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Dr. David L. Harmon, Director of Graduate Studies

EVALUATION OF A NOVEL FEEDSTUFF FOR HORSES

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture, Food and Environment at the University of Kentucky

By

Catherine Whitehouse

Lexington, KY

Director: Dr. Robert J. Coleman, Assistant Professor of Animal Science

Lexington, KY

2013

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ABSTRACT OF THESIS

EVALUATION OF A NOVEL FEEDSTUFF FOR HORSES

Determining digestibility of feed ingredients is a challenge. While forage type feeds may be fed as the sole dietary component, concentrates cannot. To determine the apparent digestibility of these ingredients a by difference method can be utilized. The study was conducted to investigate the nutritional value of corn germ dehydrated (AAFCO 48.32) for horses in comparison to two processed corns commonly fed to horses. The second objective was to compare total tract digestibility of the treatments to determine differences in digestibility of the various fiber fractions due to added concentrate and to use the difference method to determine nutrient digestibility of the three corn treatments. In addition, glycemic responses and fecal pH changes were used to assess and quantify the presence of associative effects in fiber digestibility and differences in site of digestion.

A 4x4 Latin square digestibility trial was conducted using a starch intake level of 6 g/kg BW/day, offered in three equal meals. The control diet consisted of hay cubes, alfalfa pellets, corn bran and corn oil. The treatment diets contained the control diet plus one of the three processed corn treatments, cracked, steam flaked or corn germ dehydrated. Cracked corn was selected as a negative control and steam flaked corn as a positive control based on previous research showing differences in pre-cecal starch digestibility coefficients. The four diets were formulated to have equal starch, NDF and ADF components. Each experimental period was 21 days made up of 5 days adaptation, 11 days on feed and a 5 day total fecal collection. Indirect methods for estimating small intestinal starch digestion (glycemic response) and changes in the hindgut environment (fecal pH and acid concentrations) were used due to the use of non-surgically modified experimental animals.

Mean total tract starch digestibility for all diets was high, control 92.2 ± 4.9 , cracked corn 96.6 ± 1.0 , steam flaked corn 99.2 ± 0.4 and corn germ dehydrated 98.8 ± 0.4 % ($P > 0.05$). The process of steam flaking compared to cracking or dry corn milling resulted in a greater area under the blood glucose time curve in response to 1kg of corn treatment meal, suggesting increased pre-cecal starch availability. No statistical differences were observed on an equal starch basis between the three corn products ($P > 0.05$). Cracked corn significantly lowered fecal pH compared to the control and corn germ dehydrated diets ($P < 0.05$). Fecal L-lactate was increased on the cracked corn diet compared to the control ($P < 0.05$). There were no differences in mean total tract apparent digestibility of NDF and ADF ($P > 0.05$) when comparing the control diet and the combined diets (control diet plus processed corn) and the three individual corn ingredients by the difference method. Even though mean digestibility of fiber fractions were not affected by diet,

individual horse data suggests that negative impacts on fiber fraction digestion occurred. Surprisingly, the steam flaked diet did not appear to act as a positive control in limiting changes in fermentation kinetics when fed at this intake level. The process of steam flaking improves small intestinal starch availability but the reduction in particle size may increase the rate of microbial fermentation prompting the development of acidosis. The study findings suggest the corn germ dehydrated product to be a good feedstuff for horses requiring additional calories and high feed intakes. The DE value is comparable to steam flaked corn, without the negative impacts observed on fecal fermentation end products.

KEYWORDS: Starch, digestibility, corn, grain processing, equine

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December, 2013

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Chapter 1: Introduction

The primary objectives of grain processing techniques are to maximize digestibility of the grain and the total diet (Rowe et al., 1999). Changes in diet composition have been associated with changes in the microflora of the horse's gastrointestinal tract. However, these differences observed under conditions of similar diet and management are often suggested as a possible reason why certain horses are more prone to digestive disturbances (De Fombelle et al., 1999; Perkins et al., 2012; Schoster et al., 2013).

Microbial fermentation of non-structural carbohydrates leads to increased production of VFA energetic end products and, of particular concern, lactic acid. The increases in VFA's and lactic acid can lead to the development of nutritionally induced acidosis. Hindgut acidosis results in reduced growth of acid-intolerant cellulolytic bacteria when pH is 6.0-6.1. When pH drops below 5.9, growth of these bacteria is completely inhibited, significantly depressing fiber digestibility, and is referred to as negative associative effects (Shirazi-Beechey, 2008). Associative interactions of feed ingredients on total diet digestibility may differ depending on the method of grain processing. The method of processing may have an effect on the site of starch digestion.

The study was conducted to investigate the nutritional value of corn germ dehydrated (AAFCO 48.32) for horses in comparison to two processed corns commonly fed to horses, cracked corn and steam flaked corn. Nutritional value of the corn products were evaluated by total tract apparent digestibility using the difference method. Indirect measurements of glycemic response and fecal parameters were used to assess differences in site of starch digestion of the three corn products. The second objective was to

determine differences in fiber digestibility due to a high starch intake level and if these changes in fiber digestibility could be detected by the difference method.

The study hypotheses were the corn germ dehydrated product is a suitable feedstuff for horses, as a starch source with an intermediate nutritional value between cracked and steam flaked corn. Secondly it was hypothesized that the high intake level of cracked corn would negatively affect fiber digestibility and steam flaked corn would not. The differences in fiber digestibility would be identified in the apparent digestibility of the whole diet and the corn products.

Chapter 2: Literature Review

The horse is classified as a non-ruminant roughage grazer (Ellis and Hill, 2005), and is placed into this category based on its ability to digest dietary fiber. The horse's natural diet consists of plant-based structural polysaccharides (carbohydrates), mainly cellulose, hemicellulose and pectin with smaller amounts of reserve polysaccharides, such as starch and fructan. Because the horse does not produce and secrete the enzymes required to break down structural carbohydrates, they rely on microbial fermentation to yield available energy sources, predominately by hindgut fermentation (Ellis and Hill, 2005). Normal feeding behavior involves the horse spending a minimum of 10 to 12 hours a day eating. This trickle feeding pattern prevents the horse's digestive capacity from being overwhelmed and provides a continuous supply of nutrients to the hindgut for microbial growth and energy production.

Modern horse management has resulted in significant changes in the horse's opportunity to graze. In addition to reduced grazing, the horses' role in athletic performance has led to increased dietary intakes of starch, fat and protein from non-roughage sources offered in large meals to meet energy requirements (Metayer et al., 2004).

The anatomy and digestive physiology of the horse is routinely considered as two distinct segments, with the cecum being the dividing anatomic structure. Woodward et al. (2010) describes the horse as being equipped with significant hydrolytic capability in the proximal gastrointestinal tract (stomach and small intestine) and fermentative capabilities in the distal portion (cecum and large intestine) of the tract. Recent studies have brought

into question the presumed simplicity of the stomach's role in digestive physiology. The small holding capacity (7.5 to 15 L) and high transit time have caused many to overlook the role of the stomach in digestion especially of nonstructural carbohydrates (Al Jassim and Andrews, 2009). The anatomy of the stomach produces a pH gradient, digesta in the fundus region is exposed to a higher pH and the action of microbial fermentation compared to digesta in the pylorus where hydrochloric acid secretion inhibits fermentation and initiates the proteolytic activity of pepsin. Gastric microbial fermentation has been quantified for over 40 years, and differences in gastric fermentation products have been described due to differences in nonstructural carbohydrate intakes (Kern et al., 1974). However, the implications of gastric fermentation are still largely unknown regarding starch digestion, fermentation products and gastric health.

In order to optimize the digestive capacity of carbohydrate fractions in the diet (primarily nonstructural carbohydrates (NSC)), the rate of gastric emptying must match the small intestine's rate of digestion and absorption. The chemical and physical actions of the distal stomach cause a reduction in structural integrity of the ingesta entering the small intestine. In the small intestine, enzymatic digestion occurs in two phases. The first (luminal) phase involves enzymes secreted by the gastric glands and pancreas mixing with ingesta in the lumen of the duodenum, jejunum and ileum and forming short-chain polymers. The second phase (membranous phase digestion) involves enzymes bound to surface epithelium that break down the short-chain polymers into monomers. This complete hydrolysis produces substrates that can be absorbed across the epithelium.

Dietary NSC from plant and cereal grain sources are enzymatically digested in the small intestine. Starch is digested by the actions of both luminal and membranous enzymes, whereas sugars are digested by membranous enzymes only. The enzyme involved in luminal starch digestion is alpha-amylase produced and secreted from the pancreas. Starch has two chemical forms, amylose and amylopectin, both long chain glucose polymers. Amylopectin is a branched chain polymer with β [1-6] glycosidic linkage, which cannot be hydrolyzed by alpha-amylase. Alpha amylase breaks down α [1-4] glycosidic linkage in amylose and amylopectin, producing dextrans and limit dextrans, respectively, which are further digested to produce maltose and isomaltose, respectively. Membranous enzymes sucrase, maltase and isomaltase produce fructose and glucose available for absorption via sodium monosaccharide co-transporter or via sodium independent facilitated diffusion.

Substrate digestion and utilization in the proximal gastrointestinal tract determines the composition of ingesta reaching the hindgut. Fiber fractions (neutral detergent and acid detergent fiber) of plants and cereal grains are resistant to host enzymes due to β [1-4] glycosidic linkage but on reaching the cecum and large intestines undergo the hydrolytic action of microbial enzyme referred to as cellulase. Lignin is resistant to both host and microbial enzymes, and only a small portion of lignin is digested by either process. Microbial carbohydrate digestion forms saccharides, which are not directly available to the horse but are metabolized further by the microbial flora. Hexoses are utilized by the microbes via glycolytic pathway producing pyruvate, though differences in pyruvate metabolism impact the production of energetic end products. Principal fermentation end

products are lactic acid and volatile fatty acids (acetic acid, propionic acid and butyric acid), commonly referred as their dissociated ions: lactate, acetate, propionate and butyrate. The total concentration and proportions of VFAs produced are affected by diet. Nonstructural carbohydrates that have escaped small intestinal digestion are readily available for microbial fermentation. Acetate is the principal product of fiber fermentation (65-70 %), followed by propionate (18-20 %) and butyrate (15-17 %) (Perry, 1984). As the level of NSC increases in the total diet, the proportion of acetate formation decreases, favoring propionate and lactate production and coupled by a total increase in VFA production. This dietary effect on fermentation products is a direct result of changes in microbial populations due to substrate supply, environmental pH, and buffering capacity. Due to the acidic nature of fermentation products, the horse has developed the capacity to absorb and utilize VFAs. VFAs can contribute 60-80 % of the horse's energy requirement (Rechkemmer et al., 1988).

As the horse's nutrient requirements exceed maintenance levels due to factors such as milk production, skeletal and muscle development and energy for athletic performance, the inclusion of energy dense cereal grains (oats, barley, corn and sorghum) to forage-only diets became popular based on availability, cost and nutrient composition.

Grain Processing

In order to maximize feed utilization, cereal grains are processed before they are fed to enhance their digestibility and alter site of digestion, ultimately through increasing starch availability (Rowe et al., 1999; Julliand et al., 2006). Processing is needed for all grain

types to break the seed coat and expose the endosperm, especially corn and sorghum. The endosperm matrix and starch structure determine the extent of processing required to improve starch availability. The endosperm contains individual starch granules surrounded by a matrix of protein and non-starch polysaccharides. Non-starch polysaccharides (NSP) affect starch digestibility in monogastrics by increasing digesta viscosity, which depresses pre-ileal starch digestion. In animals that utilize a combination of enzymatic and fermentative digestion, high digestible energy values are observed, though the compensatory starch fermentation may lead to inefficient digestion and energy utilization through an increased portion of VFA production and decreased intestinal absorption. The density of the protein structure surrounding the starch granules has a significant effect on digestion. A lower density protein matrix increases the accessibility of enzymes to the starch granules. The dense protein matrix and non-starch polysaccharide content (5 % DM) in corn reduces the prececal starch digestion in horses. Pretreatment of corn with proteases results in increased glucose release, suggesting the protein matrix of corn as a limiting factor of starch digestion (Hoffman and Shaver, 2008).

The physical structure of the starch granules requires disruption in order to enhance digestibility. This can be achieved mechanically (cracking or rolling) or by heat, pressure and moisture (steam flaking and extrusion). Heat and moisture causes irreversible disruption of the matrix and swelling of the internal crystalline structure of the starch granules. For each grain, there is a critical temperature at which the starch granules are gelatinized. Corn and sorghum have higher gelatinization temperatures (62-75 °C) than

wheat (52-63 °C). The magnitude of processing can be classified by the amount of disruption to the starch granule, cracking is the minimum processing required to improve starch availability over whole grain. Conventionally, cracked corn is used when comparing the effect of grain processing on cereal grains.

Mechanical processing (cracking, dry rolling and grinding) functions to break the seed coat, reduce particle size and increase the surface area for digestion. Grinding results in a smaller particle size than cracking. However, the variability in the number of starch granules released from the matrix during either process is great. Steam flaking involves the whole grain being heated with steam and subsequently rolled to varying degrees. During this process the seed coat and endosperm is disrupted, increasing the surface area. Unlike cracking, however, the whole grain remains as one. The processing techniques of steam flaking and pelleting result in greater starch gelatinization when compared to dry mechanical techniques. Steam flaking is more effective at starch gelatinization than pelleting. Pelleting allows manufactures to combine small particles normally from by-product ingredients into a larger particle using heat, moisture and pressure by means of forcing ingredients through a die.

Starch has been classified based on its digestibility. Classifications include readily digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS is hydrolyzed *in vitro* within 20 minutes with alpha amylase and amyloglucosidase compared to SDS, which includes starch that is fully hydrolyzed during prolonged incubation (20-120 minutes). RS has been defined as “the sum of starch and starch-

degradation products that, on average, reach the human large intestine,” that is starch which is not hydrolyzed after 120 minutes incubation with alpha amylase and amyloglucosidase (Perera et al., 2010). The amount of amylopectin and amylose can affect the rate and site of starch digestion. Amylopectin has a less crystalline structure and greater solubility, causing it to be broken down more readily by alpha amylase than amylose. As the amount of amylose content increases, the starch changes from rapidly, enzymatically digestible in the proximal gut to fermentable in the distal gut (Zijlstra et al., 2012). The amylose and amylopectin ratio affects the processing temperature required to gelatinize starch. Lower temperatures are required to gelatinize starch composed of high amylopectin and low amylose. This ratio can also impact the level of resistant starch, as resistant starch levels increase with increasing amylose content. Processing grains at low (83-86 °C) and high (135-145 °C) extrusion temperatures significantly increases the amount of RDS and decreases the amount of SDS and RS, with the greatest effect on SDS and RS under high temperature processing (Murray et al., 2001). Pre-ileal starch data from Kienzle et al. (1997) support this increase of RDS; with thermo-mechanical processing techniques, corn starch digestibility increased from 29 % in whole or crushed corn to 90 % in expanded (popped) corn.

There is no definitive research regarding the optimal particle size required to maintain health and function of the horse’s gastrointestinal tract. A reduction in particle size improves feed efficiency in growing-finishing pigs but also leads to a higher risk of gastric ulcers and keratinization of the gastric mucosa. In growing-finishing pigs, feed conversion was improved on finely ground diets (higher portion of particles <0.3 mm and

<0.6 mm); however, coarsely ground (higher portion 1.19-2.36 mm) and coarsely ground high fiber diets resulted in lower urease activity and reduced gastric mucosa lesion number, respectively (Millet et al., 2012). The median particle size of 0.8 mm is used in manufacturing for swine diets, due to this digestive physiology interaction (Hill, 2007). Zinn et al. (2002) reported on the importance of flake thickness and flake density from steam flaking and how this impacts feedlot cattle. Steam flaking to a density less than 0.31 kg/L (optimal) increases starch solubility, resulting in excessive ruminal starch fermentation and leading to reductions in dry matter intake, animal performance (ADG), and predisposition of cattle to acidosis and bloat. Others suggest that processing beyond 0.36 kg/L could be excessive and unnecessary relative to the benefits in starch digestion and increased cost (Sindt et al., 2006).

Perry (1984) noted that of all the concentrates used for horses, high quality oats is the first choice and corn is the second. Starch is the primary energy source in cereal grains. The amount of starch and starch availability differs between grain species. Oats have low starch (42 %, DM basis) and high fiber content compared to barley, corn and sorghum. Barley (61 %, DM basis) has an intermediate starch content compared to oats and corn. Corn and sorghum have the highest starch content (76 and 75 %, DM basis respectively) of the four commonly fed grains. Horses are very efficient at digesting starch based on total tract digestibility values, regardless of grain species. Digestion coefficients between 90 and 99% have been reported (Julliand et al., 2006; Rosenfeld and Austbø, 2009). However, enzymatic starch digestion is significantly altered by grain species and other factors. Oat starch has a prececal digestibility coefficient of 80 % compared to barley and

corn starch at 29 % (Kienzle et al., 1997). Studies have shown that further processing of oats does not significantly improve prececal starch digestibility, whereas barley and corn require thermal (steam rolling) and thermal-mechanical (flaking and popping) processing to improve starch availability for enzymatic digestion in the small intestine. Oats are considered a safer energy source for horses compared to the other grain sources.

When dietary starch is digested and absorbed as monosaccharides in the small intestine, a substantial fraction of metabolizable energy (ME) is provided and the efficiency of feed metabolizable energy utilization is 85 % (Vermorel et al., 1997). If dietary starch exceeds the horse's proximal digestive capacity, starch is fermented in the hindgut, producing VFA and lactate metabolites, which are less efficiently utilized for ME (63-68 %) (Vermorel et al., 1997). Limiting starch fermentation in the hindgut is important due to the implication that diets rich in nonstructural carbohydrates may lead to hindgut acidosis, colic and laminitis.

Studies have reported significant numbers of viable anaerobic bacteria in the fundus (pH 5.4) compared to the pyloric area (pH 2.6) of the stomach of horses. The gastric microbial community, which is predominately gram-positive and few cellulolytic bacteria, produces lactate as the principal fermentative end product (Argenzio et al., 1974; Kern et al., 1974). The amylolytic and fermentative activity of the microflora and the degree of starch availability affect gastric starch digestibility. Horses offered processed sorghum (3.26 g starch/kg BW/meal) had high lactate concentrations within the stomach, duodenum and jejunum, and the lowest levels recorded in the hindgut. The fundus (nonglandular) had

the greatest concentration of lactate (~33 to 40 mmol/L). Steam flaking sorghum versus dry rolling resulted in greater production of total lactate and D-lactate in the stomach (Al Jassim, 2006). The progressive decrease in lactate production along the gastrointestinal tract was reported by Mackie and Wilkins (1988) in grass fed horses. However, the lactate concentration ~ 1mM reported in the stomach of these grass fed horses was lower than the previous high starch diet. The presence of D-lactate (predominantly from microbial origin) is strong evidence of gastric fermentation and can be a useful parameter to assess gastric starch fermentation. Investigators have quantified digestion coefficients for NSC in the stomach of live horses with values ranging from 24.2 % (measured by nasogastric tube sampling; Varloud et al., 2006) and 60.4 and 68.6 % (measured by acid insoluble ash marker; Varloud et al., 2004) and 41.0 and 75.7 % (measured by acid-detergent lignin marker; Varloud et al., 2005).

Gastric fermentation of starch may be advantageous for improving prececal digestibility and reducing starch delivery to the hindgut; however, the organic acids produced have been implicated in the development of equine gastric ulcer syndrome (EGUS). Sampling of the gastric contents from horses fitted with chronic gastric cannulas following ad libitum bromegrass hay showed that acetic acid was the principal VFA produced, with highest concentrations immediately and up to 5 hours post feeding. Concentrations of D and L-Lactic acid in gastric contents were 0.394-10.36 and 0.57-17.8 mmol/L, respectively (Nadeau et al., 1997). A follow-up study on the implications of diet was conducted comparing a mixed diet of alfalfa hay and grain and bromegrass hay. The mixed diet produced high concentrations of acetic acid immediately and 5 hours post

feeding, high concentrations of propionic acid for 6 hours post feeding and increased concentrations of isovaleric acid. These differences between diets are likely due to the fermentation of NSC present in the grain. Even in the presence of high VFA concentrations, the pH remained higher for 5 hours post feeding of the mixed diet and gastric ulcer lesion numbers and severity were lower overall. The authors speculated that the alfalfa hay, due to its high calcium and protein content, may have had acid inhibitory and buffering effects. Feeding recommendations were made regarding continuous offering of alfalfa hay every 5-6 hours to provide a protective effect on the nonglandular squamous mucosa (Nadeau et al., 1999). The acidic environment and the formation of high concentrations of acetate, propionate, butyrate and valerate have been shown *in vitro* to cause cellular swelling as well as barrier and transporter disruption. Gastric epithelial cells can readily absorb the acids leading to accumulation in the cell rather than transport across into the blood.

Gastric emptying can be affected by meal size and composition (Metayer et al., 2004). Small meals (300 g/100kg BW) with low starch and high fiber content have significantly faster gastric emptying than small meals with high starch content when comparing half-emptying times (T_{50} , mean \pm s.d. for low starch 93 ± 7.6 mins and 143 ± 29.2 mins for high starch). Maximal gastric emptying expressed as g/minute was significantly faster for a large high starch meal (700 g/100kg BW) than the small high starch meal with a calculated mean difference of 4.2 g/minute. Feeding large meals high in starch could overwhelm the capacity of the small intestine compared to small meal sizes, as the amount of starch entering the duodenum per minute would be greater and this could lead

to starch overflow into the hindgut. Differences in gastric emptying time could be due to selective retention for adequate mixing.

Recommended feeding levels for starch are expressed on a meal basis. These recommendations are based on research investigating the capacity of the small intestine, relative to starch disappearance at the terminal ileum and suppression of microbial parameters in the hindgut. Little information is available to add to these recommendations in regard to the number of meals per day and the length of time between meals required to maintain digestive health. Potter et al. (1992) suggested horses that are fed 2 to 3 times a day should have starch intakes limited to 0.4 % of bodyweight per feeding. There is a wide reference range in the literature regarding the feeding level of starch to horses from <1.1 g/kg BW or 0.3 kg concentrate/100 kg BW with starch content 30-40 % (Vervuert et al., 2009), 2 g starch/kg BW (Kienzle et al., 1992), and up to 4 g starch/kg BW (Potter et al., 1992).

The enzymatic capacity (concentration and activity) of the horse's small intestine has been commonly thought of as the limiting factor in the horse's ability to digest starch, particularly luminal phase starch digestion (Richards et al., 2004). Pancreatic alpha amylase production is lower in the horse than other species; however, maltase activity is higher than other species, thus an unlikely limiting factor in digestion.

Richards et al. (2003) found that alpha amylase collected from the jejunum was on average 20 % less effective at digesting starch in vitro than bacterial alpha amylase

(standard). When starch was incubated for 60 minutes in vitro compared to 15 minutes digestion increased; this was enhanced by using processed grains as the starch source. The authors speculated that equine jejunum alpha amylase had a higher affinity to oat starch due to digestion values similar to the bacterial standard. The high globulin and low prolamin storage protein content of the oat endosperm matrix could result in greater susceptibility to enzymatic action (Shewry and Halford., 2002). Radicke et al. (1991) reported higher preileal oat starch digestibility mean 98 % due to higher amylase activity in jejunal chyme (40.6 U/g) when compared to corn mean starch digestibility of 70 % and amylase activity 28.9 U/g.

Dyer et al. (2009) investigated the horse's ability to adapt to increased carbohydrate load. This study compared pasture-forage fed horses to those fed diets containing oats and corn. The authors reported horses that had been maintained long term (months) on high grain diets had 1.9- and 3.7-fold higher rates of D-glucose uptake from the jejunum and ileum compared to horses maintained on pasture forage. The rate of uptake in the duodenum was not affected by diet. It was determined that the increase in D-glucose transport across the brush border was due to an increase in the number of SGLT1 transporters in the jejunum (2-fold) and ileum (5.1-fold). These data show that horses have the ability to adapt to increases in dietary carbohydrate, specifically in the jejunum and ileum of the small intestine, and this is regulated by increases in messenger RNA of the transporter protein SGLT1.

Further investigation into the horse's ability to adapt to dietary carbohydrates found that

horses transitioned from a forage-only diet to a moderate starch diet (3.3 g starch/kg BW/day, divided into 3 meals) had a 2-fold increase in SGLT1 expression in the ileum with no change in the duodenum after one week. After one month on this starch level, the SGLT1 expression in the duodenum increased 2-fold, with no further change in the ileum. Additional starch intake (6 g starch/kg BW/day, divided into 3 meals) after one month resulted in further increases of SGLT1 expression in the ileum (3.3-fold increase from expression levels on the moderate starch intake). These studies improve our understanding of the horse's ability to adapt to and tolerate dietary carbohydrates. The final starch level used in this study was 6 g starch/kg BW/day, providing 2 g starch/kg BW/meal, which is currently suggested as 'safe' feeding level per meal without horses experiencing digestive disturbances (Dyer et al., 2009).

In commercial swine production, enteric disorders such as nutrient malabsorption and diarrhea are prevalent in post weaning piglets. Two sweeteners, saccharin and neohesperidin dihydrochalcone, are frequently added to commercial piglet diets and have been found to attenuate post weaning enteric disorders and enhance growth performance. Moran et al. (2010a) found that these feed additives induced upregulation of SGLT1 expression (mRNA, protein and function) in piglet small intestine. Further work in piglets investigating the level of carbohydrate in the diet required to trigger this upregulation reported that 7.0 and 36.0 % dietary carbohydrate did not trigger a change in SGLT1 expression; however, 53.0 and 60.0 % dietary carbohydrate caused an increase in SGLT1 expression in the proximal and mid regions of the small intestine (Moran et al., 2010b).

Forage to concentrate ratios have been reported to affect site of digestion and digestion end products. Substitution of 50 % of an all hay cube diet with processed barley (rolled, extruded or micronized) showed differences in cecal fermentation parameters between the control and barley diets and between the different barley processing techniques (McLean et al., 2000). Five hours post feeding cecal pH was significantly lowered by the rolled barley diet (pH = 6.26) compared to the all hay cube diet (pH = 6.50). Comparisons between the remaining diets were not significant with extruded barley (pH = 6.36) and micronized barley (pH = 6.33). Cecal lactate concentrations were increased when barley was included in the diet, but only significantly greater lactate concentrations were recorded on the rolled barley diet. Lactate concentrations (mmol/L) ranged from 0.11 for the hay cube, 0.97 rolled barley, 0.18 micronized barley and 0.26 for the extruded barley diets. Fermentation parameters, acetate and propionate were most similar to the hay cube diet when micronized barley was fed.

The inclusion of rolled barley into a diet to produce forage:concentrate ratios of 100:0, 70:30 and 50:50 caused an increase in the apparent digestibility of the organic matter (Drogoul et al., 2001). However, the apparent digestibility of NDF decreased from 46.1 % on the forage only diet to 40.4 % (70:30) and 39.3% (50:50). ADF digestion was affected, decreasing from 42.4 % on the all forage diet to 36.1 % (70:30) and 33.9 % (50:50). The difference between NDF digestion and possibly ADF on the barley treatments may have little physiological importance in attempting to quantify starch amounts and the detrimental effects on fiber digestion. The data is supported by a decrease in numbers of cellulolytic bacteria in the large intestines (sum of cecum and

colon) with the addition of barley and changes in fermentation kinetics (Julliand et al., 2001). As the proportion of barley increased, pH decreased, acetate to propionate ratio decreased and lactate increased, suggesting a shift towards predominately amylolytic bacteria and unfavourable conditions for cellulose fermentation.

Evaluation of nutrient digestibility

The nutrient composition of feeds is commonly determined by chemical analyses. However, chemical analysis alone does not provide sufficient information about nutrient availability and how the animal will utilize the nutrients. Digestibility is a main component of determining nutritive value of a feedstuff. A commonly used in vivo method to determine total tract apparent digestibility is the comparison of the nutrient in the diet with that recovered in the feces (Forster, 1999). Digestion coefficients are simply the unrecovered fraction expressed as a percentage of intake. Apparent digestibility coefficients do not account for fecal endogenous losses of that nutrient.

In vivo digestion studies follow a general standard protocol of dietary adaptation or acclimation in which the aim is to achieve steady state nutrient intake and fecal output followed by a collection period of fecal and urine sampling. There are several methods of measuring digestibility in horses. Total collection of feces is often referred to as the gold standard but this requires horses that tolerate collection harnesses or containment in metabolism stalls. Alternative methods are the use of inert markers that allow the partial collection of feces, and digestibility calculations are based on the ratio of marker added

or naturally occurring in the feed and the feces. There is still debate over the accuracy of markers compared to total collection methods; however, the three markers commonly used are chromic oxide, acid insoluble ash and lignin. The mobile bag technique allows measurements of both total tract and partial tract digestibility if surgically modified horses are available. This method requires the preparation of mobile bags containing a measured amount of experimental feed and intubation via a nasogastric tube, followed by the collection of bags via a cannula site or in the feces.

Rosenfeld and Austbø, (2009) used the mobile bag technique to assess prececal and total tract digestibility of starch from processed grains. This method provided the opportunity to directly assess and quantify the amount of compensatory fermentation that occurred due to differences in prececal digestibility. The use of non-surgically modified animals as experimental subjects in investigating digestive capacity and obtaining representative samples of the different compartments of the gastrointestinal tract can be challenging compared to using fistulation/cannulation techniques or postmortem sample collection. Savage (1977) describes the limitations of the fecal microbiota (commonly used as an indirect assessment of the hindgut) being a good index of the true character of the gastrointestinal microbial ecosystem. Extrapolated findings from feces-only studies need to be interpreted carefully; however, the use of intact animal models is more practical and applicable to livestock systems. In a recent study (Schoster et al. (2013) using PCR-based terminal restriction fragment length polymorphism techniques, found that feces microbiota had mean similarity index (Cs) of 0.67 and 0.49 with the microbial

community in the cecum and colon respectively. Two identical profiles generate a Cs value of 1, which was reported in this study as the maximum Cs value for comparisons between cecum and colon, cecum and feces, and colon and feces. Differences in pH and bacterial counts have been observed between the right ventral colon and feces, fecal samples had lower pH and higher counts of lactate producing and utilizing bacteria (Müller et al., 2008). Studies that have investigated the gut microbiota throughout the gastrointestinal tract have reported large horse to horse variation. Differences have been observed even under conditions of similar diet and management and this may be a possible reason why certain horses are more prone to digestive disturbances (de Fombelle et al., 1999; Perkins et al., 2012; Schoster et al., 2013).

Dietary soluble carbohydrate content can be correlated with the glycemic potential of the feed ingredient. Processing techniques that increase the starch availability of the grain can elevate and exacerbate the horse's glycemic response. There are a range of methodologies for testing glycemic response. Horses are commonly adapted to the test meal, fasted for at least 10 hours prior to the test, and provided only the test meal during sampling. Blood samples are taken before feeding and for 4 to 8 hours postprandial or until baseline glucose values are recorded. Blood samples are assayed for plasma glucose and plasma or serum insulin and evaluated by the area under the curve method for these variables. The test is an artificial reference, as horses rarely consume one ingredient at a time, such as straight corn. In addition, this methodology incorporates a reduced feed intake as it is preferred that the horse consumes the test meal in a short period of time

(10-15 minutes) as to mimic meal feeding. The horse's glycemic response to a single feed ingredient or diet can be used as an indirect method to assess starch digestibility, based on the assumption that the blood glucose-time curve is a direct representation of starch digestibility in the small intestines. Both glucose and insulin parameters are required to truly evaluate the horse's glycemic response to a specific amount (dose) of starch that is being indexed (Vervuert et al., 2009).

Data presented by Verveurt and Coenen (2006) showed starch intake as a driving factor of glycemic response, with limited differences observed below a feeding level of 2 g starch/kg BW. Differences due to processing techniques were evident when the feeding level was increased. Flaked and extruded barley had a greater effect on blood glucose than rolled barley. Hoekstra et al. (1999) found that steam flaked corn produced a greater glycemic response than cracked corn, with higher plasma glucose concentrations 90 to 180 minutes post feeding, but no effect on time to peak glucose.

Vervuert et al. (2009) investigated the effect of increasing starch intake when provided as a compounded commercial concentrate on glucose and insulin responses. There was a significant difference in the insulin response as measured by area under the curve between meals providing 0.3, 0.6, 0.8 g starch/kg BW/meal and 1.1, 1.4, 2.0 g starch/kg BW/meal. Increased starch intake caused higher peak insulin values post feeding, and insulin remained elevated for longer over the 8 hour sampling period. The moderate glucose and insulin response measured for intakes <1.1 g starch/kg BW/meal prompted a feeding recommendation of <1.1 g starch/kg BW/meal or 0.3 kg/100kg BW/meal when

feeding a processed (mainly micronized cereal grains) concentrate containing 30-40 % starch with caution that starch content over 40 % may need additional meal size limitations (Vervuert et al., 2009).

Experimental design considerations

The effect of length of the dietary adaptation period on the accuracy of digestibility measurements has received little experimental attention. The current lengths of adaptation periods are somewhat arbitrary and vary among studies. In the literature, commonly reported lengths (days) include: 5, 7, 9-10, 14, and 21 days (Frape et al., 1982; Karlsson et al., 2000; Kienzle et al., 2002; Hussein et al., 2004; Varloud et al., 2004; Earing et al., 2010). A study investigating the variability in sheep digestion coefficients with zero to 60 days of adaptation did not obtain constant values but trends of decreasing or increasing coefficients from day zero with rhythmic fluctuations (Lloyd et al., 1956). It was concluded that the standard 10 day preliminary feeding period commonly used in sheep digestion trials was adequate and longer periods did not significantly increase the precision of the study. The presence of daily fluctuations in digestibility may indicate that the potential to maintain constant and maximum values of digestion in normal biological systems is questionable (Lloyd et al., 1956).

Conventionally, total fecal collection periods are 5 to 6 consecutive days with horses being adapted to the collection apparatus prior to the start of sampling (Goachet et al., 2009). Adaptation prior to sampling is used to minimize changes in fecal output due to

changes in management. Reducing the length of total collections to 3 and 4 days has been validated for measurements of DM, organic matter, NDF, ADF and hemicellulose (Goachet et al., 2009).

Additionally, some investigators incorporate wash out periods between treatment diets prior to the adaptation period to reduce carryover effects from the previous diets. Earing et al. (2010) included a seven day wash out period in which the horses had ad libitum access to hay before the start of the 14 day adaptation period to treatment diets. Warzecha et al. (2013) utilized a crossover design with 28 day treatment periods with 21 day washout in between for the investigation of dietary changes on cecal microbial populations. There is strong evidence that diet modifies the composition of the intestinal microbiota. Treatment effects on the microbial populations can have direct effects on digestibility, especially when treatment diets are significantly different chemically and physically.

By Difference method

The need to evaluate individual, non-forage feedstuffs such as cereal grains, where these cannot be fed as the sole diet due to digestive disturbances, requires at least two measurements of digestibility.

The test ingredient is combined with a reference diet (forage based) and apparent digestibility of nutrients is measured for the combined diet and the reference diet. The

coefficient of digestion of the test ingredient can be calculated using the by difference equation, which assumes that the nutrient digestibility of the combined diet is the weighted average of the reference diet and test ingredient (Crampton and Harris, 1969). The weighted mean is based on the percentages in which the reference and test ingredient are present in the combined diet (Crampton and Harris, 1969). This calculation is based on the assumption that the addition of the test ingredient to the reference diet does not alter the digestibility of either feedstuff had it been fed alone. This is complicated by the inability to separate and identify the source of the indigestible nutrient present in the feces and the inherent issue that any change in digestibility will be attributed to the test ingredient. The equation described above does not account for the relative contribution of the nutrient from the reference diet and the test ingredient to the combined diet. As a result, an alternative equation has been proposed that is weighted based on the nutrient contribution of each of these components, that reflects test ingredients with higher or lower levels of nutrient will have greater or lesser influence on the nutrient digestibility of the combined diet (Forster, 1999).

The assumption that digestibility values are unaltered is often unwarranted, as the term “associative effects” is used to describe the digestive interaction of different feed components, and such an interaction can occur when feeding conventional hay and concentrate diets. Digestive interactions are related to the simultaneous digestion of starch and cell wall fractions, observed in the rumen of foregut fermenters and the hindgut of horses when starch overload occurs, primarily causing negative associations

(Martin-Rosset and Dulphy, 1987). Kienzle et al. (2002) found unique nutrient digestibility's using the by-difference method when low digestibility straw was fed in combination with concentrates or grass meal. Assuming a constant straw value when fed in combination with grass meal and concentrates, energy digestibility increased approximately 30 % and values over 100 % were reported respectively. The inclusion of readily fermentable carbohydrates is believed to improve the growth and activity of the hindgut bacteria, thus improving fermentation and overall nutrient digestibility. Karlsson et al. (2000) observed negative associative effects between hay and oats based on curvilinearity of nutrient digestibility as the proportion of oats increased (0, 20, 40 and 60 %). Total tract NDF and ADF digestion coefficients tended to increase (39 and 32 %) at the lowest inclusion level of oats from the hay only diet (37 and 29 %) but then decline as the proportion of oats increased (35 and 24 for 40 % oats and 26 and 12 % for 60 % oats).

In comparison, Hussein et al. (2004) found the apparent total tract digestibility of NDF and ADF was not affected by the addition of 0.2 % BW total nonstructural carbohydrates offered as barley, corn and oats. Numerical increases in these fiber fractions were found for the mixed diets compared to the hay cube control. This study compared different grain sources at a fixed carbohydrate level; the level of carbohydrate used was scaled down from 0.4 % BW due to cases of gas colic and the development of acute laminitis in both the control and barley groups during the 3 week adaptation phase.

Feeding high levels of supplementary starch to production and performance animals has

been reported to negatively impact feed intake, fiber digestibility, and animal health through the development of digestive disorders (Karlsson et al., 2000; Matthé et al., 2003; Richards et al., 2006; Lean et al., 2013). Associative effects in production ruminants are well understood and changes occurring in the rumen after excessive intake of starch or NSC-containing diets have been studied in detail (Mackie and Gilchrist, 1979). Large amounts of rapidly fermentable carbohydrates lead to microbiological changes and shifts in fermentation kinetics, as conditions favorable for glycolytic and amylolytic bacteria are subsequently inhibitory to cellulose fermenters. Predominately, decreases in pH and the accumulation of the intermediate metabolite lactic acid are observed. Under normal physiological conditions, lactic acid is metabolized further to VFAs and as discussed previously measured at low concentrations in the hindgut.

The study was conducted to investigate the nutritional value of corn germ dehydrated (AAFCO 48.32) for horses in comparison to two processed corns commonly fed to horses, cracked corn and steam flaked corn. Nutritional value of the corn products were evaluated by total tract apparent digestibility using the difference method. Indirect measurements of glycemic response and fecal parameters were used to assess differences in site of starch digestion. The second objective was to determine differences in fiber digestibility due to a high starch intake level and if these changes in fiber digestibility could be detected by the difference method. A preliminary study was conducted to determine a reasonable starch intake level which would be considered high but not excessive resulting in digestive disorders to be used in the corn product's digestibility study.

The study hypotheses were the corn germ dehydrated product is a suitable feedstuff for horses, as a starch source with an intermediate nutritional value between cracked and steam flaked corn. Secondly it was hypothesized that the high intake level of cracked corn would negatively affect fiber digestibility due to low prececal starch digestibility, whereas steam flaked corn would not, due to improved starch availability from starch gelatinization during the process of steam flaking. The differences in fiber digestibility would be identified in the apparent digestibility of the whole diet and the corn products.

Chapter 3: Responses in fecal pH from low to high starch intakes

Nutritionally induced acidosis is most commonly seen during rapid changes from roughage to high grain diets and in situations that interrupt normal feed intake patterns in grain adapted animals (Goad et al., 1998). Due to the impact nutritionally induced acidosis can have on ruminant livestock systems diagnostic parameters have been established for the identification of subacute ruminal acidosis (SARA). SARA is present when the ruminal pH falls below pH 5.6 for more than 3h per day (Steele et al., 2011). There is a lack of information regarding the usefulness of fecal for the identification of hindgut acidosis in horses. It has been suggested that pH values $\leq 6.0 - 6.2$ may be indicative of subclinical acidosis (Radicke et al., 1991).

Metabolic acidosis is associated with human conditions of short bowel syndrome and ulcerative colitis. Studies have shown that pH alters the end products of fermentation and the diversity of intestinal bacteria. In continuous culture/fermentation studies using human fecal inoculum (Caldarini et al., 1996; Belenguer et al., 2007) it was reported that when pH was maintained below 6.0, VFA productions were predominately acetate. Lactate production was reported to be greatest over the pH range 5.0 -5.5, with minimal production at 5.9 to 6.5. At pH 5.9 and 6.4 lactate utilization was increased compared to pH 5.2 resulting in the production of propionate and butyrate (Belenguer et al., 2007).

A survey of racehorse trainers based in Australia identified that the average daily feeding level of concentrate was 7.3 ± 0.24 kg (range: 3.8 to 13.2 kg) (Richards et al., 2006).

These high concentrate diets contained oats, corn and a commercial mixed concentrate. There was a strong negative relationship between fecal propionate concentration and pH ($R^2 = 0.76$) in horses surveyed in this study. Mean fecal pH was 6.5 ± 0.07 (range: 5.5 to 7.9, with 27 % of samples having a pH < 6.2); mean fecal L-lactate concentration was 0.33 ± 0.06 mmol/L (range: 0.02 to 1.82 mmol/L) and fecal starch content averaged 1.2 % DM (range: 0.28 to 7.35 % DM).

Wheeler and Noller (1977) concluded from studies of concentrate fed cattle that fecal pH was a good indicator of pH in the lower portions of the gastrointestinal tract. In response to high concentrate diets a negative relationship between the amount of starch present in feces and fecal pH was observed. The use of fecal pH as the main indicator of acidosis in horses may result in erroneous assumptions of the metabolic activity in the cecum and colon. Differences in the absolute pH values from different gastrointestinal portions have been identified compared to feces. Repeated measurements over days may provide more information regarding the dynamic response of the hindgut following grain challenge (Willing et al., 2009).

In this preliminary study the starch levels investigated ranged from levels lower to equal those previously reported safe starch intake levels (Potter et al., 1992; Kienzle et al., 1992; Verveuert et al., 2009). Cracked corn was selected due to the reported low prececal starch availability, which would result in starch overflow from the small intestine into the hindgut. Increasing levels of starch were used to determine the intake level at which changes in fermentation due to the presence of starch could be detected by differences in

fecal pH. It was hypothesized that the increase in starch intake would cause a decrease in fecal pH once the starch intake exceeded 1.33 g starch/kg BW/meal offered as cracked corn in three meals per day.

Materials and Methods

Four mature thoroughbred geldings (aged 6 to 10 years; initial BW 526 ± 6 kg, mean \pm SE) were used in a longitudinal intake study. Prior to the start of the study all horses were maintained on pasture and fed grass hay and a grain mix (45 % cracked corn, 45 % oats and 10 % molasses) to maintain body weight. The non exercised horses were individually housed in 3x3m stalls bedded on shavings, all horses were given 4 to 6 hours of free exercise per day. Free exercise was provided in a dry lot with access to water. During the experimental periods all horses were offered 1 % BW of alfalfa, timothy hay cubes and cracked corn. Dietary treatments were based on cracked corn offered at levels to provide 0.67, 1.0, 1.33, 1.67, 2.0 g starch/kg BW/meal (as fed basis). Horses were fed every 8 h with cracked corn and hay cubes provided in the same meal (0600, 1400 and 2200 h). The study was extended to measure 3 g starch/kg BW/meal fed twice a day at 0600 and 1400 h (hay cube intakes were fed every 8 h). Each treatment period was 6 days in length with 2 days adaptation to the starch level and fecal samples were collected prior to each 0600 h feeding for 4 consecutive days.

Fecal pH was measured immediately post sampling using the following procedures; a fecal sample of 50 g (wet weight) was diluted with 150 ml of distilled water (3:1 ratio) and homogenized for 1 minute. The pH was measured using the probe of the pH 6 Acorn

series meter (OAKTON instruments, IL USA 60061) inserted into the homogenized solution and reading allowed to stabilize for 1 minute.

Statistical analysis used to compare mean fecal pH of each starch intake level sampled at 0600 h for four days was a one-way analysis of variance. A two-way analysis of variance was conducted to identify differences in sampling day and starch intake level. Statistical analysis was performed using Graphpad prism version 4.00 for windows, Graph Pad software, San Diego California USA, www.graphpad.com.

Results

As cracked corn intake increased mean fecal pH decreased (Table 3.1). There was no statistical difference in mean fecal pH with low starch intakes (2-5 g starch/kg BW/day). The cracked corn intake of 5.7 ± 1.0 kg provided in two meals caused a significant decrease in fecal pH (n=16). In examining the raw data there were clear differences between the four horses in their response to the increased starch intake levels. Two horses maintained pH near or above their initial pH compared to the other 2 horses that had systematic decreases in pH throughout the study. There was no statistical difference for starch intake level ($p > 0.05$) and sampling day ($p = 0.07$) when using all four horses fecal pH data. However, when comparing the data of the two horses that responded negatively to the increased starch intake level, there was a significant effect on pH due to starch level ($p < 0.05$), sampling day ($p = 0.09$) and interaction ($p < 0.05$). Tukey's test of 2-way ANOVA (n=2) showed that fecal pH on sampling day 4 was lower for cracked corn intake of 5.7 ± 1.0 kg provided in three meals than the other intake levels. Fecal pH on

sampling day 4 was significantly lower for 3, 4, 5 and 6 g starch/kg BW/day than 2 g starch/kg BW/day.

There was no significant difference in mean fecal pH across all 6 starch intake levels when using data from sampling day 4 ($p>0.05$), however the mean pH was numerically lower than using all 4 sampling days. The lowest pH values (single lowest pH value out of 16) recorded on starch intake 6 g/kg BW/day (5.8 and 5.86) fell below the level considered to cause subclinical acidosis in horses. However, mean fecal pH (4 horses for 4 days) remained close to normal values (6.4-6.8) reported in the literature (Al Jassim., 2006; Müller et al., 2008).

Discussion and conclusions

The longitudinal design of the study resulted in the slow adaptation of the horses from low to high starch intakes over 5 weeks, similar to stepwise adaptation practices used in livestock systems (Mackie and Gilchrist., 1979). Minimal decreases in mean fecal pH values indicate that as a group the four horses were not stressed by the steady increase in corn and starch intakes. There was no evidence of a significant reduction in fecal pH, until meal frequency was reduced to 2 meals per day 8 h apart. The decrease in mean pH by 0.3 units when meal frequency was reduced to 2 meals per day suggests that a greater proportion of starch entered the hindgut, resulting in exacerbated and prolonged effects on the microbial community compared to the same intake level provided in three meals. This decrease in pH was not seen when comparing only sampling day 4 data, the largest decrease in pH was seen between the first 3 intake levels and then remained constant. The

large variation in pH values may be due animal variation, one of the four horses did not respond to the treatment.

During weeks 1 to 5 the increase in cracked corn per meal (as fed) was approximately 400 g compared to week 6 when meal size increased by approximately 1 kg cracked corn, these numbers represent increases of 240 g and 600 g starch per meal, respectively.

From this study, based on the increased variability in fecal pH and pH values ≤ 6.0 a starch intake of 6g starch/kg BW/day offered in three meals was selected for the digestibility study.

Table 3.1. Dietary intake levels of cracked corn and fecal pH values (mean \pm SE)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Starch (g/kg BW/day)	2	3	4	5	6	6
Starch (g/kg BW/meal)	0.67	1.0	1.33	1.67	2	3
Mean cracked corn intake (kg/day)	1.9 \pm 0.04	2.9 \pm 0.05	3.8 \pm 0.07	4.8 \pm 0.09	5.7 \pm 1.0	5.7 \pm 1.0
Mean cracked corn intake (kg/meal)	0.63 \pm 0.01	0.95 \pm 0.02	1.3 \pm 0.02	1.6 \pm 0.03	1.9 \pm 0.03	2.9 \pm 0.05
Mean fecal pH ^{1,3}	6.89 \pm 0.05 ^a	6.89 \pm 0.08 ^a	7.0 \pm 0.12 ^a	6.73 \pm 0.08 ^{ab}	6.83 \pm 0.14 ^{ab}	6.51 \pm 0.11 ^{bc}
34 Mean fecal pH (day 4) ^{2,3}	7.06 \pm 0.09 ^a	6.75 \pm 0.15 ^a	6.58 \pm 0.08 ^a	6.65 \pm 0.15 ^a	6.57 \pm 0.37 ^a	6.54 \pm 0.18 ^a
Lowest pH ⁴	6.49	6.41	6.42	6.18	5.8	5.86

¹Mean fecal pH is for 4 horses over 4 consecutive days, number of observations = 16

²Mean fecal pH for 4 horses from sampling day 4, number of observations = 4

³Values in same row with different superscripts are different at p<0.05

⁴Lowest fecal pH = single lowest pH value out of 16 observations

Chapter 4: Materials and Methods

Four mature geldings (mean age 7 years; initial BW 552 ± 10 kg) were used in a 4x4 Latin-square design total tract digestibility experiment. Horses were maintained on a diet of cracked corn fed to provide 6 g starch/kg BW/day and 1 % BW hay cubes prior to the start of the digestibility trial. The non exercised horses were individually housed in 3x3 m stalls bedded with shavings, all horses were given 4-6 hours of free exercise per day. During the free exercise all horses wore muzzles to prevent grazing and wood chewing but allowed access to water.

Target bodyweights were chosen for each horse based on the horse's previous bodyweight and body condition score data. Average bodyweight for each horse at a BCS 5 to 5.5 was used to calculate treatment diet intakes. This target weight was required due to energy intakes that significantly exceeded maintenance requirements in the corn based treatments. The bodyweight of each horse was recorded daily before the morning feed throughout the experiment.

Dietary Treatments

Dietary treatments included a control diet and three treatment diets based on three different processed corn ingredients. The three processed treatments used were cracked corn, steam flaked corn and corn germ dehydrated (AAFCO 48.32). The corn germ dehydrated ingredient was pelleted with 15 % alfalfa meal to ensure complete consumption at the required intake levels. This treatment diet is referred to herein as corn

byproduct pellet when discussing total diet digestibility, fecal and glycemic responses; however, when discussing by difference digestibility of the grain it is referred to as corn germ dehydrated (without alfalfa meal). All four diets were formulated to provide equal intakes of neutral detergent fiber (NDF), acid detergent fiber (ADF). Starch intakes were formulated to provide 6 g starch/kg BW/day in the three corn treatment diets. The addition of 15 % alfalfa meal to corn germ dehydrated required 15 % alfalfa pellets to be included in the control, cracked and steam flaked corn diets. In order to balance starch across the three corn treatments and NDF across all four treatments, titrated amounts of cracked and steam flaked corn were used and corn bran was added to the control, cracked and steam flaked diets. Corn oil was added to the control and steam flaked diets to provide an equivalent level of fat in the cracked corn diet. The corn germ dehydrated ingredient has higher fat content than cracked and steam flaked corn. This diet was allowed to have a higher fat content than the other three diets (Table 4.1 and 4.2) as fat was not a nutrient of interest in the research question.

Treatment diet nutrient composition

Horse 1 had hay cube refusals on all three corn treatment diets and horse's 3 and 4 had a reduction of total diet intake on the corn byproduct treatment prior to the collection trial, caused dry matter intake for hay cubes to differ among treatment diets (Table 4.1) however these differences were not significant ($p=0.12$). Dry matter intake was significantly lower in the control diet ($7670 \text{ g} \pm 143$) than the three corn treatments (cracked $11221 \text{ g} \pm 334$, steam flaked $10905 \text{ g} \pm 402$ and corn byproduct $11794 \text{ g} \pm 439$)

($p < 0.05$). The control diet had the lowest crude protein level ($937 \text{ g} \pm 17$) compared to all three corn treatment diets. Within the corn treatments crude protein was not significantly different between cracked and steam flaked corn ($1231 \text{ g} \pm 39$ and $1170 \text{ g} \pm 48$, respectively), but crude protein was significantly greater in the corn byproduct diet ($1477 \text{ g} \pm 55$) ($p < 0.05$). Starch content was not significantly different between the three corn treatment diets (cracked $3195 \text{ g} \pm 61$, steam flaked $3199 \text{ g} \pm 61$ and corn byproduct $3115 \text{ g} \pm 110$) but was significantly lower in the control diet ($220 \text{ g} \pm 4$) ($p < 0.05$). The fat content of the corn byproduct diet ($663 \text{ g} \pm 24$) was double the fat content in the control ($321 \text{ g} \pm 6$), cracked ($331 \text{ g} \pm 8$) and steam flaked corn diets ($323 \text{ g} \pm 9$) ($p < 0.05$).

Experimental Design

Horses were adapted to the treatment diets over a 5 day period, with 20 % of the total treatment ration per day being removed and substituted for the new treatment diet. Each treatment diet was offered at 0700, 1430 and 2200 h with equal amounts of corn, corn bran, alfalfa pellets and corn oil per meal, horses on the corn based treatments received 2 g starch/kg BW/meal. The 1 % BW intake of hay cubes was divided into 1 kg offered at 0700 and the remaining daily hay cube intake divided equally for the 1430 and 2200 h meals.

Each period consisted of 5 days adaptation to the treatment diet, 11 days at fixed level of intake and 5 day total fecal collection. Daily intake was adjusted as necessary to be 92 % of pre collection intake approximately 7 days before collection periods to limit feed

refusals. Two days before and during the 5 day collection period, horses were fitted with total collection harnesses (Stablemaid horse hygiene and waste management, Melbourne, Australia) that allow for the complete and separate collection of urine and feces. During the collection period the horses were housed in stalls with rubber matting and walked twice daily on the mechanical walker, with no turn out. During the collection period daily feed intake was recorded, any feed refusals were measured at 7am and retained for chemical analysis. Fecal collections representing each 24 h period were weighed, thoroughly mixed and 2.5 % subsample by wet weight collected and frozen for subsequent analysis. Feed and fecal samples were analyzed at Dairy One Forage Lab (Ithaca, NY). The five daily manure samples were analyzed for individual dry matter (DM) then composited, re-dried and analyzed. Feed and composited manure samples were analyzed for DM, crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), starch, water soluble carbohydrates (WSC), crude fat (CF), and gross energy (GE). The urine was discarded daily.

Fecal pH and volatile fatty acids

At the end of the digestion collection (day 21) and prior to feeding, rectal fecal samples were taken. Fecal pH was measured immediately using the following procedures; a fecal sample of 50 g (wet weight) was diluted with 150 ml of distilled water (3:1 ratio) and homogenized for 1 minute. The pH was measured using the probe of the pH 6 Acorn series meter (OAKTON instruments, IL USA 60061) inserted into the homogenized solution and reading allowed to stabilize for 1 minute. The remainder of the wet fecal

sample was frozen until after the trial for analysis of volatile fatty acids (VFA), total lactate and L-Lactate.

Fecal samples collected on day 21 (0700, 1430 and 2200 h) of each period for each horse were thawed and composited. A 1.5 g wet sample was diluted with 5mM sulfuric acid buffer and centrifuged to remove particulates in the supernatant. Volatile fatty acid and total lactate concentration was quantified by HPLC (Dionex, Sunnyvale, Ca, USA). The column (Aminex, HP-87H, Bio-rad Hercules, CA) was operated at 50C, with a 0.4 ml/min flow rate. The VFA and total lactate in the effluent was detected using Shodex/Showa Denko refractive index (Kangagawa, Japan) and UV detector (Dionex, Sunnyvale, Ca, USA).

Fecal L-lactate was analyzed using the YSI 2700 SELECT™ Biochemistry Analyzer. The procedure used a 10 g fecal sample (wet weight) diluted with 50 ml distilled water and homogenized for 1 minute. The sample was then filtered through cheese cloth to remove particulates. The resulting solution was analyzed in duplicate for L-lactate using the L-lactate oxidase membrane and calibrated with 0.50 g/L L-lactate standard solution. YSI 2700 SELECT™ Biochemistry Analyzer oxidizes the L-lactate in the sample to hydrogen peroxide and pyruvate, with the hydrogen peroxide being detected amperometrically. The electron flow recorded at the electrode is directly proportional to the concentration of hydrogen peroxide and hence L-lactate concentration in the fecal sample.

Glycemic Response

On day 22 of the 4x4 Latin square digestibility experiment, after the completion of the total fecal collection, a 4 h glycemic response test was conducted after the horses consumed 1kg of feed as either hay cubes, cracked corn, steam flaked corn and corn byproduct pellet. Horses were fasted overnight for at least 8 h with access to water. At 0600 h indwelling jugular catheters were inserted into the right or left jugular vein. A baseline blood sample was taken 30 minutes post catheterization (pre feeding), then at feeding of 1kg of appropriate treatment meal and at 30 minute intervals post feeding until 240 minutes. Blood samples were collected into plasma tubes containing sodium heparin and centrifuged for 10 minutes within 30 minutes of sampling. Plasma glucose concentrations were analysed on the YSI 2300 STAT PLUS™ glucose and lactate analyzer using the glucose oxidase membrane and 180 mg/dL glucose calibration standard. D-glucose in the plasma sample is oxidized to hydrogen peroxide and gluconolactone. The concentration of hydrogen peroxide, hence glucose is detected amperometrically. Plasma glucose concentrations were measured the same day as the glycemic response test was conducted.

Following the completion of the digestibility experiment, all horses were returned to the pre digestibility trial diet of cracked corn and hay cubes. After three weeks on this diet due to procedures of another experiment using the same horses, a further 3x4 randomised design glycemic response tests were conducted on the same four geldings providing equal starch intakes from the three corn treatments only. Intake level of cracked, steam flaked

and corn by product pellet was calculated to provide 1 g starch/kg BW. Before the first glycemic response test horses were weighed and the actual BW was used, with the average starch value (from four chemical analyses) for each corn ingredient to calculate the quantities of corn grain to feed each horse. The corn intakes for the glycemic response tests were 975 ± 26.1 g cracked corn, 914 ± 24.5 g steam flaked corn and 1581 ± 42.1 g corn byproduct pellet (mean \pm SE). The same sampling and plasma glucose analysis protocol was followed as noted with the previous glycemic response procedure.

Digestibility calculations and statistical analysis

Apparent total tract digestibility coefficients for the four treatments were calculated for DM, CP, ADF, NDF, starch, WSC, CF and GE.

By difference digestibility coefficients for the cracked, steam flaked and corn germ dehydrated fractions (15% alfalfa meal in the byproduct pellet was calculated as a percentage of the control diet intake thus 85% of the pellet intake was calculated as the grain intake for that treatment).

The following equations were used:

$$1. \quad dG = [dT - (c \times dC)]/g$$

where dG = digestibility of concentrate, dT = digestibility of the total ration, c = percentage of control diet in total ration, dC = digestibility of control ration and g = percentage of corn grain in ration (Crampton and Harris, 1969 and De Marco et al., 2012).

$$2. \quad d \text{ nutrient corn product} = (d \text{ nutrient total} - (d \text{ nutrient control} \times a))/b$$

where d is digestibility, a is content (%) in the diet of the nutrient from the control and b is content (%) in the diet of the nutrient from the corn product (Karlsson et al., 2000).

In addition digestible energy for both the total diet and the corn fraction was estimated using the following equations:

$$(A) \text{ DE in total ration (Mcal/kg)} = [(\text{Gross energy in total ration} - \text{Fecal energy}) / \text{Total intake}]$$

$$(B) \text{ Control diet DE} \times \text{intake from control in ration} = \text{Amount of DE supplied from control}$$

$$(C) \text{ DE from corn only (Mcal/kg)} = (A - B) / \text{grain intake}$$

Data are presented as mean \pm standard error. Digestibility coefficients, fecal and glycemic parameters were compared by one-way and two-way analysis of variance with Tukey and Dunnett's posttests. All statistical analysis was performed using GraphPad Prism version 4.00 for windows, Graph Pad software, San Diego California USA, www.graphpad.com. Differences were considered statistically significant at a p-value of 0.05.

Table 4.1 Daily dry matter daily intakes of dietary ingredients and selected components of the diets

	Treatment diets ^{1,2}			
	CONTROL	CRACKED	STEAM FLAKED	CORN BY PRODUCT
DMI, kg/d				
Haycubes	4.81 ± 0.09	4.67 ± 0.22	4.60 ± 0.29	4.39 ± 0.22
Alfalfa pellets	1.18 ± 0.02	1.18 ± 0.02	1.18 ± 0.02	-
Corn bran	1.53 ± 0.03	0.94 ± 0.02	0.94 ± 0.02	-
Corn oil	0.16 ± 0.003	-	0.06 ± 0.00	-
Corn	-	4.43 ± 0.08	4.13 ± 0.08	7.41 ± 0.26
Total	7.7 ± 0.3	11.2 ± 0.7	10.9 ± 0.8	11.8 ± 1.0
Intake, g/d³				
Crude protein	937 ± 17 ^a	1231 ± 39 ^b	1170 ± 48 ^b	1477 ± 55 ^c
Acid detergent fiber	3046 ± 57 ^a	3037 ± 116 ^a	2977 ± 152 ^a	3060 ± 129 ^a
Neutral detergent fiber	4751 ± 88 ^a	4678 ± 166 ^a	4552 ± 212 ^a	4493 ± 198 ^a
Starch	220 ± 4 ^a	3195 ± 61 ^b	3199 ± 61 ^b	3115 ± 110 ^b
Crude fat	321 ± 6 ^a	331 ± 8 ^a	323 ± 9 ^a	663 ± 24 ^b

¹ CRACKED, STEAM FLAKED = cracked corn and steam flaked corn

² All four treatment diets were fortified with vitamin and mineral supplement (Micromax, 120g/day), Kentucky Equine Research, Inc. Versailles, Kentucky

³ Values in same row with different superscripts are different at p<0.05

Table 4.2 Chemical composition of feed ingredients, averaged across periods (mean % ± SE; DM basis)¹

	Alfalfa/Timothy Haycube	Alfalfa pellets	Corn bran	Corn oil²	Cracked corn	Steam flaked corn	Corn By product pellet³	Corn germ dehydrated⁴
DM	91.6 ± 0.4	89.9 ± 0.3	89.8 ± 0.5	99	87.9 ± 0.5	87.4 ± 0.4	89.4 ± 0.3	87.2
Crude protein	13.0 ± 0.2	19.7 ± 0.4	5.3 ± 0.2		7.7 ± 0.2	7.1 ± 0.3	12.3 ± 0.3	10.1
Crude fat	1.6 ± 0.05	2.4 ± 0.1	4.0 ± 0.3	99	4.3 ± 0.2	3.1 ± 0.2	8.0 ± 0.1	9.8
Starch	0.8 ± 0.1	1.0 ± 0.4	11.1 ± 0.3		68.7 ± 1.2	73.8 ± 2.2	41.6 ± 0.9	54.9
WSC	5.7 ± 0.5	6.7 ± 0.1	2.9 ± 0.1		3.9 ± 0.2	2.7 ± 0.2	7.3 ± 0.2	5.9
NSC	6.5 ± 0.5	7.7 ± 0.4	14.0 ± 0.3		72.6 ± 1.0	76.4 ± 2.4	48.9 ± 1.0	60.8
ADF	47.4 ± 1.6	38.6 ± 1.1	20.4 ± 1.2		3.9 ± 0.7	3.6 ± 0.5	13.2 ± 1.8	2.1
NDF	62.7 ± 0.5	49.2 ± 0.5	75.8 ± 1.7		10.3 ± 1.3	9.2 ± 1.2	23.5 ± 1.7	10.6
Hemicellulose	15.3 ± 1.1	10.6 ± 0.6	55.4 ± 1.0		6.4 ± 0.8	5.5 ± 0.8	10.3 ± 0.9	8.5
GE (cal/g)	4324.3 ± 47.8	4551.0 ± 15.8	4711.3 ± 11.2	9190	4526.5 ± 7.4	4486.0 ± 23.6	4715.8 ± 23.1	-

¹ Analyses conducted by Dairy One Forage Lab, Ithaca, NY (NSC = nonstructural carbohydrate, WSC = water soluble carbohydrate, ADF = acid detergent fiber, NDF = neutral detergent fiber, GE = Gross energy)(NSC = starch + WSC; Hemicellulose = NDF – ADF)

² Reference values (NRC, 2007)

³ Corn byproduct pellet (85% corn germ dehydrated and 15% alfalfa meal)

⁴ Corn germ dehydrated (No alfalfa meal)

Chapter 5: Results

Total ration digestibility

Table 5.1 and 5.2 show apparent total tract digestibility of the total ration and grain treatment respectively (mean % \pm SE). Dry matter digestibility of the diets was 48.1 \pm 1.2, 58.3 \pm 0.6, 62.8 \pm 1.1 and 65.4 \pm 0.8 for control, cracked corn, steam flaked corn and corn by product, respectively. The addition of corn grain to the diets significantly increased DM digestibility ($p < 0.05$). There was no statistical difference between the steam flaked corn diet and the corn by-product diet DM digestibility.

Acid and neutral detergent fiber digestibility coefficients were not significantly different among the four treatments (ADF $p > 0.05$ and NDF $p > 0.05$). There was a numerical increase in the mean digestibility of ADF and NDF by 5.3 and 3 %, respectively for the corn by-product diet compared to the control diet (Table 5.1). The steam flaked corn diet had a numerical decrease in mean ADF and NDF digestibility compared to the control diet (Table 5.1). Individual horse variation was observed based on numerical differences, horse 1 had numerically lower cracked corn ADF and NDF coefficients (29.6 and 42.3 %, respectively) than control diet (35.2 and 43.7 %, respectively). Horse 2 had lower ADF coefficients for all three corn treatments (cracked 38.3, steam flaked 39.1 and corn by-product pellet 34.9 %) than control (47.6 %) and lower NDF coefficients for cracked and steam flaked corn (39.1 and 45.0 % respectively) than control (46.5 %). Horse 3 had lower ADF coefficient for all three corn treatments (cracked 41.27, steam flaked 20.74 and corn by-product pellet 44.28 vs control 45.6 %) and lower NDF coefficients for

steam flaked and corn by-product pellet diets (35.4 and 42.99, respectively vs 46.69 %). Horse 4 had reduced control ADF and NDF coefficients (21.4 and 36.4 %, respectively) which caused all three corn treatment diets to be higher in ADF and NDF digestibility (cracked 41.88 and 40.18, steam flaked 38.1 and 38.98 and corn by product pellet 46.72 and 49.88 %, ADF and NDF respectively). In general the biggest differences in ADF and NDF digestibility compared to the control diet were seen in the cracked and steam flaked diets.

Starch digestibility was high in all four treatment diets as expected for the total tract measurement with no significant differences between the control, cracked, steam flaked and corn by product diets (92.2 ± 4.9 , 96.6 ± 1.0 , 99.2 ± 0.4 and 98.8 ± 0.4 %; $p > 0.05$).

The fat digestibility was greatest for the corn by-product diet (77.4 ± 1.6), this diet and the control (70.0 ± 1.6) and steam flaked corn (66.6 ± 3.5) were significantly higher than the fat digestibility of the cracked corn diet. The higher fat digestibility in the control and steam flaked diets may be attributed to the addition of corn oil in these treatments, with the highest corn oil intake in the control diet. The endogenous fat in the cracked corn diet had significantly lower digestibility (45.7 ± 5.8) than the endogenous fat in the corn by-product (77.4 ± 1.6).

The digestible energy (Mcal/kg) of the total treatment diets (calculated using gross energy intake and fecal gross energy) were significantly different between the four

treatments ($p < 0.05$). The control diet had the lowest DE 2.25 ± 0.06 , cracked corn 2.56 ± 0.04 , steam flaked corn 2.82 ± 0.06 and corn by-product diet had the highest DE value 3.06 ± 0.03 .

By difference digestibility of the corn products

Equation 1:

By difference was used to determine digestibility of the cracked, steam flaked and corn germ dehydrated (without alfalfa meal) ingredients of the total ration (Table 5.2). Dry matter digestibility of the cracked and steam flaked corn were significantly different ($p < 0.05$). The digestibility coefficients ranged from 74.0 ± 2.4 to 87.0 ± 2.2 with cracked corn having the lowest digestibility and steam flaked corn having the highest. The DM digestibility of corn germ dehydrated was in the middle of this range (80.3 ± 1.9).

Apparent crude protein digestibility of cracked corn (42.3 ± 7.9) was significantly lower compared to steam flaked corn (64.4 ± 7.6) and the corn germ dehydrated product (67.8 ± 3.7) ($p < 0.05$). There was no significant difference in acid and neutral detergent fiber (ADF $p > 0.05$ and NDF $p > 0.05$), the numerical differences between the control and the corn treatment diets observed in the total diets was repeated in the by difference digestibility coefficients. The outliers of interest on a numerical basis were horse 3 ADF (-21.6 %) and NDF (16.2 %) for the steam flaked diet.

Starch digestibility for all three corn treatments were greater than 100 % (102.2 ± 9.5 , 109.9 ± 7.5 and 104.1 ± 3.8 for cracked, steam flaked and ground corn) but not statistically different ($p > 0.05$). Crude fat digestibility was significantly lower in the cracked corn (8.03 ± 12.9) than steam flaked corn and corn germ dehydrated (61.3 ± 9.4 and 83.8 ± 3.1 , respectively). The DE value of the cracked corn (3.02 ± 0.16) was significantly lower than the DE of steam flaked corn (3.75 ± 0.15) and corn germ dehydrated (3.76 ± 0.07) ($p < 0.05$).

Equation 2: Nutrient contributions

Nutrient digestibility for the three corn products was calculated using the difference equation weighted by nutrient contribution of the control diet and corn product to the combined diet (Table 5.2B). Contribution percentages for DM and DM digestibility coefficients were the same for equation 1 and 2. Starch digestibility for all three corn treatments were lower than 100 %, when calculated by nutrient percentage (Table 5.2B), the corn products provided 96.3 % of the total starch in the diet compared to equation 1 which assumed corn provided 43.6 % of the total starch in the diet.

Fiber digestibility using equation 2 was based on the corn products providing 5.0 % ADF, this resulted in unrealistic digestion coefficients, especially for steam flaked corn (-1.5 ± 185.5 %) and corn germ dehydrated (162 ± 186.1 %). These values are due to differences in fiber digestibility of the control diet and the combined diet and the fact that any change in digestibility is credited to the test ingredient. This appears to be amplified when the nutrient contribution of the test ingredient is small. Digestibility coefficients for NDF

using all four horses data resulted in large variation and a low mean NDF value for steam flaked corn ($8.5 \pm 35.0 \%$, $n = 4$). However, if the negative outlier for cracked (-30.7%) and steam flaked (-92.8%) corn and the positive outlier for corn germ dehydrated (129.2%) is removed from the data set then the mean digestibility are reasonable (Table 5.2B). There was no statistical difference between the three corn products in ADF and NDF digestibility, $p = 0.78$ and $p = 0.63$, respectively.

Glycemic response

Plasma glucose concentrations peaked for all three corn treatments at sampling times 90 to 120 minutes, after offering 1kg treatment meal. When fed on an equal starch basis (1g starch/kg BW) horses fed cracked corn reached peak glucose values at 60 minutes and compared to horses fed steam flaked corn and corn byproduct which peaked at 120 minutes. Horses fed 1g starch/kg BW from corn byproduct pellet had elevated plasma glucose levels four hours post feeding.

When comparing the glycemic response to the three corn treatments at a fixed quantity (1kg) without accounting for the different starch compositions there was significant difference between area under the glucose curve for the corn treatments ($p < 0.05$) (Table 5.3). Steam flaked corn produced a larger glycemic response than cracked corn ($p < 0.001$) and corn byproduct pellet ($p < 0.01$). Area under the glucose concentration-time curve for 1 kg cracked, steam flaked and corn byproduct was 2867 ± 416.5 , 6198 ± 1134 and 3748 ± 1542 mg/dL·min, respectively. During the equal starch glycemic response tests horse 1

had feed refusals for the steam flaked treatment, his data for the other two treatments was removed from the data set to conduct one way ANOVA. On an equal starch basis (1 g starch/kg BW) the glucose AUC for cracked, steam flaked and corn byproduct was 2507 ± 217.1 , 5629 ± 1776 , 4104 ± 681.1 mg/dL·min, respectively, and were not statistically different ($p=0.12$) (Table 5.3).

Fecal pH, lactic acid and volatile fatty acid data

There was no effect of sampling time ($p = 0.35$), diet ($p = 0.08$) and interaction ($p>0.05$) on fecal pH. Mean fecal pH ($n=12$; Table 5.4) was significantly reduced on the cracked corn diet ($\text{pH } 6.15 \pm 0.06$) compared to the control ($\text{pH } 6.54 \pm 0.04$) and corn by-product ($\text{pH } 6.40 \pm 0.01$) diets ($p < 0.05$). Steam flaked corn ($\text{pH } 6.30 \pm 0.03$) was significantly lower than the control diet ($p < 0.05$).

Fecal L-lactate was different between treatment diets ($p = 0.056$) when comparing the corn product diets to the control diet using Dunnett's test, cracked corn produced significantly larger L-lactate concentrations than the control diet (Table 5.4). There was no significant difference in total VFA concentration on a DM basis ($p > 0.05$) between the four diets. Fecal samples with the highest VFA and L-lactate concentrations and the lowest pH were from horses fed the cracked corn diet.

Bodyweight data

The change in bodyweight over the 21 day treatment period was calculated for each treatment diet. On average horses lost 9.77 ± 8 kg on the control diet and gained 3.98 ± 6 kg on the cracked corn, 19.33 ± 4 kg on steam flaked corn and 31.83 ± 8 kg on the corn byproduct pellet diet. The difference in bodyweight balance between all four treatments was significant ($p < 0.05$) due to significant differences between control and the two corn diets, steam flaked corn and corn byproduct.

Table 5.1: Apparent total tract digestibility of total treatment diets (%; mean \pm SE, n = 4)

Nutrient	Total diet				P value ¹	Numerical difference in nutrient digestibility from control diet ²		
	CONTROL	CRACKED	STEAM FLAKED	BY PRODUCT		CRACKED	STEAM FLAKED	BY PRODUCT
DM	48.1 \pm 1.2 ^a	58.3 \pm 0.6 ^b	62.8 \pm 1.1 ^c	65.4 \pm 0.8 ^c	0.0001	10.2	14.7	17.3
CP	58.0 \pm 2.0 ^{ab}	51.9 \pm 1.9 ^a	60.6 \pm 2.2 ^{ab}	63.3 \pm 1.9 ^b	0.01	- 6.1	2.6	5.3
ADF	37.5 \pm 6.0 ^a	37.8 \pm 2.8 ^a	35.9 \pm 5.3 ^a	42.8 \pm 2.7 ^a	0.72	0.3	- 1.6	5.3
NDF	43.3 \pm 2.4 ^a	42.2 \pm 1.8 ^a	40.8 \pm 2.2 ^a	46.3 \pm 1.4 ^a	0.29	- 1.1	- 2.5	3
Starch	92.2 \pm 4.9 ^a	96.6 \pm 1.0 ^a	99.2 \pm 0.4 ^a	98.8 \pm 0.4 ^a	0.25	4.4	7	6.6
Fat	70.0 \pm 1.6 ^a	45.7 \pm 5.8 ^b	66.6 \pm 3.5 ^a	77.4 \pm 1.6 ^a	0.0003	- 24.3	- 3.4	7.4
GE	49.6 \pm 1.2 ^a	57.3 \pm 0.9 ^b	63.2 \pm 1.2 ^c	67.0 \pm 0.6 ^c	0.0001	7.7	13.6	17.4
DE	2.25 \pm 0.06	2.56 \pm 0.04	2.82 \pm 0.06	3.06 \pm 0.03	0.0043	0.31	0.57	0.81

(Mcal/kg, DM basis)

¹P value is for the whole model, values in the same row with different superscripts are different at p < 0.05

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² Difference in mean nutrient digestibility of the corn diets compared to the control diet

Table 5.2. Apparent total tract digestibility of corn products calculated by the difference method Equation 1 (%; mean \pm SE, n = 4)

Nutrient	Corn only			P value ¹
	CRACKED CORN	STEAM FLAKED CORN	CORN GERM DEHYDRATED	
DM	74.0 \pm 2.4 ^a	87.0 \pm 2.2 ^b	80.3 \pm 1. ^{9ab}	0.007
CP	42.3 \pm 7.9 ^a	64.4 \pm 7.6 ^b	67.8 \pm 3.7 ^b	0.05
ADF	38.5 \pm 12.2 ^a	32.7 \pm 20.4 ^a	47.3 \pm 9.5 ^a	0.79
NDF	40.5 \pm 4.6 ^a	36.2 \pm 6.7 ^a	48.9 \pm 4.7 ^a	0.30
Starch	102.2 \pm 9.5 ^a	109.9 \pm 7.5 ^a	104.1 \pm 3.8 ^a	0.71
CF	8.03 \pm 12.9 ^a	61.3 \pm 9.4 ^b	83.8 \pm 3.1 ^b	0.0009
GE	69.0 \pm 3.5 ^a	86.5 \pm 2.9 ^b	82.4 \pm 1.4 ^b	0.0001
DE (Mcal/kg, DM basis)	3.02 \pm 0.16 ^a	3.75 \pm 0.15 ^b	3.76 \pm 0.07 ^b	0.0043

¹P value is for the whole model, values in the same row with different superscripts are different at p < 0.0

Table 5.2 B. Apparent total tract digestibility of corn products calculated by the difference method Equation 2 (%; mean \pm SE, n = 4 except NDF)

Nutrient	Corn only			P value ¹
	CRACKED CORN	STEAM FLAKED CORN	CORN GERM DEHYDRATED	
DM	74.0 \pm 2.4 ^a	87.0 \pm 2.1 ^b	80.5 \pm 1. ^{9ab}	0.007
ADF	45.2 \pm 115.1 ^a	-1.5 \pm 185.5 ^a	162 \pm 186.1 ^a	0.78
NDF ²	56.13 \pm 10.5 ^a	42.22 \pm 13.2 ^a	41.82 \pm 10.46 ^a	0.63
Starch	96.8 \pm 1.3 ^a	99.48 \pm 0.5 ^a	98.88 \pm 0.4 ^a	0.10

¹P value is for the whole model, values in the same row with different superscripts are different at p < 0.05

²Number of observations for NDF digestibility = 3

Table 5.3 Effect of corn treatment diet on glycemic parameters (mean \pm SE)

	CRACKED	STEAM FLAKED	CORN BY PRODUCT
1kg treatment AUC ^{1,3}	2867 \pm 416.5 ^a	6198 \pm 1134 ^b	3748 \pm 1542 ^a
1kg treatment peak plasma glucose, mg/dL ¹	127.0 \pm 8.0	140.3 \pm 8.6	130.3 \pm 5.8
1g starch/kg BW AUC ^{2,3}	2507 \pm 217.1 ^a	5629 \pm 1776 ^a	4104 \pm 681.1 ^a
1g starch/kg BW peak plasma glucose, mg/dL ²	123 \pm 3.6	142.3 \pm 14.7	123.0 \pm 5.5

¹Mean \pm SE, n=4 for Glycemic response to 1 kg treatment

²Mean \pm SE, n=3 for Glycemic response to 1 g starch/kg BW

³Values in same row with different superscripts are different at p<0.05

Table 5.4 Effect of treatment diet on fecal variables (mean \pm SE, n=4)

	CONTROL	CRACKED	STEAM FLAKED	CORN BY PRODUCT
Fecal pH	6.54 \pm 0.04 ^a	6.15 \pm 0.06 ^b	6.30 \pm 0.03 ^{bc}	6.40 \pm 0.01 ^{ac}
Fecal L-lactate, mmol/L	0.53 \pm 0.09 ^a	1.20 \pm 0.26 ^b	0.92 \pm 0.16 ^{ab}	1.15 \pm 0.09 ^{ab}
Fecal Acetate, mmol/L	19.0 \pm 1.29 ^a	42.0 \pm 9.35 ^a	31.0 \pm 6.76 ^a	22.5 \pm 4.92 ^a
Fecal Propionate, mmol/L	6.0 \pm 1.41 ^a	10.0 \pm 2.71 ^a	7.0 \pm 1 ^a	6.5 \pm 0.96 ^a
Fecal Butyrate, mmol/L	2.0 \pm 0.0 ^a	5.0 \pm 1.73 ^a	3.5 \pm 0.5 ^a	2.0 \pm 0.0 ^a
Total VFA (DM), mmol/L	98.1 \pm 8.4 ^a	229.3 \pm 52.5 ^a	165.0 \pm 38.9 ^a	130.3 \pm 26.9 ^a

¹ Values in same row with different superscripts are different at p<0.05

Chapter 6: Discussion and conclusions

Total tract apparent digestibility of starch in all four diets was high which was expected based on other research reports (Varloud et al., 2004; Al Jassim., 2006; Julliand et al., 2006; Rosenfeld and Austbø., 2009). The starch digestibility of the corn products by difference were greater than 100 % using equation 1 and less than 100 % using equation 2, which is a more physiologically normal value. The addition of corn to the diet may have increased the starch digestibility of the basal diet, this improvement would be accredited to the corn when using the by difference equation. Rosenfeld and Austbø (2009) reported prececal starch digestibility for processed corn (average data of ground, pelleted, extruded and micronized) to be 66.3 % and total tract 91.0 %.

In assessing the digestibility of the treatment diets, differences in crude protein and fat digestion coefficients were observed. Crude protein digestibility was significantly lower in the cracked corn diet compared to the corn germ dehydrated by 26 % and steam flaked corn 22 % ($p < 0.05$). The corn germ dehydrated product has higher crude protein content than the other two corns and had the highest digestibility coefficient. Total diet and by difference crude protein digestibility of the treatment diets in this study were similar to prececal values (61.5 %) but lower than total tract protein digestibility 84.3 % of pooled data of processed corn (Rosenfeld and Austbø., 2009), and 88.0 % when a mixed diet of Bermuda grass hay and corn was fed (Gibbs et al., 1996). Van Weyenberg et al. (2007) reported a negative correlation between glycemic response and crude protein digestibility, observing the horses with the perceived fastest intake to have the greatest glycemic

response and speculating that this may lead to greater amounts of starch entering the hindgut and being utilized to produce bacterial protein which lowered the apparent digestibility of protein. In the current study the lower crude protein digestibility is associated with cracked corn which produced the lowest glycemic response, suggesting large amounts of starch could reach the hindgut increasing bacterial growth and endogenous protein losses in the feces. The higher crude protein digestibility for steam flaked and corn germ dehydrated may be due to processing technique.

Cracked corn also had significantly lower crude fat digestibility especially when calculated by difference this may partially be due to the artificially high fat digestibility in the control diet because of the inclusion of corn oil. However, this also indicates that the fat in cracked corn is not as available to the horses as corn oil or the fat in the other two corn products. Data from the corn byproduct diet indicates that the corn germ dehydrated product is a good fat source that is highly digestible in horses.

The crude fat digestion coefficients in this study apart from cracked corn are similar to results of Bush et al. (2001) when 5 % supplemental corn oil was fed. The lower crude fat and protein coefficients could be an indication of small intestinal bypass as seen for starch when cracked corn is fed at high intake levels. Thermal-mechanical processing of corn has been reported to improve starch, protein and fat digestibility in the horse, pig and ruminant due to disruption of the protection and structure of the starch granule enhancing enzymatic digestion (Zinn et al., 2002, Rosenfeld and Austbø., 2009; Menoyo et al., 2011).

The glycemic response to the three corn products was different when compared using a 1 kg test meal. Steam flaked corn produced a higher glycemic response, suggesting greater starch susceptibility to enzymatic digestion in the small intestines. This finding is similar to the data of Hoekstra et al. (1999) who reported that steam flaked corn produced both a greater glycemic response than cracked corn and higher peak glucose values.

Comparisons on equal weight basis are useful in implementing feed recommendations for comparing grains. When comparing the three corns on an equal starch basis there was no statistical difference in glycemic response. The magnitude of glycemic response was repeated, steam flaked corn having the highest, corn byproduct and cracked corn having the lowest. The lack of difference between treatments when fed at this level agrees with data suggesting starch intake as a driving factor of glycemic response and a minimum intake of 2 g starch/kg BW is needed to identify differences between processing techniques. The exclusion of one horse's data due to feed refusals of the steam flaked corn treatment, may have also limited the identification of treatment differences due to increased AUC variation.

Peak blood glucose values for horses fed cracked and steam flaked corn in the current study were higher than those reported by Hoekstra et al., 1999 and Pagan et al., 1999. Differences in absolute blood glucose values between studies may be due to different experimental animals (arabians and thoroughbreds compared to all thoroughbreds in the current study), study designs and rate of intake. Alternatively these differences may indicate enhanced glucose uptake and transport in the small intestine of horses

maintained on high grain diets. The responses in the small intestine of horses adapted to high starch diets in a similar way to the current study were to increase glucose transporter mRNA and protein (SGLT1) in the duodenum, jejunum and ileum (Dyer et al., 2009). Responses in the duodenum were slower than those seen in the distal small intestine. There is limited information regarding increased production of alpha-amylase in response to high grain diets which is thought of as the limiting factor in small intestinal starch digestion. However, there is evidence that increased activity of alpha-amylase requires 2-3 weeks to show a response to increased dietary NSC (Daly et al., 2012). Horses in this study had been receiving a grain meal for 12 weeks prior to the initiation of the digestion trial.

The measurement of glycemic response, the rise in blood glucose postprandial can be used as an indirect measurement of starch digestibility in the small intestine as greater increases in blood glucose suggests greater starch digestibility. The current glycemic responses suggest that steam flaked corn has the highest prececal starch digestibility followed by the corn byproduct with cracked corn having the lowest prececal starch digestibility. This indicates that there would be the potential for greater starch delivery to the hindgut when horses were fed the cracked corn diet compared to the other two corn treatments. In accordance, the cracked corn diet significantly lowered fecal pH compared to the control and corn by product. Of the three corn products, fecal pH was the highest when the horses were fed the corn byproduct diet suggesting greater prececal digestion or a moderate effect on the hindgut microflora.

Large amounts of fermentable carbohydrates can cause decreases in pH due to high rates of VFA and lactate production. Microbial changes in response to the lowered pH result in the successive replacement of acid intolerant bacteria with acid tolerant bacteria.

Surprisingly, the steam flaked corn diet had a lower pH than expected based on the higher prececal starch digestibility reported in the literature and indicated by the glyceimic response in this study. This lowered pH coupled with a high glyceimic response could suggest that on the steam flaked diet the starch that reached the hindgut was readily available to the microbes and resulted in a high rate of fermentation. This may be explained by a negative correlation between particle size and intensity of fermentation discussed by Kienzle et al. (1997). The small particle size generated by steam flaking would result in faster fermentation which may overwhelm the buffering capacity of the hindgut causing the drop in fecal pH. In comparison the larger particle size from cracking coupled with low prececal digestibility of cracked corn causes large quantities of starch to reach the hindgut per feeding providing substrate rich digesta for prolonged microbial fermentation.

Fermentation end products are the direct result of the microbial populations found in the hindgut. VFA and lactate concentrations support the fecal pH data; total VFA production was greater when horses were fed cracked corn versus the control diet with the difference being predominately acetate. Fecal VFA profile was most similar to the control diet when the corn byproduct was fed; suggesting the addition of corn germ dehydrated had little

impact on the microbial community. McLean et al. (2000) found that micronized barley produced similar cecal acetate and propionate concentrations to those observed on a hay cube diet. While the rolled and extruded barley had lower acetate and higher propionate concentrations. Butyrate concentrations were numerically higher on the cracked and steam flaked corn diets compared to the control and corn byproduct diets. Fecal L-lactate concentrations increased approximately two fold when corn was added to the diets ($p=0.056$).

The fecal pH, VFA and L-lactate data from this study fall within the ranges reported by Richards et al. (2006) for 46 Thoroughbred racehorses with mean concentrate intakes of 7.3 ± 0.24 kg. The three corn treatments in this study had lower fecal pH values (range 6.15 to 6.4) compared to fecal pH 6.75 when rolled corn was fed at 0.2% BW starch and sugars (Hussein et al., 2004). This is likely due to the higher starch intake used in the current study resulting in greater starch overload into the hindgut. The fecal pH on the cracked corn diet is within the pH range considered to cause subclinical acidosis however; none of the horses showed any clinical signs of hindgut acidosis. The drop in fecal pH is most likely the result of increased L-lactate and total VFA production. The fecal pH data support the total tract digestibility findings for ADF and NDF, the low fecal pH on the cracked and steam flaked diet may have resulted in the negative impact on fiber digestibility among individual horses. However, in ruminants fiber digestibility was not affected when ruminal pH remained higher than 6.0 for the majority of the feeding cycle (Firkins et al., 2001)

Lactate producing bacteria are considered as acid tolerant and their proliferation has been reported in horses consuming grain rich diets (Julliand et al., 2001; Al Jassim et al., 2005). Increased population sizes of Lachnospiraceae, Bacteroidetes and Bacillus-Lactobacillus-Streptococcus were identified from the colon of horses maintained on a concentrate diet (Daly et al., 2012). These microbes are capable of both fibrolytic and saccharolytic digestion that can readily adapt to changes in substrate availability. In the current study no evaluation of the changes in microbial species in response to treatment diets was made. Lactate producing bacteria, Streptococcus bovis/equinus group have been implicated in the development of lactic acidosis; these bacteria are capable of producing the L-lactate isomer only (Milinovich et al., 2010). The increased fecal L-lactate concentrations in this study may be indicative of increased numbers of S.bovis and S.equinus in response to increased dietary starch as a substrate for hindgut fermentation as reported by others.

In vitro, lactate utilization in the presence of polysaccharides resulted in the conversion of lactate to acetate and butyrate when using human inoculum, however in the absence of polysaccharides lactate was converted to propionate (Belenguer et al., 2007). De Fombelle et al. (2003) reported lower lactate concentrations and higher propionate concentrations in the stomach of horses fed a high starch pellet in association with higher numbers of lactate-utilizing bacteria, the lactate-propionate utilization pathway is well documented in production animals (Lean et al., 2013). In the current study there were no significant diet effects on fecal propionate concentrations, although cracked corn had

numerically higher concentrations, it may be speculated that the increased acetate and butyrate concentrations for the cracked and steam flaked diets may be the result of lactate utilization. The dietary response of lactate-utilizing bacteria has received limited attention and inconsistencies are present between published studies. Few studies control for all the factors which can cause changes in acid concentrations and proportions such as shifts in microbial profiles, increased microbial production, decreased absorption and or change in rate of transit time (Turnbaugh et al., 2006).

Cereal grains are principally fed to horses to provide digestible energy. The digestible energy of both cracked and steam flaked corn is reported as 3.88 Mcal/kg in the 2007 NRC. Steam flaking is considered across species to increase energy availability of corn (Zinn et al., 2002). In the current study DE values for steam flaked corn (3.75 ± 0.15 Mcal/kg) and corn germ dehydrated (3.76 ± 0.07 Mcal/kg) were similar to the NRC. The DE value measured for cracked corn (3.02 ± 0.16 Mcal/kg) is significantly lower than previously reported; this difference could be due to the lower apparent digestibility values of crude protein and crude fat and factors affecting site of digestion. The differences in DE values of the corn can be seen in the change in bodyweight over the dietary treatment periods, the cracked and steam flaked corn provided the same total diet calories (equal gross energy) but horses on average gained more weight when fed steam flaked corn (19.33 ± 4 kg) than cracked corn (3.98 ± 6 kg). The difference in bodyweight gain could reflect differences in site of digestion and efficiency in energy utilization due to differences in processing techniques. The study findings suggest starch digestion on the

cracked corn diet shifted towards microbial fermentation in the hindgut resulting in production of VFA energetic end products, which are metabolized less efficiently compared to glucose. The nutritional value of the cracked corn diet was overall depressed compared to steam flaked and corn germ dehydrated diets.

In both equine and ruminants the results of associative effects of cereal grain feeding on forage digestibility have been varied. Studies investigating the associative effects of replacing forage with increasing levels of concentrate have reported linear changes in digestive coefficients in relation to the increasing level of concentrate. In the current study a fixed level of starch from different corn products was used to investigate changes in fiber digestibility.

In this study mean digestibility of ADF and NDF was not significantly reduced when corn was fed at a level providing 14 times more starch than the control diet. Numerical increases in mean ADF and NDF digestibility (5 and 3 %) was measured on the corn by-product diet compared to the control diet. Digestibility coefficients are within range of those measured when rolled corn was fed to provide 0.2 % BW (starch and sugars) (Hussein et al., 2004). The by difference values of ADF and NDF digestion coefficients for the three corn products indicate that ADF and NDF in the corn germ dehydrated ingredient to be more digestible than cracked and steam flaked corn.

The difference method using equation 1 showed large variation in ADF digestibility for

the cracked and steam flaked corn diets, with standard errors of 12% and 20%, respectively. Equation 2 showed the same diet trend in ADF digestibility but means and variation for the steam flaked diet and corn byproduct were greatly different from equation 1. This may be the result of the numerical differences between the control diet and the combined corn diets and amplified by equation 2 as, corn provided a small fraction of the total diet ADF but the equation assumes the difference in digestibility is due to the addition of corn. Corn NDF digestibility coefficients were similar between equation 1 and 2, numerically corn byproduct had the highest NDF digestibility using equation 1 and cracked corn had the highest using equation 2.

These finding of by difference may indicate changes in fiber digestibility not associated with corn ADF and NDF. The by difference equation assumes that the digestibility of the control diet is constant, however, assuming corn ADF and NDF is 100 % digestible the decrease in total cracked and steam flaked diets has to be due to a response in fiber from the control diet notably from hay cubes and alfalfa pellets as these two ingredients provided the majority of the fiber. Hintz et al. (1971) reported a linear increase in ADF and NDF apparent digestibility with increasing concentrate suggesting concentrate and grain fiber to be more digestible than fiber from forage sources.

In the current study the high starch intake was used to create a feeding situation in which comparisons in the site of starch digestion could be made for the different corn products. Even with the moderate and high glycemic responses of the corn byproduct and steam

flaked diets there appeared to be a spillover effect into the hindgut due to lowered fecal pH and increased L-lactate concentrations. This spillover effect suggests that regardless of processing method, when the majority of the starch in the diet is supplied as corn, an intake of 6 g starch/kg BW/day may overwhelm the digestive capacity of the proximal gastrointestinal tract. The starch from the corn byproduct diet was available to enzymatic digestion in the small intestine and starch that reached the hindgut did not affect fiber fermentation or cause perturbations in hindgut. Corn germ dehydrated is a unique ingredient having a lower starch and higher crude protein and fat content than cracked or steam flaked corn although, the ground ingredient requires pelleting for commercial use, both due to visual preference and horse acceptance at high intakes. From this study the byproduct, corn germ dehydrated, is a highly digestible energy source for horses even when fed at high starch intake levels.

The by difference method is not sophisticated enough to differentiate between a change in nutrient digestibility in the basal (forage only diet) or the concentrate ingredient, thus accrediting the decrease or increase in treatment diet digestibility to the concentrate. The use of the by difference method for measuring digestibility of concentrates at high intakes may be unwarranted due to the presence of digestive interactions which will alter the digestibility of the basal diet and possibly the concentrate ingredient, invalidating the underlying assumption of the equation. Complete diet evaluations may be more useful when such associative effects are present or suspected, as feeding only concentrates is not recommended. Understanding the degree of these effects on digestibility of the total diet

is valuable in making accurate feed recommendations. The basal diet should be selected to represent normal forage diets with minimal manipulation, while the treatment diets may be manipulated to provide equal nutrient compositions and contain multiple ingredients. It may be advantageous for future *in vivo* digestibility studies using the by difference method to carry out additional *in vitro* fermentations to determine nutrient digestibility of the individual test ingredient. *In vitro* data may augment the data of the difference method by minimizing variability in the data due to animal response and digestive interactions. Information of the responses in fecal microbiota to the treatment diets would be extremely beneficial in providing a complete explanation as to the differences measured in fiber digestibility for the three corn products using the by difference method and VFA and L-lactate concentrations.

The results of this study may have been impacted by the 12 week high grain pre-study feeding period, a diet response may have occurred with the microflora shifting towards a predominately more amylolytic community. This is supported by the overall lower fecal pH values for the digestibility study compared to the preliminary starch intake study. The 5 day acclimation period for diet changes between control and corn treatments may have been too rapid to obtain reliable fiber digestibility data. Caution is warranted for feeding high starch diets to horses, even with highly processed ingredients. Factors such as the amount of starch available for fermentation and the intensity of which the starch can be fermented both in the stomach and the hindgut can have negative impacts on animal health and performance.

Literature Cited

- Al Jassim, R.A.M. (2006) Supplementary feeding of horses with processed sorghum grains and oats. *Animal Feed Science and Technology*. Volume 125, pp 33-44.
- Al Jassim, R.A.M. and Andrews, F.M. (2009) The Bacterial Community of the Horse Gastrointestinal Tract and Its Relation to Fermentative Acidosis, Laminitis, Colic and Stomach Ulcers. *The Veterinary Clinics of North America: Equine Practice*. Volume 25, pp 199 – 215.
- Al Jassim., R.A.M., Scott, P.T., Krause,D., Denman, S. and McSweeney, C.S. (2005) Cellulolytic and lactic acid bacteria in the gastrointestinal tract of the horse. *Recent Advances in Animal Nutrition in Australia*.Volume 15, pp 155-163.
- Andrews, F.M., Buchanan, B.R., Elliott, S.B., Al Jassim, R.A.M., McGowan, C.M. and Saxton, A.M. (2008) In vitro effects of hydrochloric and lactic acids on bioelectric properties of equine gastric squamous mucosa. *Equine Veterinary Journal*. Volume 40, Number 4, pp 301-305.
- Andrews, F.M., Sifferman, R.L., Bernard, W., Hughe, F.E., Holste, J.E., Daurio, C.P., Alva, R. and Cox, J.L. (1999) Efficacy of omeprazole in the treatment and prevention of gastric ulcers in horses. *Equine Veterinary Journal*. Volume 31, Issue S29, pp 81-86.
- Argenzio, R.A., Suvithworth, M. and Stevens, C.E. (1974) Sites of organic acid product and absorption in the equine gastrointestinal tract. *American Journal of Physiology*. Volume 226, pp 1043.
- Belengeur, A., Duncan, S.H., Holtrop, G., Anderson, S.E., Lobley, G.E. and Flint, H.J. (2007) Impact of pH on Lactate Formation and Utilization by Human Fecal Microbial Communities. *Applied and Environmental Microbiology*. Volume 73, Number 20, pp 6526-6533.
- Bush, J.A., Freeman, D.E., Kline, K.H., Merchen, N.R. and Fahey, Jr. G.C. (2001) Dietary fat supplementation effects on in vitro nutrient disappearance and in vivo nutrient intake and total tract digestibility in horses. *Journal of Animal Science*. Volume 79, pp 232-239.
- Caldarini, M.I., Pons, S., D'Agostino, D., Depaula, J.A., Greco, G., Negri, G., Ascione, A. and Bustos, D. (1996) Abnormal Fecal Flora in a Patient with Short Bowel Syndrome. *Digestive Diseases and Science*. Volume 41, Number 8, pp 1649-1652.

- Crampton, E.W. and Harris, L.E. (1969) *Applied Animal Nutrition*. W.H. Freeman and company, San Francisco.
- Daly, K., Proudman, C.J., Duncan, S.H., Flint, H.J., Dyer, J. and Shirazi-Beechey, S.P. (2012) Alterations in microbiota and fermentation products in equine large intestine in response to dietary variation and intestinal disease. *British Journal of Nutrition*. Volume 107, Issue 7, pp 989-995.
- De Fombelle, A., Jacotot, E., Drogoul, C. and Julliand, V. (1999) Effect of the hay:grain ratio on microbial and physio-chemical characteristics of cecal and colonic contents in ponies. Part 2. Proceedings of the sixteenth Equine Nutrition and Exercise Physiology Symposium. pp 153-154.
- De Fombelle, A., Varloud, M., Goachet, A.G., Jacotot, E., Philippeau, C., Drogoul, C. and Julliand, V. (2003) Characterisation of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets. *Animal Science*. Volume 77. Pp 293-304.
- De Marco, M., Miraglia, N., Peiretti, P.G. and Bergero, D. (2012) Apparent digestibility of wheat bran and extruded flax in horses determined from the total collection of feces and acid-insoluble ash as an internal marker. *Animal*. Volume 6, number 2, pp 227-231.
- Drogoul, C., de Fombelle, A. and Julliand, V. (2001) Feeding and microbial disorders in horses: 2: Effect of three hay:grain ratios on digesta passage rate and digestibility in ponies. *Journal of Equine Veterinary Science*. Volume 21, Number 10, pp 487-491.
- Dyer, J., Al-Rammahi, M., Waterfall, L., Salmon, K.S.H., Geor, R.J., Bouré, L., Edwards, G.B., Proudman, C.J. and Shirazi-Beechey, S.P. (2009) Adaptive response of equine intestinal Na⁺/glucose co-transporter (SGLT1) to an increase in dietary soluble carbohydrate. *Pflügers Archiv European Journal of Physiology*. Volume 458, pp 419-430.
- Earing, J.E., Cassill, B.D., Hayes, S.H., Vanzant, E.S. and Lawrence, L.M. (2010) Comparison of in vitro digestibility estimates using the Daisy II incubator with in vivo digestibility estimates in horses. *Journal of Animal Science*. Volume 88, pp 3954-3963.
- Ellis, A.D. and Hill, J. (2005) *Nutritional Physiology of the Horse*. Nottingham University Press.
- Firkins, J.L., Eastridge, N.R., St-Pierre, N.R. and Nofstger, S.M. (2001) Effects of grain variability and processing on starch utilization by lactating cattle. *Journal of Animal Science*. Volume 79. Supplement E, pp 218-238.

- Forster, I. (1999) A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquaculture Nutrition*. Volume 5, pp 143-145.
- Frape, D.L., Tuck, M.G., Sutcliffe, N.H. and Jones, D.B. (1982) The use of inert markers in the measurement of the digestibility of cubed concentrates and of hay given in several proportions to the pony, horse and white rhinoceros (*Diceros simus*). *Comparative Biochemistry and Physiology*. Volume 72A, Number 1, pp 77-83.
- Gibbs, P.J., Potter, G.D., Schelling, G.T., Kreider, J.L. and Boyd, C.L. (1996) The significance of small vs. Large intestinal digestion of cereal grain and oilseed protein in the equine. *Journal of the Equine Veterinary Science*. Volume 16, pp 60-65.
- Goachet, A.G., Philippeau, C., Varloud, M. and Julliand, V. (2009) Adaptations to standard approaches for measuring total tract apparent digestibility and gastro-intestinal retention time in horses in training. *Animal Feed Science and Technology*. Volume 152, pp 141-151.
- Goad, D.W., Goad, C.L. and Nagaraja, T.G. (1998) Ruminant microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. *Journal of Animal Science*. Volume 76, pp 234-241.
- Hill, J. (2007) Impacts of nutritional technology on feeds offered to horses: A review of effects of processing on voluntary intake, digesta characteristics and feed utilization. *Animal Feed Science and Technology*. Volume 138, pp 92-117.
- Hintz, H.F., Argenzio, R.A. and Schryver, H.F. (1971) Digestion coefficients, blood glucose levels and molar percentage of volatile acids in intestinal fluid of ponies fed varying forage-grain ratios. *Journal of Animal Science*. Volume 33, Number 5, pp 992-995.
- Hoekstra, K.E., Newman, K., Kennedy, M.A.P. and Pagan, J.D. (1999) Effect of Corn Processing on Glycemic Response in Horses. *Proceedings of the sixteenth Equine Nutrition and Exercise Physiology Symposium*. Pp 144-148.
- Hoffman, P.C. and Shaver, R.D. (2008) Corn Biochemistry: Factors Related to Starch Digestibility in Ruminants. Available from www.uwex.edu/ces/dairy/nutrition/documents/CornBiochemistryE-2.pdf
- Hussein, H.S., Vogedes, L.A., Fernandez, G.C.J. and Frankeny, R.L. (2004) Effects of cereal grain supplementation on apparent digestibility of nutrients and concentrations of

fermentation end-products in the feces and serum of horses consuming alfalfa cubes. *Journal of Animal Science*. Volume 82, pp 1986-1996.

Jansen, W.L., Geelen, S.N.J., Van Der Kuilen, J. and Beynen, A.C. (2002) Dietary soyabean oil depresses the apparent digestibility of fibre in trotters when substituted for an iso-energetic amount of corn starch or glucose. *Equine Veterinary Journal*. Volume 34, pp 302-305.

Julliand, V., de Fombelle, A., Drogoul, C. and Jacotot, E. (2001) Feeding and microbial disorders in horses: Part 3 – Effects of three hay:grain ratios on microbial profile and activities. *Journal of Equine Veterinary Science*. Volume 21, Number 11, pp543-546.

Julliand, V., de Fombelle, A. and Varloud, M. (2006) Starch digestion in horses: The impact of feed processing. *Livestock Science*. Volume 100, pp 44-52.

Karlsson, C.P., Lindberg, J.E. and Rundgren, M. (2000) Associative effects on total tract digestibility in horses fed different ratios of grass hay and whole oats. *Livestock Production Science*. Volume 65, pp 143-153.

Kern, D.L., Slyter, L.L., Leffel, E.C., Weaver, J.M. Leffel, E.C. and Samuelson, G. (1973) Pony cecum vs. Steer rumen: the effect of oats and hay on the microbial ecosystem. *Journal of Animal Science*. Volume 37, Number 2, pp 463-469.

Kern, D.L., Slyter, L.L., Leffel, E.C., Weaver, J.M. and Oltjen, R.R. (1974) Ponies vs. Steers: Microbial and Chemical Characteristics of Intestinal Ingesta. *Journal of Animal Science*. Volume 38, pp 559-564.

Kienzle, E., Fehrle, S. and Opitz, B. (2002) Interactions between the apparent energy and nutrient digestibilities of a concentrate mixture and roughages in horses. *The Journal of Nutrition*. Volume 132, Number 6, pp 1778S-1780S.

Kienzle, E., Pohlenz, J. and Radicke, S. (1997) Morphology of Starch Digestion in the horse. *Journal of Veterinary Medicine A*. Volume 44, pp 207-221.

Kienzle, E., Radicke, S., Wilke, S., Landes, E. and Meyer, H. (1992) Praeileale Stärkeverdauung in Abhängigkeit von Stärkeart und –zubereitung. *Europäische Konferenz über die Ernährung des Pferdes*. Pp 103 – 106.

Lean, I.J., Golder, H.M., Black, J.L., King, R. and Rabiee, A.R. (2013) In vivo indices for predicting acidosis risk of grains in cattle: Comparison with in vitro methods. *Journal of Animal Science*. Volume 91, pp 2823-2835.

Lloyd, L.E., Peckham, H.E. and Crampton, E.W. (1956) The Effect of Change of Ration on the Required Length of Preliminary Feeding Period in Digestion Trials with Sheep. *Journal of Animal Science*. Volume 15, pp 846-853.

Mackie, R.I. and Gilchrist, F.M.C. (1979) Changes in Lactate Producing and Lactate-utilizing bacteria in Relation to pH in the Rumen of Sheep During Stepwise Adaptation to a High-Concentrate Diet. *Applied and Environmental Microbiology*. Volume 38, issue 3, pp 422-430.

Mackie, R.I. and Wilkins, C.A. (1988) Enumeration of anaerobic bacterial microflora of the equine gastrointestinal tract. *Applied and Environmental Microbiology*. Volume 54, Number 9, pp 2155-2160.

Martin-Rosset, W. and Dulphy, J.P. (1987) Digestibility interactions between forages and concentrates in horses: Influence of feeding level – Comparison with sheep. *Livestock Production Science*. Volume 17, pp 263-276.

Matthé, A., Lebzien, P., Hric, I. and Flachowsky, G. (2003) Influence of prolonged adaptation periods on starch degradation in the digestive tract of dairy cows. *Animal Feed Science and Technology*. Volume 103, pp 15-27.

McLean, B.M.L., Hyslop, J.J., Longland, A.C., Cuddeford, D. and Hollands, T. (2000) Physical processing of barley and its effects on intra-caecal fermentation parameters in ponies. *Animal Feed Science and Technology*. Volume 85, pp 79-87.

Menoyo, D., Serrano, M.P., Barrios, V., Valencia, D.G., Lázaro, R., Argente, J. and Mateos, G.G. (2011) Cereal type and heat processing of the cereal affect nutrient digestibility and dynamics of serum insulin and ghrelin in weanling pigs. *Journal of Animal Science*. Volume 89. Pp 2793-2800.

Métayer, N., Lhôte, M., Bahr, A., Cohen, N.D., Kim, I., Roussel, A.J. and Julliand, V. (2004) Meal size and starch content affect gastric emptying in horses. *Equine Veterinary Journal*. Volume 36, Number 5, pp 436-440.

Milnovich, G.J., Klieve, A.V., Pollitt, C.C. and Trott, D.J. (2010) Microbial events in the hindgut during carbohydrate-induced equine laminitis. *Veterinary Clinics of North America Equine Practice*. Volume 26, pp 79-94.

Millet, S., Kumar, S., DeBoever, J., Meyns, T., Aluwe, M., De Brabander, D. and Ducateller, R. (2012) Effect of particle size distribution and dietary crude fiber content on

growth performance and gastric mucosa integrity of growing-finishing pigs. *The Veterinary Journal*. Volume 192, Number 3, pp 316 -321.

Moran, A.W., Al-Rammahi, M.A., Arora, D.K., Batchelor, D.J., Coulter, E.A., Daly, K., Ionescu, C., Bravo, D. and Shirazi-Beechey, S.P. (2010a) Expression of Na⁺/glucose co-transport (SGLT1) is enhanced by supplementation of the diet of weaning piglets with artificial sweeteners. *British Journal of Nutrition*. Volume 104, pp 637-646.

Moran, A.W., Al-Rammahi, M.A., Arora, D.K., Batchelor, D.J., Coulter, E.A., Daly, K., Ionescu, C., Bravo, D. and Shirazi-Beechey, S.P. (2010b) Expression of Na⁺/glucose co-transport (SGLT1) in the intestine of piglets weaned to different concentrations of dietary carbohydrate. *British Journal of Nutrition*. Volume 104, pp 647-655.

Müller, C.E., Von Rosen, D. and Udén, P. (2008) Effect of forage conservation method on microbial flora and fermentation pattern in forage and in equine colon and faeces. *Livestock Science*. Volume 119, pp 116-128.

Murray, S.M., Flickinger, E.A., Patil, A.R., Merchen, N.R., Brent, J.L. and Fahey, G.C. (2001) In vitro fermentation characteristics of native and processed cereal grains and potato starch using ileal chyme from dogs. *Journal of Animal Science*. Volume 79, pp 435-444.

Nadeau, J.A., Andrews, F.M., Mathew, A.G., Argenzio, R.A. and Blackford, J.T. (1997) Volatile Fatty Acid Concentrations in the Stomach of Horses: A pilot Study. *Proceeding of the fifteenth Equine Nutrition and Exercise Physiology Symposium*. Pp 376-377.

Nadeau, J.A., Andrews, F.M., Mathew, A.G., Argenzio, R.A. and Blackford, J.T. (1999) Implications of diet in the cause of gastric ulcer disease in horses. *Proceedings of the sixteenth Equine Nutrition and Exercise Physiology Symposium*. Pp 20-21.

Nehring, K. and Haelein, G. F. W. (1973) Feed Evaluation and Ration Calculation Based on Net Energy. *Journal of Animal Science*. Volume 36, pp 949-964.

Nutrient Requirements of Horses: sixth revised edition. (2007) The National Academies Press.

Pagan, J.D., Harris, P.A., Kennedy, M.A.P., Davidson, N. and Hoekstra, K.E. (1999) Feed Type and Intake Affects Glycemic Response in Thoroughbred Horses. *Proceedings of the sixteenth Equine Nutrition and Exercise Physiology Symposium*. Pp 149-150.

- Perera, A., Meda, V. and Tyler, R.T. (2010) Resistant starch: A review of analytical protocols for determining resistant starch and of factors affecting the resistant starch content of foods. *Food Research International*. Volume 43, pp 1959-1974.
- Perkins, G.A., den Bakker, H.C., Burton, A.J., Erb, H.N., McDonough, S.P., McDonough, P.L., Parker, J., Rosenthal, R.L., Wiedmann, M., Dowd, S.E. and Simpson, K.W. (2012) Equine Stomachs Harbor an Abundant and Diverse Mucosal Microbiota. *Applied and Environmental Microbiology*. Volume 78, Number 8, pp 2522-2532.
- Perry, T.W. (1984) *Animal Life Cycle Feeding and Nutrition*. Academic Press Inc.
- Potter, G.D., Arnold, F.F., Householder, D.D., Hansen, D.H. and Brown, K.M. (1992) Digestion of Starch in the Small and Large Intestine of the Equine. *Europaische konferenz uber die Ernahrung des Pferdes*. Pp 107-111.
- Radicke, S., Kienzle, E. and Meyer (1991) Preileal apparent digestibility of oats and corn starch and consequences for cecal metabolism. *Proceedings of the twelfth Equine Nutrition and Exercise Physiology Symposium*. Pp 43-48.
- Radicke, S., Landes, E., Kienzle, E. and Meyer, H. (1992) Aktivitat der Amylase im Darmkanal des Pferdes in Abhangigkeit von der Futterart. *Europaische Konferenz uber die Ernahrung des Pferdes*. Pp 99-102.
- Rechkemmer, G., Ronnau, K. and von Englehardt, W. (1988) Fermentation of polysaccharides and absorption of short chain fatty acids in the mammalian hindgut. *Comparative Biochemistry and Physiology: Part A*. Volume 90, Issue 4, pp 563-568.
- Richards, N., Choct, M., Hinch, G.N. and Rowe, J.B. (2003) Equine α -amylase: does it limit starch digestion in the small intestine of the horse? *Recent Advances in Animal Nutrition*. Volume 14, pp 191-196.
- Richards, N., Choct, M., Hinch, G.N. and Rowe, J.B. (2004) Examination of the use of exogenous α -amylase and amyloglucosidase to enhance starch digestion in the small intestine of the horse. *Animal Feed Science and Technology*. Volume 114, Issue 1-4, pp295-305.
- Richards, N., Hinch, G.N. and Rowe, J.B. (2006) The effect of current grain feeding practices on hindgut starch fermentation and acidosis in the Australian racing Thoroughbred. *Australian Veterinary Journal*. Volume 84, Number 11, pp 402-407.

- Rosenfeld, I. and Austbø, D. (2009) Digestion of cereals in the equine gastrointestinal tract measured by the mobile bag technique on caecally cannulated horses. *Animal Feed Science and Technology*. Volume 150, pp 249-258.
- Rowe, J.B., Choct, M. and Pethick, D.W. (1999) Processing cereal grains for animal feeding. *Australian Journal of Agricultural Research*. Volume 50, pp 721-736.
- Savage, D.C. (1977) Microbial Ecology of the gastrointestinal tract. *Annual Review of Microbiology*. Volume 31, pp 107-133.
- Schoster, A., Arroyo, L.G., Staempfli, H.R. and Weese, J.S. (2013) Comparison of microbial populations in the small intestine, large intestine and feces of healthy horses using terminal restriction fragment length polymorphism. *BMC Research Notes*. Volume 6:91.
- Shewry, P.R. and Halford, N.G. (2002) Cereal seed storage proteins: structures, properties and role in grain utilization. *Journal of Experimental Botany*. Volume 53, Number 370, pp 947-958.
- Shirazi-Beechey, S.P. (2008) Molecular insights into dietary induced colic in the horse. *Equine Veterinary Journal*. Volume 40, pp 414-421.
- Sindt, J.J., Drouillard, J.S., Titgemeyer, E.C., Montgomery, S.P., Loe, E.R., Depenbusch, B.E. and Walz, P.H. (2006) Influence of steam-flaked corn moisture level and density on the site and extent of digestibility and feeding value for finishing cattle. *Journal of Animal Science*. Volume 84, pp 424-432.
- Steele, M.A., Vandervoort, G., AlZahal, O., Hook, S.E., Matthews, J.C. and McBride, B.W. (2011) Rumen epithelial adaptation to high-grain diets involves the coordinated regulation of genes involved in cholesterol homeostasis. *Physiological Genomics*. Volume 43, pp 308-316.
- Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R. and Gordon, J.I. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*; 444: 21: 1027-1031.
- Van Weyenberg, S., Buyse, J. and Janssens, P.J. (2007) Digestibility of a complete ration in horses fed once or three times a day and correlation with key blood parameters. *The Veterinary Journal*. Volume 173, pp 311-316.

- Varloud, M., de Fombelle, A., Goachet, A.G., Drogoul and Julliand, V. (2004) Partial and total apparent digestibility of dietary carbohydrates in horses as affected by the diet. *Animal Science*. Volume 79, pp 61-72.
- Varloud, M., Guyonvarch, A. and Julliand, V. (2005) In vivo colonization of the equine stomach by commensal bacteria. *Proceedings of the nineteenth Equine Science Society*. Pp 180-181.
- Varloud, M., Fonty, G., Roussel, A., Guyonvarch, A. and Julliand, V. (2006) Postprandial kinetic of some biotic and abiotic characteristics of the gastric ecosystem of horses fed a pelleted concentrate meal. *Journal of Animal Science*. Volume 85, issue 10, pp 2508-2516.
- Vermorel, M., Vernet, J. and Martin-Rosset, W. (1997) Digestive and energy utilization of two diets by ponies and horses. *Livestock Production Science*. Volume 51, Issue 1-3, pp 13-19.
- Vervuert, I. and Coenen, M. (2006) Factors Affecting Glycemic Index of Feeds for Horses. *Proceedings of the 3rd European Equine Nutrition and Health Congress*. Belgium. Pp 17-18.
- Vervuert, I., Voigt, K., Hollands, T., Cuddeford, D. and Coenen, M. (2009) Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. *The Veterinary Journal*. Volume 182, pp 67-72.
- Warzecha, C.M., Coverdale, J.A., Janecka, J.E., Pinchak, W.E. and Wickersham, T.A. (2013) Influence of dietary grain supplementation on cecal microbial populations in horses. *Journal of Equine Veterinary Science*. Volume 33, Issue 5, pp 345.
- Wheeler, W.E. and Noller, C.H. (1977) Gastrointestinal tract pH and starch in feces of ruminants. *Journal of Animal Science*. Volume 44, Number 1, pp 131-135.
- Willing, B., Vörös, A., Roos, S., Jones, C., Jansson, A. and Lindberg, J.E. (2009) Changes in faecal bacteria associated with concentrate and forage-only diets fed to horses in training. *Equine Veterinary Journal*. Volume 41, issue 9, pp 908-914.
- Woodward, A.D., Holcombe, S.J., Steibel, J.P., Staniar, W.B., Colvin, C. and Trottier, N.L. (2010) Cationic and neutral amino acid transporter transcript abundances are differentially expressed in the equine intestinal tract. *Journal of Animal Science*. Volume 88, pp 1028-1033.

Zeyner, A., Geißler, C. and Ditrtrich, A. (2004) Effects of hay intake and feeding sequence on variables in faeces and faecal water (dry matter, pH value, organic acids, ammonia, buffering capacity) of horses. *Journal of Animal Physiology and Animal Nutrition*. Volume 88, pp 7-19.

Zijstra, R.T., Jha, R., Woodward, A.D., Fouhse, J. and van Kempen, T.A.T.G. (2012) Starch and fiber properties affect their kinetics of digestion and thereby digestive physiology in pigs. *Journal of Animal Science*. Volume 90, pp 49-58.

Zinn, R.A., Owens, F.N. and Ware, R.A. (2002) Flaking corn: Processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. *Journal of Animal Science*. Volume 80, Number 5, 1145-1156.

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Catherine moved to Versailles, KY in July 2009 as a research intern at Kentucky Equine Research. In 2011 she was the first recipient of the Larry Lawrence Fellowship, funded by Kentucky Equine Research to sponsor a M.S. student in equine nutrition. During her time at the University of Kentucky Catherine gained membership in the Honor Society of Agriculture Gamma Sigma Delta. Catherine has been involved in research studies focusing in equine nutrition and exercise physiology.

J.D Pagan, M. Lennox, L. Perry, L. Wood, L.J. Martin, C. Whitehouse and J. Lange (2010) Form of α -tocopherol affects vitamin E bioavailability in Thoroughbred horses. Proceedings of the 1st Nordic Feed Science Conference.

C. Whitehouse, J.D. Pagan and R.J. Coleman (2013) Responses in fecal pH from low to high starch intakes in healthy horses. *Journal of Equine Veterinary Science*. 33: 358.

J.D. Pagan, B.M. Waldrige, C. Whitehouse, L. Dalglish, S. Fuchs and M. Goff (2013) Furosemide reduces the energetic cost of exercise in Thoroughbreds independent of its effect on EIPH. *Journal of Equine Veterinary Science*. 33: 328.

J.D. Pagan, B.M. Waldrige, C. Whitehouse, L. Dalglish, S. Fuchs and M. Goff (2013) Furosemide administration affects mineral excretion and balance in non-exercised and exercised Thoroughbreds. *Journal of Equine Veterinary Science*. 33: 329.

J.D. Pagan, C. Whitehouse, B.M. Waldrige, A.M. Grev, S.W. Garling, O.L. Yates, S. Davis and B. James (2013) The effect of soaking or steaming timothy hay on voluntary intake and digestibility by Thoroughbreds. *Journal of Equine Veterinary Science*. 33: 350.