DIGESTIBILITY OF TWO COMPLETE PELLETED DIETS BY THE HORSE (*EQUUS CABALLUS*) AS A MODEL ANIMAL FOR NONDOMESTIC HINDGUT FERMENTERS

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ABSTRACT

Digestibility of two complete pelleted diets by the horse (*Equus caballus*) as a model animal for nondomestic hindgut fermenters

Emily Mae Schwartz

Estimating nutrient and energy requirements of exotic animals is a necessary component of nutrition management in zoos and other wildlife facilities. In the absence of species-specific data, domestic animal models are often referenced. Herbivorous hindgut fermenters, such as horses, zebra, and rhinoceros, rely on microbial fermentation in the cecum and colon to utilize dietary structural carbohydrates. The study objective was to measure the digestible energy of two (LOW, HIGH) complete pelleted diets by the horse as a model for nondomestic hindgut fermenters. Seven, individually housed, adult Quarter Horse (*Equus caballus*) geldings were assigned to one of two diets as 100% of intake in a randomized crossover design. Experimental diets both contained similar ingredients including soybean oil as an added source of supplemental fat (LOW 1.7%, HIGH 6.9%). Diets differed in predicted digestible energy (LOW 2.29 Mcal/kg, HIGH 2.85 Mcal/kg, DE), ether extract (LOW 4.00%, HIGH 7.41%, EE), and acid detergent fiber (LOW 33.7%, HIGH 26.2%, ADF). Daily feed quantities were offered at 33.3 kcal DE BW_{kg}^{-1} equally distributed over three meals to maintain target BW. Daily feed intake was quantified. Horses had *ab libitum* access to water. Horses were transitioned from all forage to 100% test diet over 14 d, acclimated to the test feed for 19 d prior to 4 d acclimation and 6 d total

fecal collection using hygiene collection harnesses (Equi-San Marketing Pty Ltd). Diet transition between periods occurred over 8 d. Total fecal output was quantified every 8 h, thoroughly mixed and 10% of measured mass output was subsampled for further analysis. Body weights (BW) recorded weekly did not change significantly throughout the trial (P = 0.420). Apparent digestibility of diet within horse and day was evaluated by a nested ANOVA (Minitab 16). The apparent digestibility of EE (P < 0.000), neutral detergent fiber (P = 0.008), and ADF (P = 0.002) differed between the two diets. Apparent digestibility of DM (P = 0.137), OM (P = 0.140), and GE (P = 0.418) were not different. Excess fat not digested and absorbed in the small intestine (by-pass fat) will enter the hindgut and may cause disruption of normal microbial activity. Additionally soybean oil, when consumed in quantities that allow by-pass to occur, has been shown to have a negative effect on fiber digestibility in hindgut fermenters. A negative effect on fiber digestibility in the higher fat diet could result in diets closer in DM, OM, and GE digestibility than initially predicted. The NRC (2007) recommends that no more than 0.7 g/kg BW/d of soybean oil be fed to the horse. The HIGH diet provided 0.91 g/kg BW/d soybean oil. Feeds that contain concentrations higher than recommended may not be appropriate as the sole dietary ingredient of hindgut fermenters. Further studies are needed to evaluate the use of soybean oil and to determine the threshold at which soybean oil will begin to suppress hindgut fiber digestion. *In vivo* measurements of digestibility in model species may provide useful benchmarks from which diets for nondomestic hindgut fermenters, as well as horses, may be formulated.

Key words: digestibility, equine nutrition, *Equus caballus*, horse, model animal, and non-domestic

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LIST OF ABBREVIATIONS

aDig	apparent digestibility
aDigDM	apparent digestibility of dry matter
aNDF	amylase neutral detergent fiber
ADF	acid detergent fiber
ADL	acid detergent lignin
ADL_{OM}	acid detergent lignin on an organic matter basis
BCS	body condition score
BW	body weight
CP	crude protein
DE	digestible energy
DM	dry matter
DMB	dry matter basis
DMI	dry matter intake
DME	dry matter excretion
EE_A	anhydrous ether extract
EE_P	petroleum ether extract
GE	gross energy
OM	organic matter

I. LITERATURE REVIEW

In Vivo Digestibility Studies

The nutritive value of a feed for one species may be completely different for another due to differences in digestive tract physiology. Measuring the concentration of nutrients in a feed itself will not alone give an accurate measure as to how those nutrients are utilized within the animal. In order to determine the nutrient composition of a feed, and how it is digested and absorbed by the animal, *in vivo* digestibility trials with animals must be employed (Schneider and Flatt, 1975). Total fecal collection trials require accurate, uncontaminated collection all of feces produced.

Total fecal collection trials that utilize non-domestic animals are uncommon. Often there are insufficient individuals in a single facility to have a statistically significant sample size. As a result, many studies use animals in several facilities. This could lead to different confounding variables, such as differing environments and management, affecting the results. Many samples from non-domestic animals are opportunistically collected from the animals' enclosure, which could lead to incomplete sample collection or contamination. Additionally, cost and labor availability are often limiting factors in these types of trials. One possible alternative to the use of exotic animals in these types of studies is the use of domestic animal models.

Another important factor in total fecal collection trials is animal selection. It is recommended that for trials not interested in lactation, castrated males should be utilized. It is also recommended that animals should not be growing due to the higher energy requirement needed for growth. During trials it is common practice to reduce the amount of exercise or activity of the animals. It is hard to give animals uniform exercise as well as the added risk of feces being lost. Animals are typically confined to a stall or crate for long periods of time. It is important that crates and stalls are cleaned daily to ensure animal health and comfort during the trial. The weight of each animal should also be measured before the trial start and multiple times throughout the trial to help ensure that treatments are not having a significant effect on the animals' weight and body condition.

Feed must also be sampled multiple times throughout the trial. Feed samples must be representative of the feed that could be potentially fed to the animals.

The Use of Horses as Model Animals

For reasons previously stated, it would be valuable, if the domestic horse was determined as an appropriate model for assessment of foods used in feeding non-domestic hindgut fermenters. This can be assessed by comparing the preferred diet types, body sizes, and evolutionary history of the model animals and non-domestic animals (Foose, 1982).

For purposes of nutrition-related research, an important aspect in the evaluation of a model animal is comparing its gastrointestinal tract anatomy to that of the non-domestic hindgut fermenter of interest (Table 1). Non-ruminant hindgut fermenters consume fibrous vegetation (Foose, 1982). Non-ruminant hindgut fermenters utilize two different strategies to consume vegetation. Horses and zebras are considered to be grazers; they consume grasses. Rhinoceros and tapirs are considered to be browsers; they consume leaves and the woody parts of trees and shrubs (Foose, 1982). Non-ruminant elephants found in Asia have been observed ingesting high fibrous vegetation when compared to ruminant animals in the same habitat (Eisenberg and McKay, 1970). The majority of the diet of wild rhinoceros consists of leaves, which are high fibrous vegetation (Clauss et al., 2003). Domestic horses evolved to eat grasses (Foose, 1982; Skipper, 2007). Foose compared the digestibility of feeds across multiple species and found that horses had similar digestibility values when compared to exotic hindgut fermenters (Table 2).

Table 1. Selected herbivorous hindgut fermenters

Scientific Name	Common Name
Equidae	
Equus caballus	Domestic Horse
Equus quagga	Plains Zebra
Rhinocerotidae	
Ceratotherium simum	White Rhinoceros
Diceros bicornis	Black Rhinoceros
Rhinoceros unicornis	Indian Rhinoceros
Tapiridae	
Tapirus indicus	Malayan Tapir
Elephantidae	
Elephas maximus	Asian Elephant

Table 2. Digestibility (%) of OM and NDF in the experimental diets and alfalfa hay in select hindgut fermenters (Foose, 1982)

Animal	Feeding Strategy	Diet	OM	NDF
Horse	Grazer	Alfalfa Hay	67.13	55.62
Wild Ass	Grazer	Alfalfa Hay	57.83	45.85
Indian Rhino	Browser	Alfalfa Hay	65.36	50.96
American Tapir	Browser	Alfalfa Hay	54.19	40.11
Grevy's Zebra	Grazer	Alfalfa Hay	66.10	45.89

Body size of non-ruminant hindgut fermenters tends to be large. The largest herbivores, elephants and rhinoceros, are hindgut fermenters (Table 3). Observational studies have noted hindgut fermenters across species do not have the same amount of body size variation as ruminant animals (Foose, 1982). Models have been produced to evaluate the relationship between diet quality, digestive processes and body weight of ungulate herbivores (Illius and Gordon, 1992). These models determined ruminant animals have more variation in body size when compared to hindgut fermenters. It was also noted that hindgut fermenters would consume more DM when compared to ruminant animals (Illius and Gordon, 1992).

Horses, rhinoceros, and zebras are all part of the Order Perissodactyla. The first recorded fossils from this order were dated at 55 million years old during the Eocene period (Ellis and Hill, 2005). Over time few species belonging to this order have survived to modern times when compared to those belonging to the Order Artiodactyla (Foose, 1982). As a result, the Order Perissodactyla is considered to be less diverse when compared to Artiodactyla (Foose, 1982).

The horse may not be a perfect representation of all non-domestic hindgut fermenters. Nutrient requirements can differ between animals within the same species due to environmental, production, and management differences. Horses, rhinoceros, and other non-domestic hindgut fermenters evolved in different parts of the world and would have had to adapt to different environments (Clauss et al., 2003).

Table 3. Average adult body weights of selected hindgut fermenters.

Scientific Name	Adult Body Mass Range (kg)
Equidae	
Equus caballus	400 – 600
Equus quagga	175 – 385
Rhinocerotidae	
Ceratotherium simum	1400 – 2300
Diceros bicornis	815 – 1300
Rhinoceros unicornis	1600 – 4600
Tapiridae	
Tapirus indicus	250 - 375
Elephantidae	
Elephas maximus	1810 – 5000

Natural Diet of Hindgut Fermenters

Horses evolved to be continuous grazers. Evidence for this can be seen in tooth structure and gastrointestinal anatomy (Skipper, 2007). Horse teeth have crowns that continue to grow for much of their lives. The high silica content of grasses produces a coarse food item resulting in continuous tooth wear. As horses evolved from their prehistoric ancestors, changes in tooth structure reflected the inclusion of grasses in their diet (Skipper, 2007). The horses' natural eating behavior should affect how they are fed in managed environments (NRC, 2007). The majority of working horses are kept in stables and not allowed to graze *ad libitum*. Typically a stabled horse fed *ad libitum* will eat on average 10 ± 3 meals

per day; each meal separated by about 3 h of other activities (Hothersall and Nicol, 2009). One study noted that the occurrence of large meals comprised primarily of concentrate feed was associated with decreased gastrointestinal retention time (Cooper et al., 2005). Another study noted that the incidence of stereotypic behavior was decreased in stabled horses were offered more meals per day (Slamova, 2011). Horses evolved on a grass diet that contained a high concentration of structural carbohydrates (Skipper, 2007). There are potential benefits to supplementing horses in a managed environment with high fat or concentrate feeds, especially if horses are engaged in disciplines that require higher energy requirement (Hothersall and Nicol, 2009). There are potential health risks associated with over supplementation. Horses with a diet high in concentrate feeds can develop health problems such as ulcers, diabetes, and laminitis (Rosenfeld and Austbø, 2009).

Overview of Non-Ruminant Hindgut Fermenter Digestive Tract

Horses, rhinoceros, and zebras are considered non-ruminant herbivorous hindgut fermenters (Pond et al., 2005; Foose, 1982). When consuming a forages, microbes will supply up to 80% of the horses energy requirement (NRC, 2007).

Tongue and Dentition

Hindgut fermenters have a tongue that is used in the collection and manipulation of feed in the mouth. The structure of the tongue of the zebra and horse are very similar in length while the rhinoceros hindgut has some adaptive differences

(Table 4). The tongue of the rhinoceros has a prominent, sagittally divided, inter-molar eminence, which is not present in the horse or tapir (Cave, 1976).

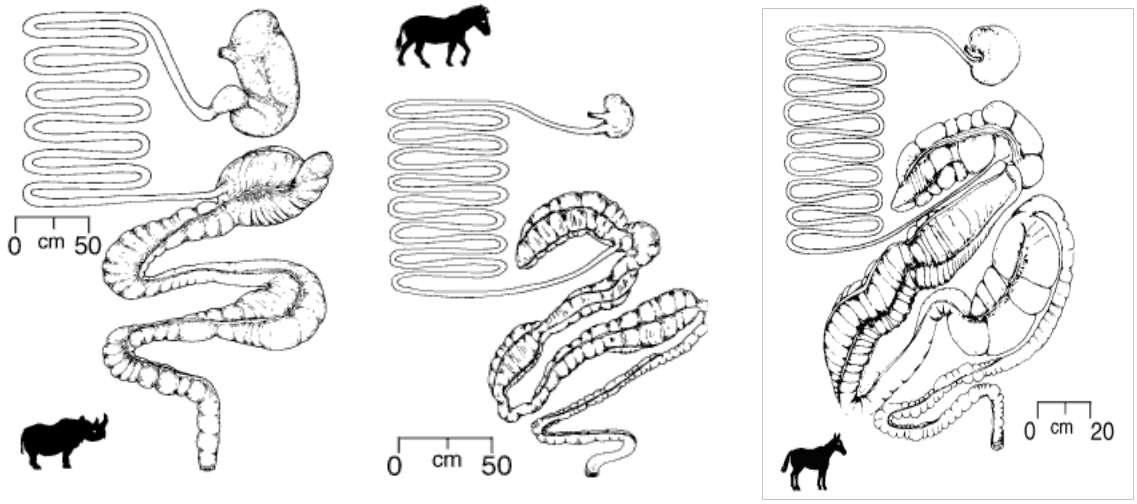

Figure 1. Illustrations of the rhinoceros, zebra, and horse gastrointestinal tract (Stevens and Hume, 1995).

Table 4. Tongue lengths of selected hindgut fermenters

Animal	Tongue Length (cm)
Equidae	
Equus caballus	12 - 20 (NRC, 2007)
Equus quagga	11 – 20 (Penzhorn, 1982)
Rhinocerotidae	
Ceratotherium simurn	30 – 58 (Cave, 1976)
Rhinoceros unicornis	54 – 55 (Cave, 1976)

The horses' teeth are classified as hypsodont, which means that their teeth are long crowned and will continually erupt from the gum as the horses grinds the

crown down over time (Klugh, 2010). The dental formulas for selected hindgut fermenters are shown in Table 5.

Table 5. Dental formulas of selected hindgut fermenters (Hillman-Smith et al., 1986; Laurie et al., 1983; Martin et al., 2011; NRC, 2007; Penzhorn, 1982)

Animal	Dental Formula
Equidae	
Equus caballus	Incisor 3/3 Canine 1/1 Premolar 3 – 4/3 Molar 3/3
Equus quagga	Incisor 3/3 Canine 1/1 Premolar 3/3 Molar 3/3
Rhinocerotidae	
Rhinoceros unicornis	Incisor 1/1 Canine 0/1 Premolar 3/3 Molar 3/3
Tapiridae	
Tapirus indicus	Incisor 3/3 Canine 1/1 Premolar 4/4 Molar 3/3

Incisors are used primarily for ripping and tearing grasses, while the premolars and molars are used to for grinding plant material (Klugh 2010). Horses fed a diet high in concentrated feed may develop sharp points associated with decreased wear on the premolar and molar occusal surfaces. Such points could potentially cause difficulty chewing and injury to the horse's mouth (Dixon and Dacre, 2005).

Saliva Composition

Jaw movement and mastication stimulate saliva secretion and saliva will be continuously secreted while the animal is eating (NRC, 2007). Typically a horse will secrete 10-12 L of saliva per day (Frape, 2004). The main role of equine saliva is to act as a pH buffer for stomach acids. Saliva will also act as a lubricant for digesta entering the stomach (Damron, 2013). It has been shown that there is

a great amount of variation in the composition of saliva between horses as daily within the same horse (Eckersall et al., 1985). Sodium chloride and sodium bicarbonate allow the pH of the digesta passing through the esophagus and entering the stomach to be alkaline. Small amounts of enzyme are present in saliva and therefore little to no enzymatic digestion occurs in the mouth. Rhinoceros saliva contains proteins that bind tannin, a plant toxin found in browse plants that make up the rhinoceros' natural diet (Clauss et al., 2007).

Esophagus

Digesta from the mouth is swallowed and moved down the esophagus into the stomach. The equine esophagus is approximately 1.2 - 1.5 m in length (Gore et al., 2008). The esophagus inner most tissue layer is lined with non-glandular stratified squamous cells (Higgins, 2006). Peristaltic muscular contractions move the digesta down the esophagus and through the cardiac sphincter muscle (NRC, 2007). Due to the incredible strength of the cardiac sphincter it is close to impossible for a horse to vomit or reflux gas (Gore et al., 2008).

Stomach

The horse's stomach has a capacity of 8 – 10 L (Kahn et al., 2010) but should not be filled to capacity in order to have optimum digestion (Gore et al. 2008). It is estimated that the stomach is only 10% of the horses' total gastrointestinal tract (Frappe, 2004). Comparisons of stomach morphologies of hindgut fermenters can be seen in Table 6. Due to the relative small size, the stomach capacity is limited to small feed quantities per meal. The stomach will never

completely empty; digesta may remain in the stomach for up to 6 h (Higgins, 2006). The cranial stomach region is lined by non-glandular, stratified squamous cells, similar to that of the esophagus (Higgins, 2006). The wall of the stomach is also coated with a protective layer of mucus, which is secreted by the mucous cells (Colville and Bassert, 2008). In this region lactobacteria convert soluble carbohydrates to lactic acid, resulting in decreased digesta (Higgins, 2006).

Table 6. Stomach measurements of selected hindgut fermenters (Clauss et al., 2003)

Animal	Length (m)	Capacity (kg)	% of Total GIT Length
Equidae			
Equus caballus	0.2 – 0.25	3 – 4	1 – 2
Equus quagga	0.2	–	1
Rhinocerotidae			
Ceratotherium simurn	1.0	–	–
Diceros bicornis	0.9 – 1.2	37	5 – 9
Rhinoceros unicornis	0.8 – 1.2	–	4
Elephantidae			
Elephas maximus	1.0 – 1.2	51 – 58	4 – 6
Tapiridae			
Tapirus indicus	0.5	–	2

The next region of the stomach, the fundus region, will relax with the swallowing of food to expand and form a pouch that will increase the space in the stomach for more digesta to enter from the esophagus (Colville and Bassert, 2008). This region of the stomach contains glandular chief cells, responsible for the secretion of pepsinogen. Pepsinogen is activated by hydrochloric acid (HCl), secreted by parietal cells, in the stomach to pepsin (Colville and Bassert, 2008). Once it is in its active form pepsin will initiate the hydrolysis of proteins into peptides (Pond et al. 2005).

The body of the stomach will expand and contract in order to facilitate mixing of the digesta and the gastric secretions (Colville and Bassert, 2008). The distal part of the stomach, called the pyloric antrum, regulates the HCl secretion. The presence of food in the pyloric antrum will cause the G-cells to release gastrin into the blood stream. The gastrin travels to proximal portion of the stomach to signal the secretion of HCl (Colville and Bassert, 2008). Swallowing of food will signal the distal part of the stomach to contract to stimulate more vigorous mixing of the digesta (Pond et al., 2005). The pyloric sphincter is muscular tissue that controls the release of chyme from the stomach into the small intestine. It also prevents chyme from reentering the stomach from the duodenum (Colville and Bassert, 2008).

Small Intestine

The small intestine (SI) is divided into 3 sections, the duodenum, jejunum and ileum. A change in the tissues on a cellular level is the only way to differentiate

between the sections of the SI (Colville and Bassert, 2008). The duodenum is located on the dorsal right side of the horse (Merck, 2010). At the dorsal midline the duodenum become the jejunum. At the end of the jejunum the wall of the intestine becomes more muscular and transitions to the ileum (Merck, 2010). Hindgut fermenters evolved to be continuous grazers with a diet low in fat and high in structural carbohydrates. Comparisons of small intestine morphologies of hindgut fermenters can be seen in Table 7.

Table 7. Small intestine measurements of selected hindgut fermenters (Clauss et al., 2003)

Animal	Length (m)	Capacity (kg)	% of Total GIT Length
Equidae			
Equus caballus	11.4 – 26.7	2	61 – 76
Equus quagga	11.4	–	66
Rhinocerotidae			
Ceratotherium simurn	13.8	–	61
Diceros bicornis	12	9	61 - 68
Rhinoceros unicornis	15.2 – 19.8	–	64 – 66
Elephantidae			
Elephas maximus	13.8 – 20.0	28 – 38	57 – 73
Tapiridae			
Tapirus indicus	21.0	–	76

Bile is produced in the liver and secreted directly into the SI (Damron, 2013). The small intestine moves the chyme forward with peristaltic waves of muscle contractions as well as segmental contractions to increase mixing of the intestinal contents (Colville and Bassert, 2008). The segmental contractions slow the movement of chyme through the SI allowing for adequate time for absorption of nutrients. Cholecystokinin (CCK) stimulates intestinal motility and is secreted by the cells when fats and proteins are present in the lumen. The surface area of the small intestine is increased dramatically because of finger-like projections that line the walls of the SI, called villi. Each villus has microvilli to further increase the surface area of the small intestine (Colville and Bassert, 2008). The cells of each villi are constantly replaced with new cells. Goblet cells present in the small intestine produce mucus that protects the intestinal wall (Freeman, 2011).

Electrolytes such as sodium, chloride, and potassium are absorbed directly through the SI wall whereas carbohydrates, lipids, and proteins must be chemically broken down further (Colville and Bassert, 2008). Carbohydrates, like starches, glycogen and sugars are broken down into disaccharides by amylase, which is secreted by the pancreas into the lumen of the duodenum. Carbohydrates are needed to help supply energy to the horses diet as well as in the synthesis of other nutrients (Cloville and Bassert, 2008). Once the carbohydrates are broken down into individual disaccharides they are further broken down into glucose units by their specific enzymes; i.e. sucrose is further broken down by sucrase (Freeman, 2011). Enzymes like sucrase are found in the cell membranes of the microvilli, and once the disaccharides are broken

down into single sugar units like, glucose, galactose and fructose, they are absorbed past the brush border (Colville and Bassert, 2008).

Protein provided in the diet must supply required amino acids (Colville and Bassert, 2008). In order for proteins to be absorbed they must be broken down further into dipeptides or single amino acids. The pancreas will secrete proteases, trypsinogen, chymotrypsinogen, elastase, aminopeptidase, and peptidase into the SI to aid with the digestion of proteins. Trypsinogen is activated to trypsin in the SI by the enterokinase, which is secreted by the mucous cells in the lining of the duodenum (Freeman, 2011). Trypsin will then activate the other proteases. Aminopepsidase will begin to breakdown the protein by cleaving off the amino end ($-NH_2$) and carboxypeptidase will cleave the carboxyl end (-COOH) (Freeman, 2011). Trypsin, chymotrypsin and elastase will breakdown the protein bonds in the middle of the protein molecule (Colville and Bassert, 2008). Chemical breakdown of proteins in completed at the brush boarder. Peptides are broken down into single amino acids or amino acid pairs by peptidases and then they are absorbed (Colville and Bassert, 2008). Protein digestibility is related to the crude protein and dry matter (DM) concentration of the feed. As DMI and crude protein concentration increase so does the protein digestibility. Amino acid profile will also give an estimate into apparent digestibility of the protein in a feed (NRC, 2007).

Fat has a large effect on feed energy density. Fat supplies more calories than protein and carbohydrate of the same weight (Schneider and Flatt, 1975). Fat in the diet is a supply of energy and dietary essential fatty acids. Fatty acids also aid in the absorption of fat-soluble vitamins. By nature, fats are hydrophobic and will form into globules in an aqueous environment (Freeman, 2011). The fats must be emulsified in order to break the globules into smaller sizes so that more of the fat is exposed bile, which is produced and secreted directly from the liver, will combine with the fat globules and will create a water-soluble compound (Colville and Bassert, 2008). Pancreatic lipases will penetrate past the bile layer attached to the fat molecule and break it down into glycerol, fatty acids, and monogylcerides (Freeman, 2011). These can readily diffuse past the brush border and are absorbed into the body (Colville and Bassert, 2008).

Fats are often used to supplement energy in the diets of horses in order to decrease the amount of rapidly fermentable carbohydrates in the form of cereal grains (NRC, 2007). The most common source of fat supplementation in the diet of the horse comes from vegetable oils, which are high in unsaturated fatty acids. Many studies have been conducted to determine beneficial effects of fat supplementation in the horse. Some benefits include increased energy utilization, increased BCS, and decreased excitability (NRC, 2007). Fat can also increase the palatability of a diet (NRC, 2007). Dietary fats also transport fat-soluble vitamins, and supply dietary essential fatty acids such as linolenic and linoleic acid (Pond, 2005). Fats added to the diet will increase the overall digestibility of

the diet (NRC, 2007). Hindgut fermenters do not have a gall bladder for bile storage and will secret bile directly into the small intestine via the common bile duct. Bile emulsifies fat into smaller particles making it more available for absorption. Bile is secreted continuously in the horse rather than just at feeding as compared to other animals (NRC, 2007). If horses are not fed for a long period of time bile will build up in the blood stream and cause a yellowing of the gums and whites of the eye (Merck, 2005). Lipids can be glycerol based or non-glycerol based. Glycerol based lipids include glycolipids, phospholipids and triglycerides. Cholesterol and fatty acid esters are examples of non-glycerol based lipids. Saturated fats are less digestible when compared to unsaturated fats. Fats with high melting points are less digestible than fats with low melting points.

Large Intestine

The large intestine of the horse is divided into the cecum, ventral colon, dorsal colon, small colon, and the rectum (Colville and Bassert, 2008). Ingesta from the small intestine will enter the cecum through the ileocecal sphincter. The majority of carbohydrates and protein are absorbed in the small intestine and the major component of the digesta entering the cecum is structural carbohydrate (Cheeke, 2005). It is estimated that the horse is only about 65% as effective at digesting fiber when compared to a ruminant animal (Cymbaluk, 1990). Furthermore ruminant animals will consume approximately 20% less feed to produce the same amount of energy (Clauss et al., 2003). This is due to the shorter retention time of the digesta in the hindgut of the horse (Damron, 2013). Efficiency of

fermentation is directly correlated to fermentation (retention) time. The longer the digesta is fermented (retained) the higher the digestibility (Clauss et al., 2003). The cecum is a large blind sac that is made up by the base, the main body, and the apex (Colville and Bassert, 2008). Comparisons of cecea morphologies of hindgut fermenters can be seen in Table 8. The cecum contains a large microbial and bacterial population, similar to that of a rumen, which will cause fermentation of the ingesta that enters the cecum (Damron, 2013). Volatile fatty acids (VFAs) are produced by the microbes in the cecum and absorbed by the horse. Horses gain the majority of their dietary energy from the VFA production in the cecum. Fermentation of structural carbohydrates can provide the majority of the horses' energy requirement (NRC, 2007). The microbes will also produce water-soluble vitamins that are absorbed from the cecum (Damron, 2013). There is some production of proteins but the horse is not able to utilize this due to the lack of enzyme secretion into the cecum (Colville and Bassert, 2008).

Diet type will also have an effect on the microbial population in the hindgut. A diet that consists solely of concentrate feed has been shown to cause the microbial population to become unstable and fluctuate. Whereas forage based diet had a microbe population that remained stable (Willing et al., 2009). The concentrate diet also produced higher amounts of bacteria that produce lactic acid, which caused a decrease in the pH of the hindgut (Willing et al., 2009). The cecum and colon are the primary sites of water re-absorption in the GI tract (Damron, 2013). Hindgut morphologies of hindgut fermenters are compared in Table 9.

Table 8. Cecum measurements of selected hindgut fermenters (Clauss et al., 2003)

Animal	Length (m)	Capacity (kg)	% of Total GIT Length
Equidae			
Equus caballus	0.7 – 1.0	4 – 5	3 – 5
Equus quagga	0.8	–	5
Rhinocerotidae			
Ceratotherium simurn	0.9	–	4
Diceros bicornis	0.7 – 1.1	40	5 – 8
Rhinoceros unicornis	0.6 – 0.9	–	3
Elephantidae			
Elephas maximus	0.5 – 1.0	75 – 86	2 – 5
Tapiridae			
Tapirus indicus	0.3	–	1

Digesta enters the right ventral colon and then flows into the left ventral colon through the sternal flexure (Colville and Bassert, 2008). Ingesta then moves caudally towards the peritoneal cavity, where the left ventral colon narrows into the pelvic flexure, which is a common site of impaction that causes colic in the horse (Damron, 2013). Once digesta has moved past the pelvic flexure it will enter the left dorsal colon. Ingesta will then move through the diaphragmatic flexure and into the right dorsal colon (Colville and Bassert, 2008). Digesta will then flow caudally into the small colon where the feces is formed and the exits through the rectum (Colville and Bassert, 2008). Comparisons of total tract length and capacity of hindgut fermenters can be seen in Table 10.

Table 9. Total colon measurements of selected hindgut fermenters (Clauss et al., 2003)

Animal	Length (m)	Capacity (kg)	% of Total GIT Length
Equidae			
Equus caballus	4.2 – 7.65	4 – 5	20 – 33
Equus quagga	4.7	–	28
Rhinocerotidae			
Ceratotherium simurn	7.2	–	32
Diceros bicornis	2.9 – 4.9	40	22 – 28
Rhinoceros unicornis	9.1	–	28 – 29
Elephantidae			
Elephas maximus	5.8 – 8.5	75 – 86	21 – 35
Tapiridae			
Tapirus indicus	5.9	–	21

GI Tract Motility and Retention Time

Transit time (TT_1) is the time necessary for digesta to pass through the gastrointestinal tract. Total tract mean retention time (R_{git}) is the amount of time the needed for the feed or fluid to be excreted. GI tract motility and feed retention time are greatly influence by the feed type (NRC, 2007). Typically a diet composed primarily of a concentrate or pelleted feed will have lower retention time and cause increased GI tract motility (Lorenzo-Figueras et al., 2005). Forage-based diets will move slower through the GI tract due to the high fiber content, which are largely undigested until the hindgut (Van Weyenberg et al.,

Table 10. Total tract measurements of selected hindgut fermenters (Clauss et al., 2003)

Animal	Length (m)	Capacity (kg)
Equidae		
Equus caballus	13.1 – 31.3	29 – 31
Equus quagga	17.2	–
Rhinocerotidae		
Ceratotherium simurn	22.8	–
Diceros bicornis	13.2 – 18.5	173
Rhinoceros unicornis	23.1 – 31.3	–
Elephantidae		
Elephas maximus	17.5 – 27.5	415 – 487
Tapiridae		
Tapirus indicus	27.4 – 27.8	–

2006). Concentrate and pelleted feeds will typically be higher in energy and fat content, the majority of the fat digestion and absorption occurs in the small intestine (Lorenzo-Figueras et al., 2005). Decreased retention time (increased transit time) is associated with decreased digestibility. Highly digestible feeds pass more rapidly through the GI tract. This could result in nutrients, such as fats or nonstructural carbohydrates, bypassing digestion and absorption in the small intestine, and moving into the hindgut fermentation regions.

Loss of these energy dense nutrients to fermentation could result in a net reduction of energy utilization in that feed when compared to those that have an higher retention time (Schneider and Flatt, 1975).

II. INTRODUCTION

Exotic animals have been kept in what is now considered the modern zoos and other wildlife facilities since the 1700's. In the United States there are over 220 zoos accredited by the Association of Zoos and Aquariums (AZA, 2015). There are over 750,000 animals in the care of these zoos (AZA, 2015). A vital aspect of their care is providing diets that supply nutrients and energy needed to meet their dietary requirements. However these animals are most often not fed foods they evolved to consume *in situ*. For practical purposes, food choices are often made based on what is locally available and cost effective. Determining the most appropriate diets for these animals can be limited by many factors. One such factor is the lack of species-specific information available for the animal in question. If the species nutrient requirements are not known, this complicates the issue of diet formulation to meet the animal's nutrient requirements. If a diet does not provide adequate nutrients the animals may develop health issues related to nutrient imbalances. It is also important to consider how wild animal's nutrient requirements will differ from a captive animal. If species-specific information is not available domesticated model animals may be used to estimate the requirements of non-domestic animals. For example, horses can be used as model animals for non-domestic hindgut fermenters such as rhinoceros, tapirs, or zebras (Nielsen et al., 2012).

Horses have recently begun to play an important role in zoo nutrition research. Captive species populations are usually limited to numbers resulting in study

populations smaller than those required to detect significant treatment differences. It is also extremely important to first evaluate the safety of the study with a model animal in order not to risk the lives of rare and endangered animals. Model animals can also be used when species-specific information is not available. These studies are vital to continue and further the care of captive exotic animals. There are several companies that produce feeds specifically produced for captive exotic animals. In order to determine how that feed will be digested in exotic hindgut fermenters, horses can be used as a model animal to estimate digestibility (Nielsen et al., 2012).

Digestibility trials are commonly used to determine how animals utilize a feed (Gordon et al., 2013). Depending on the feed, horses are often transitioned from a primarily forage based diet, to a diet that contains 100% of the experimental diet. This transition period can last for two to three weeks depending on the nature of the experimental diet. The daily intake of the horses is calculated based on ideal weight and body condition score and the daily ration is weighed and fed in a desired daily meal number, usually 2-3. The number of daily meals is most likely determined due to labor force available during the trial but may also be a factor in the experimental design. Total dietary intake (TDI) and total fecal excretion (TFE) are used to determine the digestibility of the feed mathematically.

The objective of this study was to evaluate the digestibility of two complete pelleted diets in the horse as a model animal for non-domestic hindgut fermenters. The experimental diets differed in predicted energy, crude fat (EE),

and fiber composition. It was hypothesized that the diet higher in EE (and predicted DE) would be more digestible when compared to the diet lower in EE.

III. MATERIALS AND METHODS

Ethical Considerations and Animal Welfare

This project was evaluated and approved by the California Polytechnic State University Institutional Animal Care and Use Committee (IACUC) (Protocol #1201). On d 1 of the trial, each horse was dewormed (1.87% Ivermectin, 200mcg/kg BW). Horses were hand-walked for 30 min d^{-1} with a grazing muzzle to avoid ingestion of other feeds while they were out of their stalls. Horses were groomed as needed and their hooves were picked daily. If any minor scrapes or cuts occurred during the trial they were cleaned with hydrogen peroxide and medicated ointments were applied as needed. Horses were allowed limited tactile contact with other horses on the same treatment.

Originally 8 horses were to be used for the trial but two were diagnosed with a suspected viral infection, and one horse was removed from the trial due to therapeutic oral administration of mineral oil by a licensed veterinarian. Both horses experienced fevers and were given injections of Sedazine® (Xylazine) as a sedative and Banamine® (Flunixin) an anti-inflammatory to help relieve the fever and discomfort (Munroe and Weese, 2011).

Animals, Experimental Design and Housing

Seven adult American Quarter horse geldings were fed two complete pelleted diets as 100% of intake in a randomized crossover design with two sample collection periods. Diet intake was measured throughout the trial and total fecal

output was measured during two, six consecutive day collection periods. The horses were transitioned from an all forage diet to a diet that consisted solely of one of the experimental diets (Table 11). The horses were housed in individual stalls, consisting of a covered area with rubber floor mats (3.66 x 3.66 m), and outside area with compacted decomposed granite (3.66 x 7.32 m). No bedding was used.

Experimental Diets

The experimental diets (LOW, HIGH) differed in predicted digestible energy (DE), crude fat (EE), and fiber composition (Table 12). Both diets contained similar ingredients such as soybean hulls, soybean meal, beet pulp, and oat hulls. Soybean oil was used as a supplemental source of fat in both diets. The LOW diet contained 1.7% and the HIGH diet contained 6.9% soybean oil.

Prior to the study, horses had access to grass pasture supplemented with Bermudagrass hay (*Cynodon dactylon*) and Alfalfa hay (*Medicago sativa*). Horses were randomly assigned to individual stalls. Diets were randomly assigned to treatment groups. Horses on the same treatment were kept in adjacent stalls with two empty stalls between treatment groups. Horses were gradually transitioned from a 100% forage based diet to 100% experimental diet over a period of 14 d (Table 11). Acclimation periods followed diet transitions and preceded sample collection to ensure that the samples collected represented experimental diets.

Table 11. Stages within each period during and the duration of each

Period 1	Objective	Duration (d)
Transition I	Transition from a 100% forage diet to a 100% experimental diet	14
Acclimation I	Allow horses time to acclimate to experimental diet prior initial sample collection	19
Acclimation II	Allow horses time to acclimate to harness before initial sample collection. Quantify total feed intake and total fecal output	4
Collection I	Quantify total feed intake and total fecal output and collected 30% of daily output.	6
Transition II	Transition from one experimental diet to the opposite diet	8
Period 2	**Objective**	**Duration (d)**
Acclimation III	Allow horses time to acclimate to diet prior sample collection	19
Acclimation II	Allow horses time to acclimate to harness before initial sample collection. Quantify total feed intake and total fecal output	4
Collection II	Quantify total feed intake and total fecal output	6
Transition III	Transition from a 100% experimental diet to a 100% forage diet	14

Table 12. Nutrient composition of experimental diets on a dry matter basis

Nutrient[1]	High	Low
DM%	91.70	89.90
NDF%	41.50	54.60
ADF%	29.40	36.90
OM%	83.10	82.05
EE_A%	7.41	4.00
EE_P%	5.73	3.22
CP%	15.30	14.80
Mcal DE/kg (calc)	2.78	2.25
Ash%	8.60	7.85
Ca%	0.95	0.92
P%	0.50	0.36

[1]DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, OM = organic matter, EE_A = anhydrous ether extraction, EE_P = petroleum ether extraction, CP = crude protein, Mcal = mega calorie, DE = digestible energy.

The horses were weighed prior to transitioning to experimental diets and were weighed once weekly for the remainder of the trial. Ideal body weights were determined based on the horses' initial weight and body condition score. Amount of feed offered was determined based on calculated digestible energy (DE) of the feed needed to maintain the horses' ideal weights. The horses' energy requirement was calculated as:

Energy requirement = 33.3 kcal/kg BW (NRC, 2007)

DE content was calculated as:

Dry Forage DE = 2.118 + 0.01218 CP – 0.00937 ADF – 0.00383 (NDF – ADF) + 0.04718 EE + 0.02035 NFC – 0.0262 Ash
(Where NFC = 100 – %NDF – %CP - %EE – %Ash) (NRC, 2007)

The amount of feed remained constant throughout the trial. Feed was weighed to the nearest 10 g using a digital scale (IQ+390-DC Indicator, HD3030-100 Floor Scale, Rice Lake Weighing Systems, Rice Lake, WI). Horses were fed three times daily (0700 h, 1300 h, and 1900 h) in equal portions. Orts were collected and measured prior to the 0700 feedings. All rations were offered in 265 L container placed in the covered portion of each horses' stall. The horses had *ad libitum* access to water using an automatic waterer. Waterers were checked for cleanliness daily and cleaned at least once weekly.

Total Fecal Collection

Each horse was fitted with an equine hygiene collection harness (Equisan Marketing, Ltd., South Melbourne, Victoria, Australia) prior to the first collection period. The fitted harness was assigned to that horse for the remainder of the trial unless significant repairs were needed. The use of the harnesses allows for total and uncontaminated collection of all feces produced by the horses. Horses that had never been fitted with a harness were allowed extra training to ensure it they were comfortable with the harness prior to the start of the first collection period. The horses were given a 4 d acclimation period to the harnesses. The harnesses were thoroughly cleaned and weighed prior to the start of the trial. The

harnesses were placed on the horses at 0600 h of the first day of each collection period. The inside of each harness was lined with a plastic bag and secure into the harness with duct tape. Harnesses were emptied at 0600, 1400, and 2200 h into a tared, five-gallon bucket. Samples were weighed to the nearest 10 g (IQ+390-DC Indicator, HD3030-100 Floor Scale, Rice Lake Weighing Systems, Rice Lake, WI). Each harness was cleaned after collection bag was removed from the harness and a new bag was secured inside. After weighing, fecal samples were homogenized and 10% (by mass) of the total output was collected for further analysis. Samples were transferred to a refrigerator (4°C). After the 0600 h collection, the three daily (1400 h, 2200 h, and 0600 h) samples were combined and thoroughly homogenized to create a daily composite. Composited samples were then frozen at -20°C.

Feed Sampling

Feed was sampled on d 1, 15, 35, 40, 68, and 74 of the trial. Over 1000 g of each experimental pelleted diets was collected on each day from the total amount of feed that could be potentially used in the trial. Feed was sampled using a trier (No. 76, Seedburo Equipment Co., Des Plains, IL). Feed from the first feed sampling was sent to a commercial lab for nutrient composition analysis. Further analyses, with the exception of EE, were conducted in the California Polytechnic State University, Comparative Animal Nutrition Laboratory.

Chemical Analysis

Initial Oven Dry Matter (IDM)

Frozen daily composite fecal samples were placed into aluminum pans weighed to the nearest 1 g (SB32001 Delta Range, Mettler Toledo, Columbus, OH) and placed into a forced air-drying oven set at 50 ± 5ºC (DNK600, Yamato Scientific America, Inc., Santa Clara, CA). Samples were maintained in the oven for an initial 72 h and then weighed once every 24 h until three consecutive (± 1 g) weights were recorded (IDM). Fecal samples were stored in labeled plastic bags before further processing. The following equation was used to calculate initial dry matter of the fecal samples:

$$\left(\frac{\text{Dry Weight-Pan Weight}}{\text{Fresh Weight-Pan Weight}}\right) \text{x } 100\% = \text{IDM } \%$$

Sample Processing

Fecal samples were hand crushed while they were still in the plastic bags. Both fecal and feed samples were ground using a stainless steel Thomas Wiley ED5 Mill (Thomas Scientific, Swedesboro, NJ) through a 2 mm screen. Between each sample, the mill was vacuumed and cleaned with isopropyl alcohol and acetone. Prior to grinding the next sample, the mill was inspected for cleanliness and dryness to ensure that there was no cross contamination between samples. Ground samples were stored in sealed plastic bags for further analysis.

Laboratory (Final) Dry Matter (DM)

Clean and dry crucibles (50 mL) were dried in a forced air-drying oven for at least 3 h prior to use. The crucibles were weighed to the nearest 0.0001 gram using a digital analytical balance (XS205, Mettler Toledo, Columbus, OH). Samples (2.0000 - 2.050 g) were loaded into the clean and dry crucible. Crucibles were placed into a forced-air drying oven set at 102 ± 2°C for 24 h. Samples were removed from the oven and placed into desiccators and cooled to room temperature for 1 h (minimum). Crucibles plus sample were weighed to the nearest 0.0001 g. The lab DM% was calculated with the following equation:

$$\left(\frac{(\mathbf{Crucible\ Weight} + \mathbf{Dry\ Sample\ Weight}) - \mathbf{Crucible\ Weight}}{\mathbf{Fresh\ Sample\ Weight}}\right) \mathbf{x\ 100\%} = \mathbf{DM\%}$$

Samples were run in duplicate in order to calculate a standard deviation (SD). Sample duplicates with > SD ± 0.30 were rejected and analysis was repeated.

Total DM was calculated with the following equations:

$$\left[\left(\frac{\boldsymbol{Dry\ Wt - Pan\ Wt}}{\boldsymbol{Fresh\ Wt - Pan\ Wt}}\right) \boldsymbol{x} \left(\frac{(\boldsymbol{Crucible\ Wt + Dry\ Sample\ Wt}) - \boldsymbol{Crucible\ Wt}}{\boldsymbol{Fresh\ Sample\ Wt}}\right)\right] \boldsymbol{x\ 100\%}$$

Ash

Following LDM, the dried sample residue and crucible were placed in a muffle furnace to remove all organic matter via combustion. Samples were heated to 600°C over a period of 3 h, held at 600°C for 2 h and then cooled to 200°C until they were removed from the muffle furnace and placed in a desiccator to cool for

a minimum of 1 h. Crucible plus ash residue was weighed to the nearest 0.0001 g using a digital analytical balance (XS205, Mettler Toledo, Columbus, OH). Ash % was determined using the following equation:

$$\left(\frac{(\boldsymbol{Crucible\ Weight} + \boldsymbol{Ash\ Weight}) - \boldsymbol{Crucible\ Weight}}{\boldsymbol{Dry\ Sample\ Weight}}\right) \boldsymbol{x}\ \mathbf{100}\% = \boldsymbol{Ash}\%$$

Samples were run in duplicate in order to calculate a standard deviation (SD). Samples duplicates with > SD ± 0.30 were rejected and analysis was repeated.

For energy and fiber analysis sub-composite samples were created from the dried and ground samples. Feed was equally subsampled from all of the feed samples collected during the trial. Fecal samples were subsampled proportionately based on the total output observed during the sample collection periods. Daily samples were pooled to create a period composite sample for each horse.

Energy

A Parr® adiabatic bomb calorimeter was used to determine the energy content of feed and fecal samples. Ground sample was pressed into a pellet and weighed (0.5000 - 0.5050 g) and was then weighed to the nearest 0.0001 using a digital analytical balance. The pellet was placed into a bomb capsule and placed into a bomb head. A fuse wire connecting to charges was placed onto the sample pellet. It was then loaded into the bomb and 30 atmospheres of oxygen was added to the inside of the bomb as fuel for the combustion of the sample. The

bomb was then placed into a bucket containing 2000 ± 0.5 g of water. The temperature of the jacket water was adjusted to match the bucket water and then the sample is ignited. The bomb calorimeter is run for eight min; the temperature of the bucket water in checked at minute six, seven and eight. The highest of the three temperatures is recorded. The bomb is removed from the bucket and the pressure is released. Acid is produced during the combustion of the sample. The bomb head is removed and rinsed with deionized (DI) water into a beaker. The inside of the bomb is also rinsed with DI water into the beaker. Methyl orange is added as an indicator. The mixture is then titrated with solution until the solution turns basic as indicated by the methyl orange. The remaining fuse wire is measured and the amount of wire consumed is recorded. Samples were run in duplicated and SD calculated. Critical control point was determined using a standard, benzoic acid. For this study the critical control point was determined to be 0.29. Any SD above 0.29 was rejected and analysis was conducted again. Gross energy of combustion (H_g) is calculated with the following equation:

$$\boldsymbol{H_g = \frac{((\Delta T\ x\ W) - e_1 - e_3)}{m}}$$

ΔT = The change in temperature in degrees Celsius
W = Energy equivalent of the bomb calorimeter in calories per degree Celsius
e_1 = Correction in calories for heat of formation of nitric acid (HNO_3)
e_3 = Correction in calories for heat of combustion of fuse wire
m = Weight of the pelleted sample in grams

Fiber Analysis

Fiber composition of the feed and fecal samples was determined with an Ankom 200 fiber analyzer. Fiber fractions were determined sequentially. Feed and fecal samples ground through a 2mm sieve were used for this procedure. Sample was placed into ANKOM F57 filter bags. Amylase neutral detergent fiber (aNDF) and acid detergent fiber (ADF) were determined sequentially using the Neutral/Acid Detergent Fiber in Feeds Filter Bag technique (ANKOM Technology, 2011). Acid detergent lignin (ADL) was determined using the Method for Determining Acid Detergent Lignin in Beakers (ANKOM Technology, 2011). After the ADL procedure filter bags and sample were ashed in a muffle furnace to determine the acid detergent lignin on an organic matter basis. All of weights of the fiber fractions were measured to the nearest 0.0001 d using a digital balance (XS205, Mettler Toledo, Columbus, OH). Samples were run in duplicate in order to calculate a standard deviation (SD). If samples had a SD higher than 0.35 the samples were rejected and analysis was conducted again. aNDF, ADF and ADL are calculated with the following equations:

$$\left(\frac{(W_3 - (W_1 \, x \, C_1))}{W_2 \, x \, Lab \, DM\%}\right) x \, 100\% = aNDF\%$$

$$\left(\frac{(W_3 - (W_1 \, x \, C_1))}{W_2 \, x \, Lab \, DM\%}\right) x \, 100\% = ADF\%$$

$$\left(\frac{(W_4 - (W_1 \, x \, C_2))}{W_2 \, x \, Lab \, DM\%}\right) x \, 100\% = ADL\%$$

W_1 = Empty filter bag or crucible weight

W_2 = Sample weight

W_3 = Final dry weight of filter bag or crucible containing sample residue

W_4 = Weight of organic matter (OM)

C_1 = Blank bag correction $= \frac{Final\ Oven\ Dry\ Weight}{Original\ Bad\ Weight}$

C_2 = Ash corrected blank bag = $\frac{Loss\ of\ Weight\ on\ Ignition\ of\ Bag}{Original\ Blank\ Bag}$

Table 13. Mean ± SD composition of two, nutritionally complete experimental pelleted diets (HIGH, LOW) on a dry matter basis (DMB) except for dry matter (DM).

Component	HIGH	LOW
DM%	89.05 ± 0.16	88.43 ± 0.23
OM%	90.80 ± 0.23	91.88 ± 0.29
Ash%	9.21 ± 0.23	8.12 ± 0.29
aNDF%	38.85 ± 0.07	50.74 ± 0.06
ADF%	26.21 ± 0.28	33.66± 0.05
ADL_{OM}%	2.58 ± 0.05	2.57 ± 0.09
EE_A%[1]	7.41	4.00
EE_P%[2]	5.73	3.22
GE (Mcal/kg)	4.43 ± 0.28	4.36 ± 0.14

[1]Value determined by anhydrous ether extraction by an outside laboratory
[2] Value determined by petroleum ether extraction by an outside laboratory

Digestibility Calculations

The total daily dry matter intake and daily total excretion for d 1 – 6 of the collection period was calculated for each individual as follows:

$$\boldsymbol{DMI = Total\ Feed\ Intake\ AFB\ x\ Lab\ DM\%}$$

$$\boldsymbol{DME = (Total\ Feces\ Wet\ Weight\ x\ IDM\%)\ x\ Lab\ DM\%}$$

Mean apparent digestibility (aDig, %) of DM, OM, EE, GE, aNDF, ADF, and ADL_{OM} were calculated by adding daily DMI and DME for each individual over the 6 day period with the following equations:

$$\boldsymbol{aDigDM\% = \left(\frac{DMI - DME}{DMI}\right) x\ 100\%}$$

$$\boldsymbol{aDigOM\% = \left(\frac{(DMI\ x\ OM\%DMB) - (DME\ x\ OM\%DMB)}{(DMI\ x\ OM\%DMB)}\right) x\ 100\%}$$

$$\boldsymbol{aDigEE\% = \left(\frac{(DMI\ x\ EE\%DMB) - (DME\ x\ EE\%DMB)}{(DMI\ x\ EE\%DMB)}\right) x\ 100\%}$$

$$\boldsymbol{aDigGE\% = \left(\frac{(DMI\ x\ GE\%DMB) - (DME\ x\ GE\%DMB)}{(DMI\ x\ GE\%DMB)}\right) x\ 100\%}$$

$$\boldsymbol{aDigNDF\% = \left(\frac{(DMI\ x\ aNDF\%DMB) - (DME\ x\ aNDF\%DMB)}{(DMI\ x\ aNDF\%DMB)}\right)} x\ 100\%$$

$$aDigADF\% = \left(\frac{(DMI\ x\ ADF\%DMB) - (DME\ x\ ADF\%DMB)}{(DMI\ x\ ADF\%DMB)}\right) x\ 100\%$$

$$aDigADL_{OM}\% = \left(\frac{(DMI\ x\ ADL_{OM}\%DMB) - (DME\ x\ ADL_{OM}\%DMB)}{(DMI\ x\ ADL_{OM}\%DMB)}\right) x\ 100\%$$

IV. STATISTICAL ANALYSIS

Body Weights

The initial and final BW recorded after final sample collection of each individual was entered into a General Linear Model (GLM) (Minitab 16) to determine if a significant change in BW occurred during the trial. Body weights were analyzed across diet and period using the same model.

Intake and Excretion

Intake and excretion data was analyzed for significant differences using a nested ANOVA. Individual values were calculated as a percent of BW with the following equation:

$$\frac{Component\ kg}{Average\ BW}\ x\ 100\%$$

Apparent Digestibility

Data was entered into a Nested Analysis of Variance (ANOVA) (Minitab 16, Minitab Inc., State College, PA). The 'Diet' (HIGH or LOW) was nested within 'Horse' and 'Day' (d 1 – 6). A nested ANOVA was used because the measurements of aDig are measured by two nominal variables. Horse and day are nominal variable because they are discrete categories. For horse the only possible observations can come from the individuals used in the trial. Day is a nominal variable because only observations were analyzed on specific days of the trial. Day is nested under horse because each horse will have multiple observations. The horse variable was also used as a random variable to help

account for any possible differences between the horses (Samuels et al., 2012). Significance level was set at $P < 0.05$.

V. RESULTS

Body Weight

Average BW was 507.29 ± 23.35 kg (n = 7) did not change significantly across both diets and both periods throughout the trial (P = 0.420).

Table 14. Average BW (kg) ± SE of horses consuming the experimental diets

Diet	n	BW (kg)	P-value
HIGH	7	500.35 ± 23.43	0.071
LOW	7	505.29 ± 23.25	0.094

Table 15. Average BW (kg) ± SE of horses consuming the experimental diets by period.

Period	n	BW (kg)	P-value
Period 1	7	501.79 ± 21.92	0.202
Period 2	7	503.86 ± 23.98	0.145

Feed Intake and Excretion

No significant differences were detected in the DMI and DME of DM, OM, GE, EE_A, EE_P, aNDF, ADF, or ADL_{OM}.

Table 16. Mean dry matter intake and excretion as a % of BW ± SE

Component	HIGH	LOW	P-value
DM (kg)			
DMI	1.35 ± 0.01	1.61 ± 0.02	0.997
DME	0.50 ± 0.02	0.60 ± 0.03	0.790
OM (kg)			
DMI	1.22 ±0.01	1.48 ± 0.01	0.998
DME	0.42 ± 0.02	0.53 ± 0.03	0.948
GE (Mcal/kg)			
DMI	5.98 ± 0.05	7.01 ± 0.07	0.996
DME	2.31 ± 0.11	2.74 ± 0.14	0.886
EE_A (kg)			
DMI	0.10 ± 0.00	0.06 ± 0.00	1.000
DME	0.02 ±0.00	0.01 ± 0.00	0.124
EE_P (kg)			
DMI	0.07 ± 0.00	0.05 ± 0.00	0.993
DME	0.01 ± 0.00	0.01 ± 0.00	0.131
aNDF (kg)			
DMI	0.52 ± 0.00	0.81 ± 0.01	1.000
DME	0.23 ±0.01	0.34 ± 0.02	0.929
ADF (kg)			
DMI	0.35 ± 0.00	0.54 ± 0.01	1.000
DME	0.16 ± 0.01	0.23 ± 0.01	0.853
ADL_{OM} (kg)			
DMI	0.03 ± 0.00	0.04 ± 0.00	0.997
DME	0.03 ± 0.00	0.03 ±0.00	0.570

Apparent Digestibility

There were no differences detected in the apparent digestibility of DM, OM, GE, or ADL_{OM} of the two diets by horses (Table 17). Evidence to support statistically significant differences was observed in the apparent digestibility of EE_A, EE_P, aNDF, and ADF of the two diets by horses. The HIGH diet had higher EE digestibility when compared to the LOW diet. The LOW diet had higher ANDF and ADF digestibility when compared to the HIGH diet.

Table 17. Apparent digestibility of DM, OM, GE, EE_A, EE_P, aNDF, ADF, and ADL_{OM} of the LOW and HIGH diet ± the SE

	HIGH	LOW	P-value
n	7	7	
DM%	63.93 ± 2.04	61.56 ± 2.18	0.137
OM%	65.96 ± 1.77	63.88 ± 2.18	0.140
GE%	61.55 ± 2.16	60.23 ± 2.13	0.418
EE_A%	75.05 ± 1.53	58.49 ± 2.87	< 0.001
EE_P%	76.71 ± 2.16	68.17 ± 4.27	< 0.001
aNDF%	55.80 ± 2.84	58.44 ± 2.62	0.008
ADF%	54.74 ± 3.25	57.91 ± 2.68	0.002
ADL_{OM}%	25.46 ± 4.39	21.61 ± 5.15	0.125

VI. DISCUSSION

Passage Rate

Longer retention times are associated with increased digestibility. Feeding a pelleted diet exclusively can lead to reduced retention time and increased passage rate (Cooper et al., 2005). Reduced retention time can lead to less efficient digestion (Van Weyenberg et al., 2006). A decrease in EE digestibility may lead to an increase of undigested and unabsorbed fat entering into the cecum and colon. The experimental diets were fed at 100% of intake and this could lead to less efficient digestion of the pelleted feed when compared to a diet with 50% intake of a forage and 50% intake of the pelleted diet. Forage feeds have longer retention times when compared to concentrate feeds. Feeding a diet of 50% forage and 50% pelleted diet will have a longer retention time when compared to a diet of 100% pellet. An increased retention time will lead to more efficient digestion and absorption (Cooper et al., 2005).

Fat Digestion

Fat supplies more calories than protein and carbohydrate (Schneider and Flatt, 1975). The fats must be emulsified in order to break the globules into smaller sizes so that more of the fat is exposed bile, which is produced and secreted directly from the liver (Colville and Bassert, 2008). Saturated fats are less digestible when compared to unsaturated fats (reference). Fats with high melting

points are less digestible than fats with low melting points (reference). Soybean oil has been shown to have higher saturation when compared to other commonly used vegetable oils such as corn oil (reference). This can lead to less efficient digestion and absorption of fat and the energy that would have been supplied by the fat will not be available. The amount of fat per meal should also be considered. Single doses of fat will cause a higher amount of fat entering the SI at one time. Daily fat intake should be separated into meals in order to decrease the amount of fat that enters the SI at one time. Smaller amounts of fat will be digested more efficiently and lead to more energy utilization versus a single dose.

Soybean Oil

Decreased retention time may result in fat that is not absorbed and by-passes into the cecum and colon. By-pass fat can effect the microbial population resulting in a decrease in fiber digestibility in the hindgut of horses (Hintz and Cymbauk, 1994). Soybean oil has been shown to have a negative effect on fiber fermentation in the hindgut more so than other fat source (Jansen, 2001). The NRC recommends that soybean oil intake in the horse be limited to 0.70 g/kg BW/d. The horses that consumed the HIGH diet had a daily intake of 0.91 g/kg BW/d. Reduced fat digestion in the small intestine, combined with the suppression of fiber digestibility in the hindgut may contribute to a net reduction in digestible energy of the HIGH diet resulting in the two diets being more similar in digestible energy than initially predicted.

Fiber Digestion

The disruption of the microbial population can cause fiber to not be digested efficiently. Energy from fiber fermentation will not be available to the animal and cause the diets to appear closer in digestible energy than initially predicted. Soybean oil had been shown to antagonize fiber digestion in the hindgut more so than other vegetable oils. The exact mechanism for the decrease of fiber digestibility caused by soybean oil is unknown. It is believed that fatty acids present in the hindgut will inhibit cellulolytic activity (Jansen et al., 2001). Increased fatty acids present in the hindgut can decrease the pH of the hindgut below microbe homeostasis. Another potential source of pH shift could come from bile acids that enter the hindgut (NRC, 2007).

Excess fat that is not digested or absorbed in the small intestine will enter the cecum and colon. Excess fat in the cecum and colon will disrupt the microbial population of the hindgut and lead to less efficient fiber digestion and a decrease in the availability of energy from structural carbohydrate fermentation (Jansen et al., 2001). Several studies have been conducted and found that excess fat can cause a decrease in fiber digestibility by several percentage units (NRC, 2007). It has been observed that soybean oil has a significant effect on the efficiency of fiber digestion (NRC, 2007). A study done by Jansen et al. found that for every 10g/kg DM intake of soybean oil fiber digestibility would be reduced by 0.9%. The exact mechanism that causes this disruption is not known. A possible cause is an increase in polyunsaturated fatty acids in the cecum and colon causes the pH to

decrease past the point of microbe homeostasis (Jansen et al., 2001). Another potential source of pH shift is bile acids that enter the hindgut (NRC, 2007). Another possible cause could be a combination of increased motility and increased fat in the hindgut. The less time fiber particles spend in the hindgut will decrease the amount of energy that produced from fermentation (NRC, 2007). This effect could be due to the feeding level utilized in this trial. The manufacturer recommends that these diets be fed at 33 – 50% of dietary intake with the remainder of the diet being forage. Inclusion of forage may decrease the effect of the soybean oil.

Energy Source

The gross energy of the HIGH was predicted to be greater than the LOW diet due to the addition of the soybean oil. The fiber content of the HIGH diet is decreased and the potential energy provided by the fiber that was removed was lower. By-pass fat causes the suppression of fiber fermentation and the energy gained by adding fat is lost through the suppression of fermentation. During the trial the amount of the experimental diets each horse was offered was based on the calculated energy content of the diets. The HIGH diet was lower in energy than initially predicted and the horses that were fed the HIGH diets received less energy. This could lead to the trend seen in the weights of the horses being changing over the course of the trial.

Each nutrient in the diets provides differing amounts of energy. Fat is the most energy dense nutrient and provides 9.4 Mcal/kg. Protein provides 5.65 Mcal/kg. Carbohydrates provide 4.15 Mcal/kg. Using the energy values and the amount of each nutrient in the feeds total energy values can be calculated (Table 18).

Table 18. Concentration, absolute amount and Mcal of the HIGH and LOW diets

	HIGH	LOW
Daily Intake (% BW)	1.35	1.61
Daily Intake (kg)	6.84	8.15
Protein (%)	13.0	12.0
Protein (kg)	0.89	0.98
Protein (Mcal)	5.02	5.52
aNDF (%)	38.85	50.74
aNDF (kg)	2.66	4.13
aNDF (Mcal)	6.15	10.02
EE_A (%)	7.41	4.00
EE_A (kg)	0.51	0.33
EE_A (Mcal)	3.57	1.79
EE_P (%)	5.73	3.22
EE_P (kg)	0.39	0.26
EE_P (Mcal)	2.35	1.52
NDSC (%)	31.53	25.15
NDSC (kg)	2.16	2.05
NDSC (Mcal)	8.94	8.50
Total (Mcal)[1]	23.69	25.84
Total (Mcal)[2]	22.46	25.57

[1]EE_A used to calculate total Mcal
[2]EE_P used to calculate total Mcal

Daily intake as a %BW is calculated as the average amount of feed needed to meet each horses energy requirement divided by the average body weight of the horses. The absolute amount of each nutrient was calculated with the following equation:

$$\%\ Nutritent\ x\ Daily\ intake\ (kg) = Daily\ intake\ of\ nutrient\ (kg)$$

The amount of energy supplied by the absolute amount of each nutrient was calculated using the values mentioned above.

Fat:

$$Absolute\ amount\ (kg)\ x\ 9.4\frac{Mcal}{kg}$$

Protein:

$$Absolute\ amount\ (kg)\ x\ 5.65\frac{Mcal}{kg}$$

Carbohydrate:

$$Absolute\ amount\ (kg)\ x\ 4.15\frac{Mcal}{kg}$$

Measured versus Calculated Energy Values

In vivo studies can be used to estimate the energy composition of feeds. However they are very labor intensive and require significant amounts of time and energy. Equations provided by the NRC can be used to calculate the energy composition of forages, concentrates, and fats (NRC, 2007). The formula used to

calculated the predicted energy composition of the experimental feed is shown below:

Dry Forage DE = 2.118 + 0.01218 CP – 0.00937 ADF – 0.00383 (NDF – ADF) + 0.04718 EE + 0.02035 NFC – 0.0262 Ash

(Where NFC = 100 – %NDF – %CP - %EE – %Ash)

To determine which formula is appropriate for these experimental diets the guidelines for feed classification were used. Based on the International Feed Classes guidelines these experimental diets fall under the category of dry forage. Both feeds have over 18% crude fiber on a DMB (Mazuri, 2014). These equations help to provide a way to determine the digestible energy components of the feed however they do not take into account how the diets may interact with the GI tract. Digestibility trials will give a more accurate measure of how the feed is digested and utilized by the horse. The forage calculated DE value is closer to the measured (Table 19).

Table 19. Measured versus calculated energy values for the experimental diets.

Diet	Measured DE Mcal/kg	Concentrate DE Mcal/kg	Forage DE Mcal/kg
HIGH	2.72	4.06	2.85
LOW	2.62	4.05	2.29

Concentrate DE values are calculated with the following equation from the NRC (2007).

Concentrate DE = 4.07 – 0.055 ADF

Horses as Model Animals

The use of horses as model animals to provide useful information to further the care of captive non-domestic hindgut fermenters is a relatively new practice. Horses and exotic hindgut fermenters do not have the exact same dietary requirements. This could result in predicted nutrient requirements being different than actual nutrient requirements in exotic animals. Foose was able to measure similar digestibility values of alfalfa hay in hindgut fermenters. These values are also similar to those measured in this study (Table 20).

Supplemental Fat in the Hindgut

Horse diets may be supplemented with energy dense fats and oils to increase the energy content of feeds. Performance horses that are trained frequently will have higher energy requirements. Trainers will often supplement diets with fats and oils in order to provide more energy in the horses' diet. Vegetable sources are more palatable to the horse and are used more frequently (NRC, 2007). Fat digestion and absorption occurs in the small intestine with the aid of bile. In the hindgut fermenters, bile is produced in the liver and continuously secreted directly into the duodenum via the common gall bladder, not stored for sporadic

Table 20. Digestibility (%) of OM and NDF in the experimental diets and alfalfa hay in select hindgut fermenters (Foose, 1982)

Animal	Feeding Strategy	Diet	OM	NDF
Horse	Grazer	HIGH	65.96	55.80
Horse	Grazer	LOW	63.88	58.44
Horse	Grazer	Alfalfa Hay	67.13	55.62
Wild Ass	Grazer	Alfalfa Hay	57.83	45.85
Indian Rhino	Browser	Alfalfa Hay	65.36	50.96
American Tapir	Browser	Alfalfa Hay	54.19	40.11
Grevy's Zebra	Grazer	Alfalfa Hay	66.10	45.89

secretion in a gall bladder as in other species. Fat digestion will be limited by the amount of bile that can be produced and secreted. Horses fed diets higher in fat than can be digested and absorbed due to the limited bile secretion will not be efficiently digested. The digestibility of fat is affected by the degree saturation, the melting point and fatty acid chain length (NRC, 2007). Saturated fats, such as lard and tallow, are less digestible than unsaturated fats, such as corn oil or soybean oil. Fats with higher melting points are less digestible than fats with lower melting points (Freeman, 2001). Diets higher in fat will also have a lower retention time due to increased motility when compared to diets that do not contain supplemental fats (Lorenzo-Figueras et al., 2005).

VII. CONCLUSIONS

Controlled feeding trials of diets formulated for wildlife species is not always practical. Domestic species with similar gastrointestinal tracts may provide a framework in which nutritionists may operate. It was initially predicted that the HIGH diet would be more digestible than the LOW diet. However the HIGH and LOW diet were more similar in dry matter, organic matter and, gross energy.

The HIGH diet was formulated to provide more energy to animals that required a higher amount of energy in their diets, however no difference in energy was detected when intake was at 100%. Therefore the HIGH diet fed at 100% of intake will not supply the energy it is formulated to provide. This may not be the case if these diets are fed at the recommended feeding level of no more than 50% of intake. Adding forage feeds will increase the retention time and could increase the digestibility of the diets.

It is recommended that changes in diet formulation should be considered; soybean oil should be decreased or removed and a different vegetable oil, such as corn oil, should be utilized. These diets should also not be fed above the recommended intake level of 50% of intake.

High concentrations of soybean oil may not be appropriate in the diets of hindgut fermenters. The NRC (2007) recommends that soybean oil supplementation not exceed 0.7 g/kg BW/d. Further studies are needed to evaluate the use of soybean oil and to determine the threshold at which soybean oil will begin to

suppress fiber digestion. It is possible that soybean oil provided in amounts that can be digested and absorbed prior to the hindgut may provide a useful source of supplemental fat in hindgut fermenters. A future study should evaluate how differing amounts or concentrations of soybean oil in the diet can effect fiber fermentation in the hindgut in order to determine the threshold at which fiber digestion will be effected. A study in which the pelleted diets are fed at the recommended level along with forage may have different results as influenced by increased digesta retention. Included in these studies, should be an objective measure of digesta transit and retention time.

In vivo measurements of digestibility in a model species may provide useful benchmarks from which diets for nondomestic hindgut fermenters may be formulated. These results will also help provide useful guidelines in the practical feeding of horses.

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