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Effects of Supplementing Ponies with Dietary Fat on Nutrient Digestibility and Blood Insulin, Glucose, and Fatty Acid Concentrations

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By

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Fat supplementation is a common practice to increase caloric intake in the performance horse. The effects of fat on fiber digestibility is unknown. Understanding of digestibility in the equine digestive tract is limited by sampling technique. While cecal and ileal cannulations have previously been utilized to determine equine nutrient digestibility and gastrointestinal physiology, the current research has been limited to singular portions of the equine digestive tract. The objectives of this dissertation were to determine the effects of dietary fat supplementation on nutrient digestibility and blood insulin, glucose, and fatty acid concentrations using dual cannulated ponies. The first step to this objective was establishment of a dual cannulated pony herd for research. This study resulted in a post-operation survival rate of 63%. Five of the dual cannulated ponies were fed hay and pelleted alfalfa and supplemented with vegetable oil at 0, 5, 10, or 15 % of total diet. Ileal, cecal, fecal, and blood samples were taken with blood samples analyzed for glucose, insulin and fatty acids. There was a treatment by time effect ($P < 0.1$) for apparent ileal and cecal fat digestibility and apparent cecal digestibility of crude protein. Apparent total tract digestibility of NDF, ADF and fat was affected by time ($P <$

0.1). Adding fat increased ($P < 0.1$) apparent total tract digestibility of fat. At 0 h post feeding apparent total tract digestibility of protein was greatest ($P < 0.1$) compared to other time periods. Plasma concentration of insulin increased ($P < 0.1$) over time. Ponies consuming 0 % fat diet had increased C14:0 compared to 5, 10, and 15 % diet. Adding fat at 5, 10, and 15 % of the diet increased C18:2 n-6 when the ponies were fed 0 % fat. Further research using a dual cannulated equine research herd is needed to more completely understand digestibility of other components of the equine diet.

DEDICATION

This dissertation is lovingly dedicated to my supportive family especially my mother and my grandparents, Tanya J. Whiteside and Douglas M. and Beatrice D. Whiteside. Their support, encouragement, and constant love have sustained me throughout my life. A special thought for my dad will always remain with me as he looks down from heaven. My dedication extends to a special band of ponies that made it all possible and touched my heart.

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CHAPTER I

LITERATURE REVIEW

Energy Sources and Equine Diets

Digestibility is the ability of a feedstuff to be broken down in the gastrointestinal tract (GIT) to prepare nutrients for adsorption. In horses, hindgut retention time greatly affects digestibility of the feed source (Jansen et al., 2007a). Horses, like rabbits and guinea pigs, digest and adsorb fiber in the hindgut or large intestine, most of which occurs in the cecum. Although research has been performed examining total tract digestibility in the horse, information is limited regarding sites and specific digestion mechanics of the different intestinal segments. Further knowledge of feed digestibility may be useful for ration formulization to optimize energy utilization from dietary energy sources such as carbohydrates and fats.

Adenosine Triphosphate (ATP) is the energy currency for life, and the major source of ATP production comes from carbohydrates and fats. Energy produced from the products of enzymatic digestion of starch in the small intestine have been seen to be more efficient compared to ATP from volatile fatty acids (VFA's) metabolism from fiber digested in the hindgut (Geor et al., 2013). Volatile fatty acids, such as acetic, propionic, and butyric, are released in the hindgut as fermentation occurs. Post adsorption, VFA's are available in the liver to be utilized for energy or stored as fat for energy utilization at

a later time. Fermentation of fiber in the hindgut also yields methane, CO_2 and other products.

Adding fat, as oil, into horse's diets has been used for years to increase energy intake (Dunnett, 2005). Fats provide more energy density to diets compared to carbohydrates or proteins because fats are more reduced, or the ratio of hydrogen ions to oxygen are increased, and thus, provide more energy upon oxidation. Equine diets with increased dietary fat or oils have been observed to increase muscle glycogen stores (Potter et al., 1992) and have a glycogen-sparing effect on aerobic metabolism allowing for more glycogen availability during anaerobic work (Pagan et al., 2002). Horses have been reported to have decreased plasma lactate post-exercise when fat was included in the diet (Sloet van Oldruintenborgh-Oosterbaan et al., 2002). Delayed lactate accumulation due to dietary fat results in delaying fatigue during exercise (Lewis, 1995).

Introduction to Lipids

Lipids are chemical and nutrient classifications, which are water insoluble, therefore solvents are added to the lumen of the small intestine to break down fat (Voet and Voet, 1990). Lipids break down into several classifications, but the main separation is saturation amount or structure and binding affinity. Amount and types of bonds within lipids determine how they can be utilized or the form that they are presented, such as solid (fats) or liquid (oils). Saturated fatty acids are filled with hydrogens at every carbon of the polar carbon chain. Unsaturated lipids, such as those found in oil, indicate the carbons are not completely filled with hydrogens, and thus, the polar carbon chain contains double carbon bonds. Polyunsaturated fatty acids include multiple double bound carbons. As the degree of unsaturation is increased, fluidity of the lipid increases and

melting point decreases. Degree of saturation, length, and position of double bonds determines the function of the lipid in a biological system.

Polyunsaturated fatty acids, some of which are essential fatty acids, are required in the diet for body functions (Voet and Voet, 1990). Equine require 2 fatty acids, linoleic (18:2) and linolenic (18:3), to be included within the diet. Fatty acids are grouped into 3 categories and used for functions such as the production of prostaglandins and other steroid hormones. Omega-6 fatty acids are found in animal products such as meat, dairy, and shellfish. Flaxseed oil, pine nuts, pistachios, sunflower seeds, primrose oil, corn, safflower, sunflower, soybean, and cottonseed oils have omega-6 fatty acids as well. Omega-6 fatty acids possess a double bond between the sixth and seventh carbon, hence the term "omega-6". Omega-3 fatty acids are found in cold water fish and plant oils including olive, canola, linseed, and hemp oil. Omega-3 fatty acids are similar to omega-6, but have a double bonds between the third and fourth carbons.

Lipid Metabolism

Digestion of lipid, consisting of a multi-step process, includes emulsification of large lipid particles, saturation of lipids through enzymatic mechanics, and transformation of lipid to allow water solubility for absorption. Voet and Voet (1990) summarized lipid digestion occurring in the small intestine. Digestion of water insoluble triacylglycerol (TAG) occurs at the lipid water interface where digestive enzymes, which are water soluble, exist.

Through the first step of lipid digestion, the mechanical emulsification of lipid begins with chewing and is continued in the stomach by churning and a decrease of pH. Surface area of lipid droplets and peristaltic movement greatly affect emulsification of

bile salts on lipids during digestion. By this process, large lipids are broken down to small droplets resulting in a larger surface area for enzymes to attach (Voet and Voet, 1990).

The second segment of lipid digestion occurs through enzymatic digestion of lipids in the stomach. Although complete mechanics are unknown, hydrolysis of triacylglycerol was assumed to begin when the zymogen cells in the stomach's fundic mucosa layer secrete gastric lipase (Geor et al., 2013). Little research has been completed to determine mechanics of fat digestion in equids; meaning most of the knowledge has been derived from comparable species. Generally, in comparable monogastrics, fatty acids exiting the stomach trigger release of hormones to aid in digestion through the small intestine, specifically the duodenum. Gastric acid in the small intestine causes secretin to be released, triggering the pancreas to release bicarbonate. Bicarbonate, in the small intestine, neutralizes chyme and enzymes, and lipase separated fatty acids from triacylglycerol. Bicarbonate, allows emulsification, hydrolysis preparing for digestional uptake by the intestinal mucosa layer (Harris et al., 1999). Once chyme in the duodenum, cholecystokinin (CCK) is released from the mucosa, which initiates the release of pancreatic enzymes and bile to further aid digestion of lipids.

Bile salts have also been seen to aid in the process of breaking down large particles. Horses unlike most mammals do not store bile in the gallbladder. Horses lack a gallbladder, and thus, continuously excrete bile acids directly from the liver to aid in lipid digestion. Increased fat in the diet of horses stimulated the secretion of bile (Meyer et al., 1997); therefore, some hepatic regulation of bile excretion must be present.

Pancreatic lipase has been seen to cleave 2 fatty acids from triacylglycerol, which results in monoacylglycerol and 2 nonesterified fatty acids (NEFA; Goer et al., 2013). During the third stage, monoacylglycerol, NEFA, and bile salts form a more water soluble micelle. Nonpolar lipid products, within the micelles, are delivered to the brush border where the mucosa cells transport the lipid across the intestinal wall. Inside the enterocyte, fatty acids bind to proteins, such as intestinal fatty acid binding protein (I-FABP), and are transported through the cytosolic fatty acid transporter. Once absorbed into the enterocyte, fatty acids and monoacylglycerols are converted to TAG, which is then enveloped by lipoproteins to form chylomicrons. Chylomicrons are made of an inter triacyglycerol core with an outer layer of phospholipids, protein, and cholesterol, and are released into the lymphatic system to be distributed throughout the body.

Triacylglycerol lipoprotein does not enter directly into the bloodstream; rather, the lipid is released into the lymphatic system, and then, enters the bloodstream. A small number of short chain fatty acids may not be re-esterified, and are absorbed into capillary blood and transported to the liver via blood. Shorter chained fatty acids are transported as free fatty acid, through circulation, rather than, the lymphatic system by lipoproteins (Harris et al., 1999).

In other species, increased dietary fat has been reported to inhibit gastric emptying compared to carbohydrates. Digestive products in the duodenum are considered to influence gastric empting, yet research results on horses are conflicting. Increased fat diets have been seen to reduce consumption rate of hay intake (Zeyner et al., 2002). Consumption rate and amount of fiber intake within the hindgut could also affect gastric

emptying. Thus, fat intake decreased which effected rate of gastric emptying due to the fiber retention in the hindgut.

Lipoproteins

Dietary fat was observed to be absorbed in the duodenum and jejunum, after which, lipids are transported to the liver, adipose tissue, or other tissues via lipoproteins, such as chylomicrons or very low density lipoproteins (VLDL). Lipoproteins have been thought to transport triacylglycerol and cholesterol within blood plasma. Voet and Voet (1990) described lipoproteins as proteins that were non-covalently bonded to a lipid; often, the fatty acid binds to the carboxyl terminus of the protein.

Very low density lipoproteins and low density lipoproteins (LDL) contain triacylglycerols, which are synthesized by the liver and released into the blood. Little has been discovered about lipoproteins or whether VLDL plays more of a role as both are based from triacylglycerol. High density lipoproteins (HDL) have been seen to transport cholesterol from the tissue to the liver (Voet and Voet, 1990).

Fatty Acids

Although required dietary concentrations have yet to be determined, equine require essential fatty acids including linoleic (n-6) and linolenic (n-3; Harris et al. 1999). Essential fatty acids, which cannot be synthesized by the body, are key for biomembranes structural components, lipid transport and are precursors for prostaglandins. Sallmann et al. (1992) observed no long term impacts of decreased linoleic acid concentrations in the diet suggesting C18:2 had a long half-life due to the horse's stored fatty acids.

Role of Lipids in the Body

Fats are required for many body functions beyond metabolizable energy. For example, unsaturated fatty acids, such as 20 carbon arachidonate, are precursors for intercellular control, thromboxane, and prostaglandin. Prostaglandin mechanics are not fully known, but are thought to be important for growth regulation (Voet and Voet, 1990), tissue repair, central nervous system activity, and mechanics of inflammation.

Physiology of Fat in Equine

Although equids developed a digestive system geared around fiber digestibility, horses, for the most part, have been observed to be proficient at adapting to utilize fat in their diet. Kronfeld et al. (2004) reported fat digestibility peaked when between 100 and 150 fat g / kg of diet and peak digestibility remained up to 230 fat g / kg DM when horses had been adapted to these amounts of fat intake. Kronfeld et al. (2004) observed horses to utilize up to 20 % or greater of dietary fat, which was hypothesized to be possible from increased lipase secretion from the pancreas.

Horses digest fat in the small intestine where bile has been observed to be secreted from the liver to aid in fat digestion. Estimates of apparent digestibility of ether extract by ponies are 42 to 49 % for forage and 88 to 94 % of supplemented fat and oil (Kronfeld et al., 2004). According to Goer et al. (2013), the most difficult fat for horses to digest is from forages (5 to 57 % apparent digestibility) and cereal grains (55 to 57 % apparent digestibility); yet, fat added to the diet has a much greater digestibility, between 64 and 96% (Kane et al., 1979). Differences of fat from forage and concentrate sources are most likely due to structural differences. Fat in forage and concentrates are within the cell wall of the plant, which makes it more difficult for lipase to reach the fat. Enzymatic

hydrolysis of lipids is possibly reduced with decreased fat intake involving forage and grain (Kronfeld et al., 2004).

Horses, similar to other mammals, store fat metabolized from excess carbohydrates, proteins and fats as saturated fat. Triacylglycerols are nonpolar structures that are insoluble in water and serve as energy storages for the body. Triacylglycerols, the most abundant form of lipid in the body, are found in both fats and oils. Fats are solid at room temperature whereas oils are liquid at room temperature. In the body, TAG is stored in adipose tissue within the subcutaneous layers of the body wall. Horses have been seen to absorb medium chain fatty acids (6 to 12 C) well and transport TAG to the liver (Geor et. al., 2013).

Nonesterified fatty acids are small sections of fat that are unbound to glycerol and also known as free fatty acids. Hiney and Potter (1996) suggested an effect of fat supplementation was an increase of plasma NEFA concentrations resulting with an increase of FA oxidation and a decrease in muscle glycogen and blood glucose utilization. Added dietary fat affected NEFA, lipoproteins and free fatty acids utilized for exercising muscles with increased NEFA resulting in increased fat oxidation (Lawrence, 1994).

Fat and Glucose Conservation

Often, fat reserves in the body have been seen to be utilized for energy to conserve glucose. Zeyner et al. (2002) reported increased concentrations of glucose in blood plasma when horses were fed increased fat and decreased starch. As stated previously, added dietary fat has been reported to delay gastric emptying of carbohydrates. Increased dietary fat stimulates the secretion on CCK, which is

responsible for decreased gastric motility and delayed gastric emptying (Dunnett, 2005). According to Frappe (2004), the delayed emptying increases plasma glucose concentration due to the decreased post feeding plasma glucose response peak.

According to Goer et al. (2013), horses fed oil had increased lipoprotein lipase activity in the small intestine, which aided in breakdown of HDL and LDL. Increased lipase activity in cells of skeletal muscle or liver is linked to an increase in citrate synthases and beta-hydroxy acyl-CoA dehydrogenase. Citrate synthases drives the reaction for the citrate product in the citric acid cycle. Beta-hydroy acyl-CoA dehydrogenase was key for the third of 4 reactions in beta-oxidation for the breakdown of TAG for energy. Fat supplementation stimulates β-oxidation and fat catabolism, which results in decreased plasma TAG. Increased muscle LDL activity and citrate production was seen to be a result of increased fat catabolism. Increased citrate production inhibits phosphofructokinase, which is a rate limiting enzyme in glycolysis.

Glucose is converted into 2 pyruvates and ATP is released for energy through glycolysis. Glucose is preferentially utilized, rather than fat, for several reasons: muscles cannot utilize fat as quick as glucose from glycogen and fat cannot be metabolized anaerobically. Zeyner et al. (2002) reported blood glucose remained within expected range with horses on an increased starch diet, but increased glucose concentration more than expected with the increased fat diet. Results may be due to the alleviation of glucose turnover by the increased fat oxidation or related to an increased insulin resistance from the increased fat diet (Zeyner et al., 2002), although no insulin resistance was reported by Harris et al. (1999).

Role of Fiber in Equine Digestion

Carbohydrates from plants, which include structural or non-structural, are the predominant component in equine diets. Nonstructural carbohydrates, including starch, fructans, and simple sugars, are substances that can be digested easily by enzymes in the small intestine (Voet and Voet, 1990). Structural carbohydrates are comprised of the fibrous portion that provides rigidity to the plant. Structural carbohydrates have been measured using NDF, including cellulose, hemicellulose and lignin, or ADF, which measures cellulose and lignin. One issue that persists with NDF is the test includes a large amount of amylase digestible starch. To remove starch, the sample can be acid treated, but that also removes some hemicellulose. During NDF, amylase can be added, instead of acid, to remove starch. Loss of hemicellulose causes an underestimate of insoluble fiber from ADF because the horse digests almost all hemicellulose from the plant. To establish the hemicellulose content of a feed source the difference between the NDF content and ADF content is determined.

Previously, 3 factors have been considered to affect fiber digestion and utilization: rate of passage through the digestive tract; gastrointestinal tract (large intestine) microbial activity; and diet composition, especially comparing nonstructural to structural carbohydrates. Rate of passage and microbial activity are specifically related to hindgut fermentation, the cecum in particular. Structural carbohydrates, such as fiber, contain cell wall components, consisting of cellulose, hemicellulose, lignin and pectin, which must be broken down via digestion into smaller particles for adsorption. Cellulose reinforces the cell wall of the plant where the hemicellulose resides. Fiber structures have beta bonds between the monosaccharaide glucose units, as with cellulose, which only enzymes from

microbes in the hindgut are able to breakdown. Horses have been observed to be more efficient at breaking down hemicellulose than cellulose, whereas lignin cannot be utilized. Microbes have to break beta bonds between the monosaccharides in the hindgut converting fibrous carbohydrates into VFA that are absorbed and used for energy. The VFA's, consisting of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids, are short chain fatty acids containing two to five carbons. Volatile fatty acids have been confirmed to provide, on average, 30 to 70% of the horse's total maintenance energy needs (Lewis, 1995).

Stated previously, VFA's are a result of the digestion of fiber by cecal microbes. Goer et al. (2013) summarized cecal environment including ciliate protozoa, fungal zoospores and bacteria and reported that fungi and bacteria were responsible for fiber digestion as protozoa did not appear to contribute to cellulolysis. Cecal microbes bind to fiber particles, specifically cell walls of forage, and excrete enzymes to break down fiber particles. Hemicellulose is digested by xylanasic activity and cellulose is degraded by carboxymethylcellulasic activity. The second step of fiber digestion is the greater β-D-glucosidase activity, which further degrades cellulose with hemicellulose degradation occurring through α-L-arabinosidase activity (Geor et al., 2013). After hemicellulose and cellulose are broken down to monomeric sugars (primarily glucose), these sugars are hydrolyzed by bacterial cells. Bacteria utilize the Embden-Meyerhoff pathway through glycolysis with the end product of pyruvate (Voet and Voet, 1990).

Protozoa in the equine digestive hindgut includes 6 different genus and 50 different species ranging from 102 to 105 depending on the section of hindgut (Voet and Voet, 1990). Although some fungal strains, including Piromyces genus, exist, bacterial

population of the large intestine is seen to play the largest role and has the largest biomass. Bacterial population includes 72% Firmicutes phylum and 20% Bacteroidetes. *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* are the microbes mostly responsible for cellulolytic activity. For years, the cecum was assumed to be the site for fiber digestion, although more recent research has demonstrated a possible contradiction (Geor et al., 2013). Bacteria in the hindgut are responsible for fiber digestion; yet the population of anaerobic microbes for celluloytic digestion in the cecum is less compared to the colon (Julliand et al., 2001).

Cecal contents of glycolytic and amylolytic bacteria include streptococci, lactobacilli, and enterococci. Lactate utilizing bacteria, such as *Megasphaera* sp. and *Veillonella* sp., are greatly affected by undigested starch, which enters the hindgut. When undigested starch reaches the hindgut and begins to ferment, proliferation of bacteria occurs resulting in possible microbe die-off (Linberg and Palmgren Karlsson, 2001).

Microbe population balance was essential in previous fiber studies, but microbe environment has been difficult to determine. Julliand et al. (2001) stated that neither fecal nor colon samples accurately represent the cecal ecosystem, especially when hay was provided. Ponies fed solely hay had different concentrations of streptococci, lactobacilli, and lactate-utilizing bacteria in the cecum compared to the colon. Diets have been observed to play a major role in hindgut microbe concentrations. Grains with fibrous seeds, such as oats or barley, were seen to increase concentrations of total anaerobic and aero-anaerobic bacteria as a result of the increase in amylolytic bacteria concentrations. However, ponies fed hay had increased concentrations of cellulytic bacteria. Lactobacilli and streptococci have been determined to function best when horses were fed

predominantly starch diets resulted in acid conditions of the cecum and were improved by hindgut fermentation of carbohydrates due to the decreased pH. As amylose was fermented, lactate, a product of fermentation, was increased. However, fiber based diets, such as hay, had similar lactate concentrations in both the cecum and colon.

Animals fed fibrous grain diets had reduced cellulytic bacteria resulting with a decrease in fibrolysis, and therefore, a decrease in NDF and ADF digestibility (Geor et al., 2013). Decreased fiber digestibility resulted with decreased proportional cecal acetate production, which, in turn, decreased the ratio (acetate+butyrate)/propionate. Ratio between acetate, butyrate and propionate is important for the citric acid cycle. Acetate and butyrate enters the cycle as actyl-CoA, whereas propionate enters as succinyl CoA which is gluconeogenic. Geor et al. (2013) recorded average cecal VFA percentages of acetate at 74.9%, propionate at 18 %, and butyrate at 6 % fiber diet.

Microbial efficiency has been seen to be increased by fat due to the reduced protozoal populations in cattle (Clark et al., 1992). Kern et al. (1973) described the difference between cecum and rumen fiber digestion. Unlike in the rumen, protozoa were similar whether ponies were fed oats with forage or only forage, meaning no predatory activity of protozoa on bacteria existed in the cecum. Compared to the rumen, equine had increased concentration of aerobic microbes due to either a greater supply of oxygen or less active fermentation. Timothy hay fed to ponies did impact protozoa with an increase of *Cycloposthium bipalmatum*. When clover hay was fed, an increase of total cecal VFA concentration was observed (Kern et al., 1973). Gram-positive cocci, *Streptococcus bovis*, was the most common bacteria overall and hydrolyzed lactate. An increase of both *Streptococcus bovis* and *Streptococcus equinus* was observed when ponies were fed

timothy hay. Overall, 54% of the cecal proteolytic bacteria were gram-negative rods and 19.5 % were gram-positive cocci.

Diets have also been seen to effect digestibility due to a cecal pH change. Julliand et al. (2001) stated that including barley into a hay diet caused an increase of cecal lactate concentration and a decrease of cecal pH. Volatile fatty acid concentrations overall were not altered with the addition of barley; yet acetate decreased and propionate increased, thus decreasing the (acetate+butyrate):propionate ratio. Goodson et al. (1988) observed increased total viable anaerobic bacteria with increased concentrate compared to a primarily hay diet. Feeding a concentrate based diet decreased the number of genera and total protozoan number. Kern et al. (1973) observed, on average, 52 % of the acid-producing bacteria generated acetic acid. The remaining bacteria yielded propionic, butyric, isobutyric, valoric and isovaloric acid; although, diet may influence these.

Equine Digestion and Utilization of Fat and Fiber

Historically, fats have been added to animal diets to increase growth, athletic performance, milk production, and reproductive performance. Increased energy diets decrease DM intake; thus, reduced weight of the gastro-intestinal contents and gut fill, which was beneficial to performance horses (Jansen et al., 2007[b]). Both fat and concentrates increase energy of the diet, but an increase in grain to meet energy needs can lead to colic and laminitis in horses. Fat has been used to achieve the same amount of total energy with decreased total daily intake compared to concentrates preventing grain overload and associated health risks. The amount of energy in the form of fat in the body is 30 to 60 times that of stored carbohydrates (Geelen et al., 1999).

Lipid metabolism and Glycogen Metabolism for Equids

Energy from lipids is utilized during aerobic activities as mitochondrial oxidations of lipids occur. Due to the oxygen requirement for fat metabolism, slow-twitch muscle cells during long distance endurance activities are most efficient. As the oxygen in the cell becomes inadequate for lipid metabolism, cells become more reliant on anaerobic glycolysis for ATP. In contrast, glucose, stored as glycogen in the cell, provides energy for short term anaerobic activity. Linberg and Palmgren Karlsoon (2001) reported blood glucose supply was supplied by glycogen reserves within the body and only slightly from gluconeogenesis. Occasionally, glycogen in cells depletes, which causes muscles to stop relaxing. To reduce depletion of glycogen, fat can be a fuel source utilized to extend depletion of glycogen, meaning the duration of exercise before exhaustion may be increased. Prevention of glycogen depletion has been associated with glucose sparing effect. Glucose sparing effect has been reported with horses during and post exercise within resting and fasting conditions (Meyer and Sallmann, 1996; Duren et al., 1999; Zeyer et al., 2002). Addition of dietary fat has been seen to have beneficial effects on exercise with an increase in muscle glycogen and decrease in blood lactate post exercise (Hiney and Potter, 1996). Added fat as part of a decreased starch diet decreases peak glucose concentrations and insulin response (Linberg and Palmgren Karlsson, 2001).

Equids also utilize dietary fat for absorption of fat-soluble vitamins (A, D, E, and K) and to meet requirement of the unsaturated fatty acid linoleic and linolenic acid. Although 0.5 % linoleic acid has been suggested based on the total diet, the actual dietary requirement is unknown (Morris et al., 1991; McCan et al., 2000; Hall et al., 2004) Providing fat as a feed additive has recently become more popular due to research

(McCan et al., 2000) regarding the benefits to performance animals and immune response by horses, but research is limited.

Determining Rate of Passage and Fiber Digestibility

Rate of nutrient passage through the digestive tract of equine species is notable for determining fiber digestibility. Hindgut rate of passage has been observed to alter cecal digestion (Jansen et al., 2007[a]). Jansen et al. (2007[a]) documented fiber being retained in the cecum and colon for up to 15 to 24 h, which explains the importance the role of passage rate plays on digestion of fiber. Rate of passage through the cecum, when too slow, has caused impaction colic. Conversely, rapid rate of passage through the hindgut has caused a reduction in optimal adsorption of nutrients and water.

According to Drogoul et al. (2001), the time available for digestion and rate of microbial fermentation determines the extent of fiber utilization in the GI tract. Increase in digestibility is directly related to an increase in retention. Drogoul et al. (2001) also observed adding concentrates to the diet resulted in decreased fiber degradation, which may be similar to the impact of fat to rate of passage and fiber digestion.

Cannulation assists in understanding rate of passage in the equine and often fiber digestibility. Nevertheless, measuring digestion of cannulated horses has been seen to have different factor affects such as altered rate of passage, depending on the cannulated segment, exercise, feeding amount, and composition of diet (Pagan et al., 1998). Although previously hypothesized, the size of the animal (i.e. size variation between ponies or horses) did not affect apparent digestibility or greatly change rate of passage when animals were fed forage (Drogoul et al., 2001). Pulse et al. (1973) indicated an increase of total gastrointestinal tract retention time post cannulation, which conflicted

with Drogoul et al. (2000) who reported a decrease in total tract retention. However, most researchers utilizing cannulated horses reported, through the mobile bag technique, a similar rate of passage and digestion of nutrients to an intact animal (Austbø and Volden, 2006). Austbø and Volden (2006) found an age-dependent effect of cannulation with increased retention time from 7.3 h to 9 h. Total mean retention time was estimated to be 26.9 h when dry matter intake was 1.7% of BW. As little as one hour post feeding, ponies fed large meals have been reported to have upper alimentary secretions, which induced a rapid transfer of fluid to the lumen of the digestive tract (Drogoul et al., 2001).

Theories about How Fat Affects Fiber Digestion

Although previous research has been reported investigating the effects of additive dietary fat on fiber utilization, the conclusions are conflicting. First examined in ruminants, unsaturated fatty acids were seen to inhibit ruminal microbes and decreased fiber digestion (Jenkins, 1993). Lipids, like other nutrients, have been reported to reach the hindgut if provided in large amounts. According to Goer et al. (2013), little difference was observed for ether extract content between ileal and fecal samples; thus, fat that reached the hindgut was not absorbed, but was lost via defecation.

Several equine studies (Bowman et al., 1979; Kane et al., 1979; Bush et al., 2001) examined the effects of adding up to 15% corn oil to the total diet concluding that additional fat had no effect on digestibility of fiber. However, the addition of soy oil, up to 15% of DM, increased fiber digestibility of mixed hay and concentrate (Zeyner, 2002). Kronfeld et al. (2004) reported no negative effect on DM, CP, or fiber digestibility when fed 23 % diet of fat, differing from Meyer et al. (1997) and Jansen et al. (2002) who observed a decreased CP digestibility in horses fed fat. Delobel et al. (2008) observed no

influence from added fat. Conflicting reports of fat supplementation on fiber digestibility may be due to differences in experimental protocols i.e. different types of fat, mode of fat inclusion, forage:starch ratio, time of feed or collection. Most previous studies fed a concentrate with fat as a top-dressing to mimic typical owner feeding habits, yet grain has been seen to negatively affect fiber digestibility. Therefore, negative fiber digestibility results could be attributed to possible grain effects. Due to these variables it is difficult to determine precise effect fat has on fiber digestibility and give recommendations for maximum fat supplementation.

Fat has been documented as decreasing fiber digestibility (Jansen et al., 2002; Zeyner et al., 2002), but the mechanics behind this effect of fat on fiber digestibility are unknown. Poor fiber digestion has been observed to cause large intestine impactions and cecal microbial disorders. When incorporated at small concentrations of the diet, there have been conflicting reports of fat affecting the digestibility of fiber. Three main theories exist to explain the negative effect of dietary fat on fiber digestibility, which include 1) fat coats the fiber particle; 2) fat increases rate of passage, and thus, fiber passes before completely digested; and 3) large amounts of fat negatively impact cecal microbes.

Fat Coating Fiber Particles

In ruminants, fat has been reported to coat fiber particles, and thus, reducing fiber digestibility (Hess et al., 2008). Researchers reported that amount of dietary fat negatively affected digestibility of crude fiber, NDF, and ADF in horses. In contrast, the inclusion of fat at 8% of the diet had a positive effect on NDF and no effect on ADF in horses (Delobel et al., 2008). Providing additional fat to the equine diet at 15 to 28%

negatively affected digestibility of fiber when the amount of fat, not digested in the duodenum or jejunum, possibly exceeded the lipolytic capacity in the ileum, thus more fat was present in the cecum (Jansen et al., 2002). The study reported a decrease in the digestibility of fiber in the hindgut due to coating of fiber particles. As previously stated, fat is digested in the small intestine, and thus, there should be no effect of fat on digestibility of fiber in the cecum. However, several researchers have postulated that increased amount of fat, such as >10 to 12 %, would allow some fat to bypass small intestinal absorption similar to excess cereal grains, leading to decreased hindgut fermentation and therefore a decrease in fiber digestion.

Fat Affecting Rate of Passage

Fat has also been observed to increase rate of passage when included in the diet without appropriate transition time. Stated previously, added concentrates to the diet of horses have been observed to increase rate of passage, and thus, decreasing fiber digestibility (Drogoul et al., 2001). Added grain load, particularly in large amounts, was suspected to reduce fiber fermentation in the hindgut by impacting the microbial environment. Goer et al. (2013) stated fat added at 11 % increased rate of passage through the stomach and small intestine. Increased rate of passage through the stomach and small intestine also caused more fat to reach the hindgut due to the reduced digestion rate in the small intestine.

Fat Affecting Cecal Microbes

Overconsumption of fat has been observed to negatively affect many species, particularly animals with fermentative ability, such as horse's cecum and cattle's rumen.

According to Jenkins (1993), unsaturated fatty acids impact ruminal microbes, and thus, decreased fiber digestion. Type of fat fed seemed to affect fiber digestion as well because unsaturated fat had a greater impact on rumen microbes than saturated (Hess et al., 2008). An increase of fermentable carbohydrate to the hindgut was hypothesized to proliferate bacteria, thus increasing rate of fermentation (Linberg and Palmgren Karlsson, 2001). Increased rate of fermentation would cause a large amount of gas production and decrease pH of the cecum overall negatively affecting cecal digestibility. Decreased pH could cause damage to the mucosal layer and microbial death resulting in endotoxic release or decrease fluid absorption. Bypassed fat could possibly result in similar negative effects to microbial populations in the cecum.

Previous researchers have confirmed fiber utilization by horses was greatly dependent on microbial fermentative activity in the cecum. The addition of fat may influence the fermentation of forage varieties by the microbial community and fermentation rate and pattern in the hindgut of the horse. Increased dietary fat of large amounts such as soy oil at 22 to 32% of DM have been shown to decrease digestibility, resulting from a decrease in cecal fermentation activity by microbes (Jansen et al., 2000). It has been hypothesized that by adding fat to diet, it would reduce cecal microbial degradation, which, in turn, would decrease fiber digestion. Large amounts of fat that bypassed small intestinal digestion, possibly reaching the hindgut, would alter cecal microbe ecosystem. In vitro research has observed dietary fat utilization and cecal bacteria and protozoa counts (Bush et. al., 2001).

Inclusion of > 9% soybean oil has caused decreased gas production in the large intestine, which, in turn, decreased fermentation capacity of fiber (Jansen et al., 2007[b]).

However, the decreased fermentation of fiber led to the decreased in gas production. Several factors may have affected gas production, and thus, fiber digestibility. Factors included differing microbial amounts, whether due to differing metabolic pathways or differing from horses to horse, or possible compounding effects from increased rate of passage. Jansen et al. (2007[b]) observed ponies fed increased amount of fat in their diet had less gas production in the colon from cellulose and reduced xylan gas production from cecal and fecal output. These researchers suggested the decrease in apparent fiber digestibility may be an inhibitory effect of the cellulolytic activity of the microflora, especially when undigested fat reaches the hindgut, especially, when fed a large amount of fat.

Fat has been observed to stimulate bile secretion, which was originally considered a factor in decreased fiber digestibility. Bile acids including deoxycholic and chenodeoxycholic acids have been seen to have antimicrobial activity, and thus, decreasing fiber digestibility by decreasing bacterial activity (Binder et al., 1975). In contrast to previous results, dietary fat had no effect on bile acids including deoxycholic or chenodeoxcholic acid from fecal matter (Jansen et al., 2007[b]).

Another possible effect on cecal fermentation of fiber due to additional dietary fat could be attributed to linoleic acid and cholesterol. Jansen et al. (2007[b]) reported an increased amount of sterols in fecal matter of horses from increased dietary fat. Cholesterol has been observed to have antagonistic effects on lytic activity of linoleic acid. Results suggested that increased cholesterol were due to a direct increase of fat intake. Although the extra fat reached the hindgut, Jansen et al. (2007[b]) concluded fat did

not affect NDF or ADF digestibility nor did it affect cellulose digestibility. Thus, Jansen et al. (2007[b]) suggested that extra linoleic acid did not inhibit fiber fermentation.

Future of Fat Supplementation Research

Dietary fat has many benefits such as providing essential fatty acids and more energy while reducing total DM intake. Studies have been conducted to assess the effects of fat supplementation to horses, but no study has analyzed the type or amount of fat, in conjunction with digestibility through different segments of the equine digestive tract and this type of research may assist in proving or disproving some of the theories associated with the relationship between fat and fiber digestion in the equine. Furthermore, cannulation research has proven useful in understanding the digestive process of the different segments of the equine digestive tract that have been observed, but these studies have been conflicting as they have been limited to observation of one segment at a time. Understanding the digestibility of fat and its relationship to fiber digestibility begins with research using a cannulated equine herd, but this research requires a herd with multiple cannulations, this type of herd is lacking and procedures for establishing such a herd is limited (Horney et al., 1973).

Effects of fat on equine diets are not clearly defined and more research is required. Previous research of how fat affects nutrients, especially fiber, digestibility has been conflicting. Therefore, the objectives of this study were to:

1. design an equine model for determining digestion by:
    a. addressing ileal cannula design via molding, dual cannula surgical procedures, extensive post-operative recovery, and to offer suggestions to rectify complications

b. developing post-surgery maintenance of a band of ponies for future nutrient absorption studies.

2. determine effects of feeding fat, at differing amounts up to 15%, on digestibility and (or) utilization of fiber with no added starch to a forage diet.
3. determine the effects of fat supplementation on fiber utilization through the analysis of plasma glucose, insulin and fatty acid profiles of equids.

CHAPTER II

A NOVEL SURGICAL METHODOLOGY FOR DUAL CANNULA PLACEMENT OF THE ILEUM AND CECUM IN EQUIDS: ASSESSMENT OF POST-OPERATIVE MANAGEMENT AND CLINICAL OUTCOME

Abstract

Cecal and ileal cannulations have previously been utilized to determine equine digestibility and define gastrointestinal physiology. However, research has been limited to singular portions of the equine digestive tract limiting analysis of the total digestive tract. The current study evaluated a unique methodology for cannulation of dual sections, ileum and cecum, of the equine digestive tract. The purpose of this study was to: 1) address ileal cannula design via molding, dual cannula surgical procedures, extensive post-operative recovery care, and to offer suggestions to minimize complications; and 2) develop and maintain a band of ponies for future nutrient absorption studies. The current study provides information for the academic community to better plan and implement dual cannulation of equids, while also demonstrating the benefits of such an endeavor by detailing a successful establishment of a dual cannulated equine herd. Dual cannulation of the ileum and cecum utilizing the described methodology was considered to be successful with a survival rate of 63%.

Introduction

Nutritionally, horses are an anomaly compared to other livestock species having similarities to both monogastrics and ruminant animals. As such, empirical data regarding equine nutrient utilization is limited and has been primarily based on extrapolation of data obtained from monogastrics and ruminant animals. Nutrient absorption and rate of passage in specific sections of the equine GI tract have been evaluated using ileal cannulas, cecal cannulas, and (or) horses slaughtered for segmental evaluation of the digestive tract (Kienzle et al., 1992).

Drogoul et al. (1995) performed a cannulation of the cecum and the colon for analysis of fiber fermentation. Limitation of the previous research (Drogoul et al., 1995) was the inability to evaluate pre-fermentation digesta. Flexible (Peloso et al., 1993) and rigid polyvinyl chloride (Coleman at el., 1998) ileal cannulas have been successfully placed in equids for sampling digesta to determine utilization of protein, soluble carbohydrates, and fat, but again, this research was limited to one aspect of the digestive tract. Despite the use of ileal and cecal cannulation, equine nutrition research, specifically nutrient absorptive ability, is lacking compared to other livestock.

The current study evaluated a unique methodology for cannulation of dual sections, ileum and cecum, of the equine digestive tract. The objectives were to: 1) address ileal cannula design via molding, dual cannula surgical procedures, extensive post-operative recovery, and to offer suggestions to rectify complications; and 2) develop post-surgery maintenance of a band of ponies for future nutrient absorption studies.

Materials and Methods

Animal Selection

Surgical and animal care procedures were in accordance with the Mississippi State University Institution Animal Care and Use Committee. Eight Hackney/Shetland pony cross females, ranging from 1 to 6 years of age, were selected for development of a band of dually cannulated ponies. Ponies ranged in BW from 200 to 250 kg. Ponies were selected, rather than horses, for ease of post-operative handling due to their relatively small size. Surgeries ranged from July to November 2013 and were monitored for 2 wks to 1 mo post-surgery.

Ileal Cannula Development

From previous cannulations, ileal cannulation has proven to be the most challenging due to increased pressure of digesta flow and the small diameter compared to cecum cannulation (Taniguchi et al., 2003). Coleman et al. (1998) stated that improvements to cannula design would reduce complications and increase lifespan of the animal. To address the challenge, a novel design was proposed to advance upon existing ileal cannula designs (Weed et al., 2014). The novel ileal cannula needed to be a single piece design in a T-shape similar to previous functioning cannulations, but modified to account for leakage at the base/stem junction (Coleman et al. 1998, Taniguchi et al. 2003, Peloso et al, 1993). Ileal cannulas were constructed of a biologically compliant silicone and included a flange to assist with surgical anchoring. Taniguchi et al. (2003) stated that although flexible cannulas had slightly more leakage, flexible cannulas decreased inflammation and lessened reaction to tissue compared to rigid cannulas. Computer Aided Design (CAD) software (Autodesk Inventor 2014, San Rafael, CA) was used to

create a model ileal cannula printed on low quality using a MakerBot Replicator 2X extrusion printer (MakerBot Industries, Brooklyn NY). The T-shaped model design was used to print a negative in CAD, and small vent channels were added to allow molded material to vent. Separated into 3 parts, the design was 3D printed and the interior mold was treated using acetone to create a smooth surface model with the 2 outer sections unsmoothed to create a rougher surface improving grip during surgery. A two-part silicone putty, Silicone Plastique (Culinart Inc, Cincinatti OH), was compressed into the mold and set for 2 h. Each cannula was plugged using a 3D printed Agriculture and Biological Science designed plug.

Surgical Procedure; Ileal Cannula Placement

Ponies were withheld from feed for 12 h prior to the surgical procedure. Procaine penicillin G (22,000 IU/kg intramuscular; Vetone, Newry, North Ireland) was administered intramuscularly and gentamicin (6.6 mg/kg intravenously; Vetone, Newry, North Ireland) intravenously, with flunixin meglumine (1.1mg/kg intravenously; Merck & Co Inc. Whitehouse Station, NJ) administered 30 min prior to surgery. Ponies were sedated with (1.0 mg/kg intravenously) xylazine (Lloyd, Shenandoah, IA). Anesthesia was induced with ketamine (2.0 mg/kg intravenously; Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO) and diazepam (0.1 mg/kg; Hospria, Inc, Lake Forest, IL), anesthesia was maintained with isoflurane inhalant (1.75% minimum alveolar concentration) throughout the procedure. Each pony was placed in left lateral recumbency with the right paralumbar fossa clipped and surgically prepped with chlorohexidine and alcohol and draped sterile material around procedure site. A 15-cm vertical skin incision was made with a No. 10 scalpel blade half way between the last rib and the tuber coxae 10-cm distal

to the transverse vertebral processes of the lumbar vertebrae. The external abdominal oblique, internal abdominal oblique and transverse abdominus muscles were bluntly dissected in a grid fashion until the peritoneum was exposed. Peritoneum was penetrated with blunt dissection and the abdomen entered. Upon entry to the abdomen, the small intestine was palpated and exteriorized and replaced into the abdomen in the oral to aboral direction until the antimesenteric band of the ileum was identified. All gastrointestinal contents were stripped into the cecum and a 30-cm segment of ileum was isolated and packed with moist laparotomy sponges. A 5-cm incision was made into the antimesenteric surface ileum approximately 25-cm from the ileocecal junction. Cannula base was folded and both ends were simultaneously inserted into the incision. Starting at the oral end the enterotomy of the ileal stoma was closed with 0-2 polydiaxanone with a simple continuous pattern, and then, oversewn with Cushings pattern until the stem of the cannula fit snuggly against the aboral end of the incision. A polypropylene mesh was applied over the intestine and secured to the cannula with 0-prolene. Mesh was further adhered to the small intestine with multiple simple interrupted partial thickness sutures of 0-2 Polydiaxanone incorporated with the mesh, serosa and submucosa. A small circular incision was made through the body wall at the flank fold and the ileal cannula was pulled through the body and secured in place with 0 prolene sutures from the cannula to the skin and a plastic washer. Lateral band of the cecum and 10-cm fold of the base of the cecum was then secured in the flank incision with 0-Polydiaxanone with a three layer closure serosa to muscle, serosa to subcutaneous tissue and serosa to skin, in a circumferential pattern. Remaining length of the flank incision was closed with 0-polydiaxanone in the external abdominal oblique fascia. Subcutaneous tissue was closed

with 0-2 polydiaxanone with a simple continuous pattern and the remaining skin was apposed with stainless steel staples.

Prior to recovery from anesthesia, silver sulfadiazine cream was applied to the exposed cecum and bandaged. During recovery from anesthesia, personnel assisted when pony's attempted to stand. Each pony was hospitalized until it could withstand consumption of feed without gastrointestinal upset (colic, impaction, etc.) of a complete feed (mean= 5 d). Procaine penicillin (22,000 IU/kg intramuscularly; Vetone, Newry, North Ireland) and gentamicin (6.6mg/kg intravenously; Vetone, Newry, North Ireland) were continued until 3 d post-surgery. Upon the third post-operative day, ponies' antimicrobial therapy was changed to trimethoprim sulfadiazine (25 mg/kg orally; Aurobindo Pharma USA, Inc, Dayton NJ) every 12 h for 2 wk.

Placement of Cecal Cannula

Each pony was transported to the College of Veterinary Medicine at Mississippi State University 14 d after surgery and sedated with detomidine (0.01 mg/kg intravenously; Orion Pharma, Espoo, Finland) and butorphanol (0.01 mg/kg intravenously; Wyeth, New York, NY) to remain restrained within stocks. The right paralumbar flank and exposed cecum were surgically prepped with iodine solution. Stainless steel staples were removed. Cecal stoma was created using a number 10 scalpel blade sharply incised into the cecum. All remaining exposed cecum was excised and a small cannula (flexible rumen cannula, #7C; 3.8 cm center diameter and 8.9 cm wall thickness; Bar Diamond, Parma, ID) was placed into the cecal fistula (Beard et al., 2011). Trimethoprim sulfadizeane (25mg/kg orally; Aurobindo Pharma USA, Inc, Dayton NJ)

and flunixin meglumine (1.1mg/kg intravenously; Merck & Co Inc. Whitehouse Station, NJ) were discontinued 48 h after placement of the cecal cannula.

Pony Recovery

All ponies stayed in the Mississippi State University College of Veterinary Medicine Animal Health Center for 3 to 14 d post-surgery. After released from veterinary intensive care, ponies were relocated to the Mississippi Agriculture Forestry and Experiment Station Leveck Animal Research Center Horse Unit. Animals were housed in individual stalls, set up in a hoop barn structure. Ponies were fed Equine Senior (Purina Mills, St. Louis, MO) mixed with water or dry for approximately 2 mo post-surgery. Ponies were also allowed to graze or fed chopped herbage. Fescue or dallasgrass hay was slowly introduced at d 10 post-surgery. After ponies had been introduced to hay for 1 mo, coastal bermudagrass hay was substituted. Neck cradles or muzzles were utilized for at least 4 wk post-surgery to prevent biting at surgery site. Ponies were hand walked at least 45 min / d starting on d 14 post-surgery.

Results

Complications

At 6 mo after surgery, 5 of the 8 ponies recovered well with slight complications; however, 3 of the 8 ponies (Ponies 2, 6, 7) were euthanized (Table 1). The most common complication, which every pony experienced, was increased rectal temperature of 38.9 to 40.9 ºC from 4 to 10 d post-surgery (Figure 1a, 1b, and 1c). One pony (Pony 1) continued to have rectal temperature greater than 38.9 ºC until 19 d post-surgery. When rectal temperatures exceeded 39 ºC, an alcohol bath was administered along with an oral lavage

of electrolytes to encourage hydration. Ponies were observed as dehydrated when elevated rectal temperatures persisted or during daytime heat and often experienced an increased heart and respiration rate (Figure 2a, 2b, 2c). Thirty milliliters of electrolytes, twice to thrice daily, were provided when pony showed signs of dehydration.

Three of the ponies (Ponies 5, 6, 7), 2 of which required euthanasia, herniated the ileal-jejunal junction between the abdominal body wall and skin within 24 h post-surgery. The 2 ponies that were euthanized (Ponies 6 and 7) herniated within 1 h post-surgery while trying to stand during recovery of anesthesia. After herniation, 2 ponies where immediately placed back under anesthesia and bowel was manually reset. All 3 ponies herniated, individually, 2 to 3 times. Bowel was reset standing while ponies were under sedation with xylazine (0.5 mg/kg intravenously; Lloyd, Shenandoah, IA) and butorphanol (0.01mg/kg intravenously; Wyeth, New York, NY).

One pony (Pony 5), which herniated 3 times, had a dislodged ileal cannula at d 15 post-surgery. Full adherence of body wall with complete ileal fistula formation was observed. A replacement cannula (11E-S esophageal cannula; 2.2 cm width, 2.5 cm diameter Bar Diamond, Parma, ID) was placed. Although neck cradles were used on all ponies, 2 ponies (Ponies 2 and 8) removed the ileal cannula via biting. One pony (Pony 2) removed the ileal cannula while grazing 4 d post-surgery and the silicon cannula was replaced in the ileum. Due to lack of adherence, peritonitis resulted and the mare was euthanized on d 7 post-surgery. The 3 ponies that were euthanized, had intestinal leakage into the abdominal cavity, due to lack of adherence of ileum to the body wall, resulting in peritonitis. Another pony (Pony 8) removed her ileal cannula 3 times (d 24, d 37, and 5

mo post-surgery), resulting in the ileal cannula being replaced by an esophageal cannula, a successful procedure due to full adherence of the stoma from intestine to body wall.

Five of the 8 ponies (Pony 1, 3, 5, 8) appeared to show signs of colic several times periodically during post-surgical recovery and through 3 mo post-surgery. Most colic signs were due to impaction, which was rectified with electrolytes orally (30 mL up to 3x daily). Ponies with replacement ileal (esophageal model) cannulas were un-impacted manually by removing hay at the junction of the intestine and cannula. When colic persisted, the animal was admitted to the College of Veterinary Medicine at Mississippi State University, where intravenous fluids were provided and a nasogastric tube was passed to facilitate gastric reflux.

All ponies showed signs of mild lameness up to 5 mo post-surgery. Lameness was evaluated at the walk as an asymmetrical gait and reduction in stride length in the right rear limb. Lameness varied from constant to sporadic, depending on the specific pony.

Post-Operative Outcome

Current dual cannulation resulted in 5 of the 8 ponies (Pony 1, 3, 4, 5, and 8) remaining healthy after 9 mo. Success rate of healthy animals was 63% survival of healthy animals. Adherence of the ileal stoma was seen after 14 d and the cecal stoma adhered from 10 to14 d post-surgery. Intestinal herniation had a substantial impact on post-surgical death in all but 1 pony.

Discussion

Research is limited regarding the methods of equine intestinal cannulation (Coleman et al., 1998), specifically concerning cannula design, surgical procedure, and

post-operative needs. The dual cannulation developed in this study will allow for the analysis of nutrient absorption and rate of passage through the total digestive tract. Multiple cannulations generate a comprehensive evaluation of absorption in the digestive tract. Specifically, the dual cannulation of the jejunal/ileal junction and the cecum would provide a possible means of sampling digesta and determination of pre-ileal, ileal, and post-cecal interactions. Dual cannulation allows for better analysis of mixed feed nutrient utilization. Determining digestibility within different sections of the gastro-intestinal tract will advance feed source evaluation, allowing for improved decisions regarding nutritional impact on animal performance and economic strategies.

Compared to other tubing or polyvinyl chloride options, the current novel ileal cannula design when paired with the surgical technique was considered successful due to the formation of a stoma and easy maintenance. Few complications were encountered with the cecal cannula with the exception of increased rectal temperature and external leakage. The novel ileal cannula design through 3D printing was less expensive than other models, yet still provided the benefits of a replica with an exact fit for future ingesta collection.

Only 3 of the 8 ileal cannulas became dislodged, mirroring previous reports (Peleso et al., 1993 and Coleman et al., 1998). Three ponies whose ileal cannulas dislodged or herniated were of less BW than the non-herniating ponies.

Increased rectal temperatures were caused by several cannulation issues. Obvious cause of fevers, as seen in any intestinal surgery, was peritonitis. A clear trend of increased body temperatures was seen from 5 to 6 d post-surgery. Once granulation tissue covered the exposed serosal surface, rectal temperature abated with the exception of 1

pony. Peloso et al. (1993) reported signs of dehydration where the ponies became anorectic and had excessive leakage. The current study provided intravenous fluids and electrolytes when dehydration persisted.

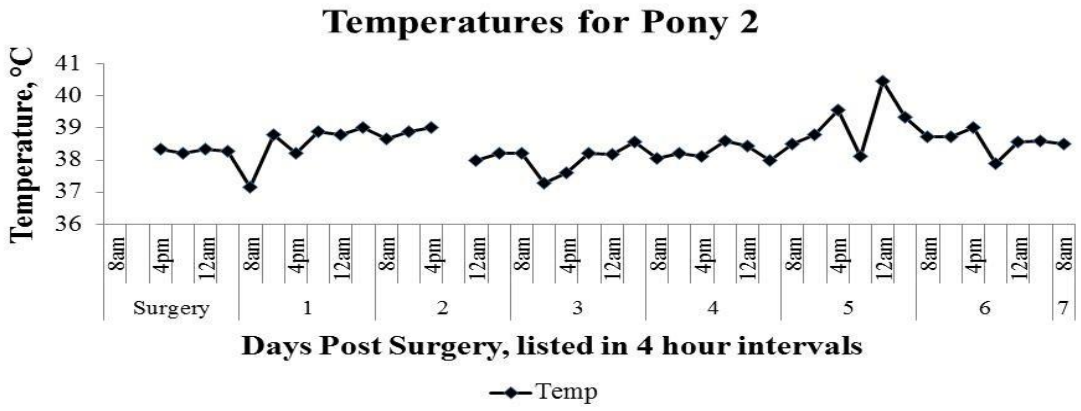
Colic was more common in the current study when compared to prior ileal cannulations with Peleso et al. (1993) reporting 2 of 3 ponies displaying colic signs. Colic symptoms were attributed to several factors, such as the age of a pony in combination with heat from the summer season. Horses had difficulty with thermoregulation in hot, humid conditions because evaporation becomes less effective (Johnson, 2009).

All cannulation surgeries, whether cecal or ileal, reported excessive external abdominal leakage around cannulation site (Taniguchi et al., 2003 and Peleso et al., 1993). Although no previous studies reported lameness, slight gait adjustments were observed in all ponies. Within the current study, all ponies showed signs of mild lameness evaluated at the walk in the right rear limb. Gait adjustment in the right rear limb was not a result of orthopedic lameness, confirmed by the lack of response to non-steroidal anti-inflammatory drug administration. Evaluation of gait changes appears to be compensation in locomotion caused by the formation of the stoma in the right lower flank which slightly altered range of motion of the stifle in the right rear limb.

Methodology of the current dual cannulation of the ileum and cecum of ponies was considered successful. Overall, survival rate of the current pony research band was 63% with only 3 ponies euthanized. A novel cannula design was selected as a decreased cost and flexible cannula option. Both cannula designs and surgical technique were considered successful.

Table 1 Results summary table of all complication, including signs and symptoms, with a healthy pony 21 d post-surgery considered successful.

Pony	Rectal temperature greater than 38.9 ºC past 14 d	Removed ileal via biting	Colic	Slight Lameness	Herniated	Lack of stoma	Successful	Euthanized
1	X		X				X	
2		X				X		X
3			X				X	
4				X			X	
5			X		X		X	
6					X	X		X
7					X	X		X
8		X	X				X	



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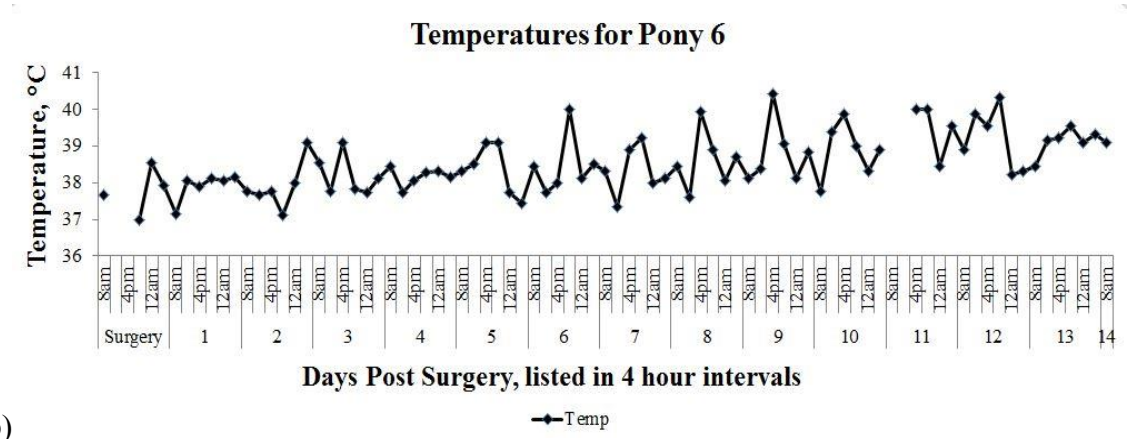

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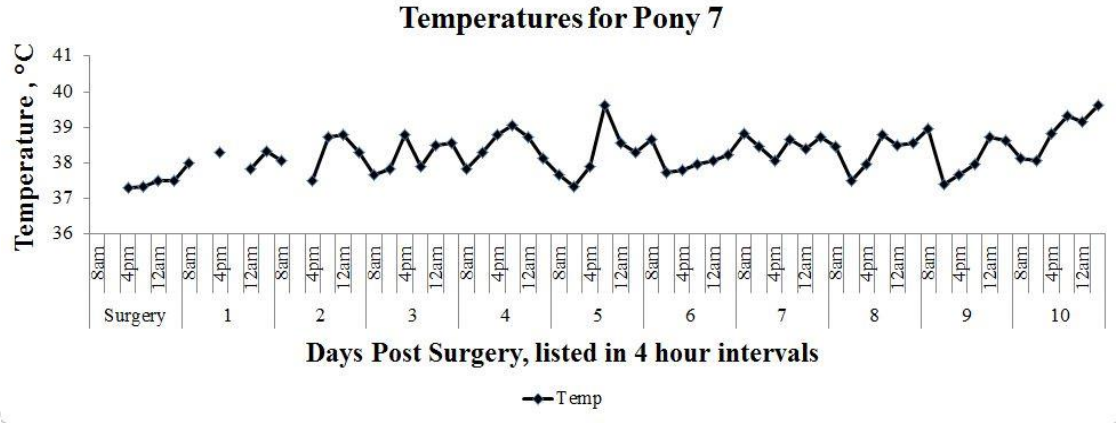
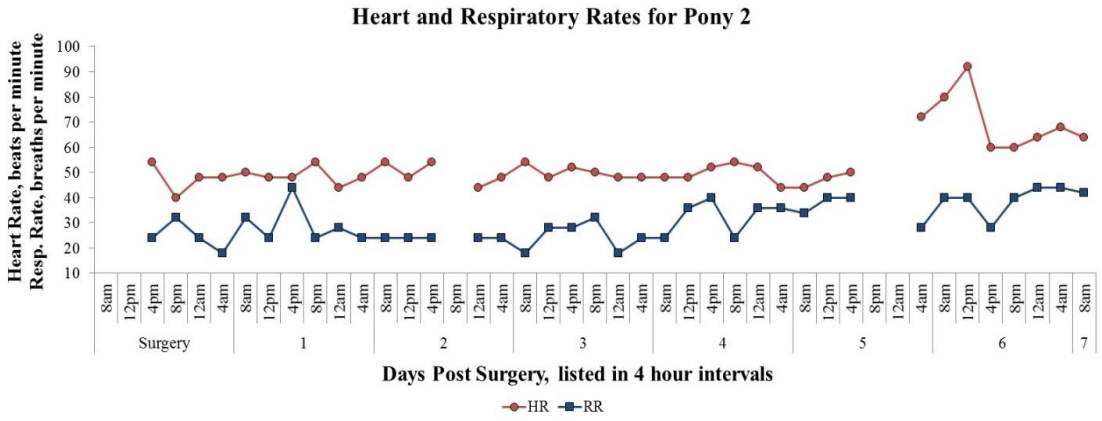


c)

Figure 1 Temperature summary taken every 4 h from surgery until euthanasia for the 3 ponies (Pony 2, 6, and 7) which were unsuccessful.



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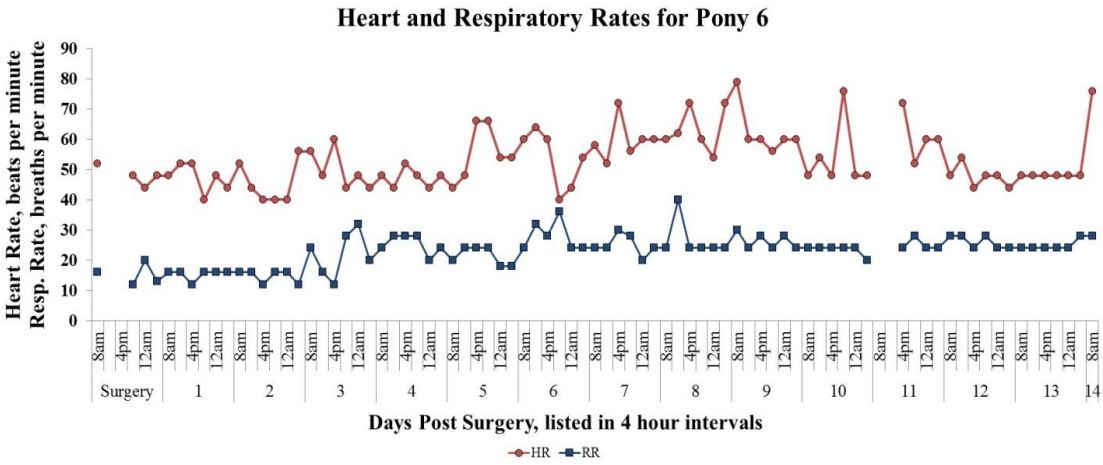

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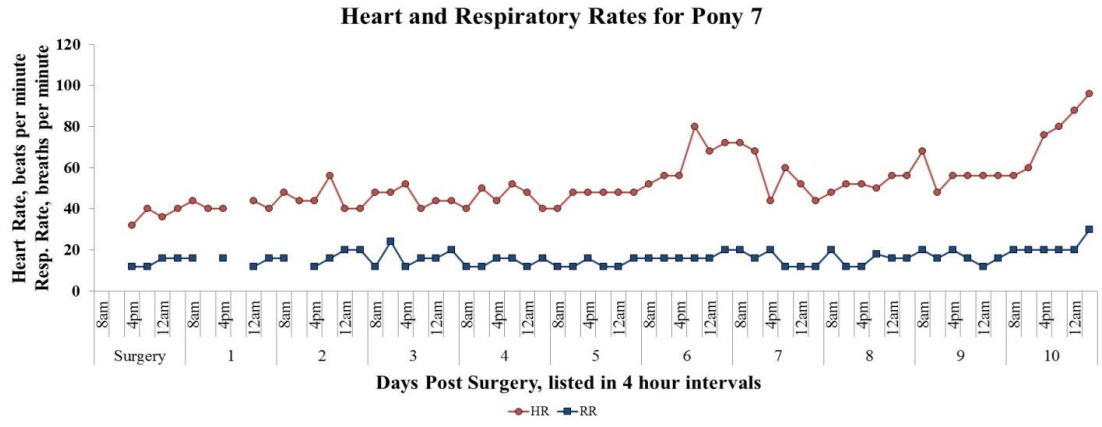

c)

Figure 2 Heart and Respiratory Rate Tables taken every 4 h from surgery until euthanasia of the 3 ponies (Pony 2, 6, 7) considered unsuccessful

CHAPTER III

EFFECTS OF FEEDING FAT ON NUTRIENT DIGESTIBILITY BY CANNULATED PONIES

Introduction

Providing supplemental fat has recently become more popular due to research results indicating benefits for performance horses. Although previous research has investigated the effects of additive dietary fat on fiber utilization, results are conflicting. The addition of soy oil, up to 15% of DM, increased fiber digestibility of mixed hay and concentrate (Zeyner, 2002). However, several studies (Bush et al., 2001; Bowman et al., 1979; Kane et al., 1979) examined the effects of corn oil, up to 15% of the total diet, finding no effect on digestibility of fiber. Jansen et al. (2000) reported that amount of dietary fat negatively affected digestibility of crude fiber, NDF, ADF, and nitrogen-free extract, in contrast, the inclusion of fat at 8% had a positive effect on NDF and no effect on ADF apparent digestibility according to Delobel et al. (2008).

Several theories exist regarding the reason fat may affect fiber digestibility including increased rate of passage and decreased number of cecal microbes. Increased fat content from added soy oil at 22 to 32% of DM have been shown to decrease digestibility of fiber possibly due to decreased cecal fermentation activity of decreased number of microbes (Jansen et al., 2000). Additional fat in the diet, at 15 to 28%, also negatively affected digestibility of fiber when the amount of fat possibly exceeded the

lipolytic capacity in the ileum (Jansen et al., 2002). Jansen et al. (2002) reported a decreased digestibility of fiber in the cecum due to fat coating of fiber particles. When incorporated at small concentrations of the diet, there have been conflicting reports of fat affecting the digestibility of fiber. Varying results could be due to the differences between experiments such as the forage: concentrate ratio, mode of fat inclusion, or the fat coating fiber particles.

Results regarding supplemental fat also suggest effects on apparent CP and ether extract digestibility, but again the reports have been conflicting. Meyer et al. (1997) and Jansen et al. (2002) reported a decrease CP digestibility when dietary fat was increased. In contrast Webb et al. (1987), Meyer et al. (1987), and Bush et al. (2001) reported no effect of fat on CP digestibility.

Although research has examined the effects of additional fat on nutrient digestibility, determining apparent total tract digestibility would allow horse owners to make better performance and economic decisions. Previous research utilized some starch in diets with additional fat which may be a confounding factor to determine fiber digestibility. The objective of the current study was to determine effects of feeding fat, at differing amounts from 0 to 15%, on digestibility of nutrients with no added starch to a forage diet.

Material and Methods

Animals and Diets

Five Shetland/Hackney cross pony mares, aged 2 to 10 years of age, were utilized with BW ranging from 156 to 199 ± 17.26 kg. At the start of each of 4 trial periods, animals were weighed, and ad libitum access to hay was provided. Diets, fed twice a day

(0600 and 1600), consisted of ad libitum access to 80% Bermudagrass (Paspalum dilatatum) and 20 % Dallisgrass (Cynodon dactylon) hay (Table 2), a trace mineral salt block, and water. Due to the large amount of fat in some diets, 2 kg / d of alfalfa pellets (Pride and Pleasure, Faithway Guntersville, AL) were provided to all ponies. Alfalfa pellets acted as a carrier for oil consumption to ponies at differing fat amounts. Vegetable oil was provided at 0, 5, 10, or 15 % of total diet, fatty acid composition (Table 3). Nutrient composition of trace mineral block is provided in Table 4. Once hay and pellet intakes were determined by assuming a 1.2 % BW DM intake, supplemental fat was adjusted as a percent of the total diet. Hay, pellets, and oil offered/ refusals, and thus intake, were measured daily during the entire experiment (Table 5 and 6).

Ponies were randomly assigned so that each pony was given 1 of 4 diets during each period. Each 5 d trial period, with a 3 d sample collection, was separated by a 14 d period where the ponies were adjusted slowly to the new diet. The four experimental diets consisted of hay paired with alfalfa and oil (fat added at 0%, 5%, 10%, and 15 % of the total diet). Experimental period recurred such that every diet was fed to one pony in each of the 4 periods.

Each of the 4 periods consisted of a 5 d collection separated by a 14 dietary adaption period during which ponies were slowly adjusted to their new diet. The 4 experimental diets consisted of ad libitum access to 80 % Bermuda (*Paspalum dilatatum*) / 20% dallisgrass (*Cynodon dactylon*) hay paired with alfalfa pellets and oil (0,5,10,15 % added fat). Three additional experimental periods were implemented such that every animal randomly received each of the 4 diets.

Ileal, and cecal samples were collected during the 5 d trial period. Samples on d 1 were collected at 0, 90, 180, and 270 min, and h 7 and 10 post 0600 feeding of supplement. Collections on d 3 were obtained at 30, 120, and 210 min, and 5 and 8 h post feeding. Lastly, collections on d 5 occurred at 60, 150, 240 min and 6 and 9 h post feeding such that samples for each week were obtained at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270 min. Ileal and cecal samples were collected via cannula. Fecal samples were hand collected via immediate collection after defecation and ileal, cecal and fecal samples were stored at -20°C. Hay, pellets, and oil samples were collected over each trial period. Hay, oil, pellets, ileal, cecal, and fecal samples were dried for 36 h in a 70°C forced air oven, and then ground through a 2 mm screen with a Wiley Mill (model 4, Thomas Wiley).

Ponies were fed and housed in individual stalls within the same barn at the equine unit of the Leveck Animal Research Center of the Mississippi Agriculture and Forestry Experiment Station at Mississippi State University. Ponies were hand walked for 45 min / d for exercise. All animal procedures were approved by the Mississippi State University IACUC.

Laboratory Analysis

Crude protein was determined by Kjeldahl-N (AOAC, 2000) technique and CP calculated by nitrogen (%) X 6.25. Fat by either extract was determined by standard AOAC methodology (2000). Acid-detergent fiber, NDF, ADL were determined according to Van Soest et al. (1991) procedures.

Apparent digestibility of dry matter (DM), crude protein (CP), either extract (EE), neutral-detergent fiber (NDF), and acid-detergent fiber (ADF) were analyzed using acid-

detergent lignin (ADL) as an internal marker. Digestibility was determined by the following equation:

Digestibility = 100-100 (% ADL of diet consumed / % ADL of location) X (% nutrient in Location / % nutrients in diet consumed).

Statistics

Due to lack of samples at each time period (no ileal, cecal, or fecal at all time points), after laboratory of all obtained samples, data were pooled into "common" time references. These time references were hourly for apparent ileal and cecal digestibility (0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 h post feeding of alfalfa pellets and oil). For apparent total tract digestibilities these times references were every 1.5 h post feeding of alfalfa pellets and oil (0, 1.5, 3, 4.5, 6, 7.5, and 9 h).

The current study was analyzed based on an incomplete randomized block design. Ponies were designated as the block, treatment was considered an effect and repeated measure was the collection time (reference period, described above). Trial period were not significant and was removed from the model. Data were analyzed according to the mixed model for repeated measures (V. 9.1, SAS Inst,. Inc., Cary, NC). Means were separated when significant ($P < 0.1$) using lsd.

Results

Two ponies required substitution for individual trial periods as a result of increased supplemental fat at 15 % of the diet. Lethargy and anorexia, or feed refusal, were observed for all ponies fed 15 % fat diet, but at 2 points required substitution due to

weight loss. Ponies were removed due to weigh loss and lack of feed intake for more than 3 d.

Apparent ileal CP, NDF or ADF digestibility was not affected ($P > 0.1$) by treatment or time (Table 7 and 8). There was a treatment by time interaction ($P < 0.1$) for apparent ileal fat digestibility (Table 9). For the 0 % fat diet, from h 0 to 1 post feeding, a decrease in apparent ileal fat digestibility from 61.09 to 41.06 % occurred, and then was not different until 7 h post feeding when apparent digestibility increased to 132.16 %. From 7 to 8 h post feeding, apparent ileal digestibility of fat decreased to 34.2 %, and then increased to 78.86 % by 9 h post feeding. Diet with additional fat (5, 10, 15 %) had greater apparent ileal digestion of fat compared to no additional fat, but were not affected by time ($P > 0.1$)

Apparent cecal digestibility of fiber, NDF and ADF were not different ($P > 0.1$) among time or treatment (Table 10 and 11). Apparent cecal digestibility of crude protein decreased ($P < 0.1$) from 0 to 2 h post feeding, then increased until 4 h post feeding, decreased at 5 h post feeding, then increased at 6 and 7 h post feeding and decreased from 8 to 9 h (Table 10). As expected, added dietary fat affected ($P < 0.1$) apparent cecal digestibility of fat from 0 % fat at 19 to 83, 83, and 89 % apparent digestibility (Table 11) for diets with added fat (5, 10, 15 %; respectively).

Apparent total tract digestibility of NDF, ADF or fat (Table 12) was affected by time ($P < 0.1$). Apparent total tract digestibility of NDF and ADF were not affected ($P > 0.1$) by added fat; however, adding fat (5, 10, 15 %) increased ($P < 0.1$) apparent total tract digestibility of fat (Table 13). There was treatment by time interaction for apparent total tract protein digestibility (Table 14). At 0 h post feeding apparent total tract

digestibility of protein was greatest ($P < 0.1$) compared to other time periods. Adding fat to the diet decreased apparent total tract protein digestibility at 0 h post feeding. However, at 3 h post feeding only 10 % additional fat decreased protein digestibility compared to 0 % additional fat. Also, 5 and 15 % additional fat apparent total tract protein digestibility was an intermediate.

Discussion

In the present experiment, supplementation of vegetable oil did not affect digestibility of fiber. Previous reports regarding supplemental fat effect on fiber digestibility have been conflicting. Kane et al. (1979), McCann et al. (1987), Meyer et al. (1989), Hughes et al. (1995), and Bush et al. (2001) reported no effect on fiber digestibility with the inclusion of up to 15 % additional fat. However, Jansen et al. (2000, 2002) reported decreased apparent digestibility of CF, NDF, and ADF when fat was included in the diet at 0.09 %.

As expected, dietary fat had a significant effect on apparent EE digestibility in ileum, cecum and total tract. Bush et al. (2001) reported the largest fat digestibility was observed when horses were fed 15 % dietary fat, while treatments of 5 and 10 % fat were not different from 0 %. Delobel et al. (2008) concluded that the addition of linseed oil increased fat digestibility by 26.4 %. Estimates of apparent digestibility of fat by ponies were 42 to 49 % for forage and 88 to 94 % of supplemented fat and oil (Kronfeld et al., 2004). However, Meyer et al. (1987) and Webb et al. (1987) reported no effect of diet on fat digestibility when horses were fed similar fat amounts. Digestibility differences could be due to overall diet composition with Meyer et al. (1987) including starch within the diet.

Vegetable oil, which is dense in triglycerides, increased the proportion of digested true fat (Delobel et al., 2008). Increased dietary fat resulted in the increase of preileal fat digestibility and of jejuno-ileal flow (Meyer et al., 2007). This, lipolysis should be complete in the small intestine with no significant amount of fat entering the cecum to affect fiber digestibility (Delobel et al., 2008).

Digestibility of CP was similar to previous result with comparable supplemental fat. Although ileal CP digestibility was not affected by added fat, slight decrease in CP was observed at 4 to 5 and 7 to 8 h post, which indicated a time effect in the cecum. Meyer et al. (1997) and Jansen et al. (2002) reported a decrease in CP with the inclusion of fat into the diet. Meyer et al. (1997) hypothesized the large amount fat decreased apparent digestibility in the small intestine because of the increased endogenous nitrogen flow related to the stimulation of digestive secretions. Although Delobel et al. (2008) reported no effect on CP when vegetable oil was added to the diet, concentrate was also presented. Oil presented with concentrate could be less likely to coat fiber particles or could allow fat to be more digestible when compared to fiber only diet.

Jansen et al. (2000) hypothesized increased lipid digestibility from the diet would increase lipid amounts in the large intestine, thus decrease total tract output of microbial nitrogen due to decreased cecal-colic microbial growth. In the current study, approximately 95 % of the dietary fat was digested before the ilium with minimal fat entering the cecum. Therefore fat does not effect microbial environment in the cecum because the amount to cecum is negligible.

Results from the current study would conflict with reports on altered microbial affect or decreased CP or fiber digestibility due to inclusion of increased dietary fat.

Conclusion

Performance horses are often supplemented with dietary fat to increase caloric intake. However, previous effects of fat on fiber digestibility in equine have been conflicting. Without determination of proper inclusion rates of fat, dietary fat could be detrimental to fiber which is the main component in equine diets. In summary, this current research found fat to not affect apparent fiber or CP digestibility when included up to 15 % of diet DM. Lack of additional fat effects, from 5 to 15 %, means that performance horses can be fed oil for highly digestible energy. Effects of fat at different inclusion rates on fiber digestibility and impacts of differing dietary nutrients could be the cause for the differing results. Future research could be conducted to examine the possible impacts of fat on microbial populations.

Table 2 Nutrient composition of hay, alfalfa pellets and oil fed to ponies.

Offered	DM (%)	CP (% DM)	NDF (% DM)	ADF (% DM)	EE (% DM)
Hay	84.46	10.52	79.40	33.85	1.21
Alfalfa Pellets	89.23	16.39	48.52	32.08	10.17
Oil	89.61	0	0	0	100.00

Table 3 Fatty acids in vegetable oil provided to ponies on diets at 5, 10, and 15 % diets (μg/mL and % of total fatty acids in oil).

Fatty Acids	$^{\mu g}/_{mL}$	%
C14:0	63.68	0.57
C14:1	3.23	0.03
C16:0	381.39	3.42
C16:1	150.47	1.35
C18:0	457.57	4.10
C18:1	1052.21	9.43
C18:2	930.60	8.34
C18:3	7000.38	62.77
C20:0	413.95	3.71
C20:1	428.93	3.85
C20:3	12.14	0.11
C20:4	36.73	0.33
C20:5	1.47	0.01
C22:1	7.82	0.07
C24:0	204.32	1.83
C24:1	7.91	0.07

Table 4 Trace mineral salt block nutrient composition provided to the ponies at ad libitum access.

Mineral	%
Sodium Chloride	98.236
Ferrous Carbonate	0.526
Zinc Oxide	0.486
Manganese Oxide	0.334
Iron Oxide	0.252
Cupric Sulfate	0.120
Ethylenediamine Dihydriodide	0.035
Mineral Oil	0.020
Cobalt Carbonate	0.011
Calcium Iodate	0.011
Artificial Flavor	0.005

Table 5 Hay and total dry matter intake (DMI) by 0, 5, 10, and 15 % oil of the total diet (kg/d).

	Diet (% fat)				
	0	5	10	15	s.e.m.
Hay DMI, kg/d	3.00	2.42	2.43	2.35	1.081
Total DMI, kg/d	5.04	4.78	4.81	4.72	0.683
s.e.m.	0.633	1.081	1.010	0.755	

Table 6 Hay and total diet dry matter intake (DMI) for 0, 5, 10, 15 % oil of the diet (% BW/d).

	Diet (% fat)				
	0	5	10	15	s.e.m.
Hay DMI, % BW /d	1.59	1.32	1.31	1.29	1.081
Total DMI, % BW /d	2.66	2.63	2.63	2.60	0.683
s.e.m.	0.633	1.081	1.010	0.755	

Table 7 Apparent ileal protein, NDF and ADF digestibilities from 0h before feeding throughout a 10 h collection period after alfalfa pellet consumption with different amount of fat (0, 5, 10 or 15 %).

Time (h post-feeding)	Apparent Ileal Digestibility (as a % of total diet)		
	Protein	NDF	ADF
0	60.60	32.75	42.50
1	36.66	34.27	20.24
2	29.06	21.92	7.30
3	33.53	29.76	19.18
4	31.73	21.17	12.50
5	27.26	18.96	3.72
6	37.12	20.90	8.75
7	37.80	27.53	9.24
8	17.42	27.92	9.78
9	30.97	28.75	7.01
s.e.m.	20.021	9.643	11.632

Means within columns are not different ($P > 0.1$)

Table 8 Apparent ileal protein, NDF, and ADF digestibility for ponies fed different amount of fat (0, 5, 10 or 15 %).

Diet (% additional fat)	Apparent Ileal Digestibility (as a % of total diet)		
	Protein	NDF	ADF
0	38.70	26.03	21.10
5	35.87	26.79	17.79
10	28.48	27.24	23.94
15	31.80	27.03	19.14
s.e.m.	11.064	5.981	12.042

Means within columns are not different ($P < 0.1$)

Table 9 Apparent ileal EE digestibility as a % of total diet for all ponies between diet (% fat) and time post feeding.

Time (h)	Diet (% fat)				s.e.m.
	0	5	10	15	
0	$61.09^{b,y}$	91.23^{z}	94.49^{z}	95.05^{z}	7.710
1	$41.06^{a,y}$	91.98^{z}	86.46^{z}	91.46^{z}	6.473
2	$20.93^{a,y}$	86.48^{z}	93.26^{z}	93.89^{z}	10.922
3	$34.35^{a,y}$	91.95^{z}	93.01^{z}	95.01^{z}	7.750
4	$40.20^{a,y}$	90.81^{z}	93.83^{z}	97.32^{z}	6.461
5	$28.98^{a,y}$	88.09^{z}	92.79^{z}	91.51^{z}	10.930
6	$35.16^{a,y}$	94.04^{z}	88.55^{z}	97.37^{z}	10.923
7	$132.16^{c,z}$	90.99^{y}	84.99^{y}	92.88^{y}	10.924
8	$34.20^{a,y}$	94.44^{z}	95.23^{z}	95.03^{z}	10.920
9	$72.86^{b,y}$	85.07^{yz}	91.77^{yz}	97.83^{z}	10.921
s.e.m.	10.930	10.841	10.881	7.710	

$^{a, b, c}$Least squares means within columns with same superscript letters were not different ($P < 0.1$)

$^{y, z}$Least squares means within rows with same superscript letters were not different ($P < 0.1$)

Table 10 Apparent cecal protein, NDF, ADF, and EE digestibility throughout a 10 h collection period for ponies fed different amount of fat (0, 5, 10 or 15 %).

Time (h post-feeding)	Apparent Cecal Digestibility (as a % of total diet)			
	Protein	NDF	ADF	EE
0	33.27[c]	27.75	13.08	70.06
1	27.84[bc]	16.99	7.25	71.18
2	24.35[ab]	19.15	11.24	66.38
3	27.35[bc]	19.64	5.34	68.38
4	33.42[c]	22.92	7.56	64.40
5	23.46[ab]	23.72	7.89	69.57
6	26.96[abc]	19.73	13.40	66.92
7	35.04[c]	19.21	6.73	74.93
8	19.67[a]	24.88	11.24	70.15
9	21.14[ab]	17.36	6.01	66.87
s.e.m.	4.64	6.51	5.85	3.35

[a, b, c]Least squares means within columns with same superscript letters were not different ($P < 0.1$)

Table 11 Apparent cecal protein, NDF, ADF, and EE digestibility for all ponies fed different amount of fat (0, 5, 10 or 15 %).

Diet (% additional fat)	Apparent Cecal Digestibility (as a % of total diet)			
	Protein	NDF	ADF	EE
0	31.74	16.17	4.68	19.10[a]
5	30.69	23.34	11.40	83.02[b]
10	28.72	21.57	5.48	83.17[b]
15	22.21	22.59	11.71	89.01[b]
s.e.m.	5.445	2.072	3.120	4.671

[a, b]Least squares means within columns with same superscript letters were not different ($P < 0.1$)

Table 12 Apparent total tract NDF, ADF, and EE digestibility throughout a 9 h collection period for all ponies fed different amount of fat (0, 5, 10 or 15 %).

Time (h post-feeding)	Apparent Total Tract Digestibility (as a % of total diet)		
	NDF	ADF	EE
0	51.00	37.72	85.91
1.5	40.98	21.73	78.83
3	36.86	18.70	76.53
4.5	36.23	19.32	81.38
6	44.82	28.93	85.02
7.5	34.58	22.67	81.45
9	36.02	20.22	86.27
s.e.m.	8.720	12.613	6.201

Means within columns are not different ($P < 0.1$)

Table 13 Apparent total tract NDF, ADF, and EE digestibility for all ponies fed different amount of fat (0, 5, 10 or 15 %).

Diet (% additional fat)	Apparent Total Tract Digestibility (as a % of total diet)		
	NDF	ADF	EE
0	30.14	16.43	9.51[a]
5	39.32	21.04	87.72[b]
10	38.14	17.72	83.94[b]
15	47.52	33.66	88.05[b]
s.e.m.	3.472	4.501	2.391

[a, b]Least squares means within columns with same superscript letters were not different ($P < 0.1$)

Table 14 Apparent total tract CP digestibility displayed in 90 min increments from 0 to 10.5 h post feeding for all ponies.

Time (1.5 h)	Diet (% fat)				s.e.m.
	0	5	10	15	
0	78.25[b,z]	31.53[y]	24.46[y]	37.20[y]	10.922
1.5	45.29[a]	43.58	33.61	39.69	8.621
3	52.23[a,z]	41.12[yz]	35.44[y]	41.77[yz]	8.642
4.5	48.53[a]	44.38	38.07	43.62	8.950
6	47.93[a]	42.41	34.29	33.57	10.175
7.5	50.04[a]	45.32	36.67	42.99	8.724
9	47.36[a]	31.44	32.66		10.364
s.e.m.	10.170	10.361	10.151	10.960	

[a, b]Least squares means within columns with same superscript letters were not different ($P < 0.1$)

[y, z]Least squares means within rows with same superscript letters were not different ($P < 0.1$)

CHAPTER IV

EFFECTS OF FEEDING FAT ON EQUINE PLASMA GLUCOSE, INSULIN AND FATTY ACID ANALYSIS

Introduction

Fats have often been included in equine diets to increase growth, energy density, milk production, and inflammatory response (Delobel et al., 2008). Fat has also been included to performance horse's diets due to its glucose sparing effect (Duren et al., 1999). In endurance horses, dietary fat protected against the decrease in blood glucose due to sparing of over utilization of glucose (Hintz, 1982). . Glucose, insulin, and fatty acids play an important role during exercise specifically during equine competition. Clear determination of fat effects on fiber digestibility and the utilization of glucose, insulin, and fatty acids could improve feeding practices to benefit equine metabolism. Nevertheless, reports on dietary fat's influence on glucose and insulin are conflicting. As fat was added, thus decreasing dietary starch, blood glucose and insulin concentrations were also decreased (Linberg and Palmgren Karlsson, 2001). Supplemental dietary fat reduced blood glucose and insulin concentrations directly after feeding (Ott and Kivipelto, 1999). Plasma glucose was increased compared to horses fed increased starch diet (Zeyner et al., 2002). An unexpected increase in concentrations of glucose were observed from increased fat diets (Zeyner et al., 2002), but the increased glucose was likely related to increased insulin resistance (Zeyner et al., 2002). Harris et al. (1999) and

Duren et al. (1999) reported no insulin effect or resistance in horses fed diets with additional fat.

Fat in the diet increased flux of fatty acids, which facilitated oxidation of fatty acids, especially during exercise (Zeyner et al., 2002; Geelen et al., 1999). Increase of plasma concentrations of cholesterol and phospholipids were also reported. Although the exact requirements have yet to be determined, equine require essential fatty acids including linoleic (n-6), and linolenic (n-3; Harris et al., 1999). Essential fatty acids, which cannot be synthesized by the body, has been noted to play a role in biomembrane structural components and, lipid transport and were precursors for prostaglandins. Therefore, the objective of this study was to determine the effects of fat supplementation on nutrient utilization through the analysis of plasma glucose, insulin and fatty acid profiles of equids.

Material and Methods

Animals and Diets

Five Shetland/Hackney cross pony mares, aged 2 to 10 years of age, were utilized with BW ranging from 156 to 199 ± 17.26 kg. At the start of each of 4 trial periods, animals were weighed, and ad libitum access to hay was provided. Diets, fed twice a day (0600 and 1600), consisted of ad libitum access to 80% Bermudagrass (Paspalum dilatatum) and 20 % Dallisgrass (Cynodon dactylon) hay, a trace mineral salt block (Table 15), and water. Due to the large amount of fat in some diets, 2 kg / d of alfalfa pellets (Pride and Pleasure, Faithway Guntersville, AL) were provided to all ponies. Alfalfa pellets acted as a carrier for oil consumption to ponies at differing fat amounts. Nutrient composition of hay, alfalfa, and oil listed is provided in Table 16. Vegetable oil

was provided at 0, 5, 10, or 15 % of total diet, fatty acid composition (Table 17). Once hay and pellet intakes were determined by assuming a 1.2 % BW DM intake, supplemental fat was adjusted as a percent of the total diet. Hay, pellets, and oil offered/ refusals were measured daily during the entire experiment (Table 18 and 19).

Ponies were randomly assigned so that each pony was given 1 of 4 diets during each period. Each 5 d trial period, with a 3 d sample collection, was separated by a 14 d period where the ponies were adjusted slowly to the new diet. The four experimental diets consisted of hay paired with alfalfa and oil (fat added at 0%, 5%, 10%, and 15 % of the total diet). Experimental period recurred such that every diet was fed to one pony in each of the 4 periods.

During each of the periods, blood samples were collected at 0, 90, 180, and 270 min, and 7 and 10 h post feeding on d 1. Blood samples on d 3 were collected at 30, 120, and 210 min, 5 and 8 h post feeding. Similarly, d 5 samples were collected at 60, 150, and 240 min, and 6 and 9 h post feeding such that samples for each collection week were 0, 30, 60, 90, 120, 150, 180, 210, 240, and 270 min, and 5, 6, 7, 8, 9, and 10 h post feeding. Blood samples were collected by indwelling jugular catheter. From the blood samples, plasma (Li-heparin) were kept on ice until centrifuged at 3000 X *g* and stored then in -80°C for later analysis. Hay, pellets, and oil samples were collected over the trial periods and dried for 36 h in 70° C until laboratory analysis.

Ponies were individually stalled, due to the need to individually feed their specific diet, within a hooped barn at the equine unit at the Leveck Animal Research Center of the Mississippi Agriculture and Forestry Experiment Station at Mississippi State University.

Ponies were hand walked for 45 min per day for exercise. Animal procedures used for this research were approved by the Mississippi State University IACUC.

Blood samples analysis

Blood samples were analyzed for glucose, insulin and fatty acids. Fatty acids were determined using gas chromatograph-mass spectrometry using previously published procedures (Vickers, A. K., 2007). Plasma fatty acid standard in lipid fraction (as a percentage of the methyl ester by weight) is displayed in Table 20. Once extracted, in a procedure similar to Lepage and Roy (1986), samples were evaporated and reconstituted with 100 µL of hexane (Agilent Technologies., Santa Clara, CA).

Glucose was measured by a colorimetric assay (Cayman Chemical, Procedure 10009582) validated for equine plasma. Glucose assay included glucose oxidase-peroxide reaction to determine glucose concentrations.

Plasma insulin was determined by Equine Insulin ELISA (Mercodia, Procedure 10-1205-01). The insulin ELISA was a solid phase two-site enzyme immunoassay. Duplicate assay were performed for each plasma sample and CV < 6 % were accepted.

Statistics

The current study was analyzed as a randomized incomplete block design. Data was analyzed according to the Mixed procedures for repeated measures (SAS Inst., Inc., Cary NC, 2000). Treatment was considered as an effect, sample collection times as repeated measure and the pony was designated as the block. Class variables were defined as pony, treatment, and time. Time was the repeated variable and significance was defined at $P < 0.10$. The model included treatment X time interactions and the error term

was pony X treatment. Ponies were randomly assigned so that each pony was given 1 of 4 diets during each period. Each 5 d trial period, with a 3 d sample collection, where the 5 ponies were assigned to the 4 experimental diets.

Results and Discussion

Differing from previous results (Zeyner et al., 2002), additional amounts of fat did not impact ($P > 0.1$) glucose (Table 21 and 22). Plasma concentration of insulin increased ($P > 0.1$) over time increasing from 0 to 2 h post feeding remaining similar until about 6 h post feeding, and then, remained similar through 10 h post feeding (Table 21). Dietary supplementation of fat did not affect blood plasma insulin concentration (Table 22). Harris et al. (1999) and Duren et al. (1999) did not observe insulin resistance when horses' fat consumption increased. Similar to the current results, Ott and Kivipelto, (1999) reported no effect on blood glucose concentrations, but did report a decrease insulin concentration after horses were fed increased dietary fat. Although time of fiber consumption affected glucose concentrations due to altered passage rates, no insulin effect by time of fiber consumption has been reported (Ott and Kivipelto, 1999). Conflicting results from these previous studies evaluating insulin sensitivity may be a result of differing blood collection times, rather than insulin resistance. Insulin has been reported to control glucose homeostasis (Lindberg and Karlsson, 2001), which may have affected the current glucose concentrations by increasing cell uptake resulting with decreased blood glucose (Kronfeld et al., 2005). Thus, the effect of insulin was triggered by increased glucose, but the stabilization of glucose concentration was immediate. These results could be influenced by the fact that ponies consumed hay during the collection

period. Future studies may need to take plasma samples more often to observe glucose affects and possible oral glucose challenges when horses are fed different fat types.

Ponies consuming 0 % fat diet had increased ($P < 0.1$) C14:0 2.66 mg / mL compared to 5, 10, and 15 % diet (1.62, 1.59, and 1.41 mg / ml; respectively). Adding fat at 5, 10, and 15 % of the diet increased C18:2 n-6 (Table 23) from 5.28 mg / mL when the ponies were fed 0 % fat to 7.12, 7.61, and 8.06 mg / mL respectively. Plasma fatty acids (% of total FA) had an increase in C18:0, C18:1, C18:2, and C20:1 (Table 24) as fat was included in the diet. A decrease in C14:0, C18:3, and C20:3 was observed in plasma fatty acids (% of total diet) as dietary fat increased. Plasma fatty acid concentrations (Table 25) were not affected by time ($P > 0.1$). Plasma fatty acids (% of total diet) had a significant time effect with C20:5 decreasing from 4 to 5 h post feeding (Table 26). Decrease in blood FA as oil was increased could mean the FA were stored in adipose tissue for energy. Conversely, the ponies on no additional fat diet had greater amounts in the blood as they utilized it for energy at the time. Rich et al. (1981) reported no effect of diet on linoleic acid (18:2) in blood serum of ponies fed fat at 10 % of the diet. However, Harris et al. (1999) observed a slight variation in fatty acids between increased unsaturated fat and saturated sources with the exception of a slight increase of linoleic acid in the blood of horses fed unsaturated fat diets. Harris et al. (1999) also observed an increased C 14:0 in the blood of horses fed saturated fat diet compared to unsaturated. Harris et al. (1999) theorized feeding saturated fat could increase deposition in adipose tissue and decrease the mobilization of NEFA which negatively affected performance horses overall.

Conclusion

Dietary fat effects on nutrient digestibility have been observed to affect glucose, insulin, and fatty acid concentration in the blood of horses. Although glucose and insulin were not affected by supplemental dietary fat, insulin increased from 0 to 1 h. Overall, total plasma fatty acids were not influenced by dietary fat amount with the exception of C14:0, which increased when ponies were fed 0 % fat, and C18:2 n-6, which decreased from 0 % fat compared to diets containing 5, 10, and 15 % fat of the diet. Plasma fatty acids (% of total FA) had an increase in C18:0, C18:1, C18:2, and C20:1 (Table) with a decrease in C14:0, C18:3, and C20:3 as dietary fat increased. By feeding an increased fat diet, 5 or 10 %, performance horses can store the excess fat for energy.

Table 15 Trace mineral salt block nutrient profile provided to the ponies in *ad libitum* access.

Mineral	%
Sodium Chloride	98.236
Ferrous Carbonate	0.526
Zinc Oxide	0.486
Manganese Oxide	0.334
Iron Oxide	0.252
Cupric Sulfate	0.120
Ethylenediamine Dihydriodide	0.035
Mineral Oil	0.020
Cobalt Carbonate	0.011
Calcium Iodate	0.011
Artificial Flavor	0.005

Table 16 Nutrient composition of hay, alfalfa pellets (feed) and oil fed to ponies.

Offered	DM (%)	CP (% DM)	NDF (% DM)	ADF (% DM)	EE (% DM)
Hay	84.46	10.52	79.40	33.85	1.21
Alfalfa Pellets	89.23	16.39	48.52	32.08	10.17
Oil	89.61	0	0	0	100.00

Table 17 Fatty acid profile of dietary vegetable oil added to the 5, 10, and 15 % diets (μg/mL and % of the total FA in the diet) provided to ponies.

Fatty Acid	$^{\mu g}/_{mL}$	%
C14:0	63.68	0.57
C14:1	3.23	0.03
C16:0	381.39	3.42
C16:1	150.47	1.35
C18:0	457.57	4.10
C18:1	1052.21	9.43
C18:2	930.60	8.34
C18:3	7000.38	62.77
C20:0	413.95	3.71
C20:1	428.93	3.85
C20:3	12.14	0.11
C20:4	36.73	0.33
C20:5	1.47	0.01
C22:1	7.82	0.07
C24:0	204.32	1.83
C24:1	7.91	0.07

Table 18 Hay and total dry matter intake (DMI) by 0, 5, 10, and 15 % oil of the total diet (kg/d).

	Diet (% fat)				
	0	5	10	15	s.e.m.
Hay DMI, kg/d	3.00	2.42	2.43	2.35	1.081
Total DMI, kg/d	5.04	4.78	4.81	4.72	0.683
s.e.m.	0.633	1.081	1.010	0.755	

Table 19 Hay and total diet dry matter intake (DMI) for 0, 5, 10, 15 % oil of the diet (% BW/d).

	Diet (% fat)				
	0	5	10	15	s.e.m.
Hay DMI, % BW /d	1.59	1.32	1.31	1.29	1.081
Total DMI, % BW /d	2.66	2.63	2.63	2.60	0.683
s.e.m.	0.633	1.081	1.010	0.755	

Table 20 Fatty acid profile of external standard by weight (%).

Fatty Acid	% by weight
C14:0	1.0
C14:1	0.5
C16:0	21.0
C16:1	1.0
C18:0	7.0
C18:1	17.0
C18:2	39.0
C18:3	0.5
C20:0	0.5
C20:1	0.5
C20:3	1.5
C20:4	8.0
C20:5	0.5
C22:1	0.5
C24:0	0.5
C24:1	0.5

Table 21 Plasma glucose and insulin concentration throughout a 9 h collection period for all ponies fed different amount of fat (0, 5, 10 or 15 %).

Time (h post-feeding)	Concentration	
	Glucose ($^{mg}/_{dL}$)	Insulin ($^{milU}/_{mL}$)
0	71.87	0.08[a]
1	71.10	0.14[bcd]
2	73.07	0.20[ef]
3	75.50	0.19[ef]
4	84.61	0.23[f]
5	81.94	0.17[def]
6	75.12	0.12[abcd]
7	71.10	0.16[cde]
8	77.48	0.10[abc]
9	68.01	0.13[abcd]
10	82.49	0.09[ab]
s.e.m.	8.194	0.042

[a, b, c, d, e, f]Least squares means with same superscript letters were not different ($P < 0.1$)

Table 22 Plasma glucose and insulin concentration of ponies fed varying amount of supplemental fat (0, 5, 10, and 15 % fat of diets DM).

Diet (% additional fat)	Concentration	
	Glucose ($^{mg}/_{dL}$)	Insulin ($^{milU}/_{mL}$)
0	72.46	0.12
5	71.99	0.15
10	80.58	0.19
15	75.00	0.15
s.e.m.	4.060	0.021

Means within columns were not different ($P < 0.1$)

Table 23 Plasma fatty acids concentration (µg/ml) of ponies fed varying amounts of supplemental fat (0, 5, 10, and 15 % diets DM).

	Diet (% fat)					
Fatty Acid	0	5	10	15	s.e.m.	p =
C14:0	2.66[b]	1.62[a]	1.59[a]	1.41[a]	0.301	0.0452
C14:1	0.73	0.50	0.47	0.54	0.100	0.2043
C16:0	3.20	3.21	3.16	3.31	0.362	0.9926
C16:1	2.54	1.51	1.09	1.89	0.591	0.3795
C18:0	9.94	11.79	12.16	12.69	1.204	0.3963
C18:1	2.56	2.76	3.03	3.23	0.401	0.6612
C18:2	5.28[a]	7.12[b]	7.61[b]	8.06[b]	0.595	0.0197
C18:3	24.03	16.51	17.89	17.56	3.712	0.4819
C20:0	4.56	4.64	5.10	5.17	0.554	0.7941
C20:1	2.32	2.42	2.98	3.45	0.423	0.2126
C20:3	1.52	1.28	1.20	1.09	0.140	0.2344
C20:4	1.03	0.95	1.17	0.92	0.240	0.8518
C20:5	1.37	1.03	1.47	1.13	0.281	0.6406
C22:1	4.54	4.22	4.18	4.30	0.933	0.9923
C24:0	3.18	3.70	3.66	3.84	0.574	0.8385
C24:1	4.85	5.77	5.50	6.19	0.792	0.6059
Total Fatty Acid	74.31	69.03	72.26	74.78		
s.e.m.	3.631	3.710	3.650	3.662		

[a, b]Least squares means within rows with same superscript letters were not significant ($P < 0.1$)

Table 24 Plasma fatty acids (%) of ponies fed varying amounts of supplemental fat (0, 5, 10, and 15 % diets DM).

	Diet (% fat)					
Fatty Acid	0	5	10	15	s.e.m.	p =
C14:0	3.81[b]	2.47[a]	2.28[a]	2.02[a]	0.230	0.0004
C14:1	1.23	0.81	0.75	0.73	0.291	0.5305
C16:0	4.60	4.83	4.64	4.73	0.197	0.5263
C16:1	3.41	1.94	1.36	2.31	0.562	0.1091
C18:0	14.18[a]	17.71[b]	17.71[b]	17.49[b]	0.602	0.0019
C18:1	3.57[a]	4.17[b]	4.38[b]	4.48[b]	0.151	0.0019
C18:2	7.60[a]	10.66[b]	11.09[b]	11.12[b]	0.554	0.0013
C18:3	33.26[b]	24.53[a]	24.78[a]	23.49[a]	2.391	0.0430
C20:0	6.44	6.93	7.47	7.08	0.310	0.1468
C20:1	3.23[a]	3.51[ab]	4.19[bc]	4.57[c]	0.362	0.0478
C20:3	2.25[b]	2.00[ab]	1.81[a]	1.61[a]	0.180	0.0966
C20:4	1.45	1.46	1.56	1.37	0.210	0.9076
C20:5	1.87	1.58	1.91	1.61	0.216	0.5181
C22:1	6.14	5.88	5.53	5.92	0.641	0.9101
C24:0	4.16	5.42	4.87	5.17	0.473	0.1576
C24:1	6.54	8.51	7.74	8.22	0.792	0.2113
s.e.m.	2.351	2.391	2.364	2.373		

[a, b, c]Least squares means within rows with same superscript letters were not different ($P < 0.1$)

Table 25 Plasma fatty acids concentration (µg/ml) throughout a 10 h collection period for all ponies fed different amount of fat (0, 5, 10 or 15 %).

Fatty Acid	Time (h post-feeding)												
	0	1	2	3	4	5	6	7	8	9	10	s.e.m.	p =
C14:0	1.76	1.85	1.66	1.88	1.74	1.70	2.00	1.83	1.84	2.08	1.79	0.390	0.6065
C14:1	0.49	0.46	0.49	0.52	0.57	0.72	0.56	0.58	0.70	0.51	0.58	0.273	0.9895
C16:0	3.48	3.33	3.14	3.46	3.03	3.23	3.00	3.29	3.38	3.55	3.60	0.544	0.7781
C16:1	2.23	1.98	1.59	1.71	1.32	1.64	1.38	1.52	2.08	1.96	1.97	0.829	0.7974
C18:0	13.13	11.46	11.55	12.93	10.77	12.06	10.56	12.01	12.12	12.74	13.63	2.255	0.7447
C18:1	3.07	2.87	2.94	3.09	2.77	2.81	2.75	3.05	3.05	3.23	3.16	0.538	0.8765
C18:2	7.89	6.79	7.44	7.81	6.55	7.31	6.35	7.09	7.40	7.32	8.06	1.387	0.8306
C18:3	18.64	21.52	18.69	19.12	19.91	16.85	18.76	19.74	20.00	20.74	21.89	5.012	0.9528
C20:0	5.35	4.62	4.98	5.40	4.56	5.04	4.46	4.91	5.21	5.34	5.72	0.991	0.8146
C20:1	3.23	2.99	2.83	3.16	2.46	2.89	2.63	2.98	3.11	3.01	3.20	0.750	0.8955
C20:3	1.29	1.29	1.15	1.43	1.21	1.34	1.21	1.25	1.36	1.32	1.47	0.214	0.6804
C20:4	1.00	0.97	0.92	0.98	0.94	0.99	0.97	0.98	0.92	1.04	0.99	0.638	0.9908
C20:5	1.15	1.03	0.97	1.19	1.41	1.03	1.38	1.29	0.89	1.44	1.14	0.623	0.3753
C22:1	5.71	4.36	3.97	4.55	3.45	4.23	4.51	3.93	3.93	5.30	4.71	1.606	0.9482
C24:0	3.22	3.78	2.99	4.25	3.05	4.12	3.54	3.92	3.50	4.22	3.56	1.012	0.4921
C24:1	5.94	5.63	5.25	6.18	4.81	5.92	5.35	6.01	5.80	6.76	6.60	1.439	0.7884
Total Fatty Acid	77.58	74.93	70.56	77.66	68.55	71.88	69.41	74.38	75.29	80.56	82.07		
s.e.m.	3.053	3.078	3.072	2.987	3.085	3.519	3.054	3.076	3.021	5.012	3.088		

Least squares means were not significant ($P < 0.1$)

Table 26 Plasma fatty acids (% of total fatty acid) of ponies throughout a 10 h collection period for all ponies fed different amount of fat (0, 5, 10 or 15 %).

Fatty Acid	Time (h post-feeding)												
	0	1	2	3	4	5	6	7	8	9	10	s.e.m.	p =
C14:0	2.37	2.56	2.71	2.63	2.75	2.37	2.89	2.63	2.56	2.64	2.32	0.398	0.2139
C14:1	0.74	0.63	0.73	0.82	0.91	1.22	0.81	0.81	1.45	0.71	0.88	0.605	0.9669
C16:0	4.71	4.65	4.77	4.74	4.84	4.70	4.55	4.68	4.76	4.65	4.66	0.334	0.9959
C16:1	2.48	2.48	2.20	2.27	1.69	2.07	1.85	1.86	2.64	2.23	2.12	0.729	0.5332
C18:0	17.04	15.92	16.88	17.31	17.05	17.51	16.13	16.91	17.07	16.54	17.32	1.243	0.9371
C18:1	4.13	3.97	4.38	4.09	4.30	4.07	4.14	4.21	4.20	4.17	4.02	0.268	0.7690
C18:2	10.29	9.64	10.65	10.40	10.60	10.56	9.75	9.98	10.44	9.23	10.31	0.921	0.7671
C18:3	25.66	28.60	27.95	25.04	28.30	23.44	27.00	26.48	26.26	26.39	26.92	2.790	0.7514
C20:0	7.04	6.38	7.31	7.15	7.15	7.24	6.75	6.75	7.31	6.89	7.22	0.578	0.6596
C20:1	3.92	4.18	3.91	3.96	3.64	4.12	4.04	4.10	4.24	3.66	3.77	0.665	0.9824
C20:3	1.89	1.76	1.87	2.00	1.95	1.98	1.83	1.84	1.97	1.80	2.07	0.323	0.8573
C20:4	1.36	1.38	1.42	1.36	1.45	1.43	1.46	1.44	1.32	1.32	1.32	0.514	0.9889
C20:5	1.59^{ab}	1.39^{ab}	1.52^{ab}	1.59^{ab}	2.13^{b}	1.33^{a}	1.95^{ab}	1.80^{ab}	1.24^{a}	1.73^{ab}	1.61^{ab}	0.582	0.0978
C22:1	7.47	5.79	5.35	5.86	4.95	5.95	6.59	5.22	5.08	6.74	5.32	1.532	0.7765
C24:0	4.14	5.02	3.93	5.24	4.38	5.68	5.32	5.28	4.55	5.25	4.56	0.994	0.1319
C24:1	7.78	8.07	7.13	7.85	7.29	7.50	8.42	8.11	8.31	7.47	8.88	1.437	0.9193
s.e.m.	1.704	2.028	1.733	1.787	1.769	1.795	1.771	1.790	1.787	1.754	1.796		

[a, b]Least squares means within rows with same superscript letters were not different ($P < 0.1$)

CHAPTER V

OVERALL CONCLUSION OF EFFECTS OF SUPPLEMENTING PONIES WITH DIETARY FAT ON NUTRIENT DIGESTIBILITY AND BLOOD INSULIN, GLUCOSE, AND FATTY ACID CONCETRATION

Although further research is needed to determine different fat on differing equine all objectives for this study were completed. A band of dual cannulated ponies were successfully developed to determine fat effects on nutrient digestibility, blood glucose, insulin and fatty acid concentration.

Previous research, including Gerhards et al. (1991), reported a death loss of 25 to 45 % with only ileal cannulation. Two surgical stressors were presented in the current study, a dual cannulation of the cecum and ileum; thus, a decreased success rate was anticipated. Death loss was 37%, which compares to previous methods when dual cannulation was taken into account. Death was influenced by lack of adherence of stoma and timing of trauma to surgery site. When the stoma had not formed, causing a lack of adherence, and the pony sustained trauma, recovery was negatively impacted and chance of survival decreased.

Dietary fat effects on nutrient digestibility have been observed to affect glucose, insulin, and fatty acid concentration in the blood of horses. Although glucose and insulin were not affected by supplemental dietary fat, insulin increased from 0 to 1 h. Overall, total plasma fatty acids were not influenced by dietary fat amount with the exception of

C14:0, which increased when ponies were fed 0 % fat, and C18:2 n-6, which decreased from 0 % fat compared to diets containing 5, 10, and 15 % fat of the diet.

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