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Effects of Transitioning from a Free Choice Tall Fescue (Lolium Arundinaceum) Hay Diet in Late Winter to a Free Choice Spring Tall Fescue Pasture Diet on Plasma Fructosamine Concentrations, Body Weight, and Body Condition Scores of Stock Horse Mares

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EFFECTS OF TRANSITIONING FROM A FREE CHOICE TALL FESCUE (*LOLIUM ARUNDINACEUM)* HAY DIET IN LATE WINTER TO A FREE CHOICE SPRING TALL FESCUE PASTURE DIET ON PLASMA FRUCTOSAMINE CONCENTRATIONS, BODY WEIGHT, AND BODY CONDITION SCORES OF STOCK HORSE MARES

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EFFECTS OF TRANSITIONING FROM A FREE CHOICE TALL FESCUE (*LOLIUM ARUNDINACEUM)* HAY DIET IN LATE WINTER TO A FREE CHOICE SPRING TALL FESCUE PASTURE DIET ON PLASMA FRUCTOSAMINE CONCENTRATIONS, BODY WEIGHT, AND BODY CONDITION SCORES OF STOCK HORSE MARES

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Approximately half of all reported laminitis cases are the result of "grass founder" (laminitis associated with long-term over consumption of lush, early spring pastures). Elevated body weights (BW), body condition scores (BCS), and blood glucose concentrations have all been associated with the onset of grass founder. Plasma fructosamine concentrations (PFC) have recently been used as an indicator of long-term (14–21 d), mean blood glucose concentrations in horses and numerous authors have reported that elevated PFC were observed in laminitic horses (Murphy et al., 1997; Keen et al., 2004; Knowles et al., 2012). This study was conducted to evaluate the long-term effects of transitioning from an ad-libitum tall fescue hay diet in late winter to an ad-libitum tall fescue pasture diet in early spring on parameters associated with grass founder in horses. Five mature stock horse mares were given free choice access to good quality tall fescue hay for 18 weeks before initiation of data collection. Each horse acted as their own control. PFC were determined on day 1, day 128, and then monitored at 14 d intervals for the following 84 d (February 26th through May 21st). Three trained lab technicians evaluated BW and BCS on day 1, 128, 170 and 212 of the trial. The horses were placed in a 20 acre field where their diets consisted of free choice access to hay only

for 156 days (DTP1) followed by free choice access to tall fescue hay with minimal access to some early emerging tall fescue pasture for 14 days (DTP2), and finally to free choice access to lush spring tall fescue pasture only for the final 42 days (DTP 3) when the animals refused to eat offered hay. Mean PFC were highest ($P < 0.01$) for DTP1 and decreased with each successive transition to DTP2 and DTP3. This may have been due to increased insulin secretion associated with the pasture only diet. Mean BCS at the beginning of the trial was 5.7 and increased throughout the trial ($P < 0.001$) to a value of 7.8. BCS associated with the pasture only diet were higher ($P < 0.001$) than those associated with the free choice hay and hay plus pasture diets. Mean BW increased ($P < 0.05$) from 1199 lbs. to 1268.3 lbs. during the 12-week trial. Mean weight gain was 113 lbs. with an average daily gain of 1.35 lbs./day. No incidence of laminitis was observed.

Introduction

The wild horse evolved as a foraging animal and was constantly on the move, traveling great distances while consuming small frequent meals of fibrous forage. The horse's unique digestive anatomy and physiology were perfectly adapted to this roaming lifestyle providing a nearly perfect environment for the digestion, absorption, and utilization of nutrients present in the diet. Horses therefore rarely developed metabolic or digestive disorders associated with diet.

Upon domestication, the horse was relegated to a life of strenuous labor, often working up to 14 hours per day six days per week. The intensive work load energy requirements could not be met by forage alone. This was especially true since these horses had only limited access to forage during the night when they were turned out to pasture after a hard day of intense activity. Relatively large quantities of high-energy grains fed once, twice, or even three times per day in addition to free choice hay and pasture diets were often fed to meet the massive energy requirements of the hard-working draft horse.

These high starch, grain based diets did result in occasional metabolic anomalies such as "Blackwater disease" or "Monday morning sickness". Access to reasonable quantities of forage coupled with the extreme energy expenditure associated with high levels of activity did result, however, result in horses that tended to be healthy, extremely fit, and well-conditioned.

During the decade between 1940 and 1950, the "light riding horse" replaced the draft horse as the most prevalent equid in the U. S. While a limited number of these

animals were and are still utilized for work, the activity level of the typical light riding horse of today could perhaps best be defined as that of a “lethargic yard ornament”.

This modern light riding horse receives very little, if any, forced daily exercise and rarely if ever, is subjected to any type of strenuous work. Coupled with the fact that the horse is typically either; 1. Confined in a small stall and receives large, infrequent, meals of high starch, high-energy, grain-based diets, or 2. Turned out to pastures and allowed to consume unlimited quantities of lush, high energy forage. It should be clear that the modern light riding horse can easily far exceed its maintenance energy needs. Most knowledgeable horsemen recognize that either of these mismanagement scenarios will yield lethargic, overly fat, poorly conditioned, and generally unhealthy riding horses.

Many misinformed first-time horse owners, however, are under the mistaken impression that light riding horses must be fed grain-based diets to meet their energy requirements, and that forage only diets are insufficient to meet their animal’s energy requirements. These misinformed owners are simply unaware of the negative consequences of overfeeding and under exercising their horses. These well-meaning people are thus, unknowingly, “killing their horses with kindness” by setting them up to develop one of the most devastating metabolic diseases known to afflict the horse, laminitis!

Literature Review

Laminitis

Laminitis, more commonly referred to as founder, is a metabolic disease that has plagued the light riding horse industry since its inception. It has been observed to accompany many metabolic anomalies not thought to be related to diet, such as retained placentas, abortion, and stress from exercise. Horsemen have long observed that founder most often results from feeding mismanagement (Garner et al., 1975; King and Mansmann, 2004; Pass et al., 1998, Kronfeld and Harris, 2003; Treiber et al., 2006).

Laminitis is an exceptionally debilitating disease that leaves the horse to deal with extremely painful, chronic lameness and often results in euthanasia of the affected horse. The exact physiological mechanisms involved in the onset of laminitis are unknown. One day an animal appears to be perfectly normal, the next day, for no apparent reason, it shows all of the classic symptoms of laminitis (Hood et al., 1993).

A basic understanding of the functional anatomy of the horse's lower limb is essential in order to understand the intricacies of laminitis. The coffin bone is the most distally positioned bone of the horse's fore and hind limbs. It is suspended directly above the sole by laminar tissue which connects it directly to the hoof wall inside the hoof. The coffin bones, therefore, bear all of the horse's body weight and transfers it to the hoof wall in the normal healthy horse. Laminae are the delicate, finger-like tissues that attach the inner surface of the hoof wall to the coffin bone. The sensitive laminae interlock with insensitive laminae lining the hoof wall to effectively

suspend the coffin bone in a hammock-like manner above the sole. The sensitive laminar tissue, therefore, provides a connective support mechanism which protects and suspends the coffin bone to keep it from falling down onto, or actually protruding through, the sole of the foot when weight is brought to bear on the structure (Pollit and Davies, 1998; Hood, 1999; Pollitt, 2003; Bailey, 2004).

While development of founder is thought to be the result of some type of allergic reaction to some toxic substance or substances ingested by the affected animal (Heymering, 2010). It is not known what exact factors triggers this sudden dramatic change, nor is it understood what physiologic parameters are affected. It is known that laminitis results after vasoconstriction of laminar tissues brought about by the inflammation and resulting fever generated in the tissues of the affected horse's foot (Bailey et al., 2004). If the fever and inflammation are not immediately dissipated, partial to complete death and destruction of the supporting laminar tissues is inevitable. The coffin bone, therefore, detaches from the hoof wall and the apex collapses down onto the sole of the foot (Pollit and Davies, 1998; Hood, 1999; Pollitt, 2003; Bailey, 2004). The affected horse then displays the common "dropped sole" conformation and lameness characteristic of founder. In fact, further rotation may result in the coffin bone actually protruding through the sole of the foot. The pain associated with severe founder is excruciating, chronic, and the tissue damage is irreparable. Euthanasia is often the kindest treatment for horses exhibiting these symptoms. (Pollitt and Davies, 1998; Hood, 1999; Pollitt, 2003; Bailey, 2004).

No one knows why, but laminitis most commonly affects only the fore limbs.

Rarely are all four feet affected (Pollitt, 2008). Symptoms of the disease are all generated when the horse attempts to eliminate the extreme pain that occurs in the front feet. Symptoms include extremely hot, feverish feet; refusal to move; leaning backward with the front feet extended out in front of the animal; and lying down, refusing to rise. The disease is so debilitating simply because accurate diagnosis is most often attained only after irreversible structural tissue damage has already occurred. (Hood et al., 1993)

While there is little known of the etiology of founder, very little is known about the physiological, metabolic, or genetic factors which actually predispose a horse to any type of founder. No single factor has been identified as the one, easily quantified parameter that pre-disposes horses to the disease. Without a clear understanding of why or how the physical and metabolic changes occur with any type of founder, it has been impossible to predict, prevent, or effectively treat animals afflicted with this debilitating disease. Clearly, additional founder research, especially grass founder research, is warranted. That is why the American Veterinary Medical Association, the United States Humane Society, the American Association of Equine Practitioners, and the American Horse Council named laminitis as their top priority for research funding in 2016.

GRASS FOUNDER VERSUS GRAIN FOUNDER

Early founder research studies focused on the relationship of overconsumption of grain-based diets with the immediate onset of founder in the horse. Garner et al. and King and Mansmann (1975, 2004) first reported that over consumption of grain-based diets was the primary predisposing factor associated with the onset of laminitis.

Pass et al., Kronfeld and Harris, and Treiber et al. (1998, 2003, 2006) reported that short-term overconsumption of these high starch grain diets certainly can and will quickly generate the onset of founder quickly; and further and most trainers, nutritionists, and veterinarians are well aware that if horses are given free choice access to grain, they will exhibit symptoms of founder within as few as 12 – 48 hours of the overconsumption (Pollit and Davies, 1998; Pass et al., 1998). It is also well known that overfed horses consuming lesser quantities of grain/hay combination diets will have a tendency to become fatter and eventually develop founder (Pass et al. 1998; Kronfeld and Harris, 2003).

Professionals in the horse industry also agree that grazing horses consuming ad libitum quantities of lush spring pasture grasses and legumes as their only food source for extended periods of time do frequently founder. In a recent study, Hintz (2000) reported that the single most commonly reported cause of founder is not the overconsumption of grain as reported by Garner et al. (1975), but rather the overconsumption of early springtime grass and legume pastures. Hintz (2000) found that grass founder accounts for nearly half (45.6%) of all reported cases of laminitis in the United States while grain/hay combination diets account for 7.4% of reported cases. Hintz also noted that this "grass founder" laminitis occurs with two and a half times greater frequency during the spring as opposed to winter and summer months. The disparity between Hintz and Garner's findings are most likely due to the fact that horses fed grain/hay combination diets were, in the past, and still are observed today, typically with greater frequency and intensity than horses consuming only forage pastures. This may be because they are commonly fed

by hand twice daily. It is always easier, as is well known by veterinarians, to diagnose the possible cause of founder when you find the lame horse standing in the grain bin.

The above referenced studies all indicate that grass founder and grain founder are similar in their effects on the horse. Both forms of the disease appear to be the result of overconsumption. Grain founder appears to be induced by mere overconsumption of non-structural carbohydrates (starch, glucose, and fructose). While grass founder, on the other hand, appears to be due to the overconsumption of lush, early spring pastures. These early spring pastures may or may not contain high concentrations of non-structural carbohydrates.

Garner et. al (1975) conducted one of the earliest concerted efforts to determine the physiological cause of founder in horses. They noted that short-term blood serum glucose and insulin concentrations dramatically increased following the onset of experimentally induced laminitis generated through carbohydrate overload. They concluded that chronically laminitic equines developed hyperglycemia and hyperinsulinemia in response to overconsumption of grain-based diets, while blood glucose and insulin concentrations were not affected in un-foundered horses. Their results were substantiated by Coffman and Colles and Stull and Roedieck (1983, 1988). This led to the conclusion that hyperglycemia resulted in the insulin resistance observed in foundered horses. Trieber et al. (2006) suggested that hyperglycemia was a possible cause of founder. They also reported that previously foundered fat ponies and horses were far less tolerant of oral glucose loading than normal horses and ponies. It is significant to note that none of these studies monitored pre-laminitic blood glucose or

blood insulin concentrations in previously foundered or un-foundered horses and that all the horses studied were fed grain-based diets.

Garner et al., Coffman and Colles, Stull and Roedieck, and Trieber et al. (1975, 1983, 1988, 2006) indicated that grain founder is the result of overconsumption of high starch diets containing large concentrations of glucose and other easily digested and absorbed sugars and starch. Stull and Roedieck (1988) noted that following overconsumption, hyperglycemia was generated in response to four different diets. One diet consisted of alfalfa; another of 50% alfalfa, 50% corn; another of complete corn diet; another of 90% corn and 10% corn oil. The metabolic mechanisms of grass founder have not been elucidated yet, however, it appears that free choice grazing and the resultant overconsumption of grass pastures are closely related to the onset of grass founder in horses. More research needs to be done to determine the metabolic roles of grain and forage in the development of founder.

EQUINE METABOLIC SYNDROME LAMINITIS VERSUS HUMAN METABOLIC SYNDROME TYPE II DIABETES

Early studies indicated that blood glucose metabolism anomalies occurred when horses foundered, however, few studies focused on the relationship of long-term hyperglycemia and laminitis. Johnson (2002) theorized that no single physical parameter was responsible for the onset of laminitis. Many scientists noticed that pre-laminitic horses often displayed similar phenotypic characteristics prior to the onset of laminitis. Johnson (2002) observed that obese, overfed, under exercised horses and ponies often

developed laminitis with far greater frequency than their leaner, more fit, properly fed and exercised counterparts. They, therefore, proposed that obesity, hyperinsulinemia, and insulin resistance (IR) were components of a clinical syndrome they had observed in pre-laminitic and laminitic equines. He coined the term "Equine Metabolic Syndrome" (EMS) to describe horses that displayed these characteristics because they are identical to those displayed by human patients who were diagnosed as having "Human Metabolic Syndrome" (HMS).

Medical doctors implicate hyperglycemia as the primary cause of the obesity, hyperinsulinemia, and insulin resistance displayed by type II diabetics (Reaven, 1988). EMS researchers were unwilling initially to pursue the idea that hyperinsulinemia, insulin resistance, and obesity were primarily due to hyperglycemia in the horse. In their review of research on EMS, Frank et al. (2010) stated that "Hyperglycemia is rarely detected in horses with EMS because most animals maintain an effective compensatory insulin secretory response in the face of IR. However, blood glucose concentrations trend toward the higher end of the reference range indicating a partial loss of glycemic control". They also stated that "If persistent hyperglycemia is detected, a diagnosis of diabetes mellitus should be considered." They concluded that "Type 2 diabetes mellitus occurs in horses and may be more common than previously thought and that this diagnosis should be considered when hyperglycemia cannot be attributed to other causes such as stress, recent feeding, administration of α-2 agonist drugs, or inflammatory processes."

The primary difference between the two syndromes is that EMS has become the

widely accepted term to describe a collection of symptoms associated with the onset of laminitis that has developed in the absence of other recognized causes (such as grain overload, colic, colitis, Cushing's disease or retained placentas) in horses. HMS, on the other hand, has become the widely accepted term for a collection of risk factors that medical doctors use to assess the propensity of human patients to develop type 2 diabetes mellitus (chronic uncontrollable hyperglycemia) (Haller, 1977).

Some early studies suggested that EMS and Cushing's disease were one and the same disease. Walsch et al. and Frank et al. (2009, 2010) determined that they are two separate and distinct diseases. These studies determined that Cushing's disease, also now known as pituitary pars intermedia dysfunction (PPID), occurs when cancerous cells invade the anterior pituitary resulting in hyperadrenocorticism. Both authors also concluded that foundered horses resulting from Cushing's disease displayed the phenotypic similarities of obesity, hyperglycemia, hyperinsulinemia and insulin resistance found in EMS horses. They both concluded, however, that the laminitis resulting from Cushing's disease is not the result of EMS but is rather the result of abnormal cancerous cells.

Numerous human type II diabetic nutrition studies have provided insight into possible causes and relationships associated with laminitis in the horse. For instance, Sinha et al. and Shen (1996, 1970) have shown a positive correlation between caloric intake and blood glucose concentrations in humans. Akiyana et al. (1996) found that rats fed twice the caloric intake of controls also gained twice as much weight during a 27-day feeding period.

Rats fed the higher caloric diet were determined to have become hyperglycemic and insulin resistant. These studies showed that over consumption of high energy diets resulted in elevated blood glucose concentrations and possible insulin resistance in humans and rats, and suggested that similar results may be seen in other species. It should be noted that both of these conditions are highly correlated with the onset of type II diabetes in humans.

Sinha et al., (1996) used body mass index (BMI) as their indicator of obesity to compare blood glucose metabolism of lean, obese and type II diabetic human subjects. Male subjects having a BMI of 27.3 and female subjects having a BMI of 27.8 or greater were classified as obese. Blood glucose concentrations averaged 100.4, 103.3, and 196.1mg/dl, respectively, for the thin, obese and, type II diabetics consuming identical diets, while blood insulin concentrations were 14.4, 25.6, and 30.7µU/mL for the same groups, respectively. Shen et al. (1970) reported similar results and found that glucose levels were 52% higher in type II diabetic versus normal patients. These data clearly show that type II diabetic human subjects produce much greater blood glucose and insulin concentrations than thin or even fat, non-diabetic humans, and that obese individuals produced greater insulin concentrations than thin individuals. Their data clearly show that obese and type II diabetics humans tend to display hyperglycemia, hyperinsulinemia and the resultant insulin resistance (IR) when compared to normal human patients. Monfort and Akers (2009, 2011) both found that fat horses displayed hyperglycemia and hyperinsulinemia when compared to horses possessing moderate and thin body condition scores. These results led the authors to conclude that EMS horses

were not only hyperinsulinemic and IR but also hyperglycemic.

The relationship between obesity and long-term hyperglycemia and type II diabetes in humans is now well-established. (Boshell et al., 1968; DeFonzo et al., 1991; Kahn et al., 2000). Boshell et al. (1968) observed that 59.30% of obese human subjects tested displayed higher than normal blood glucose levels when subjected to glucose tolerance tests. In their study, non-obese, non-diabetic subjects averaged blood glucose concentrations of 176mg/dl while the obese diabetic group averaged 321mg/dl. Insulin averages were 131µU/mL and 555 µU/mL for the normal and the obese groups, respectively. Results of these studies indicate that type II diabetics and obese human subjects, in fact do display elevated blood glucose and insulin concentrations when compared with normal individuals who are fed identical diets. These results led equine scientists to develop studies to determine if blood glucose and insulin metabolism in EMS horses is similar to that observed in obese and type II human diabetic patients. Monfort (2009) fed a free choice tall fescue pasture diet to thin, moderate, and fat horses. They found that blood insulin concentrations were twice as high for horses having moderate body condition scores when compared to thin individuals. They also found that the fat horses required 50% more insulin to maintain normal blood glucose concentrations than horses possessing a moderate body condition score. Akers (2011) obtained similar results. These studies clearly indicate that horses do display hyperinsulinemia and IR when allowed to overconsume tall fescue grass pastures. Results of these studies clearly indicate that overweight, overfed horses display glucose anomalies similar to those of the obese pre-diabetic patients.

Boshell et al., DeFonzo et al., and Kahn et al. (1968, 1991, 2000) studied the effects of weight loss on blood insulin and glucose metabolism in obese non-diabetic and obese type II diabetic human patients. They determined that when adipose cell size decreased during weight loss, insulin sensitivity increased and blood glucose concentrations decreased. They concluded that hyperglycemia and IR diminished as a direct result of excess weight loss. Their results showed conclusively that prediabetic and type II diabetic hyperglycemia and insulin resistance could be controlled through weight loss and forced exercise. Their results led to further studies into the interrelationships between the effects of obesity and weight loss on blood chemistry and metabolic disorders in horses displaying EMS and laminitis. It should be noted that while no study has focused on the effects of weight loss on glucose metabolism in horses, equine nutritionists routinely recommend reduced caloric intake and increased activity levels for obese horses as a means of preventing the onset of both grass and grain founder.

The World Health Organization has as estimated that by the year 2025, 300 million people will have been diagnosed with type II diabetes (Zimmet et al., 2001). Therefore the relationship between type II diabetes and insulin resistance has been extensively studied in attempts to determine ways to prevent or decrease the severity of the debilitating effects of type II diabetes. Biddinger and Kahn (2006) reported that 27% of adults and 50% of obese children exhibit insulin resistance in the United States. Kahn (1978) defined IR as occurring when normal concentrations of insulin produce a less than normal biological response (a decline in blood glucose concentration into the normal range). Reaven (1988) found that humans develop type II diabetes because they first

develop hyperinsulinemia (excess insulin is secreted from the pancreas in response to hyperglycemia) resulting in ever-increasing elevated levels of circulating blood insulin concentrations until insulin resistance occurs. These and other studies (Ravussin and Smith, 2002; Özcan et al., 2004) have confirmed the positive correlation that exists between excessive energy intake, elevated blood glucose concentrations, obesity, insulin resistance and type II diabetes in humans. The similarities between HMS and EMS are so marked that scientists have begun to attempt to determine if long-term hyperglycemia might be a major factor in the onset of laminitis in the horse.

As noted by Monfort and Akers (2009, 2011), under normal conditions, when energy is consumed, glucose is rapidly absorbed into the blood stream resulting in elevated blood glucose concentrations. In response to the elevated circulating blood glucose concentration, the pancreas secretes insulin from the beta cells. The insulin then activates cellular receptors on skeletal muscle cells, liver cells, and fat cells, driving insulin into the tissue. Therefore, blood glucose concentrations are lowered into the normal range (approximately 80 mg/dL) (Yalow and Berson, 1960). An increase in blood glucose concentration will always be followed by a blood insulin spike which in turn allows the blood glucose concentration to quickly return to and remain within the "normal" range at any given time. If too much insulin is present, the pancreas instantly reacts by releasing glucagon which activates receptors in the liver and skeletal muscle to release glycogen that elevates blood glucose concentrations back into the "normal" range. These two pancreatic metabolic systems regulate the normalization of free glucose and insulin concentration in the blood stream at any given time. Hyperglycemia can only

occur when: 1.) Cellular receptors malfunction as with insulin resistance associated with type II diabetes or, 2.) When insulin is no longer produced as with type II diabetes.

Numerous authors have attempted to determine the cause or causes of the onset of insulin resistance associated with type II diabetes. Martin et al. (1992) first found that a pre-diabetic condition could be detected prior to the actual onset of full-blown type II diabetes. They determined that pre-diabetic patients displayed slightly elevated blood glucose concentrations (130 to 150 mg/dL) and were prime candidates for the development of full-blown type II diabetes (consistently elevated blood glucose concentrations of over 150 mg/dL).

Ogata et al. (2007) monitored blood glucose concentrations of type II diabetic and non-diabetic patients continuously throughout a 24-hour period. They concluded that diabetic patients exhibit continuous hyperglycemia and insulin resistance throughout the day. They also concluded that diabetic hyperglycemia was due to an impaired negative feedback mechanism that yielded insulin resistance. They theorized that the chronically elevated circulating blood glucose concentrations displayed by type II diabetics resulted in chronically elevated blood insulin. The cellular receptors are bathed constantly in ever increasing quantities of insulin. The result is decreased insulin receptor sensitivity. Thus, the result of constant hyperglycemia is insulin resistance (IR), hyperinsulinemia, increased adiposity, and obesity (hyperlipidemia).

Inflammation

Inflammation, interestingly enough, is a major side effect of type II diabetes. The

relationship of body fat, insulin sensitivity, and the incidence of inflammation in insulin-resistant patients was studied by Festa et al. (2000). They found that one in three insulin resistant patients had impaired glucose tolerance, indicating that there is chronic subclinical inflammation present as part of insulin resistance. Rossi et al. (1998), found that a majority of patients who had been diagnosed with diabetes for more than eight years suffered from vasoconstriction, resulting in limb amputations. The vasoconstriction seen in humans prior to limb amputations is similar to the mechanisms that cause vasoconstriction and the resultant inflammation observed in the limbs of foundered horses (Marso and Hiatt, 2006).

Glycemic Index

Jenkins et al. (1980) at first observed that different foods and nutrients were digested and absorbed with different rates of efficiency. They theorized that this might have an effect on blood glucose concentrations in prediabetic and type II diabetic patients. Therefore, they developed what is known as the glycemic index. The glycemic index or glycaemic index (GI) is a numerical system indicating the effect of different foods on the consuming animal's short-term blood glucose concentration. The higher the glycemic index of a given food, the greater effect it will have on increasing the blood glucose concentration. Subsequent research has shown that the glycemic index is of great value for controlling blood glucose concentrations of overweight, prediabetic and type II diabetic patients (Brand-Miller et. al, 2003; Rizkalla et. al, 2004; Thomas and Elliott, 2009). For instance, Brand-Miller et. al and Rizkalla et. al, (2003, 2004) found that

overweight pre-diabetic patients had reduced blood insulin and glucose concentrations when they converted from a diet containing high glycemic index foods to a diet containing low glycemic index foods.

Seidell **(**2000) meanwhile studied the effects of low glycemic index foods combined with forced exercise on blood glucose and insulin parameters and found that IR can be controlled or even reversed in prediabetic and type II diabetic patients by incorporating forced exercise regimens and low glycemic index foods to reduce body weight and blood glucose concentrations. In a subsequent overview of numerous diabetic studies, Reaven (2005) reported that many studies indicate a moderate amount of weight loss will result in decreased blood glucose and insulin concentrations as well as insulin resistance thereby diminishing the chances of developing the onset of type II diabetes. It is interesting to note that Geor (2010) observed that dietary restrictions similar to those suggested by Seidell and Reaven (2000, 2005) could help reduce and/or prevent the onset of laminitis in horses.

Insulin Resistance in the Horse

Kahn (1978) first observed that insulin resistance occurs in the horse. He defined IR as an abnormal response by the body to "normal" concentrations of blood insulin. Kronfeld et al. (2005) later defined insulin resistance in the horse as a decline in insulin sensitivity at the cellular surface resulting in hyperglycemia in equine. They all theorized this was likely caused by a disruption in the glucose metabolism inside the cell.

Johnson et al. (2004) meanwhile, observed that feeding high glycemic index feeds

to unexercised horses led to obesity in horses. He theorized that long-term obesity could predispose a horse to insulin resistance, which in turn, might predispose a horse to laminitis. Kronfeld et al. (2005) also noted that insulin resistance and laminitis are positively correlated. Hoffman et al. (2003) noted that insulin resistance is much more commonly seen in obese horses. They also observed that fat horses fed diets containing large quantities of non-structural carbohydrates (NSC) (feeds with high glycemic indexes) developed greater insulin resistance than thin horses fed identical diets. Monfort and Akers (2009, 2011) observed similar results when feeding iso-caloric tall fescue pasture diets to fat and thin horses.

Coffman and Colles (1983) compared 6 laminitic ponies to 6 non-laminitic ponies in an earlier study. They observed decreased sensitivity to insulin injections was common in ponies that were affected with chronic laminitis. They also observed that decreased sensitivity was greater in the spring than in the fall of the year. In a similar study, Treiber et al. (2006) noted that insulin resistance could be responsible for the onset of laminitis in horses.

Abnormal glucose and insulin metabolism are both trademarks of both EMS and HMS. Thus, it is reasonable to assume that EMS horses may respond to long-term hyperglycemia and hyperinsulinemia in a fashion similar to diabetic humans.

Body Condition Score (BSC) as a measure of obesity in the horse

As noted earlier, obesity is clearly linked to the onset of laminitis. Therefore,

numerous studies have attempted to link the degree of obesity exhibited by a horse to his tendency to develop laminitis (Carter et al., 2009; Geor, 2008; Frank, 2009; Monfort 2009; Akers, 2011).

Henneke et al. (1983) developed the current, most commonly used system to measure the degree of obesity exhibited by equines. They determined that body fat is not evenly distributed across the body and is best measured by palpation and visualization of the amount of adipose tissue deposited on the neck, withers, ribs, hips, loin, and tail head. Based on these observations, they then developed a numerical system (the Body Condition Scoring system) to accurately assess degree of body fat exhibited by equine. They determined that BCS of all equines range between 1 to 9. They defined the ideal, normal, healthy horse as having a 5. Horses displaying a 1 are extremely thin and emaciated with no fatty tissue present. All skeletal processes are clearly visible. Horses displaying a 9 are at the opposite end of the spectrum and are defined as extremely obese with bulging, patchy fat covering the skeleton so that no skeletal structures can be felt through palpation.

ADDITIONAL THOUGHTS

Most recent EMS studies have focused only on obesity, IR, and hyperinsulinemia as the major causative factors associated with laminitis in EMS horses. Many of the earliest studies, however, indicated hyperglycemia was responsible for these three metabolic anomalies. None of these authors attempted to distinguish causative factors associated with the onset of the most common form of laminitis, "grass founder". Frank

et al. (2010) in their consensus statements for the American College of Veterinary Internal Medicine regarding EMS stated that "EMS is a complex disorder for which there are more questions than answers at present. The principal components of EMS are, they stated, increased adiposity, IR, and laminitis", but they also found that "this syndrome likely encompasses a much wider spectrum of problems that affect energy metabolism in the horse". They further stated that "hyperglycemia is rarely detected in horses with EMS because most animals maintain an effective compensatory insulin secretory response in the face of IR". It is clear that they are implying that we do not see hyperglycemia in most horses simply because they are so highly hyperinsulinemic for such long periods of time due to IR that the excessive blood glucose concentration in the blood cannot possibly be due to hyperglycemia. These statements appear to be the antithesis of each other. Furthermore, no studies have been conducted to determine if long-term hyperglycemia is a possible causative factor associated with EMS or grain or grass founder. In fact, no effective means have been developed to monitor long-term blood glucose metabolism in the horse.

Meanwhile, results of studies with type II diabetic humans has progressed beyond merely attempting to determine causative factors associated with the onset of type II diabetes. It is well-established in the medical community that both type I and type II diabetes result from the inability of the pancreas to produce sufficient insulin levels to regulate blood glucose concentrations within the normal range.

In type I diabetes, also known as childhood-onset diabetes, the beta cells in the pancreas stop producing insulin so that blood glucose concentration regulation can only

be accomplished through exogenous insulin injections. It is well-established that blood glucose concentration regulation is only possible when insulin is either secreted by the pancreas or exogenously injected. In either case, insulin activates cellular receptors of the liver, skeletal muscle, and fat tissue to allow blood glucose to enter the cells and therefore diminish blood glucose concentrations.

The cause of type II diabetes is likewise well known. It is most commonly seen in overweight to obese, lethargic individuals who consume excessive quantities of glycemic index diets. These patients develop hyperglycemia simply due to the fact that they overeat and under exercise. Over time the pancreas is called upon to produce ever increasing quantities of insulin (hyperinsulinemia) in response to the hyperglycemia. Eventually, the receptor cells of the skeletal muscle, liver, and fat tissues become desensitized to and cannot be regulated by the ever-increasing quantities of circulating insulin. At that point, they are insulin resistant.

These facts explain the roles that obesity, overeating, the lack of exercise, hyperinsulinemia, IR, and most importantly hyperglycemia play in development of type II diabetes. They indicate that the major symptoms of human metabolic syndrome and the resultant type II diabetes in humans are the result of long-term hyperglycemia. These observations indicate that equine nutritionists might find similar results by studying the role of long-term hyperglycemia in EMS and the development of laminitis.

Long-Term Hyperglycemia Indicators

The medical profession determined early in the 20th century that prolonged

elevated blood glucose concentrations led to cardiovascular disease, hypertension, retinopathy, kidney disease and a shortened lifespan. However, initially there was no way to accurately monitor short or long term blood glucose concentrations in response to their treatments.

Midway through the 20th century, small, hand-held, patient operated blood glucose monitors were developed. These tools allowed doctors and patients to accurately assess blood glucose concentrations in a patient at any given time and were useful for determining the short-term effects of food intake and insulin injections on controlling blood glucose concentrations in diabetic patients. Monfort (2009) later established their effectiveness and accuracy and value for monitoring blood glucose concentrations in horses.

Hemoglobin A1c (HbA1c) as an effective indicator of long-term blood glucose concentrations

While HbA1c is an effective tool to determine short-term fluctuations in blood glucose concentrations, doctors knew that the only means of effectively treating type I and type II diabetics was to develop methods that would allow patients a means of determining long-term average blood glucose concentration responses to medications and treatments. They needed to develop a testing procedure that accurately assessed average long-term blood glucose concentrations in their patients. Fortunately, combined efforts of researchers allowed for the development of such a test.

Huisman et al. (1958) first separated Hemoglobin A1c (HbA1c) from other forms

of hemoglobin using chromatography. The reactions leading to its formation were characterized by Bunn and coworkers (1976) and the use of HbA1c for monitoring the degree of control of glucose metabolism in diabetic human patients was proposed in 1976 by Koenig et al.

Rahbar (1968) determined that abnormal hemoglobin (HbA1c) is formed when developing red blood cells are exposed to elevated blood glucose concentrations. These abnormal cells contain large quantities of sugar and are then referred to as glycated hemoglobin. Bunn et al. (1976) reported that the lifespan of a red blood cell is approximately 120 days. Gabbay et al. (1977) used Bunn's data to develop a method of measuring circulating blood glycosylated hemoglobin concentrations over time to determine long-term (previous 90-120 day) average blood glucose concentrations in human patients. The resultant test is referred to as hemoglobin A1c (HbA1c).

Coffman and Colles (1983) first theorized that laminitic horses display hyperglycemia and insulin resistance. Treiber et al. (2006) suggested that their theory is correct. These factors have led to expanded studies of methods to determine if short-term hyperglycemia is merely the result of founder or if long-term hyperglycemia is a possible cause of founder (Treiber et al., 2006; Pollitt, 2003; Walsch et al., 2009; Monfort, 2009; Akers, 2011).

The HbA1c test is used to derive numerical values that are indicative of average, long-term blood glucose concentrations in diabetic patients during the previous 120 days. While normal, non-diabetic patients develop 5% or lower glycated hemoglobin concentrations, uncontrolled diabetes yields substantially higher HBA1C concentrations

(7- 16 % and even higher) (Koenig et al., 1976). They also noted that non-diabetic patients having HbA1c scores of 5% averaged blood glucose concentrations of 80 mg/dl, while poorly controlled diabetics having 10% HbA1c averaged blood glucose concentrations of 343 mg/dl. Nathan, D.M. et. al. (1984) subsequently derived values presented in equilibration table 1 to accurately portray blood glucose concentrations represented by numerical HBA1C values ranging from 5-15%. He noted a glucose of 90 mg/dL correlated to a fructosamine concentration of 212.5 μmol/L and HbA1c of 5% while a glucose concentration of 390 mg/dL correlated with a fructosamine concentration of 587.5 μmol/L and a HbA1c of 15%. Currently, HBA1C is the primary test used by physicians to accurately assess the previous three month average plasma glucose concentration of human diabetic patients.

Shahbazkia and Nazifi (2005) attempted to substantiate the value of HBA1C for blood glucose concentration analysis in the horse. They reported that blood glucose concentrations in 20 horses were accurately determined through cation exchange chromatography testing for HBA1C. They also reported that their horses averaged 3.2 ± 0.84% glycosylated hemoglobin concentrations. In a later study, Shahbazkia et al. (2010) reported glycated hemoglobin percentages observed in 193 crossbred horses. In this study, Males averaged 3.21 ± 0.56% while females averaged 3.34 ± 0.72% in females. Fasting plasma glucose concentrations were 81.3 ± 7.6 mg/dL and 84.2 ± 14.5 mg/dL in males and females, respectively. They reported HbA1c and plasma glucose concentrations were highly correlated ($r = 0.81$, $P < .01$). They concluded that glycated

hemoglobin A1c can be considered as a reliable indicator of blood glucose status in the horse.

Monfort and Akers (2009, 2011) in pursuant studies, found that this test projected inconsistent and inaccurate long-term blood glucose concentrations in light riding horses. Further study must be done in order to substantiate the results of these studies.

Plasma Fructosamine concentration (PFC) as an effective indicator of long-term blood glucose concentrations

While HBA1C equine studies have yielded widely varied results, PFC testing has been utilized by veterinarians as an accepted method of determining fluctuations in long-term blood glucose concentrations in companion animals (Reusch et al., 1993).

PFC were first noted as possible long-term indicators of average blood glucose concentrations in diabetic humans by Armbruster (1987). He observed that elevated blood glucose concentrations resulted in increased formation of a blood protein/glucose complex called fructosamine. Reusch et al. (1993) later determined the lifespan of fructosamine to be much lower than that of HBA1C. They reported that serum albumin, which constitutes approximately 80% of fructosamine, has a half-life of 14 to 21 days. Both Staudacher and Reusch et al. (1990, 1993) reported that by using the half-life of plasma proteins as a reference, it was easy to determine through a single PFC measurement the average blood glucose concentration of an individual over the previous 2 to 3 week period.

Staudacher (1990) first observed PFC in horses. He found that non- laminitic horses averaged 257.3 µmol/L while Knowles et al., (2012), in a subsequent study, noted that normal fructosamine concentrations averaged a similar value of 248.7 µmol/L with a normal range of 195.5 to 301.9 µmol/L. Murphy et al. (1997) reported fructosamine concentrations of 288 µmol/L in foundered ponies and Knowles et al. (2012) also reported that laminitic horses in their study had higher mean fructosamine concentrations than normal horses. They also reported that 13 of 30 laminitic horses displayed fasting hyperinsulinemia and that fructosamine concentrations were higher (261.2±39.2 µmol/L) in laminitic, PPID horses verses normal horses (234.5±32.9 µmol/L). Keen et al. (2004) also reported that EMS horses in their study exhibited higher fructosamine concentrations than normal horses. These studies all noted wide variation in PFC values for laminitic and non-laminitic horses. It is important to note that all of these authors reported that laminitic horses had higher circulating blood glucose concentrations than non-laminitic horses, as evidenced by their differences in PFC.

Nathan et al (1984) compared glucose, fructosamine and glycated hemoglobin (HbA1c). They determined that there was a positive correlation between the three. Gardner and Shoback (2011) also reported that HbA1c and fructosamine are highly correlated. They determined the relationship between circulating fructosamine and the HbA1c in humans through linear regression analysis to be as follows:

HbA1c = 0.017 X fructosamine level (µmol/L) + 1.61

As noted by the American Diabetes Association (2014), in practice, PFC is rarely measured clinically because PFC tests produce much higher variability than A1c tests.

The HbA1c test is very well standardized and trusted due to its nearly universal use, however, it must be remembered that the hemoglobin A1c test is a measurement of average blood glucose concentrations during a 120-day period. Conversely, fructosamine is indicative of blood glucose concentrations during a 14 to 21 day period. A variety of more advanced forms of the HbA1c test have been utilized more recently (e.g. some types of HPLC, immunoassay and capillary electrophoresis) because they can more accurately assay A1c levels during complex hemoglobinopathies and other conditions. The overwhelming majority of reported PFC values are derived from HbA1c values utilizing the formula below that was derived by the United States National Quality Measurements Clearinghouse. They observed that, on average, each change of 3.3 mmol (60 mg/dl) in average blood sugar levels will give rise to changes of 2% HbA1c and 75 µmol fructosamine values. However, this formula overemphasizes the upper-limit of many laboratories' reference ranges of 285 µmol/L as equivalent to HbA1c 7.5% rather than 6.5%. Nonetheless, many laboratories utilize this formula rather than using the formula of Garner and Showback for conducting chemical analysis to determine PFC values:

Fructosamine= (HbA1c - 1.61) x 58.82

These factors alone account for a major quantity of the variability seen in reported PFC values.

An additional source of variation is due to the fact that there is presently no standard reference range available for PFC testing. The reference values depend upon the factors of age, gender, sample population, concentration of testing substrates and testing

methods. Hence, each laboratory report will need to include the patient's specific reference range for a given particular chemical analyzer for the test to be accurate.

Results of these studies indicate that single value fructosamine testing cannot be used to accurately predict an exact average blood glucose concentration value for a given period of time in a given horse. The real value of fructosamine testing, as noted by Knowles and the American Diabetes Association (2012, 2014), is the ability to portray the general tendencies of plasma glucose concentrations to remain constant or fluctuate up or down, during any given three-week time period. Malmström et al. (2014) noted that fructosamine testing is rarely used in clinical practice. They observed that the main advantage of fructosamine testing is that it can be used as an indicator to detect long-term overall changes in blood glucose concentrations within a few weeks, rather than months (like HbA1c). The American Diabetes Association (2014) recognizes the usefulness of both fructosamine and HbA1c tests as monitoring tools to help people with diabetes control their long-term average blood glucose concentrations. They noted that the HbA1c test is much more well-known and widely accepted simply because there are firm data that clearly correlate exact blood glucose concentrations with exact HbA1c concentrations. There are many studies that positively correlate chronically elevated HbA1c levels with increased risk for certain diabetic complications such as retinopathy, kidney disease, and blindness. They further stated that fructosamine may be considered a viable HbA1c substitute and noted that monitoring the trends of fructosamine values may be a more important indicator of glycemic control than any one fructosamine value.

The one factor that researchers agree upon is that an increase in PFC values determined over time in laboratory testing results is indicative of an increase in blood glucose concentration. However, the high variability among single PFC values renders them of little use when considered alone. Repetitious values can be used to conclusively indicate the tendency of a single individual to maintain, raise, or lower their blood glucose concentrations over time.

Objectives

With the aforementioned factors in mind, the following study was conducted to determine the effects of converting aged, stock horse mares from an ad libitum tall fescue hay diet, in late winter, to an ad libitum, lush, spring tall fescue pasture diet. Parameters observed were long-term fructosamine values as indicated blood glucose concentrations, body condition scores as indicators of body fat composition, body weight, and functional soundness.

Materials and Methods

Preliminary Research

A preliminary study was conducted to validate the accuracy and repeatability of PF values derived using the Beckman Coulter AU 480 chemical analyzer.

Duplicate blood samples were collected from five very thin, emaciated mares (BCS 2-3) fed a diet consisting of limited quantities of poor quality fescue hay and five moderate (BCS 5-6) mares fed ad libitum quantities of average quality fescue hay. Immediately following collection, the samples were analyzed in the Beckman AU 490 chemical analyzer to determine if paired PF values were different.

In the Beckman Coulter AU 480 chemical analyzer procedure, glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). A photometer in the analyzer tests the change in absorbance of a diluted blood plasma sample at 340/380 nm to determine the amount of glucose present in the sample (PFC).

Upon verification that the Beckman AU 490 generated repeatable PF results ($P > 0.01$), the data were further analyzed by paired t-tests to determine the effects of diet and BCS on PFC values in adult stock horse mares.

Primary Research

Five healthy, adult stock horse mares with moderate BCS (5-6) ranging in age from 8 to 13 years were placed in a 20-acre field, devoid of actively growing vegetation.

The horses were then given free choice access to three separate consecutive treatment diets for three distinct, different, specific periods of time (determined by the emergence and growth of spring pastures). Dietary treatment one, hereafter referred to as DTP1,consisted of ad-libitum (free-choice) access to average quality fescue hay that was offered for a period of 128 days (October 22- February 25). Dietary treatment period two, hereafter referred to as DTP2, was initiated when test subjects were first observed to begin grazing on emerging fescue pasture and consisted of a 28-day feeding period (February 26- March 26), in which the horses had ad-libitum access to average quality fescue hay along with limited quantities of available emerging fescue pasture. The third and final dietary treatment period, hereafter referred to as DTP3, consisted of 42 days (March 27 -May 8) of ad-libitum access to 20 acres of recently seeded (during the previous fall) lush, spring tall fescue pasture. Test subjects received free access to water, trace mineralized salt, macro-minerals and exercise throughout the three dietary treatment periods.

The horses were body condition scored by three trained technicians on the first day of DTP 1 and on the final day of each dietary treatment period. A scale was not available on the first day of DTP1, however, the horses were weighed on the final day of dietary treatment periods 1, 2, and 3. The horses were monitored daily throughout the trial to assess any visible symptoms of lameness, colic, or other anomalies. Duplicate blood samples were collected on the final day of DTP 1 and at 14-day intervals throughout the remainder of the trial. The samples were immediately taken to the

laboratory and analyzed for PF values using the Beckman Coulter AU 480. Resultant BCS, body weight, and PF values were subjected to paired t-test analysis to determine the effects of dietary treatment periods on these parameters.

Results and Discussion

Preliminary Research

Statistical analyses of derived PF values (µmol/L) from the 10 horses used in the preliminary research are shown in Table 1. These data indicate that duplicate PFC were not different ($P > 0.1$) for any of the 10 test subjects and that the Beckman AU490 chemical analyzer yielded highly repeatable results. Further, these data showed that PFC values differed widely between horses (Table 2) ($P < 0.1$). The PFC values for this group of un-foundered mares ranged from 270 to 372 µmol. These results are similar to those of Staudacher (1990), Murphy et al. (1997), Keen et al. (2004), and Knowles et al. (2012). Similarly, these researchers noted PFC variations of approximately 100 µmol/L. The mean PFC value of the horses was 315.2 µmol/L. Other researchers have reported average PFC values ranging from less than 180 µmol/L to more than 330 µmol/L. There was a great deal of variation in reported mean values. This variation is most likely due to variations in testing procedures, mathematical calculation, and variation in laboratory procedures such as the quantity and concentration of substrates, enzymes, etc. used to derive PFC values as noted by Knowles (2012), The American diabetes Association (2014), and Malmström et al., (2014). These researchers concluded that analysis to determine a single PFC is of little use in determining glycemic control and they all agreed that trends of PFC collected over time are a much better indicator of actual blood glucose concentration than any given single PFC sample. Results of this research were similar to theirs, and we must, therefore, concur with their findings and realize that general trends of our reported PFC values are a much better indicator of glycemic variation and blood

glucose concentrations in test subjects.

Observation of PFC values, BCS values, and type of diet presented in Table 2 and Table 3 indicated these three parameters were correlated. The data were therefore further analyzed by grouping the horses according to their BCS and diet. The fit, moderate (BCS 5 to 6) well-fed horses (Group B) were compared to the underfed, very thin, emaciated horses (BCS 2 to 3) (Table 2). Group B exhibited higher PFC than the very thin, poorly conditioned, underfed horses (Group A) ($p<0.05$). These underfed, poorly conditioned horses were in starvation mode and unable to maintain a healthy BCS. It is not surprising then, that their PFC along with their BCS indicated they were experiencing hypoglycemia. Their diet did not supply enough glucose to meet the maintenance requirements of mature adult stock horse mares with moderate BCS and weights. It appears that some quantity of energy was obviously being generated through catabolic mechanisms (i.e. muscle atrophy and ketosis) to sustain life. Regardless of the cause, all five animals in the well-fed, desirable body conditioned group exhibited circulating PF values in excess of 320 µmol/L, while the less than desirable body condition scored group all fell below 301 µmol/L. These results are similar to those of Murphy et al. (1997), Monfort (2009), and Knowles et al. (2012). PF values tended to be higher as body condition score increased. It appears then, that heavier, well-conditioned and well- fed horses exhibited higher blood glucose concentrations than the poorly conditioned underfed horses. These mares consuming ad libitum quantities of average quality tall fescue hay were supplied with adequate energy to maintain a desirable body condition score and blood glucose concentrations in excess of their maintenance requirements.

These horses were much more capable of doing work than the thin emaciated horses simply because their circulatory system contained more energy. These results are in agreement with National Research Council. (1989) suggesting that ad libitum average quality tall fescue hay intake by mature horses will fulfill all their maintenance requirements and supply enough energy to meet the animal's requirements for light work.

Primary Research

A scale was not available to assess initial body weights of the horses used in our primary study. Therefore, body weights were not attained on the first day of DTP 1 (October 22). The horses were body condition scored on day 1 and on the final day of each dietary treatment period (Table 5). The data conclusively show that the body condition scores of the mares did not change ($P < 0.05$) during the 128 days of DTP 1. These healthy, non-pregnant, mature stock horse mares maintained their moderate body condition scores throughout the winter months of November, December, January, and February while consuming an ad libitum average quality fescue hay diet as their only source of energy containing nutrients. These data suggest that these mares did not exhibit appreciable body weight gains or losses during DTP 1 confirm that a diet consisting of ad libitum access to average quality fescue hay as the only source of energy supplies adequate nutrient intake to meet the maintenance requirements of adult stock horse mares throughout the duration of a typical Kentucky winter. These results are in agreement with the National Research Council's (1989) dietary recommendations but are contrary to the recommendations of many company representatives who foster the idea that ad libitum fescue hay diets must be supplemented with grain in order to meet the maintenance

requirements of adult stock horses during a typical Kentucky winter. These mares not only met their maintenance requirements but also exited the winter months with the most desirable BCS and body weight possible. Clearly, an average quality tall fescue hay diet with high energy grains is not only unnecessary, but it may have the detrimental effect of horses exiting winter with unhealthy obesity that may generate other anomalies, such as founder or colic.

Results of data collected during DTP 2 and DTP 3 on the body weight of test subject mares are reported in Table 4. When the horses were transitioned from the ad libitum hay only diet (DTP1) to the ad libitum fescue hay plus limited fresh, fescue pasture diet (DTP2) for a period of 28 days, A dramatic increase in body weight was observed in all five of the test subjects ($P < 0.05$). These mares each gained an average of 53 pounds during the 28 days of DTP2. The average daily gain (ADG) of these mares during DTP2 was 1.9 pounds per day. Such massive weight gains are normally only observed in very young, rapidly growing weanling horses that are gaining most of their weight in the form of muscle and bone tissue. In the case of these under-exercised mature, adult horses, however, a change in body weight or BCS is the result of a change in body fat composition. The body mass and metabolism observed in these mares can be attributed only to one thing, the addition of the limited quantities of high energy, emerging lush fescue grass to the diet. Considering that these were mature horses that were not forced to exercise, coupled with the associated change in average body condition score observed to during DPT 2 (Table 5), it is clear that their rapid body weight gain is due to increased adiposity. These horses maintained their moderate body

condition scores while consuming average quality fescue hay as their only source of energy containing nutrients (Table 4). However, when they were converted to a diet consisting of limited quantities of emerging tall fescue grass in addition to free choice tall fescue hay (DPT2) for 28 days their BCS increased by 9% to a value of 6.2 ($P < 0.05$). The increased body condition scores and body weights of these mares can be explained by the fact that fresh tall fescue pasture contains approximately 15% more total digestible nutrients (TDN) than average quality tall fescue hay (Hannaway et al., 1999). Therefore, the addition of fresh tall fescue pasture to the tall fescue hay diet obviously increased the energy density of the diet by 15% for each pound of pasture substituted for hay. In addition, horses typically consume greater quantities of the more palatable, fresh tall fescue than hay, which explains why energy intake is greatly increased with the addition of the fresh forage. When the spring tall fescue pasture began to emerge, the horses simply began to consume greater quantities of more energy dense fresh tall fescue and to consume lesser quantities of the higher fiber, lower energy tall fescue hay. The increased body condition scores and body weights associated with the addition of greater quantities of more energy dense feeds were expected.

The American Diabetes Association (2004) and Betts et al., (2005) substantiated the fact that a major symptom of HMS, increased body weight, in the form of adiposity, commonly precedes the onset of type II diabetes. Similarly as noted by Johnson et al. (2004), Geor and Harris (2008), Frank (2009) and Carter et al. (2009) the degree of obesity and body weight is directly correlated with the onset of laminitis in EMS horses. Johnson (2002) theorized that no single physical parameter was responsible for the onset

of laminitis but, like many other scientists, they observed that lack of exercise combined with overfeeding led to obesity, which in turn led to the development of laminitis. It is important to note that the mares in this primary research exhibited increased body weight (Table 4), and obesity as indicated by BCS (Table 5), in response to DTP 2 and DTP 3, none of the horses in this research indicated any evidence of the onset of founder. Since none of the subjects ever attained the maximum obesity score of 9, it is unclear whether these horses may have developed laminitis had they achieved the maximum BCS. The data indicate that trends for these horses were continuing toward increasing body weight gains and BCS, while PFC and blood glucose concentrations decreased. If the horses had continued on the trial for additional periods of time, the maximum obesity body condition score most likely would have been attained. Based on body condition scores and body weights observed (Tables 4 and 5) the horses would have had to gain an additional 9% of body weight in the form of fat to reach the maximum BCS of 9 (extremely obese). Evidence of laminitis prior to the development of an extremely obese body condition score would not be expected. The effect of the development of the maximum BCS of 9 on predicted PFC values is conjecture based on this research data. These data do show that DTP 3 resulted in increased body fat composition, and that these test mares might continue to have a tendency toward increased body weight and body condition scores over time and that they may have eventually reached a body weight of approximately 1450 pounds (a gain of 250 pounds of excess fat above their moderate body condition score weight of 1200 pounds, if they were to achieve a BCS of 9).

DTP 3 consisted of a 42-day period in which the test subjects consumed ad libitum quantities of lush spring tall fescue pasture as their only source of energy-containing nutrients.

The effects of DTP 3 on body weight (table 4), are similar to those of DPT 2. Once again, all five of the test subjects gained weight during DTP 3 ($P < 0.05$). Their mean weight gain was 63 pounds per subject with an ADG of 1.5 pounds per day. All five mares exhibited increased body condition scores ($P < 0.05$) (Table 5). This would be expected with the increase in body weight. The test subjects averaged an increase of 1.6 body condition scores over the 42 days of DTP 3 ($P < 0.01$). The subjects gained an average of 4.4 % body fat during DTP 2, and an additional 5.2 % body fat during DTP3. Therefore, these mares averaged an overall increase of a 9.4 % or 113 lb. of body fat during the 70 days that they were allowed free choice access to spring tall fescue pasture. All five horses were in an anabolic state throughout the duration of DTP 2 and DTP 3. Increased adiposity was evident from the time that the horses transitioned from an ad libitum average quality tall fescue hay diet to an ad libitum lush, spring tall fescue pasture diet. These data are in agreement with Geor (2008, 2010), Geor and Harris (2010), and Frank et al. (2010) Monfort (2009) and Akers (2011). The horses in this trial gained additional body weight and BCS with each successive dietary treatment period, and that they may have continued this trend had the duration of the trial continued longer into the spring.

Dietary treatment periods 1, 2, and 3 had effects on the trends of PFC values of the test subjects over time as they transitioned from DTP1 to DTP2 and then to DTP3 (Table

6). These data are representative of the tendencies of blood glucose concentrations of the test mares during DTP 1, DTP 2, and DTP 3 (Figure 3). When either the general trends Table 6 and Figure 3 are considered, as suggested by the ADA, or the actual mean values of each dietary treatment period, it is that PFC values decreased ($P < 0.05$) during each successive dietary treatment period ($P < 0.05$). Thus, blood glucose concentrations were decreasing as the horses transitioned from the ad libitum tall fescue hay only diet to the diets containing tall fescue pasture.

Upon initial observation, it might be assumed that these results were not expected or even corrupted. It is not logical to assume that when horses that are gaining weight and consuming greater quantities of higher energy diets, they would exhibit lowered blood glucose concentrations. The fact that these horses gained weight with each successive dietary treatment and became fatter, as evidenced by their increasing BCS with each transition, indicated that the horses were merely exhibiting the early signs of hyperinsulinemia. These findings are similar to those of Frank et al. (2010) who stated that "Hyperglycemia is rarely detected in horses with EMS because most animals maintain an effective compensatory insulin secretory response in the face of IR". They also stated that if persistent hyperglycemia is detected, a diagnosis of diabetes mellitus should be considered. These mares were gaining weight in the form of body fat as evidenced by their increasing body condition scores while blood glucose concentrations decreased. The most likely explanation is that these horses were responding to the increased blood glucose concentrations of DTP 2 and DTP 3 by generating greater and greater quantities of insulin as suggested by Reaven (1988). The test subjects were

apparently becoming hyperinsulinemic, as suggested by Eiler et al., (2005), in order to deal with the increasing quantities of dietary glucose supplied during DTP 2 and DTP 3.

Apparently, the horse's pancreas had adapted to the intake of a consistent quantity of nutrients supplied by the tall fescue hay diet during the 128 days of DTP 1. They were secreting a consistent quantity of insulin sufficient to maintain blood glucose concentrations within the normal realm while consuming the hay only diet. When the horses were transitioned to DTP 2, the pancreas was shocked and jumpstarted into emergency mode because blood glucose concentrations began to increase rapidly when the horses were introduced to DTP 2. Hyperinsulinemia could be the explanation for the rapid massive weight gains observed during DTP 2 and 3. The pancreas immediately responded by secreting more insulin in an attempt to drive excessive blood glucose out of the circulating blood stream and into skeletal muscle, liver, and fat cells. The effect would be to lower circulating blood glucose concentrations into the normal range. The horses, however, continued to consume increasing quantities of sugar during DTP 2 and DTP 3. This over stimulated the horse's pancreas to produce so much insulin that hyperinsulinemia developed. Hyperinsulinemia then appears to be responsible for the rapid response of lowered blood glucose concentrations associated with DTP 2 and DTP 3. These results are consistent with those of Reaven (1988), Ravussin and Smith (2002), and Özcan et al.,(2004). It is evident from these data that glycemic control of circulating blood glucose concentrations during DTP 2 and DTP 3 could only be controlled through hyperinsulinemia. Blood glucose concentrations did decrease in response to hyperinsulinemia generated by the increased glucose content of DTP 2 and DTP 3,

indicating that the horses were able to generate sufficient insulin through hyperinsulinemia to prevent evidence of hyperglycemia for the 70 days of DTP 2 and DTP 3. Further research should be undertaken to determine whether horses are able to produce adequate insulin to contain hyperglycemia over a longer period of time.

To summarize, these mature, adult, stock horse mares developed all the symptoms of EMS equine metabolic except laminitis during a very short period of time. During the 70 days they were allowed access to limited and later unlimited quantities of average quality tall fescue hay, they gained a great deal of weight, developed obesity, and became hyperinsulinemic in response to DTP 2 and DTP 3. It is unclear whether or not trends would have continued long-term if the trial had lasted longer into the spring. It is not clear what effect the hyperinsulinemia would have had on BCS, body weight, blood glucose concentrations or the onset of founder in these mares. It would be interesting to know these points and additional research is required.

Conclusions

1. The Beckman AU 480 chemical analyzer produces highly repeatable PFC values using blood samples collected from mature stock horse mares.

2. Moderate (BCS 5 – 6) mature stock horse mares consuming ad libitum quantities of average quality tall fescue hay exhibited higher plasma fructosamine concentrations, therefore higher blood glucose concentrations, than very thin, emaciated (BCS 2 – 3) underfed stock horse mares.

3. Moderate (BCS 5 – 6) mature stock horse mares consuming ad libitum quantities of average quality tall fescue hay as their only source of energy containing nutrients maintained their body weight and body condition score throughout winter months of November, December, January, and February in Kentucky.

4. When mature adult stock horse mares were transitioned from an ad libitum tall fescue hay diet to an ad libitum tall fescue hay plus limited emerging tall fescue pasture diet in early spring, obesity increased as evidenced by their increasing body condition scores and body weight.

5. When mature adult stock horse mares were transitioned from an ad libitum tall fescue hay diet to an ad libitum tall fescue hay plus limited emerging tall fescue pasture diet in early spring, plasma fructosamine concentrations and therefore blood glucose concentrations decreased in response to dietary induced hyperinsulinemia.

6. The addition of tall fescue pasture in DTP 2 and DTP 3 resulted in increased sugar and calorie content of these dietary treatment periods, which in turn led to the development of hyperinsulinemia.

7. These mature stock horse mares exhibited massive weight gains and body condition score changes during DTP 2 and DTP 3, however no horse gained enough weight in the form of fat to achieve the maximum body condition score of nine.

8. No horse in the primary trial expressed any lameness or tendency toward laminitis.

9. The mature adult stock horse mares in this study developed all the symptoms of equine metabolic syndrome except laminitis during the 70 days they were allowed access to limited and then unlimited quantities of average quality tall fescue hay.

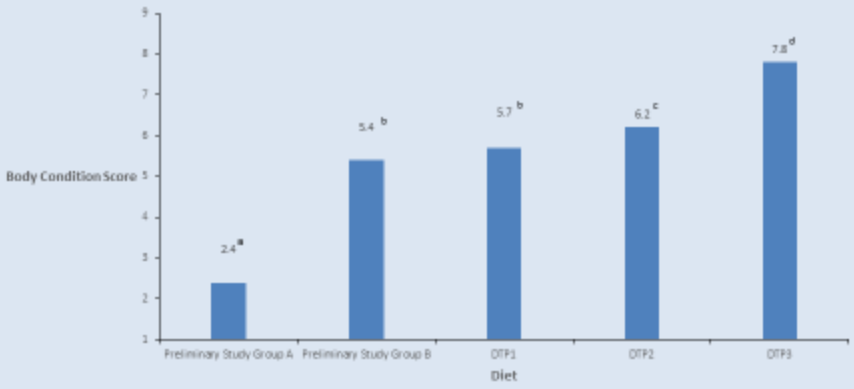
10. The trends observed in this study indicate that continuation of ad libitum intake of fresh spring tall fescue pasture for an extended period of time may have resulted in additional increased body weights and body condition scores, along with additional decreases in plasma fructosamine and blood glucose concentrations as the result of hyperinsulinemia.

11. Parametric trends of data from this study indicated that continuation of DTP 3 for an extended period of time may have generated maximum body weights and body condition scores along with further insulin resistance and hyperglycemia. The only way to determine if these parameters would have been affected is to conduct further research.

Figure 1. Body Weight, in lbs., of Stock Horse Mares on the final day of Dietary Treatment Periods 1, 2, and 3

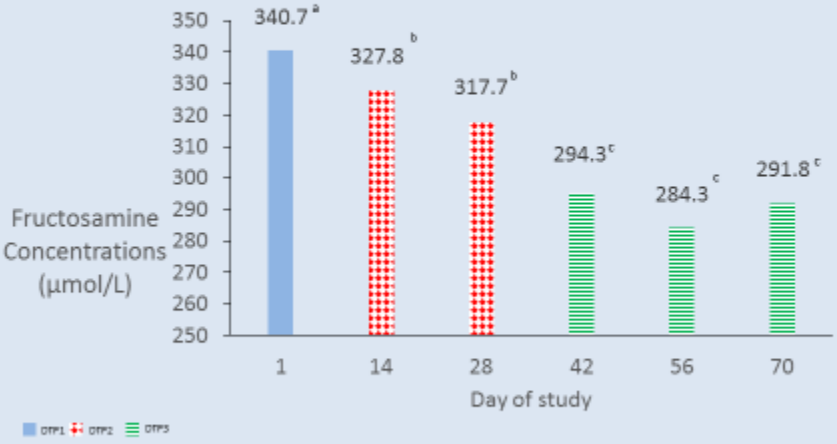
a,b,c Means with different superscripts differ $P < 0.05$

Figure 2. Body Condition Score of Stock Horse Mares on the final day of Dietary Treatment Periods 1, 2, and 3



[a,b,c] Means with different superscripts differ $P < 0.05$

Figure 3. Plasma Fructosamine Concentrations, µmol/L, of Stock Horse Mares on the final day of Dietary Treatment Period 1 and at 14 day intervals throughout the remainder of the trial



a,b,c Means with different superscripts differ $P < 0.05$

Table 1. Preliminary research results of duplicate Plasma Fructosamine concentrations, µmol/L.

Horse	First Blood Sample Plasma Fructosamine Concentration	Second Blood Sample Plasma Fructosamine Concentration
1	299[a]	300[a]
2	300[a]	298[a]
3	292[a,b,c]	295[a,b,c]
4	273[b]	270[b]
5	285[c]	284[c]
6	325[d]	323[d]
7	372[e]	370[e]
8	320[d]	321[d]
9	362[f]	360[f]
10	327[d]	327[d]
Mean	315.5	314.8

[a,b,c,d,e,f] Values in the same row with the same superscripts are not different ($P > 0.05$)

Table 2. Preliminary Research of Plasma Fructosamine concentrations, µmol/L, of fit, well-fed stock horse mares verses thin, underfed stock horse mares.

Group A		Group B	
Emaciated BCS 2-3 consuming limited quantity poor quality fescue hay diet		Moderate BCS 5-6 consuming ad libitum fescue hay diet	
Horse	PFC (µmol/L)	Horse	PFC (µmol/L)
1	299.5[a]	6	324[b]
2	299[a]	7	371[b]
3	293.5[a]	8	320.5[b]
4	271.5[a]	9	361[b]
5	284.5[a]	10	327[b]
Mean	289.6[a]	Mean	340.7[b]

[a,b] Values with different superscripts are different ($P < 0.05$)

Table 3. Preliminary Research of Body Condition Score of fit, well-fed stock horse mares verses thin, underfed stock horse mares.

Group A		Group B	
Emaciated BCS 2-3 consuming limited quantity poor quality fescue hay diet		Moderate BCS 5-6 consuming ad libitum fescue hay diet	
Horse	BCS	Horse	BCS
1	3[a]	6	5.5[b]
2	2.5[a]	7	5[b]
3	2[a]	8	5[b]
4	2.5[a]	9	5.5[b]
5	2[a]	10	6[b]
Mean	2.4[a]	Mean	5.4[b]

[a,b] Values with different superscripts are different ($P < 0.05$)

Table 4. Body Weight, lbs., of stock horse mares on the final day Dietary/Treatment Period 1, 2, and 3 (DTP 1, DTP 2, DTP 3, respectively).

Horse	DTP1	DTP2	DTP3
1	1210[a]	1290[b]	1400[c]
2	1200[a]	1290[b]	1400[c]
3	1115[a]	1130[b]	1190[c]
4	1220[a]	1280[b]	1310[c]
5	1250[a]	1270[b]	1290[c]
Mean	1199[a]	1252[b]	1318[c]

[a, b, c] Values in the same row with the different superscripts are different ($p<0.01$)

Table 5. Body Condition Score of stock horse mares on the final day of each Dietary/Treatment Period.

Horse	DTP1	DTP2	DTP3
1	6^{a}	6.5^{b}	8^{c}
2	5^{a}	5.5^{b}	8.5^{c}
3	5^{a}	5^{b}	7^{c}
4	6^{a}	7^{b}	8^{c}
5	6.5^{a}	7^{b}	7.5^{c}
Mean	5.7^{a}	6.2^{b}	7.8^{c}

[a, b, c] Values within the same line with the same superscripts are not different ($p<0.05$)

Table 6. Mean Plasma Fructosamine Concentrations, µmol/L, of stock horse mares at 14 day intervals throughout Dietary/Treatment Periods 1, 2, and 3.

Horse	Dietary/Treatment Period					
	1		2		3	
1	320.5	318	310	282.5	285	287.5
2	367	360	343.5	327.5	298.5	314.5
3	322.5	301	306	279	262	281.5
4	365	340	311.5	293	299	296
5	328.5	320	317.5	289.5	277	279.5
Mean	340.7^{a}	327.8^{b}	317.7^{b}	294.3^{c}	284.3^{c}	291.8^{c}

^{a,b,c} Values with different subscripts are different ($p<0.05$)

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