PSYLLIUM LOWERS BLOOD GLUCOSE AND

INSULIN CONCENTRATIONS IN HORSES

by

Jyme Lynn Peterson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal and Range Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2010

APPROVAL

of a thesis submitted by

Jyme Lynn Peterson

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency and is ready for submission to the Division of Graduate Education.

Dr. Shannon John J. Moreaux

Approved for the Department of Animal and Range Science

Dr. Bret E. Olson

Approved for the Division of Graduate Education

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

ACKNOWLEDGEMENTS

Thank you to my wonderful parents, Mike and Tammy Peterson, for their love and continued support. The principles that you have bestowed upon me of patience, determination, and dedication have been invaluable throughout my academic career but especially with this research and thesis work. I would like to thank Kyla Hendry for her assistance in the lab and in the field; the mornings were certainly brighter having someone to share them with! Thank you also to Bryan Nichols for your support, constructive criticism, and friendship. Your enthusiasm for research and obtaining new knowledge helped me strive for more with my research and in the classroom. Also, I would like to express my sincerest gratitude to my major professor, Dr. Shannon J. Moreaux. Your guidance, patience, knowledge, dedication, and positive attitude made this an amazing experience. Your high standards made me want to get up earlier, work later, and try harder throughout the course of my graduate training. Thank you for your friendship and mentorship; you are truly an asset to the equine program at Montana State University. I would also like to thank Dr. Jim Berardinelli for the instruction and training in the physiology lab, and finally, thank you to my graduate committee members, Dr. Jan Bowman and Dr. Jane Ann Boles, for your valuable time, assistance, and encouragement during the course of my studies at Montana State University.

TABLE OF CONTENTS

TABLE OF CONTENTS – CONTINUED

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES – CONTINUED

LIST OF FIGURES – CONTINUED

ABSTRACT

The metabolic effect of feeding psyllium daily to horses is unknown. Eight 11- to 16-yr-old (Trial 1) and sixteen 7- to 16-yr-old (Trial 2; Trial 3) light breed stock horses were used in a completely randomized design to determine the effects of psyllium supplementation on BW, BCS, neck circumference, tailhead fat, plasma glucose, insulin, leptin, ghrelin, and adiponectin concentrations. An intravenous glucose challenge was also performed and the same variables were measured (Trial 3). Horses were stratified by sex and BW and put on trial for 42 d (Trial 1) and 60 d (Trial 2). Horses were individually fed a grain ration at 0.5% BW (Trial 1) and 0.25% BW (Trial 2), hay at 1.5% BW (Trial 1;Trial 2), and a psyllium treatment. Psyllium treatment levels for Trial 1 were: 1) 90 g/d psyllium or 2) an isocaloric control and 0 g/d psyllium. In Trial 2 levels of 180 and 270 g/d psyllium were added. Trial 3 took place the day after Trial 2 ended; horses were administered an intravenous dextrose solution at 0.5% BW after a 12 h fasting period. In Trial 1, horses fed psyllium had a greater ($P = 0.01$) increase in neck circumference than those not fed psyllium. Trial 2 revealed treatment by time interactions for glucose ($P < 0.001$) and insulin ($P = 0.03$). Glucose was lower 90 min ($P = 0.05$) and 120 min ($P < 0.001$) after a meal in horses fed psyllium compared to those not fed psyllium. Insulin concentrations were lower 90 min ($P = 0.002$) and 300 min ($P < 0.001$) in horses fed psyllium compared to those that were not. There was no difference in glucose ($P = 0.48$) or insulin ($P = 0.15$) concentrations between horses on 90, 180, or 270 g psyllium treatments. In Trial 3, horses fed psyllium had lower glucose ($P = 0.03$) and adiponectin ($P = 0.02$) concentrations than horses not fed psyllium. Supplementing horses with any level of psyllium appears to lower concentrations of plasma glucose and serum insulin after a meal.

CHAPTER 1

INTRODUCTION

Obesity and insulin resistance have become major health concerns in the equine

industry primarily due to their link with the development of laminitis (Frank et al., 2006).

Reaven (1988) suggests diets high in carbohydrate content may intensify genetic

predispositions to insulin resistance. Insulin resistance is characterized as a decreased

response to insulin-mediated uptake of glucose by body tissues. Insulin resistant horses

are prone to developing laminitis because the laminae within the hoof cannot tolerate the

hyperinsulinemic condition that results from insulin insensitivity (Asplin et al., 2007).

Laminitis is a painful and potentially career ending condition where the laminar tissues,

which connect the bone to the hoof wall, become inflamed and the attachment weakens.

Frank et al. (2006) compared healthy non-obese horses to obese horses with

insulin resistance and found that obese and insulin resistant horses had higher resting

plasma glucose concentrations, higher resting serum insulin concentrations, a higher

resting glucose-to-insulin ratio, and higher area under the curve concentrations for both

glucose and insulin.

Insulin resistance is a concern in human research as well because of its link to

type II diabetes mellitus. In an effort to manage insulin and glucose concentrations in

humans, Sierra et al. (2001) administered *isphaghula husk* (commonly referred to as

psyllium), a viscous water-soluble fiber obtained from the seed husk of *Plantago ovata*,

to healthy female patients. Mean glycemic values and glucose area under the curve were

lower in patients who received psyllium compared to those who did not (Sierra et al., 2001).   Mean serum insulin concentrations were lower 30 to 90 min after a meal in patients consuming psyllium compared to those who were not; furthermore, insulin area under the curve was 36% lower in patients who consumed psyllium (Sierra et al., 2001).

Psyllium is readily available to horse owners as a supplement for horses and is commonly used to treat and prevent sand colic because of its water-soluble and viscous nature. Research has not yet identified the metabolic response of a horse to psyllium. If horses respond to psyllium in the same manner as humans, psyllium could be used to manage blood glucose and insulin concentrations. This would be especially important in managing horses that are genetically predisposed to insulin resistance and ultimately predisposed to episodes of laminitis. The objective of this study was to determine the effects of psyllium supplemented to mature healthy horses on body mass and serum metabolites by measuring body weight, body condition score, neck circumference, tailhead fat, glucose, insulin, cortisol, leptin, ghrelin, and adiponectin.

CHAPTER 2

LITERATURE REVIEW

Psyllium

Psyllium is the common name for the seed husk of *Plantago ovata.* The psyllium

seed husk is water soluble and gelatinous in nature, and is the portion of the plant that is

commercially harvested and used as a dietary fiber. Psyllium is a major ingredient in

high fiber products and bulk laxatives.

Psyllium is commonly used in horses as a treatment and preventative for sand

colic. The psyllium seed husk forms a gel-like substance in the gastrointestinal tract that

helps the horse pass ingested sand and dirt entrapped in the large colon. Research

supports the use of psyllium in the treatment and prevention of sand colic (Hotwagner

and Iben, 2007; Landes et al., 2008), but the metabolic effects of feeding psyllium to

horses has not been documented.

Research has established metabolic and physical benefits of psyllium

supplementation to human patients (Sierra et al., 2001; 2002). When administered to

people, psyllium elicits a lowered glycemic response, improves glucose homeostasis,

decreases serum insulin concentrations, and increases insulin sensitivity. The ability to

administer an oral supplement and decrease glucose and insulin concentrations after a

meal is an important finding for the prevention and management of insulin resistance.

The prevalence of obesity, insulin resistance, and type II diabetes in human populations

of developed countries is rapidly rising. Horses and humans face the same epidemic:

obesity and insulin resistance. Human researchers have found that psyllium is useful in managing obesity and insulin resistance (Sierra et al., 2001; 2002), and if horses suffer from similar conditions it's possible that psyllium could help them as well.

Obesity in Horses

Obesity in horses has not been universally defined among equine researchers and practitioners, but it can be described as an excessive accumulation of adipose tissue in the body (Geor, 2008). It is estimated that 51% of horses in the United States are overweight (Thatcher et al., 2008). Obesity is important because it is the first visible sign that a horse could be developing insulin resistance. Obese horses are 80% less insulin sensitive (Hoffman et al., 2003) meaning they are much more likely to develop insulin resistance. Obesity appears to be an important contributing factor to insulin insensitivity in both horses and humans (Carter et al., 2008).   Regional adiposity is also linked to insulin insensitivity, or an insulin resistant condition. Horses that have enlarged fat deposits on the neck, thoracic, or tailhead regions, but have BCS less than 7, are predisposed to insulin resistance according to research by Geor (2008).

Insulin Resistance in Horses

Obesity and insulin resistance are two conditions that are closely inter-related; however it is possible to have one condition without the other. An estimated 10% of horses in the United States are insulin resistant (McGowan et al., 2008). Insulin resistance is generally defined as a state in which normal concentrations of insulin fail to

elicit a normal physiological response (Kahn, 1978). Insulin is responsible for maintaining glucose homeostasis in the body. Insulin resistance occurs when insulin receptors on cells within the body become insensitive, or resistant, to increasing insulin concentrations in the bloodstream. The insulin receptors do not sense and respond to insulin in the bloodstream as readily as they should.

After a normal, insulin sensitive, horse consumes a meal, blood glucose concentrations would increase as glucose is absorbed through the intestinal wall into the bloodstream. Beta cells in the pancreas would then secrete insulin in response to the elevated glucose concentrations. Insulin receptors on cells would recognize increased insulin concentrations and signal GLUT-4 transport proteins to allow glucose into the cell clearing it from the bloodstream. In an insulin resistant horse, the GLUT-4 transport protein would not be signaled in an efficient manner and blood glucose concentrations would remain elevated. The pancreas would secrete additional insulin in an effort to elicit a response from the insulin receptors until the GLUT-4 proteins are triggered to allow glucose into the cell. This series of events results in abnormally high blood glucose concentrations and a hyperinsulinemic condition. A review (McGowan, 2008) of recent research on endocrinopathic laminitis stated that hyperinsulinemia is a result of insulin resistance and may cause laminitis in horses; however the exact mechanisms still remain elusive.

Laminitis in Horses

Laminitis is defined as inflammation of the laminae within the hoof. When the laminae become inflamed dermal-epidermal lamellar separation occurs. In severe or prolonged cases the coffin bone rotates or sinks downward and in extremely severe cases can sink completely through the sole. Laminitis can be caused by varying situations such as a retained placenta or carbohydrate overload resulting in endotoxemia. Endocrinopathic laminitis, resulting from abnormal hormone secretion or function, has been a major focus in equine research over the last decade.

Obesity and insulin resistance have become major health concerns in the equine industry primarily due to their link with the development of laminitis (Frank et al., 2006). Laminitis is a painful and potentially career-ending disease that is thought to be a direct result of insulin resistance (McGowan 2007; Asplin et al., 2007a,b; Treiber et al., 2005). A survey of 113,000 horses in the United Kingdom estimated 7.1% of horses suffer from laminitis (Rendle, 2006).

A study using hoof explants proved that laminae within the hoof have an absolute requirement for glucose, and rapid dermal-epidermal separation occurs when glucose is deprived (Pass et al., 1998). One common theory for the cause of laminitis through insulin resistance was that adequate amounts of glucose were not being taken up by the laminar tissue. However, recent research concluded that GLUT-4 is not detectable in laminar tissue, and glucose uptake in lamellar explants occurs without insulin (Asplin et al., 2007a). The hoof explant study (Pass et al., 1998) initially leads one to conclude that laminitis is caused by glucose deprivation; however, insulin was not a factor in the

glucose transport into the cells. Therefore, GLUT-4 transport was not being used, yet glucose was still able to permeate the laminar cells within the hoof. There is another mechanism facilitating glucose transport into the cells. When glucose transport proteins within the hoof were examined, there was a predominance of insulin-independent GLUT-1 transport proteins, meaning glucose can passively permeate cells in the hoof and insulin is not needed (Asplin et al., 2007a). The theory that laminitis is caused by glucose deprivation has been disproved.

Insulin resistance is linked to laminitis, but it is not glucose deprivation that causes the onset, it is hyperinsulinemia. A landmark study by Asplin et al., (2007b) induced laminitis in 100% of ponies when exposed to high insulin concentrations (mean, 1290 ± 46 µIU/mL) while maintaining normal blood glucose concentrations (5.2 ± 0.1 mmol/L) using a modified euglycemic-hyperinsulinemic clamp technique. Hyperinsulinemia creates a toxic condition that stimulates laminitis; however, the physiologic mechanism is not yet fully understood. We now know that in order to prevent endocrinopathic laminitis we must prevent hyperinsulinemia which is a result of insulin resistance, which is linked with increases in fat mass and obesity.

<u>Measures of Fat Mass in Horses</u>

<u>Body Condition Score</u>

For the last quarter century, the standard method of assessing body fat mass in horses has been the body condition scoring system (BCS) set forth by Henneke et al. (1983). The Henneke scoring system assigns a subjective numerical grade on a scale

from 1 to 9 based on observable or palpable subcutaneous fat deposits. According to the Henneke system, a horse with BCS 7 (fleshy) would be considered overweight and horses in BCS 8 (fat) and 9 (extremely fat) might be considered obese (Geor, 2008). The subjective nature of this system and the inability to assess differences in fat mass deposits are problematic. Regional adiposity may actually be a better indicator of the potential for insulin resistance in horses than overall body fat (Geor, 2008). It seems that an objective, convenient, and repeatable method for measuring fat mass in horses is needed.

Neck Circumference

Measurements of neck circumference were adapted by Frank et al. (2006) in an effort to obtain an objective measure of the adipose tissue that accumulates along the neck, commonly termed "cresty neck." Horses are to be restrained so that the head and neck are maintained in a normal upright position, and the distance along a straight line from the poll to the cranial aspect of the withers is measured. Neck circumference is measured perpendicular to that line at 3 equidistant points between the poll and withers. Mean neck circumference values are then calculated. Although this seems to be a step in the right direction, the measurement is extremely variable based upon the position of the horse's head and neck. Even the slightest movement of the horses head shortens or lengthens the distance from poll to withers.

Tailhead Fat

Ultrasonic measurement of tailhead fat might be the most reliable and objective physical measurement of fat mass in horses. Subcutaneous fat measurements and BCS

are correlated best at the tailhead area (Gentry et al., 2004). Fat thickness is measured 10 cm lateral to the spine and 11 cm cranial to the tailhead origin (Kane et al., 1987). Tailhead fat is an objective physical assessment of fat mass in horses; it will be compared to BCS and metabolic assessments of fat mass (leptin, adiponectin, and ghrelin concentrations).

Leptin

Leptin is a hormone, secreted by adipocytes, that is positively associated with fat mass in horses (Kearns et al., 2005). Leptin is a 16 kDa protein product of the obese gene that acts as an indicator of energy balance (Zhang et al., 1994; 2002).   Leptin crosses the blood brain barrier and signals receptors in the hypothalamus to 1) decrease appetite; 2) increase energy expenditure by increasing metabolic rate; and 3) decreases energy storage by decreasing insulin secretion (Guyton and Hall, 2006). Basically, high circulating leptin increases energy expenditure while decreasing food intake and vice versa (Schwartz et al., 2000).

Humans can have mutations that prevent fat cells from producing leptin or cause defective leptin receptors in the hypothalamus; those people will have an increase in appetite, increased consumption of food, and morbid obesity (Guyton and Hall, 2006). Leptin resistance is thought to be associated with obesity when leptin receptors or signaling pathways are defective, causing obese people to eat even though there are high levels of leptin (Guyton and Hall, 2006). Leptin insensitivity and hyperleptinemia are related to obesity in horses much like they are related to obesity in humans (Radin et al., 2009).

One study concluded plasma leptin concentrations decrease in horses when an oral grain challenge is administered, but do not change when dextrose is infused intravenously (Gordon and McKeever, 2006). Another study reported that plasma leptin levels are lowered by a 24 h fasting period in horses (McManus and Fitzgerald, 2000). Leptin is secreted in proportion to fat mass (Maffei et al., 1995); therefore leptin may be useful as an objective measurement of body condition in horses.

Adiponectin

Adiponectin was first identified in 1996 (Maeda et al., 1996) as a 244 amino acid protein which is abundantly expressed in adipose tissue. Low adiponectin concentrations are related to insulin resistance and type II diabetes mellitus in humans (Díez and Iglesias, 2003) and increased adiponectin has insulin sensitizing effects (Berg et al., 2002). Plasma adiponectin concentrations in humans are negatively correlated with glucose, insulin, and body mass index (Díez and Iglesias, 2003).

Adiponectin concentrations are inversely related to fat mass in horses (Gordon et al., 2005), and are reported as having no change over a 240 min sampling period in response to an oral grain challenge or to an intravenous glucose challenge (Gordon and McKeever, 2006). Adiponectin also does not change in response to exercise in horses (Gordon et al., 2007).

High adiponectin was a stronger protective factor against development of type II diabetes than low waist circumference, fasting glucose, 2-h glucose or fasting insulin levels in humans (Lindsay et al., 2002), and low plasma adiponectin levels in humans are predictive of future development of insulin resistance and diabetes (Lindsay et al., 2002;

Daimon et al., 2003; Yamamoto et al., 2004). Adiponectin increases insulin sensitivity by stimulating phosphorylation of AMPK which promotes translocation of GLUT-4 to the cell surface to allow glucose uptake (Radin et al., 2009). Furthermore, activation of AMPK by adiponectin increases fatty acid oxidation (Radin et al., 2009).

Adiponectin is an important factor to consider because research suggests a critical link between this hormone and metabolic conditions associated with insulin resistance. Adiponectin concentrations could give insight to a horse's insulin resistant status before physical signs could be seen. Adiponectin is the most abundant adipose specific protein (Matsuzawa, 2005); therefore it could also be used as an objective assessment of fat mass and body condition in horses.

Ghrelin

Ghrelin is a satiety related hormone that is inversely correlated to body composition in horses (Gordon et al., 2005) and humans (Tschop et al., 2001). Ghrelin is a peptide hormone released mostly by the stomach, through oxyntic cells, and partly through the intestines (Guyton and Hall, 2006), but small amounts are also produced in the placenta, kidney, pituitary, and hypothalamus (Meier and Gressner, 2004).

Ghrelin secretion by the stomach activates GHS-Rs in the pituitary gland and GH-releasing neurons in the hypothalamus which stimulate the release of GH (Petersenn, 2002). The activation of GHS-Rs by ghrelin on the neurons in the hypothalamus stimulate food intake (Hagemann et al., 2003). Ghrelin receptors are also present in the heart and adipose tissue (Casanueva and Dieguez, 2002) suggesting ghrelin has many pathways in the body, not just stimulation by the digestive tract.

During fasting periods ghrelin concentrations increase but quickly decrease immediately after a meal. Ghrelin stimulates food intake in rodents and humans (Drazen and Woods, 2003) and is extremely influential in the regulation of energy homeostasis (Gualillo et al. 2003). Ghrelin increases hunger resulting in an increase in food intake; ghrelin also decreases fat oxidation which increases fat tissue and consequently increases BW. Ghrelin is inversely related to fat mass, so during an obese state ghrelin concentrations would be low.

Ghrelin concentration increases with meal anticipation or initiation in humans (Cummings et al., 2001; 2004) and exogenous ghrelin infusion increases feed intake in rats and humans (Wren et al., 2000; 2001a, b). Increases in ghrelin concentration with the initiation of feeding do not seem to occur in horses (Gordon and McKeever, 2006). Ghrelin was suppressed during both an intravenous and oral carbohydrate challenge suggesting ghrelin is inversely related to glucose and insulin concentrations in horses (Gordon and McKeever, 2006). Ghrelin is important in this study because it is related to glucose and insulin concentrations in horses and is involved in the control of appetite, food intake, and fat mass.

<center>Glucose Metabolism</center>

Glucose

Glucose is the final common pathway for transport of nearly all carbohydrates into tissue cells within the body (Guyton and Hall, 2006). Glucose is one of the body's main sources of energy and is essential for cell health and vitality. After consuming a

meal, carbohydrates are broken down to the monosaccharides glucose, fructose, and galactose. Glucose represents about 80% of those monosaccharides and much of the fructose and galactose are converted to glucose in the liver, so more than 95% of circulating monosaccharides are glucose (Guyton and Hall, 2006).

The amount of glucose that can passively diffuse to the insides of cells is generally not sufficient for energy metabolism. Insulin, which is secreted by the pancreas, increases the rate of transport of glucose into the cell by 10 times (Guyton and Hall, 2006); therefore the rate at which cells utilize carbohydrates is highly dependent upon the amount of insulin secreted.

Average normal fasting plasma glucose concentration in horses is estimated at 86.8 mg/dL (June et al., 1992), and can increase 56% after oral carbohydrate administration and 432% after intravenous dextrose administration (Gordon and McKeever, 2006). The average fasting glucose concentration in horses (n = 61) estimated by the Montana State University Animal Science Physiology Lab is 90.0 ± 8.6 mg/dL.

Insulin

Insulin is a small protein that functions as part of the feedback system controlling blood glucose concentration (Dickson, 1970). Insulin is synthesized by the ß-cells of the pancreas and is responsible for facilitating glucose uptake by cells in the body for energy use or storage. At rest, insulin is responsible for the transport of glucose into the cell; however during exercise muscles are capable of taking up glucose without insulin (Willmore and Costill, 1994).

Insulin stimulation has many effects within the body. Within seconds after insulin receptors sense insulin in the bloodstream, 80% of the cells in the body increase their uptake of glucose and cell membranes become more permeable to many amino acids, potassium ions, and phosphate (Guyton and Hall, 2006). Excess carbohydrates that cannot be stored as glycogen are converted under the stimulus of insulin into fats and stored in the adipose tissue (Guyton and Hall, 2006). Insulin promotes fat synthesis and storage by increasing the use of glucose by tissues throughout the body, which decreases utilization of fat (Guyton and Hall, 2006). Insulin also promotes the synthesis of fatty acids in the liver which are used to form triglycerides (the usual form of fat storage) when excess carbohydrates are ingested (Guyton and Hall, 2006).

In the absence of insulin the body works in reverse by resorting to fat stores for energy. The enzyme *hormone-sensitive lipase* is activated which causes hydrolysis of stored triglycerides, releasing large amounts of fatty acids and glycerol into the circulating blood (Guyton and Hall, 2006). The free circulating fatty acids are then used by all body tissues (except the brain) for energy. The liver converts some circulating fatty acids into phospholipids and cholesterol which, along with excess triglycerides, are discharged into the blood in lipoproteins (Guyton and Hall, 2006). High lipid concentration in the blood, especially cholesterol, promotes the development of atherosclerosis in people with serious diabetes (Guyton and Hall, 2006).

Insulin concentrations in horses can increase 802% in response to an oral carbohydrate load and 395% in response to an intravenous dextrose challenge according to one study by Gordon and McKeever (2006). Actual serum insulin concentrations can

vary between labs; the average fasting serum insulin concentration in horses (n = 61)

assayed by the Montana State University Animal Science Physiology Lab is 5.5 ± 1.8

μIU/mL.

Increasing insulin sensitivity in the body is a key factor for preventing insulin

resistance and hyperinsulinemia. Simply put, increasing insulin sensitivity is a matter of

decreasing the amount of insulin needed to elicit a response by the insulin receptors.

CHAPTER 3

STATEMENT OF THE PROBLEM

Laminitis is a painful and potentially career ending condition in horses that weakens the dermal-epidermal union within the hoof. Recent research (Asplin et al., 2007) suggests that extremely high concentrations of insulin (hyperinsulinemia) in the body may cause laminitis. Hyperinsulinemia is a result of insulin resistance, a condition that nearly 10% (McGowan et al., 2008) of horses in the United States suffer. Insulin resistance and obesity are strongly inter-linked, each exasperating the other, and ultimately have the potential to cause laminitis (Frank et al., 2006).

In an effort to prevent laminitis one must eliminate the hyperinsulinemic situation. Hyperinsulinemia is caused by insulin insensitivity which is a result of ongoing insulin resistance and obesity. In order to avoid laminitis it is essential to prevent obesity and insulin resistance, which can be accomplished by lowering blood glucose and insulin concentrations and reducing fat mass in the horse.

Human research (Sierra et al., 2001; 2002) reported that oral psyllium supplementation reduced the blood glucose and insulin response after a meal and reduced body weight in patients. Psyllium is a marketed plant product that is readily available to horse owners because it is commonly fed as a preventative for sand colic. If horses react to psyllium in the manner that humans do, it could be used as a preventative for laminitis by decreasing blood glucose and insulin concentrations as well as reducing body weight.

The goals of this research were: 1) to determine if feeding a daily dose of psyllium would suppress plasma glucose and insulin concentrations in normal horses, 2) to determine if psyllium fed daily to normal horses would decrease physical measurements of body weight or fat mass, 3) to determine if psyllium would alter concentrations of fat related hormones such as leptin, adiponectin, and ghrelin, and 4) to determine what dose of psyllium would elicit the greatest physical and/or metabolic response.

CHAPTER 4

MATERIALS AND METHODS

Trial 1 (2008)

Objective & Hypotheses

The objective of trial 1 was to determine the effects of supplemental psyllium fed to mature quarter horses not in training on body mass and serum metabolites. Hypothesis (H_o): horses supplemented with psyllium or an isocaloric control will not differ in: body mass or serum metabolites.

Horse Selection

Procedures were approved by the Montana State University Institutional Animal Care and Use Committee (protocol #AA-048). Eight (4 mares and 4 geldings) 11- to 16-yr-old Quarter Horses (535.8 ± 25.3 kg initial BW) with BCS of 5 or 6 out of 9 (Henneke et al., 1983), were used in the trial. All horses had the same sire and were selected from the Montana State University equitation herd. Horses were first stratified by sex and BW then randomly assigned to 1 of 2 treatment groups.

Treatment

Horses were allowed a 12-d adaptation period to hay and grain (Table 1). During the following 42 d supplementation period, horses were individually fed a mixed grain (corn, oats, barley and molasses) ration at 0.5% BW, grass/alfalfa hay at 1.5% BW, and 1 of 2 treatment supplements. Treatments were: 90 g·horse^{-1}·d^{-1} psyllium pellets (90G) or

an isocaloric grain based control supplement (0G). The amount of psyllium fed was
extrapolated from the dose given to human patients in a similar study by Sierra et al.
(2002) and was consistent with the Psyllium EQ® (Vetri-Science Laboratories; Essex
Junction, Vermont) label recommendations for prevention of sand colic in horses.
Treatments were administered June 13, 2008 to July 25, 2008. Horses were allowed *ad*
libitum access to clean water and a plain white salt block. All feed was consumed by
every horse each day throughout the study. Horses were housed in individual 4 x 7 m
outdoor runs with shelter and allowed a 5 h turnout period each day into a larger dry lot
pen.

Table 4.1. Feed analysis[1] of hay[2], grain[3], and psyllium[4]

Item	Hay	Grain	Psyllium
DM, %	94.4	94.1	94.0
CP, %	11.1	14.8	12.5
ADF, %	25.9	8.1	11.4
NDF, %	55.2	-	-
Starch, %	2.0	44.8	17.2
Sugars, %			
Water soluble	7.8	4.4	7.2
Ethanol soluble	2.0	3.0	2.2
NSC,[a] %	28.8	-	-
Crude fat, %	0.9	3.9	3.7
Ash, %	8.9	7.5	7.3
DE, Mcal/kg	2.6[b]	3.9[c]	3.7[c]

[1] Analyzed by O.O. Thomas Nutrition Center, Bozeman, MT and Equi-Analytical
Laboratories, Ithaca, NY.
[2]Grass/alfalfa mixed hay.
[3]Comprised of corn, oat, barley, and molasses.
[4]Psyllium seed husk, manufactured by Vetri-Science Laboratories of Vermont.
[a]Nonstructural carbohydrate (NSC = 100 – CP – NDF – crude fat – ash).
[b] DE, Mcal/kg = $4.22 – 0.11(\%ADF) + 0.0332(\%CP) + 0.00112(\%ADF^2)$; NRC, 2007.
[c] DE, Mcal/kg = $4.07 – 0.055(\%ADF)$; NRC, 2007.

Data Collection

Measurements of BW, tailhead fat, neck circumference, and plasma leptin were taken on d 0 and 42. Blood samples were collected for determination of plasma glucose and serum insulin concentrations on d 42. For blood collections, indwelling jugular catheters were placed at least 2 h prior to the first blood draw which began at 0730 (30 min before feeding) and continued every 30 min for 6 h. Horses were kept in individual pens and left undisturbed with the exception of blood collection. The feed used during blood collection was the same treatment protocol described in the treatment section and every horse consumed all feed.

Sample Analysis

Body weight was measured with an electronic scale (Tru-Test AG 500; Tru-Test, Te Kuiti, New Zealand). Body condition scoring was performed by three independent, trained technicians and determined by palpation of body fat using the Henneke et al. (1983) rating system. Tailhead fat thickness was measured ultrasonically by a multi-frequency linear array transducer (Sonovet 600; Universal Ultrasound, Bedford Hills, New York) 10 cm lateral to the sacral spinous processes and 11 cm cranial to the tailhead origin (Kane et al., 1987). Mean neck circumference was calculated using 3 equidistant measurements between the poll and withers with horses restrained in stocks and the head held in a normal upright position (Frank et al., 2006).

Blood samples were collected into BD Vacutainer® tubes (Franklin Lakes, New Jersey, USA) containing sodium fluoride and potassium oxalate for analysis of plasma glucose and leptin and into Monoject™ tubes (Tyco Healthcare Group LP; Mansfield,

Massachusetts, USA) without additives for analysis of serum insulin and cortisol. Within

1 min of collection, vacutainers were placed on ice. Samples were centrifuged (1,600 x

g) at 4 ºC, and plasma and serum were decanted and stored at -20ºC. Glucose analysis

was performed in duplicate using a spectrophotometric method based on glucose

hexokinase (Glucose hexokinase kit; Sigma Diagnostics, St. Louis, MO). The intra- and

interassay CV were 7.7 and 8.4%, respectively, for a pool of plasma that contained a

mean of 113.6 mg/dL glucose. The low pool plasma contained a mean of 36.3 mg/dL

and the intra- and interassay CV were 6.6 and 12.8%, respectively. The sensitivity of the

assay for glucose was 20 mg/dL. Serum insulin and cortisol were determined in

duplicate by use of commercially available solid phase radioimmunoassay kits (Coat-a-

Count Diagnostics, Los Angeles, CA) previously validated for use in horses (Freestone et

al., 1991). The intra- and interassay CV were 20 and 22%, respectively, for serum insulin

concentrations of 16 µIU/mL, and limit of detection was 3.3 µIU/mL. Plasma leptin

concentrations were determined using a commercially available mulit-species

radioimmunoassay (Multi-Species Leptin RIA kit; Linco Research, St. Charles, MO) that

had been validated for measuring leptin in equine serum and plasma (Fitzgerald and

McManus, 2000). Samples were run in duplicate. The intra-assay CV for leptin high and

low pools were 0.76 and 0.31%, respectively. In the absence of purified equine leptin,

results are expressed as human equivalents of immunoreactive leptin. The kit utilized

[125]I-labeled human leptin with a specific activity of 135 µCi/µg, a guinea pig anti-leptin

serum, and a goat anti-guinea pig IgG serum in the precipitating reagent. Samples were

run in duplicate and counted for 1 min in a gamma counter (Cobra Auto-Gamma,

Meriden, CT). Data from the manufacturer indicated a specificity of 100% for human, 67% porcine, 61% rat, 73% mouse, and 3% canine leptin.

Hay, grain, and psyllium were analyzed for nutrient composition (O.O. Thomas Nutrition Center, Bozeman, MT). Starch and sugar content were also analyzed (Equi-Analytical Laboratories, Ithaca, NY). Sugar content was analyzed as water soluble carbohydrate and ethanol soluble carbohydrate. Non-structural carbohydrate for the hay was calculated with the following equation:

NSC= 100 - CP - NDF - crude fat - ash

Digestible energy was calculated with the following equations (NRC, 2007):

For hay: DE, Mcal/kg = 4.22- 0.11(%ADF) + 0.0332(%CP) + 0.00112(%ADF^2)

For grain and psyllium: DE, Mcal/kg = 4.07 - 0.055(%ADF)

Analyzed and calculated nutrient composition of feeds is presented in Table 4.1.

Statistical Analysis

Each horse was considered an experimental unit. Plasma glucose and serum insulin concentrations were analyzed using a mixed model with repeated measures with treatment, time, and the interaction of treatment and time as fixed effects, horse as random effect, and compound symmetry as the covariance structure (SAS Inst. Inc., Cary, NC). Areas under the concentration-time curve were calculated by the trapezoidal method (Gibaldi and Perrier, 1982) for plasma glucose and serum insulin and were analyzed by ANOVA of SAS. Changes in plasma leptin concentrations, neck circumference, BW, and tailhead fat between d 0 and 42 were also analyzed using

ANOVA of SAS. Least square means were separated using the LSD method when $P <$ 0.10.

Trial 2 (2009)

Objective & Hypotheses

The objective of trial 2 was to determine the effects of different levels of supplemental psyllium fed to mature light breed stock horses not in training on body mass and serum metabolites. Three hypotheses were used to test this objective. Hypothesis 1 (H_{o1}): horses supplemented with 90 g psyllium or an isocaloric control will not differ in: body mass or serum metabolites. Hypothesis 2 (H_{o2}): horses supplemented with 180 g psyllium or an isocaloric control will not differ in: body mass or serum metabolites. Hypothesis 3 (H_{o3}): horses supplemented with 270 g psyllium or an isocaloric control will not differ in: body mass or serum metabolites.

Horse Selection

Procedures were approved by the Montana State University Institutional Animal Care and Use Committee (protocol #AA-048). Sixteen (8 mares and 8 geldings) 7- to 16-yr-old Paint and Quarter Horses (515.3 ± 37 kg initial BW) with BCS between 4.5 and 5.5 out of 9 (Henneke et al., 1983), were used in the trial. All horses were selected from the Montana State University equitation herd. Horses were first stratified by sex and BW then randomly assigned to 1 of 4 treatment groups.

Treatment

Horses were allowed a 16-d adaptation period to pen assignment, hay and grain (Table 2). During the following 60 d supplementation period, horses were individually fed a mixed grain (corn, oats, barley and molasses) ration at 0.25% BW, grass/alfalfa hay at 1.5% BW, and 1 of 4 treatment supplements. Treatments were: 1) 0 g·horse^{-1}·d^{-1} psyllium, 2) 90 g·horse^{-1}·d^{-1} psyllium, 3) 180 g·horse^{-1}·d^{-1} psyllium or 4) 270 g·horse^{-1}·d^{-1} psyllium. Psyllium was fed in pellet form and obtained from Vetri-Science Laboratories (Essex Junction, Vermont). The amount of psyllium fed was extrapolated from the dose given to human patients in a similar study by Sierra et al. (2002) and then doubled and tripled. Treatments were administered June 11, 2009 to August 11, 2009. Horses were allowed *ad libitum* access to clean water and a plain white salt block. All feed was consumed by every horse each day throughout the study. Horses were placed in individual 4 x 7 m pens long enough to consume entire meal. When all horses had finished all feed they were turned out into a dry lot with access to water and salt only.

Table 4.2. Feed analysis[1] of hay[2], grain[3], and psyllium[4]

Item, %	Hay	Grain	Psyllium
DM	92.2	93.4	94.0
CP	8.5	16.2	11.7
ADF	35.9	8.2	11.0
NDF	57.1	15.2	-
Starch	0.4	34.7	16.1
Sugar			
WSC[5]	14.2	8.6	6.7
ESC[5]	5.4	2.8	2.1
NSC	17.4	53.0	-
Ash	8.4		6.8
DE, Mcal/kg[6]	1.91	3.3	3.5

[1]Analyzed by Equi-Analytical Laboratories, Ithaca, NY.
[2]Grass/alfalfa mixed hay.
[3]Comprised of corn, oat, barley, and molasses.
[4]Psyllium seed husk, manufactured by Vetri-Science Laboratories of Vermont.
[5]WSC = water soluble carbohydrate; ESC = ethanol soluble carbohydrate.
[6]DE, Mcal/kg = 4.07 - 0.055(%ADF).

Data Collection

Measurements of BW, tailhead fat, and neck circumference as well as plasma leptin, adiponectin, and ghrelin were taken on d 0 and 60. Blood samples were collected for determination of plasma glucose and serum insulin concentrations on d 60. For d 60 blood collections, indwelling jugular catheters were placed the evening prior to the first blood draw which began at 0700 and continued every 30 min for 6 h. Horses were kept in individual pens and left undisturbed with the exception of blood collection and morning feeding, which took place between 0700 and 0730. The feed used during blood collection was the same treatment protocol described in the treatment section and every horse consumed all feed on the blood draw day.

<u>Sample Analysis</u>

Body weight was measured with an electronic scale (Tru-Test AG 500; Tru-Test, Te Kuiti, New Zealand). Body condition scoring was performed by four independent, trained technicians and determined by palpation of body fat using the Henneke et al. (1983) rating system. Tailhead fat thickness was measured ultrasonically by a multi-frequency linear array transducer (Sonovet 600; Universal Ultrasound, Bedford Hills, New York) 10 cm lateral to the sacral spinous processes and 11 cm cranial to the tailhead origin (Kane et al., 1987). Neck circumference was calculated as the mean of 3 equidistant measurements between the poll and withers with horses restrained in stocks and the head held in a normal upright position (Frank et al., 2006).

Blood samples were collected into BD Vacutainer® tubes (Franklin Lakes, New Jersey, USA) containing sodium fluoride and potassium oxalate for analysis of plasma glucose, into Monoject™ tubes (Tyco Healthcare Group LP; Mansfield, Massachusetts, USA) containing 15% EDTA for leptin, ghrelin, and adiponectin, and into Monoject™ tubes without additives for analysis of serum insulin and cortisol. Samples were centrifuged (1,600 x g) at 4 ℃, and plasma and serum were decanted and stored at -20℃. Blood samples intended for analysis of ghrelin were acidified with 50 μl of 1 N HCl and 10 μl of PMSF (phenylmethylsulfonly fluoride) per one mL of plasma prior to freezing.

Glucose analysis was performed in duplicate using a spectrophotometric method based on glucose hexokinase (Glucose hexokinase kit; Sigma Diagnostics, St. Louis, MO). The intra- and interassay CV were 7.0 and 6.0 %, respectively, for a pool of plasma that contained a mean of 90.5 mg/dL glucose. The low pool plasma contained a

mean of 47.7 mg/dL and the intra- and interassay CV were 7.8 and 10.0 %, respectively.

The sensitivity of the assay for glucose was 20 mg/dL. Serum insulin and cortisol were

determined in duplicate by use of commercially

available solid phase radioimmunoassay kits (Coat-a-Count Diagnostics, Los Angeles,

CA) previously validated for use in horses (Freestone et al., 1991). The intra- and

interassay CV were 9.4 and 13.9 %, respectively, for a pool of serum that contained a

mean of 191.3 µIU/mL insulin. The low pool serum contained a mean of 5.6 µIU/mL

insulin and the intra- and interassay CV were 31.8 and 48.0 %, respectively. The

sensitivity of the assay for insulin was 6.1 µIU/mL. The intra- and interassay CV were

7.8 and 33.8%, respectively, for a pool of serum that contained a mean of 62.3 ng/mL

cortisol. The low pool serum contained a mean of 27.8 ng/mL insulin and the intra- and

interassay CV were 6.3 and 20.1%, respectively. The sensitivity of the assay for cortisol

was 1.95 ng/mL.   Serum concentrations of leptin were determined in triplicate and were

quantified by using a competitive liquid-liquid phase, double-antibody leptin RIA

procedure described previously (Delavaud et al., 2000). The intra and inter assay

coefficients of variation were 2.01% and 3.22%, respectively.

Plasma active ghrelin and adiponectin concentrations were determined using

commercially available radioimmunoassay kits (Linco Research/Millipore). The active

ghrelin kit was previously validated for use in horses (Gordon and McKeever, 2005).

Linearity and parallelism were established using horse plasma to validate partially the

adiponectin kit used in this study. Parallelism of the adiponectin assay kits were

established using a serial dilution of horse plasma and the adiponectin (Figure 4.1)

standard from the assay kit. In the absence of purified equine active ghrelin and adiponectin, results are expressed as human equivalents of immunoreactive ghrelin and adiponectin. Samples were run in duplicate and counted for 1 min in a gamma counter (Cobra Auto-Gamma, Meriden, CT). The active ghrelin kit utilized ^{125}I-labeled ghrelin with a specific activity of 302 µCi/µg, a guinea pig anti-ghrelin serum, and a goat anti-guinea pig IgG serum in the precipitating reagent. Data from the manufacturer indicated a specificity of 100% for human, rat, and canine active ghrelin and $< 0.1\%$ for des-octanoylghrelin (total ghrelin). The the intra- and interassay CV was 24.9 and 25.7 % respectively for a pool of serum that contained 13.3 pg/mL active ghrelin. The sensitivity of the assay for active ghrelin was 7.3 pg/mL. The adiponcectin kit utilized ^{125}I-labeled adiponectin with a specific activity of 67.7 µCi/µg, 30% normal rabbit serum, and goat anti-rabbit IgG serum in the precipitating reagent. Data from the manufacturer indicated a specificity of 400% for mouse adiponectin and $< 0.01\%$ for human C1q. The the intra- and interassay CV was 1.4 and 10.4% respectively for a pool of serum that contained 29.5 ng/mL adiponectin. The low pool serum contained a mean of 19.4 ng/mL adiponectin and the intra- and interassay CV were 5.1 and 10.0%, respectively. The sensitivity of the assay for adiponectin was 0.78 ng/mL.

Hay and grain were analyzed for nutrient composition, starch, and sugar content (Equi-Analytical Laboratories, Ithaca, NY). Sugar content was analyzed as water soluble carbohydrate and ethanol soluble carbohydrate. Analyzed and calculated nutrient composition of feeds is presented in Table 4.2.
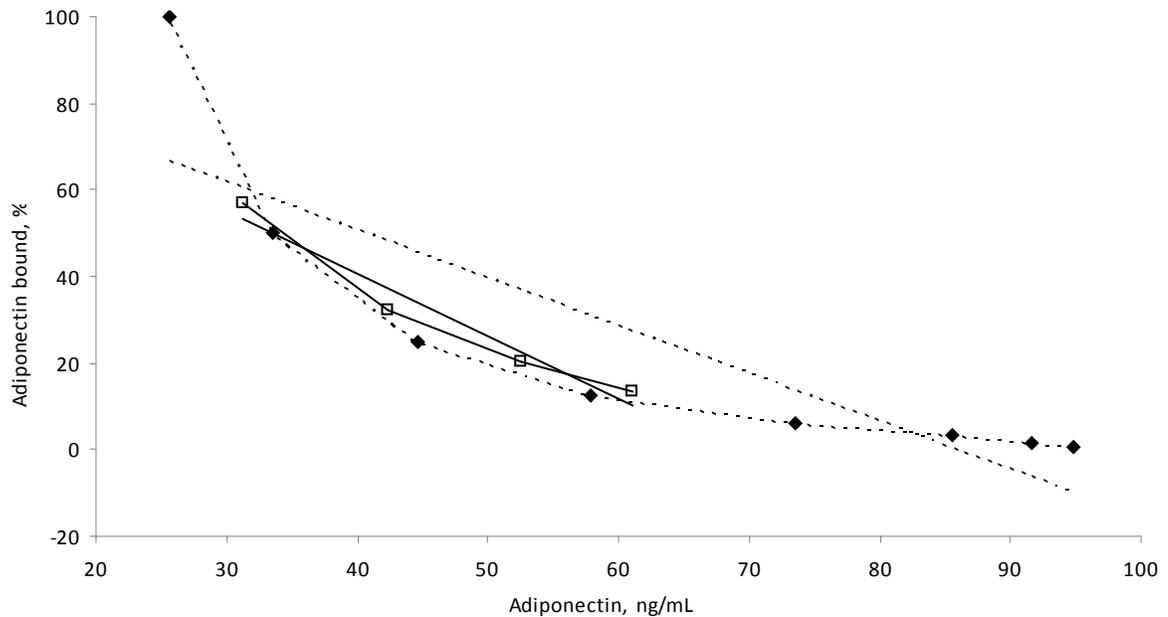
Figure 4.1. Dose response curves using adiponectin standard (closed diamonds) and equine plasma (open sqares). Plasma samples of 25, 50, 100, and 200 μL were used to generate each point on the graph. Linear regression lines were fit to the adiponectin standard (dashed line) and equine plasma (solid line).

Statistical Analysis

Each horse was considered an experimental unit. Serum glucose, insulin, and cortisol concentrations were analyzed using a mixed model with repeated measures. Treatment, time, and the interaction of treatment and time were set as fixed effects, horse as random effect, and compound symmetry as the covariance structure (SAS Inst. Inc., Cary, NC). Changes between d 0 and 60 for plasma leptin, ghrelin, and adiponectin concentrations, BW, BCS, neck circumference, and tailhead fat were analyzed using ANOVA of SAS. Least square means were separated using the LSD method when $P < 0.10$.

Trial 3 (2009) – Glucose Challenge

Objective & Hypotheses

The objective of trial 3 was to determine the effects of different levels of supplemental psyllium fed to mature light breed stock horses not in training on metabolic response to an intravenous glucose load compared to an oral glucose load. Three hypotheses were used to test this objective. Hypothesis 1 (H_{o1}): horses supplemented with 90 g psyllium or an isocaloric control will not differ in clearance of an intravenous glucose load. Hypothesis 2 (H_{o2}): horses supplemented with 180 g psyllium or an isocaloric control will not differ in clearance of an intravenous glucose load. Hypothesis 3 (H_{o3}): horses supplemented with 270 g psyllium or an isocaloric control will not differ in clearance of an intravenous glucose load.

Horse Selection

All horses from Trial 2 were used for Trial 3. Trial 2 lasted 60 d and Trial 3 took place on d 61. Procedures were approved by the Montana State University Institutional Animal Care and Use Committee (protocol #AA-048).

Treatment

All horses were treated as described in Trial 2 from June 11, 2009 to August 11, 2009. On, August 12, 2009, a glucose challenge was performed on each horse. Horses were administered an intravenous dose of dextrose at 0.5% BW. Horses were withheld from hay, grain, and psyllium for 12 h prior to the glucose challenge and were not

allowed to eat until blood sampling had finished. Horses remained in their respective pen during the challenge.

Data Collection

Blood samples were collected for determination of serum glucose, insulin, corisol, leptin, ghrelin, and adiponectin concentrations. Indwelling jugular catheters that had been placed the day prior were used for blood sampling. The first blood sample took place at 0900 h and dextrose was infused immediately after. Sampling continued at 5, 15, 30, 60, 90, 120, 180, and 240 min after dextrose infusion. Horses were kept in individual pens and left undisturbed with the exception of blood collection. Horses were allowed access to water but not hay or grain during data collection.

Sample Analysis

Blood samples were collected into BD Vacutainer® tubes (Franklin Lakes, New Jersey, USA) containing sodium fluoride and potassium oxalate for analysis of plasma glucose, into Monoject™ tubes (Tyco Healthcare Group LP; Mansfield, Massachusetts, USA) containing 15% EDTA for leptin, ghrelin, and adiponectin, and into Monoject™ tubes without additives for analysis of serum insulin and cortisol. Samples were centrifuged (1,600 x g) at 4 ºC, and plasma and serum were decanted and stored at -20ºC. Blood samples intended for analysis of ghrelin were acidified with 50 μl of 1 N HCl and 10 μl of PMSF (phenylmethylsulfonly fluoride) per one mL of plasma prior to freezing.

Serum samples were analyzed with the samples collected on d 60 (Trial 2), therefore all procedures, assay kits, and percent CV are the same as reported previously in Trial 2.

Statistical Analysis

Each horse was considered an experimental unit. Serum glucose, insulin, cortisol, leptin, ghrelin, and adiponectin concentrations were analyzed using a mixed model with repeated measures. Treatment, time, and the interaction of treatment and time were set as fixed effects, horse as random effect, and compound symmetry as the covariance structure (SAS Inst. Inc., Cary, NC). Least square means were separated using the LSD method when $P < 0.10$.

CHAPTER 5

RESULTS AND DISCUSSION

Trial 1- 2008 Results:

Glucose

There was no treatment or treatment by time interaction for glucose

concentrations on d 42 (Figure 5.1). The mean glucose concentration for a 6 h

postprandial period was 99.5 mg/dL for horses fed psyllium and 97.8 mg/dL for horses

not fed psyllium.

Figure 5.1. Mean plasma glucose concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line). Effects of treatment and treatment x time ($P \geq 0.34$). SEM = 7.02.

Insulin

Insulin concentrations 6 h after a meal did not differ between treatments, but there was a tendency for a treatment by time interaction ($P = 0.09$; Figure 5.2). This appeared to be caused by an increase in insulin concentrations from 60-120 min after feeding in horses fed psyllium, whereas, insulin concentrations decreased during this time in horses not fed psyllium. Insulin concentration tended to be greater ($P = 0.10$) at 120 min after feeding for horses fed psyllium (92.7 µIU/mL) compared with horses not fed psyllium (56.6 µIU/mL; Figure 5.2). The mean insulin concentration for a 6 h postprandial period was 45.4 µIU/mL for horses fed psyllium and 33.0 µIU/mL for those not fed psyllium.
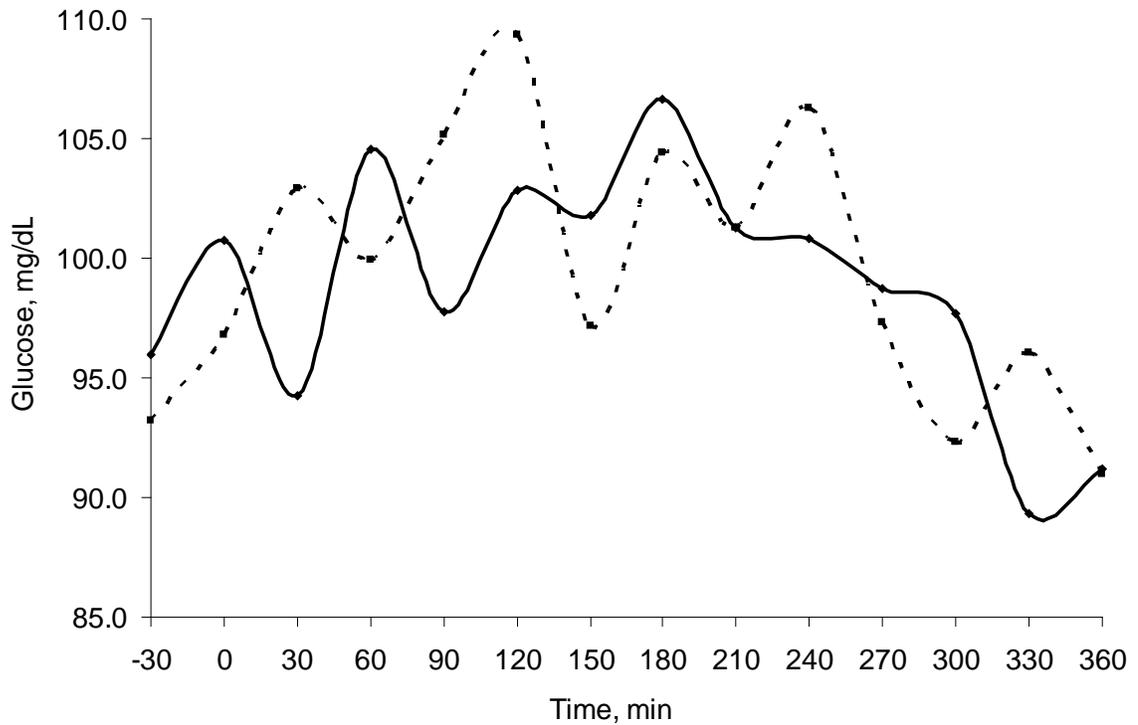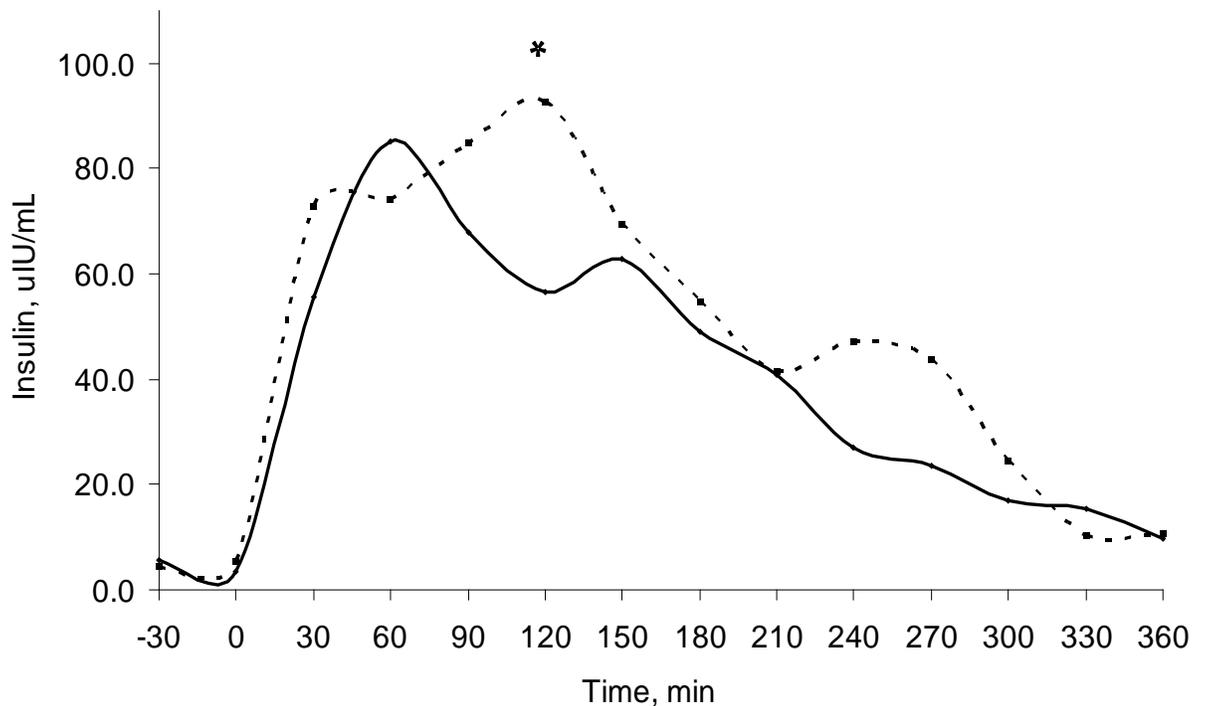

Figure 5.2. Mean serum insulin concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line). Effect of treatment x time ($P = 0.09$). SEM = 21.85. Asterisks indicate a tendency in difference between means at 120 min ($P = 0.10$).

Leptin and Body Mass

The change in leptin concentration between d 0 and 42 was not different between horses supplemented with psyllium and those that were not (Table 5.1). Change in BW, BCS, and tailhead fat did not differ between treatments, but there was a tendency for BW and BCS to increase (Table 5.1). Neck circumference increased from d 0 to d 42 in horses for both treatments, however, there was a greater increase ($P = 0.01$) in neck circumference of horses fed psyllium than horses not fed psyllium (Table 5.1).

Table 5.1. Changes (d 0 to 42) in fasting plasma leptin concentration, BW, BCS, neck circumference, and tailhead fat for mature Quarter Horses fed 0g and 90g psyllium[1]

Variable	0g Psyllium	90g Psyllium	SEM	P-value
n	4	4		
Leptin, ng/mL	-0.1	-0.4	1.20	0.74
BW, kg	19.2	27.0	13.48	0.12
BCS	0.45	0.89	0.11	0.10
Neck circumference, cm	1.1	2.8	0.27	0.01
Tail-head fat, mm	1.9	2.4	1.95	0.73

[1]Changes in these variables were calculated as the difference between measurements taken on d 0 and 42.

Trial 1- 2008 Discussion

In this trial, there was a tendency in horses fed psyllium to have delayed and higher peak serum insulin concentrations from 60-120 min after a meal. These results contrast with human psyllium research (Sierra et al., 2001) which reported lower glucose and insulin concentrations after a meal. The psyllium seed husk is water-soluble and forms a gel-like substance in the intestines. Therefore, it may have slowed the passage

rate of ingesta through the small intestine delaying glucose absorption and the resulting insulin response.

All horses in this trial gained weight. It is known that obesity and insulin resistance are interrelated (Geor, 2008). Horses in both treatments gained weight and increased body condition score, but the horses that were fed psyllium had the higher gains and increases. All horses started the trial with a body condition score between 5 and 6 out of 9 (Henneke et al., 1983), and horses in both treatments increased in body condition by the end of the trial. This suggests fat mass was increasing in the horses. There were also increases in tailhead fat and neck-circumference, both places of regional adiposity that are characteristic of horses prone to developing insulin resistance (Kane et al., 1987; Frank et al., 2006). The trial consisted of only 4 horses in each treatment which means one or two horses could skew the results dramatically. Some horses have a genetic predisposition to developing insulin resistance that phenotype may not appear until the horse starts to gain fat mass (Geor, 2008). It's possible that some horses in the psyllium fed group may have had a genetic predisposition to developing insulin resistance that was not evident prior to the trial. When the group started to gain fat mass, those particular horses may have become less insulin sensitive which would explain the higher glucose and insulin concentrations in the psyllium treated group.

It may be a more likely assumption that the 42 d trial period was simply not long enough, or that the 90 g psyllium dose was not high enough to see metabolic effects in normal horses like researchers have found in human patients (Sierra et al., 2001; 2002).

Trial 2- 2009 Results:

Glucose

On d 60, horses not fed psyllium had higher ($P < 0.01$) mean blood glucose concentrations (95.0 mg/dL ± 4.7) than horses that were fed psyllium. Mean blood glucose concentrations were similar ($P = 0.48$) in horses supplemented with psyllium at 90 g (87.5 mg/dL ± 4.7), 180 g (89.6 mg/dL ± 4.7), or 270 g (89.3 mg/dL ± 4.7). Since there was no difference in psyllium treatment level, all psyllium treatments were averaged together so that horses not fed psyllium (n = 4) could be compared to horses that were fed psyllium (n = 12).

Blood glucose concentration had a treatment by time interaction ($P < 0.001$) during the postprandial period (Figure 5.3). Ninety minutes after feeding, blood glucose concentrations were higher in horses not fed psyllium compared to horses that were fed psyllium (101.0 ± 22.0 versus 88.6 ± 7.6 mg/dL; Figure 5.3). Glucose was higher ($P < 0.01$) in horses not fed psyllium 120 min after feeding than in horses that were fed psyllium (123.4 ± 21.0 versus 85.1 ± 5.1 mg/dL; Figure 5.3).

Sex had no effect on the results. Mean blood glucose concentration on d 60 was similar for mares and geldings (90.25 mg/dL ± 16.0 versus 90.24 mg/dL ± 14.5). There was no difference in mean blood glucose concentrations between sex ($P = 0.96$), treatment ($P = 0.63$), or the sex by treatment interaction ($P = 0.41$) on d 60.

Figure 5.3. Mean plasma glucose concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line). Effect of treatment x time ($P < 0.001$). SEM = 4.73. Asterisks indicate a difference between means at 90 min ($P = 0.05$) and 120 min ($P < 0.001$) after feeding.

Insulin

On d 60, horses not supplemented with psyllium had higher ($P < 0.01$) mean insulin concentrations (24.3 µIU/mL ± 2.9) than horses that were supplemented with 90 g (17.4 µIU/mL ± 2.8), 180 g (18.7 µIU/mL ± 2.9), or 270 g (14.3 µIU/mL ± 2.8) psyllium. The mean insulin concentration in non-psyllium supplemented horses was 23.8 µIU/mL ± 17.1 and the mean of all three psyllium treatments combined was 16.7 µIU/mL ± 14.2. Mean insulin concentration was similar ($P = 0.15$) among psyllium treatment levels of 90, 180, and 270 g; therefore, the 3 psyllium treatments were averaged together so that horses not fed psyllium (n = 4) could be compared to horses that were fed psyllium (n = 12).

After 60 d of psyllium supplementation, there was a difference in insulin concentrations between treatments ($P = 0.04$) and in the treatment by time interaction ($P = 0.03$) in blood sampled over a 6 h period of time after feeding (Figure 5.4). Ninety minutes after feeding, insulin concentrations were higher ($P < 0.01$) in horses not fed psyllium (50.3 µIU/mL ± 12.8) compared to horses that were fed psyllium (23.9 µIU/mL ± 13.5). Insulin was higher ($P < 0.01$) in horses not fed psyllium (33.9 µIU/mL ± 11.0) 300 min after feeding than horses that were fed psyllium (10.5 µIU/mL ± 0.7).
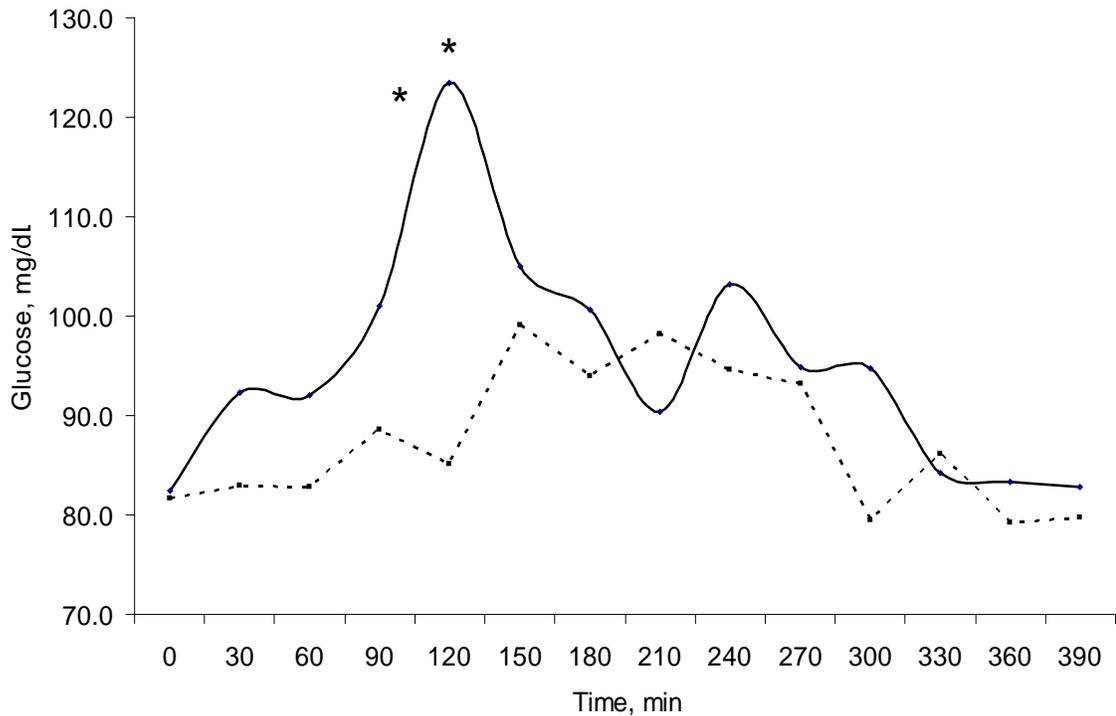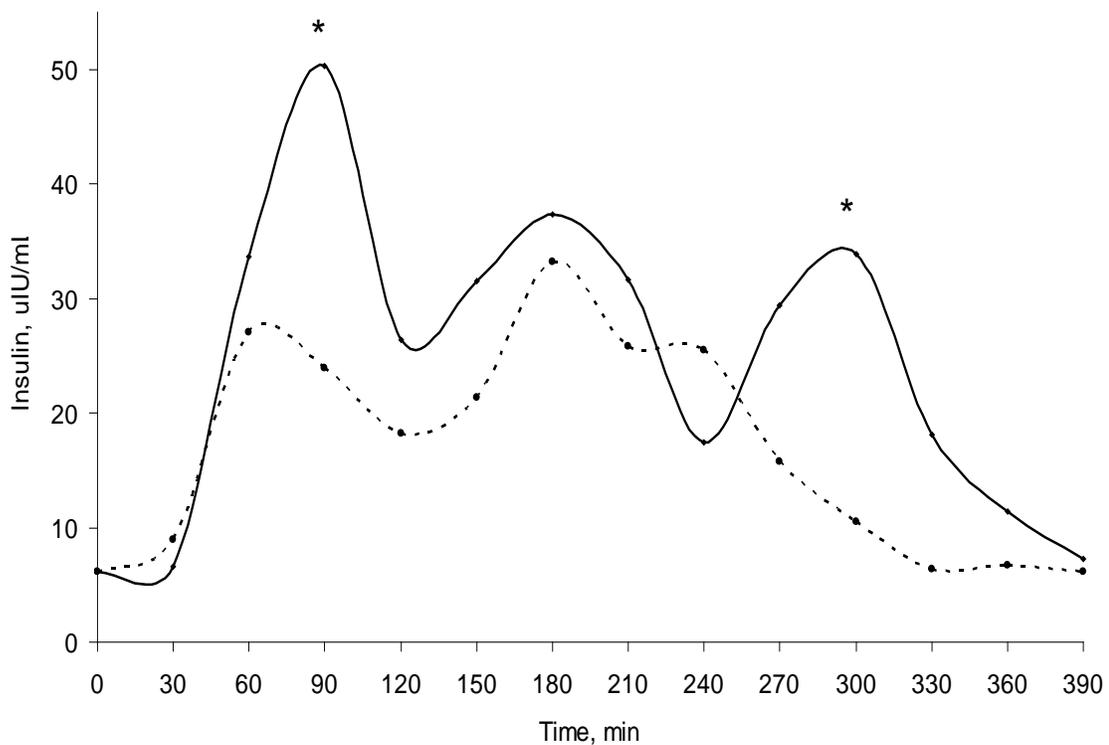

Figure 5.4. Mean serum insulin concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line). Effect of treatment x time ($P = 0.03$). SEM = 2.84. Asterisks indicate a difference between means at 90 min ($P = 0.002$) and 300 min (P < 0.001) after feeding.

Cortisol

Cortisol was similar ($P > 0.40$) among treatments (57.4 ± 19.1 versus 54.0 ± 16.4 versus 46.5 ± 14.7 versus 56.9 ± 24.7 ng/mL) and among the treatment by time interactions during the postprandial blood sampling period on d 60. Cortisol was also similar ($P = 0.42$) between treatments when non-psyllium supplemented horses (n = 4) were compared to the composite of psyllium supplemented horses (n = 12).

Leptin

Fasting leptin concentrations were similar ($P = 0.95$) among horses assigned to 0, 90, 180, and 270 g treatments at the beginning of the trial (2.1 ± 2.9 versus 1.7 ± 1.0 versus 1.7 ± 1.9 versus 1.3 ± 0.7 ng/mL, respectively). Fasting leptin concentration was similar ($P = 0.85$) among treatments at the end of the trial (0.8 ± 0.8 versus 1.3 ± 0.8 versus 1.1 ± 0.9 versus 1.3 ± 1.0 ng/mL, respectively). The change in fasting leptin concentrations from d 0 to d 60 did not differ ($P = 0.63$) between treatments (Table 5.2).

Ghrelin

Fasting ghrelin concentrations were similar ($P > 0.50$) among horses assigned to 0, 90, 180, and 270 g treatments at the beginning of the trial (20.8 ± 9.9 versus 22.9 ± 9.1 versus 19.5 ± 7.6 versus 14.1 ± 5.3 pg/mL, respectively). Fasting ghrelin concentrations were also similar ($P > 0.74$) among treatments at the end of the trial (20.3 ± 15.7 versus 12.8 ± 9.1 versus 17.5 ± 7.1 versus 14.8 ± 5.5 pg/mL, respectively). The change in fasting ghrelin concentration over the 60 d trial tended to differ ($P > 0.13$) between treatments (Table 5.2). The tendency likely resulted from the 90 g psyllium group having

a greater decrease in fasting ghrelin concentration compared to the other treatments (-10.1 versus -0.5, -2.0, and 0.8 pg/mL).

Adiponectin

Fasting adiponectin was higher (P = 0.02) before the trial began in horses assigned to the 180 g treatment compared to horses assigned to the 270 g treatment (35.8 ± 2.4 versus 26.1 ± 1.9 ng/mL). At the end of the trial, fasting adiponectin was higher (P = 0.02) in horses fed 0 and 180 g psyllium (32.9 ± 5.9 and 32.8 ± 2.7 ng/mL, respectively) compared to horses fed 90 and 270 g psyllium (25.7 ± 2.4 and 25.9 ± 3.1 ng/mL, respectively). The change in fasting adiponectin from d 0 to d 60 did not differ (P = 0.78) between any of the treatments (Table 5.2).

Body Mass

Psyllium did not effect body mass. All treatment groups were similar in body mass at the beginning of the trial: BW (P = 0.99), BCS (P = 0.81), neck circumference (P = 0.99), and tailhead fat (P = 0.18). All treatment groups were similar in body mass at the end of the trial: BW (P = 0.95), BCS (P = 0.96), neck circumference (P = 0.99), and tailhead fat (P = 0.77). The change in body mass from d 0 to 60 of the trial was similar among treatments: BW (P > 0.42), BCS (P > 0.90), tailhead fat (P > 0.69), and neck circumference (P > 0.89) did not differ in the change from d 0 to d 60 (Table 5.2).

Table 5.2. Changes (d 0 to 60) in fasting plasma leptin, adiponectin, and ghrelin concentration, BW, BCS, neck circumference, and tail-head fat for mature light breed stock horses fed 0, 90, 180, and 270g psyllium[1]

Variable	0g Psyllium	90g Psyllium	180g Psyllium	270g Psyllium	SEM	P-value
n	4	4	4	4		
Leptin, ng/mL	-1.3	-0.4	-0.6	-0.1	0.25	0.63
Adiponectin, ng/mL	-0.9	-2.2	-3.0	-0.2	0.63	0.78
Ghrelin, pg/mL	-0.5	-10.1	-2.0	0.8	2.45	0.13
BW, kg	20.5	17.8	9	9	3.0	0.42
BCS	0	-0.1	0.1	-0.1	0.05	0.90
Neck circumference, cm	-2.4	-1.0	-1.7	-1.8	0.29	0.89
Tail-head fat, mm	2.3	1.8	2.6	0.6	0.44	0.69

[1] Changes in these variables were calculated as the difference between measurements taken on d 0 and 60.

Trial 2- 2009 Discussion

The results from this trial parallel the results from similar studies on psyllium supplementation in humans (Sierra et al., 2001; 2002). Blood glucose and insulin concentrations were lower in horses that were fed psyllium, regardless of the level of psyllium. It appears that 90, 180, and 270g psyllium, when fed daily, are all effective at reducing overall blood glucose and insulin responses after a meal.

Cortisol is a stress hormone that effects glucose and insulin concentrations; it was measured to assess accuracy of glucose and insulin data in this study. Cortisol concentrations were not abnormal for any horse and were similar between treatments so we are confident that the glucose and insulin concentrations reported are truly results of the psyllium supplement.

The mean postprandial glucose concentrations of mares and geldings (regardless of treatment) were compared to assure that changes were not a result of sex. Sex had no

effect; postprandial mean glucose concentration was similar between mares and geldings (90.25 ± 16.0 versus 90.24 ± 14.5 mg/dL).

Body weight and fat mass of the horses in Trial 2 did not differ statistically. Although not significant, horses in all treatments gained weight, and horses fed 0 or 90 g psyllium gained 50% more weight than horses that were fed higher doses (180 or 270 g) of psyllium (Table 5.2). It should be noted that horses in this study were not exercised, and that the experiment only lasted 60 d. In that short time, psyllium did lower blood glucose and insulin concentrations so we expect that over a longer period of time BW and fat mass would differ statistically between treatments and that exercise would also intensify BW and fat mass differences (Raben et al., 1998).

Body condition score is a subjective measurement used to assess fat mass. Fasting leptin, adiponectin, and ghrelin concentrations were measured as an objective assessment of fat mass. Leptin is positively related, and adiponectin is inversely related, with fat mass in horses (Kearns et al., 2006). Ghrelin, a satiety hormone secreted primarily by the stomach, is negatively related to fat mass in horses (Gordon et al., 2005).

Changes from d 0 to d 60 in fasting leptin, adiponectin, and ghrelin concentrations did not differ significantly between treatments. The absence of change in those fat related hormones corroborates with the absence of change in the physical measurements. There was no significant change from d 0 to d 60 in BW, BCS, neck circumference, or tailhead fat between treatments.

Leptin, an adipocyte derived hormone, decreases feed intake, increases energy expenditure and metabolism, and decreases insulin secretion (Guyton and Hall, 2006).

Leptin is proportional to fat mass in horses (Kearns et al., 2005). Fasting leptin was lower at the end of the trial in all treatments, however not to a statistically significant degree. Decreases in leptin would indicate a negative energy balance. Leptin may be influenced by day length, with lower leptin concentrations occurring when nights are shorter (Radin et al., 2009). These horses were fed an estimated 15-20% above maintenance requirements. The absence of a significant change in leptin confirms that these horses were not underfed and the slight decreases may have been due to the longer summer days.

Ghrelin is a satiety hormone secreted primarily by the stomach and has an inverse relation to fat mass in horses (Gordon et al., 2005). Although the change in the fasting ghrelin concentration was not significant between treatments, the general decrease in ghrelin coincides with the general, non significant, increase in BW.

Adiponectin is an adipocyte derived hormone that is inversely related to fat mass (Kearns et al., 2005) and has insulin sensitizing properties (Berg et al., 2001). Horses in all treatment groups had a lower fasting adiponectin concentration and increased tailhead fat after the 60 d trial, however there was no significant differences between treatments.

<div align="center">Trial 3- Glucose Challenge Results:</div>

Glucose

Glucose concentrations were lower after intravenous dextrose infusion in horses fed higher doses of psyllium. Glucose concentrations were lower 5 minutes after dextrose infusion in horses fed 180 g (303.2 mg/dL; $P = 0.024$) and 270 g (296.8 mg/dL;

$P = 0.007$) psyllium compared to those that were fed 0 g (334.4 mg/dL) or 90 g (313.1 mg/dL) psyllium (Figure 5.5). Glucose concentrations at 15 min were lower ($P < 0.04$) in all horses fed psyllium than in horses not fed psyllium (252.7, 251.4, and 257.2 versus 285.4 mg/dL; Figure 5.5). Horses fed 270 g psyllium had lower ($P = 0.04$) glucose concentrations 30 min after dextrose infusion compared to all other treatments (216.7 versus 244.9, 221.1, and 227.9 mg/dL; Figure 5.5). Horses supplemented with 90 g psyllium had lower glucose concentrations at 60 min (188.6 mg/dL; $P = 0.01$), 90 min (154.3 mg/dL; $P < 0.01$), and 120 min (128.1 mg/dL; $P < 0.01$) compared to all other treatments (Figure 5.5). The average glucose concentration during the glucose challenge was 173.9, 152.1, 159.6, and 158.1 mg/dL for 0, 90, 180, and 270 g psyllium treatments, respectively; SEM = 6.60.
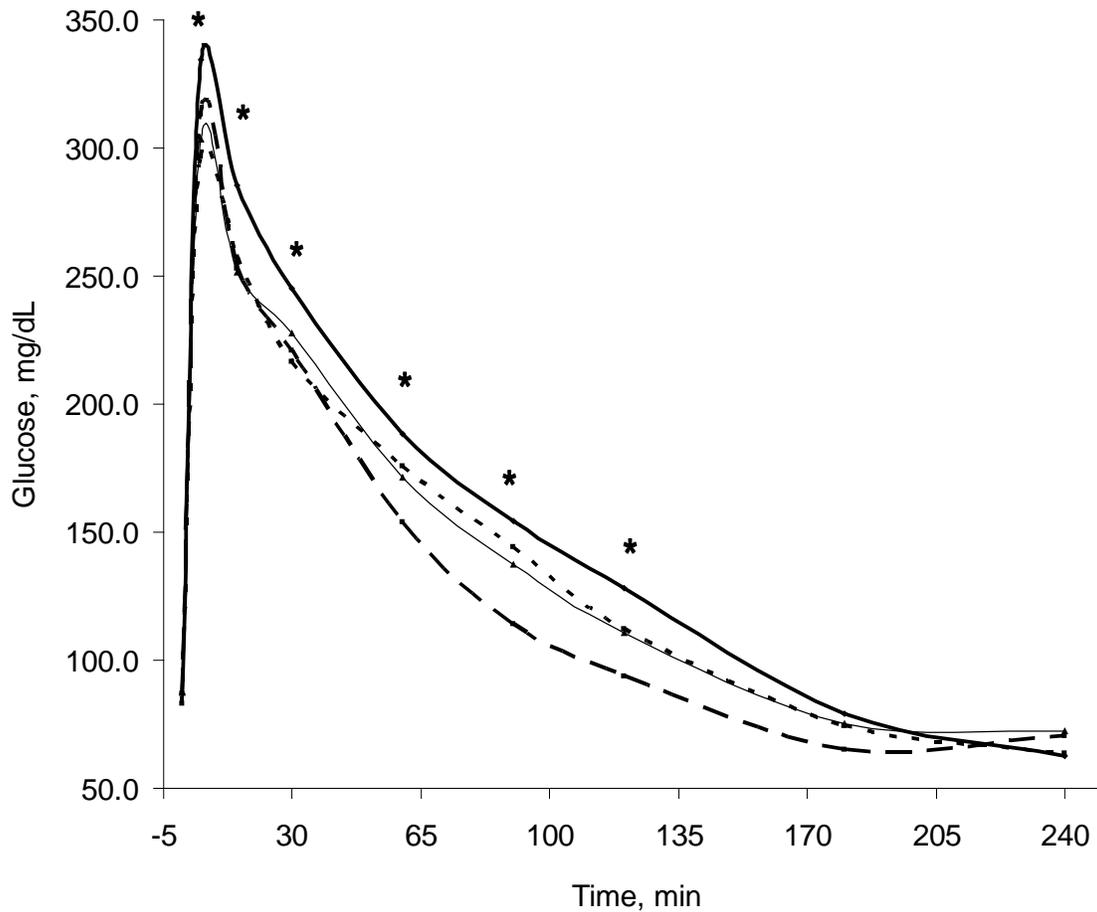
Figure 5.5. Mean plasma glucose concentrations of horses not fed psyllium (dark solid line), horses fed 90 g psyllium (dashed line), horses fed 180 g psyllium (light solid line), and horses fed 270 g psyllium (dotted line). Effect of treatment x time ($P = 0.049$). SEM = 6.60. Asterisks indicate a difference between means ($P < 0.05$).

When the three psyllium treatments (90, 180, and 270g psyllium) were averaged together to represent one psyllium group (n = 12) and compared to the control group (n = 4), there was a treatment by time interaction ($P = 0.024$) and a treatment effect ($P = 0.032$) on glucose concentrations (Figure 5.6). The average glucose concentration was 173.9 ± 6.3 for horse not fed psyllium and 156.6 ± 3.6 mg/dL glucose for horses fed psyllium.
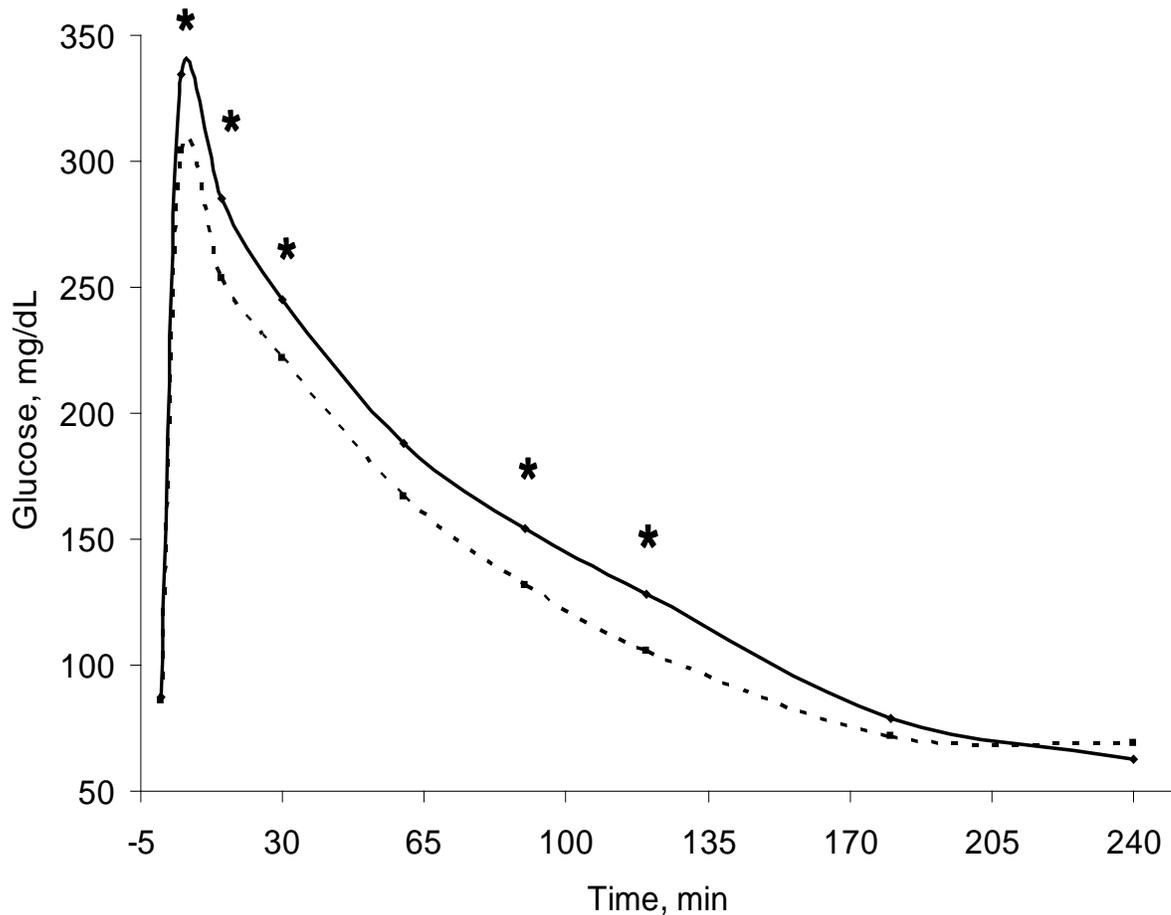
Figure 5.6. Mean plasma glucose concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line). Effect of treatment x time ($P = 0.02$). SEM = 6.29. Asterisks indicate a difference between means at 5 min ($P = 0.007$), 15 min ($P = 0.005$), 30 min ($P = 0.036$), 90 min ($P = 0.040$), and 120 min ($P = 0.040$) after feeding.

Insulin

The treatment by time interaction was not significant ($P = 0.25$) between treatments of 0, 90, 180, and 270 g of psyllium (Figure 5.7). The effect of treatment alone was not different ($P = 0.97$). The average insulin concentration during the glucose challenge was 17.1 ± 10.3, 16.6 ± 18.8, 15.3 ± 11.6, and 15.3 ± 16.0 µIU/mL for 0, 90, 180, and 270 g psyllium treatments, respectively.

Figure 5.7. Mean serum insulin concentrations of horses not fed psyllium (dark solid line), horses fed 90 g psyllium (dashed line), horses fed 180 g psyllium (light solid line), and horses fed 270 g psyllium (dotted line). Effect of treatment x time ($P = 0.25$).

When the three psyllium treatments (90, 180, and 270g psyllium) were averaged together to represent one psyllium group (n = 12) and compared to the control group (n = 4), there was no treatment by time interaction ($P = 0.77$) and no treatment effect ($P = 0.71$) on insulin concentrations between treatments (Figure 5.8). The average insulin concentration was 17.1 ± 10.3 µIU/mL for horses not fed psyllium and 15.7 ± 15.6 µIU/mL for horses fed psyllium.

Figure 5.8. Mean serum insulin concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line) after intravenous infusion of dextrose. Effect of treatment x time ($P = 0.77$).

Cortisol

Cortisol treatment by time interaction was not significant ($P = 0.96$) between treatments of 0, 90, 180, and 270 g of psyllium (Figure 5.9). The effect of treatment alone was not different ($P = 0.13$). Mean cortisol concentrations were 46 ± 17.3, 49.9 ± 12.7, 39.3 ± 13.3, and 50.9 ± 15.1 ng/mL for 0, 90, 180, and 270 g treatments, respectively.
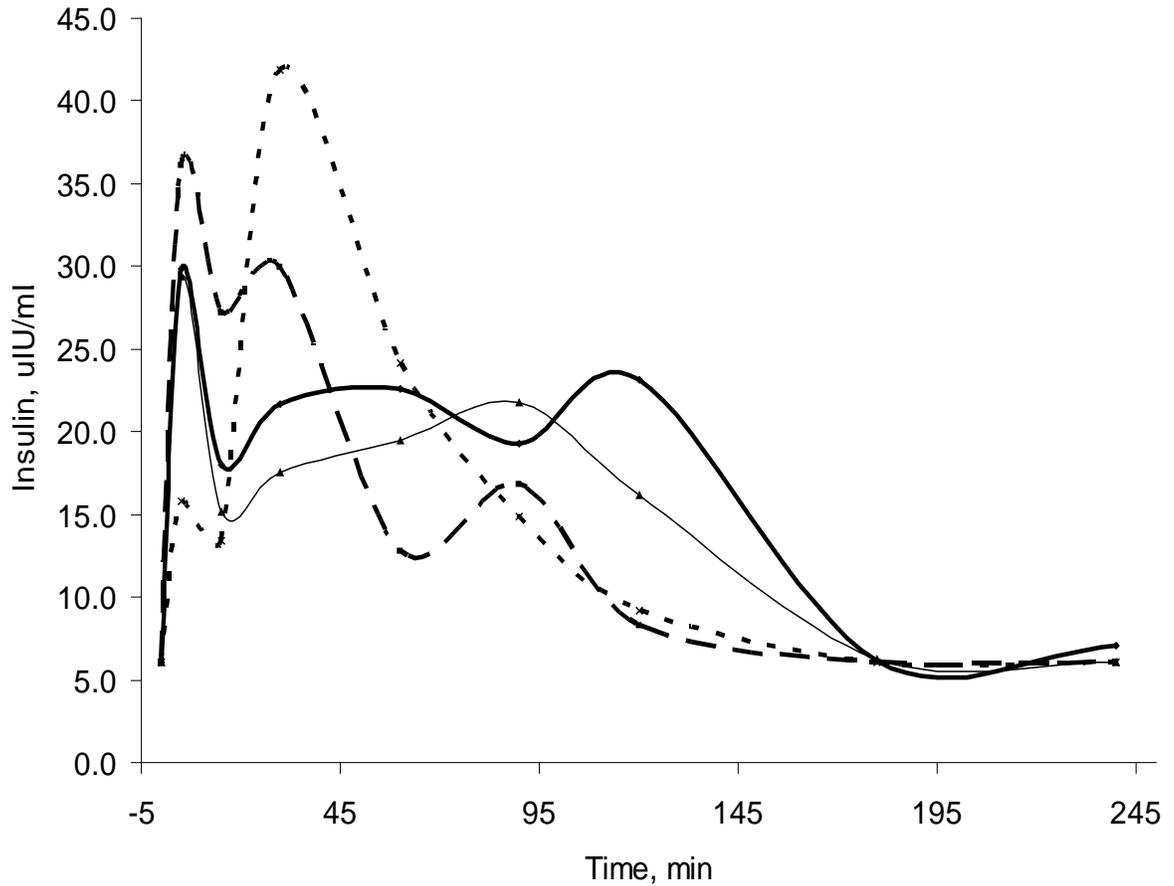
Figure 5.9. Mean serum cortisol concentrations of horses not fed psyllium (dark solid line), horses fed 90 g psyllium (dashed line), horses fed 180 g psyllium (light solid line), and horses fed 270 g psyllium (dotted line). Effect of treatment x time ($P = 0.96$).

When the three psyllium treatments (90, 180, and 270g psyllium) were averaged together to represent one psyllium group (n = 12) and compared to the control group (n = 4), there was no treatment by time interaction ($P = 0.54$) and no treatment effect ($P = 0.92$) on cortisol concentrations between treatments (Figure 5.10).
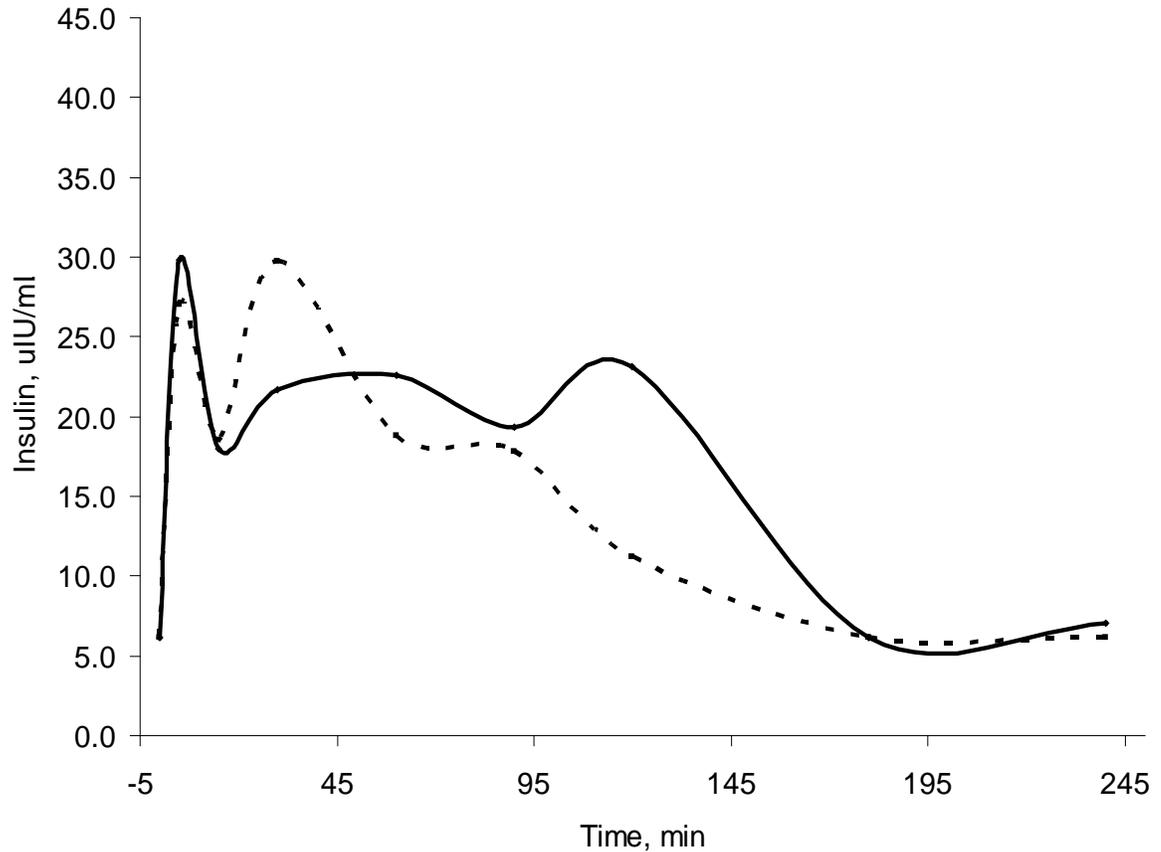
Figure 5.10. Mean serum cortisol concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line) after intravenous infusion of dextrose. Effect of treatment x time ($P = 0.54$).

Ghrelin

Ghrelin concentrations were not different among psyllium treatments. The treatment by time interaction was similar ($P = 0.75$) among treatments of 0, 90, 180, and 270 g of psyllium (Figure 5.11). The effect of treatment alone was also similar ($P = 0.76$). Mean ghrelin concentrations were 13.5 ± 12.1, 16.8 ± 23.1, 13.3 ± 8.8, and 12.2 ± 9.7 pg/mL for 0, 90, 180, and 270 g treatments, respectively.
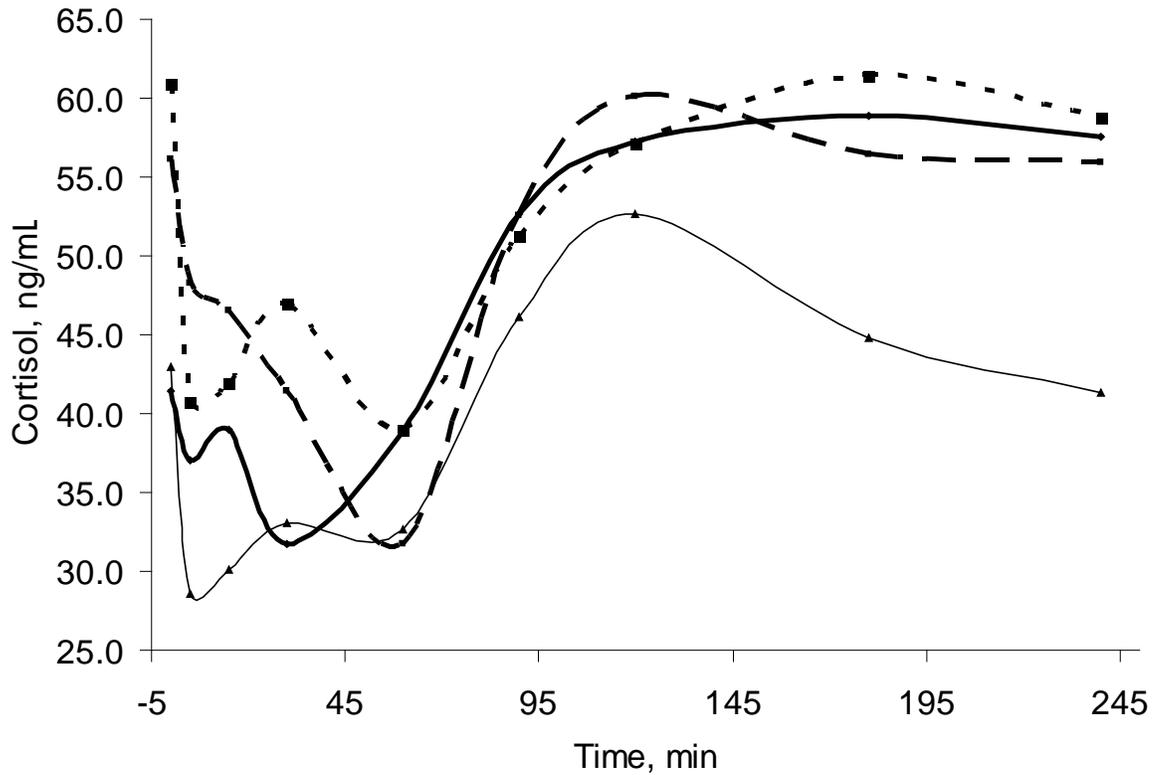
Figure 5.11. Mean active ghrelin concentrations of horses not fed psyllium (dark solid line), horses fed 90 g psyllium (dashed line), horses fed 180 g psyllium (light solid line), and horses fed 270 g psyllium (dotted line). Effect of treatment x time (P = 0.75).

When the three psyllium treatments (90, 180, and 270g psyllium) were averaged

together to represent one psyllium group (n = 12) and compared to the control group (n =

4), no treatment by time interaction (P = 0.82) and no treatment effect (P = 0.18) on

ghrelin concentrations was identified between treatments (Figure 5.12). It appears that

higher levels of psyllium may maintain a more homeostatic ghrelin response after
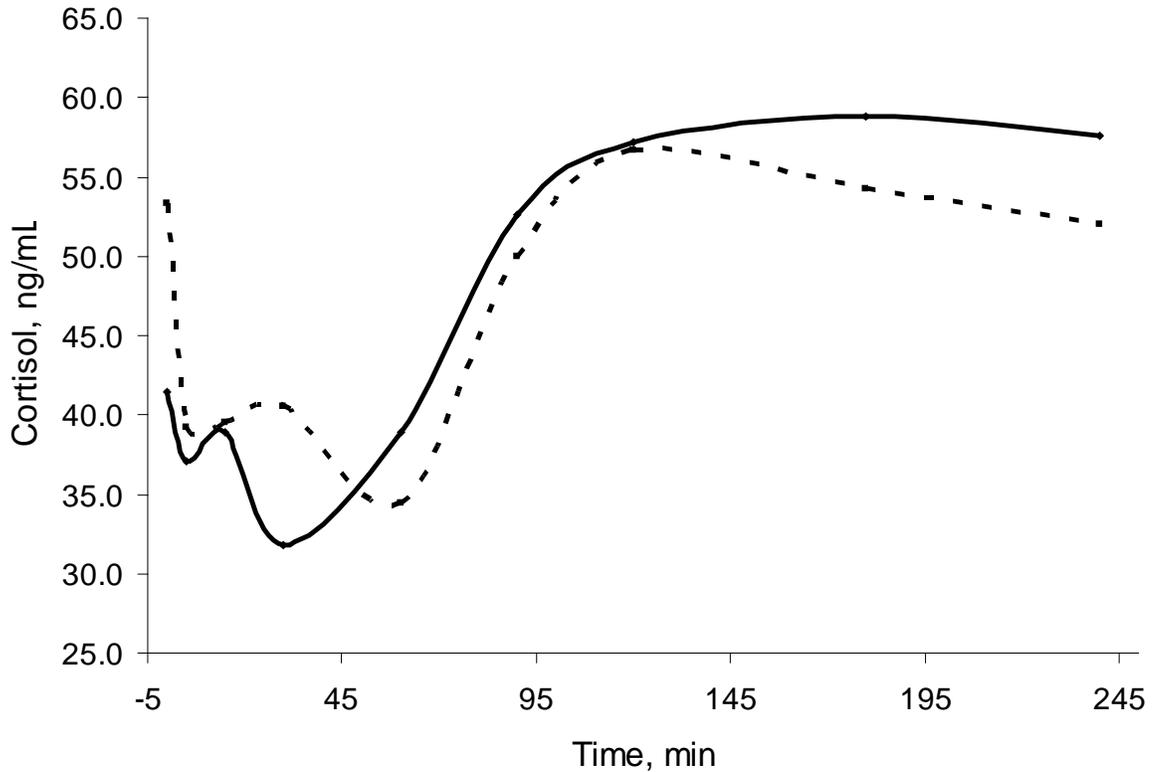
intravenous dextrose infusion.

Figure 5.12. Mean active ghrelin concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line) after intravenous infusion of dextrose. Effect of treatment x time ($P = 0.82$).

Leptin

Leptin concentrations were similar among treatments. The treatment by time interaction was not significant ($P = 0.38$) for leptin concentrations between treatments of 0, 90, 180, and 270 g of psyllium (Figure 5.13). The effect of treatment alone was not different ($P = 0.79$). Mean leptin concentrations were 1.7 ± 2.3, 1.6 ± 2.0, 1.0 ± 0.9, and 0.8 ± 0.8 ng/mL for 0, 90, 180, and 270 g treatments, respectively.
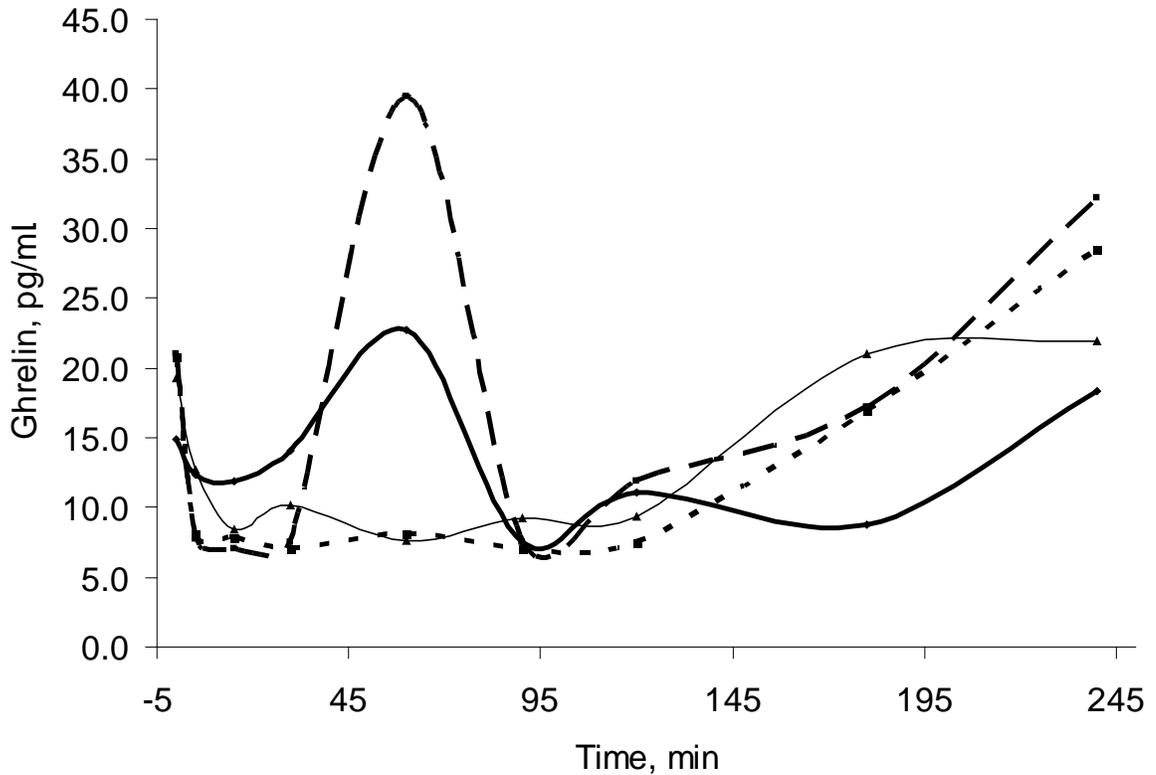
Figure 5.13. Mean plasma leptin concentrations of horses not fed psyllium (dark solid line), horses fed 90 g psyllium (dashed line), horses fed 180 g psyllium (light solid line), and horses fed 270 g psyllium (dotted line). Effect of treatment x time ($P = 0.38$).

When the three psyllium treatments (90, 180, and 270g psyllium) were averaged together to represent one psyllium group (n = 12) and compared to the control group (n = 4), there was a tendency for a treatment by time interaction ($P = 0.07$) and no treatment effect ($P = 0.53$) on leptin concentrations between treatments (Figure 5.14).
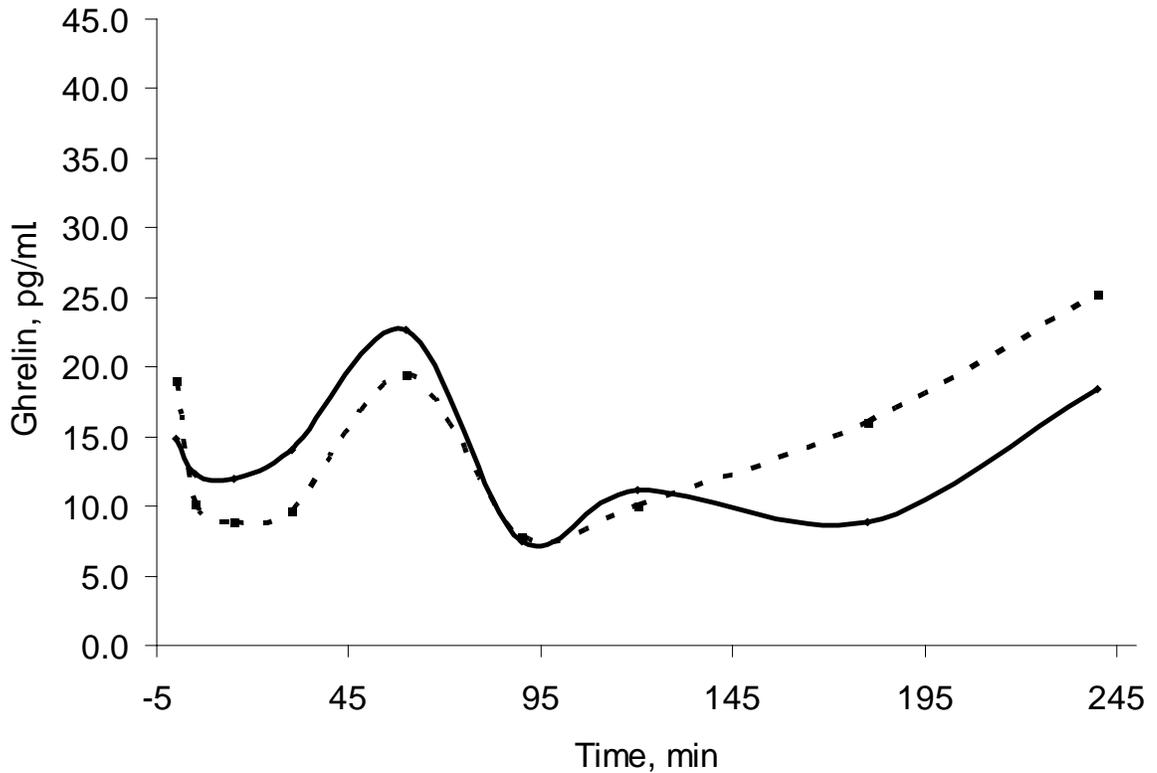
Figure 5.14. Mean leptin concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line) after intravenous infusion of dextrose. Effect of treatment x time (P = 0.07).

Adiponectin

The treatment by time interaction was not significant (P = 0.92) between treatments of 0, 90, 180, and 270 g of psyllium. The effect of treatment alone was different (P = 0.017; Figure 5.7). Adiponectin concentrations differed (Figure 5.15) in horses fed 90 g psyllium at 15 min (24.9 ± 3.3; P = 0.01), 90 min (27.5 ± 2.2; P = 0.04), 120 min (27.7 ± 2.6; P = 0.01), and 240 min (26.9 ± 3.4; P = 0.02). Horses fed 270 g psyllium had adiponectin concentrations that differed (Figure 5.7) at 0 min (26.5 ± 3.6; P = 0.04), 15 min (25.6 ± 2.7; P = 0.02), 60 min (27.9 ± 3.2; P = 0.05), and 120 min (28.8 ± 6.7; P = 0.02). The average adiponectin concentration during the glucose challenge

was 33.4 ± 5.7, 27.0 ± 2.7, 34.6 ± 2.9, and 27.4 ± 3.9 ng/mL for 0, 90, 180, and 270 g

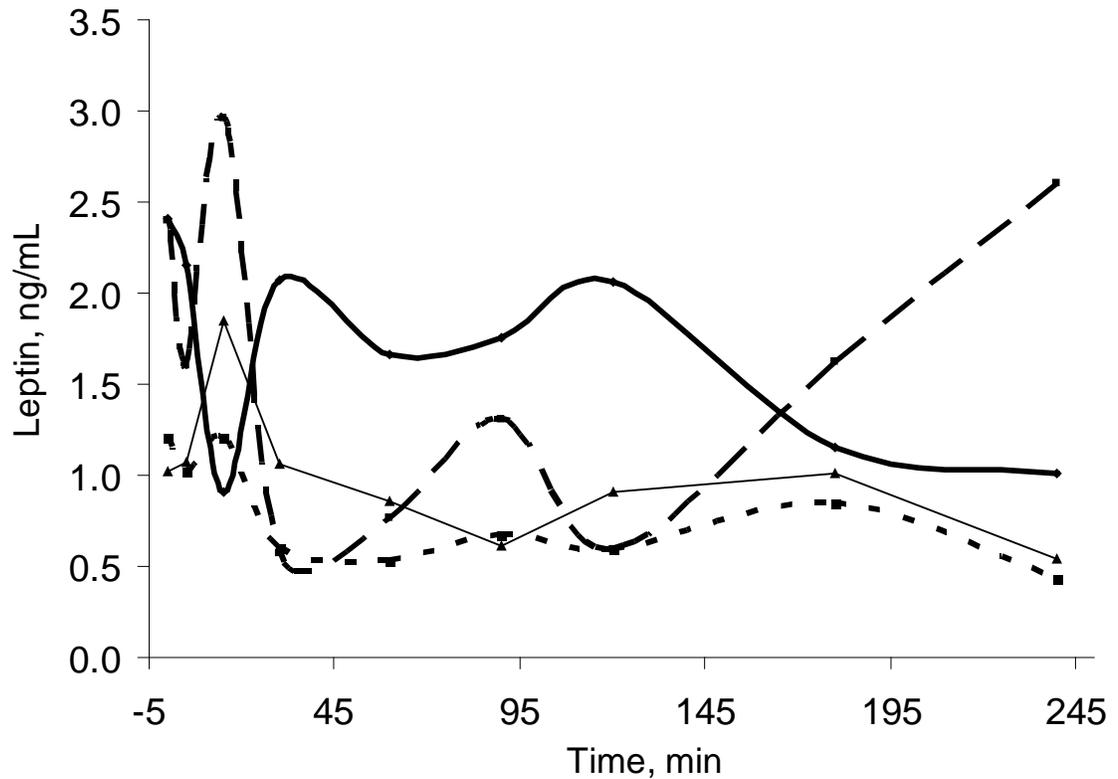psyllium treatments, respectively.

Figure 5.15. Mean plasma adiponectin concentrations of horses not fed psyllium (dark solid line), horses fed 90 g psyllium (dashed line), horses fed 180 g psyllium (light solid line), and horses fed 270 g psyllium (dotted line). Effect of treatment ($P = 0.017$). SEM = 1.76. Asterisks indicate a difference between means ($P < 0.05$).

Adiponectin concentrations, when the three psyllium treatments (90, 180, and 270

g psyllium) were averaged together to represent one psyllium group (n = 12) and

compared to a control group (n = 4), were not different in the treatment by time

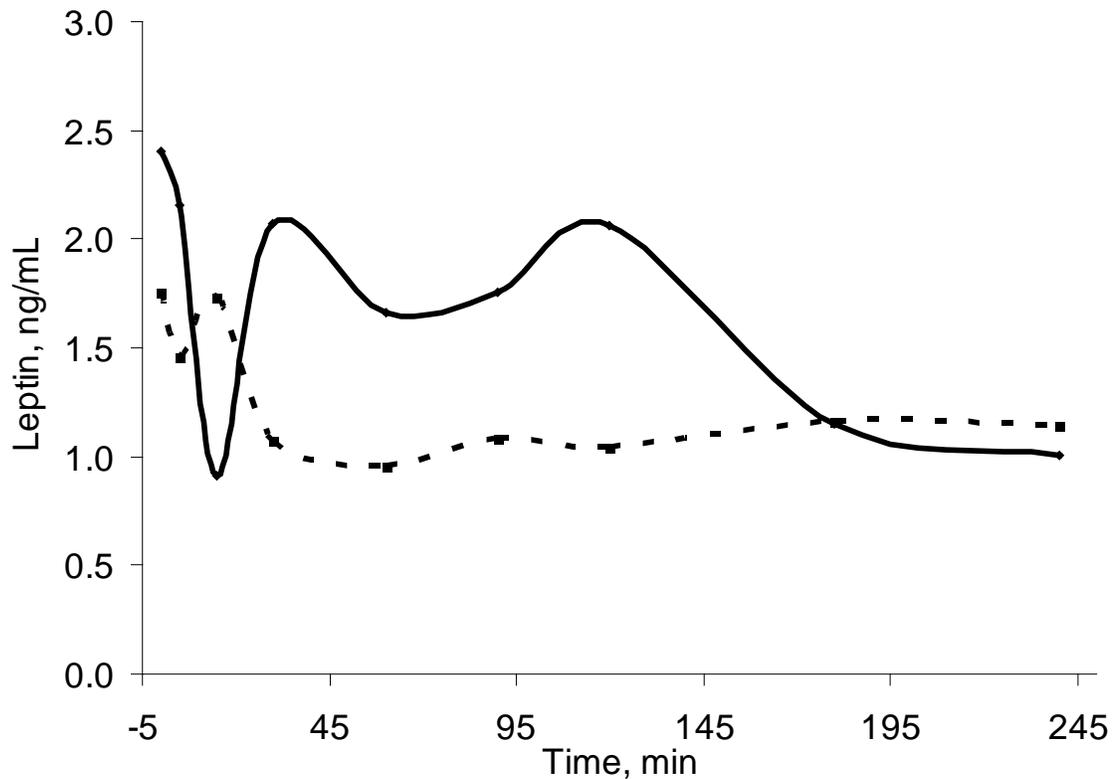interaction ($P = 0.77$) and there was no treatment effect ($P = 0.18$; Figure 5.16).
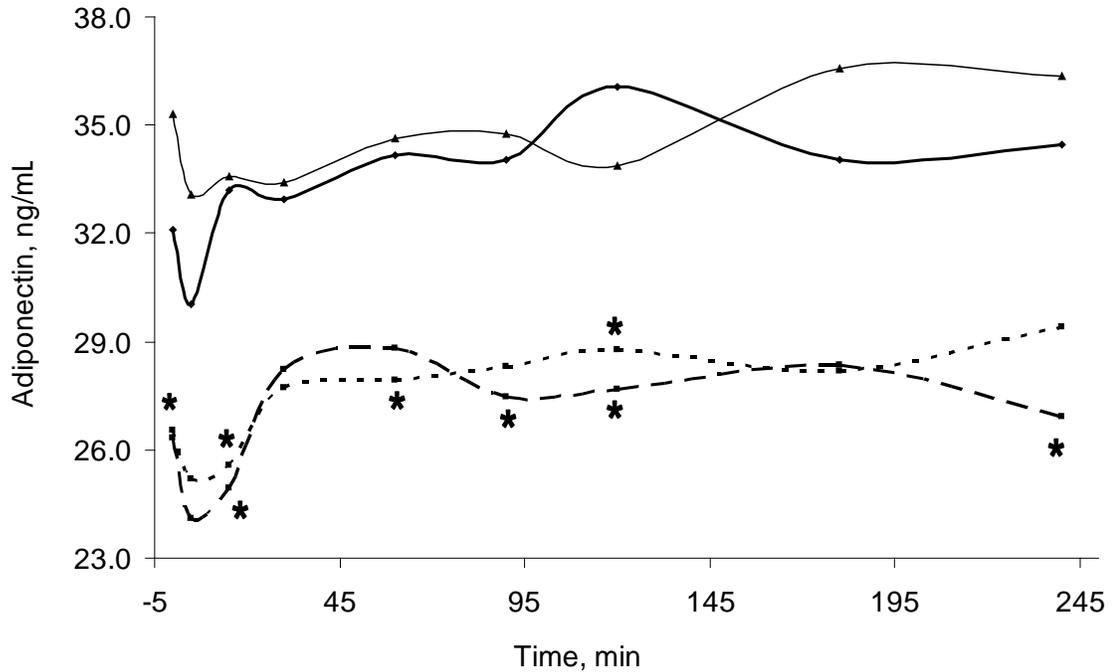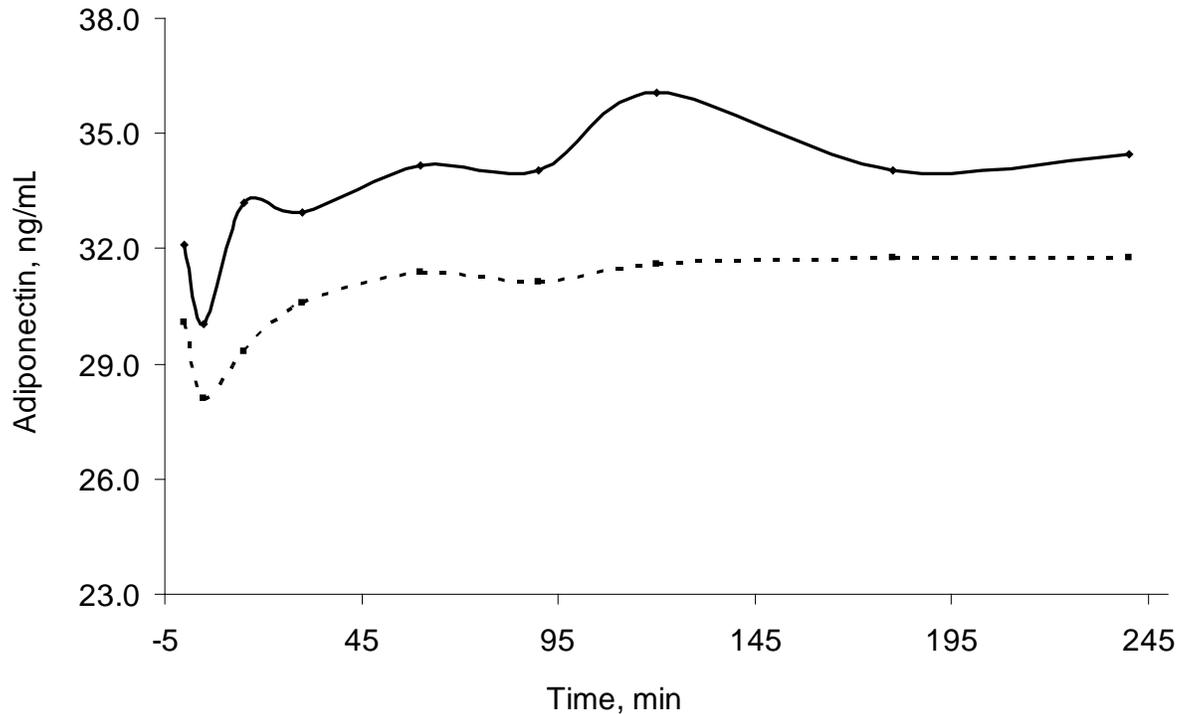
Figure 5.16. Mean adiponectin concentrations of horses not fed psyllium (solid line) and horses fed psyllium (dashed line) after intravenous infusion of dextrose. Effect of treatment x time ($P = 0.77$).

Trial 3- Glucose Challenge Discussion

Results from Trial 3 suggest that psyllium does have an effect on glucose metabolism. All horses were fasted for 12 h before the intravenous dextrose was infused. Feed was withheld from the horses until all samples had been collected as well so there were not any digestive influences on the results.

Peak glucose was 9% lower after infusion in horses that had been supplemented with psyllium. The mean glucose concentration (or glycemic index) during the glucose challenge was 10% lower in horses that had been fed psyllium compared to those that had not. Feeding psyllium for 60 d (Trial 2) appears to modify the metabolic response of a horse making them more sensitive to insulin secretions. Increases in insulin sensitivity

allowed the horses on psyllium to be more efficient at clearing an infused dextrose load than the horses that were not previously on a psyllium treatment, hence the lower peak and lower overall glucose concentration. Efficient glucose clearance would also explain why there was no difference in the insulin concentration between horses fed psyllium and those that were not. Horses fed psyllium were able to clear the glucose load quicker with the same amount of insulin secretion as the horses that were not fed psyllium. Horses on the psyllium supplement were more efficient at maintaining glucose homeostasis during the intravenous glucose challenge which suggests they were more insulin sensitive.

Mean fasting insulin concentration (0 min) was at or below the sensitivity (6.1 μIU/mL) of the insulin RIA assay, confirming that all horses had normal insulin concentrations and no horse was insulin resistant. Mean cortisol concentrations throughout the glucose challenge were 46.0 ± 17.3 and 46.8 ± 14.6 ng/mL for control and psyllium fed horses, respectively. The mean cortisol concentration for each group was in the normal range for horses, and cortisol was lower in this trial than has been reported in similar glucose challenge trials (Gordon and McKeever, 2006). Normal cortisol concentration ensures accuracy of glucose and insulin concentrations. Cortisol is secreted in response to stress. High cortisol concentrations stimulate gluconeogenesis, which causes blood glucose concentration to rise, which triggers an increase in insulin secretion. In this trial, no single horse or treatment had a high or abnormal cortisol concentration; therefore we are confident that the glucose and insulin concentrations reported are indeed accurate.

Ghrelin peaked 60 min after infusion in horses fed 0 and 90 g of psyllium whereas ghrelin remained constant through that time in horses fed higher doses of psyllium. Horses fed 0 g psyllium had a 66% higher ghrelin concentration and horses fed 90 g psyllium had an 80% higher ghrelin concentration at 60 min than horses fed 180 or 270 g psyllium (Figure 5.11). The results from Trial 3 suggest that high doses of psyllium (180 or 270 g) may suppress ghrelin. Ghrelin did not peak in horses at any point during a similar glucose challenge study; in fact it was instead suppressed (Gordon and McKeever, 2006). Ghrelin concentrations in our trial did increase toward the end of the glucose challenge in all treatments which is consistent with data previously reported (Gordon and McKeever, 2006). Gordon and McKeever (2006) suggest that there is an inverse relationship between ghrelin and glucose/insulin. This trial only demonstrated that inverse relationship in horses that were treated with high doses of psyllium (180 and 270 g).

Ghrelin concentration increases with meal anticipation or initiation in humans (Cummings et al., 2001; 2004). Ghrelin remained consistently lower in horses supplemented with 180 and 270 g psyllium, so psyllium may affect satiety. Psyllium may suppress meal anticipation and make horses feel less hungry.

Leptin tended to be lower in all horses that were fed psyllium. Leptin is a hormone secreted by adipocytes and is positively related to fat mass in horses (Kearns et al., 2005). Even though horses were all similar in BCS (Trial 2), the lower leptin concentration in psyllium supplemented horses suggests that the horses had less fat mass even though decreases in fat were not visibly detectable or physically palpable. This

further supports the idea that BCS is a subjective measurement, and maybe measurements of leptin would be a more valid assessment of fat mass in horses.

It appears that higher doses of psyllium (180 and 270 g) elicit lower concentrations of circulating leptin and maintain lower leptin concentrations during an intravenous glucose challenge. Gordon and McKeever (2006) report increases in leptin beginning 180 min after dextrose infusion; those results are consistent with the results of 90 g psyllium horses in this trial while 0 g psyllium horses maintained leptin concentration and 180 and 270 g psyllium fed horses had decreasing leptin concentrations during that time. It's possible then that psyllium may even have a leptin sensitizing effect. The tendencies in the leptin data support the statistically significant results of the glucose data, and are consistent with the idea that psyllium increases the efficiency of glucose metabolism in horses by increasing insulin sensitivity. Increasing leptin sensitivity might help explain the insulin sensitizing response by the horses fed higher doses of psyllium because leptin is partially responsible for decreasing insulin secretion.

Adiponectin concentrations during the glucose challenge were higher in horses that had been fed 0 and 180 g psyllium than horses that had been fed 90 or 270 g psyllium. Adiponectin is inversely related to fat mass in horses (Kearns et al., 2005) and it directly sensitizes the body to insulin (Kadowaki et al., 2006). Body weight was not different between treatments so the higher adiponectin concentrations in the 180 g psyllium fed horses was most likely because those horses had statistically higher fasting adiponectin concentrations before psyllium supplementation ever began (Trial 2). Adiponectin concentrations did remain relatively stable after intravenous dextrose

infusion which is consistent with the stable pattern reported previously by Gordon and

McKeever (2006).

CHAPTER 6

CONCLUSION

Horses can be challenging to feed. The ideal situation for a horse's physical,

mental, and digestive state is to graze pasture grass at will throughout the day. Human

intervention, space availability, and the risk of becoming obese inhibit most horses from

living in that ideal environment. Obesity is a major concern because it predisposes

horses to insulin resistance and alters metabolic pathways that could ultimately result in

laminitis. Caretakers must provide adequate nutrition to maintain health and avoid

creating an obese horse or aggravating an insulin resistant predisposition. Maintaining a

low glycemic response (the degree to which the glucose concentration rises after a meal)

is a primary goal for feeding horses (Frank et al., 2008).

It appears psyllium has an effect on glucose metabolism. After Trial 1 we

concluded that psyllium delayed glucose absorption through the small intestine because

the peak in glucose and insulin was delayed. Trial 2 suggests psyllium inhibited glucose

absorption through the small intestine because overall blood glucose and insulin were

lower in horses fed psyllium. Results from Trial 3 indicate that psyllium may have an

overall metabolic effect in the horse because higher doses of psyllium resulted in lower

concentrations of blood glucose in response to dextrose infusion.

The results of these trials suggest that adding psyllium to the diet may be an

important component in reducing the glycemic response to a meal and maintaining

insulin sensitivity. Psyllium fed daily to horses delays peak glucose by 30 min, lowers

the glycemic response to a meal by 7%, and decreases overall postprandial insulin concentrations by 30%. Postprandial peak glucose and insulin were 20% and 46% lower, respectively, in horses fed psyllium compared to those that were not. Statistically, psyllium elicits this same response when fed at any of the following levels: 90, 180, or 270 g·horse^{-1}·d^{-1}. However, the statistical trend in change of BW suggests psyllium fed at 180 and 270g may be most effective. Trial 2 suggests feeding psyllium for 60 d stimulates insulin sensitizing responses whereas a 42 d supplementation period (Trial 1) appears to have no effect.

Feeding higher doses of psyllium elicits a greater metabolic response according to the significantly lower glucose concentrations after dextrose infusion in horses fed 180 g ($P = 0.02$) and 270 g ($P < 0.01$) psyllium (Trial 3). It appears that high doses of psyllium are best at lowering blood glucose levels and increasing insulin sensitivity. Both of which are important in the management of obesity and insulin resistance to prevent laminitis in horses.

The insulin sensitizing effect that psyllium had on these horses is even more remarkable when one considers the absence of exercise in the trials. Muscle contraction from exercise stimulates an increase in insulin sensitivity. The horses were not exercised during any of the trials, meaning the insulin sensitizing effects that psyllium elicited were direct results of treatment. Exercise in combination with a high dose daily psyllium supplement may have even more profound positive effects on increasing insulin sensitivity in horses.

LITERATURE CITED

Asplin, K. E., C. M. McGowan, C. C. Pollitt, M. N. Sillence. 2007a. Role of insulin in glucose uptake in the equine hoof. J. Vet. Int. Med. 21:668 (abstr).

Asplin, K. E., M. N. Sillence, C. C. Pollitt, C. M. McGowan. 2007b. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. Vet J 174:530-535.

Berg, A. H., T. P. Combs, and P. E. Scherer. 2002. ACRP30/adiponectin: An adipokine regulating glucose and lipid metabolism. Trends Edocrinol. Metab. 13:84-89.

Carter, R. A., L. J. McCutcheo, T. A. Burns, J. K. Belknap, N. Frank, and R. J. Geor. 2008. Increased adiposity in horss is associated with dereased insulin sensitivity but unchanged inflammatory cytokine expression in subcutaneous adipose tissue. J. Vet. Intern. Med. 22:735(abstr).

Casanueva, F. F., C. Dieguez. 2002. Ghrelin: the link connecting growth with metabolism and energy homeostasis. Rev Edocrinol Metab Disord. 3:326-338.

Combs, T.P, J.A. Wagner, J. Berger, T. Doebber, W.J. Wang, B.B. Zhang, M. Tanen, A.H. Berg, S. O'Rahilly, D.B. Savage, K. Chatterjee, S. Weiss, P.J. Larson, K.M. Gottesdiener, B.J. Gertz, M.J. Charron, P.E. Scherer and D.E. Moller. 2002. Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. Endocrinology 143:998–1007.

Cummings, D. E., R. S. Frayo, C. Marmonier, R. Auber, and D. Chapelot. 2004. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cures. Am. J. Physiol. Endocrinol. Metab. 287:E297-E304.

Cummings, D. E., J. Q. Purnell, R. S. Frayo, K. Schmidova, B. E. Wisse, and D. S. Weigle. 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714-1719.

Daimon, M., T. Oizumi and T. Saitoh. 2003. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese Population: the Funagata study. Diabetes Care 26:2015–2020

Delavaud C, Bocquier F, Chilliard Y, Keisler DH, Gertler A and Kann G. 2000. Plasma leptin in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep Journal of Endocrinology 165 519-526.

Dickson, W. M. 1970. Endocrine glands. Pages 1189-1252 in Dukes' Physiology of Domestic Animals. Swenson, M. J., ed. Cornell University Press, Ithaca, NY.

Díez, J. J., P. Iglesias. 2003. The role of the novel adipocyte-derived hormone adiponectin in human disease. European J. of Endocrinology 148; 3:293-300

Drazen, D. L., S. C. Woods. 2003. Peripheral signals in the control of satiety and hunger. Curr Opin Clin Nutr Metab Care 6:621-629.

Fitzgerald, B. P., and C. J. McManus. 2000. Photoperiodic versus metabolic signals as determinants of seasonal anestrus in the mare. Biol. Repro. 63:335-340.

Frank, N., R. J. Geor, S. Adair. 2008. Management of insulin resistance. Pages 35-36 in Guide to Insulin Resistance and Laminitis for Equine Practitioners. Lloyd Inc., Shenandoa, IA.

Frank, N., S. Elliott, L. Brandt, and D. H. Keisler. 2006. Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. J. Am. Vet. Med. Assoc. 228:1383-1390.

Freestone, J. F., K. J. Wolfsheimer, S. G. Kamerling, G. Church, J. Hamra, and C. Bagwell. 1991. Exercise induced hormonal and metabolic changes in Thoroughbred horses: Effects of conditioning and acepromazine. Equine Vet. J. 23:219-223.

Gentry, L. R., D. L. Thompson, G. T. Gentry, R. P. Del Vecchio, K. A. Davis, and P. M. Del Vecchio. 2004. The relationship between body condition score and ultrasonic fat measurements in mares of high versus low body condition. J. of Equine Vet. Sci. 24:198-203.

Geor, R. J. 2008. Metabolic predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. J Equine Vet Sci 28;12:753-759.

Gibaldi, M., and D. Perrier. 1982. Pharmacokinetics, 2nd rev. ed. Marcel Dekker, NY.

Gordon, M. E., K. H. McKeever. 2006. Oral and intravenous carbohydrate challenges decrease active ghrelin concentrations and alter hormones related to control of energy metabolism in horses. J. Anim. Sci. 84:1682-1690.

Gordon, M. E., K. H. McKeever. 2005. Diurnal variation of ghrelin, leptin, and adiponectin in Standardbred mares. J. Anim. Sci. 83:6365-6371.

Gordon, M. E., K. H. McKeever, C. L. Betros, and C. Manso Filho. 2007. Exercise-induced alterations in plasma concentrations of ghrelin, adiponectin, leptin, glucose, insulin, and cortisol in horses. The Veterinary Journal 173:532–540.

Gualillo, O., F. Lago, J. Gomez-Reino, F. F. Casanueva, C. Dieguez. 2003. Ghrelin, a widespread hormone: insights into molecular and cellular regulation of its expression and metabolism of action. FEBS Lett 552:105-109.

Guyton, A. C. and J. E. Hall. 2006. Page 870-871 in Textbook of Medical Physiology. 11th ed. Elsevier Saunders, Philadelphia, PA.

Hagemann, D., J. J. Meier, V. Gallwitz, W. E. Schmidt. 2003. Appetite regulation by ghrelin- a novel neuro-endocrine gastric peptide hormone in the gut-brain-axis. Z Gastroenterol 41:929-936.

Henneke, D. R., G. D. Potter, K. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15:371-372.

Hoffman, R. M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, and P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. J. Anim. Sci. 81:2333-2342.

Hotwagner K., and C. Iben. 2007. Evacuation of sand from the equine intestine with mineral oil, with and without psyllium. J. Anim. Physiol. Anim. Nutr. 92: 86-91.

June, V., V. Soderholm, H. F. Hintz, and W. R. Butler. 1992. Glucose tolerance in the horse, pony, and donkey. J. of Equine Vet. Sci. 12(2):103-105.

Kadowaki, T., T. Yamauchi, N. Kubota, K. Hara, K. Ueki, and K. Tobe. 2006. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J. Clin. Investigation 116:7:1784-1792.

Kahn, C. R. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 27:1893-1902.

Kane, R. A., M. Fisher, D. Parrett, and L. M. Lawrence. 1987. Estimating fatness in horses. Pages 127-131 in Proc. 10th Equine Nutr. Physiol. Soc., Ft. Collins, CO.

Kearns, C. F., K. H. McKeever, V. Roegner, S. M. Brady, and K. Malinowski. 2005. Adiponectin and leptin are related to fat mass in horses. Vet J. 172:460-465.

Landes A. D., D. M. Hassel, J. D. Funk, and A. Hill. 2008. Fecal sand clearance is enhanced with a product combining probiotics, prebiotics, and psyllium in clinically normal horses. J. Equine Vet. Sci. 28: 79-84.

Lindsay, R.S., T. Funahashi and R.L. Hanson. 2002. Adiponectin and development of type 2 diabetes in the Pima Indian population, Lancet 360**:**57–58.

Maeda, K., K. Okubo and I. Shimomura. 1996. cDNA cloning and expression of a novel adipose-specific collagen-like factor, apM1 (adipose most abundant gene transcript 1), Biochem. Biophys. Res. Commun. 221:286–289.

Maffei, M., J. Halaas, E. Ravussin. 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1:1155-1161.

Matsuzawa, Y. 2005. Adiponectin: Identification, physiology and clinical relevance in metabolic and vascular disease. Atheroscler. Suppl. 6:7–14.

Meier, U., A. M. Gressner. 2004. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chmical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem. 50:9:1-15.

McGowan, C. 2008. The role of insulin in endocrinopathic laminitis. J. of Equine Vet. Sci. 28; 10:603-607.

McManus, C. J., B. P. Fitzgerald. 2000. Effects of a single day of feed restriction on changes in serum leptin, gonadotropins, prolactin, and metabolites in aged and young mares. Domest. Anim. Endocinol. 19:1-13.

NRC. 2007. Nutrient Requirements of Horses. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Pass, M. A., S. Pollitt, C. C. Pollitt. 1998. Decreased glucose metabolism causes separation of hoof lamellae in vitro: a trigger for laminitis? Equine Vet. J. Suppl vol. 6:133-138.

Petersenn, S. 2002. Structure and regulation of the growth hormone secretagogue receptor. Minerva Endocrinol 27:243-256.

Raben, A., E. Mygind & A. Astrup. 1998. Lower activity of oxidative key enzymes and smaller fiber areas in skeletal muscle of postobese women. Am. J. Physiol. 275:E487–E494.

Radin, M. J., L. C. Sharkey, and B. J. Holycross. 2009. Adipokines: a review of biological and analytical principles and an update in dogs, cats, and horses. Vet. Clin. Pathology. 38(2):136-156.

Reaven, G. M. 1988. Role of insulin in human disease. Diabetes. 37:1595-1607.

Rendle, D. 2006. Equine laminitis 1. Management in the acute stage. In Pract. 28: 434-443

Sierra, M., J. J. Gracia, N. Fernández, M. J. Diez, A. P. Calle, A. M. Sahuagún, and Farmafibra Group. 2001. Effects of ispaghula husk and guar gum on post prandial glucose and insulin concentrations in healthy subjects. European. J. Clin. Nutr. 55:235-243.

Sierra, M., J. J. Gracia, N. Fernández, M. J. Diez, A. P. Calle, and Farmafibra Group. 2002. Therapeutic effects of psyllium in type 2 diabetic patients. European J. of Clin. Nutr. 56: 830-842.

Schwartz, M. W., S. C. Woods, D. Porte Jr., 2000. Central nervous system control of food intake. Nature 404:661-671.

Tschop, M., C. Weyer, P. A. Tataranni, V. Devanarayan, E. Ravussin, and M. L. Heiman. 2001. Circulating ghrelin levels are decreased in human obesity. Diabetes 50:707-709.

Willmore, J. H., D. L. Costill. 1994. Pages 122-143 in Human Kinetics. Physiology of Sport and Exercise, hormonal regulation of exercise. Willmore and Costill, ed. Champaign, IL.

Wren, A. M., L. J. Seal, M. A. Cohen, A. E. Brynes, G. S. Frost, K. G. Murphy, W. S. Dhillo, M. A. Ghatei, and S. R. Bloom. 2001b. Ghrelin enhances appetite and increases food intake in humans. J. CLin. Endocrinol. Metab. 86:5992.

Wren, A. M., C. J. Small, C. R. Abbott, W. S. Dhillo, L. J. Seal, M. A. Cohen, R. L. Batterham, S. Taheri, S. A. Stanley, M. A. Ghatei, and S. R. Bloom. 2001a. Ghrelin causes hyperphagia and obesity in rats. Diabetes 50:2540-2547.

Yamamoto, Y., H. Hirose, I. Saito, K. Nishikai and T. Saruta. 2004. Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: two-year follow-up study in Japanese population, J. Clin. Endocrinol. Metab. 89:87–90.

Yamauchi, T., J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B.B. Kahn and T. Kadowaki. 2002. Adiponectin

stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase, Nature Medicine 8**:**1288–1295.

Zhang, Y., R. Proenca, M. Maffei. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432.

Zhang, Y., M. Matheny, S. Zolotukhin. 2002. Regulation of adiponectin and leptin gene expression in white and brown adipose tissues: influence of beta 3-adrenorgic agonists, retinoic acid, leptin and fasting. Biochem. Biophys Acta. 2002. 1584(2-3):115.