METABOLIC AND MORPHOMETRIC EFFECTS OF PSYLLIUM SUPPLEMENTATION IN HORSES GRAZING RAPIDLY GROWING COOL SEASON GRASSES

by

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ABSTRACT

Digestion of non-structural carbohydrates (NSC) from cool season pasture grasses can result in increased adiposity, insulin resistance, and laminitis in horses. Lowering blood glucose levels and increasing insulin sensitivity can reduce disease risk. Supplementing horses with psyllium reduces blood glucose and insulin concentrations in meal fed horses. The objective of this study was to evaluate the effects of psyllium supplementation in horses grazing rapidly growing cool season grass.

Eleven lightbreed stock horses (7 mares, 4 geldings, Age 13.5 ± 2.5; mean ± SD) were individually confined in dry lots overnight and strip grazed for 8 hours daily for 30 days. Psyllium-supplemented horses (n = 6) received 180 g of psyllium daily. All horses received an isocaloric protein supplement. Forage intake was calculated using a previously published equation based on bodyweight. Forage was analyzed for nutrient content every other week. Changes in metabolic characteristics were assessed by assay of glucose, insulin, leptin and adiponectin concentrations in blood samples collected on days 0, 8, 15, 22, and 29 at 0700, 0800, 0900, 1100, 1300, and 1500 hours. Changes in morphometric characteristics were assessed using bodyweight, body condition score, mean neck circumference, and tailhead fat mass on days 0 and 29. Significance accepted at $P < 0.05$.

Psyllium supplementation lowered mean glucose, glucose AUC and increased time to peak glucose. There was a treatment by gender interaction for mean insulin, peak insulin, and insulin AUC. These variables were lowered to a greater extent in geldings than in mares. Higher NSC intake resulted in higher peak insulin in all horses. Older horses had higher peak insulin and decreased time to peak glucose and insulin. All characteristics of glucose and insulin decreased over the 30 day interval spent grazing pasture. Mares had higher leptin concentrations. Adiponectin concentrations increased with NSC intake. No significant differences occurred for morphometric characteristics.

These results indicate that supplementing psyllium in horses grazing cool season grasses lowered systemic glucose and insulin concentrations and these effects may reduce the risk of metabolic diseases, such as laminitis. However, systemic glucose and insulin concentrations were affected to a greater extent in males than females.

INTRODUCTION

A common management practice among equine owners is to graze their horses on pasture. This can be cost effective if disease does not develop, however pasture associated laminitis appears to be due to excess consumption of grasses high in non-structural carbohydrate (NSC) content [1]. Cool season grasses use NSCs as reserve carbohydrates during excessive growth [2]. The NSC fraction is composed of the sum of the simple sugars, fructan, and starch, with no self-limiting mechanism [2]. Consequently, there is no limit to the amount of NSC that can be stored by these grasses during periods of growth. Normal digestion of NSCs can result in excess glucose absorption into the blood which is generally available to be taken up by adipose tissue. Increased adiposity can lead to insulin resistance and laminitis [3]. Since horses cannot digest excessive amounts of NSC in the foregut, a high consumption rate of NSCs will result in dynamic metabolic changes in the large intestine [1] and can ultimately cause a serious condition known as laminitis, as well as insulin resistance [4, 5].

Laminitis is a painful condition that is characterized by the hoof wall becoming detached from the underlying tissue. This can become so severe that the hoof will no longer support the animal's weight, resulting in a crippled horse [6]. While the etiology of this disease is not fully understood it is known that 54% of laminitis cases were due to pasture associated laminitis [7]. Recent research has expanded the understanding of insulin in relation to laminitis by implicating hyperinsulinemia as an important factor in this process [8], and points to the significance of insulin resistance in the development of laminitis in horses.

Insulin resistance was originally defined as existing when a normal concentration of insulin produces “a less than normal biologic response” [9]. For horses, this means an ever increasing amount of insulin is needed to trigger the glucose transport protein (GLUT4) necessary for tissues to absorb glucose [6]. Insulin resistance is not only a factor in the etiology of laminitis, it is also closely associated with obesity and equine metabolic syndrome. According to Frank et al., in 2010 [10], the main diagnostic signs of Equine Metabolic Syndrome (EMS) are laminitis, insulin resistance, and increased adiposity. Even during normal digestion, intake of NSCs can make horses more prone to the development of EMS (due to increased adiposity). In addition, a horse that is currently suffering from EMS has an increased risk of developing pasture-associated laminitis [11].

The recommendation to prevent laminitis is to restore glucose tolerance and maintain insulin concentration within the normal range [6]. The most appropriate way to do this is to reduce blood glucose concentrations which may in turn increase insulin sensitivity [1]. Lowering blood glucose concentrations, and increasing insulin sensitivity, is important for horses at risk of, or currently afflicted with, insulin resistance, EMS, or obesity. One way to accomplish this may be to supplement horses with psyllium.

Psyllium is a commercially marketed product that has a history of being used as a supplemental dietary fiber in humans and a treatment to remove large colon sand from horses [12]. Moreaux et al., in 2011 [13] reported that psyllium supplementation in horses consuming a twice daily ration of grain and hay had lower concentrations of postprandial blood glucose and insulin than control horses. However, delaying the rate of nutrient

absorption (as would occur in continual grazing versus fixed feeding) along with the addition of higher concentrations of NSC may alter carbohydrate metabolism [14].

This review of literature will encompass: 1) an overview of the digestive physiology in the horse including glucose and insulin dynamics, 2) NSC accumulation in cool season grass and NSC digestion in the horse, 3) definition, detection, and risk assessment of metabolically associated diseases in horses, and 4) psyllium supplementation.

LITERATURE REVIEW

Equine Digestive Physiology

Horses evolved as grazers with their main energy source coming from a range of both hydrolysable and fermentable carbohydrates [for reviews see 15, 16]. The fermentation of carbohydrates in the horse occurs in the hindgut, or cecum [for review see 15]. These carbohydrates, such as hemicellulose, soluble fibers, and cellulose, are fermented to produce volatile fatty acids which are absorbed across the intestinal wall through passive diffusion [for review see 15] [17].The digestive tract of the horse is adapted to utilize high levels of fiber as well as large quantities of forage in a continuous grazing fashion [for review see 16]. Horses are therefore classified as non-ruminant 'roughage-grazers' and are able to utilize forage of lower nutrient content as compared to cattle [for review see 18]. The main site for breakdown and absorption of starch, sugar, lipids and protein is the small intestine [for review see 18]. Since horses evolved as continuous grazers their digestive system is not adapted to being meal fed. Feeding two large meals a day of grain supplement can cause starch overload and "set up a feeding-fasting cycle of metabolites and hormones" [for review see 15]. Therefore, large meals of grain can result in the digestive capacity of the stomach and small intestine being inundated since the digestive tract of the horse is designed to process feed in a continuous fashion [for review see 16]. Despite these complications meal feeding has become common practice in modern equine husbandry [19].

When the digestive capacity of the small intestine is overloaded, i.e. when a horse is fed a meal of grain, starch and complex sugars can escape small intestine digestion and

spill over into the large intestine [for review see 18]. Overload can cause the mean retention time, which is the time available in herbivores for fiber digestion to occur, to decrease. This can result in starch escaping to the cecum and depressing fibrolytic activity by disrupting the microbial ecosystem [20]. When nutrients escape to the large intestine and cecum, alterations occur in both the microflora and fermentation products [21]. This disruption of normal digestive processes results in a predisposition to gastrointestinal disturbances and can potentially lead to laminitis [21, for review see 16]. The digestive capacity for starch in the small intestine of horses has been determined as 0.4% of body weight [22]. Although there is a compensating effect by the large intestine, resulting in almost complete starch digestion over the entire tract, excessive fermentation of carbohydrate in the large intestine can lead to colic and laminitis via disruption of the microbial ecosystem in the hindgut [22].

A grazing, or free choice feeding system, may decrease the incidence of digestive disturbance thereby decreasing the risk of associated diseases such as colic, laminitis, and potentially insulin resistance. As seen in humans, nibbling (eating 17 snacks throughout the day) as opposed to gorging (eating 3 meals throughout the day) decreases postprandial oscillations in glucose concentration and decreases mean serum insulin levels [23]. Thus, horses grazing instead of being meal fed may avoid many of the complications associated with starch overload in the large intestine and maintain a more steady state of blood glucose throughout the day.

Nonstructural Carbohydrate in Grasses and Digestion in the Horse

Under certain circumstances grazing horses still face the threat of starch overload and blood or systemic elevation of glucose concentrations. Nonstructural carbohydrates (NSC), defined as the sum of simple sugars, fructans, and starch, are the reserve carbohydrate during times of excess energy for cool season grasses that dominate the Rocky Mountain region [for review see 2]. Examples of cool season grasses include brome (*Bromus* sp.), fescue (Festuca sp.), perennial rye (*Lolium* perenne), and timothy (*Phleum* sp.) [for review see 24]. The NSC reserve of these plants are moved from the leaf vacuole to the stem for storage, resulting in high concentrations of NSC in the stem. Further exacerbating the issue of NSC accumulation within the plant is the lack of a self-limiting mechanism for this accumulation. Once a seed head develops on the grass the reserve NSC moves to the seed head for the purpose of growth and development, further increasing the concentration of NSC in the pasture [for review see 2].

There is a diurnal pattern of NSC concentration since sugar production, or photosynthetic capacity, is light dependent [for review see 24]. Concentrations are lowest in the early morning and increase until maximal values are reached in the afternoon with a decrease occurring overnight. This means horses grazing in the afternoon can ingest two to four times more NSC than if they graze in the morning [for review see 25]. In addition, long summer days in high latitudes, such as the Rocky Mountain region, result in a greater opportunity for sugar production in grass. The same can be said of longer spring days. This season-dependent variation in NSC production makes spring the most

dangerous season for development of pasture associated laminitis in horses [for review see 1]. NSC can still be found in grasses during the fall in the Rocky Mountain Region. Grasses with the ability to survive extremely cold winters require greater amounts of NSC in order to survive [for review see 1]. In this regard, both seasonal and circadian patterns of glucose and insulin concentrations have been detected in grazing horses [26].

NSC includes both non-hydrolysable, rapidly fermentable, carbohydrates which account for 62% NSC in a pasture, and hydrolysable carbohydrates which account for 38% NSC in pasture [17]. In the horse the hydrolysable fraction is digested in the small intestine. However, if starch intake exceeds 0.4% of body weight per feeding, the portion remaining of the hydryolysable fraction, as well as the nonhydrolysable fraction, are fermented in the hindgut [17, for review see 3]. This occurs due to the enzymatic digestive capacity of the small intestine being overloaded [17]. Rapid fermentation in the hindgut can lower the pH in the cecum causing it to become more acidic, change the microbial population via the death of Gram negative bacteria, and release trigger factors, such as amines and endotoxins, that may ultimately lead to laminitis [for reviews see 27, 28]. Even if the small intestine is not overloaded, consumption of NSCs can lead to excess glucose in the system, i.e. circulation. As discussed previously, excess glucose can lead to increased adiposity, and ultimately, insulin resistance.

Glucose Metabolism in the Horse

Glucose is essential to carbohydrate metabolism in the horse since only simple monomeric compounds, such as glucose and fructose, can be absorbed across the intestinal mucosa [for review see 18]. Glucose can also enter intermediary metabolism

through enzymatic digestion of di- and oligosaccharides, or the fermentation of carbohydrate to volatile fatty acids which are then converted to glucose in the liver. Glucose, stored as glycogen in muscle and fat tissue, is the main energy source for the horse during intense exercise [for review see 18], and serve as a source of cellular energy by providing ATP through glycolysis and the Krebs Cycle.

Horses have a baseline glucose concentration that rises after meals [29]. The baseline concentration, postprandial rise, and the time to peak postprandial concentration all give important information about the status of the horse such as degree of insulin sensitivity and ability to clear glucose from the blood [29, 30]. June et al., reported mean baseline glucose of 90.8 mg/dL and a rise to 163.5 mg/dL two hours after a glucose challenge of 1g/kg BW [30]. Williams et al., reported mean baseline glucose of 74.7 ± 10.9 mg/dL in 12 Thoroughbred mares and a peak of approximately 160 mg/dL after being fed a pelleted concentrate feed [31]. Moreaux et al. reported mean postprandial blood glucose in light breed stock horses to be 95.0 ± 18.9 mg/dL [13]. In addition peak glucose, after a 10% dextrose solution at 0.5% of body weight, was reported to be 334.4 mg/dL [13].

In order to maintain homeostasis between stored glycogen and blood glucose from food intake, glucose sensors within the blood monitor blood concentrations and adjust the use of both stored and produced glucose [for review see 32]. One cell type that contains glucose sensors are Pancreatic β cells which induce insulin secretion when blood glucose concentrations rise. This results in the uptake of glucose by fat and muscle as well as blocking the production and/or release of glucose from the liver [for review see 32]. Uptake of glucose through insulin stimulation is carried out by the activation of GLUT 4,

a glucose transporter, that is localized in intracellular vesicles and recruited to the surface by insulin [for review see 33]. While there are 7 known glucose transporters, GLUT 4 is the only insulin-dependent transporter [for review see 33]. Transporters are necessary, whether insulin independent or dependent, due to the impermeability of lipid bilayers in cell membranes [for review see 34]. This makes glucose transport "the rate-controlling step in skeletal muscle glucose metabolism" [for review see 34].

Interest in the dynamics of glucose metabolism has increased along with the rising obesity epidemic that is occurring in humans and horses. It has been shown that horses adapted to a high-glycemic diet are at increased risk of developing insulin resistance, which has been associated with laminitis and obesity [35, 19]. Excess amounts of glucose in the equine diet are converted to fat and can be stored in skeletal muscle, liver and pancreatic tissues when the storage capacity of adipose tissue is exceeded [for review see 36]. Therefore, excess glucose can lead to either obesity, or regional adiposity, both of which can lead to the development of insulin resistance, Equine Metabolic Syndrome, and laminitis [19, for review see 36].

Insulin Dynamics in the Horse

Insulin is a hormone that is secreted by the pancreas, specifically from islet β cells. Release of insulin is stimulated by a rise in blood sugar, which principally occurs after food ingestion [for reviews see 11, 37, 1]. This release facilitates glucose entry into skeletal muscle and adipose tissue [for reviews see 1, 38]. Once insulin is released it stimulates disposal of glucose through a cascade of events. This cascade involves autophosphorylation of the insulin receptor, phosphorylation of insulin receptor

substrates, and ultimately stimulates GLUT 4 to translocate to the surface of the cell [for review see 38]. This receptor, as described previously, is insulin dependent and allows the uptake of glucose into the responsive cell. In addition to stimulating the uptake of glucose, insulin also decreases glycogenolysis and gluconeogenesis in the liver, and lipolysis, thereby reducing production of glucose and suppressing the release of nonesterified fatty acids [for review see 38]. Through these mechanisms of insulin stimulated glucose uptake, insulin is able to control the blood glucose concentration (in the healthy state) within a range to protect the body from glucotoxicity [for review see 39].

Baseline concentrations of insulin have been reported as 5.86 ± 1.8 mIU/L in Thoroughbred mares [31], and 29.7 ± 27.4 μU/mL in three geldings and two mares of unreported breed [40]. The half-life of insulin is only 2 to 3 minutes and is rapidly cleared from the blood once glucose concentrations are no longer elevated [for review see 41]. Therefore, insulin concentrations reflect acute changes in energy status, such as ongoing metabolism, as well as a direct proportion between fluctuations of insulin concentration and total body fat [for review see 41].

Insulin also functions as an adiposity signal to the brain, entering from the plasma [for review see 41]. As an adiposity signal, insulin becomes a regulator of both food intake and energy balance by inhibiting energy intake and increasing thermogenesis via signaling through the central nervous system [for reviews see 39, 37]. Transport of insulin into the central nervous system occurs over several hours making it a long-term

regulator of body adiposity through a negative feedback signal of body adiposity and recent energy intake [for review see 37].

Both sites of insulin-stimulated glucose uptake, skeletal muscle and adipose tissue, can become affected by increased lipid storage [for review see 11]. Excess energy (specifically glucose) is stored in adipose tissue, which can increase in both volume and number of adipocytes as needed [for review see 11]. When adipocyte tissue can no longer increase, lipid can accumulate within skeletal muscle [for review see 11]. Therefore, maintenance of normal insulin concentrations is important in horses and other species, as a positive association exists between adiposity and basal insulin concentrations [4]. In 2007 Geor et al., reported the prevalence of hyperinsulinemia (increased concentrations of insulin secretion), in a random sample of 300 horses from the Virginia-Maryland Regional College of Veterinary Medicine Equine Field Service practice, to be 10.0%, with 18 of the 30 hyperinsulinemic horses being obese [4]. Freestone et al., also found that development of hyperinsulinemia was positively correlated with back fat thickness and body score, both indications of obesity [42].

Metabolic Hormones in the Horse

Ghrelin Function in Mammals and Horses

Ghrelin is a peptide hormone produced primarily in the epithelial cells that line the fundus of the stomach [for review see 43]. Smaller quantities are produced in the hypothalamus, kidney, placenta, and pituitary [for review see 43]. When ghrelin was originally discovered the focus was on the potential stimulus of growth hormone from the

anterior pituitary, which it does both *in vivo* and *in vitro*, however further research has shown that ghrelin may be a link connecting metabolism and energy homeostasis with growth [for reviews see 43, 44]. One possible link is the regulation of ghrelin by insulin. Saad et al., reported that changes in insulin concentration, within normal physiologic ranges, caused reciprocal changes in ghrelin in humans [45]. In addition, hyperglycemia induced through an oral or intravenous administration of glucose decreases ghrelin concentrations in humans [46]. Whether it is the action of insulin or glucose, or both, that regulates ghrelin is yet to be fully determined.

Ghrelin increases food intake and body weight, as well as adipose tissue, by decreasing fat oxidation, enhancing the use of carbohydrates, increasing acid secretion and gastric motility and reducing locomotor activity [for reviews see 43, 47]. In addition, ghrelin may down-regulate anti-inflammatory molecules and increase immune response [for review see 43]. The effects of ghrelin are in direct opposition to the actions of leptin which is further shown by the negative correlation of plasma concentrations of these hormones in humans [48]. Ghrelin is also negatively correlated with body weight, percent body fat, fat mass, and insulin in humans [48]. In horses ghrelin is negatively correlated with body condition score, leptin, glucose, and insulin [49, 50].

Administration of ghrelin to rats, either peripherally or centrally, stimulated food intake making it unique among orexigenic peptides which do not have peripheral effects [for review see 44]. When ghrelin was administered to rats, at 2.4 $\mu molkg^{-1}d^{-1}$ once daily for two weeks, it induced a metabolically efficient state that led to increased fat mass and body weight [51]. However, in obese humans ghrelin concentrations were down

regulated, showing lower concentrations than lean age-matched controls [48]. This may be a product of elevated insulin or leptin [48].

In addition to the previously listed functions of ghrelin it has been postulated to play a role in meal initiation in humans; increasing an average of 78% one to two hours before a meal [52]. Concentrations of ghrelin then fell to trough concentrations within one hour of meal initiation [52]. In this study conducted by Cummings et al., it should be noted that subjects were given a meal at the same time each day throughout the study which may have affected ghrelin concentrations [52]. Conversely, the pre-meal rise in ghrelin has not been reported in horses. Gordon et al., showed ghrelin concentrations rising 20 minutes after meal initiation in Standardbred mares [53]. However, in this study horses had almost twenty-four hour access to hay and active, instead of total, ghrelin was measured [53]. Either of these differences may explain the lack of a rise in ghrelin before meals were given.

Leptin Function in Mammals and Horses

Leptin, a peptide hormone discovered in the late 1990s, is secreted from adipocytes [for reviews see 41, 43]. This particular adipokine is secreted in direct correlation to amount of adiposity, specifically reflecting the ongoing metabolic activity of the fat cell it is secreted from [for review see 41]. Leptin can therefore be an indicator of body fat, under normal conditions, and is very stable due to a 45 minute half-life [for review see 41]. During negative and positive energy balances leptin serves as a signal of the energy imbalance [for review see 43].

The overall function of leptin, as currently understood, is two-fold: leptin conveys to the brain the status of body energy stores, and serves as a sensor of energy balance [for review see 54]. As part of its function to convey energy store status to the brain, leptin serves to reduce food intake and increase energy expenditure [for review see 54]. This effect of leptin also occurs when it is administered exogenously [for review see 41]. Leptin is partially regulated by a negative feedback loop that prevents further leptin gene expression [for review see 54].

Insulin and leptin work in concert to communicate total fat mass of the body to the brain [for review see 41]. In horses, plasma leptin is negatively correlated with both adiponectin and ghrelin [49]. Plasma leptin has also been found to correlate positively with body weight, body condition score, and percent fat in horses [49].

Adiponectin Function in Mammals and Horses

Adiponectin, also called Acrp30, AdipoQ, apM1and GBP28, is a fat derived protein that affects energy homeostasis [for review see 55]. In animal models adiponectin increases insulin sensitivity, inhibits inflammatory pathways, improves glucose tolerance, increases β-oxidation in muscle, and decreases free fatty acids and plasma triglycerides [for review see 43, 55]. Increased insulin sensitivity in animal models as a result of adiponectin is believed to result as a secondary consequence of decreased free fatty acids and triglycerides circulating in the blood [for review see 55]. Negative correlations were reported between adiponectin and body size, as well as adiponectin and intra-abdominal fat in a study of 76 men and 106 women who were apparently healthy [56]. A positive correlation was found between adiponectin and insulin sensitivity in the same study [56].

In addition, adiponectin concentrations showed a positive correlation with age and female gender [56].

In humans adiponectin decreases with obesity and is positively associated with insulin sensitivity [for review see 43]. While adiponectin concentrations decreased with progressively worsening insulin resistance, it is not known if this is an effect of altered metabolism or a cause of insulin resistance [for review see 55]. In wild-type mice with intraperitoneal adiponectin injections, which increased circulating adiponectin approximately four-fold, transient glucose was reduced 4 hours later [for review see 55]. The authors proposed that the liver became more sensitive to circulating insulin as a result of the adiponectin injection, which was the reason for the suppression of glucose [for review see 55]. If this situation also occurs in horses it is possible that increased adiponectin concentrations might play a role in improving insulin sensitivity and decreasing systemic glucose concentrations.

In horses, adiponectin decreases in proportion to increasing adiposity, thus being inverse to leptin concentrations [57]. This inverse relationship may mean these two adipocyte derived hormones work in concert during insulin resistance or obesity development [57]. Gordon et al. reported an adiponectin range for young fit Standardbred horses of 0.6-2.3 ng/mL, while mature unfit Standardbreds showed a range of 1.8-7.8ng/mL [49]. The adiponectin concentrations in this study were negatively correlated with body condition score, body weight, and percent fat [49].

Metabolically Associated Diseases in Horses and Related Disease Factors

Insulin Resistance in the Horse

Insulin resistance, as originally defined by Kahn in 1978, occurs when normal concentrations of insulin "produce a less than normal biologic response" [for review see 9]. Essentially this means that greater amounts of insulin have to be secreted in order for glucose to be taken up by the cell. It can also be understood as a decreased response to insulin by those cells, such as muscle, adipose and liver tissues, that are insulin sensitive [35]. Some horses are able to compensate for decreased insulin sensitivity (or increased insulin resistance) by secreting greater amounts of insulin. However, when this situation is coupled with obesity the excess insulin will cause an increase in adipocyte fat accumulation, thus exacerbating the situation [for review see 41].

Treiber et al., reported that Thoroughbred weanlings adapted to a high-glycemic diet showed greater insulin resistance than weanlings adapted to a diet rich in fat and fiber [35]. Treiber et al., further reported that insulin resistance may develop within horses as an adaption to increasing content of starch in spring pasture [7]. While this provides a nutritional guideline and furthers the understanding of insulin resistance development it does not describe the mechanism at a cellular level. The exact mechanism by which insulin resistance occurs has not been elucidated however, there are several possible factors.

One possible mechanism is an absence of colonic metabolism leading to insulin resistance through changes in adipose tissue as described in the review by Robertson et al. [58]. Insulin has been implicated as a key component in the regulation of adipose

tissue, which further establishes this mechanism [for review see 59]. In human type 2 diabetes patients, decreased glucose transport to adipocyte tissue and increased lipolysis are an early component of insulin resistance [for review see 59]. As previously discussed, GLUT 4 is the insulin dependent glucose transporter. If GLUT 4 is lost in cells, not sequestered or translocated to the plasma membrane properly, insulin resistance can occur [for review see 33]. GLUT 4 may still be abundant and functioning properly within the cell but a disruption in the pathway that recruits GLUT 4 to the cell surface could also lead to insulin resistance [for review see 60]. In humans a dysfunction in the phosphatidyl-inositol-3 (PI-3) kinase activation, part of the pathway used to translocate GLUT 4 to the cell surface after insulin has bound to its receptor on the cell, has been cited as a possible mechanism of insulin resistance [for review see 60]. In rats the development of insulin resistance has been associated with the activation of a pattern recognition receptor, Toll-like receptor-4 (TLR4) caused by consumption of a high saturated fat diet [61]. TLR4 may be involved in the mechanism of insulin resistance through inflammation [61]. An individual mechanism, or all of these mechanisms working in concert either described above or not yet elucidated, could be the cause of insulin resistance developing in the horse or other species.

Detection of insulin resistance can be done with a single fasting blood sample, however conditions such as age, breed, recent nutrition, and fitness can all complicate the findings of a single blood draw [for review see 62]. Insulin resistance can often be diagnosed by measuring basal insulin and glucose concentrations. An insulin resistant horse will show hyperinsulinemia and either normoglycemia or slightly elevated glucose

concentrations. A baseline insulin concentration greater than 300 pmol/l can diagnose insulin resistance [for review see 63]. Basal glucose remains in normal range due to the compensatory secretion of excess insulin from the pancreas [for review see 1]. While this compensation mechanism continues to lower blood glucose concentrations it does not decrease the risk of associated diseases. Insulin resistance in horses is associated with pituitary pars intermedia dysfunction, diabetes mellitus, equine metabolic syndrome, and laminitis [for review see 62]. Insulin resistance and obesity are also strongly linked due to the higher concentrations of free fatty acids that occur when insulin can no longer regulate lipolysis [for reviews see 10, 64]. If insulin resistance is not coupled with hypoinsulinemia (compensatory insulin secretion) the cells can become starved of glucose, as a result of GLUT4 not being recruited to the cell surface, causing cellular death or damage [for review see 65].

Insulin Measurement Methods for Determining Insulin Sensitivity in the Horse

Determining whether a horse is insulin resistant can be difficult due to lack of a single standardized method. There are several different techniques to measure insulin which include measurement of basal samples, basal proxies, and quantitative methods such as tolerance tests and clamp techniques.

Basal Samples for Determination of Insulin Sensitivity in the Horse:

A single sample can be taken to determine basal blood glucose and insulin concentrations in order to determine hyperglycemia or hyperinsulinemia [66]. Caution must be used when interpreting the results of single

samples because glucose and insulin concentrations have variation due to diurnal changes, stress, time of feeding and type of feed consumed [for review see 62].

Interpretation of hyperglycemia determined using a single sample will not provide a distinction between decreased insulin sensitivity of tissues and pancreatic β-cells secreting insufficient amounts of insulin [66]. Hyperinsulinemia can be used to determine if a horse is insulin resistant because the decreased insulin sensitivity by tissues is compensated by increased insulin secretion [66]. When this compensation is no longer adequate, which occurs in later stages of insulin resistance, hyperinsulinemia declines making insulin resistance detection difficult [27, 66].

Hyperinsulinemia is defined differently between diagnostic labs. The University of Tennessee defines hyperinsulinemia as serum insulin concentrations above 30 µunits/ml while the Diagnostic Center for Population and Animal Health at Michigan State University defines hyperinsulinemia as serum insulin concentrations above 300 pmol/l (43 µunits/ml) [for review see 63]. Resting insulin, as a measurement of insulin resistance, has a reported within-horse coefficient of variation greater than 15% [40]. Resting plasma glucose has a reported within-horse coefficient of variation of 5.5% [40].

Ratios of glucose-to-insulin and insulin-to-glucose from single basal blood samples can also be used. Glucose-to-insulin is positively correlated to insulin sensitivity and insulin-to-glucose is positively correlated to insulin secretions [66, 40]. Use of these ratios to determine insulin resistance has a reported within-horse coefficient of variation greater than 15% [40].

Basal Proxies for Determination of Insulin Sensitivity in Horses: Surrogate tests, or basal proxies, have also been established in order to further elucidate insulin resistance in the face of hyperinsulinemia occurring in the non-fed state, which is followed by decreasing insulin due to β-cell decompensation. These proxies were developed by Treiber et al. in 2006 using basal plasma insulin (mU/L) and glucose (mg/dL) concentrations assessed by the minimal model [7]. The proxies measure insulin sensitivity (RISQI) and pancreatic β-cell response (MIRG) [7]. RISQI=1/√basal insulin concentration = basal insulin concentration-0.5 and MIRG = [800 – 0.3 X (basal insulin concentration – 50)^2]/(basal glucose concentration – 30) [7]. Insulin resistance is diagnosed when RISQI is less than 0.32 [mU/L]-0.5 [7]. The use of reference quintiles allows comparison of individual animal response to a larger population [67]. Reported reference quintiles for RISQI are: 0.152-0.295, 0.296-0.335, 0.336-0.393, 0.394-0.470, and 0.471-0.953 [67]. Reported reference quintiles for MIRG are: 1.20-2.12, 2.13-3.48, 3.49-4.54, 4.55-5.27, and 5.27-10.67 [67]. Basal proxies are currently the most practical insulin resistance test for population studies [for review see 68].

Quantitative Methods for Determination of Insulin Sensitivity in Horses: Quantitative methods to assess presence or absence of insulin resistance include the insulin suppression test, clamp methods, and the minimal model of glucose and insulin dynamics. Tolerance tests assess glucose and insulin responses to oral or IV administration of a specific dose of glucose. When glucose response, measured as incremental area under the curve, is exaggerated the cause can be pancreatic β-cells secreting insufficient amounts of insulin, glucose sparing due to

fat adaptation (which does not decrease insulin sensitivity), or impaired glucose-mediated or insulin-mediated glucose disposal [66]. When glucose tolerance tests are conducted using an oral dose of glucose complications arise from administration rate, gastric emptying rate and intestinal absorption rate [66]. These gastrointestinal complications can be avoided with IV administration [66]. An insulin tolerance test can be conducted by performing two oral glucose tolerance tests, one with concurrent insulin administration and one without. Decreased glucose response, again measured as the area under curve, can indicate insulin tolerance; however, response of endogenous insulin and other hormones which regulate hypoglycemia can affect the results [66].

An insulin suppression test is conducted by suppressing endogenous insulin via drug administration (typically the use of somatostatin or octreotide) and infusing insulin at a fixed-rate for 150 minutes [66]. During the last 60 minutes of the test 6 blood samples are collected and the mean is taken as the steady-state plasma glucose concentration [66]. The assumption for this test is that insulin sensitivity and steady-state plasma glucose concentration are negatively correlated [66]. This test can become difficult to accomplish if the drugs used to suppress insulin create adverse effects [66].

The combined glucose-insulin test (CGIT) requires infusing glucose at a 150 mg/kg bodyweight dose followed by insulin at dosage of 0.1 U/kg. Blood samples are collected via catheter at 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135 and 150 minutes after the insulin injection. Insulin resistance is determined by the time it takes blood glucose concentrations to return to pre-injection concentrations [69]. According to Frank et al., seven obese insulin resistant horses (2 geldings and 5 mares) had the following

results from a CGIT: resting plasma glucose of 83.9 mg/dL, resting serum insulin of 50.5 μU/mL, glucose AUC of 15.5 $mg \cdot dL^{-1} \cdot min^{-1}$, and insulin AUC of 23.2 $\mu U \cdot mL^{-1} \cdot min^{-1}$ [69]. Insulin resistance is diagnosed using this test when blood glucose concentrations are above the baseline value for ≥ 45 minutes [for review see 63].

The euglycemic-hyperinsulinemic clamp (EHC), historically considered the "gold-standard" in multiple species, measures insulin-mediated glucose disposal by determining the amount of glucose required to maintain euglycemia when a supraphysiologic concentration of insulin is administered [for reviews see 68, 70] [40]. The technique requires inducing a controlled state of hyperinsulinemia to stimulate glucose disposal and measures the amount of glucose infused to achieve a steady state, typically 5 mmol/L [66]. A priming dose of insulin is administered and followed by continual insulin infusion to reach a given concentration of hyperinsulinemia [66]. Insulin infusion rate is usually based on body weight but should ideally be based on body surface area [66]. The test can be conducted for a total of 120 or 180 minutes, but should last longer than 60 minutes as endogenous glucose production will be suppressed during this time [66]. Glucose disposal rate can then be calculated as the mean infusion rate during the last 60 minutes of the test. This test is very labor intensive, requires horses to be safely confined for a period of 3 to 4 hours, and requires horses to be fitted with two jugular catheters [66]. These limitations make the EHC difficult to use in clinical settings, for daily monitoring, or for a study with a large number of animals [for review see 70].

The minimal model of glucose and insulin dynamics uses data obtained from a frequent-sampling IV glucose tolerance test (FSIGT) to calculate glucose clearance per unit of insulin, or insulin sensitivity [66]. An intravenous insulin dose is superimposed on the FSIGT after a delay [for review see 68]. The data is fitted to two differential equations which describe the glucose-time curve: 1) glucose-mediated glucose disposal (single-rate constant) and 2) insulin-mediated glucose disposal (rate constant with the right side of the equation including insulin sensitivity [66]. Use of the minimal model can determine the insulin sensitivity index (SI), defined as change in glucose clearance rate in response to an exogenous insulin dose, the insulin-independent glucose clearance component (Sg), response of endogenous insulin to an exogenous glucose dose (AIR_g) which measures pancreatic β-cell response, and the disposition index (DI) which compares insulin secretion to insulin sensitivity [for review see 68]. The minimal model is advantageous over other methods because it measures insulin activity at a physiological level and is the only method to differentiate between insulin-independent and dependent glucose clearance [for review see 68].

Obesity Development and Disease Consequences in the Horse

Obesity has become a rising epidemic among the human population, which is of growing concern because of the many disease associations such as type II diabetes, heart disease, and arthritis [for review see 47]. Obesity has also become a growing concern within the equine population for the same reason; the diseases that it is associated with. Defined as “the excessive accumulation of adipose tissue in the body,” obesity in horses

is usually identified through the body condition scoring (BCS) system developed by Henneke et al. [71] [for review see 72]. According to the BCS, which appraises the fat covering at 6 different sites on the equine body, a horse that scores 8 or 9 (on a scale of 9) is considered obese [71]. In 2005 the USDA conducted a National Animal Health Monitoring System survey that represented 78.0% of the equine population in the US. According to this survey 0.9±0.1% of all horses in the US are currently overweight or obese [73]. Thatcher et al. conducted a body condition study on a subpopulation of light breed horses in Virginia and found a much higher obesity rate of 19% [74]. Since the Thatcher study was conducted using personnel trained in body condition scoring and the USDA utilized owner self-reported information there is a very strong possibility that the obesity rate of horses in the US is higher than the 0.9% reported.

There are several disease associations that occur with obesity, some being due to the pro-inflammatory state it induces [for review see 10]. In addition, insulin sensitivity is 80% lower in obese horses compared to non-obese horses, forcing them to rely on glucose mediated disposal of glucose [19]. If insulin resistance develops in conjunction with obesity resting glucose, insulin, and leptin concentrations can be significantly greater than those in healthy non-obese horses [69]. Development of obesity may be due to environmental factors, genetic factors, or most likely a combination of the two [69, for review see 75].

It has recently been discovered that adipose tissue is not simply a storage organ, but rather an active endocrine organ composed of adipocytes and other structures [for review see 75]. Within adipose tissue, adipokines are produced by adipocytes [for review

see 10]. Adipokines are hormones that have both paracrine and endocrine effects on tissues [for review see 10]. Leptin and adiponectin are the two best known adipokines and were discussed previously in this review. Altering the balance of adipokine production can potentially have negative effects on the overall health of the animal as these adipokines impact cardiovascular function, inflammation, and glycemic control [for review see 75].

Not only does obesity alter the production of adipokines, it also can contribute to the development of insulin resistance (IR) and laminitis. Some horses may tolerate obesity better than others and be able to maintain normal insulin sensitivity [for review see 10]. For those that develop IR after becoming obese the direct relation occurs through the effects on insulin-sensitive tissues that become subjected to higher concentrations of free fatty acids (FFA) that occur with nutrient excess [for review see 10]. During nutrient excess FFAs increase their movement into tissues which causes skeletal myocytes to accumulate diacylglycerols which interfere with insulin signaling [for review see 10]. Development of IR while the horse is in an obese state is increased if too many calories are fed or the horse is being fed a grain-based meal rich in starch and sugar [for review see 5] [19].

Obesity, as well as regional adiposity, has been identified by Geor as a possible independent risk factor for the development of pasture-associated laminitis [for review see 25]. The connection between obesity and laminitis has not been fully elucidated. Obesity may predispose to laminitis because of the pro-inflammatory state, the development of IR, or a factor yet to be discovered. What is known, however, is that

allowing a horse to become obese greatly increases the risk of laminitis development [for review see 1]. In addition, obesity can further exacerbate the issue because of increased downward forces on dermoepidermal attachments [for review see 10].

Fortunately, obesity can be managed through weight reduction. Weight reduction can be accomplished through eliminating any grain from the diet, limiting or eliminating pasture access, and increasing exercise [for review see 10]. Not only will exercise increase the rate of weight reduction, but 7 consecutive days of exercise in obese mares has been shown to increase insulin sensitivity by 60% as compared to obese sedentary mares [76].

Morphometric Characteristics Related to Obesity and Insulin Resistance in the Horse

As with any animals there are several physical indications in horses for overall health and specifically for obesity and insulin resistance. The physical identifiers of health include: body weight, body condition score, tailhead fat mass, and mean neck circumference. Body condition score, based on the Henneke et al. system [77], utilizes several areas of the horse to portray "a more accurate indicator of stored body fat than weight, height or heartgirth." The Henneke body condition scoring system, or BCS, gives a score of 1 to 9 based on the observation and palpation of 6 different areas on the horse: shoulder, ribs, neck, withers, back, and tailhead [77]. Within this nine digit scoring system 1 is emaciated, 2 is very thin, 3 is thin, 4 is moderately thin, 5 is moderate, 6 is moderately fleshy, 7 is fleshy, 8 is fat, and 9 is extremely fat [77]. An ideal condition can vary among breeds and disciplines, but a score of 5-6 is usually considered ideal and 8-9

is considered obese. The BCS was verified to correlate well with blood variables, such as hyperinsulinemia, that relate to the diagnosis of insulin resistance and Equine Metabolic Syndrome [78]. This scoring system, however, does not account for regional adiposity which is a strong indicator of Equine Metabolic Syndrome and insulin resistance. In order to account for regional adiposity both tailhead fat mass and mean neck circumference can be measured [69, for review see 11].

Tail head fat mass is measured ultrasonically 10 cm lateral to the sacral spinous processes and 11 cm crainial to the tailhead origin [79]. This site of measurement was determined to have the strongest correlation between ultrasound measurement and actual fat [79]. Measurement of tail head fat mass has been shown to correlate with changes in BCS, showing that the tailhead area serves as a fat repository [80]. Mares with a BCS of 8 to 8.5 measured 1.96 cm of tailhead fat mass while mares with a BCS of 3 to 3.5 only measured 1.52 cm [69]. Therefore, this measurement can serve as an indicator of regional adiposity even if the horse does not have an obese body condition score.

Mean neck circumference is defined as the mean of three measurements along a line perpendicular to “a straight line from the poll to the cranial aspect of the withers” [69]. The placement of the three measurements occurs at 0.25, 0.50 and 0.75 of the distance from the poll to the withers. Enlargement of the neck, as measured by mean neck circumference, can be a risk factor for insulin resistance. The mean neck circumference for a sample population of non-obese horses was 87.7 cm, while the mean neck circumference for a sample population of obese insulin resistant horses was 105.8 cm [69].

Equine Metabolic Syndrome

Equine metabolic syndrome (EMS) is a metabolic and endocrine disease [for review see 11]. The components of EMS include increased adiposity (either in the form of generalized obesity or regional adiposity), insulin resistance, and laminitis [for review see 36]. It can be diagnosed through the clinical signs of regional adiposity, obesity, evidence of previous laminitic episodes, or current lameness due to laminitis [for review see 36]. Regional adiposity can occur through increased subcutaneous adipose tissue surrounding the nuchal ligament in the neck (also known as a cresty neck), fat accumulation near the tail head, fat accumulation in or around the prepuce or mammary glands, or fat accumulation caudal to the scapula [for review see 36]. However, some horses with EMS do not exhibit either obesity or regional adiposity, which makes insulin sensitivity the most useful screening test to diagnose this disorder [for review see 36]. Insulin concentrations that are greater than 15 μU/ml combined with adiposity is considered diagnostic [for review see 81]. Most horses with EMS are described by their owners as "easy keepers." These horses seem to subsist on fewer calories while maintaining their body weight [for review see 11]. There is speculation that this is a genetic predisposition, especially since EMS is more common in metabolically efficient breeds such as Morgan horses, Mustangs, and American Quarter Horses [for review see 10].

Wild horses evolved to utilize lower quality forages, as compared to ruminants, with a natural diet containing little fat [for reviews see 36, 18]. In their natural state the diet of a horse mainly consists of complex structural carbohydrates such as cellulose,

hemicellulose and lignin [for review see 1]. Current processed diets and improved pasture contain high amounts of starch and sugar which can be detrimental to the horse [for review see 18]. Frank et al. reported that certain aspects of EMS may not be exhibited until carbohydrate intake is increased [for review see 36]. While this doesn't elucidate the exact mechanism that causes EMS, which is still unknown, it does provide a management opportunity.

Horses with EMS can be managed through either limited access to pasture or completely eliminating pasture, increased exercise in order to decrease glucose concentrations and therefore increase insulin sensitivity, reduced caloric intake, and removing as much starch and sugar from the diet as possible [for review see 10] [76]. It is also recommended that the non-structural carbohydrate component of the diet should fall below 10% of dry matter intake [for review see 36]. The major strategy to improve the health of obese horses with EMS should be to increase insulin sensitivity and decrease body weight [for review see 10]. If a horse with EMS has previously, or currently, suffered from laminitis the decision to return the horse to pasture is very crucial. The horse should only be returned to pasture if it is exhibiting normal insulin sensitivity and there is a low likelihood of a recurrent laminitic episode [for review see 36]. The horse should not be grazed on fast growing grass in the spring, or grass that has suffered from cold stress in the fall in preparation for winter, as the carbohydrate and starch contents, respectively, will be high [for reviews see 10, 36].

Laminitis Pathology in the Horse

Laminitis, a debilitating disease affecting the equine hoof, has been reported since ancient times [for review see 82]. This disease is characterized by the lamellae, folded leaf-like tissue that makes up the inner hoof wall, failing [for review see 82]. The lamellae serve as the suspensory apparatus for the distal phalanx (pedal or coffin bone) and failure can result in the distal phalanx being detached from the hoof wall [for reviews see 82, 28]. The failure of the suspensory apparatus in acute laminitis occurs between the connective tissue of the dermis, or corium, and the basal layer of the epidermal lamellae [for review see 82]. Once detachment occurs forces of locomotion can then drive the distal phalanx into the hoof capsule [for review see 82]. If the distal phalanx penetrates the hoof capsule arteries and veins are crushed and damage occurs to the corium of the coronet and sole [for review see 82]. The pain that results is unrelenting, sometimes resulting in euthanasia as the only humane option [for reviews see 82, 1].

Laminitis progresses in three phases: developmental, acute, and chronic. The developmental phase lasts 30-40 hours, or 40-48 h when caused by excessive NSC ingestion, and results from a triggering factor [for reviews see 82, 1]. This factor is usually a result of a problem with a specific organ system, such as endocrine, gastrointestinal, renal, or reproductive [for review see 82]. Pain in the foot, and other clinical signs, do not appear during the developmental phase making it very hard to detect [for review see 82]. In naturally occurring cases of laminitis the developmental phase is unnoticed. Most owners and/or managers do not detect laminitis until the acute phase begins. The acute phase is characterized by signs of pain in the foot, lameness at the walk

and trot, a bounding digital pulse, and increased hoof temperature [for reviews see 82, 65]. Some horses are fortunate enough to end their bout with laminitis in the acute phase. However, if the disease progresses into the chronic phase it means there is some displacement of the distal phalanx [for review see 82]. The chronic phase is indefinite with signs ranging from persistent, mild lameness, to penetration by the distal phalanx of the hoof sole [for review see 82].

Laminitis can also be characterized by different grades of lameness, specifically Obel grade I through IV [for review see 82]. Obel grade I is defined as weight shifting with relatively free movement. Obel grade II is defined as more obvious lameness, a stilted and shuffling gait, but one foot can still be lifted without contralateral foot discomfort. Obel grade III is defined as reluctance to move and a refusal to lift a foot because of contralateral foot pain. Obel grade IV is defined is immobile [for review see 82]. While these lameness grades were created based on clinical observations they have been shown to correlate well with lamellar histopathology [for review see 82].

Despite laminitis existing for as long as humans have been associated with horses and grain, it is still the second biggest killer of horses, with only colic causing more deaths [for review see 82]. In the 1998 National Animal Health Monitoring System's Equine study, sponsored by the U.S. Department of Agriculture: Animal and Plant Health Inspection Service: Veterinary Services, $50 \pm 3.2\%$ of horse operations surveyed reported at least one horse in the previous 12 months having a case of laminitis [83]. Additionally, the survey did not find any significant differences in laminitis occurrence between regions of the country or primary use of horses [83].

The exact mechanism of laminitis has yet to be elucidated; however, it has become clear that there may be several different ways in which the disease can be triggered. These triggers can be drug or diet related or connected to another stressful medical condition [6]. Some horses may have underlying causes, such as genetic or metabolic disorders, that increase susceptibility to laminitis trigger factors [6]. Laminitis can be caused by mechanical overload [for review see 65], trauma, nutritional factors, and sepsis [for review see 84]. Gastrointestinal disturbances, such as mucosal damage that follows ischemia or acidic changes caused by excess carbohydrate fermentation, lead to trigger factors gaining access to systemic circulation [for review see 85].

Carbohydrate overload, an experimental model of laminitis induction, has revealed important information about the bacterial population within the equine cecum. Following carbohydrate overload *Lactobacillus* spp increased, while decreases were seen in *Bacilli* spp, anaerobic *Streptococci* spp, *Clostridia* spp, and *Streptococci* spp. *Clostridia* spp and *Streptococci* spp did have mean values near control mean at 24 hours after carbohydrate overload. However, the increase in lactic acid producing bacteria resulted in a pH decline in the cecum, of 7 to 4, within 24 hours post overload [86]. A change in pH leads to the death of Gram negative bacteria and results in endotoxins (vasoactive bacterial lipopolysaccharides) being released into the cecum. Lactic acid absorption into the circulation can result in generalized lactic acidosis and increased endotoxin levels have been detected in horses with Obel grade III laminitis showing that laminitis onset is related to endotoxemia [86, 87]. Obel grade III laminitis was achieved

in 13 of 20 horses 44 hours after carbohydrate overload with increased endotoxin levels detected in 11 of the horses [87].

The enzymatic theory of laminitis development, originally proposed by the Australian Equine Laminitis Research Unit, holds that an increase in digital blood flow allows delivery of trigger factors, such as cytokines, that lead to production and activation of zinc-containing proteases called minimal metalloproteinase (MMP) [for reviews see 65, 85]. MMPs may be the active component that destroys the bond between the interlocking lamellar leaflets termed the basement membrane [for review see 85]. However, it is yet to be confirmed if MMP activation occurs only in the hoof or if it is a response of systemic tissue [for review see 85]. It has been confirmed that endocrinopathic laminitis is not completely due to a vascular mechanism as vasodilation for 48 hours did not cause any damage to the lamellae [88]. Whether this is the case for gastrointestinal derived trigger factors as well is uncertain.

Endocrinopathic laminitis, which does not occur as a result of gastrointestinal disturbances but rather hormone dysfunction, is associated with insulin resistance and hyperinsulinemia [for review see 89] [90]. The equine hoof has been shown to have a glucose demand higher than the head and explants of equine hoof have been shown to separate under pressure after 12 hours of culture in glucose-free media [for review see 32] [91]. Explants maintained adhesion of basal epidermal cells to the basement membrane for 8 days in glucose containing medium [92]. These reports led many authors to believe endocrinopathic laminitis was due to a reduction of glucose entering the equine hoof, or glucose uptake impairment [for review see 65]. However, the strong expression

of GLUT1 mRNA in the coronary band, as opposed to only some appearance of GLUT4 mRNA, shows that the laminar layer or epidermal cells depend on insulin-independent glucose transport to meet the high energy demand for glucose [6] [for review see 32]. Even in an insulin resistant state glucose can still reach the equine digit because GLUT4 is not the main source of glucose transport. Glucotoxicity, created by an insulin resistant state, will not induce laminitis as hyperglycemia induced over 48 hours did not cause the development of laminitis or any clinical signs of lameness [88].

Induction of laminitis has occurred with hyperinsulinemia and euglycemia, independent of dietary factors and gastrointestinal disturbances [for review see 65] [6]. Lamellar changes have been detected as early as 6 hours after the onset of hyperinsulinemia in young Standardbred horses, with basement membrane disruption beginning at 24 hours after hyperinsulinemia onset [88]. Subclinical lamellar pathology, the beginning of the developmental phase of laminitis, begins at approximately 200µIU/mL serum insulin concentration. Laminitis induced with hyperinsulinemia does not cause an increase in MMP activity unlike laminitis induced by gastrointestinal disturbances, which means laminitis may not have a pathophysiology common to all forms of the disease [88].

Experimental induction of laminitis has also been accomplished with oligofructose (OF), a commercially available form of fructan extracted from chicory (*Cichorium intybus*) roots [93]. Fructan is a storage oligosaccharide in grass that cannot be digested in the equine small intestine and is therefore fermented in the cecum [94]. Six clinically normal, mature Standardbred horses were dosed with 7.5, 10.0 or 12.5 g/kg

body weight (BW) of OF. The doses chosen reflect possible intakes of horses grazing pasture since fructan concentrations can range from 30 to 50% of pasture grass and horses can consume 15 kg of pasture daily [95]. All dosing levels resulted in clinical and histological laminitis in the 48 hour testing period without signs of colic. Increased dosing rates were associated with increased severity of laminitis [93]. Dosing with 10 g/kg BW has also been reported to induce separation of *lamina densa* from epidermal basal cells in a separate study [96]. Attachment between the basement membrane and lamellar epidermal basal cells is compromised within the first 24 hours of OF induced laminitis [97]. The pathophysiology of OF induced laminitis is very similar to carbohydrate induced laminitis with a proliferation of Gram-positive bacteria in the hind gut [95]. Pathology of OF induced laminitis has been proposed as activation of MMPs and glucose deprivation; however, glucose deprivation is unlikely for the reasons discussed previously [96]. The lesions that occur in OF induced laminitis were reported to be very similar to the lesions detected in explants of equine hoof that underwent insulin-induced laminitis [88]. OF laminitis induction provides a link between laminitis and pasture consumption.

Pasture-associated laminitis was reported as 54% of the initiating laminitis cause, where the cause could be identified [7]. Rapidly growing grass contains high concentrations of non-structural carbohydrates as discussed in the previous section. Susceptibility to pasture induced laminitis differs among horses and may be explained by differences in the gut wall and responses to pH change, however this theory has yet to be verified [for review see 28]. Even when laminitis does not occur, the consumption of

large amounts of energy through grazing can contribute to obesity, especially in breeds that are more metabolically efficient [for review see 5]. Not only does the horse experience an excess of energy, but lush pasture can present gastrointestinal issues because this large amount of energy can be consumed in a short time period [for review see 5]. As previously discussed, gorging as opposed to nibbling increases glucose and insulin concentrations when there is a high concentration of carbohydrates, which occurs in grass when sunlight and nutrients are abundant [for review see 5]. Obesity is also a contributing factor to laminitis, therefore even if a horse is not highly susceptible to pasture induced laminitis it could still potentially occur through obesity-related mechanisms.

Based on the known risk factors for laminitis, such as obesity, IR, and carbohydrate overload, a pre-laminitic metabolic syndrome (PLMS) has been identified [98]. The risk factors that are incorporated into the PLMS include: insulin resistance, increased insulin secretory response, obesity, and hypertriglyceridemia (high concentrations of triglycerides) [98]. The total predictive power of the PLMS was determined as 65% in a study of 76 pony mares [98]. The pre-laminitic phenotype can be expressed as a result of summer pastures inducing abnormal metabolic responses [99]. In a closed herd of Dartmoor and Welsh, ponies in Virginia it was reported that basal insulin and leptin concentrations, reciprocal of the square root of insulin (RISQI), generalized obesity measured using BCS, and localized obesity measured with cresty neck score, also predict laminitic episodes with reproducible diagnostic accuracy better than the original PLMS [100]. However, it is uncertain if these tests would predict laminitis with

diagnostic accuracy in cases where metabolism has not been altered by obesity [100]. In addition, these tests were applied specifically to prediction of laminitic episodes related to consumption of nutrient dense pasture high in NSC content [100]. Since there may also be a genetic component to laminitis predisposition, horses or ponies predisposed to laminitis, such as those expressing PLMS, may be able to avoid laminitis development through pasture consumption when starch, sugar, and fructan concentrations are low [7, 101].

Psyllium Supplementation in Humans and Horses

Psyllium fiber is the seed husk of *Plantago ovata*, containing three fractions denoted as A, B, and C [102]. These fractions contain a mixture of acid and neutral polysaccharides with the ratio of 70/30 soluble to insoluble fiber [103]. The active portion, fraction B, is not only resistant to intestinal microflora digestion but also forms a gel [104, 102]. Fischer et al. determined that although the enzymes (exoxylosidases and xylanases) possessed by intestinal flora in humans can cleave the terminal α-L-arabinofuranose residues from fraction B that these do not affect the ability of the fraction to a form a gel [104].

Previous use for psyllium has included fecal sand clearance in horses, a bulk laxative in humans, and a treatment for type II diabetes patients to decrease postprandial glucose [12, 105, 106]. The gel forming fraction B, which is resistant to digestion, is the active component of psyllium and comprises >80% of the husk [102]. Colectomized rats fed psyllium showed an increase in bile acid secretion and mice fed psyllium showed reduced glucose and insulin concentrations as well as weight attenuation in a diet-induced

obesity model [102, 107]. Human research has shown similar results. Healthy subjects consuming psyllium at a dosage of 10.5g mixed in an aqueous solution with 50g of a glucose load, after an overnight fast, showed an 11.1% decrease in glucose area under the curve (AUC) and a 36.1% decrease in insulin AUC [105]. In type 2 diabetes, patients consuming 14g of psyllium per day (divided into 4 doses of 3.5g before each meal and an afternoon snack) postprandial blood glucose concentrations were significantly decreased and insulin AUC was decreased by 5% [106]. The exact mechanism by which psyllium decreases postprandial glucose has not been elucidated, however, the gel it forms may slow glucose absorption in the small intestine [103]. The mechanism of psyllium may also be a delay in gastric emptying created by the soluble fiber portion which would slow the rate of carbohydrate uptake [103]. The final possible mechanism is slowing the access of digestive enzymes to carbohydrates ingested in the meal as a result of those carbohydrates being sequestered [103].

Until recently psyllium research in horses has been limited to the fiber's ability to clear fecal sand [12, 108]. However, in 2011 Moreaux et al. reported that psyllium has similar effects on glucose and insulin concentrations in horses as it does in human subjects [13]. Stalled horses fed a twice daily mixed grain and hay ration supplemented with 90, 180, and 270g of psyllium for 60 days were reported to have lower postprandial glucose concentrations compared to controls [13]. Horses also had lower peak glucose concentrations when given an intravenous 10% dextrose solution at 0.5% body weight after 60 days of psyllium supplementation [13]. Insulin concentrations were lower, as compared to controls, with 270g psyllium supplementation for 60 days [13].

STATEMENT OF THE PROBLEM

Digestion of non-structural carbohydrates (NSC) from cool season pasture grasses can result in increased adiposity, risk of insulin resistance, and laminitis in horses [for review see 5]. In a USDA study in 2000, 13% of horse operations surveyed (which represented 89.3% of horses in 28 states) reported having a horse with laminitis in the previous 12 months [109]. Sillence et al., reported that laminitis was induced in 5 healthy ponies, without history of laminitis, by infusion of insulin while maintaining a normal resting concentration of glucose [6]. Their conclusion was that infusion of insulin induced insulin toxicity by causing severe hyperinsulinemia that exaggerated the insulin response to a glucose challenge in these ponies [6]. Although the exact biological mechanism for the separation of hoof lamellae, or laminitis, is not known their recommendation to prevent laminitis is to restore glucose tolerance and maintain insulin concentration within the normal range [6]. Lowering blood glucose concentrations, and increasing insulin sensitivity, is important for horses at risk of, or currently afflicted with, insulin resistance, EMS, or obesity. Changes in blood hormone concentrations and morphometric measurements can be used to assess risk of developing these diseases.

Psyllium, a supplement that could aid in decreasing glucose concentrations in grazing horses, contains a gel forming fraction of non-nutrient polysaccharides that resist digestion [104]. Sierra et al., in 2001[106] showed that consumption of psyllium, the seed husk of *Plantago ovata*, decreased postprandial blood glucose concentrations in humans with type 2 diabetes. Psyllium has also been shown to reduce blood glucose and insulin concentrations in horses on a twice daily feeding schedule [13]. If psyllium lowers blood

glucose concentrations and increases insulin sensitivity in grazing horses, as it did during a fixed feeding schedule [13], then supplementation with psyllium may provide a powerful tool in the management of laminitis and related diseases for horses grazing cool season grasses.

Therefore, the objective of this study was to evaluate metabolic and morphometric effects of psyllium supplementation in horses grazing rapidly growing cool season grass. Changes in metabolic characteristics were determined by assessing changes in systemic characteristics of glucose, insulin, leptin, ghrelin and adiponectin concentrations. Changes in morphometric characteristics were used to measure changes in adiposity and as overall indicators of health to ensure adequate nutrition. Body weight, body condition score, mean neck circumference, and tail head fat mass were measured to detect any changes in regional or overall adiposity.

MATERIALS AND METHODS

Animals and Treatment

All procedures were approved by the Montana State University Agriculture Animal Care and Use Committee (AACUC 2011-AA05). Twelve light breed stock horses were selected from the Montana State University equitation herd. One horse was removed from the study after becoming unapproachable on day 0 resulting in 11 horses being used for sample collection (Table 1). Horses were stratified by gender and breed, and then randomly allocated to one of two treatments: 1) 180 g psyllium, or 2) an isocaloric protein supplement (0.85 ± 0.02 DE Mcal/kg).

Table 1. Gender, age, breed, bodyweight (BW), and body condition score (BCS) of horses used in study

	Collar No.											Mean	SD
	1	3	4	5	8	12	13	14	15	16	17		
Gender	M	G	M	G	M	M	G	M	M	M	G		
Trt[1]	C	C	C	C	C	T	T	T	T	T	T		
Age	15	16	12	11	11	15	18	12	14	15	10	13.55	2.50
Breed[2]	QH	QH	QH	Paint	Paint	Paint	QH	QH	QH	QH	Paint		
BW (kg)	555	575	577	553	513	525	557	493	537	548	523	541.45	26.23
BCS[3]	6.2	6.7	6.3	6.0	5.2	5.7	5.8	5.3	6.5	6.8	5.2	5.97	0.60

[1]Trt; Treament of 180 g/d/horse of psyllium supplement (T) or no psyllium supplement (C)
[2]QH; quarter horse
[3]BCS; body condition score [77]

All horses were fed a ration balancing supplement consisting of a protein, vitamin and mineral pellet (Nutrena Empower® Balance Horse Supplement) each morning to ensure proper protein, vitamin, and mineral intake (Table 2). Six horses received a 180 gram psyllium pellet (Psyllium Pellets EQ, Vetri-Science® Laboratories of Vermont), 1.93 DE Mcal/kg and 222.26 g of protein supplement. Psyllium dosage level is an

extrapolation of human psyllium fiber supplementation and has been previously shown to decrease post-prandial glucose absorption in horses [13]. The other five horses received 453.6 grams of protein supplement. All horses received an additional 125 g of whole oats to encourage psyllium consumption in psyllium-supplemented horses. Caloric intake from the supplement was 0.937 ± 0.07 Mcal/kg of digestible energy for psyllium supplemented and non-psyllium supplemented horses.

Table 2. Nutrient analysis of Empower supplement[1], psyllium supplement[2] and whole oats

	Oats	Empower[1]	Psyllium[2]
ADF, %	16.15	36.62	11.72
NDF, %	30.34	14.83	85.84
DM, %	91.61	92.95	93.09
Ash, %	2.61	17.50	3.00
CP, %	13.97	29.78	9.08
Fat, %	3.65	4.57	3.15
NSC, %[3]	49.43	33.33	0.00
DE, mcal/kg[4]	1.08	1.62	2.33

[1]Nutrena Empower® Balance Horse Supplement
[2]Psyllium Pellets EQ, Vetri-Science® Laboratories of Vermont
[3] NSC = 100- CP - Fat - NDF – Ash [110]
[4]DE (Mcal/kg) = 2118 + 12.18(CP%) -9.37(ADF%) -3.83(hemicellulose %) + 47.18(Fat %) + 20.35(NSC%) - 26.3(Ash %) [110]

Horses were individually confined in dry lots (4 x 7 M) overnight where they had partial cover and free access to a plain white salt block. Feed was given at 0800 daily and horses were given 30 minutes for consumption. Refusals were weighed and recorded daily. Horses were then turned out at 0830 to graze for 8 hours. Horses had 24 hour access to water that was provided in individual 20 gallon buckets in dry lot pens and pasture allotment.

Pasture Allotment

The study was conducted on an 8.5 ha pasture at the Bozeman Agriculture Research and Teaching Farm in Bozeman, MT. Forage in the pasture consisted primarily of the following cool-season grasses: Timothy (*Phleum pretense* L.), smooth brome (*Bromus inermis* Leyss.) and Kentucky bluegrass (*Poa pratensis* L.). The most common forb was dandelion (*Taraxacum officinale* F.H. Wigg).

Horses were gradually acclimated to a diet of growing cool season pasture grass over the course of 20 days. Grazing time began at 3 hours per day and was increased by one hour until a total of 8 hours a day was reached. The time allotment of 8 hours grazing was chosen based on industry standard [111]. Horses were acclimated as a herd over the entire 8.5 ha for the first 17 days and were separated into individual grazing strips for the final 3 days of acclimation. During the last 10 days of acclimation every horse received 100 g of protein supplement to ensure adaptation. Pasture was analyzed at the beginning of the acclimation period for nutrient content and available forage to determine size of individual grazing allotments (Table 3). Total forage availability in the study pasture was determined by clipping plant standing crop within ten 0.50-m^2 quadrats randomly located throughout the pasture. Clipped samples were dried in a forced-air oven at 55° C until weight was constant to determine available forage (kg/ha) [112, 113]. This preliminary clipping showed a production level of 3,146.42 kg/ha on a DM basis and was used to determine individual grazing allotment size. Each horse strip grazed on approximately .61 ha. Strips were separated with electric tape to decrease effects of herd behavior on grazing pattern and intake. Individual allotment was determined as follows:

Equation 1.

Mcal/day Requirement of Horse ÷ DE value Mcal/kg of Pasture = kg/day to Consume

Equation 2.

Kg/day to Consume X 30 Days of Grazing= kg Allotment/Horse

Equation 3.

Kg Allotment/Horse ÷ Forage Production in kg/hectare = hectares/Horse

Table 3. Initial nutrient composition of study pasture.

	Grass	Forbs	Composite
ADF, %	23.38	16.83	23.91
NDF, %	51.96	26.85	53.82
DM, %	96.00	96.00	96.00
Ash, %	11.19	11.19	11.19
CP, %	17.57	18.97	19.23
Fat, %	3.69	5.31	4.24
NSC, %[1]	15.58	37.68	11.52
DE, mcal/kg[2]	2.03	1.51	2.10
Production[3]			3,146.40

[1]NSC = 100- CP - Fat - NDF – Ash [110]
[2]DE (kcal/kg DM) = 2,118 + 12.18 (CP%) – 9.37 (ADF%) - 3.83 (hemicellulose %) + 47.18 (fat %) + 20.35 (NSC) – 26.3 (Ash %) (R^2 =0.88) [110]
[3]kg/ha DM basis

Pasture was analyzed every other week during the study to determine nutrient content to ensure that all horses would meet or exceed the 2007 NRC requirements for maintenance [114]. Individual grazing strips were large enough that no adjustment was necessary during the course of the study.

Intake was determined for each horse using an equation based on BW created by Dowler and Siciliano in 2009 (Equation 4) (111).

Equation 4. Pasture dry matter intake = 0.166 ± 0.015 kgDM $\cdot$ 100 $kgBW^{-1} \cdot h^{-1}$

Blood Collection and Analyses

Preliminary blood tests were conducted to: ensure none of the horses used were currently insulin resistant, determine baseline glucose concentrations, and determine baseline insulin concentrations. On days -1, 7, 14, 21, and 28 jugular vein catheters were placed and used for blood sample collections the following day. A total of 16 milliliters of blood was collected five times (0700, 0800, 0900, 1100, 1300) on days 0, 8, 15, 22, and 29. A total of 26 ml of blood was collected at a sixth time (1500) on days 0, 8, 15, 22, and 29. Blood samples for analyses of glucose and insulin were collected into BD Vacutainer tubes (Franklin Lakes, NJ, USA) containing sodium fluoride and potassium oxalate. Blood samples for assay of leptin, ghrelin, and adiponectin concentrations were collected into Monoject^{TM} Tubes (Covidien; Mansfield, Massachusetts, USA) containing 15% EDTA. All vacutainers were placed on ice within one minute of collection and subsequently centrifuged (2,000 X g) at 4° C. Plasma was decanted into 12 X 75 mm plastic tubes and stored at -20° C. Plasma for ghrelin analysis was acidified with 50 uL of 1 N HCl and 10 uL of phenylmethylsulfonyl fluoride (PMSF) per one mL of plasma prior to freezing.

Glucose Analyses

Analysis of glucose concentration was determined colorimetrically using a commercially available hexokinase reagent (Infinity glucose hexokinase kit; Thermo Scientific, Hampton, NH, USA). The glucose kit had an intra- and interassay CV of 7% and 16%, respectively for a sample containing a mean glucose concentration of 129.2

mg/dL, and an intra- and interassay CV of 15% and 21%, respectively, for a sample containing a mean glucose concentration of 62.9 mg/dL.

Insulin Analyses

Serum insulin concentration was determined in duplicate using a commercially available solid phase radioimmunoassay kit (Siemens Healthcare Diagnostics Inc., Los Angeles, CA). Insulin RIA kit was previously validated for use with equine insulin by Freeston et al., in 1991 [115]. The insulin kit has a sensitivity of 1.5 µIU/mL. Intra- and interassay CV for a sample with a mean concentration of 102 µIU/mL was 17% and 16%, respectively.

Ghrelin Analyses

Plasma active ghrelin concentrations were determined using a commercially available radioimmunoassay kit (EMD Millipore, Billerica, MA), which was previously validated for use in horses [53]. The sensitivity of the assay was 7.8 pg/mL and the intra- and interassay CVs were 5% and 38%, respectively. In the absence of purified equine active ghrelin results are expressed as human equivalents (HE) of immunoreactive ghrelin. Samples were assayed in duplicate.

Leptin Analyses

Plasma concentrations of leptin were determined using a commercially available kit (EMD Millipore, Billerica, MA) previously validated in horses [116]. Sensitivity of this kit was 0.801 ng/mL HE and the intra- and interassay CVs were 11% and 20%, respectively for a sample containing a mean concentration of 1.5 ng/mL HE, and 8% and

14%, respectively, for a sample containing a mean concentration of 13.8 ng/mL HE. The manufacture reports specificity of 100% for human, 67% for porcine, 61% for rat, 73% for mouse, and 3% for canine. Samples were assayed in duplicate.

Adiponectin Analyses

Plasma concentrations of adiponectin were determined using a commercially available RIA kit (EMD Millipore, Billerica, MA). Kit has been previously validated in horses [117]. In the absence of purified equine adiponectin results are expressed as human equivalent of immunoreactive adiponectin. Sensitivity of this kit was 1 ng/mL and the intra- and interassay CVs were 8% and 4%, respectively for a mean concentration of 40.5 ng/mL, 6% and 19%, respectively, for a mean concentration of 23 ng/mL and 3% and 22% , respectively, for a mean concentration of 14.4 ng/mL. The manufacture reports specificity of 400% for mouse and <0.01% for Human C1q. Samples were assayed in duplicate.

Morphometric Characteristics

On days 0 and 29 morphometric characteristics were measured in all horses. Body weight was measured using an electronic scale (TruTest AG500). Body condition score was recorded as the mean of three trained observers that were blind to treatment [77]. Mean neck circumference was measured as the mean between three measurements along a straight line from the poll to the withers [69]. Tail head fat mass was measured ultrasonically 10 cm lateral to the sacral spinous processes and 11 cm cranial to the tailhead origin [79].

Forage Collection and Analyses

Forage analyses of individual grazing allotments were conducted every other week throughout the grazing portion of the study. Forage was collected by hand clipping plant standing crop within three 0.25-m^2 quadrats located at 30-m intervals along a transect within each individual paddock. Clipped samples were dried in a forced-air oven at 55°C for 48 hours, and then weighed. Total weight was converted to kg/ha to determine available forage [112, 113]. Samples were ground through a 0.5 mm screen in a Wiley Mill and stored for later nutrient analyses.

Plant samples from individual pens were analyzed for water-soluble carbohydrates (WSC), ethanol-soluble carbohydrates (ESC), NSC, DM, DE, CP, fat, ash, ADF, and NDF. WSC and ESC was analyzed by Equi-Analytical Laboratires, Ithaca, NY. DM, Crude protein and fat were analyzed at the Bozeman Fish Technology Center, Bozeman, MT. NSC was determined using the following equation: 100 - CP - Fat – NDF - Ash [110]. DE was determined using the following equation: DE (kcal/kg DM) = 2,118 + 12.18 (CP%) – 9.37 (ADF%) – 3.83 (hemicellulose%) + 47.18 (Fat%) + 20.35 (NSC%) -26.3 (Ash%) (R^2=0.88) [110]. ADF and NDF were analyzed in the C.M. Bair Family Ranch Nutrition Laboratory at Montana State University using the methods of Van Soest et al., 1991 [118]. Ash was analyzed at the Oscar Thomas Nutrition Center, Bozeman Agriculture Research and Teaching Farm, Bozeman, MT. All samples were assayed in duplicate.

Statistical Analyses

Data was analyzed using a repeated measure model with a compound symmetric correlation structure in the statistical software R [119]. Mean glucose, peak glucose, time to peak glucose, glucose area under the curve (AUC), mean insulin, peak insulin, time to peak insulin, and insulin AUC were analyzed with potential explanatory variables of treatment (supplement/non-supplement), time, treatment by time interaction, gender, treatment by gender interaction, age, and NSC intake. A stepwise regression procedure was used to reduce the model. At each step the variable that had the largest p-value > 0.05 was removed. For the morphometric characteristics data the difference for each response variable was modeled using a 2-way ANCOVA model with a stepwise regression procedure to reduce the model and included the potential explanatory variables of treatment (supplemented/non-supplemented), gender, interaction between treatment and gender, age, leptin, insulin, and adiponectin. Leptin and adiponectin data was fitted to a repeated measure model with a compound symmetric correlation structure. Potential explanatory variables included were treatment (supplemented/non-supplemented), time, treatment by time interaction, gender, treatment by gender interaction, age, NSC intake, and morphometric characteristics. A stepwise regression procedure was used to reduce the model removing the variable that had the largest p-value >0.05 at each step.

RESULTS

Forage Nutrient Composition and Consumption by Horses

Pasture was analyzed for nutrient composition on Days 0 (Table 4), 13 (Table 5), and 27 (Table 6). There were no differences in NDF, fat, NSC, WSC or DE between pens or days. A day effect (Table 7) was found for ADF ($P < 0.05$), ash ($P < 0.05$), and CP ($P < 0.05$). ADF and ash were higher on day 27 compared to day 0 and 13. CP was lower on day 27 compared to days 0 and 13. An effect of pen was found for CP ($P < 0.05$) and ESC ($P < 0.05$) in the model. However, the Bonferroni-adjusted p-values for the pairwise differences, for mean CP and mean ESC values for the pens, did not show a significant difference. Estimated NSC intake of pasture and supplement was determined for all horses (Table 8) and analyzed. A treatment by day interaction occurred for NSC intake ($P < 0.05$). The treatment by day interaction seems to be driven by a difference in NSC intake on day 29 (Figure 1). A reduced data set, excluding day 29, was analyzed to determine if day 29 was in fact driving the treatment by day interaction. For the reduced data set only day was a significant effect ($P < 0.05$). Bonferonni adjusted p-values for all pairwise comparisons of day for NSC intake show that day 0 NSC intake was greater than day 15 and day 22, and day 8 NSC intake was greater than day 15 and day 22 (Table 9).

Table 4. Nutrient composition of individual strip grazing allotments; pen, approximately .61 ha each, in cool season pasture on day 0 (June 4th, 2012) of study.

	Pen[1]										
	1	3	4	5	8	12	13	14	15	16	17
ADF, %	29.80	32.15	30.32	30.76	31.26	27.09	29.17	29.85	30.85	32.51	32.18
NDF, %	57.67	59.40	60.20	60.99	58.80	51.93	51.17	58.23	56.48	60.06	59.44
DM, %	93.55	97.49	97.11	97.22	96.66	96.47	97.83	97.89	97.36	97.76	97.60
Ash, %	11.53	10.88	11.09	10.58	11.47	10.51	10.94	10.80	11.54	10.98	11.97
CP, %	10.48	9.37	12.67	13.06	14.60	15.93	15.92	14.85	14.03	14.42	13.32
Fat, %	1.86	3.29	2.79	2.75	2.68	4.71	4.51	3.91	3.44	3.65	4.47
NSC, %[2]	18.46	17.06	13.26	12.62	12.46	16.92	17.46	12.21	14.51	10.88	10.80
ESC, %[3]	18.10	14.00	9.50	12.30	9.80	15.30	12.10	14.00	13.70	11.30	7.60
WSC, %[3]	9.20	8.70	8.10	10.20	8.70	8.90	7.90	7.60	8.30	7.90	6.10
DE, mcal/kg[4]	1.91	1.82	1.99	2.00	2.03	1.86	1.84	1.98	1.94	1.99	1.97

ADF, acid detergent fiber; NDF, neutral detergent fiber; DM, dry matter; CP, crude protein; NSC, nonstructural carbohydrate; ESC, ethanol-soluble carbohydrates; WSC, water-soluble carbohydrates; DE, digestible energy

[1]Pen number corresponds to animal ID number

[2]NSC = 100- CP - Fat - NDF – Ash [110]

[3]ESC and WSC analyzed by Equi-analytical Laboratires, Ithaca, NY

[4]DE (kcal/kg DM) = 2,118 + 12.18 (CP%) – 9.37 (ADF%) - 3.83 (hemicellulose %) + 47.18 (fat %) + 20.35 (NSC) – 26.3 (Ash %) (R^2 =0.88) [110]

Table 5. Nutrient composition of individual strip grazing allotments; pen, approximately .61 ha each, in cool season pasture on day 13 (June 17th, 2012) of study.

	Pen[1]										
	1	3	4	5	8	12	13	14	15	16	17
ADF, %	30.81	41.39	32.29	31.87	31.07	34.91	31.62	29.75	27.54	37.80	37.71
NDF, %	56.81	59.21	60.17	62.37	61.07	60.63	62.52	58.63	53.44	57.33	55.43
DM, %	89.73	92.13	93.17	94.74	93.87	94.08	94.11	94.38	93.80	93.58	93.67
Ash, %	10.92	12.04	12.03	12.48	13.36	11.32	12.00	12.13	12.36	12.66	13.53
CP, %	10.16	9.91	8.20	12.51	13.36	11.76	10.03	12.51	15.67	13.24	19.72
Fat, %	3.34	2.81	2.66	4.32	2.80	3.67	3.30	3.94	3.65	2.79	3.17
NSC, %[2]	18.76	16.02	16.94	8.33	9.41	12.63	12.15	12.78	14.89	13.98	8.14
ESC, %[3]	14.20	12.40	11.90	13.00	11.60	17.10	14.00	12.40	12.70	11.10	8.90
WSC, %[3]	8.20	3.70	7.10	7.60	6.20	8.60	6.90	6.90	8.60	8.20	6.10
DE, mcal/kg[4]	1.80	1.78	1.87	2.04	2.13	1.90	1.97	1.98	2.00	1.92	2.11

ADF, acid detergent fiber; NDF, neutral detergent fiber; DM, dry matter; CP, crude protein; NSC, nonstructural carbohydrate; ESC, ethanol-soluble carbohydrates; WSC, water-soluble carbohydrates; DE, digestible energy

[1]Pen number corresponds to animal ID number

[2]NSC = 100- CP - Fat - NDF – Ash [110]

[3]ESC and WSC analyzed by Equi-analytical Laboratires, Ithaca, NY

[4]DE (kcal/kg DM) = 2,118 + 12.18 (CP%) – 9.37 (ADF%) - 3.83 (hemicellulose %) + 47.18 (fat %) + 20.35 (NSC) – 26.3 (Ash %) (R^2 =0.88) [110]

Table 6. Nutrient composition of individual strip grazing allotments; pen, approximately .61 ha each, in cool season pasture on day 27 (July 1st, 2012) of study.

	Pen[1]										
	1	3	4	5	8	12	13	14	15	16	17
ADF, %	39.09	34.99	40.14	43.56	43.63	34.48	40.27	35.79	34.44	36.52	38.18
NDF, %	54.98	62.93	57.15	57.91	62.89	64.76	65.45	66.25	64.14	65.41	59.97
DM, %	94.52	93.86	94.47	94.61	94.76	94.14	94.32	93.58	93.73	94.66	94.90
Ash, %	12.08	12.95	1306	12.90	12.51	11.20	11.36	12.31	12.67	11.74	14.15
CP, %	9.21	7.66	8.51	9.27	7.87	9.26	9.60	9.61	10.03	10.72	11.60
Fat, %	3.15	3.48	3.65	2.74	4.13	3.68	2.93	2.52	3.65	3.18	2.68
NSC, %[2]	20.58	12.97	17.63	17.18	12.60	11.10	10.66	9.31	9.51	8.95	11.59
ESC, %[3]	12.30	12.80	11.50	9.00	10.10	12.50	11.80	9.60	12.40	10.00	9.40
WSC, %[3]	9.30	7.00	8.30	8.00	9.20	7.30	9.90	6.80	6.90	6.40	7.50
DE, mcal/kg[4]	1.68	1.90	1.72	1.74	1.76	1.92	1.90	2.03	2.00	1.99	2.00

ADF, acid detergent fiber; NDF, neutral detergent fiber; DM, dry matter; CP, crude protein; NSC, nonstructural carbohydrate; ESC, ethanol-soluble carbohydrates; WSC, water-soluble carbohydrates; DE, digestible energy
[1]Pen number corresponds to animal ID number
[2]NSC = 100- CP - Fat - NDF – Ash [110]
[3]ESC and WSC analyzed by Equi-analytical Laboratires, Ithaca, NY
[4]DE (kcal/kg DM) = 2,118 + 12.18 (CP%) – 9.37 (ADF%) - 3.83 (hemicellulose %) + 47.18 (fat %) + 20.35 (NSC) – 26.3 (Ash %) (R^2 =0.88) [110]

Table 7. Significant day effects on nutrient composition of cool season grass pasture from June 4th (day 0) to July 1st (day 27) (mean ± SD).

	Day 0	Day 13	Day 27
ADF, %	30.54 ± 1.57a[1]	32.41 ± 2.69a	37.10 ± 2.40b
Ash, %	11.06 ± 0.53a	11.83 ± 0.91a	12.27 ± 0.78b
CP, %	13.40 ± 2.20a	12.06 ± 3.2a	9.17 ± 1.24b

ADF, acid detergent fiber; CP, crude protein
[1]Means within each row with differing lowercase letters are significantly different (P<0.05)

Table 8. Mean NSC intake for psyllium-supplemented; psyllium, (n = 6) and non-supplemented (n = 5) horses on days 0 (June 4th), 8, 15, 22, and 29 (July 3rd) for morning supplement[1,2] and cool season pasture grazing for 8 h/d (mean ± SD).

	Day				
Mean NSC Intake, kg DM	0	8	15	22	29
Psyllium[1]	1.86 ± 0.60	1.87 ± 0.61	1.38 ± 0.49	1.38 ± 0.49	0.816 ± 0.46
Non-psyllium supplemented[2]	1.85 ± 0.44	1.86 ± 0.44	1.22 ± 0.69	1.22 ± 0.69	2.271 ± 0.77

[1]Psyllium; morning supplemented was 180 g/d/horse of psyllium (Psyllium Pellets EQ, Vetri-Science® Laboratories of Vermont), 222.26 g/d/horse protein supplement (Nutrena Empower® Balance Horse Supplement), and 125 g/d/horse whole oats.
[2]Non-psyllium supplemented; morning supplement was 453.6 g/d/horse protein supplement (Nutrena Empower® Balance Horse Supplement), and 125 g/d/horse whole oats.

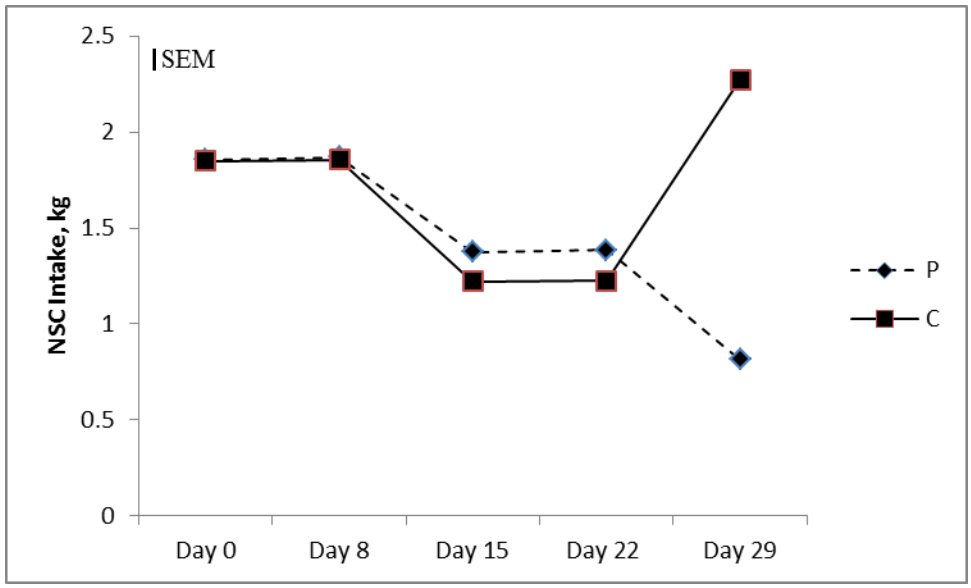

Figure 1. Treatment by day interaction of mean NSC intake of psyllium-supplemented; P, 180 g/horse/d (n = 6) and non-psyllium supplemented; C (n = 5) horses on days 0, 8, 15, 22, and 29, SEM; pooled standard error of the mean = 0.13, P < 0.05.

Table 9. Mean NSC intake for all horses (n = 11) on cool season pasture and morning supplement[1,2] on days 0, 8, 15, and 22 of grazing (mean ± SD).

	Day			
Mean NSC Intake, kg DM	0	8	15	22
All horses	1.85 ± 0.51a[3]	1.86 ± 0.51a	1.31 ± .56b	1.31 ± 0.56b

[1]Psyllium; morning supplemented was 180 g/d/horse of psyllium (Psyllium Pellets EQ, Vetri-Science® Laboratories of Vermont), 222.26 g/d/horse protein supplement (Nutrena Empower® Balance Horse Supplement), and 125 g/d/horse whole oats.
[2]Non-psyllium supplemented; morning supplement was 453.6 g/d/horse protein supplement (Nutrena Empower® Balance Horse Supplement), and 125 g/d/horse whole oats.
[3]Means differing lowercase letters are significantly different (P<0.05)

Glucose: Mean Concentrations, Peak Concentrations, Time to Peak and AUC

Mean glucose, peak glucose, time to peak glucose, and glucose area under the curve were analyzed. Mean glucose was effected by psyllium supplementation (P<0.0001) (Figures 2 and 3) (Table 10), and day (P<0.0001), (Figure 4). On average,

psyllium supplemented horses have mean glucose concentrations that are 11.62 ng/mL lower than control horses according to the point estimate derived from the regression model. On average, for each increase in day mean glucose decreases by 0.72 ng/mL for horses grazing 8 hours daily on cool season pasture grass, according to the point estimate derived from the regression model.

Peak glucose was effected by time ($P<0.0001$). The point estimate derived from the linear regression model predicts that, on average, for each increase in day for horses grazing cool season grass eight hours daily peak glucose decreases by 2.52 ng/mL. Time to peak glucose was effected by psyllium ($P = 0.0015$), (Table 10), and age ($P<0.0001$). Psyllium supplemented horses, on average, take 1.15 hours longer to reach peak glucose than non-supplemented horses according to the point estimate derived from the regression model. On average, for each year increase in age the time to peak glucose decreases by 0.25 hours according to the point estimate derived from the regression model. Glucose AUC was effected by time ($P<0.0001$) and psyllium ($P<0.0001$) (Table 10). The point estimate derived from the regression model predicts that, on average, for each increase in day glucose AUC will decrease by 6.73 ng/mL, hour, for horses grazing 8 hours daily on cool season pasture grass. Psyllium supplementation, on average, decreases glucose AUC by 98.43 ng/mL, hour, according to the point estimate derived from the regression model.

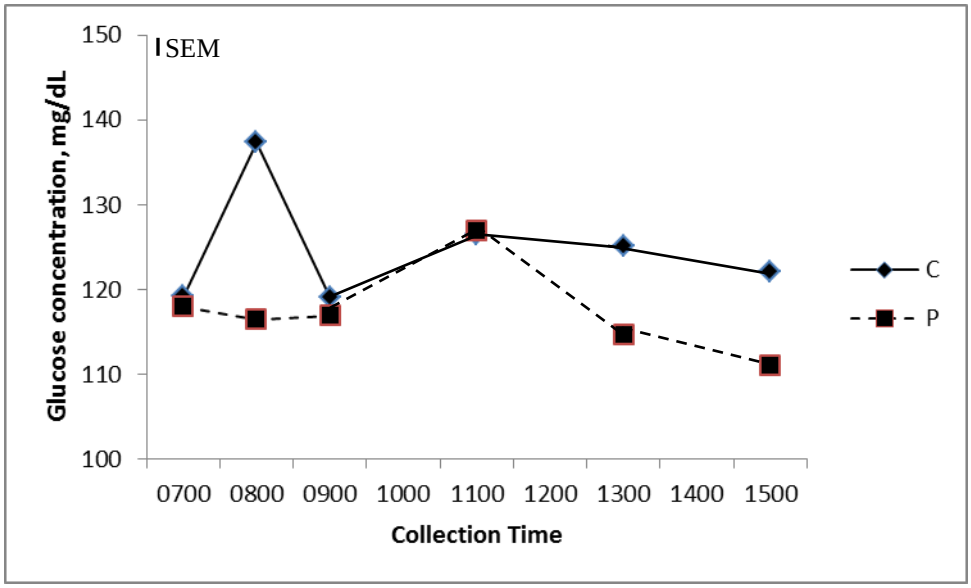

Figure 2. Mean glucose concentrations of psyllium-supplemented; P, 180 g/horse/d (n = 6) and non-psyllium supplemented; C (n = 5) horses at times -1 (0700), 0 (0800), 1 (0900), 3 (1100), 5 (1300) and 7 (1500). SEM; pooled standard error of the mean = 2.11.

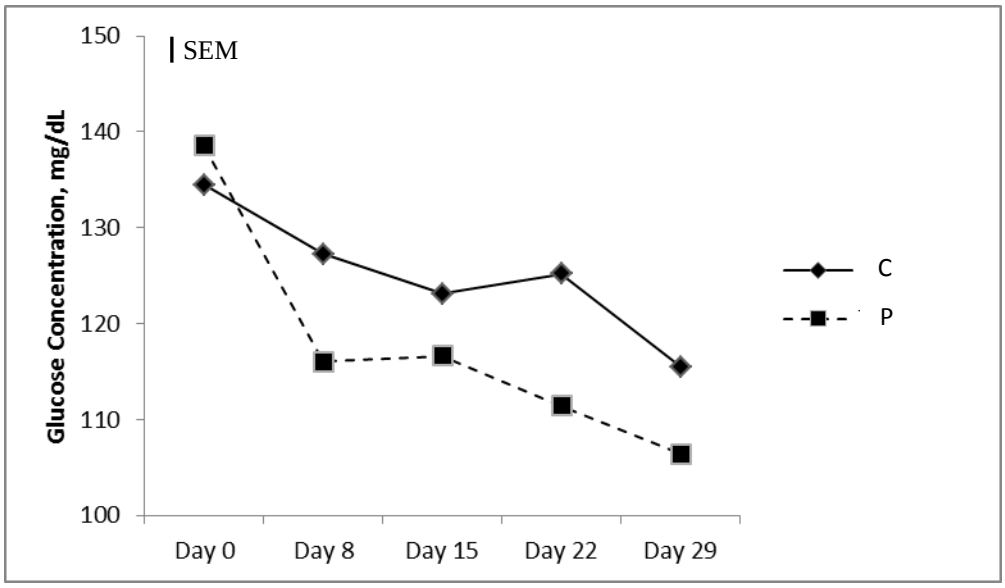

Figure 3. Mean glucose concentrations for psyllium-supplemented; P, 180 g/horse/d (n = 6) and non-supplemented; C, (n = 5) horses on days 0 (start of supplemented diets on day 1), 8, 15, 22, and 29. SEM; pooled standard error of the mean = 3.06. $P < 0.05$.

Table 10. Mean glucose concentration, time to peak glucose, and glucose area under the curve (AUC) of psyllium supplemented; psyllium, 180 g/horse/d, (n = 6) and non-supplemented; control (n = 5), horses over 29 day period of grazing cool season pasture 8 h/d (mean ± SD)

	Psyllium	Control
Mean glucose concentration, mg/dL	115.08 ± 9.82	126.64 ± 12.08
Time to peak glucose, h	3.30 ± 2.37	2.4 ± 2.48
Glucose AUC, (mg/dL), h	946.90 ± 169.42	1003.57 ± 131.58

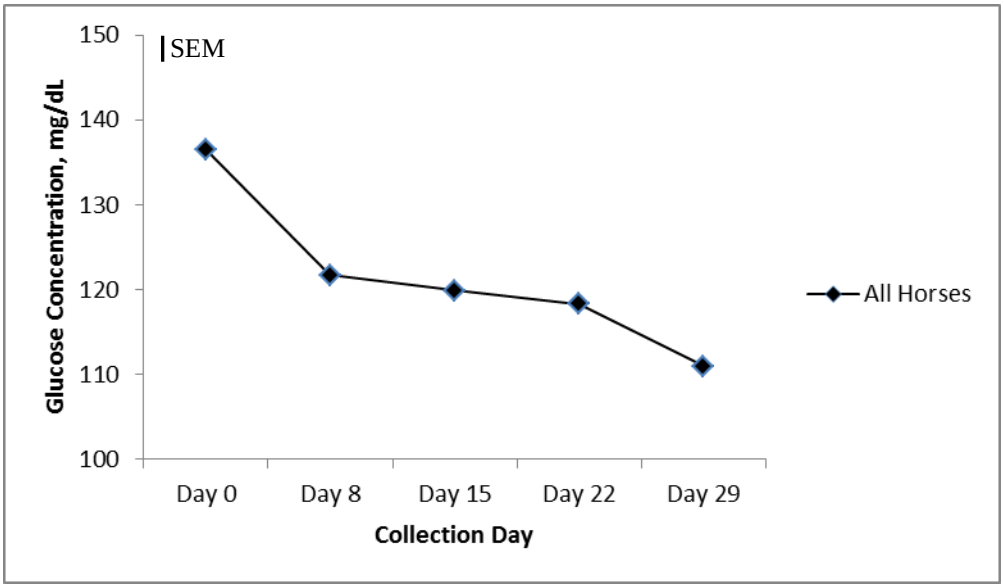

Figure 4. Mean glucose concentration of all horses, psyllium-supplemented; 180 g/horse/d, and non-supplemented, (n = 11) on days 0 (start of supplemented diets on day 1), 8, 15, 22, and 29. SEM; pooled standard error of the mean = 2.82, $P < 0.05$.

Insulin: Mean Concentrations, Peak Concentrations, Time to Peak, AUC and Chi-Square

Mean insulin, peak insulin, time to peak insulin, and insulin AUC were analyzed. Mean insulin concentrations were affected by time ($P<0.0001$) (Figures 5 and 6), and an interaction between psyllium and gender ($P = 0.0101$). On average, for each increase in

day mean insulin concentrations decrease by 0.77 µIU/mL, for horses grazing 8 hours daily on cool season pasture grass, according to the point estimate of the regression model. On average, psyllium supplementation decreases mean insulin concentrations by 19.45 µIU/mL compared to non-supplemented horses, according to the point estimate of the regression model.

On average, the concentration of mean insulin is 10.06 µIU/mL lower for mares than for geldings, according to the point estimate derived from the regression model. On average, concentrations of mean insulin increase by 0.33 µIU/mL for each year increase in age according to the point estimate derived from the regression model. The interaction of treatment and gender indicates that the effect of psyllium differs depending on gender. In this study mares in the control group had lower mean insulin concentrations than geldings, but the reverse effect occurred in the treatment group with psyllium supplemented geldings having lower mean insulin concentrations than treatment mares (Figure 7).

Peak insulin concentrations were effected by day ($P<0.0001$), age ($P = 0.0077$), NSC intake ($P = 0.0477$), and an interaction between psyllium and gender ($P = 0.0121$). On average, peak insulin decreases by 1.19 µIU/mL for horses grazing cool season grass for eight hours daily with each increase in day, according to the point estimate derived from the regression model. On average, peak insulin concentrations decrease by 22.50 µIU/mL in psyllium supplemented horses compared to non-supplemented horses as determined by the point estimate derived from the regression model. On average, peak insulin concentrations were increased by 1.23 µIU/mL for every year increase in age, as

determined by the point estimate derived from the regression model. On average, peak insulin concentrations increase by 9.66 µIU/mL with each kg increase in NSC intake, as determined by the point estimate derived from the regression model. The interaction of treatment and gender indicates that the effect of psyllium differs depending on gender. In this study mares in the control group had lower peak insulin concentration than geldings, but the reverse effect occurred in the treatment group with psyllium supplemented geldings having lower mean insulin concentrations than treatment mares (Figure 8).

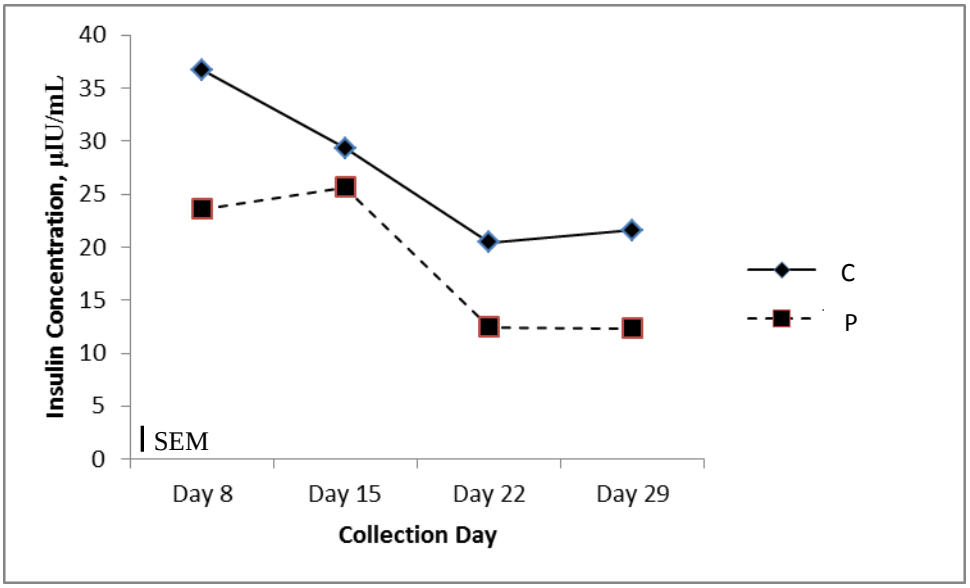

Figure 5. Mean insulin concentration for psyllium-supplemented; P, 180 g/horse/d (n = 6) and non-supplemented; C, (n = 5) horses on day 8 (start of supplemented diets on day 1), 15, 22, and 29. SEM; pooled standard error of the mean = 2.47. $P < 0.05$.

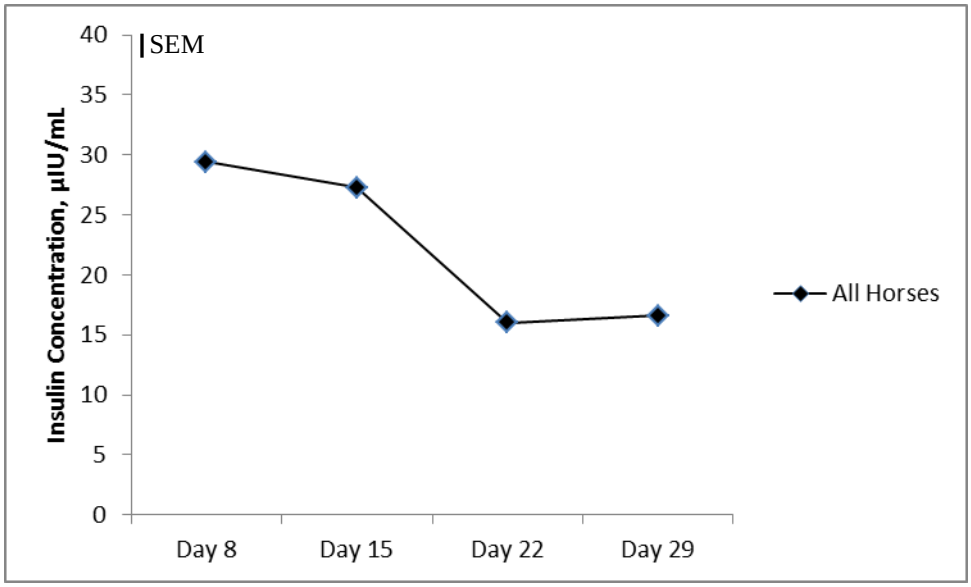

Figure 6. Mean insulin concentration of all horses, psyllium-supplemented; 180 g/horse/d, and non-supplemented, (n = 11) on days 8 (start of supplemented diets on day 1), 15, 22, and 29. SEM; pooled standard error of the mean = 2.11, $P < 0.05$.

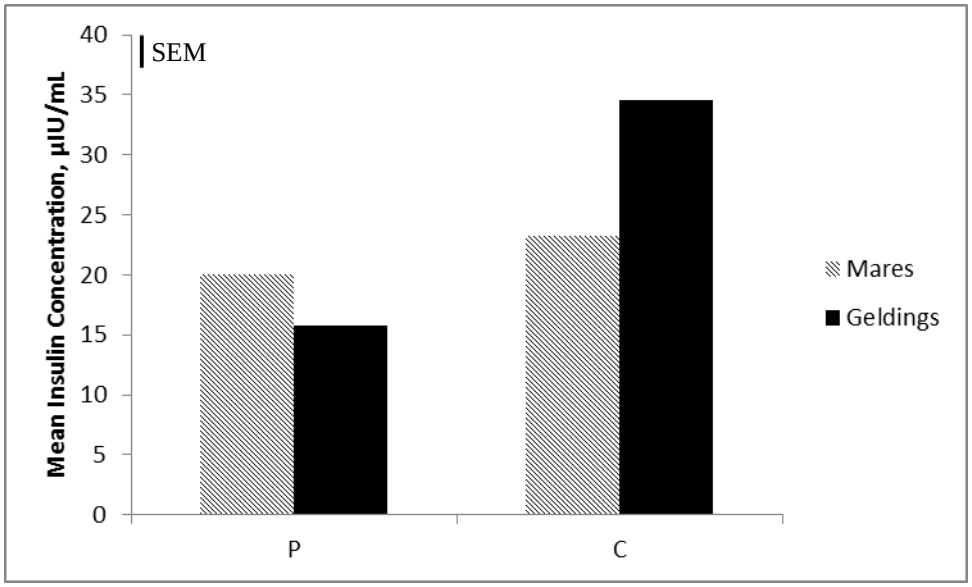

Figure 7. Treatment by gender interaction for mean insulin concentrations for psyllium-supplemented; P, 180 g/horse/d, mares (n = 4), psyllium-supplemented; P, 180 g/horse/d, geldings (n = 2), non-supplemented; C, mares (n = 3) and non-supplemented; C, geldings (n = 2). SEM; pooled standard error of the mean = 2.42, $P < 0.05$.

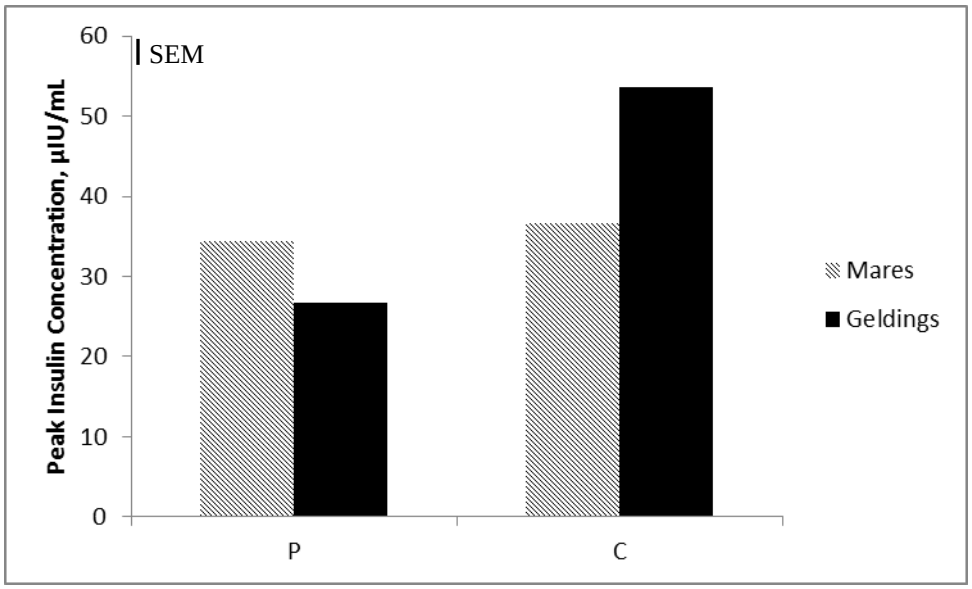

Figure 8. Treatment by gender interaction for peak insulin concentrations for psyllium-supplemented; P, 180 g/horse/d, mares (n = 4), psyllium-supplemented; P, 180 g/horse/d, geldings (n = 2), non-supplemented; C, mares (n = 3) and non-supplemented; C, geldings (n = 2). SEM; pooled standard error of the mean = 3.42, $P < 0.05$.

Time to peak insulin was affected by gender ($P = 0.0009$), 3.96 ± 1.97 h and 2.44 ± 1.79 h in mares and geldings, respectively, and age ($P = 0.0005$). On average, time to peak insulin is increased by 1.63 µIU/mL in mares compared to geldings, as determined by the point estimate derived from the regression model. On average, time to peak insulin increases by 0.33 µIU/mL for each year increase in age, as determined by the point estimate derived from the regression model. Insulin AUC was affected by time ($P<0.0001$), and an interaction between psyllium and gender ($P = 0.0129$). On average, insulin AUC is lowered by 6.50 µIU/mL, hours, for each day increase for horses grazing cool season pasture for 8 hours daily, as determined by the point estimate derived from the regression model. On average, insulin AUC is lowered by 161.06 µIU/mL, hours, for psyllium supplemented horses compared to non-supplemented horses, as determined by

the point interaction derived from the regression model. The interaction of treatment and gender indicates that the effect of psyllium differs depending on gender. In this study mares in the control group had lower insulin AUC than geldings, but the reverse effect occurred in the treatment group with psyllium supplemented geldings having lower insulin AUC concentrations than treatment mares (Figure 9).

Due to the high variation between assays and the limited sensitivity of the kit insulin was also analyzed using a chi-square. Insulin concentration for each horse on each collection day (days 8, 15, 22, 29) was averaged and grouped by high (values ≥21 µIU/mL) and low (values <21 µIU/mL). No significant differences were detected (Figure 10).

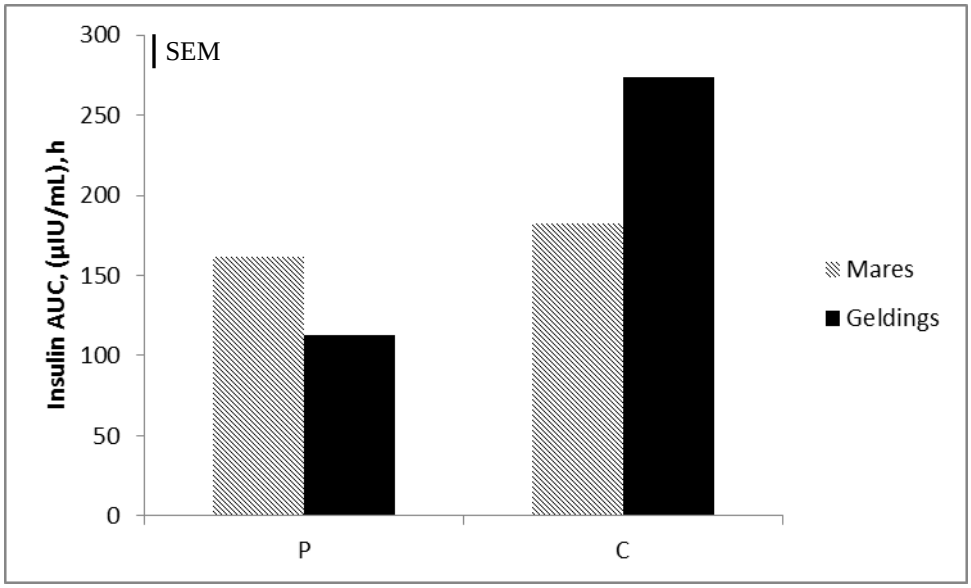

Figure 9. Treatment by gender interaction for insulin AUC for psyllium-supplemented; P, 180 g/horse/d, mares (n = 4), psyllium-supplemented; P, 180 g/horse/d, geldings (n = 2), non-supplemented; C, mares (n = 3) and non-supplemented; C, geldings (n = 2). SEM; pooled standard error of the mean = 20.34, $P < 0.05$.

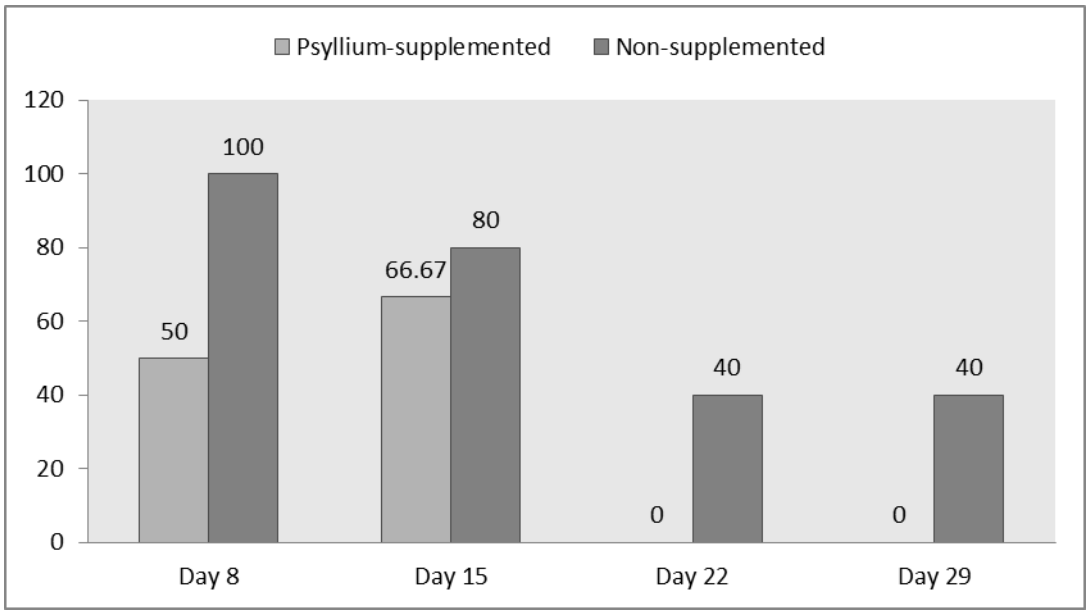

Figure 10. Percentage of psyllium-supplemented; 180 g/d/h (n = 6) and non-supplemented horses; C, (n = 5) with high insulin concentrations (mean concentration >21 µIU/mL) on days 8, 15, 22, and 29.

Digestive Hormone Concentrations

Ghrelin

All measures of ghrelin concentration in equine plasma were below the sensitivity of the kit (7.8 pg/mL), therefore, ghrelin was not analyzed for this study.

Leptin

Leptin was affected by gender (P = 0.005) (Figure 11). On average, mares have leptin concentrations 0.69 ng/mL HE lower than geldings, according to the point estimate derived from the regression model. Mares in this study over the entire 29 day period had a mean leptin concentration of 1.51 ng/mL HE ± 0.67. Geldings in this study over the entire 29 day period had a mean leptin concentration of 2.19 ng/mL HE ± 0.61. Pearson correlations for day 29 measurements were analyzed for leptin and the morphometric

characteristics of BW, BCS, mean neck circumference and tail head fat mass. Leptin was positively correlated with BW, mean neck circumference, BCS and negatively correlated with tail head fat mass; however, none of the correlations were significant.

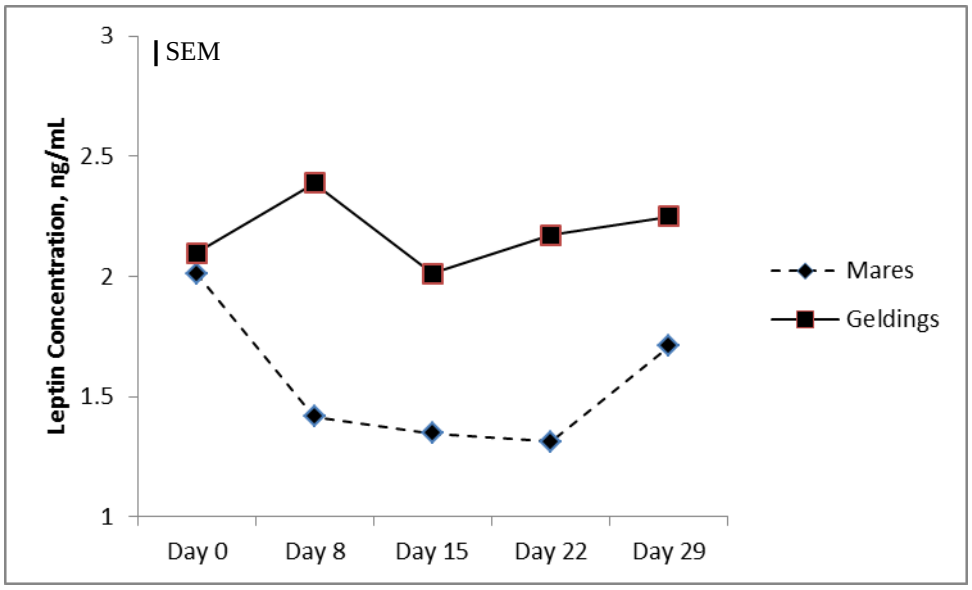

Figure 11. Mean leptin concentrations of mares (n = 7) and geldings (n = 4) on Days 0, 8, 15, 22, and 29. SEM; pooled standard error of the mean = 0.12, $P < 0.05$.

Adiponectin

Adiponectin was affected by NSC intake ($P = 0.0371$). According to the point estimate derived from the regression model, on average, adiponectin concentrations decrease by 1.88 ng/mL HE for every 1 kg increase in NSC Intake. Pearson correlation coefficients for day 29 measurements were analyzed for adiponectin and the morphometric characteristics of BW, BCS, mean neck circumference and tail head fat mass. Adiponectin was negatively correlated with mean neck circumference, BCS, and positively correlated with tail head fat mass; however, none of these correlations were significant. Adiponectin was significantly correlated to BW ($r = -0.6806$, $P < 0.05$).

Morphometric Characteristics

Morphometric characteristics of BW, BCS, mean neck circumference, and tail head fat mass were measured on days 0 and 29. Although the majority of measurements changed over the course of the 29 day study no change was statistically significant.

DISCUSSION

Forage Nutrient Composition and Consumption by Horses

The decrease in CP and increase in ADF and ash on the last collection day is in accordance with the growth cycle of the cool season grass species within the pasture. Smooth brome (*Bromus inermis* Leyss.), one of the dominant species in the study pasture, is a good representation of cool season grass growth. Smooth bromegrass reaches peak standing crop in late July with 41 to 57% of growth occurring by late May or early June. Grazing time is suggested as early May to early June for smooth bromegrass as CP decreases, and ADF increases, after seed set near June 5th. In addition, smooth bromegrass grows best with 16 or more inches of precipitation [120]. In April, May and June of 2012 precipitation at the study site was 7.24 inches, compared to 9.93 inches the previous June. Year to date precipitation was 13.43 inches compared to 18.12 inches the previous year [121]. During the study period the growing season of the grass may have been shortened slightly due to this decrease in annual precipitation. There were no significant differences in nutritive values for pasture grass between individual strip grazing allotments, therefore all horses had access to pasture of similar nutritive value. The mean NSC content of the pasture, for all allotments over the entire study period, was 13.42 ± 3.35%. This is similar to previously reported mean pasture NSC of 13.5 ± 2.37% for mid-March to end of May [122], 13.85 ± 0.3% for May, and 9.2 ± 0.5% for August [123]. The supplement given to horses each morning was isocaloric, but contained different amounts of NSC (Table 11). Despite this difference in NSC content of the

morning supplement the only significant difference between psyllium supplemented and non-psyllium supplemented horses in estimated total NSC consumption from supplement and pasture occurred on day 29. This suggests that pasture intake by psyllium supplemented horses was slightly greater for the majority of the study period with the exception of the last week, as measured by the previously given equation based on body weight [111], where pasture consumption was similar resulting in greater total NSC intake by the control group.

Table 11. Nonstructural carbohydrates (NSC) in morning supplement for psyllium supplemented[1] (n = 6) and non-psyllium supplemented[2]; control, (n = 5) horses.

	NSC, kg DM
Psyllium Supplemented Horses[1]	132.85
Control Horses[2]	212.97

[1]Psyllium; morning supplemented was 180 g/d/horse of psyllium (Psyllium Pellets EQ, Vetri-Science® Laboratories of Vermont), 222.26 g/d/horse protein supplement (Nutrena Empower® Balance Horse Supplement), and 125 g/d/horse whole oats.
[2]Non-psyllium supplemented; morning supplement was 453.6 g/d/horse protein supplement (Nutrena Empower® Balance Horse Supplement), and 125 g/d/horse whole oats.

Glucose Concentrations in Grazing, Psyllium Supplemented Horses

The mean glucose concentrations for psyllium supplemented (115.08 ± 9.82 mg/dL) and non-psyllium supplemented (126.64 ± 12.08 mg/dL) horses in this study are slightly higher than previously reported glucose concentrations. McIntosh, et al. reported glucose concentrations for grazing horses to be 110.9 ± 2.1 mg/dL and 98.1 ± 3.8 mg/dL for May and August, respectively [26]. Mean baseline glucose concentration for non-pregnant Thoroughbred broodmares grazing cool season grass were reported to be 100 ± 6.2 mg/dL [124]. Mean glucose concentration for 6 unfit Quarter Horse geldings on a

meal fed diet of grass hay and Purina Mills Strategy, fed to reach intake of 0.3 g NSC/kg BW, was 88.9 ± 0.77 mg/dL [125].The slightly higher mean glucose concentrations in this study could be related to breed, mixing of gender, or the amount of NSC consumed.

Horses in this study had a mean peak glucose concentration of 154.14 mg/dL, which is higher than previously reported values. Peak glucose concentrations for six unfit Quarter Horse geldings fed timothy grass hay and Purina Mills Strategy in two meals daily in pelleted, oval, and extruded forms were 94.56 ± 2.36, 95.94 ± 2.36, and 98.39 ± 2.36 mg/dL, respectively [126]. Mean peak glucose concentration in Thoroughbred broodmares grazing cool season grass was reported to be 104 ± 6.2 mg/dL [124]. The higher peak glucose concentration reported in the current study is most likely due to a higher NSC intake and a higher body condition (which indicates more adipose tissue) in the horses used as compared to previous research.

The results of the glucose analyses show blood glucose concentrations were effected by two distinct factors: psyllium and continuous grazing. Mean glucose, time to peak glucose and glucose AUC were all lowered in psyllium supplemented horses compared to control horses. However, psyllium supplementation did not decrease peak glucose concentrations. This suggests that the gel formed by psyllium decreases the absorption rate of glucose but not the total amount absorbed.

The time effect in the analysis, which occurred for mean glucose, peak glucose, and glucose AUC, showed that glucose concentrations decreased in all horses over the 29 day study period. A decreased glucose concentration as a result of continuous grazing was not a specific hypothesis of this study, however this result was expected. Horses

evolved as continuous grazers and as a result are designed for continuous intake throughout the day. This continuous intake results in a fairly steady state of glucose throughout the grazing period. The industry standard of feeding horses in meals results in peaks and valleys of glucose concentrations that can be damaging to the horse by leading to disease states such as insulin resistance [15]. Despite the horses being given a morning supplement, and only grazing for 8 hours daily, continuous grazing still resulted in an overall decrease in glucose concentrations.

In this study older horses reached peak glucose faster than younger horses. A decrease in insulin sensitivity will result in blood glucose being cleared at a slower rate, and therefore peak glucose will be reached at a faster rate. This is in accordance with other studies which have found decreased insulin sensitivity in older horses [127].

Insulin Dynamics in Grazing, Psyllium Supplemented Horses

The sensitivity of the insulin kit used in this study was lower than previously reported. The decreased sensitivity may have been a result of using the human insulin standards supplied with the kit instead of equine insulin. Due to this decreased sensitivity insulin concentrations were analyzed with a chi-square in addition to the regression analysis. For the chi-square analysis insulin concentrations for each horse were averaged by day and grouped as high or low. The high category was assigned to mean daily insulin concentrations greater than 21 µIU/mL. Although this is slightly lower than the insulin concentration used to designate hyperinsulinemia (30 to 43 µIU/mL), it was the closest standard to this designation, with the next standard being 102 µIU/mL. The chi square analysis did not detect statistically significant differences in insulin concentrations

between psyllium supplemented and non-supplemented groups. However, a difference may be detected if the study were replicated as day 8 ($P = 0.06$), day 22 ($P = 0.09$), and day 29 ($P = 0.09$) were approaching significance.

In the regression analysis insulin was affected by psyllium, continuous grazing, gender, age, and NSC intake. While psyllium is not capable of a direct physiological impact on insulin the treatment affected insulin as a result of decreasing glucose concentrations. The insulin response needed to clear glucose from the blood will be decreased with a slower rate of glucose absorption, which occurred with psyllium supplementation. This treatment effect on insulin occurred for mean insulin and peak insulin. However, time to peak insulin was not impacted by psyllium supplementation. This suggests that while supplementation to decrease glucose concentrations can decrease the amount of insulin secreted, time to peak insulin is either a reflection of time to peak glucose or is caused by other regulatory factors, such as fat mass.

Mean insulin concentration for psyllium supplemented mares (19.99 ± 9.49 µIU/ml), psyllium supplemented geldings (15.78 ± 9.55 µIU/mL), non-psyllium supplemented mares (23.26 ± 10.37 µIU/ml), and non-psyllium supplemented geldings (34.52 ± 10.38 µIU/mL), are similar to previously reported insulin concentrations in grazing horses [26, 124]. Peak insulin concentration for psyllium supplemented (31.84 ± 18.76 µIU/ml) and non-psyllium supplemented horses (43.41 ± 15.60 µIU/ml) were similar to previously reported peak insulin concentrations in grazing horses (43 ± 8.9 µIU/ml) [124].

There was a time effect on insulin concentrations which occurred as a result of this same effect on glucose concentrations. Under a continuous grazing setting glucose enters the circulation at a steadier rate in slower concentrations throughout the grazing period. The alteration in glucose pattern results in the same pattern occurring with insulin: a slower steadier release throughout the day.

Insulin concentrations were also affected by gender, age, and NSC intake. In this study mean insulin concentrations were lower in mares. Mean insulin concentrations, peak insulin concentrations, and time to peak insulin, in this study, increased in older horses. This result corresponds with previous research and shows that insulin sensitivity decreases with increasing age in the horse [127]. Due to this age effect, which impacts glucose clearance, special care should be taken to ensure the diets of older horses are low in NSC content with energy coming from fat. This type of diet will help protect older horses from developing insulin resistance as a result of their natural decline in insulin sensitivity with age. Care should be taken when grazing older horses.

Peak insulin concentrations were increased with increasing NSC intake. The mean NSC intake for all horses during the study was 2.5 ± 0.64 kg per day (4.6 g/kg BW). This is below the 10 g/kg BW used to induce lamellar separation [96], but appears to be great enough to increase adipose deposition. Since NSC intake did not affect glucose concentrations, the increase in peak insulin due to increased NSC intake must be from a different mechanism. Insulin functions to clear glucose from the blood so that it can be taken up by muscle and adipose tissue. However, insulin also operates as an adiposity signal to the brain [41]. When excess glucose enters the system, such as occurs with high

rates of NSC intake, it is deposited in adipose tissue. Increasing NSC intake in this study resulted in glucose deposition in adipose tissue, as evidenced by the increase in peak insulin. This increase in fat deposition, and resulting increase in insulin secretion, may also be the cause of psyllium not affecting peak insulin concentrations.

Digestive Hormone Concentrations

Ghrelin

Blood samples for ghrelin analysis were collected at 1500 on days 0, 8, 15, 22, and 29. Due to collection at this time point ghrelin concentrations in equine plasma were too low to be detected with the RIA kit used. Ghrelin has been previously analyzed in horses, however, not in a grazing setting. Ghrelin has been shown to be suppressed during an intravenous dextrose challenge and an oral grain challenge [50]. If ghrelin is suppressed due to a glucose challenge there is a strong possibility that ghrelin may also be suppressed in a continuous grazing setting where glucose is able to enter the circulation throughout the course of the day. In addition, previous research into ghrelin concentrations in horses has cautioned that both time of day when blood is collected and timing of a meal in relation to collection will have an effect on ghrelin concentrations [53].

Leptin Concentrations in Grazing, Psyllium Supplemented Horses

Leptin concentrations in this study were lower in mares (1.51 ng/mL HE ± 0.67) than geldings (2.19 ng/mL HE ± 0.61). This is similar to research in humans, where women were found to have 40% higher leptin concentrations than men for any level of

adiposity [128]. However, leptin has been reported to be greater in stallions and geldings than in mares [129]. The reason for this discrepancy is unclear, however it may show that the relationship between leptin and fat mass has a stronger effect than gender. Although morphometric characteristics were measured in this study, percent body fat was not, so the discrepancy cannot be solved by the results of the current study.

Leptin was positively correlated with BW, mean neck circumference, and BCS, which corresponds to previous research comparing fit and unfit Standardbred horses [49]. Mean leptin concentrations in the previously mentioned study were 4.38 ± 0.69 ng/mL for unfit horses and 1.05 ± 0.10 ng/mL for fit horses [49].

Adiponectin Concentrations in Grazing, Psyllium Supplemented Horses

Adiponectin concentrations in this study decreased with increasing NSC intake. This fits well with the hypothesis that increasing NSC intake results in increasing amounts of fat deposition, since adiponectin has been shown to decrease with increasing fat deposition [80]. Adiponectin was negatively correlated to BW, mean neck circumference, and BCS, which has been previously reported in other studies [49].

Morphometric Characteristics of Grazing, Psyllium Supplemented Horses

No significant differences were found for any of the measures of morphometric characteristics in this study. This suggests that, for grazing horses not undergoing regular exercise or training, psyllium supplementation will not compromise the horses ability to maintain body condition. Increases in tailhead fat mass were positively correlated with adiponectin and negatively correlated to leptin. The difference in fat deposition by horses

used in this trial may be due to a difference in breed than previously reported studies [80], or a genetic difference as all horses used in this study have a common genetic pool Therefore, when assessing fat deposition via morphometric measurements care should be taken to ensure what is being measured is in accordance with the individual horse fat deposition pattern.

Implication of Psyllium Supplementation for Metabolically Associated Disease Risk in the Horse

A slowed glucose absorption rate will result in a decreased insulin response, thus horses supplemented with psyllium are less likely to develop insulin resistance or laminitis. Additionally, the decreased glucose and insulin concentrations as a result of grazing in all horses suggest that horses at risk of developing insulin resistance would benefit from smaller, more frequent meals throughout the day instead of the industry standard of two meals a day.

CONCLUSION

The results of this study indicate that supplementing psyllium in horses grazing cool season grasses lowered systemic glucose and insulin concentrations. These effects may reduce the risk of metabolic diseases, such as laminitis, insulin resistance, and Equine Metabolic Syndrome. However, systemic glucose and insulin concentrations were effected to a greater extent in males than females.

In conclusion, horses grazing cool season grass could benefit from being supplemented with psyllium. Decreased glucose and insulin concentrations, with no significant difference in morphometric characteristics, suggests that psyllium supplementation will decrease the risk of developing insulin resistance and laminitis in horses grazing cool season grass without compromising their ability to maintain body condition. However, the effects of psyllium supplementation for a period longer than 30 days, as well as the effects of psyllium supplementation in exercising horses, needs to be explored.

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