, or ingestion of carrots, apples, or improperly soaked beet-pulp (Chiavaccini and Hassel, 2010). In a retrospective study of 34 cases of esophageal obstruction treated at the Clinic of Veterinary Internal Medicine and Veterinary Surgery at the University of Zurich, Feige et al. found the most common signs of choke reported by the owners represented in the study were nasal discharge containing

ingesta, coughing, gulping, excess salivation, and extension of the head and neck (Feige et al, 2000). Twenty-eight of the cases presented had no anatomical cause associated with the incidence of choke, and in 21 out of these 34 cases, the cause of impaction was determined and was attributed to pelleted or cubed feed, grass or hay, carrot pieces, or apple pieces in 9, 5, 4, and 3 of the cases, respectively (Feige et al, 2000).

In an analysis of 109 cases of esophageal obstruction presented at the Equine Hospital at Colorado State University, Chiavaccini and Hassel found that 62.2% of horses who underwent edoscopy had tracheal contamination of ingesta present in the esophagus. Further, 51.4% of admitted horses had developed complications associated with esophageal obstruction, including aspiration pneumonia, esophageal strictures, and esophageal diverticula or rupture, and 11.9% of horses died or were euthanized as a result of the obstruction (Chiavaccini and Hassel, 2010).

Therefore, rapid ingestion of feedstuffs is considered a risk factor of choke and the complications following episodes of choke can be life threatening to horses. The presence of ingesta in nasal discharge and the esophagus is commonly associated with esophageal obstructions, and pelleted or cubed feed is the most frequent source of impaction when the cause is determined.

RESEARCH PURPOSE AND HYPOTHESIS

Current Methods to Reduce Risk Factors of Insulin Resistance and Associated Conditions by Management of Meal Administration

If it is deemed necessary to include meal feeding of concentrated feeds to horses, it is advisable to enact management methods which reduce the likelihood of insulin resistance (IR) development. Proposed methods to reduce the glycemic or insulineremic response to feeding and thus reduce the risk of IR development include altering the primary energy sources in the diet (Hoffman et al., 2003; Stull and Rodeik, 1987; Williams et al., 2001) and increasing the number of meals per day and subsequently reducing the size of each meal (Gordon et al., 2007).

Hoffman et al. published a report in 2003 discussing the effects of feeding 2 isoenergetic diets either rich in starch and sugar or high in fat and fiber. Glucose effectiveness and tissue sensitivity to insulin were measured after 8 weeks of feeding the diets to adult horses of various body condition scores (BCS) ranging from 5 to 9 (Henneke et al., 1983). Sensitivity to insulin and the acute insulin response to glucose both tended to decrease when the horses had consumed the diet high in sugar and starch when compared to the post-prandial response under the diet higher in fat and fiber, regardless of BCS (Hoffman et al., 2003). Williams et al. also investigated the effects of either a diet rich in starch and sugar or a diet high in fat and fiber but reviewed the effects in lactating mares. The diet high in fat and fiber resulted in a lower peak and a lower area under the curve (AUC) of both

glucose and insulin (Williams et al., 2001). Therefore, diets high in fat and fiber are able to decrease the peak glucose and insulin concentrations following feeding as well as the total glucose and insulin response to feeding when compared to diets high in starch and sugar, and diets high in starch and sugar tend to reduce tissue sensitivity to insulin and the responsiveness of insulin to glucose.

Stull and Rodiek reported in 1987 regarding feeding 4 different pelleted isoenergetic single meals to 2 year old horses to evaluate the variations in glucose and insulin concentrations when different feedstuffs comprised the meal. The 4 treatment meals included 100% alfalfa, 50% alfalfa and 50% corn, 100% corn, and 90% corn and 10% corn oil. Therefore, conclusions can be drawn regarding increasing levels of high starch feedstuffs in a diet, since the corn used in this experiment was 66.4% starch and the alfalfa was 1.6% starch, as well as the inclusion of added fats in the diet (corn oil) on post-prandial glucose and insulin levels. The diets which were 50% corn or 100% corn resulted in significant differences in the pre-feed glucose and insulin concentrations and the peak in glucose and insulin concentrations, indicating glucose and insulin levels increased dramatically following feeding, but the 100% alfalfa diet and the diet which included corn oil did not have a significant difference in the baseline and peak in glucose or insulin (Stull and Rodiek, 1987). Further, the 100% alfalfa diet resulted in a lower AUC of insulin than any of the diets which included corn (Stull and Rodiek, 1987). This is likely due to lower amounts of glucose absorption from the low starch alfalfa feed (as compared to the high starch content of the corn) since glucose is less available for digestion and absorption from higher fiber feeds like alfalfa. Further, the diet which included oil also would have lower starch content

than a corn only diet and therefore less glucose was available for absorption from the feed. Therefore, diets void of high starch grains like corn and diets in which added oils comprise a portion of the calories can prevent exaggerated peaks glucose and insulin levels following feeding, and diets void of high starch grains lower the total insulin response to feeding.

Both the effects of starch content and meal size on glucose and insulin dynamics were reported in a 2 part experiment by Gordon et al. in 2007. In the first experiment, 2 isocaloric feeds of lower (9.4%) and higher (18%) non-structural carbohydrate (NSC) were fed to mature horses for 6 days, and on the seventh day blood samples were taken to assess metabolite concentrations. All horses received the same amount of feed per meal, and thus the horses on the higher NSC diet received nearly twice the amount of NSC per meal. The high NSC diet resulted in higher average glucose and insulin, higher AUC glucose and insulin, and higher peaks in glucose and insulin (Gordon et al., 2007). In the second experiment, the same feeds were used as described in Exp. 1, but meal size was calculated so that the horses received the same amount of NSC in each meal. Therefore, both experimental meals provided each horse 0.3 g/kg body weight of NSC, requiring the low NSC meal to include approximately twice the amount of feed as the high NSC meal. There were no differences in the glucose response parameters between the 2 feeds when the grams of NSC intake of the horses was the same, but the larger meal resulted in higher average insulin and higher AUC insulin following consumption (Gordon et al., 2007). Therefore, when meal sizes are the same but one diet provides more NSC, both glucose and insulin responses are heightened under higher NSC intake. However, when grams of NSC intake are the same and the size of the meal is different, a larger meal will induce an exaggerated insulin response to

feeding, suggesting the physical size of the meal alone may somehow influence the insulin response to the meal.

The proposed effectiveness of increasing number of meals per day, and thereby decreasing the size of the meal, is related to limiting the amount of concentrated feed entering the digestive tract at a given time, reducing the volume of digesta within a given portion of the digestive tract, and also decreasing the total amount of glucose absorption per meal, and thus attenuating the insulin response following a single meal. Limiting the amount of feed entering and within the tract by feeding multiple small meals throughout the day is more similar to the feed intake rate and digesta volumes a grazing horse would experience when compared to the fluctuations in digestive status for a horse who consumes only two large meals per day.

Hypothesis

Uninhibited concentrate ingestion normally allows the horse to consume large amounts of feed in a short period of time. If increasing the number of meals per day (and thus decreasing the amount of concentrated feed entering the digestive tract and limiting the amount of digesta present within a given time) is effective at decreasing the insulin response to feeding, it is conceivable that simply slowing the rate of ingestion could demonstrate similar results. Specifically, feed delivery methods which slow the rate of concentrate feed intake (and therefore prolong the total time taken to consume a meal) may attenuate the insulin response to feeding when compared to uninhibited concentrate feed consumption.

Slowing feed intake and prolonging the progression of concentrate feed into the digestive tract would be similar to the intake method of a grazing equine, as the amount of feed consumed and swallowed while a horse grazes is limited by the physical structure of the grass and the capacity of the mouth. Therefore, this author hypothesizes that feed delivery methods which successfully prolong feed intake time and may limit extreme post-prandial metabolite responses.

Current Feed Intake Time Research and Methods Shown to Slow Feed Intake

A variety of research has measured the time needed to consume various feedstuffs in horses, but little research has been conducted to determine how time to consume feed physiologically affects the horse. Bergero and Nardi reported in 1996 that consuming 1 kg of feed took horses 557 s (9 min 17 s) for whole oats, 548 s (9 min 8 s) for whole barley, and 827 s (13 min 47 s) for whole corn, but pelleting, crushing, adding water, and flaking all decreased the time needed to consume these grains (Bergero and Nardi, 1996).

Brökner et al. (2008) compared the total amount of time spent eating and the number of jaw movements (JM) needed to consume different concentrate types and found that pelleted feed (17 min/kg DM) was consumed faster than ground oats (26 min/kg DM) or whole oats (25 min/kg DM) (Brökner et al, 2008). Further, either oat form was consumed faster than the loose chaff mix (30 min/kg DM), and, while there was no difference between time to consume ground and whole oats, fewer jaw movements were involved in consuming ground oats (272 JM) than for whole oats (399 JM) (Brökner et al, 2008). In 1976, Meyer et

al. compared feed consumption time of 1 kg of medium quality long cut hay, 1 kg of either oat grains or oat pelleted mixtures, and 1 kg of the concentrate feeds with up to 20% chopped forage added. One kilogram of long cut hay only took approximately 2400 s (40 min) to consume, 1 kg of oat grains or oat pelleted mixtures took approximately 600 s (10 min) to consume, and the concentrate and chopped hay mixture took between approximately 780 to 1200 s (13 to 20 min) to consume (Meyer et al, 1975).

Therefore, processing, adding water, and pelleting grains is associated with faster feed concentrate consumption, while, alternatively, leaving grains in a whole state, delivering them as a loose chaff mix, or adding chopped hay increases time taken to consume feed. However, processing grains to a greater extent, such as through micronizing, increases prececal digestibility of starches in horses (Rosenfeld and Austbø, 2009), indicating a reduced risk of by-pass starch reaching the cecum and large intestine. Also, consumption of barley which has been only physically processed unfavorably alters the environment of the cecum (McLean et al., 2000). More involved processing, like micronizing and extrusion of barley, maintains cecal fermentation parameters generally associated with forage only diets, including pH and lactic acid formation, and micronizing results in similar volatile fatty acid production profiles associated with hay only diets (McLean et al., 2000). Further, when grains and hay are mixed together, fiber digestion decreases when compared to a hay only meal and rate of passage through the digestive tract increases when hay is added to a grain meal (Drougal et al., 2001), indicating a mixed meal of hay and grain reduces potential digestion of both feed types. Therefore, alternative methods to slow feed intake are needed,

and currently no research has investigated the impacts of increasing time to consume feed on glucose and insulin responses to feeding.

A Novel Method to Slow Feed Intake

Physical obstacles included in a feed delivery method may be an alternative way to slow feed intake in horses, thereby prolonging time needed to consume feed. Some potential feed bucket designs which slow feed intake could involve obstacles placed above the feed, obstacles built in to the bucket base which allow obstacles to be underneath the feed, or mechanisms which limit the amount of feed the animal has access to, such as a timed-release or trickle feeders.

Feed bowls with built in obstacles and obstacles designed to be placed above feed are commercially available for use in both dogs and cats (Greedy Pup, LLC, New York City, NY; Hagen, Inc., Montreal, Canada; JW Pet Company, Inc., Teterboro, NJ; Omega Paw, Inc., Ontario, Canada; Petco Animal Supplies, Inc., San Diego, CA). Few commercial buckets are available for horses with similar construction concepts, but one commercially available bucket with molded cups in the base, which allows feed to settle into the lowered portion is available (Pre-vent Group, LLC, Houston, TX), and this bucket has been shown to significantly increase time to consume meals when compared to standard, flat bottomed commercial feed buckets (Carter, et al., 2012). However, the use of physical obstacles as a means to prolong feed intake time in order to exert effects on post-prandial metabolites has not been specifically investigated.

CONCLUSION

Consumption of concentrate feeds can increase the risk of a variety of metabolic and physiological disease states. Current methods to reduce metabolic disorders as a result of concentrate consumption require marked changes in feed type and managerial operations. Instead, it is proposed that increasing feed intake time may result in similar beneficial effects on post-prandial metabolite concentrations. While limited concentrate processing and adding forage to concentrates has been shown to increase feed intake time, both of these methods are also associated with reduced digestibility and unprocessed feeds can lead to unfavorable alterations in microbial profiles. Therefore, the intent of this research was to determine if physical obstacles in a feed bucket, either above or below feed, effectively increase time to consume feed in horses and if there are alterations in post-prandial glucose and insulin when time to consume feed is increased.

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CHAPTER TWO

Research Trials

(Formatted in style of the Journal of Animal Science)

The effect of different feed delivery methods on time to consume feed and the resulting changes in post-prandial metabolite concentrations in horses¹.

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ABSTRACT: Management techniques that reduce the insulin response to feeding in horses have application in preventing insulin resistance (IR) and potential associations (e. g., laminitis). Eight mature idle horses of BCS between 5 and 6.5 and with no previous indication of IR were fed a meal of concentrate feed under 4 feed delivery treatments in a repeated Latin Square design. Treatments were all based on a bucket of equal dimensions. The treatments included a control (CON) and 3 treatments hypothesized to increase time to consume feed (TIMEfeed): mobile obstacles above the feed (BALL), stationary obstacles below the feed (WAFF), and feed with water added (WTR). Jugular venous blood samples were taken at feed delivery, every 10 min for the first hour, and then every 30 min until 300 min post feed delivery. TIMEfeed was greater ($P = 0.004$) for BALL and WAFF when compared to CON and WTR. Compared to CON and WTR, average glucose and insulin concentrations tended to decrease due to BALL ($P = 0.059$) and WAFF ($P = 0.072$) and the peak glucose concentration was decreased ($P = 0.049$). Compared to all other treatments, peak insulin concentrations ($P = 0.030$) and area under the curve of insulin ($P = 0.051$) were decreased and time to peak insulin was increased ($P = 0.031$) due to BALL. Therefore, feed delivery methods that include obstacles effectively increase TIMEfeed and attenuate post-prandial glucose and insulin parameters. A second experiment was designed to determine if the TIMEfeed changes associated with BALL and WAFF in Exp. 1 remain effective over 4-d periods. Four horses with no recent or regular history of consuming meals were fed concentrate meals for 4 consecutive days using the same treatments described in Exp. 1 under a Latin Square design. Horses were subject to a 4-d adaptation period (ADP) and were randomly assigned to 4-d treatment periods using the 4 previously described treatments.

During ADP, TIMEfeed decreased ($P = 0.018$). However, following adaptation, TIMEfeed did not decrease significantly over 4 days of any treatment feed delivery method, but BALL and WAFF had higher TIMEfeed when compared to CON and WTR ($P < 0.001$) and maintained prolonged TIMEfeed after 4 d of use ($P = 0.006$). Utilizing obstacles to increase TIMEfeed on a daily basis may be an effective method to reduce post-prandial glucose and insulin concentrations, thereby decreasing the risk of insulin resistance development in horses.

Key Words: feed management, horse, insulin resistance, meal

INTRODUCTION

Daily inclusion of concentrate meals is a regular management practice for equines (United States Department of Agriculture, 1998; Richards et al., 2006; Hoffman et al., 2009). Concentrated feeds can be high in non-structural carbohydrates (NSC), and high NSC diets have been attributed to reduced insulin sensitivity in horses (Pratt et al., 2006, Quinn et al., 2008) and increased post-prandial insulin responses to feeding (Stull and Rodiek, 1987). Repeated or long-term exposure to heightened insulin concentrations reduces tissue sensitivity to insulin (Kopp, 2003). This increases the risk of developing insulin resistance (IR), defined as a decrease in liver, skeletal, and adipose tissue sensitivity to insulin and a resulting decrease in the effectiveness of insulin directed uptake of glucose into these tissues

(Kronfeld et al., 2005). Also, IR is a risk factor for development of laminitis and is therefore of concern for horse owners (Treiber et al., 2006).

Despite these potential risks, substantial concentrate intake is often a necessary component of the equine diet for horses with high energy requirements or when quality forage is not available, so it is of interest to investigate methods that reduce the risks associated with feeding daily concentrate meals. Various diet and management changes have been proposed to reduce post-prandial insulin levels, including use of feeds high in structural carbohydrate (fiber), replacing NSC with fats as the primary energy source, and feeding more frequent, smaller meals (Kronfeld et al., 2005).

The following experiments aimed to investigate the effects of novel feed delivery methods on the glucose and insulin response to feeding without diet or extensive management changes. It was hypothesized that feed delivery methods which force equines to take longer to consume a concentrate meal would attenuate the glucose and insulin response to feeding when compared to uninhibited concentrate feed consumption.

MATERIALS AND METHODS

All research techniques were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Exp. 1 was designed to determine if different feed delivery methods could increase time to consume concentrate feed (**TIMEfeed**) and subsequently decrease post-prandial glucose and insulin responses to feeding.

Experiment 1

Eight mature idle horses (six geldings and two mares) ranging in age from 6 to 12 years and of Quarter Horse, Thoroughbred, and Arabian lines were utilized in this trial. The horses had a mean BW of 544 ± 53 kg and BCS ranging from 5 to 6.5 out of 9 (Henneke et al., 1983), and no horse used had any history or indication of IR. Once per week, each horse was fed a single concentrate meal in 1 of 4 feed delivery methods as part of a 4 by 4 repeated Latin Square design. Thus, the trial lasted 4 weeks with 2 sampling days per week. Horses were randomly assigned to a sampling day group (Tuesday or Thursday) before being randomly assigned to treatments, and each horse had 6 d without concentrate meal intake between sampling days when they were maintained solely on grass hay mix of Bermudagrass, Fescue, and alfalfa hay, described in greater detail below.

Each delivery method was based in a commercially available 43 cm diameter by 20 cm depth rubber bucket which was hung by 3 points at approximately 1 m off the ground. The control delivery (**CON**) involved an unaltered bucket and unaltered feed. The ball delivery method (**BALL**) involved an unaltered bucket and unaltered feed with 4 balls placed in the bucket. The balls used were bocce style (Bocce Standards Association of the United States), chosen for their smoothness, uniform size (10.7 cm diameter), and heavy weight (0.92 kg each). The waffle delivery method (**WAFF**) also involved unaltered feed but had an insert attached to the bucket base constructed from 1.25 cm plywood and 2.5 cm and 5 cm polyvinyl chloride pipes. These pipes were transversely cut and attached to the plywood at intersecting angles, forming crossed raised bars that were 1.25 and 2.5 cm tall and creating

dips that were approximately 2.5 by 5 cm that the feed would settle into. The water delivery method (**WTR**) consisted of an unaltered bucket containing the weighed feed plus an equal weight portion of water. The feed soaked for approximately 20 min prior to feeding.

Prior to the start of the trial, all horses were housed in a mixed grass and white clover pasture with unlimited access to water. Seven days before the trial began, the horses were contained individually in partially covered 5 m by 15 m runs without access to pasture, and horses were offered approximately 7 kg of Bermudagrass and Fescue hay mix and 3 kg of alfalfa hay per day, given to each horse in 2 meals at 0800 and 1600 hr. Additionally, all horses had free access to a trace mineral salt block and water.

On treatment days, horses were weighed and fitted with a jugular catheter (14 gauge, 12 cm) and extension line to facilitate blood sampling approximately an hour before treatments were administered. Blood samples were collected and placed into evacuated tubes containing either no additive for the collection of serum or EDTA for the collection of EDTA-plasma (BD Diagnostics, Franklin Lakes, NJ). After collection, EDTA blood samples were cooled in a refrigerator and samples containing no additive were allowed to clot at room temperature for approximately 1 h. All samples were centrifuged at approximately 1500 x g for 15 min at 5°C. The serum and plasma were then harvested and frozen at -20°C until subsequent analysis.

The horses were given 1 kg/500 kg of BW of commercially available pelleted feed (minimum guaranteed analysis: 14% crude protein, 6.5% crude fat, 12.5% crude fiber, and 26% non-structural carbohydrate on an as-fed basis) via 1 of 4 delivery methods on sample collection days. The horses consumed only the pelleted feed on the morning of sample

collections and were not allowed access to any other feed, water, or trace mineral block throughout sample collections, as horses remained tied individually in runs during concentrate feeding and the duration of sample collections. As feed was poured into feed buckets, a blood sample was drawn and this sample was considered time 0. Time to consume feed started with the first bite of food and time until all loose pellets were consumed and only feed fines remained was recorded as TIME_{feed}. Additional blood samples were initially taken every 10 min post-feed introduction up to 60 min post-feed introduction. Samples were then taken every 30 min up to 300 min post-feed introduction.

Plasma was analyzed for glucose concentrations in triplicate using a commercially available kit (Autokit Glucose C2, Wako Chemicals USA Inc., Richmond, VA) and spectrophotometry (Pratt et al., 2007; Nielsen et al., 2010). Serum was analyzed for insulin concentrations in duplicate using a radioimmunoassay kit (Coat-A-Count, Siemens Healthcare Diagnostics Inc., Terrytown, NY) previously validated for use in horses (McGowan et al., 2008). For glucose and insulin, the number of assays needed to analyze all samples was $n = 16$ and $n = 5$, respectively; the mean intra-assay CV was 5.99% and 4.93%, respectively; the inter-assay CV was 6.06% and 8.54%, respectively; and the minimum detectable concentration was 0.07mg/dL and 1.2 uU/mL, respectively.

Data were analyzed using SAS 9.2 (SAS Institute, Inc., Cary, NC) with the MIXED procedure. Metabolite concentrations following feeding (plasma glucose (**GLU**) and serum insulin (**INS**)) were analyzed with a repeated measures mixed model of SAS with Horse within Day*Week as the experimental unit. Classes included Horse, Sample Day, Week, Treatment, and Time after feeding. The model included Treatment, Time after feeding, and

Treatment*Time after feeding, with Horse, Day, and Week considered random effects. Area under the curve (**AUC**) of glucose (**AUCglu**) and insulin (**AUCins**) were calculated using the trapezoidal method. The peak concentration (**PEAK**) and the time to reach the peak concentration (**TIMEPEAK**) of each metabolite was determined as are noted as the peak in glucose (**PEAKglu**) and insulin (**PEAKins**) and time to reach the peak in glucose (**TIMEPEAKglu**) and insulin (**TIMEPEAKins**). The AUC, PEAK, and TIMEPEAK of each metabolite were analyzed using the mixed model of SAS. For this analysis, Horse within Day*Week was the experimental unit. Classes included Horse, Day, Week, and Treatment. The model only included Treatment, with Horse, Day, and Week considered random effects. TIMEfeed, INS, and the AUC of each metabolite were not normally distributed and were therefore log transformed for statistical purposes. Statistical significance was accepted at $P < 0.05$ and trends accepted at $P < 0.10$. Differences among treatments means were determined by the lsmeans/pdiff option for both mixed model analyses, and if an effect of treatment was at least a trend ($P < 0.10$), the least squares means (**LSM**) of the concentrations are reported to compare treatments. All means are reported \pm the SE.

Experiment 2

As a result of the findings from Exp. 1, Exp. 2 was designed to assess if TIMEfeed changes significantly in the first 4 d of introducing meals in an equine management plan and if the methods hypothesized to increase TIMEfeed in Exp. 1 can continue to be effective after

4 d of use. Exp. 2 took place approximately 1 year after Exp. 1. Four mature idle geldings between 6-12 years of age, of Quarter Horse and Thoroughbred lines, and with an average weight of 610 ± 32 kg were utilized in this trial. Horses utilized were considered of average temperament and had no history of regular or recent concentrate meal feeding, as the horses had been kept on pasture for the 6 months prior.

A 4 d adaptation period (**ADP**) was implemented in Exp. 2 to accustom the horses to concentrate meal feeding from a bucket and to determine if TIMEfeed changed over the first 4 days of implementing concentrate meals in a daily feeding schedule. Following the adaptation period, 4 feed delivery methods (previously described in Exp. 1) were given to the horses in a Latin Square Design with each treatment being administered for 4 consecutive days. Upon the completion of each 4 day treatment period, horses moved to the next treatment the following day, so there were no days between periods. When ADP is included, the horses consumed concentrate meals for a total of 20 d, and TIMEfeed was recorded each day for each horse.

During the trial, the horses grazed ad libitum on mixed grass and white clover pastures from 800 h to 1600 h and were contained individually in 5 m by 15 m runs without access to pasture the remainder of the day. The horses had ad libitum access to water at all times. Treatments were administered each day at 1700 h, and after completion of data collection, the horses received approximately 8 kg of Fescue and Bermudagrass mix hay.

The first day of each period, the horses were weighed after coming in from pasture and the amount of feed to be given for the 4 d period was calculated. The administered feed was commercially available pelleted feed (previously described in Exp. 1) offered at a rate

of 1kg/500 kg body weight. At 1700 hrs, after stall confinement for an hour, the treatment buckets and feed were placed in the stalls. TIMEfeed started with the first bite of food and time until all loose pellets were consumed and only feed fines remained was recorded as TIMEfeed.

Exp. 1 data were analyzed using SAS 9.2 (SAS Institute, Inc., Cary, NC) with the mixed procedure. One-way ANOVA was used to analyze TIMEfeed during the four days of ADP, but data from ADP was not included in treatment data analysis, as it represented the 4 days prior to the commencement of the randomly assigned treatment periods. TIMEfeed over the 4 days of each treatment period was analyzed as a repeated measures mixed model of SAS with Horse within Period as the experimental unit. Classes included Horse, Day of treatment (**DOT**), Period, and Treatment. The model included Treatment, DOT, and Treatment*DOT, with Horse and Period considered random effects. Unlike our TIMEfeed data in Exp. 1, TIMEfeed was normally distributed for this trial and therefore did not require transformation for analysis. Statistical significance was accepted at $P < 0.05$ and trends accepted at $P < 0.10$. Differences among Treatment, DOT, and Treatment*DOT were determined by the lsmeans/pdiff option for the mixed model analysis, and if an effect of treatment or DOT was at least a trend ($P < 0.10$), the LSM of TIMEfeed are reported to compare the differences among treatments, DOT, and treatment*DOT.

RESULTS

Experiment 1

TIMEfeed. The average TIMEfeed was 693.91 ± 324.36 s (11.57 ± 5.41 min). There was a significant effect of treatment on logTIMEfeed ($P = 0.014$) (Figure 1). Feed consumption time for BALL was greater than CON ($P = 0.049$) and WTR ($P = 0.029$). Similarly, WAFF was greater than CON ($P = 0.012$), and WTR ($P = 0.007$). There was no difference between WAFF and BALL ($P = 0.495$) or between CON and WTR ($P = 0.802$). Specifically, 7 out of 8 horses had the shortest TIMEfeed under the CON or WTR treatments and 6 out of 8 horses had the longest TIMEfeed under BALL or WAFF treatments (data not shown).

Plasma Glucose. There was a significant effect of time ($P < 0.001$) and a trend for the effect of treatment ($P = 0.059$) on GLU, but there was no interaction between time and treatment ($P = 0.994$) (Figure 2). The LSM glucose concentration of BALL was lower than CON ($P = 0.022$), and the LSM glucose concentration of WAFF was lower than CON ($P = 0.032$). There was no statistical difference between the LSM glucose concentration of BALL and WAFF ($P = 0.850$), CON and WTR ($P = 0.459$), or WAFF and WTR ($P = 0.130$).

There was a significant effect of treatment on PEAKglu ($P = 0.049$) (Table 1). PEAKglu for CON was greater than BALL ($P = 0.008$) and WAFF ($P = 0.047$). There was no difference in the PEAKglu of BALL and WAFF ($P = 0.386$), BALL and WTR ($P =$

0.246), or WAFF and WTR ($P = 0.758$). There was no significant effect of treatment on TIMEPEAKglu ($P = 0.823$) or logAUCglu ($P = 0.385$) (Table 1).

Serum Insulin. Insulin concentrations over time are shown in Figure 3. There was a significant effect of time ($P < 0.001$) and a trend for treatment ($P = 0.072$) on logINS, but no interaction between time and treatment was found ($P = 0.624$). The LSM logged insulin concentration of BALL was lower than WTR ($P = 0.031$), and the LSM logged insulin concentration of WAFF was lower than WTR ($P = 0.045$). There was no statistical difference between the LSM logged insulin concentration of BALL and WAFF ($P = 0.850$), CON and WAFF ($P = 0.113$), or CON and WTR ($P = 0.633$).

There was a significant effect of treatment on PEAKins ($P = 0.030$) (Table 1). PEAKins for BALL was lower than CON ($P = 0.013$) and WAFF ($P = 0.007$). There was no statistical difference in the PEAKins of CON and WAFF ($P = 0.795$), CON and WTR ($P = 0.446$), and WAFF and WTR ($P = 0.311$).

There was a significant effect of treatment on TIMEPEAKins ($P = 0.031$) (Table 1), with BALL taking longer to reach TIMEPEAKins than WAFF ($P = 0.025$), and WTR taking longer to reach TIMEPEAKins than WAFF ($P = 0.008$). There was no statistical difference in TIMEPEAKins of BALL and CON ($P = 0.177$), BALL and WTR ($P = 0.581$), or CON and WAFF ($P = 0.316$).

There was a trend for the effect of treatment on logAUCins ($P = 0.051$) (Table 1). AUCins of BALL was less than CON ($P = 0.020$) and WTR ($P = 0.014$), and tended to be lower than WAFF ($P = 0.061$). There were no statistical differences between AUCins of CON and WAFF ($P = 0.582$), CON and WTR ($P = 0.871$), or WAFF and WTR ($P = 0.478$).

Experiment 2

The average TIMEfeed over Exp. 2 was 578.50 ± 186.92 s (9.64 ± 3.16 min). The effects of treatments and DOT on TIMEfeed are shown in Figure 4. Data from ADP is included in this graph but was not included in statistical analysis of the treatment periods. During ADP, there was a significant effect of DOT on TIMEfeed ($P = 0.018$) with TIMEfeed of Day 1 being significantly greater than TIMEfeed of Day 4 of ADP ($P = 0.034$). Day 2 and Day 3 were not statistically different from any other days of ADP.

During the treatment periods, there was a significant effect of treatment on TIMEfeed ($P < 0.001$) and a significant effect of DOT on TIMEfeed ($P = 0.006$), but no interaction was found between treatment and DOT ($P = 0.300$) (Figure 4). Specifically, the TIMEfeed of WAFF (865.94 s) was greater than the TIMEfeed of BALL ($P = 0.001$), CON ($P < 0.001$), and WTR ($P < 0.001$) for every DOT. TIMEfeed of BALL (598.75 s) tended to be greater than CON ($P = 0.064$), and TIMEfeed of BALL was greater than WTR ($P = 0.001$) for every DOT. The TIMEfeed of CON (510.62 s) was greater than WTR ($P = 0.011$) for every DOT, and therefore WTR had the lowest TIMEfeed of all treatments (371.56 s).

The effect of DOT resulted in a decrease in TIMEfeed from 603.12 s on Day 1 to 573.44 s Day 2, an increase from Day 2 to 601.25 s on Day 3, and a decrease from Day 3 to 569.06 s on Day 4 across all treatments. Specifically, TIMEfeed of Day 1 was greater than Day 2 ($P = 0.014$) and Day 4 ($P = 0.005$), and TIMEfeed of Day 3 was greater than Day 2 ($P = 0.021$) and Day 4 ($P = 0.008$). There was no difference in Day 1 and Day 3 ($P = 0.871$) or Day 2 and Day 4 ($P = 0.705$). Therefore, the average TIMEfeed of each DOT did not

steadily increase or decrease across 4 days of treatments and instead regularly fluctuated up and down (Figure 4).

DISCUSSION

The major findings of these studies are that feed delivery methods that include obstacles, such as mobile balls or inserts that create dips in the bucket base, can successfully increase TIME_{feed}, even after 4 days of acclimation to the obstacles, and this can decrease post-prandial metabolite concentrations. These findings suggest that simple managerial changes to feeding protocols may be an effective way to reduce some risk factors for development of insulin resistance in horses.

The obstacles in BALL and WAFF both prolonged the time horses needed to consume concentrate but worked in very different ways. The WAFF design allowed food to settle between grooves, making feed retrieval difficult and prolonging the time until all feed particles were consumed. The BALL treatment required the horses to continuously maneuver the balls to access the food, forcing the horses to eat more slowly. The bocce balls were chosen to mimic rocks often used by horse owners to slow feed intake but were considered a safer, more ideal choice with a uniform size and weight. It is notable that 1 horse in each experiment was able to remove 1 ball from the bucket, indicating a limitation and potential negative aspect of this method. Though the removed ball was quickly returned during the experiments, falling bocce balls pose a risk for physical harm to horses' legs, and

once even a single ball is removed, this feed delivery method would become less effective at increasing TIMEfeed.

In Exp. 1, TIMEfeed was significantly increased for both BALL and WAFF, but the largest number of significant and beneficial effects on post-prandial glucose and insulin concentrations was seen with the BALL treatment. The average glucose and insulin concentrations and the PEAK glucose of BALL and WAFF were decreased compared to other treatments, but under the BALL treatment, PEAKins and logAUCins were also reduced and TIMEPEAKins was increased. The fact that these findings were not associated with WAFF may be a function of how effective these 2 treatments could be at altering the specific rate of feed intake (such as grams of feed consumed per minute) as opposed to their respective effectiveness of simply increasing the total TIMEfeed.

The nature of the BALL treatment, with obstacles above the feed, may be more successful in forcing the horses to consume feed at a slower rate throughout the entire meal. The WAFF treatment involved fixed obstacles that were a maximum of 2.5 cm in height, so when the pelleted feed was poured on the insert, the bottom layer of feed settled into the dips but the top layer of feed was unobstructed by the insert. Because feed settled into the dips of the waffle insert, the WAFF treatment likely only slowed feed consumption towards the end of the meal and therefore had little impact on how quickly the horses consumed feed at the beginning of the meal. The overall flatter insulin concentration curve of the BALL treatment compared to the peak and then slow decline of the WAFF treatment supports this theory. It is possible that the actual rate of feed consumption is the key factor to producing the greatest

extent of desirable changes in insulin parameters. However, actual bite rates and feed consumption rates were not determined in either study.

Treatments which include obstacles significantly increased TIME_{feed}, and both of these treatments tended to decrease average glucose, peak glucose, and average insulin concentrations following feeding. This finding has significant implications for horses at risk of developing metabolic diseases related to insulin resistance, as the amount and duration of tissue exposure to insulin is a factor in reducing tissue sensitivity to insulin (Kopp, 2003), which is implicated in the pathogenesis of IR (Kronfeld et al., 2005). It is therefore of interest to develop feed delivery methods which are even more effective at increasing TIME_{feed}, preferably by decreasing the actual rate of feed intake, as this may be an even more effective means of beneficially altering glucose and insulin parameters following concentrate meals.

The average post-prandial concentrations of glucose in Exp. 1 ranged from 101.14 to 111.23 across treatments, with CON having the highest average glucose and BALL and WAFF having the lowest averages. The average for CON is similar to findings by Stull and Rodiek in 1987 when feeding 1.33 kg of a 50:50 mixture of pelleted alfalfa and corn, which had an average post-prandial glucose response of 112.0 mg/dL (Stull and Rodiek, 1987). However, the average post-prandial insulin response reported by Stull and Rodiek for the pelleted alfalfa and corn mixture was 22.9 uU/mL (Stull and Rodiek, 1987), and the average post-prandial insulin response of this trial ranged from 34.39 to 54.23 uU/mL. However, it is of interest that the amount of time to consume meals in the experiment by Stull and Rodiek is reported as between 15 and 60 min and that up to 15% of feed was reported as unconsumed

(Stull and Rodiek, 1987), which may be reasons average insulin was considerably lower for their pelleted alfalfa and corn mixture.

Glucose concentrations are controlled by a variety of homeostatic mechanisms within horses (Hyypä, 2005), so it was not surprising that glucose parameters were less subject to substantial differences among the treatments when compared to the more dynamic response found for insulin. This is especially unremarkable given the moderate non-structural carbohydrate percentage of our treatment feed (26%). However, the reduction in average glucose and PEAKglu due to treatments which include obstacles demonstrates that glucose concentrations can be altered through feed delivery methods.

Despite having the lowest TIMEfeed, WTR also tended to decrease PEAKglu when compared to CON. It is possible the dilution of concentrated feed by water, which increased the volume of the feed, may have altered transit rates or digestion and absorption rates. This concept may also explain how WTR prolonged TIMEPEAKins when compared to CON and WAFF. However, the effects of digesta volume on digestion or absorption rates were not investigated in this experiment. Though TIMEfeed of CON and WTR were only statistically different from each other in Exp. 2, WTR had the lowest average TIMEfeed in both experiments, which may have been because the inclusion of water in the feed reduced the amount of chewing required by the horses and made swallowing the pelleted feed easier and faster.

In Exp. 2, TIMEfeed decreased over the 4 days of ADP when horses were first introduced to regular, daily meals. However, over 4 days of each feed delivery method, TIMEfeed did not decrease significantly and instead fluctuated up and down, albeit within 45

s across all DOT, with some regularity. Therefore, TIMEfeed can be considered relatively stable following a phase of adaptation to consuming meals. This is visually evident when ADP over time is graphed adjacent to CON over time, since both are based on an unaltered bucket with unaltered feed, and TIMEfeed clearly plateaus with the beginning of CON.

BALL and WAFF both significantly increased average TIMEfeed when given with a single meal in Exp. 1. In Exp. 2, WAFF increased TIMEfeed when compared to all other treatments and BALL increased or tended to increase TIMEfeed when compared to WTR and CON, respectively, when given for 4 consecutive days. Therefore, following an adaptation period, since TIMEfeed does not decrease significantly with 4 days of feeding under any specific treatments, obstacles in a feed bucket can be considered effective at maintaining increased TIMEfeed after multiple days of use. It is possible that 4 d treatment periods were insufficient to find substantial changes in TIMEfeed, particularly given the pattern of change in TIMEfeed over the 4 DOT. More days of feeding may allow horses to decrease TIMEfeed, either with or without the presence of obstacles, and thus even longer term studies are warranted.

Because rapid feed intake has been identified as a risk factor for choke in horses (Chiavaccini and Hassel, 2010), the ability of BALL and WAFF to increase TIMEfeed may indicate obstacles in a feed bucket as useful tools to reduce feed intake rates in horses prone to choke. However, choke did not occur in any of the horses in the experiments, therefore specific recommendations regarding choke prevention are not available from this experiment.

While a variety of commercially available feeder designs propose to slow feed intake in canines and felines (Greedy Pup, LLC, New York City, NY; Hagen, Inc., Montreal,

Canada; JW Pet Company, Inc., Teterboro, NJ; Omega Paw, Inc., Ontario, Canada; Petco Animal Supplies, Inc., San Diego, CA), only one commercial feeder with similar design has been found by this author that is marketed as slowing feed consumption time in horses (Prevent Group, LLC, Houston, TX). This bucket has molded cups in the base which allows feed to settle into the lowered portion, making this design similar to the WAFF treatment, and has been shown to significantly increase time to consume meals when compared to standard, flat bottomed commercial feed buckets (Carter, et al., 2012). However, this study only determined time to consume feed and did not measure the post-prandial metabolic response to feeding under the different treatments.

Both BALL and WAFF treatments effectively increased TIME_{feed} in Exp. 1 when given with a single meal, but WAFF was clearly more effective at increasing TIME_{feed} when given under a more regular, daily feeding schedule in Exp. 2. Yet, BALL resulted in a greater number of changes in insulin concentrations in Exp. 1. Each treatment worked in different ways to increase TIME_{feed}, which seems to be related to their respective ability to effectively increase TIME_{feed} after multiple days of feeding and to beneficially alter post-prandial metabolism.

BALL is less effective at promoting long-term heightened TIME_{feed} and potentially poses safety risks due to falling balls, and WAFF does not promote as many desirable alterations in metabolite concentrations following concentrate meals. As a result, additional research and development is necessary to produce a feed delivery method that is more effective at both increasing the time it takes for horses to consume a meal and simultaneously attenuating post-prandial glucose and insulin concentrations.

Conclusions

The time it takes horses to consume feed can be effectively increased by inclusion of obstacles in the feed buckets, and obstacles either above or below the feed also tends to decrease the average glucose and insulin concentration following feeding and the peak glucose concentration. However, additional effects are found with obstacles placed above the feed, including decreased peak and total insulin concentrations and increased time to peak insulin concentration following feeding. This may be due to the location of the obstacles above the feed continuously restricting feed intake. Further research into even more effective and safe feed delivery methods that increase the time to consume feed in equines is warranted. However, this research demonstrates the potential of simple management techniques to reduce the risk of horses developing insulin resistance and associated conditions such as laminitis.

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TABLES AND FIGURES

Table 1. The effects of four feed delivery methods¹ on glucose and insulin

Parameter	Treatment Mean				SE	P-Value
	Control ²	Ball ³	Waffle ⁴	Water ⁵		
Glucose						
PEAK ⁶ , mg/dL	144.52 ^b	123.60 ^a	129.71 ^a	131.85 ^{ab}	7.38	0.049
TIMEPEAK ⁷ , min	153.75	141.50	157.50	123.75	26.10	0.750
logAUC ⁸ , (mg/dL)/min	5.40	5.01	5.04	5.10	0.18	0.385
Insulin						
PEAK ⁶ , uU/mL	111.96 ^b	62.94 ^a	116.64 ^b	98.17 ^{ab}	34.79	0.030
TIMEPEAK ⁷ , min	157.50 ^{ab}	195.00 ^b	130.00 ^a	210.00 ^b	31.14	0.031
logAUC ⁸ , (uU/mL)/min	5.78 ^b	5.28 ^a	5.67 ^{ab}	5.81 ^b	0.41	0.051

^{a, b, c} Within a row, means without a common superscript letter differ ($P < 0.05$).

¹ All feed delivery methods were based on the same commercially available bucket with a depth of 20 cm and diameter of 43 cm

² Control treatment was an unaltered bucket with unaltered feed

³ Ball treatment had 4 bocce style balls (10.7 cm diameter, 0.92 kg) placed above the feed to act as mobile obstacles

⁴ Waffle treatment had an insert attached to the base to act as stationary obstacles under the feed as the feed was poured on top of the insert

⁵ Water treatment had an unaltered bucket and an equal weight water portion added to feed

⁶ The highest concentration measured within 300 min post-feeding

⁷ Time until the highest concentration was measured

⁸ Area under the curve of the metabolite concentrations up to 300 min post-feeding as determined by the trapezoidal method

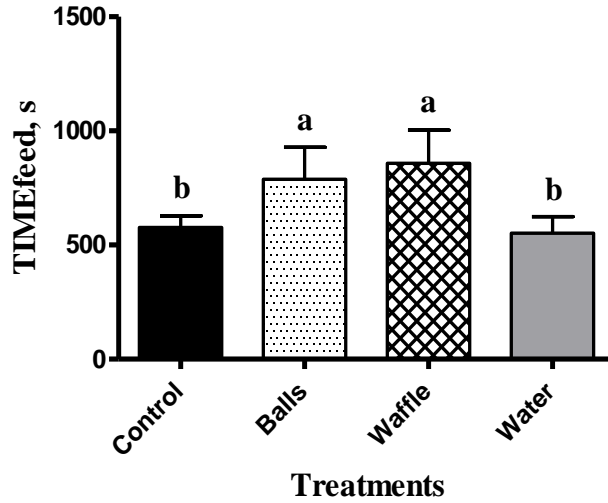


Figure 1. Time to consume feed (TIMEfeed) across treatments in Experiment 1. Eight horses were fed a concentrated feed in 4 different feed delivery methods, including 2 methods which contained obstacles (Balls and Waffle) and 2 methods which did not contain obstacles (Control and Water), and the TIMEfeed was recorded. Bars represent mean \pm SE. A significant effect of treatment was found on logTIMEfeed ($P = 0.014$). Treatment means without a common letter differ at $P < 0.05$ when TIMEfeed was log transformed.

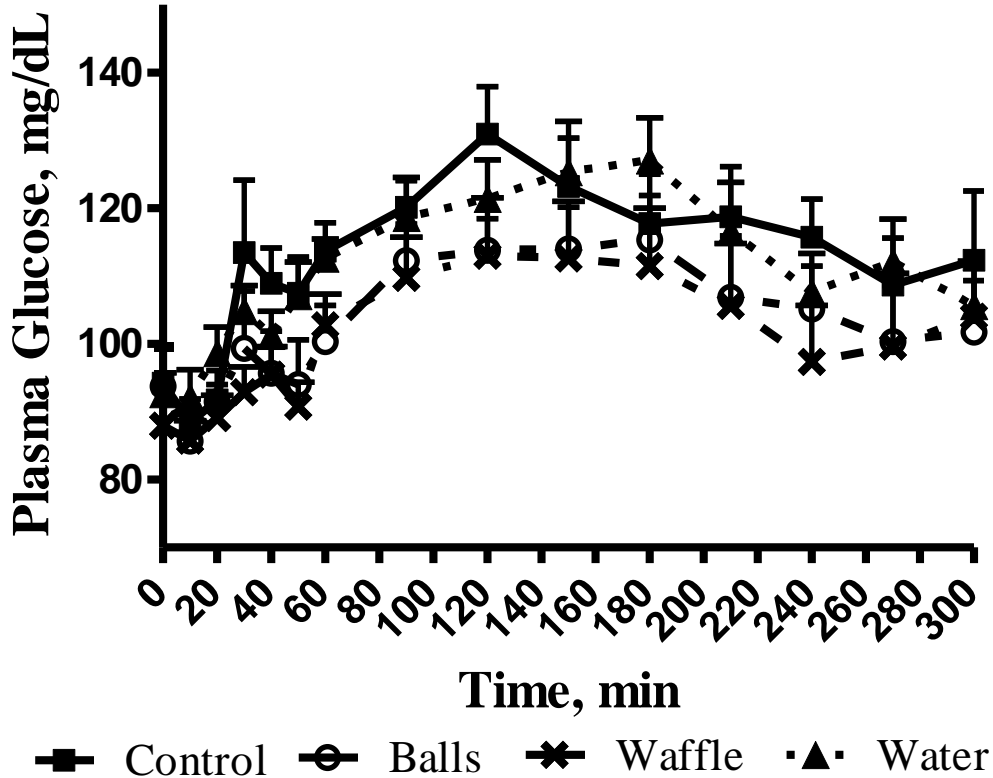


Figure 2. Plasma glucose concentrations over time in Experiment 1. Eight horses were fed a concentrated feed containing 26% non-structural carbohydrate in 4 different feed delivery methods, including 2 methods which contained obstacles (Balls and Waffle) and 2 methods which did not contain obstacles (Control and Water), and post-prandial plasma glucose concentrations were determined. A significant effect of time after feeding ($P < 0.001$) and a trend for the effect of treatment ($P = 0.059$) was found on plasma glucose concentrations. The least squares mean (LSM) glucose concentration of Balls was lower than Control ($P = 0.022$) and tended to be lower than Water ($P = 0.093$), and the LSM glucose concentration of Waffle was lower than Control ($P = 0.032$).

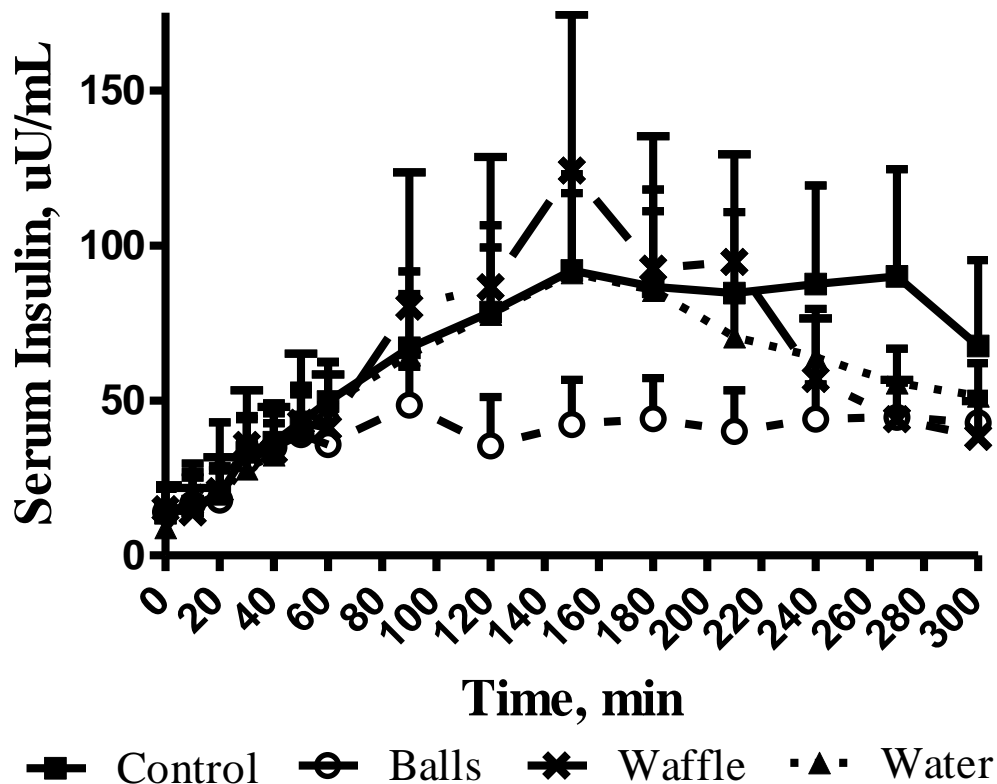
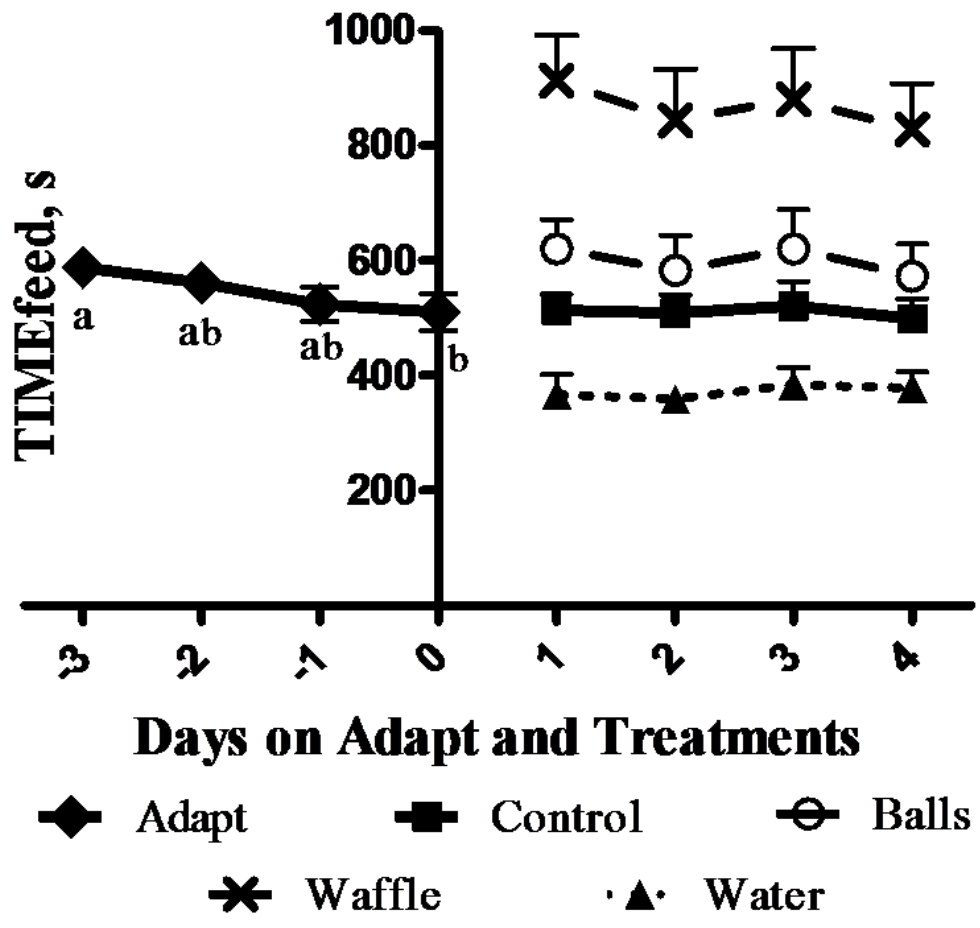


Figure 3. Serum insulin concentrations over time in Experiment 1. Eight horses were fed a concentrated feed containing 26% non-structural carbohydrate in 4 different feed delivery methods, including 2 methods which contained obstacles (Balls and Waffle) and 2 methods which did not contain obstacles (Control and Water), and post-prandial serum insulin concentrations were determined. A significant effect of time after feeding ($P < 0.001$) and a trend for the effect of treatment ($P = 0.072$) was found on serum insulin concentrations. The least squares mean (LSM) insulin concentration of Balls was lower than Water ($P = 0.031$) and tending to be lower than Control ($P = 0.080$), and the LSM insulin concentration of Waffle was lower than Water ($P = 0.045$).

Figure 4. Time to consume feed (TIMEfeed) differences by treatment and over days on treatments (DOT) in Experiment 2. Four horses were fed a concentrate meal once per day for 20 days, with the first 4 days representing an adaptation phase (ADP) and the remaining 16 days representing 4 treatment periods of 4 days each. Each period, horses received 1 of 4 feed delivery methods, including 2 methods which contained obstacles (Balls and Waffle) and 2 methods which did not contain obstacles (Control and Water), and TIMEfeed was recorded for each horse every day. Data from ADP is included in the graph but was analyzed separately from the treatment periods. A significant effect of DOT during ADP was found ($P = 0.018$). Data from ADP with different lowercase letters differ ($P < 0.05$). During the treatment periods, a significant effect of treatment ($P < 0.001$) and a significant effect of DOT was found ($P = 0.006$), with no interaction between treatment and DOT ($P = 0.298$). The average TIMEfeed of Waffle was greater than all other treatments, the average TIMEfeed of Water was lower than all other treatments, and Ball tended to have a higher average TIMEfeed than Control. The average TIMEfeed of Day 1 and Day 3 were both greater than Day 2 and Day 4, but there was no difference between the average TIMEfeed of Day 1 and Day 3, nor between the average TIMEfeed of Day 2 and Day 4.



CHAPTER THREE

Summary of Research in Relation to Literature Review

SUMMARY

The findings of these research trials demonstrate that feed delivery methods which include obstacles increase how long it takes horses to consume a pelleted concentrate meal. Additional results of obstacles being included in a feed bucket include decreased average glucose and insulin concentrations and peak glucose concentrations following feeding. When obstacles are mobile and located primarily above pelleted concentrate feed, additional desirable changes include a decrease in the peak and total insulin response to feeding, as calculated by the area under the curve of the insulin response, and prolonged time until the peak in insulin is detected. Because obstacles in a feed delivery method are capable of both increasing time to consume feed and simultaneously altering the glucose and insulin response to feeding in ways that may reduce the risk of insulin resistance, it can be concluded that any method which increases time to consume feed deserves investigation as a means to reduce development of insulin resistance.

Multiple ways to prolong time to consume feed were discussed in the literature review, including addition of forages to feed and leaving grains unprocessed, but it has also been demonstrated in other research trials that processing grains improves digestibility and decreases resistant starch which could alter microbial populations when compared to whole or less processed grains. Also, inclusion of forages with a concentrate diet decreases potential digestion of each feed type. Therefore, methods which do not negatively affect digestion should be used to increase time to consume feed, and there is no reason to currently suspect that use of obstacles in a feed bucket has a negative impact on digestibility.

While concentrate meal feeding can be associated with a variety of potential risks, such as development of insulin resistance, obesity, laminitis, choke, behavioral issues, and gastric ulcers, often concentrate meal feeding is necessary as a means to ensure horses consume sufficient nutrients and energy to maintain body condition and performance. For each of these conditions, the cause is often multi-factorial. However, in each case, prolonging time to consume feed has potential as a simple and inexpensive method to reduce the risk of the condition's development.

As insulin resistance, obesity, and laminitis have all been associated with heightened glucose and insulin concentrations and prolonging time to consume feed can reduce these concentrations, obstacles in a feed bucket may reduce the risk of these conditions developing. Choke is often caused by rapid feed ingestions, so any method which slows rate of feed intake has potential to reduce choke. Behavioral issues in meal fed horses are often associated with the time in between meals when horses begin chewing on or consuming undesirable material. Prolonging time to consume feed would reduce the amount of time between meals when the horse doesn't have access to feed material. Gastric ulcers can develop as a result of decreased pH of the stomach, particularly in meal fed horses that consume feed quickly and then have prolonged time between meals where they do not chew and therefore do not stimulate saliva production, which would normally buffer the pH of the stomach. Prolonging time to consume feed would prolong time spent chewing, increasing time saliva is flowing to the stomach, and potentially maintain higher gastric pH.

Other methods shown to reduce development of insulin resistance, including feeding diets higher in fat and lower in non-structural carbohydrates or increasing the number of

meals per day, could both increase costs associated with maintaining horses. Replacing common concentrate diets with specialty feeds low in starch and high in fat could increase feed costs. While more commercial feeds like this are being developed and marketed by feed companies, slowly decreasing the cost of such feeds, it is unlikely that low starch, high fat feeds will equal the cost of moderate starch and fat feeds any time soon. Increasing the number of meals fed per day would increase the cost of labor in a professional equine setting, and private horse owners often have careers that make increasing the number of meal feedings impossible. Alternatively, including obstacles in a feed bucket is inexpensive and can decrease the glucose and insulin response to feeding without needing additional meals.

It is possible the cumulative effects of a low starch, high fat diet, increased number of meals per day, and feed delivery methods which prolong time to consume feed could cause the greatest reduction in glucose and insulin concentrations following feeding. The effects of such combinations should be explored, particularly for horses at increased risk of developing insulin resistance or who already have insulin resistance.

While the methods used in these experiments were successful at increasing time to consume feed and creating desirable changes in glucose and insulin concentrations, they were not equal in their effects on glucose and insulin and both have room for improvement before being a reasonable method to implement for daily feeding of horses. Research and development is warranted for a feed delivery method that safely and consistently increases time to consume feed while also reducing post-prandial glucose and insulin concentrations. However, the feed delivery methods used here have clearly demonstrated a prospective method for reducing risk of insulin resistance development in horses, increasing our

awareness of the potential for simple managerial changes to decrease the risk of disease and improve the quality of life for horses.