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Effects of Early Spring Growth Annual Ryegrass Pasture Consumption on Parameters Associated with Laminitis in Horses

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EFFECTS OF EARLY SPRING GROWTH ANNUAL RYEGRASS PASTURE CONSUMPTION ON PARAMETERS ASSOCIATED WITH LAMINITIS IN HORSES

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Ten adult Quarter Horses (5 mares and 5 geldings) were placed in dry lot for 90 days and allowed free choice access to a diet consisting of average quality orchard grass hay, salt and water. The horses were then allowed free choice access to early-growth annual ryegrass pasture, salt and water for a 28 day period. Random hay and grass samples were analyzed for nutrient content. Blood samples were collected at 6 am, 8 am, 6 pm, and 8 pm on the final day of hay consumption and on the 4^{th}, 9^{th} and 28^{th} days of grass consumption. Samples were subsequently analyzed to determine the effects of diet type on circulating blood glucose, insulin, and triglyceride concentrations. Body weights and body condition scores were monitored on the first and last day of blood collection.

Ryegrass pasture consumption by horses in this trial resulted in body weight gains, increased body condition scores, and elevated insulin secretions. While blood glucose levels varied depending on day of sampling, there was no effect of diet type and

blood glucose concentrations. Sex of test subject did not affect any of the parameters measured. Nutrient content analysis of the forages fed was attempted, however results were skewed therefore further correlations could not be determined.

Chapter 1

INTRODUCTION

The horse evolved as a grazing animal, consuming small quantities of forage as he wandered, however performance horses today often exhibit high metabolic rates and therefore have greater nutrient requirements than an all forage diet can provide. In order to meet their requirements the traditional all forage diets have been supplemented with sometimes exceedingly large amounts of grain. These changes in feeding management practices have often been associated with laminitis, colic, founder, obesity and other debilitating metabolic disorders.

During the past decade research has been done in attempts to study effects of type of diet on the metabolic processes in horses. Recent studies have focused on the interrelationships between diet type, blood chemistry, other physiological parameters, and metabolic disorders (Glade et al. 1984; Stull and Roedieck 1988; Hoffman et al. 2003; Bailey et al., 2007).

Several authors have reported that obesity is associated with the development of insulin resistance and type II diabetes in humans (Boshell et al. 1968, Kahn et al. 2000). Recent research has shown that fatter horses also have higher insulin and glucose concentration spikes after the consumption of a meal as compared to thin horses (Hoffman et al., 2003; Johnson et al., 2004). Other studies with horses have also shown that overweight horses tend to develop insulin resistance (Hoffman et al., 2003) which may result in metabolic disorder such as founder (Jeffcott et al., 1986; Pass et al., 1998).

While type II diabetics have been shown to exhibit higher concentrations of plasma triglycerides than that of non-diabetics (Jain, 1980), several studies have reported

the type of diet a horse consumes has no affect on the circulating plasma triglycerides (Glade et al., 1984; Bailey et al., 2007).

Many studies have shown that the fatter horses are, the more likely they are to founder, regardless of type of diet consumed (Jeffcott et al., 1986; Alford et al., 2001; Kronfeld et al., 2005; Treiber et al., 2005). Numerous studies have evaluated the physiological effect of traditional grain/forage diets on various physiological parameters (Garner et al., 1975; Williams et al., 2001; Kronfeld and Harris, 2003). Obesity in horses may well be a factor in insulin resistance leading to laminitis (Treiber et al., 2005; Frank et al., 2006). Large amounts of non-structural carbohydrates ingested by obese horses intensifies insulin resistance (Hoffman et al., 2003; Treiber et al., 2005). However, more research is needed on the relationship of blood chemistry, physiology and metabolic disorders in horses consuming strictly all forage diets.

Identifying and eliminating conditions and factors that predispose horses to laminitis and founder would definitely be a valuable preventative nutrition management tool for horse owners.

Chapter 2

LITERATURE REVIEW

Laminitis and Founder

Laminitis is an extremely debilitating disease to the horse. It is the result of an allergic reaction in the body that settles in the hoof. It produces fever in the foot due to vasodilatation and results in inflammation of the laminea causing vasoconstriction and swelling (Pollit and Davies, 1998; Hood, 1999; Pollitt, 2003; Bailey, 2004). This causes an insufficient amount of blood flow to the foot and its supportive connective tissues. These supportive tissues hold together the P3 bone (coffin bone) to the hoof wall. Inflammation and lack of blood flow to the tissues damage and weaken the support structures which begins to die. The P3 bone rotates in a downward motion towards the sole of the horse's foot, yielding the conformation of a "dropped sole" an appearance characteristic of founder. The coffin bone often rotates so far that it protrudes through the sole of the horse's foot. There are different degrees of severity of laminitis and founder. The "dropped sole" occurs in the most debilitating cases of founder. The acute stage of laminitis is the easiest to treat and begins when the animal first exhibits lameness. The animal will only have inflammation of laminea at this stage. With corrective shoeing, preventing founder at this stage is possible. When complete rotation of the coffin bone occurs, the most humane option is to euthanize the animal. Animals who have previously foundered are predisposed to reoccurrence of the condition (Kronfeld, 2006).

There are several different theories as to the exact physical mechanism of founder in horses; but there is no cure for the disease. Ingestion of excessive quantities of nonstructural carbohydrates (as in a high grain diet), overgrazing of lush pastures, and

stress are the most common reasons that founder occurs in horses. Regardless of the cause, the result of all three conditions is inflammation in the laminea.

Pollit and Davies (1998) established that inflammation could happen within 16-40 hours after the consumption of an excessive amount of carbohydrates. Kane (2000) reported an increase in the number of cases of founder reported in certain regions of the United States during the spring and summer months as compared to fall and winter seasons when pasture is not as readily available. A study by Hintz (2000) revealed that 45.6% of lameness issues were due to the horse grazing lush pastures. Hoffman and coworkers (2003) found that grains contained higher levels of fermentable carbohydrates as compared to forages. Spring pastures contained higher fermentable carbohydrate concentrations than fall pastures. Most of pasture laminitis cases are reported in the spring. These research findings suggested that the hydrolysable and fermentable carbohydrates likely cause the laminitis.

Nearly 2% of the entire horse population in the U.S. founder each year (USDA 2000). The frequency increases to 5% in the spring and summer months, with almost half of these occurring in horses on pasture (Kane et al., 2000; USDA, 2000). These frequencies indicate the extent to which laminitis affects the horse industry.

Insulin and Glucose Metabolism

Insulin is the most potent anabolic hormone in the animal body. It is secreted from the beta islet cells in the pancreas and under normal conditions acts in conjunction with glucagon to regulate circulating glucose concentrations. Insulin binds with receptor

sites on skeletal muscle cells creating a channel to allowing glucose to enter the cell where it can be oxidized in the glycolytic pathway, or converted to glycogen for later use as an energy source. Insulin also stimulates the fat cells to store any excess glucose by utilizing it for fat synthesis.

The most common form of abnormal glucose metabolism in humans results in the disease known as type II diabetes, the non-insulin dependent, or adult-onset diabetes. The disease occurs most commonly in obese, inactive adults who habitually over eat diets consisting of excessive quantities of sugars and starch which are absorbed as blood glucose. This in turn places a great demand on the pancreas to produce larger and larger quantities of insulin in order to clear the sugar from the blood. If this pattern continues for extended periods of time, the receptors on muscle and fat cells, which are constantly being bathed in ever increasing insulin concentrations, begin to lose their sensitivity to insulin. They become "resistant" to the normal stimulus provided by insulin and no longer activate the removal of insulin from the blood stream. Long term "insulin resistance" will eventually lead to blood glucose concentrations of 5 to 10 times the normal levels requiring the injection of exogenous sources of insulin as the only means of regulating blood glucose concentrations in a "normal" range. If blood glucose levels are allowed to remain excessively elevated for an extended period of time, damage to the eyes, nerves, heart or kidneys may result. Over time the nerve damage expresses itself in loss of sensitivity in the lower extremities. Insulin has been shown to cause vasodilatation in the extremities (Middleton and French, 1974). The increase blood flow to the horses hoof associated with insulin resistance may therefore be partially responsible for the fever and the resulting damage done to the laminea. This allows more

blood to travel to the hoof causing swelling, heat, and tissue damage resulting in lameness. Since blood that enters the foot can only leave the hoof when hydraulic pressure is applied to the sole of the foot, the lame horse is unable to walk and therefore unable to force blood back up the leg resulting in compounding the effects of swelling and heat on laminar destruction.

Sinha and others (1996) found a positive correlation between what humans ingest and the circulating blood glucose concentrations. Raskin and others (1975) tested plasma glucagon levels infused with insulin and glucose in fasting diabetics and non diabetics. Insulin was administered at 0.03 U/kg min, for 2 hours, raising the mean insulin to 25-35 microU/ml, in the type II diabetics. The glucagon declined from its baseline at 71+2 (SEM) to 56±1 pg/ml at 120 min ($P < 0.001$). Non-diabetics subjects required infusion with much larger amounts of glucose to generate hyperglycemia than did the diabetic subjects. Their results showed that insulin resistance was a major factor in the inability of diabetics to normalize blood glucose concentrations following ingestion of a meal.

Sinha and others (1996) observed glucose and insulin levels in lean, obese, and obese type II diabetics. They were categorized by their body mass index (BMI) according to the National Institutes of Health Consensus Development Panel (NIHCDP, 1995). The lean, obese, and type II diabetics had BMI's of 24.3, 38.8, and 41.5 (kg/m^2); respectively, and blood glucose concentrations of 100.4, 103.3, and 196.1 (mg/dl); respectively. The insulin concentrations were found to be 14.4, 25.6, and 30.7 (microU/ml); respectively, for lean, obese, and obese type II diabetics. These values are similar to reports of non-diabetics for insulin of Raskin and others (1975). These findings are similar to those of Shen and others (1970). Shen et al. reported that glucose

concentrations in diabetics compared to non-diabetics was 52% higher. The above studies indicated that non-diabetics have lower blood insulin and glucose levels than those of obese type II diabetics. The non-diabetics also had lower glucose and insulin concentrations than that of obese non-diabetic individuals. These results indicate that humans who are type II diabetic and those who are obese tend to demonstrate insulin resistance.

Researchers have studied the relationship between obesity and type II diabetes and insulin resistance for some time now (Boshell et al., 1968; DeFonzo et al., 1991; Kahn et al., 2000). Boshell and others (1968) reported that 59% of the 231 obese test subjects displayed abnormally high blood glucose concentrations following glucose tolerance tests. Similar results were reported by Paullin and Sauls (1922) and John (1929). Boshell and others (1968) continued his study by observing two obese diabetics and three non-diabetic and healthy weight subjects following glucose infusion. It was found that the blood glucose concentration of the obese diabetic group was 321 mg/dl (four times the normal amount) while the insulin concentration was 555 microU/ml. The non-diabetic group averaged concentrations of only 176 mg/dl (twice the normal) for glucose and 131 microU/ml. Therefore, it appears that obese diabetics often projected high blood glucose and insulin levels. They continued to study what the effects weight loss would have on the levels of insulin and glucose in both groups, and found that weight loss results in adipose cell size. Glucose tolerance tests showed a decrease in blood glucose concentrations while insulin sensitivity increased. This study was in agreement with Boshell et al. (1968) who found a positive correlation between obesity and insulin resistance.

The relationship between type II diabetes and insulin resistance has been studied extensively. Type II dibetes is wide spread and seemingly controllable with exercise, proper diet and weight. The percent body fat and insulin sensitivity of children was reported to be highly correlated (Arslanian et al., 1996). Up to 50% of severely overweight children exhibit insulin resistance, and 27% of adults in the United States have an insulin resistance syndrome (Biddinger and Kahn, 2006).

Insulin and Glucose in the normal (non-insulin resistance) horse

Stull and Roedieck (1988) researched the basal blood glucose and insulin levels of four, 2 year old Quarter Horse geldings consuming four separate diets. The trial was done in a one month period, allowing one week for each trial. The diets were 100% alfalfa, 50% alfalfa and 50% corn, 100% corn, and 90% corn and 10% corn oil (diet CO). Diets consisting of alfalfa and of corn and corn oil produced more consistent plasma glucose levels than those of the half alfalfa and half corn diet, and the 100% corn diet. Blood glucose concentrations of horses consuming the two diets peaked at the 1100 hr at 140-150 mg/dl. Although the basal and postprandial plasma glucose values for all diets were not different, the mean basal insulin value of horses consuming all diets was 4.7+/- 1 microU/ml and did not differ (P>05) with respect to diet. Blood insulin levels of horses consuming the diets consisting of 100% alfalfa and the corn and corn oil did not differ from basal value. However, the corn and alfalfa diet produced an increase in plasma insulin concentrations of twelve fold and the all corn diet generated a seven fold increase. The peak plasma insulin concentrations were at 75 to 95 minutes following a meal, which coincides with the plasma glucose peak.

Glade and coworkers (1984) compared blood insulin and glucose levels after the

ingestion of two different diets, one being 80% and the other being double that at 160% of the recommended energy and protein requirements suggested by the National Research Council. The diets consisted of corn and a pelleted hay-concentrate at the appropriate ratio to formulate the above diets. The horses' insulin concentrations peaked 2 hours sooner when fed the 160% diet, as well as declined sooner. At the 8th hour after ingestion of the meals, all insulin concentrations returned to their original level. The glucose concentrations were not affected by either diet.

These results suggested that horses metabolized glucose and insulin in similar fashion to humans. They also suggested that circulating blood glucose and insulin concentrations are virtually identical in horses and humans.

Williams and coworkers (2001) compared plasma insulin and glucose concentrations in thoroughbred mares fed a pelleted concentrate (a conventional sweet feed high in sugar and starch), or a high fat and fiber diet. The baseline values for all diets were 74.7 ± 10.9 mg/dl for glucose and 5.86 ± 1.80 mIU/L for insulin. The peak concentration for plasma insulin and glucose was higher in the group fed the high sugar and starch diet. Thus, the amount of sugar and starch in a diet rather than the fat and fiber moderates the fluctuations in blood glucose and insulin that could be linked to grain-based metabolic disorders.

Insulin resistance in the horse

Insulin resistance in the horse is the insensitivity of muscle, liver and adipose tissues at the cell's surface to the activity of the hormone insulin (Kronfeld et al., 2005). Researchers have studied insulin resistance, obesity, founder and other metabolic

disorders and their interweaving relationships. Insulin resistance has been associated with laminitis in horses (Kronfeld et al., 2005). Johnson and coworkers (2004) recognized that obesity might predispose horses to insulin resistance which can lead to laminitis.

Bailey and coworkers (2007) compared plasma insulin and glucose concentrations of two groups of ponies, one group being previously laminitic and the other non-laminitic, eating grass pasture followed by hay. They did a second study adding inulin (a commercial form of fructan) to the hay. The non-laminitic ponies showed no difference in their serum insulin concentrations on either of the diets. The previously laminitic ponies had a significant decrease in their serum insulin concentrations when changed from a pasture grass diet to a hay diet, 23.8 mU/L to 15.6 mU/L, which was greater than the non-laminitic ponies. By the time the previously laminitic ponies had adjusted to the hay diet, there was no longer a difference in blood insulin concentrations between the two groups consuming the hay diets. At the beginning of the second study there was no difference in the serum insulin concentrations in either of the groups on the hay. Addition of inulin to the diet the previously laminitic horses' resulted in serum insulin levels approximately six fold higher than the non-laminitic ponies. These results indicated that the laminitic ponies were insulin resistant and had an inflated insulin response to the high fructan carbohydrate concentration.

Coffman and Colles (1983) compared insulin and glucose concentrations of 6 laminitic and 6 control ponies. They were subjected to an insulin sensitivity test. The trial was done twice, once in the fall and then the next spring. In the fall trial the laminitic ponies' glucose levels were higher than the non-laminitis ponies at 30, 60, 90,

120, 180 and 240 minutes post insulin injection. During the spring trial however the laminitic ponies had higher glucose levels at 120 min. post insulin injection. These results indicated that once again, the laminitic ponies were less sensitive to the injected insulin.

Vick and coworkers (2007) compared the relationship of insulin sensitivity, body condition score, body weight, and percent body fat. They used 60 mares of mixed light breeds and found reported that the body condition score of a horse and the percent body fat were negatively correlated with insulin sensitivity. Their data plural further corroborate the conjecture that the more obese a horse is the more likely (he/she) is to be insulin resistant.

Triglycerides

Following absorption, dietary fatty acids join together with glycerol to form triglycerides which are transported in blood plasma for deposition in muscle, fat, and liver tissue cells. The horse may utilize excess dietary carbohydrates, fats, and protein to generate endogenous triglycerides.

Excessively high concentrations of blood triglycerides have been implicated as possible causes of metabolic disorders in humans. If left untreated, high levels of triglycerides can lead to many diseases such as myocardial infarction and arthrosclerosis (Stampfer et al., 1996; de Man et al., 1996). Type II diabetics typically exhibit elevated levels of blood triglycerides (Chait and Brunzell, 1996). Zargar and coworkers (1995) conducted a study to determine the serum lipid profile of type II diabetes patients. 50

obese type II diabetics and 20 obese controls with normal glucose tolerance tests were used. A BMI of 27.8 and 27.3 kg/m^2 for males and females, respectively, were considered obese. They reported that obese type II diabetics had higher levels of circulating plasma triglycerides when compared to obese non-diabetics. This evidence supports the findings that higher levels of circulating plasma triglycerides in diabetics versus non-diabetic controls (Jain et al., 1980; Santen et al., 1970; Sharma, 1970).

Glade and coworkers (1984) subjected eight thoroughbred 6 to 8 month old weanlings two different diets consisting of corn and a pelleted hay-concentrate, one being 80% and the other 160% of the NRC (1978) for protein and energy requirements. They found no significant increase in the plasma triglyceride levels on either diet, which is similar to findings of Bailey et al., (2007) who fed a grass and hay diet,

These results indicated that horses are efficient in removing triglycerides from the circulatory system, and depositing them in the adipose tissue. Therefore, circulating triglyceride concentrations are probably not adequate indicators of nutrient intake, energy utilization, body fat deposition, or weight gain.

The Equine Digestive Tract

Foraging practices of wild horses probably led to fewer metabolic disorders than are observed in domestic horses today. Modern management practices often confine horses to stalls, allowing limited access to exercise and feed grain based diets. These changes in their diet and activity level have led to the development of ailments other than laminitis and founder, such as colic and ulcers.

Many studies have been conducted to determine the ideal diet for horses. One

factor of considerable consideration has been to determine the best glycemic index for horse diets. (Glade et al., 1984; Stull and Roedick, 1988; Pagan et al., 1999; Groff et al., 2001; Williams et al., 2001; Roediek, 2003; Jose-Cunilleras et al., 2004). The glycemic index is a numerical value system that is used to measure the extent to which consumption of a given feed elevates blood sugar levels of the animal that consumes it. The glycemic index for horses has been used as a parameter to measure the effect of diet type and on blood glucose concentration. Kronfeld et al., (2004) observed that circulating blood glucose concentrations are affected by dietary intake, however they also determined that the sugar content of horse diets is not linearly related to the glycemic index. Jose-Cunilleras and coworkers (2004) also found the glycemic index to be important when attempting to moderate the blood glucose concentration spikes associated with high sugar diets. Therefore, it appears that the glycemic index provides a useful management tool in the prevention of metabolic diseases in horses (Kronfeld et al., 2005). High sugar diets produce much higher glycemic indices than low sugar diets.

Body Condition Score

A system was developed by Henneke and coworkers (1983) to score horses based on the amount of finish (fat) that is exhibited by the animal. It is a numerical scoring system in which horses are assigned a values ranging from 1 to 9. The system involves measurement of condition on six anatomical structures of the horse's body: the neck, withers, ribs, hip, loin and tail head. The amount of fat deposited is measured by palpation and visualization of these different sites. An animal receiving a score of 1 is extremely emaciated and will have protruding hip bones and ribs, with their skeletal structure being easily visible. This animal is near death due to starvation. An animal that

is moderately fleshy receives a score of 5. This horse will have structures that blend smoothly with no visible skeletal delineation; however, the examiner will still be able to palpate the animal and feel its ribs. Horses receiving a body condition score of 9 are extremely obese. They exhibit a significant amount of fat deposition over all structures, and skeletal structures cannot be discerned through palpation.

This body condition scoring system has become the industry standard and is a valuable feeding management tool. Owners can easily monitor their horse's body condition and quickly determine the type and amount of feed needed to maintain the ideal body condition score of 5 to 6.

It has been reported that the body condition of the horse has an effect on the horse's metabolic response to a diet. Hoffman et al., (2003) observed that obese horses tend to exhibit insulin resistance much more frequently than moderately fleshy horses. They also observed that obese horses fed high sugars and starch diets tended to be less sensitive to insulin than thin horses fed the same diet.

Pasture Nonstructural Carbohydrates

Virtually half of all cases of animals foundering in the spring and summer months occur when horses are consuming an all pasture diet (USDA 2000). When photosynthesis is occurring, grasses take in atmospheric carbon dioxide with energy from light to produce oxygen and carbohydrates, such as glucose or starch. Extra sugars are stored as "reserve" carbohydrates. The C_3 grasses store fructan as this "reserve" carbohydrate in their vegetative tissues until needed. High concentrations of fructans are

frequently observed in C_3 grasses, particularly during early periods of rapid growth. C_4 grasses and legumes do not produce fructans, however they produce more readily available glucose in the form of starch which yields a high glycemic index when consumed by horses. These C_4 grasses and legumes are more prevalent and productive during the early summer months. Immature C_4 plants yield higher starch concentrations than mature high fiber plants.

Lush growth of immature plants appears to produce high sugar concentrations in commonly consumed grass and legume pastures. These sugar concentrations have been shown to be positively correlated with laminitis. Pollitt et al. (2003) found that administration of fructan boluses induced laminitis in horses. It appears that the increased incidence of laminitis and founder associated with grazing early spring and summer pastures may is due to the elevated sugar concentrations.

Objective

The objective of the present study was to determine the short term effects on horses following the conversion from an ad-libitum orchard grass hay diet to an ad-libitum early spring growth annual ryegrass pasture diet. The parameters measured were body condition score, body weight, blood insulin, glucose, and triglyceride concentration.

Chapter 3

MATERIALS and METHODS

Test Subjects

Ten mature adult American Quarter Horses (five mares and five geldings) were adapted to a diet which consisted of free choice access to orchard grass hay from November until April, supplemented with trace mineralized salt, and water. On April 1, 2008, their diet was then changed to ad-libitum ('Marshall') annual ryegrass pasture. The horses were supplemented with trace mineralized salt and water. They were not forced to exercise during the trial. Each horse was assigned an identification number that remained throughout the study. All values reported are considered significant or nonsignificant at the $P<0.05$ level.

Body weight and body condition scores were recorded on day 1 (last day on hay consumption) and on day 28 (the final day of trial on annual ryegrass pasture). The weights were taken on day 1 and 28 using a portable agricultural scale (model MTI-500WB, MTI Weigh Systems, Inc. North Kingstown, Rhode Island). The three daily weights obtained for each horse were averaged to establish mean weights. Body condition scores were evaluated by three independently trained technicians following the system developed by Don Henneke. Scores were averaged to derive a mean body condition.

Blood Sampling

Blood samples were obtained on day 1 (the final day of hay consumption), and on days 4, 9, and 28 of annual ryegrass pasture consumption. Samples were collected via jugular veinapuncture in vacutainers tipped with 20 gauge needles by trained technicians. Samples were drawn at the feeding site during the 6^{th}, 8^{th}, 18^{th}, and 20^{th} hour from alternating sides of the neck to avoid unnecessary stress associated with veinapuncture. The horses were released immediately to continue feeding. Blood glucose concentrations were determined using an Ascensia Contour blood glucose monitoring system and corresponding test strips (Bayer Health CareLLC, Mishawaka, Indiana) as per Monfort (2007). Samples were immediately centrifuged for five minutes. Serum was collected using a disposable pipette, and placed in marked test tubes and frozen at -1^{o}C.

Sample Analysis

One cc of serum from each sample was analyzed for blood triglyceride concentrations by an Advia-1200 Chemistry System Photometric Analysis (Deerfield, Illinois). The procedure is a GPO Trinder, Endpoint reaction. Analysis was conducted by the University of Georgia Diagnostics and Invest Laboratory, an AAVLD certified laboratory.

One cc of serum from each sample was analyzed to determine insulin concentrations using a radioimmunoassay insulin kit manufactured by Diagnostics Systems Laboratory (Webster, Texas) at the Diagnostic Center for Population and Animal Health at Michigan State University.

Forage Sampling

Samples for analysis were taken from all forages being fed to the hores. Each 400 kg bale of hay presented for consumption was core sampled using a forage probe in five different locations prior to feeding. Samples from the annual ryegrass pasture were taken on the days that blood was drawn (days 4, 9 and 28). Three triangular enclosures, each consisting of three 6 foot tall, 8 foot long panels, were randomly placed in the pasture. On the days that blood was collected, grass in the enclosures was cut at the same level as the remaining pasture. These grass samples were then dried in a microwave oven and stored in brown paper bags until analysis could be rendered. The enclosures were randomly placed in new locations within the pasture following each collection period.

Forage Analysis

The dried forage samples were analyzed using near infrared radiation (Cumberland Valley Analytical Services Inc, Maugansville, Maryland).

Statistical Analysis

Analysis of data was done using analysis of variance and paired t-tests on Microsoft Excel.

Chapter 4

RESULTS and DISCUSSION

Body Weight

Table 1 includes the initial and final mean weights, and changes in body weight of the horses. Initial mean body weights ranged from 501.2 kg to 588.8 kg. Final mean body weights ranged from 531.3 kg to 623.1 kg. Gelding 4 lost weight during the 28 days of pasture consumption; all other animals had significant weight gain. There was no logical explanation for the weight loss exhibited by this one animal. The data indicate that there was an increase ($P < 0.05$) in body weight of horses that can be attributed to the change in diet. The total mean weight gain of all horses during the 28 days on the annual ryegrass diet was 23.6 kg, giving an average daily gain of 0.94 kg. The weight gains indicated that energy of the ryegrass pasture diet was sufficiently high to jeopardize health of adult moderately fleshy or obese horses since we know that obesity is positively correlated with insulin resistance (Vick et al., 2007), laminitis, and founder (Hoffman et al., 2003).

These results on weight gain support those of weight and body condition associated with consumption of early growth lush pastures in the spring as opposed to hay diets provided during the fall and winter. (Pollock, 1982; Housley and Pollock, 1993) They found that gains were due to the large quantities of sugars and starch derived from young vegetation. They suggested that the horse's body responds by rapidly expanding the adipose tissues, yielding increases in weight and body condition score.

Although there was variation in individual responses to dietary change, there was no effect of the sex of the test subject on body weight on day 1 or on day 28. These

results are consistent with those of (Monfort, 2007) and indicate that sex of the animal has no effect on the utilization of nutrients supplied by grass hay and pasture diets.

Table 1

Mean body weight (kg) change of horses from day 1 to day 28 of horses converted from an orchard grass hay diet to an annual ryegrass pasture diet

Horse	Day 1	Day 28	Net Weight Change
G1	543.3	560.2	16.9
G2	535.7	566.5	30.8
G3	501.2	542.2	41
G4	570.2	567.8	-2.4
G5	588.8	623.1	34.3
M6	514.8	531.3	16.5
M7	533.6	554.2	20.6
M8	541.5	592.4	50.9
M9	576.5	579.1	2.6
M10	539.3	564.3	25
Mean	544.5	568.1	23.6_a

a. indicates a difference at $P < 0.05$ in weight change among the horses

Body Condition Score

Body condition scores were recorded on day 1, the last day of hay consumption and day 28, the final day of pasture consumption. Initial body condition scores ranged from 4 to 8, while final body condition scores ranged from 5.5 to 8.5. The mean values and changes in body condition score are listed in Table 2. While 9 out of the 10 body condition scores remained constant or increases, gelding 4 exhibited a 0.5 body condition score decrease. This is expected since he lost 2.4 kg of body weight. The mean body condition score of all horses in the trial increased ($P<0.05$) by 0.4 during the 28 days that they consumed the ryegrass pasture diet. An increase in body condition score that large

in a 28 day period indicates that moderately fleshy horses could be expected to raise their body condition score by a factor of approximately 1 every 60 days when consuming this pasture diet and thus change physically from a moderately fleshy animal to a fleshy or fat horse in the same time period. By the same token an obese horse (body condition score of 8 or 9) could be expected to become morbidly obese during the same time frame. One of the test subjects had an increase of 1.5 in his body condition score in 28 days. Changes of this magnitude in such a short time frame would be advantageous for thin, emaciated and debilitated horses; they could produce devastating results in overly fat animals. These data indicated that the horses were rapidly accumulating adipose tissue in response to consumption of the annual ryegrass pasture.

The sex of the test animal had no effect on body condition scores of animals consuming either diet (Table 2). These results are consistent with the findings of Monfort (2007) on sex of the test subject and body condition score.

Table 2

Mean body condition score changes of horses on day 1 and day 28 of horses being converted from an orchard grass hay diet to an annual ryegrass pasture

Horse	Day 1	Day 28	Change
G1	7	8	1
G2	7.5	7.5	0
G3	4	5.5	1.5
G4	7	6.5	-0.5
G5	7	7.5	0.5
M6	8	8	0
M7	6.5	6.5	0
M8	7	7.5	0.5
M9	6	6.5	0.5
M10	8	8.5	0.5
Mean	6.8	7.2	0.4_a

a. indicates a difference at $P < 0.05$ in the change of body condition score among horses

Glucose

Mean blood glucose concentrations of all horses in the trial are shown in Table 3. The mean glucose concentrations for days 1, 4, 9, and 28 were 57.5 mg/dl, 59.7 mg/dl, 65.6 mg/dl, and 59.8 mg/dl respectively. Day 9 mean was higher ($P < 0.05$) than those for other test days, the values were within the normal expected values for horses consuming a grass pasture diets as reported by other authors (Stull et al., 1988; Hoffman et al., 2003). Since food passes through the equine digestive track within 7 days, glucose samples were drawn on day 9 to accurately assess the body's response to the change from a hay to pasture diet. The resultant day 9 blood glucose concentrations ranged from 48.75 mg/dl to 72 mg/dl. The elevated blood glucose concentrations associated with day 9 of collection may indicate that the horses endocrine systems had not adapted to the high sugar content of the pasture diet. Normal blood glucose concentrations in a healthy adult horse range from (60-120 mg/dl) depending on their diet (Stull et al., 1988; Hoffman et

al., 2003). All mean values in the present study were within that range. These concentrations are similar to those observed in non-diabetic humans (Sinha et al., 1996) and horses observed in other studies (Monfort, 2007). Sinha et al. (1996) found a positive correlation between sugar content and glycemic index of the diet with circulating blood glucose concentrations. Similar results were reported by (Stull and Roedick, 1988; Hoffman et al., 2003) in studies done with horses. Bailey et al. (2007) found that previously laminitic, and non-laminitic ponies exhibited normal glucose concentrations (82.8mg/dl) when consuming a grass diet.

Mares had a lower blood glucose concentration on day 4 of the trial, they were within the normal expected range reported in other studies. Sex of the test subject had no effect on blood glucose concentrations of any other sampling day. These results support the findings of Monfort (2007) that blood glucose concentrations of mares and geldings consuming grass pastures are not different and that female steroid hormones do not affect the blood glucose concentrations of horses (Monfort,2007).

Table 3

Mean blood glucose concentrations of horses (mg/dl) from sampling days consuming different diet types

Horse	Day 1 Hay	Day 4 Grass	Day 9 Grass	Day 28 Grass
G1	60.3	66.5	66	54.4
G2	69.8	59.8	71.4	58.2
G3	58	59	66.4	63.2
G4	56.8	62.8	66.6	64
G5	55.3	62	63.8	60.6
M6	48.8	54.5	61.8	54.2
M7	55.3	57.8	67.8	69
M8	61.5	60.5	72	65.6
M9	49	51.5	54.2	49
M10	60.5	62.8	66.4	59.4
Mean	57.5 $_{a}$	59.7 $_{a}$	66.6 $_{b}$	60.5 $_{a}$

a. indicates no difference in change of blood glucose among horses
b. indicates a difference at P <0.05 in change of blood glucose among horses

Insulin

Blood insulin concentrations of the test animals are presented in Table 4. The mean blood insulin concentrations for days 1, 4, 9, and 28 were 61.4, 176.2, 183.4, and 123.8 pmol/L; respectively. Blood insulin concentrations of horses consuming the orchard grass were found to be lower (P<0.05) than for any days the horses were consuming the annual ryegrass pasture. Horses secrete insulin in response to the diets being consumed (French and Pollit, 2004). These data suggest that blood glucose values were held constant through increases in insulin secretion by the pancreas. These observations are further supported by the earlier noted increase in body condition score and body weight associated with the elevated insulin values. Elevated sugar and or starch concentrations in the ryegrass diets likely were responsible for the resulting elevated blood insulin concentrations observed in the horses consuming the ryegrass pasture diet. These results are similar to those of other researchers (Hoffman et al., 2003; Bailey et al.,

2007).

` In humans, insulin resistance is provoked by long term ingestion of high carbohydrate concentration diets, (Reaven, 1988), French and Pollit (2004), reported similar results in horses. Our data suggest that horses on ryegrass pasture diet were consuming large quantities of carbohydrates predisposing them to insulin resistance, laminitis and founder as reported by Hoffman et al. (2003).

Kane, (2000) and USDA, (2000) observed that horses founder more frequently and readily when grazing lush pastures. The high insulin concentrations along with the gain in weight and increased body condition scores of horses consuming the grass pasture diet in the present study all point toward an increased predisposition to insulin resistance and founder. If the horses in this trial had been allowed to consume the pasture for a longer period of time, they may have exhibited laminitis and a resultant founder.

Monfort (2007) found that obese horses consuming grass pasture in August had a mean plasma insulin concentration of 95.8 pmol/L. The obese horses had body condition scores of 7 and 8. In the present study horses with a mean body condition score of 6.8 which was low because gelding 3 had a body condition score of 4. The blood insulin concentrations on grass were 176.2 pmol/L, 183.4 pmol/L, and 123.8 pmol/L which is much higher than that observed by Monfort (2007). These data suggested that horses consuming ad libitum quantities of lush grass pasture may exhibit signs of insulin resistance.

Statistical analysis indicated that gender of the test subject had no effect on blood insulin concentrations ($P < 0.05$) and further substantiate the findings of Monfort (2007)

that female steroid hormone patterns appear to have no affect on blood insulin concentrations.

Table 4

Mean blood insulin concentrations (pmol/L) of horses from sampling days consuming different diet types

Horse	Day 1 Hay	Day 4 Grass	Day 9 Grass	Day 28 Grass
G1	46.5	117.3	127.8	66.4
G2	104.3	423	585.2	323
G3	35	77	67.6	95.6
G4	75	97.3	130.4	132.6
G5	33.5	79.3	88	83.8
M6	50.3	114.5	98.4	81.6
M7	82.8	345.5	230.8	149.2
M8	58.3	147.3	193.6	156.6
M9	55.5	88.5	113.6	55.6
M10	73.3	272	199	93.2
Mean	61.4 [a]	176.2 [b]	181.1 [b]	130.2 [b]

a. indicates a significantly lower insulin concentration at the $P < 0.05$ among horses
b. indicates no difference in insulin concentrations among horses

Triglyceride

Results in table 5 show that mean blood triglycerides concentrations were not affected by type of diet throughout the duration of the study. These findings are consistent with other studies by Treiber et al., 2005b who fed a mixed grass/legume pasture with either a corn and molasses diet or a corn oil and fiber diet, and those of Bailey et al., 2007 who fed ponies of mixed breeds a grass diet and a hay diet. While there was no increase in blood triglycerides, the increases in body condition scores and body weight of these horses indicated that excess energy was most likely converted to and deposited as white adipose tissue.

Table 5

Mean blood triglyceride concentrations (mg/dl) of horses from sampling days consuming different diet types

Horse	Day 1 Hay	Day 4 Grass	Day 9 Grass	Day 28 Grass
G1	33.8	39.5	39.8	44.2
G2	40.8	40	65	55
G3	29.5	18.3	25	27
G4	36.3	37	37.8	31.4
G5	26.5	27.3	28.8	38
M6	26.3	24	24.2	26.6
M7	44.0	35	42.4	39.8
M8	45.8	36	38	41
M9	30.5	35.5	31.2	31
M10	32.3	29.5	35.2	27.4
Mean	34.6	32.2	36.5	37.2

No differences were found

Forage Analysis

Statistical analysis of forage data was not completed due to apparent faulty laboratory analysis methodology. Table 6 presents forage nutrient data received. While the data presented are the calculated values obtained by an NIR machine, the crude protein values are approximately twice values expected for Kentucky orchard grass hay, ryegrass pastures and the TDN values represent energy values that are 20 to 30 percent higher than values normally observed for Kentucky orchard grass hay and ryegrass pastures. These inconsistencies indicate the analysis is flawed to the point that results are inaccurate and of no value.

Table 6

Forage analysis means from the compilation of samples

	Crude Protein	Available Protein	TDN	ADF	NDF	Crude Fat	Starch	Sugar
Hay	17.7	16.1	60.6	35.2	62.2	3.4	2.3	4.9
Day 4	22.0	21.0	67.5	24.9	46.6	4.3	3.9	11.2
Day 0	19.1	17.7	61.2	32.7	60.1	3.6	2.7	5.8
Day 28	19.9	19.1	65.5	27.0	50.7	4.4	2.7	13.9

Chapter 5

IMPLICATIONS

Horses that were adapted to an orchard grass hay diet throughout the winter and then given free choice access to lush annual ryegrass pasture in April exhibited physiological changes that may be predisposing factors in the development of laminitis and founder. These changes included significantly elevated blood glucose concentrations, subsequently significant increases in circulating insulin levels, weight gain (0.94 kg per day) and significant increases of body condition scores (0.1 per week), all occurring in a very short time period (4 weeks).

The combined effect of these changes indicates that horses in this study may have been developing the initial symptoms of insulin resistance which has been shown to be associated with laminitis and founder. The data further suggest that horses allowed to freely graze early growth annual ryegrass pasture need to be closely monitored for weight gain and that obese horses (animals with body condition scores of 7 or more) may be considered at too "high risk" to graze these pastures.

The most effective means of eliminating laminitis and other metabolic disorders associated with obesity is simply to prevent its occurrence. This is most easily done by limiting "high risk" horses access to lush pastures.

The rapid weight gain, increased body condition scores and affects on blood parameters of horses indicated that annual ryegrass pasture would be suitable for feeding horses that are thin, debilitated, and/or underweight. The high sugar content of the ryegrass pastures facilitated rapid body tissue restoration resulting in significant gains in

body weight and condition. Additional research would be beneficial to further elucidate the relationship between pasture consumption and the parameters measured in this study.

Table Appendix

Table 1

Body Weights (kg) of Horses Consuming an Orchard Grass Hay Diet

Horse	Weight 1	Weight 2	Weight 3
G1	545.7	546.6	537.5
G2	527.5	528.9	550.7
G3	499.4	502.6	510.7
G4	574.2	568.8	567.4
G5	580.1	581.1	605.1
M6	515.3	513.0	516.2
M7	531.2	531.2	538.4
M8	545.2	540.7	538.4
M9	579.2	578.3	572.0
M10	533.3	531.2	553.8

Table 2

Body Weights (kg) of Horses Consuming an Annual Ryegrass Pasture Diet for 28 Days Weights Taken on Day 28

	Weight 2	Weight 3
	558.4	561.1
	565.2	567.4

		543.4	536.1
		561.1	566.1
		620.1	625.5
		529.3	533.0
		553.8	556.6
		592.8	591.5
		576.1	580.6

	566.1	562.9

Table 3

Body Condition Scores for Day 1

Horse	Score 1	Score 2	Score 3	Day 1
G1	7	7	7	7
G2	7	7.5	8	7.5
G3	4	4	4	4
G4	7	6.5	7.5	7
G5	7	7	7	7
M6	8	8	8	8
M7	6.5	6.5	6.5	6.5
M8	7	7	7	7
M9	6	6	6	6
M10	8	8	8	8

Table 4

Body Condition Scores for Day 28

Horse	Score 1	Score 2	Score 3	Day 28
G1	8	8	8	8
G2	7	7.5	8	7.5
G3	5	5.5	6	5.5
G4	6.5	6.5	6.5	6.5
G5	7.5	7.5	7.5	7.5
M6	8	8	8	8
M7	6	6.5	7	6.5
M8	7.5	7.5	7.5	7.5
M9	6.5	6.5	6.5	6.5
M10	8	8.5	9	8.5

Table 5

Mean blood glucose concentrations (mg/dl) of horses from sampling days 1 vs day 4 consuming different diet types

Horse	Day 1 Hay	Day 4 Grass	Change
G1	60.3	66.5	6.2
G2	69.8	59.8	-10.0
G3	58	59	1.0
G4	56.8	62.8	6.0
G5	55.3	62	6.7
M6	48.8	54.5	5.7
M7	55.3	57.8	2.5
M8	61.5	60.5	-1.0
M9	49	51.5	2.5
M10	60.5	62.8	1.3
Mean	57.5	59.7	2.2_a

a. indicates no difference in change of blood glucose among horses

Table 6

Mean blood glucose concentrations (mg/dl) of horses from sampling day 1 vs day 9 consuming different diet types

Horse	Day 1 Hay	Day 9 Grass	Change
G1	60.3	66	5.7
G2	69.8	71.4	1.6
G3	58	66.4	8.4
G4	56.8	66.6	9.8
G5	55.3	63.8	8.5
M6	48.8	61.8	13
M7	55.3	67.8	12.5
M8	61.5	72	10.5
M9	49	54.2	5.2
M10	60.5	66.4	5.9
Mean	57.5	66.6	9.1_a

a. indicates a difference at $P < 0.05$ in change of blood glucose among horses

Table 7

Mean blood glucose concentrations (mg/dl) of horses from sampling days 1 vs day 28 consuming different diet types

Horse	Day 1 Hay	Day 28 Grass	Change
G1	60.3	54.4	-5.9
G2	69.8	58.2	-11.6
G3	58	63.2	5.2
G4	56.8	64	7.2
G5	55.3	60.6	5.3
M6	48.8	54.2	5.4
M7	55.3	69	13.7
M8	61.5	65.6	4.1
M9	49	49	0
M10	60.5	59.4	1.1
Mean	57.5	60.5	3 [a]

a. indicates no difference in change of blood glucose among horses

Table 8

Mean blood glucose concentrations (mg/dl) of horses from sampling day 4 vs day 9 consuming different diet types

Horse	Day 4 Grass	Day 9 Grass	Change
G1	66.5	66	-0.5
G2	59.8	71.4	11.6
G3	59	66.4	7.4
G4	62.8	66.6	3.8
G5	62	63.8	1.8
M6	54.5	61.8	7.3
M7	57.8	67.8	10
M8	60.5	72	11.5
M9	51.5	54.2	2.7
M10	62.8	66.4	3.6
Mean	59.7	66.6	6.9 [a]

a. indicates a difference at $P < 0.05$ in change of blood glucose among horses

Table 9

Mean blood glucose concentrations (mg/dl) of horses from sampling days 4 vs day 28 consuming different diet types

Horse	Day 4 Grass	Day 28 Grass	Change
G1	66.5	54.4	-12.1
G2	59.8	58.2	-1.6
G3	59	63.2	4.2
G4	62.8	64	1.2
G5	62	60.6	-1.4
M6	54.5	54.2	-0.3
M7	57.8	69	11.2
M8	60.5	65.6	5.1
M9	51.5	49	-2.5
M10	62.8	59.4	-3.4
Mean	59.7	60.5	0.8 [a]

a. indicates no difference in change of blood glucose among horses

Table 10

Mean blood glucose concentrations (mg/dl) of horses from sampling day 9 vs day 28 consuming different diet types

Horse	Day 9 Grass	Day 28 Grass	Change
G1	66	54.4	-11.6
G2	71.4	58.2	-13.2
G3	66.4	63.2	-3.2
G4	66.6	64	-2.6
G5	63.8	60.6	-3.2
M6	61.8	54.2	-7.6
M7	67.8	69	1.2
M8	72	65.6	-6.4
M9	54.2	49	-5.3
M10	66.4	59.4	-7.0
Mean	66.6	60.5	-6.1 [a]

a. indicates a difference at $P < 0.05$ in change of blood glucose among horses

Table 11

Mean blood insulin concentrations (pmol/L) of horses from sampling day 1 vs day 4 consuming different diet types

Horse	Day 1 Hay	Day 4 Grass	Change
G1	46.5	117.3	70.8
G2	104.3	423	318.7
G3	35	77	42
G4	75	97.3	22.3
G5	33.5	79.3	45.8
M6	50.3	114.5	64.2
M7	82.8	345.5	262.7
M8	58.3	147.3	89
M9	55.5	88.5	33
M10	73.3	272	198.7
Mean	61.4	176.2	114.8 [a]

a. indicates no difference in insulin concentrations among horses

Table 12

Mean blood insulin concentrations (pmol/L) of horses from sampling day 1 vs day 9 consuming different diet types

Horse	Day 1 Hay	Day 9 Grass	Change
G1	46.5	127.8	81.3
G2	104.3	585.2	480.9
G3	35	67.6	32.6
G4	75	130.4	55.4
G5	33.5	88	54.5
M6	50.3	98.4	48.1
M7	82.8	230.8	148
M8	58.3	193.6	135.3
M9	55.5	113.6	58.1
M10	73.3	199	125.7
Mean	61.4	181.1	119.7 [a]

a. indicates no difference in insulin concentrations among horses

Table 13

Mean blood insulin concentrations (pmol/L) of horses from sampling day 1 vs day 28 consuming different diet types

Horse	Day 1 Hay	Day 28 Grass	Change
G1	46.5	66.4	19.9
G2	104.3	323	218.7
G3	35	95.6	60.6
G4	75	132.6	57.6
G5	33.5	83.8	50.3
M6	50.3	81.6	31.3
M7	82.8	149.2	66.4
M8	58.3	156.6	98.3
M9	55.5	55.6	0.1
M10	73.3	93.2	19.9
Mean	61.4	130.2	68.8 [a]

a. indicates no difference in insulin concentrations among horses

Table 14

Mean blood insulin concentrations (pmol/L) of horses from sampling day 4 vs 9 consuming different diet types

Horse	Day 4 Grass	Day 9 Grass	Changes
G1	117.3	127.8	10.5
G2	423	585.2	162.2
G3	77	67.6	-9.4
G4	97.3	130.4	33.1
G5	79.3	88	8.7
M6	114.5	98.4	-16.1
M7	345.5	230.8	-114.7
M8	147.3	193.6	46.3
M9	88.5	113.6	25.1
M10	272	199	-73.0
Mean	176.2	181.1	-5.1 [a]

a. indicates no difference in insulin concentrations among horses

Table 15

Mean blood insulin concentrations (pmol/L) of horses from sampling day 4 vs 28 consuming different diet types

Horse	Day 4 Grass	Day 28 Grass	Change
G1	117.3	66.4	-50.9
G2	423	323	-100
G3	77	95.6	18.6
G4	97.3	132.6	35.3
G5	79.3	83.8	4.5
M6	114.5	81.6	32.9
M7	345.5	149.2	-196.3
M8	147.3	156.6	9.3
M9	88.5	55.6	-32.9
M10	272	93.2	-178.8
Mean	176.2	130.2	-46 [a]

a. indicates no difference in insulin concentrations among horses

Table 16

Mean blood insulin concentrations (pmol/L) of horses from sampling day 9 vs 28 consuming different diet types

Horse	Day 9 Grass	Day 28 Grass	Change
G1	127.8	66.4	-61.4
G2	585.2	323	-262.2
G3	67.6	95.6	28.0
G4	130.4	132.6	2.2
G5	88	83.8	-4.2
M6	98.4	81.6	-16.8
M7	230.8	149.2	-81.6
M8	193.6	156.6	-37.0
M9	113.6	55.6	-58.0
M10	199	93.2	-105.8
Mean	181.1	130.2	-50.9 [a]

a. indicates no difference in insulin concentrations among horses

Table 17

Mean blood triglyceride concentrations (mg/dl) of horses from sampling day 1 vs 4 consuming different diet types

Horse	Day 1 Hay	Day 4 Grass	Change
G1	33.8	39.5	5.7
G2	40.8	40	-0.8
G3	29.5	18.3	-11.2
G4	36.3	37	-0.7
G5	26.5	27.3	-0.8
M6	26.3	24	-2.3
M7	44.0	35	-9.0
M8	45.8	36	-9.8
M9	30.5	35.5	5.0
M10	32.3	29.5	-2.8
Mean	34.6	32.2	-2.4

No differences were found

Table 18

Mean blood triglyceride concentrations (mg/dl) of horses from sampling day 1 vs 9 consuming different diet types

Horse	Day 1 Hay	Day 9 Grass	Change
G1	33.8	39.8	-6.0
G2	40.8	65	24.2
G3	29.5	25	-4.5
G4	36.3	37.8	1.5
G5	26.5	28.8	2.3
M6	26.3	24.2	-2.1
M7	44.0	42.4	-1.6
M8	45.8	38	-7.8
M9	30.5	31.2	0.7
M10	32.3	35.2	2.9
Mean	34.6	36.5	1.9

No differences were found

Table 19

Mean blood triglyceride concentrations (mg/dl) of horses from sampling day 1 vs 28 consuming different diet types

Horse	Day 1 Hay	Day 28 Grass	Change
G1	33.8	44.2	10.4
G2	40.8	55	14.2
G3	29.5	27	-2.5
G4	36.3	31.4	-4.9
G5	26.5	38	11.5
M6	26.3	26.6	0.3
M7	44.0	39.8	-4.2
M8	45.8	41	-4.8
M9	30.5	31	0.5
M10	32.3	27.4	-4.9
Mean	34.6	37.2	2.6

No differences were found

Table 20

Mean blood triglyceride concentrations (mg/dl) of horses from sampling day 4 vs 9 consuming different diet types

Horse	Day 4 Grass	Day 9 Grass	Change
G1	39.5	39.8	0.3
G2	40	65	25
G3	18.3	25	16.7
G4	37	37.8	0.8
G5	27.3	28.8	1.5
M6	24	24.2	0.2
M7	35	42.4	7.4
M8	36	38	2.0
M9	35.5	31.2	-4.3
M10	29.5	35.2	5.7
Mean	32.2	36.5	4.3

No differences were found

Table 21

Mean blood triglyceride concentrations (mg/dl) of horses from sampling day 4 vs 28 consuming different diet types

Horse	Day 4 Grass	Day 28 Grass	Change
G1	39.5	44.2	4.7
G2	40	55	15
G3	18.3	27	8.7
G4	37	31.4	-5.6
G5	27.3	38	10.7
M6	24	26.6	2.6
M7	35	39.8	4.8
M8	36	41	5
M9	35.5	31	4.5
M10	29.5	27.4	2.1
Mean	32.2	37.2	5.0

No differences were found

Table 22

Mean blood triglyceride concentrations (mg/dl) of horses from sampling day 9 vs 28 consuming different diet types

Horse	Day 9 Grass	Day 28 Grass	Change
G1	39.8	44.2	4.4
G2	65	55	-10
G3	25	27	2.0
G4	37.8	31.4	-6.4
G5	28.8	38	9.2
M6	24.2	26.6	2.4
M7	42.4	39.8	2.6
M8	38	41	3.0
M9	31.2	31	-0.2
M10	35.2	27.4	-7.8
Mean	36.5	37.2	0.7

No differences were found

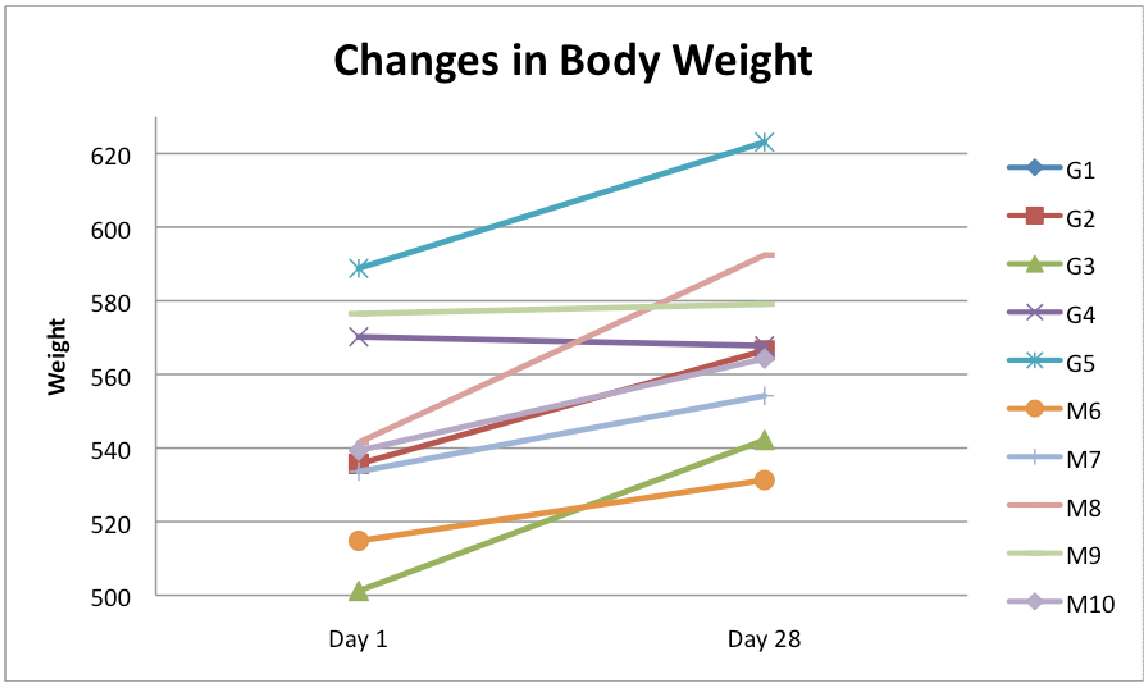

Figure 1 - Changes in Mean body weight between day 1 to day 28.

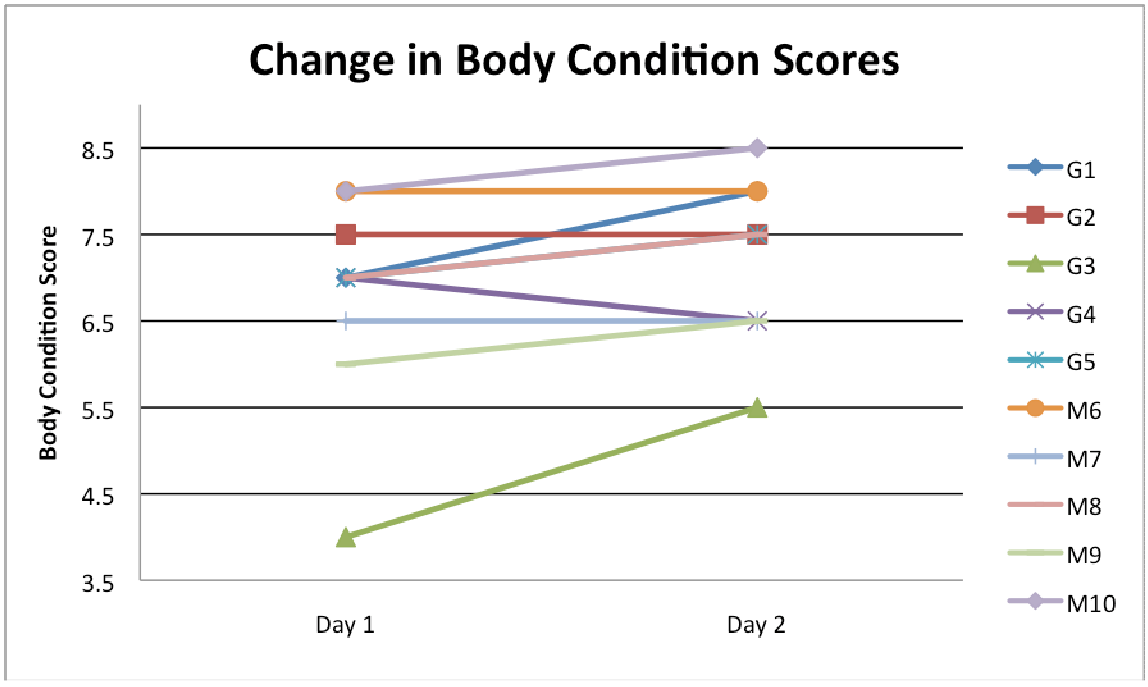

Figure 2 – Changes in body condition scores of horses from day 1 to day 28. Each value is a mean determined by three separate values.

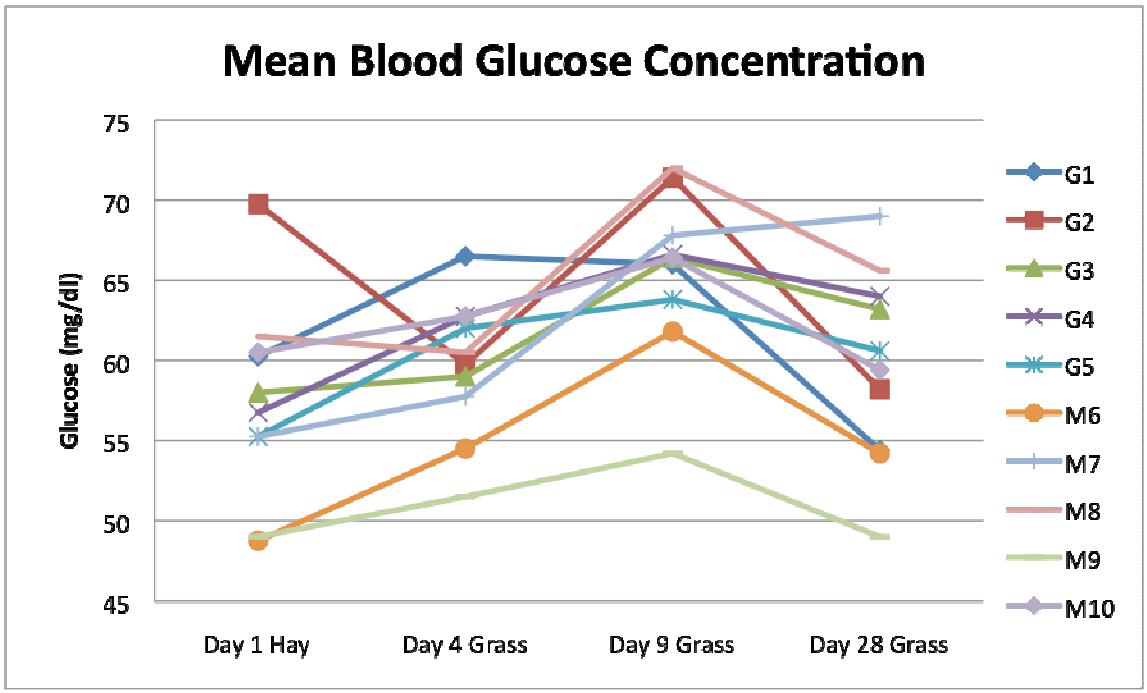

Figure 3 – Mean glucose concentrations of horses from each sampling day.
* indicates a significant difference at the $P < 0.05$

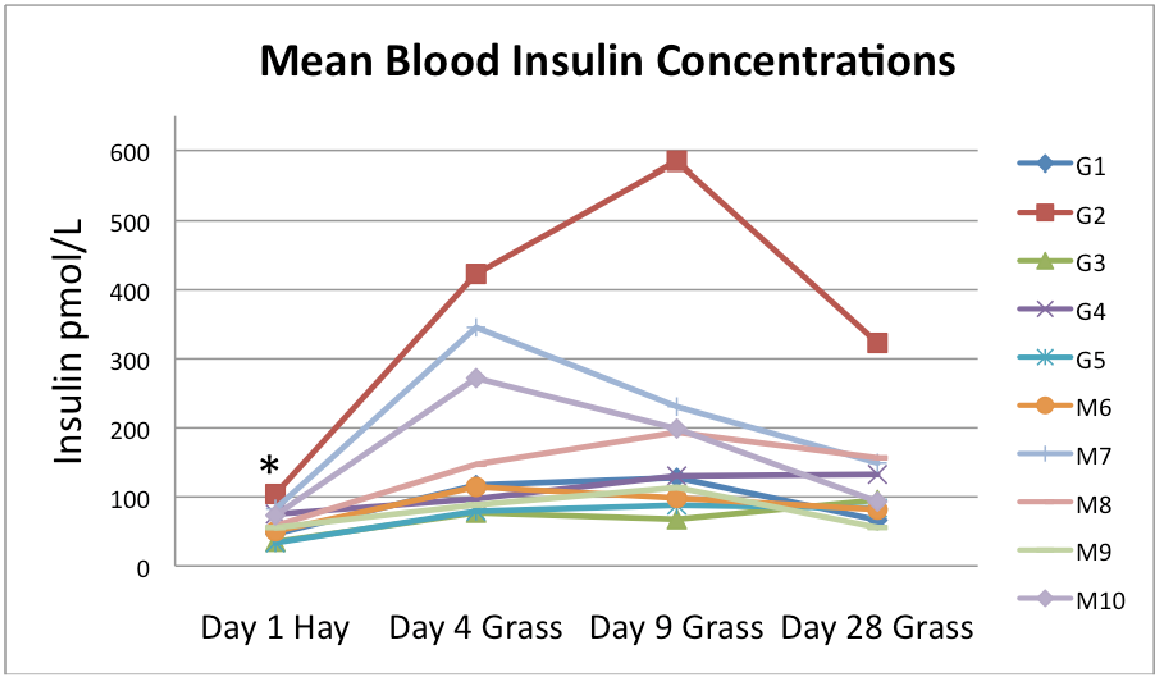

Figure 4 – Mean blood insulin concentrations of horses from each sampling day.
* Indicates a significantly lower concentration at the P <0.05

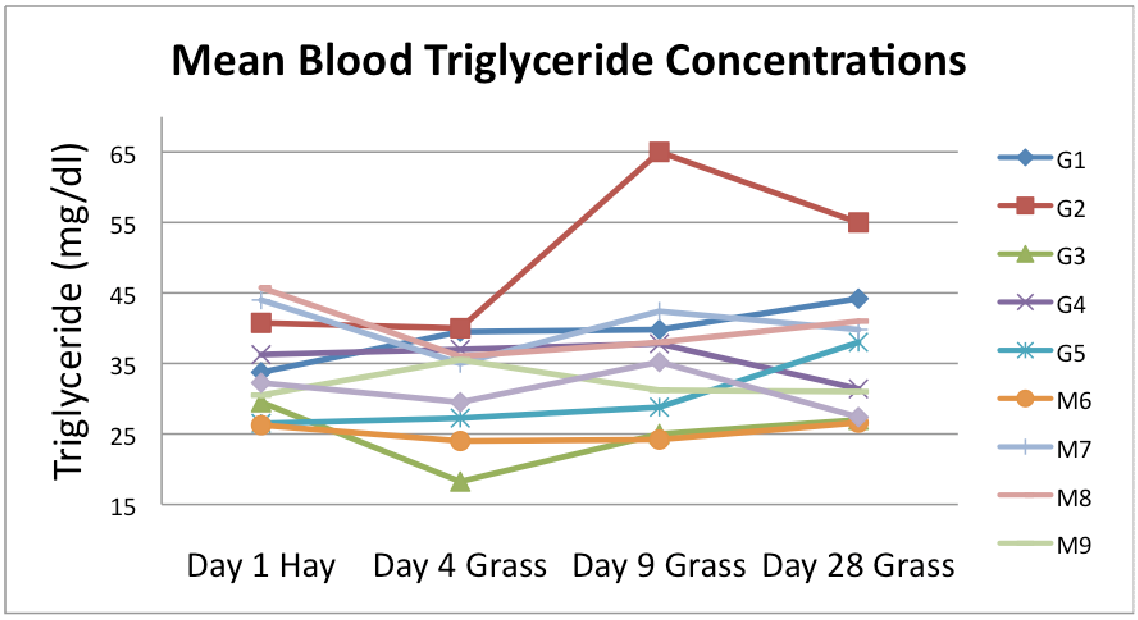

Figure 5 – Mean blood triglyceride levels of horses from each sampling day. No differences were found.

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