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thesis entitled

THE INFLUENCE OF VARIOUS CONTRATE TO
ROUGHAGE RATIOS ON DIETARY INTAKE AND
NUTRIENT DIGESTIBILITIES OF

presented by

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has been accepted towards fulfillment
of the requirements for the

M.S. degree in Animal Science

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8-8-03

Date

THE INFLUENCE OF VARIOUS CONCENTRATE TO ROUGHAGE RATIOS ON DIETARY INTAKE AND NUTRIENT DIGESTIBILITIES OF WEANLINGS

By

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A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Animal Science

2003

ABSTRACT

THE INFLUENCE OF VARIOUS CONCENTRATE TO ROUGHAGE RATIOS ON DIETARY INTAKE AND NUTRIENT DIGESTIBILITIES OF WEANLINGS

By

Susan Kathleen Turcott

The objective of this study was to compare the feed intake and the apparent digestibilities of three different diets varying in concentrate to roughage ratios in weanling horses (n=24) at 5 and 8 months. Horses were stratified by breed, gender, birth date, and bodyweight and were assigned to one of three dietary treatments containing the following concentrate to roughage ratios: 70:30 (High Con), 50:50 (Equal), and 30:70 (Low Con). All horses were fed their respective diets for a 10-d adaptation period and a 4-d collection period at 5 and 8 mo of age. There were no differences in body weights or daily feed intake between treatments with all treatments during both trials (5 and 8 mo) consuming between 28 and 29 ± 1 g/kg BW/d. The horses consuming Low Con had a greater amount of fecal output than High Con at both 5 mo and 8 mo ($P<.01$). At 5 mo, High Con had the highest crude protein digestibility ($P<.05$). At 8 mo, High Con had a higher crude protein digestibility than Low Con ($P<.01$) and a trend to be higher than Equal ($P=.07$). Digestibility of ADF did not differ between treatments; however, horses fed the Low Con tended to digest a higher percentage of NDF than both the Equal and High Con treatments ($P=0.09$). Horses in the High Con treatment tended to digest a higher percentage of energy than those in the Low Con treatment ($P=0.06$). Weanlings seem to digest protein more thoroughly when fed high concentrate diets, and may digest fiber more efficiently when fed diets higher in fiber.

DEDICATION

I dedicate this thesis to my parents and Tim for understanding my quest for completion and supporting my many hours of writing and riding.

ACKNOWLEDGEMENTS

Thank you to my parents, who really thought that horses would be a "passing phase." Thank you for supporting me and encouraging me to follow my dreams. The last two years have been full of challenges: starting graduate school, being diagnosed with thyroid cancer, undergoing surgery and treatment for thyroid cancer, buying a new horse, getting engaged, getting married, graduating, accepting a job in Pennsylvania, and moving across the country. These challenges have helped create the person I am as I write this, and I expect to be a different person by the time I read this again. Thank you for teaching me about challenges and change. Your energy is an inspiration to me and you both have such optimistic views of life and all this it brings.

Tim; thank you for being you. You are patient, caring, hard working, fun, and absolutely wonderful. I truly appreciate you moving back to be with me, understanding my many long nights at work, and my need to ride and work with horses. It is exciting to start our journey together and just know that we will have so much fun in the future: moving into a new house, running together, training our dogs, trying new sports and activities, learning to dance, and just spending time together. Thank you for inspiring me to be a better person and "just relax," sometimes.

Thank you Cara; your help was instrumental in completion of all my work. Thank you for going far above and beyond the requirements of work to help me with my research, equestrian team, and all those little things I just must get done. I know there were times when you dreaded my long phone messages and urgent E-mails, but you always came through. Thank you for all of your support and friendship. Good luck with Solo; I will ride him anytime (and try not to get him too "jazzed up"). You will have a great time teaching the draft horse class and just remember that you know what you know through hard work and your resourceful nature.

Thank you to my committee for their work in preparing me to succeed at my defense and learn about research and horse while in school. My graduate program was perfect for me. I loved the balance of teaching, research, classes, and opportunities to participate in educational events and extension work.

Thank you to Angie and my other graduate school friends for making me laugh and realize that I am not the only one struggling through some of the tough times. You all truly helped me keep my sanity! The best roommate awards go to David Mello and Katie Swartz. What a fun two years we had. I really didn't think that things would go as great as they did. We all balanced each other out so well and you will always be welcome at the home of Tim and I (even if it is a trailer in the hills). I had a great time at our "big party."

Thank you to Dr. Nielsen for giving me the opportunity to get my master's degree. I am a different person now than when I started, and am prepared to take on my dream job. Thank you for always being available, even for my "quick questions" that often aren't so quick. I had a wonderful experience and truly value the growth personally and professionally that I acquired while at Michigan State University.

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LIST OF ABBREVIATIONS

ADF – Acid detergent fiber

Arab – Arabian

BW – Body weight as weighed by mechanical scale

Cannon C – Cannon bone circumference

CP- Crude protein

CV – Coefficient of variance

DE – Digestible energy

DM – Dry matter

Equal – Diet comprised of equal amounts of concentrate and roughage feeds

High Con – Diet comprised of 70% concentrate and 30% roughage

HTRC – Michigan State University Horse Teaching and Research Center

Low Con – Diet comprised of 30% concentrate and 70% roughage

MEC – MSU Merillat Equine Center

MSU – Michigan State University

NDF – Neutral detergent fiber

NRC – National Research Council

SE – Standard error

QH – Quarter Horse

INTRODUCTION

The 1989 NRC recommends feeding a ration consisting of a concentrate to roughage ratio of 70 to 30. This ratio is based upon the amount of feed that a weanling will consume due to a small gut size and their limited hindgut development. The limited development of the caecum and colon in juvenile horses should theoretically result in decreased digestibility and utilization of the fibrous portion of the diet compared to a mature animal. The ratio of concentrate to roughage that is consumed is expected to change throughout the growth period of the horse. In contrast to the high concentrate diet recommended for weanlings, a diet consisting solely of roughage is recommended for the non-working, mature equine at maintenance. Both the anatomical and the physiological makeup of the mature horse's digestive tract are designed for continual grazing throughout the day with the majority of the digestion occurring in the hindgut by the bacteria, protozoa, and fungi microbial inhabitants of the caecum and large intestine (Gibbs et al., 1988; Julliard et al., 2001).

Though the NRC (1989) recommends a high concentrate diet for weanlings, many horse owners do not feed this way. A recent study of racehorse farms reported that 98% of farms fed weanlings diets containing less concentrate than the NRC (1989) recommended 70% concentrate (Gibbs and Cohen, 2001). Furthermore, 62% of the farms fed less concentrate than roughage, in sharp contrast to the recommendations of the 1989 NRC. The majority of the horses surveyed (87%) were being fed for moderate growth, presumably because of a fear regarding the potential for a high-concentrate diet to maximize, though potentially not "optimize," growth. Concerns exist over whether feeding high levels of concentrate may contribute to musculoskeletal problems, behavior problems, and increases in management costs. High concentrate diets have been associated with a variety of musculoskeletal problems, in addition to digestive system dysfunctions such as laminitis and decreases in caecal and rumen pH values in both horses and ruminants (Willard et al., 1977).

The question remains as to whether young horses are able to adequately digest high forage diets to aid in maintaining normal growth rates. Our study begins to answer these questions in the quest to determine the proper concentrate to forage ratio to be fed to weanling horses.

The objective of the present study was to compare the feed and water intake and the apparent digestibilities of three different diets varying in concentrate to roughage ratios in weanling horses at 5 and 8 mo of age. The hypothesis is that a high concentrate diet is more thoroughly digested by weanlings compared to a high forage diet, and that this difference decreases with age.

CHAPTER 1

LITERATURE REVIEW

Roughages and concentrates

Horse feeds can generally be divided into two primary classes of feeds: roughages and concentrates. Roughages include four main categories of feeds: long and dry hays and straws, ground and pelleted hay, straw, or oat feed, ensiled long grass and comparable succulent forages, and chopped succulent ensiled material (Frape, 1998). Roughages generally contain at least 20 % crude fiber (air-dry basis), and contain high proportions of cell wall components. Typically, roughages are fed to provide nutrients, reduce dry matter intake when mixed with other feeds, decrease the net energy intake of the total diet, maintain an active microbial population in the large intestine and caecum, and promote equine welfare through decreasing possible diet-associated behavior problems. Nutrient quality of roughages can vary and depends on type of roughage, climate effects, soil fertility, and stage of maturity when harvested (Hintz, 1983).

Concentrates are the portion of the horse's diet comprised of feeds rich in starch, protein, or both, and typically contain less than 15 to 17 % crude fiber (Frape, 1998). They are most often fed to increase the energy and nutrient concentration of the total diet in growing, breeding, or working horses. Cereal grains, milling by-products, and other formulated supplements are usually referred to as concentrates. Nutrient content and formulation must be thoroughly investigated when feeding mixed concentrate diets because the concentrate ingredients in the diet can drastically vary in nutrient concentrations (Pond, 1995).

Evolution of the horse

There is sizeable variation in management and feeding practices of domestic horses across the world and throughout history. This is reflective of the many uses for horses and the availability and costs of suitable environments and feedstuffs. The horse has developed specific digestive physiology, anatomy, and feeding behaviors associated with a high fiber diet consumed through grazing and browsing. Prior to domestication, horses survived on a diet consisting solely of available forages containing relatively large amounts of water, soluble proteins, lipids, sugars, and structural carbohydrates, but very little starch (Frape, 1998). Additionally, wild horses consume multiple meals throughout the day and night during short, frequent feedings.

In domesticated horses, horsemen control the feedstuffs consumed, the frequency of meals, and the feeding pattern. Domestic horses are commonly fed infrequent, large meals consisting of starchy cereals, high protein concentrates, and dried forages for restricted periods of time. Clearly, the art of feeding domestic horses of all classes is to provide the needed nutrients for service and performance while avoiding digestive and metabolic upsets resulting from feeding methods and ingredients.

The gastrointestinal tract of the horse is comprised of a small, simple stomach, a volumetrically limited small intestine, a large caecum, and a sacculated hind gut. This anatomy allows the horse, a nonruminant herbivore, to digest fibrous diets. However, at birth the foal is completely dependent on milk for nutrients. The digestive system changes in the first year to enable the horse to digest starches and eventually fiber. The age when these changes to the digestive system occur, and the factors that initiate these changes have not been definitively determined.

Equine digestion

Digestion refers to the preparation of food for absorption, and absorption is the processes of transporting molecules to the blood system to be metabolized (Pond, 1995). Digestibility of feedstuffs is evaluated to determine how the digestive system works, how efficiently an animal retains nutrients, and how effective diet formulations are for different classes of horses. Digestion coefficients have been developed to measure efficiency of various feeds (Evans, 1999). Apparent digestibility is calculated according to the following equation:

$$\text{Apparent digestibility} = 100 \times \frac{\text{nutrient intake} - \text{nutrient in feces}}{\text{Nutrient intake}}$$

True digestibilities are calculated by subtracting the endogenous nutrient sources from the total amount measured in the feces. However, true digestibility calculations involve invasive insertion of fistulas and thus they are not determined as often as apparent digestibilities (Slade et al., 1979).

Protein digestion

Protein can be found in both the roughage and concentrate portions of the diet. It is generally accepted that the quality of the protein and its amino acid profile determine the value of a protein source. The quality of the protein may be more important than the quantity of protein in the diet. While there is limited information available regarding the amino acid requirements of horses, it is known that lysine is the first limiting amino acid (Ott et al., 1981). The lysine content of the total diet has been shown to influence rate of gain in weanlings and foals even more than the intake of crude protein (Hintz et al., 1971; Ott et al., 2002; Staniar et al., 1999). Dietary crude protein must be digested and yield products, amino acids, that can be easily absorbed and utilized.

About 70 % of protein digestion occurs in the stomach and duodenum (Evans et al., 1999). Interestingly, endogenous fecal nitrogen loss is proportional to bodyweight and not to the amount of nitrogen consumed, however apparent nitrogen digestibility increases as the nitrogen content of the diet increases (Slade et al., 1970). Gibbs et al. (1988) fed three different hay sources to ponies fitted with cannulas in the posterior ileum to determine true protein digestibility. They found that the large intestine was the major site for apparent digestion of hay nitrogen, but the role of the small intestine increased as higher quality hay was fed (Gibbs et al., 1988). Thus, protein from concentrates seems to be digested more in the upper gastrointestinal tract, while protein from hay sources are digested mostly in the lower gastrointestinal tract.

Carbohydrate and energy digestion

Carbohydrates are used as energy sources and are present in both roughages and concentrates. Carbohydrates are molecules composed of carbon, hydrogen, and oxygen. They include storage carbohydrates, such as sugars and starches, and structural carbohydrates such as cellulose, hemicellulose, pectins, gums, and lignin (Frape, 1998). Similar to protein, the soluble storage carbohydrates are primarily digested in the small intestine, while the insoluble structural carbohydrates are primarily digested in the caecum and large intestine (Evans et al., 1999).

Carbohydrates are the primary source of energy, but energy can also come from fats and protein in either roughages or concentrates. The gross energy of a given feed is the total energy contained in the feed. Upon consumption, energy is either digested or released as fecal energy.

Fiber digestion

Fiber is the major carbohydrate in equid diets. Starch, cellulose, hemicellulose, pectin, and sugars make up carbohydrates that vary in digestibility. Fiber is often measured by determining acid detergent fiber (ADF) and neutral detergent fiber (NDF) to describe components that are mostly available, incompletely available, and mostly unavailable (Van Soest, 1975). The NDF portion contains the plant cell wall: hemicellulose, cellulose, and lignin, while ADF is representative of cellulose and lignin. The soluble portion of the sample is composed of cell contents and pectin and is made up of soluble carbohydrates, starch, organic acids, protein, and pectin (Maynard et al., 1979). Thus, the soluble portions are the most available, while the insoluble portions are partially available or completely unavailable.

Horses digest relatively the same amount of fiber as mules, donkeys, zebras, and elephants; yet they digest less fiber than kangaroo, chinchillas, and ruminants (Evans, 1999). Fiber digestion in the horse is directly related to the microbial activity in the caecum and large intestine for the breakdown of structurally complex carbohydrates. Bacteria are the most important of the symbiotic organisms and different types of bacteria are present throughout the digestive tract of the horse. The microbial action forms primarily acetic, propionic acid, and small amounts of other acids. The amount of acids produced per unit dry-matter intake, and their relative proportions, depends directly on the nature of the feed ration in horses (De Fombelle et al., 2001). Although it is a common fibrous constituent of most equine diets, Lignin is most resistant to microbial attack and indigestible (Evans, 1999).

Cellulose is more easily broken down than lignin, and the hemicelluloses are the most easily digested fibrous cell wall component. The addition of easily digestible carbohydrates such as starch, cane sugar or molasses to equine rations may reduce the digestibility of the fiber. Martin-Rosset and Dulphy (1987) did not find any associative affects on fiber digestion when varying levels of concentrate were added to a roughage diet. However, Drogoul et al. (2001) reported that the fiber digestibility is reduced as the grain to forage ratio is increased. This is explained by the premise that the bacteria attack the simpler carbohydrates by preference. It is commonly accepted that bulky concentrates, those that are high in fiber, are "safer" to feed to horses in high quantities as compared to concentrates that contain lower levels of fiber and more energy. The equid has evolved to efficiently utilize a high forage diet, but it is not completely clear at what stage of maturation the digestive system is developed enough to fully utilize the nutrients available in high fiber diets.

Weanling digestive system

The equine digestive tract continues to develop from birth through maturity. During the time after weaning, the large intestine increases in volume faster than the remainder of the gastro-intestinal tract to accommodate the increased consumption of fiber in the diet (Frape, 1998). Although the proximal regions, stomach and small intestine, proportionately grow very little after birth, the distal regions, caecum and large intestine, continue to grow and develop until the horse is nearly 20 years old (Frape, 1998). Digestion in the caecum and ventral colon depends almost entirely on the activity and vitality of the microflora of this region. The inoculation and development of fiber-digesting microflora in the young equid is an area with limited researched data available at the current time.

Coprophagy in foals is considered normal behavior, but the purpose of its occurrence is still uncertain. Fransis-Smith and Wood-Gush (1977) have suggested that coprophagy may serve to introduce bacteria into the foal's gut and thus supply needed nutrients, such as vitamins, in which the foal may be deficient. There is either a trend or direct correlation for foals to consume feces only from their mother (Fransis-Smith and Wood-Gush, 1977; Crowell-Davis and Houpt, 1985). However, research seems to indicate that any coprophagy that supplies foals with nutrients occurs four to six times per day during the first few weeks of life and thus it would be difficult for the foal to consume sufficient nutrients that are needed in large quantities (Crowell-Davis and Houpt, 1985). Furthermore, in rat pups, which also exhibit coprophagy before being weaned, active ingestion of maternal feces was not necessary for inoculation of the pup's gastrointestinal tract with normal flora (Galef, 1979). Coprophagy may serve multiple purposes in the foal, but inoculation of flora is probably not significant.

Factors affecting digestion

Digestion in the herbivorous animal is a much slower process than in simple-gutted animals. For instance, pigs pass feed through the gastrointestinal tract in as little as 24 h (Pond et al., 1995). In horses, it has been reported that 95% of feed particles consumed pass through the digestive system in 140 h when all roughage diets are consumed, and 75 h when hay-grain diets are consumed (Maynard et al., 1979; Evans et al., 1999). This difference in passage rates between the horses is primarily due to the differences in the fiber content resulting from the concentrate to roughage ratio of the diets studied and the feedstuffs fed.

The processing of feeds is a common practice in the equine industry presumably to increase the digestibility of the feeds. Although pelleted feeds are often more expensive because of additional processing, they are easier to weigh, feed, store, and transport (Earle, 1950). Concentrates are often processed into pellets of mixed ingredients, though complete diets consisting of concentrate and roughage combined are also fed in the industry. Wolter et al. (1974) found that digesta of horses consuming ground hay passed more quickly through the digestive system than long-stem hay, and the horses showed decreased digestibility of nutrients. Pelleting presses ground hay into pellets and similarly decreases digestibility by increasing the rate of passage through the digestive system (Cympaluk and Christensen, 1986). However, pelleting of concentrated and concentrate-hay diets has been reported to either increase digestibility of the ingredients or not change the digestibility when compared to the same diet in an unpelleted form (Frape, 1998, Hintz et al., 1972, Hintz et al., 1966).

Feed intake is regulated by intragastric regulators that monitor distension of the stomach, presence of soluble carbohydrates, rate of gastric emptying, and the osmolar concentration of infused or ingested solutions (Ralston et al., 1982). The mechanisms controlling appetite in horses are still unclear. Equine satiety regulators are different than the mechanism in ruminants, which are more directly related to the classical factors such as crude protein and crude fiber contents (Faverdin et al., 1995). Horses are not able to regulate appetite by sensitive physical appetite-regulating mechanisms (Ralston and Baile, 1982; Laut et al., 1985). Ralston and Baile (1982) suggest that there are four main stimuli which may be responsible for the generation of cues in the gastrointestinal system that determine feeding behavior: distension of the stomach or proximal gut or both, presence of soluble carbohydrates or other nutrients in the proximal gastrointestinal tract, the rate of gastric emptying (may be dependent on upon nutrient concentrations and volume of gastric contents), and the osmolar concentration of infused or ingested solutions.

Increasing the frequency of feeding meals and large feed intakes consumed at a rapid rate will increase the rate of passage of ingesta through the digestive system, and slightly reduce roughage digestibility (Pearson and Merritt, 1991). Interestingly, light exercise improves digestibility, while heavy work decreases digestibility (Olsson and Ruudevere, 1955). However, individuality also is an important factor when studying digestibility. Fonnesbeck et al. (1968) found differences in the ability of mature horses to digest protein even when consuming the same diets. Time of feeding and watering may also influence digestibility in horses.

Behavior problems associated with nutrition

Diets high in concentrate and low in forage offer a variety of possible welfare concerns. Two important concerns pertinent to weanlings are gastric ulcers and oral behavioral stereotypies. There is a high prevalence of gastrointestinal ulcers found in foals. Reportedly up to 51% of foals fed high concentrate – low fiber diets show some gastric lesions (Murray, 1989). Oral stereotypies such as crib-biting and wood-chewing in adult horses have been associated with high concentrate and low forage diets (McGreevy et al., 1995; Willard et al., 1977). Nicol et al. (2001) found crib-biting in foals to be associated with gastric inflammation and ulceration. Furthermore, it has been suggested that wood chewing may result from low fiber diets (Naenlein, 1969), boredom (Ralston et al., 1979), or malnutrition (Schurg et al., 1978). Clearly nutrition and feeding management are directly related to behavior welfare, but additional research is needed in this area to determine the best feeds and methods of feeding for weanling horses of varying uses.

Physiological problems associated with nutrition

Limiting nutrients in young, growing horses can be detrimental to both quantity and quality of growth. It is commonly agreed that for maximum growth rates, roughage or pasture must be supplemented with concentrates. Dawson et al. (1945) found a delayed maturity and stunted skeletal development in horses fed only Western range forages from birth through three years of age. Similarly, foals fed a diet containing 9% protein from 120 d of age to 260 d showed very low average daily gain, low average daily feed intake, high feed to gain ratio, and decreased height and cannon bone circumference compared to young horses fed diets consisting of 14 and 20% protein (Schryver et al., 1986). Protein values below the NRC recommendations cause decrease growth parameters (average daily gain, wither height, hip height, cannon bone circumference), but levels fed above the NRC requirements have not shown any differences in growth if energy and other nutrients are sufficient (Schryver et al., 1986). Although excess protein can be used for energy, non-protein nitrogen such as urea can be lethal if fed at high levels (Hintz et al., 1970).

Underfeeding the weanling may result in problems associated with mineral and vitamin deficiencies (Hintz, 1996). Furthermore, energy deficiency in weanlings results in a slow rate of growth and a general unthrifty appearance (Ellis and Lawrence, 1979). Feeding restrictions may also delay closure time of the epiphyseal cartilage of proximal and intermediate phalanges and third metacarpus (Ellis and Lawrence, 1978; Rezende et al., 2000). However, 6-mo-old horses, malnourished for 4 mo, can recover when fed properly through compensatory gains (Ellis and Lawrence, 1979). The young horses reportedly were able to attain body weights and heights similar to the horses fed adequate levels of energy and protein. It is unclear what, if any, long-term problems may be associated with early malnutrition.

In addition to obesity, overfeeding energy may result in problems associated with the integrity of growth. Overfeeding young, growing horses can lead to horses that may appear healthy, but develop severe problems from excess strain on their developing frames. Weanlings that grow and mature quickly are often prized in the equine industry because of the possibility of beginning training and competitive careers at a younger age. The growth potential is further embellished by feeding high concentrate diets with high concentrations of energy.

A high nutritional plane and rapid growth have been associated with developmental orthopedic diseases (Hintz and Kallfelz, 1981; Wright and Pickles, 1991). Ideally, growth of a foal should follow a smooth curve, being steepest in the first few months of age and gradually leveling out after about 2 years of age (NRC, 1989). Most breeds today reach about 90% of their mature body weight by 18 mo of age (Frape, 1986). Jelen et al. (1996) describes growth from birth to 20 mo in four phases: birth to 1, 1 to 12, 12 to 15, and 15 to 20 mo. Since the average daily gain changes during each phase of growth, this may be especially important when formulating diets for growing equines.

High starch, low fiber diets

High starch, low fiber diets are associated with rapid growth and often increased weight gain and strain on the growing bone structure. Diets high in starch cause changes in serum insulin, thyroxine, and triiodothyronine, which slow cartilage maturation (Glade and Belling, 1984; Glade and Luba, 1987). Rezend et al. (2000) found foals supplemented with a concentrate mix prior to weaning until 12 mo had lower frequency of epiphyseal cartilage alterations and better cortical index when compared to foals not receiving the concentrate mix until after weaning. This is presumably because of mineral deficiencies and their association with epiphysitis. Interestingly, the amount of concentrate in the diets of the weanlings from weanling until 12 mo was still comprised of less than 70% concentrate in the total diet.

There may also be a link between above-average weight gains and the onset of epiphysistis and other related disorders (Thompson et al., 1987). The increased force on the bone structure from the heavy body weights may impair normal cartilage and bone development. An 8-year study in dogs found that maintaining body condition scores of dogs at or slightly below what is currently considered to be optimal had decreased incidence of degenerative joint disease throughout the lives of the dogs (Smith et al., 2001). Developmental orthopedic disorders in horses can have huge economic repercussions in the horse industry such as lower sale prices of young horses and decreased performance potential (Thatcher, 1991).

Feeding weanlings

The optimal growth rate for horses to produce maximal soundness and future performance remains unclear. The NRC (1989) is the most frequently used compiling of equine nutritional research and associated recommendations for feeding classes of horses. Producers feeding weanlings can feed for either moderate or fast growth with the primary difference between diets being the level of energy intake. A weanling that is expected to reach a 500 kg mature weight should consume 15 Mcal DE, 750 g crude protein, 29 g calcium, and 16 g phosphorous daily to meet the requirements if being fed for moderate growth. On the other hand, a weanling that is being fed for fast growth is expected to consume 17.2 Mcal DE, 860 g crude protein, 36 g calcium, and 20 g phosphorous. Feeding these values will have a daily expected gain of 0.65 and 0.85 kg/d, respectively.

The expected feed consumption per day for weanlings is 2.0 to 3.5% body weight with recommendations of 0.5 to 1% BW consumed as forage and 1.5 to 3% BW consumed as concentrate (NRC, 1989). These values were created as guides for managers, but factors such as processing of feeds, level of intake, disease, previous nutritional status, work, and individuality can have an affect on the individual equine to increase or decrease these requirements.

More recently, Ott et al. (2002) provided evidence that the NRC (1989) values for dry matter intake for weanling horses are 4 to 13% too high and the DE requirements are 4 to 12% too high. The protein requirements may also be 2 to 20% in excess of the actual needs of weanlings (Ott et al., 2002). Monitoring intake and determining digestion of different feedstuffs in weanling horses will aid in the determination of more specific recommendations for feeding weanlings for growth which promotes healthy, functional horses.

CHAPTER 2

MATERIALS AND METHODS

Animals and Management

Twenty-four horses (12 Quarter Horses and 12 Arabians) at 5 mo of age and again at 8 mo of age (1 Quarter Horse was no longer available) were used to study the digestibility of three diets. Arabians (Arabs) and Quarter Horses (QH) were obtained from and housed at the Michigan State University Horse Teaching and Research Center (HTRC) and the MSU Merillat Equine Center (MEC), respectively. Digestibility studies consisted of a 10-d pre-trial adjustment period followed by a 4-d total fecal collection. The study was approved by the Michigan State University Animal Care and Use Committee (05/02-018-00).

Weanlings were stratified by breed, date of birth, and gender to avoid confounding factors previously identified by Heusner (1992) and Hintz et al. (1979) and were randomly assigned to one of three treatments (Table 1). Weanlings were age-matched in four groups of five to seven horses, such that each group entered the study at a mean age of 5 mo ± 16 d.

Before the start of the digestibility studies, both QH and Arabs were group-fed and group-housed in mixed grass pastures with free access to water, mineralized salt blocks, and shelter. Prior to the start of the 5 mo digestibility study, all QH (n=12) were consuming approximately 3.77 kg commercial concentrate mix (Strategy; Purina Mills Inc., St. Louis, MO) and 2 kg alfalfa-grass mixed hay (Table 2). Before the 8 mo digestibility study, all QH (n=11) were consuming approximately 2 kg commercial concentrate mix (Strategy; Purina Mills Inc., St. Louis, MO), 3 kg mixed grass pasture, and 2.5 kg grass hay (Table 2). Arabs (n=12 throughout) at both 5 and 8 mo were consuming free choice mixed grass hay, whole oats, and protein pellets in addition to the mixed grass pastures available (Table 2).

At the start of both digestibility trials, weanlings were separated into treatment groups and moved into dry-lots for a 10-d adjustment period. This period allowed for the elimination of any feed eaten prior to the start of the 4-d fecal collection period and let the weanlings become accustomed to the feeds and feeding methods used in the study. Quarter horses were moved into 992 to 1,488 m^2 dry-lots with one to three horses per lot, while Arabians were moved into 4,046 to 6,070 m^2 dry-lots with two horses per lot. During this time, all horses had free access to water, mineralized salt blocks, shelter, and their respective treatment diet. Metal, three-sided sheds with dirt flooring were provided for shelter. Feed was placed into large rubber feed tubs located inside the sheds. Horses and feed were checked twice daily and a minimum of 12 kg feed was maintained in each tub to assure free access to feed.

Following the adjustment period, horses were moved into individual stalls for a 4-d fecal collection period. Quarter Horses were moved to 3.7 x 3.7 m stalls at MEC while Arabians were moved into 4.0 x 3.4 m stalls at HTRC. All stalls were swept, vacuumed, and completely lined with clean rubber mats to remove dirt and debris. Stalls were also hand-swept a minimum of three times each day during the 4-d collection. All horses had free access to water, feed, and a mineralized salt block. Water was provided in 19 l buckets filled ½ to ¾ full, while feed and the mineralized salt block were placed in plastic feeders mounted in the stalls.

Treatments

The treatment diets were custom-formulated, mixed, and pelleted for this research project by Hamilton Feeds (Hamilton, MI). Complete diet pellets (3.18 mm diameter) for each treatment diet were prepared for the entire project in three respective batches. The diets consisted of concentrate and roughage in the following ratios: 70:30 (High Con); 50:50 (Equal); 30:70 (Low Con). The roughage portion of each diet contained an equal amount of alfalfa hay and timothy grass hay acquired from the same source for all diets. The concentrate portion of the diets was formulated using primarily corn, soybean meal and oats to best represent feeds commonly used in the industry (Table 3). Each treatment diet was nutritionally balanced to meet the NRC (1989) requirements for weanlings. Diets were not isonitrogenous or isocaloric; however, nutrient to calorie ratios of Mcal, CP, lysine, Ca, and P to DE were constant across treatments (Table 4).

Measurements and Sample Collection

Horses were weighed on a digital scale at the start of the adjustment period and at the start and end of the collection period. Wither height, hip height, and cannon bone circumference were also measured at the end of the collection period, as indicators of the size of the horses at different ages and locations (Appendix Table 1A). Feed consumption during the adjustment period was calculated by subtracting the total weight of the orts from that of the total feed given over the 10-d period (Appendix Table 2A).

Horses were monitored continuously throughout the 4-d (96 h) fecal collection and stalls were checked at least every 15 min. Each 24-h period began at 0900. The time of each defecation and urination was recorded for each horse. Urine was removed from stalls via a commercial wet-dry vacuum to avoid contamination of feces. Fecal matter was immediately collected from the stall floor with a treatment specific plastic dustpan and rubber gloves, weighed on a digital scale to the nearest 0.01 kg, and placed in an airtight labeled plastic bag on ice in a cooler. At the end of each 24-h period, fecal material collected from each horse was thoroughly hand-mixed and a 1 kg sample was retrieved and frozen in a commercial freezer (below -4°C) for future analysis.

During the 4-d fecal collection period, feed, a mineralized salt block, and water were provided free choice as in the 10-d adjustment period. Water consumption was gravimetrically measured every 8 h. Feed or water were removed from the stall for weighing a maximum of 15 min to limit the time horses did not have access to them. At the start of the 4-d collection period, horses were fed their respective treatment diets in amounts equal to 3.5% of their body weight (BW). Daily feed intake was determined at the end of each 24 h and each horse was then fed 5% more feed than they had consumed on the previous day to ensure that feed was not restricted. Feed buckets in each stall were checked when fecal matter was collected or urine was removed. If a horse had consumed all of the feed presented, such that only feed particles (no full pellets) remained in the feeder before 0900, more feed was added in 1 kg increments.

Laboratory Analyses

Sample preparation and dry matter

Feed samples were ground in a Wiley mill and passed through a 2 mm screen and fecal samples were ground in a Cyclone mill and passed through a 1 mm screen. Feed samples from each sample period were analyzed on an as fed-basis, while fecal samples were individually freeze dried prior to grinding. Initial analysis showed no difference in nutrient analysis between fecal samples collected each day during the fecal collection period, thereafter d 3 and 4 fecal samples were combined for each horse by taking sub-samples from d 3 and 4 in equal proportions to the total amount of fecal matter defecated (total g DM) each day. All samples were analyzed in duplicate. Values were averaged and coefficient of variance (CV) was accepted at less than 5%, 2%, 2%, and 5% for CP, ADF, NDF, and energy, respectively. If CV was greater than the accepted rate, two additional samples were analyzed, then the highest and lowest values were dropped and the two remaining values were averaged to obtain a new CV. This process was repeated until the CV was at an acceptable level for the given measurement. Dry matter (DM) values for all feed and fecal samples during each trial were determined by drying 2.5 to 3.0 g samples in a laboratory oven at 105°C for 24 h.

Crude protein

Crude protein of feed and fecal samples were determined by a non-dispersive, infrared, microcomputer based machine, designed to measure nitrogen content in samples (LECO® FP-2000 Nitrogen/Protein Analyzer). Samples were weighed (0.4 to 0.5 g) and placed in a ceramic boat prior to being placed into the machine. The machine combusted each sample by pushing samples into a combustion chamber filled with oxygen gas and thus converting elemental nitrogen into NO_x gasses. This gas entered a series of chambers for cooling before two gases (Lecosorb® and anhydrone) were released to remove CO_2 and H_2O, respectively, leaving N_2 and helium. The gasses were then measured and compared to determine a voltage which was then processed and given as a nitrogen measurement by the computer. The ceramic boats were later removed from the machine and cleaned. Nitrogen content was automatically calculated to CP values by the machine.

Fiber

Wet chemistry processes were used to determine ADF and NDF of the samples. ADF was determined as a fraction of dry matter (Goering and Van Soest, 1970).

$$\text{ADF (g)} = \frac{\text{(crucible and ADF residue weight)} - \text{(crucible and ash residue weight)}}{\text{g of sample dry matter}}$$

Samples (0.5 and 1.0 g for fecal and feed, respectively) were placed into 600 ml Berzelius beakers. Acid-detergent solution (100 ml) was then added prior to beakers being set on a refluxing apparatus and heated to boiling for 60 min. Beaker contents were then poured into crucibles containing 5 ml sand that were vacuumed and rinsed with hot (90 to 100°C) water. Each crucible was then washed with acetone and allowed to air dry before being placed in a laboratory oven (100°C) for 8 h. Crucibles were hot weighed before they were ignited in a muffle oven kept at 500°C for 5 h. After cooling to 100°C, crucibles were again hot weighed.

Similarly, NDF was determined as a fraction of dry matter (Goering and VanSoest, 1970; Robertson and VanSoest, 1977; Cherney et al., 1989).

$$\text{NDF (g)} = \frac{\text{(crucible and NDF residue weight)} - \text{(crucible and ash residue weight)}}{\text{g of sample dry matter}}$$

Samples (0.5±0.01 g) were placed into 600 ml Berzelius beakers. Sodium sulfite (0.5 g), neutral-detergent solution (100 ml), and heat stable amylase (2 ml) were then added prior to beakers being set on a refluxing apparatus and heated to boiling for 60 min. Two ml of working amylase was added to each beaker after being removed from the refluxing apparatus. Beaker contents were then poured into crucibles containing 5 ml sand that were vacuumed and rinsed with hot (90 to 100°C) water. Each crucible was then washed with acetone and allowed to air dry before placing crucibles in a laboratory oven (100°C) for 8 h and then hot weighed. Crucibles were then ignited in a muffle oven kept at 500°C for 5 h. After cooling to 100°C, crucibles were hot weighed.

Energy

An oxygen bomb calorimeter (Parr Instrument Company Inc., Moline, Il.) was used to determine the energy content of feed and fecal samples. Wiley milled samples were pressed into pellets (0.7 to 1.1 g for fecal and 0.9 to 1.1 g for feed) and weighed just prior to loading into the bomb calorimeter. Bensoic acid, the standard, was analyzed before the first sample and each time a group of samples were analyzed more than one week apart. Samples were loaded into the combustion capsule with a 10 cm fuse wire in contact with the sample loaded into the bomb head. Capsules were then filled with oxygen to 32 atm.

The combustion capsule was placed in a calorimeter bucket filled with 2,000 ml distilled water and placed into the calorimeter. Two ignition wires were attached to the terminal sockets on the bomb head, the calorimeter was covered, and an initial temperature was taken. At least 6 min after igniting the calorimeter, the temperature was taken. The unburned pieces of fuse wire were removed from the bomb electrodes, straightened, and the length measured. The energy equivalent of the bombs A and B were 2,400 and 2,398 cal/°C, respectively.

$$\text{Heat of combustion (cal/g)} = \frac{tW - \text{(calories of wire burned)}}{\text{g of pellet sample}}$$

t = temperature final – temperature initial
W = energy equivalent of the bomb used

Apparent Digestibility

Apparent digestibility of CP, ADF, NDF, and energy was calculated by the following equations on a DM basis:

$$\text{CP, ADF, or NDF Digestibility (\%)} = \frac{\text{g in feed} - \text{g in feces}}{\text{g in feed}} \times (100)$$

g in feed = g consumed/d * (% nutrient in feed sample)
g in feces = g defecated/d * (% nutrient in feces sample)

$$\text{Energy digestibility (\%)} = \frac{\text{Mcal energy in feed} - \text{Mcal energy in feces}}{\text{Mcal energy in feed}} \times (100)$$

$$\text{Digestible Energy (Mcal/kg)} = \frac{\text{Mcal energy in feed} - \text{Mcal energy in feces}}{\text{Mcal energy in feed}} \div (BW)$$

Mcal energy digested = Mcal energy in feed – Mcal energy in feces

Energy in feed = Mcal consumed/d * (% energy in feed samples)
Energy in feces = Mcal defecated/d * (% energy in fecal samples)

Statistical Analyses

Significance of differences between means were determined for treatments, age, breed, sex, treatments within age, and breeds within age were determined using PROC MIXED (SAS, 2001). A repeated-measure design was used with treatment, breed, and sex as the main effects and time as the repeated effect. The LSMEANS test was used to compare means. A P-value of less than 0.05 was considered significant, and a P-value of less than 0.10 was considered a trend.

Table 1: Horses used in digestibility studies: horse number, breed, date of birth (DOB), sex, and treatment

Horse Number	*Breed*	*DOB*	*Sex*	*Treatment*
1	QH	1/19/02	Colt	Low Con
2	QH	1/30/02	Colt	Equal
3	QH	2/2/02	Filly	Low Con
4	QH	2/23/02	Colt	High Con
5	QH	2/23/02	Filly	High Con
6	QH	2/25/02	Filly	Equal
7	QH	3/3/02	Colt	High Con
8	QH	3/20/02	Colt	Equal
9	QH	3/26/02	Filly	Low Con
10	QH	4/3/02	Filly	Low Con
11	QH	4/10/02	Colt	High Con
12*	QH	4/20/02	Colt	Equal
13	Arab	3/31/02	Colt	Equal
14	Arab	4/2/02	Filly	High Con
15	Arab	4/5/02	Colt	High Con
16	Arab	4/8/02	Colt	Equal
17	Arab	4/19/02	Filly	Low Con
18	Arab	4/19/02	Filly	Low Con
19	Arab	5/9/02	Filly	Equal
20	Arab	5/13/02	Filly	Equal
21	Arab	5/14/02	Filly	Low Con
22	Arab	5/18/02	Colt	Low Con
23	Arab	5/21/02	Colt	High Con
24	Arab	5/23/02	Filly	High Con

* Horse not available, therefore not used in 8 mo digestibility study

Table 2: Diets fed to Quarter Horses (QH) and Arabians (Arab) prior to starting the digestibility studies at 5 and 8 mo.

Feed Ingredient (%)	*QH, 5 mo*	*QH, 8 mo*	*Arabs, 5 mo*	*Arabs, 8 mo*
Concentrate				
Whole Oats	-	-	45	30
Protein Pellet	-	-	10	10
Purina Strategy	65	20	-	-
Roughage				
Grass Pasture	-	40	45	5
Grass Hay	-	30	-	50
Mixed Hay*	35	-	-	-

* Mixed refers to grass and alfalfa mix

Table 3: Percentage of ingredients and laboratory analysis of CP, ADF, and NDF for each treatment diet, High Con, Equal, and Low Con

Ingredients (%)	*High Con*	*Equal*	*Low Con*
Corn	19.00	30.00	29.15
Oats	43.00	14.60	0.00
Soybean Meal	6.00	4.00	0.00
50:50 Hay Mix	30.00	50.00	70.00
Lysine	0.09	0.15	0.23
Buckeye® Vit/Min	0.15	0.15	0.15
Salt	0.06	0.05	0.08
Dicalcium Phosphate	0.55	0.55	0.00
Limestone	1.15	0.45	0.00
Phosphate	0.00	0.05	0.40
Nutrients (%)			
CP	14.00±0.20	15.00±0.20	12.80±0.20
ADF	16.40±0.60	17.50±0.60	22.90±0.60
NDF	29.80±0.40	29.20±0.40	35.40±0.40

Table 4: Calculated nutrient to calorie ratios for CP, Lysine, Ca, and P of the treatment diets: High Con, Equal, and Low Con

Measurements	*High Con*	*Equal*	*Low Con*
g CP/ DE Mcal	51.9	52.0	52.0
g Lysine/ DE Mcal	2.2	2.3	2.2
g Ca/ DE Mcal	3.1	3.1	3.1
g P/ DE Mcal	1.5	1.5	1.5

CHAPTER 3

RESULTS

<u>Treatment effects (age, breed, and sex combined)</u>

Body weight, feed intake, and water intake were not different (P=0.89, P=0.96, and P=0.37, respectively) between treatments when age, breed, and sex were combined (Table 5). However, fecal DM was greater in the High Con group compared to the Equal and Low Con groups (P<0.05). There was a trend of greater fecal output by the weanlings consuming the Low Con diet compared to those consuming the High Con diet (P=0.09). Although the number of times per day horses urinated was not related to treatment, the frequency of defecation was related to treatment (P<0.01); horses in the Low Con treatment defecated more often than those consuming High Con and Equal diets (Table 5).

Crude protein apparent digestibility was affected by treatment (P<0.01), with horses consuming the High Con digesting more protein than those consuming Low Con (Table 6). Apparent digestibility of ADF did not differ between treatments with values of 32.6 ± 2.9%, 33.3 ± 2.9%, and 39.7 ± 3.0% for High Con, Equal, and Low Con, respectively (P=0.21; Table 6). However, horses fed the Low Con tended to digest a higher percentage of NDF than both the Equal and High Con treatments (P=0.09; Table 6). Horses in the High Con treatment tended to digest a higher percentage of energy than those in the Low Con treatment (P=0.06; Table 6).

Age effects (breed and sex combined)

The body weight of weanlings increased from 5 mo to 8 mo ($P<0.01$; Table 7). There were no differences in body weight among treatments ($P=0.97$), with an average body weight for all weanlings at 5 and 8 mo of 200 ± 4 kg and 259 ± 4 kg, respectively. Feed intake was the same across treatments and ages, but weanlings drank more water at 5 mo than 8 mo ($P<0.01$). When treatments were combined, feces from weanlings at 5 mo contained a higher fecal dry matter, although there were no differences in fecal output. Weanlings urinated and defecated more frequently at 5 mo than 8 mo, regardless of treatment ($P<0.05$; Table 7). At 8 mo, there was a trend for the horses consuming High Con to urinate more frequently than horses consuming Equal and Low Con diets.

Weanlings fed the Low Con diets digested more CP at 5 mo than at 8 mo ($P<0.05$; Table 8). Weanlings at 5 mo fed High Con digested more protein than those fed Equal and Low Con diets. Similarly, weanlings at 8 mo fed the High Con diet digested more CP than the Low Con diet. In contrast to CP, the apparent digestibility of ADF, NDF, and energy did not differ with age when treatments were compared.

Breed effects (treatment and sex combined)

The body weight of QH was 10% and 23% greater than Arabs at 5 and 8 mo, respectively (Table 9). At 5 mo, the Arabs consumed more than the QH, while at 8 mo the QH consumed more than the Arabs. Water intake was similar in Arabs and QH at 5 and 8 mo. In Arabs, percent fecal dry matter was higher (27.0±0.4%) than in QH (24.7±0.4%; P<0.01). Although the Arabs showed a decrease in the percent fecal dry matter from 5 mo to 8 mo (P<0.01), the QH did not show a change in percent fecal dry matter with age (P=0.96). Interestingly, Arabs at 5 mo had a higher fecal matter output than at 8 mo, but the QH had a higher fecal matter output at 8 mo than at 5 mo (P<0.01). Although QH urinated more frequently than Arabs, defecation was similar, with the Arabs at 8 mo defecating less frequently than Arabs at 5 mo, QH at 5 mo, and QH at 8 mo.

Arabs digested more CP than QH when ages were combined (P<0.05), and tended to digest more energy than QH (P=0.07; Table 9). The digestibility of ADF and NDF did not differ between breeds at 5 and 8 mo.

Sex effects (treatment, age, and breed combined)

Body weight (P=0.69), feed intake (P=0.23), and water intake (P=0.66) were not different between fillies and colts (Table 10). Fillies at 8 mo consumed more feed (29±1 g/kg BW/d) than fillies at 5 mo (26±1 g/kg BW/d). Fillies had higher fecal dry matter than colts, but the fecal output was similar between sexes. QH fillies urinated more often (15±1 times/d) than QH colts (11±1 times/d), Arab fillies (7±1 times/d), and Arab colts (8±1 times/d; P<0.05). Quarter Horse colts also urinated more often than Arab colts (P<0.05). Overall, fillies and colts urinated the same number of times each day, but colts defecated more often than fillies.

CP digestibility tended to be higher in fillies than in colts (P=0.08; Table 10). There was no difference in digestibility of ADF, NDF, or energy between fillies and colts.

Table 5: Mean ± SE body weight, feed intake, water intake, fecal output, frequency of urination, and frequency of defecation of all weanlings in High Con, Equal, and Low Con treatments with age, breed, and sex combined.

Measurement	*Treatment*	*Mean ± SE*
Body Weight (kg)	High Con	229±7
	Equal	233±7
	Low Con	228±7
Feed Intake (g/ kg BW/d)	High Con	29±1
	Equal	29±1
	Low Con	28±1
Water Intake (g/ kg BW/d)	High Con	55±5
	Equal	63±5
	Low Con	63±5
Fecal DM (%)	High Con	27.6±0.5[a]
	Equal	25.4±0.5[b]
	Low Con	24.6±0.5[b]
Fecal Output (g/ kg BW/d)	High Con	8.0±0.4[y]
	Equal	8.6±0.4[xy]
	Low Con	9.3±0.4[x]
Urination (times/day)	High Con	11±1
	Equal	10±1
	Low Con	10±1
Defecation (times/day)	High Con	11±1[b]
	Equal	12±1[b]
	Low Con	16±1[a]

[ab]Values within a measurement lacking common superscripts differ ($P<0.05$)

[xy]Values within a measurement lacking common superscripts tend to differ ($P<0.10$)

Table 6: Mean ± SE CP, ADF, NDF, and energy apparent digestibility of all weanlings in High Con, Equal, and Low Con treatments with age, breed, and sex combined.

Measurement	*Treatment*	*Mean ± SE*
	High Con	73.9±1.6[a]
CP Digested	Equal	68.8±1.6[ab]
(%)	Low Con	63.9±1.6[b]
	High Con	32.6±2.9
ADF Digested	Equal	33.3±2.9
(%)	Low Con	39.7±3.0
	High Con	34.8±2.3[y]
NDF Digested	Equal	34.7±2.2[y]
(%)	Low Con	41.4±2.3[x]
	High Con	72.1±1.4[x]
Energy Digested	Equal	69.7±1.3[xy]
(%)	Low Con	67.0±1.4[y]

[ab]Values within a measurement lacking common superscripts differ ($P<0.05$)

[xy]Values within a measurement lacking common superscripts tend to differ ($P<0.10$)

Table 7: Mean ± SE body weight, feed intake, water intake, fecal dry matter, fecal output, and frequency of urination and defecation of horses in High Con, Equal, and Low Con treatments at 5 and 8 mo.

Measurements	*Treatments*	*5 mo*	*8 mo*
	High Con	199 ± 7^{a}	259 ± 7^{b}
Body Weight*	Equal	203 ± 7^{a}	259 ± 7^{b}
(kg)	Low Con	199 ± 7^{a}	259 ± 7^{b}
	High Con	28±1	29±1
Feed Intake	Equal	28±1	29±1
(g/ kg BW/d)	Low Con	28±1	29±1
	High Con	61±4	50±4
Water Intake*	Equal	69±4	59±4
(g/ kg BW/d)	Low Con	64±4	62±4
	High Con	28.7±0.5	26.9±0.5
Fecal DM*	Equal	25.5±0.5	25.4±0.5
(%)	Low Con	25.0±0.5	24.1±0.5
	High Con	7.9±0.3	8.1±0.3
Fecal Output	Equal	8.5±0.3	8.3±0.3
(g/ kg BW/d)	Low Con	9.5±0.3	9.1±0.3
	High Con	12 ± 1^{x}	11 ± 1^{x}
Urination*	Equal	11 ± 1^{x}	9 ± 1^{y}
(times/day)	Low Con	12 ± 1^{x}	9 ± 1^{y}
	High Con	11±1	10±1
Defecation*	Equal	13±1	11±1
(times/day)	Low Con	16±1	15±1

^{ab} Values within a measurement lacking common superscripts differ ($P<0.05$)

^{xyz} Values within a measurement lacking common superscripts tend to differ ($P<0.10$)

* Overall 5 mo different than 8 mo, regardless of treatment: $P<0.05$

Table 8: Mean ± SE CP, ADF, NDF, and energy apparent digestibility of horses in High Con, Equal, and Low Con treatments at 5 and 8 mo.

Measurements	*Treatments*	*5 mo*	*8 mo*
	High Con	74.6±1.8[a]	73.2±1.8[ab]
CP digestibility*	Equal	68.7±1.8[bc]	69.0±1.8[bc]
(%)	Low Con	67.7±1.8[c]	60.1±1.9[d]
	High Con	29.4±3.4	36.0±3.3
ADF digestibility	Equal	30.1±3.4	35.9±3.3
(%)	Low Con	40.3±3.4	40.0±3.6
	High Con	32.6±2.9	37.0±2.8
NDF digestibility	Equal	33.2±2.9	36.3±2.9
(%)	Low Con	41.5±2.8	41.3±3.1
	High Con	71.5±1.2	72.6±1.2
Energy Digested	Equal	69.2±1.2	71.1±1.2
(%)	Low Con	67.3±1.2	66.7±1.3

[abcd] Values within a measurement lacking common superscripts differ (P<0.05)

* Overall 5 mo greater than 8 mo, regardless of treatment: P<0.05

Table 9: Mean ± SE body weight, feed intake, water intake, fecal dry matter, fecal output, frequency of urination and defecation, and CP, ADF, NDF, and energy apparent digestibility of Arabians and Quarter Horses at 5 and 8 mo.

Measurements	*Arabians*		*Quarter Horses*	
	5 mo	*8 mo*	*5 mo*	*8 mo*
Body Weight (kg)*	190±6[d]	246±6[b]	211±7[c]	273±7[a]
Feed Intake (g/kg BW/d)	30±1[a]	27±1[b]	26±1[b]	31±1[a]
Water Intake (g/kg BW/d)	59±4	54±4	70±4	59±4
Fecal DM (%)	27.8±0.4[a]	26.2±0.4[b]	24.7±0.4[c]	24.7±0.4[c]
Fecal Output (g/kg BW/d)	9.1±0.3[a]	8.0±0.3[b]	8.1±0.4[b]	9.3±0.4[a]
Urination (times/day)*	9±1	6±1	14±1	12±1
Defecation (times/day)	14±1[a]	11±1[b]	13±1[a]	13±1[a]
CP digested (%)*	73.0±1.5	69.4±1.5	67.6±1.5	65.5±1.5
ADF digested (%)	36.0±2.9	39.8±2.6	31.0±2.8	34.2±2.9
NDF digested (%)	37.9±2.4	40.4±2.2	33.6±2.4	36.0±2.5
Energy digested (%)[†]	70.6±1.2	71.7±1.2	67.6±1.2	68.4±1.2

[abcd] Values within a measurement lacking a common superscript differ (P<0.05)

* Overall differences between breeds when 5 and 8 mo combined, regardless of age: P<0.05

[†] Overall trend in differences between breeds, regardless of age: P<0.05

Table 10: Mean ± SE body weight, feed intake, water intake, fecal dry matter, fecal output, frequency of urination and defecation, and CP, ADF, NDF, and energy apparent digestibility of fillies and colts (Arabs and QH combined).

Measurements	*Fillies*	*Colts*
Body Weight (kg)	228±6	232±6
Feed Intake (g/kg BW/d)	26±1	30±1
Water Intake (g/kg BW/d)	59.3±3.8	61.7±3.7
Fecal DM (%)	26.5±0.4[a]	25.2±0.4[b]
Fecal Output (g/kg BW/d)	8.4±0.3	8.9±0.3
Urination (times/day)	11±1	10±1
Defecation (times/day)	12±1[a]	14±1[b]
CP digested (%)	70.6±1.3	67.1±1.3
ADF digested (%)	36.3±2.4	34.1±2.3
NDF digested (%)	38.6±1.9	35.4±1.9
Energy digested (%)	70.6±1.1	68.5±1.1

[ab] Values within a measurement lacking a common superscript differ ($P<0.05$)

CHAPTER 4

DISCUSSION

Weight gain of horses did not differ between treatments or between sexes. This similarity in body weights between colts and fillies is consistent with past research in which differences are usually not found until the animals are at least 18 mo of age (Saastamoinen, 1990). QH were heavier than Arabs at 5 and 8 mo; probably because QH will reach a mature weight of 450 to 600 kg, while Arabs will reach a mature weight of 363 to 450 kg (Evans, 1999). Thoroughbred foals from Canada, Ireland, and Kentucky at 5 and 8 mo are reported to average 234±10 and 280±10 kg, respectively (Pagan et al., 1996; Jelan et al., 1996). In the current study the QH and Arabs weighed less than the Thoroughbreds at 5 mo, although the QH weighed about the same as the Thoroughbreds at 8 mo, while the Arabs still weighed less.

Treatment diets were formulated with feedstuffs frequently used in the industry, such as corn, oats, and alfalfa-timothy grass hay. In addition, the ratios of concentrate to roughage were determined to mimic those recommended by the NRC, commonly fed in the industry, and representing a high roughage diet. Providing young horses ad libitum access to feed is commonly used in the industry to encourage consumption and growth. Furthermore, providing ad libitum access to feed may also decrease the intake of large glucose-yielding carbohydrates over short time periods and help decrease hyperinsulinaemia, which is often reported in horses that are meal-fed (Frape, 1989).

Decreasing hyperinsulinaemia may aid in preventing developmental orthopedic disorders in young horses (Murphy et al., 1997).

Cymbaluk (1989) found that the energy density of the diet was an important determinant of feed intake with horses. Weanlings fed a high fiber diet ate up to 26% more feed than those fed a high concentrate diet (Cymbaluk, 1989). However, in the current study, the nutrient composition of the treatment diets did not alter the amount of feed consumed by the weanlings. Additionally, a preference study done by Hawkes et al. (1985) found that even though ponies demonstrated preferences, the total intake per meal was not greatly decreased when the less preferred feed is fed alone. Voluntary dry matter intake of ingested feeds may be difficult to predict from simple characteristics such as protein and fiber levels (Dulphy et al., 1997).

The primary difference between the previous study and the present study is the pelleting of the diets. Cymubaluk and Christensen (1986) found no difference in intake rates when mature horses were fed unpelleted and pelleted diets of alfalfa. However, rate of passage is faster with diets higher in fiber (Ellis, 2002), which may also increase the dry matter intake. Over a 63-d study, Hintz (1966) found that the pelleting of feed did not have an effect on the growth of young horses, and the diets were consumed, digested and utilized similarly to diets that were not pelleted.

Intake in horses is generally perceived to be regulated primarily by physical qualities of the forage such as taste, odor, toughness, and ease of sorting. Mature horses will ingest about 20.8 g DM/kg BW/d (Dulphy et al., 1997), while the growing horses in the current study consumed approximately 29 g/kg BW/d. These weanlings consumed the same amount of feed when fed different treatment diets, suggesting that they relied primarily on gut distension to regulate feed intake since the treatment diets had the same DM and similar weights per volume, but different energy, protein, and fiber concentrations. Feed intake was not different in horses at 5 and 8 mo, perhaps because weanlings at 5 and 8 mo are still growing at nearly the same rate (Jelan et al., 1996).

There were some factors in the study that could not be controlled; temperature, location, and diets fed between digestibility trials. Thus, some of the related data may not be biologically significant. For instance, weanlings drank more water at 5 mo than 8 mo, however, the ambient temperature probably had a greater influence than the age of the horses (Crowell-Davis et al., 1985; Scheibe et al., 1998). Horses were 5 mo of age during the months of July through October in Michigan with temperatures averaging 22°C, and horses were 8 mo of age during the months of October through January with temperature often below freezing (www.weatherchannel.com).

In the present study, water intake during the 4-d digestibility studies was not affected by the treatment diets. This is in contrast to a study by Fonnesbeck (1968) in which adult horses fed all-roughage diets consumed more water than those fed mixed concentrate-roughage diets presumably because of the varied fiber content in the diets as opposed to the percent DM in the diets. In the present study, all treatments contained varied fiber levels, but there were no differences in the percent DM of the treatments as there was in the Fonnesbeck (1968) study. In the current study, dietary fiber content did not affect water intake, but perhaps if the horses on the current study were fed the diets for a longer time or fed a non-pelleted diet, water intake would correlate with previous research.

Water excretion in feces has been associated with protein, mineral, and fiber content of the diet (Fonnesbeck, 1968). The current study found percent fecal DM was greatest in horses fed High Con which had the highest amount of protein digested and the least amount of fiber in the diet.

The quantity of feces is the main factor contributing to fecal water excretion; furthermore the quantity of feces depends on DM intake, DM digestibility, and DM of feces (Fonnesbeck, 1968). In the present study, weanlings consuming Low Con tended to have a greater fecal output than High Con due to the increased metabolic components (endogenous excretions) and residual (undigested) feed components (Maynard et al., 1979). This agrees with Fonnesbeck (1968) and more recently Gunter (1993).

Diets higher in fiber generally produce greater amounts of feed residue which require an increased amount of water for excretion and cause retention of more water as the bulky matter passes through the digestive system (Frape, 1998). Horses fed the Low

Con may have defecated more often simply to allow for the passage of the increased fecal matter as compared to those horses fed Equal and High Con.

Similar to Gibbs and Potter (2002), the High Con had higher apparent crude protein digestibility than Low Con at both 5 and 8 mo. This could be due to the increased availability of the protein in the high concentrate diet as opposed to the low concentrate diet, which was formulated to derive a higher percentage of its protein from the hay (roughage) portion of the complete feed. Interestingly, Slade (1970) reported that while apparent digestibility studies reported an increase in apparent nitrogen digestibility when nitrogen in the diet is increased, true digestibility studies (endogenous N is subtracted from the fecal N) report similar digestibility values for a range of dietary nitrogen concentrations. A correlation may exist between fecal DM and crude protein digestibility. In the present study, there was an increase in DM of the feces as crude protein digestibility increased. This trend has also been reported by Grenet et al. (1984).

Digestibility of ADF did not differ between treatments; however, horses fed the Low Con tended to digest a higher percentage of NDF than both the Equal and High Con treatments. This is in agreement with past research that indicates when grain proportions were increased in the total diet, the efficiency of fiber utilization decreased in adult horses (Martin-Rosset and Dulphy, 1987). Similarly, Drogoul et al. (2001) found that although the mean retention time of the ingesta in the gastrointestinal tract was higher in horses fed grain (concentrates) in addition to hay (roughages), the efficiency of fiber utilization was decreased in those horses fed a diet of both hay and grain. This most likely is partly due to the faster the passage of ingesta through the entire gastrointestinal tract as the hay to grain ratio consumed is increased (Drogoul et al., 2001). The extent of fiber digestion is determined by the time the fiber stays in the hindgut region, the rate and amount of microbial fermentation activity in the hindgut, the ratio of roughage to concentrate consumed, and the type and quality of forage fed.

Potentially the 10-d diet adaptation period was sufficient to encourage microbial growth and allow greater ADF digestion in horses fed the Low Con. Furthermore, Grenet et al. (1984) reported that the percentage of fecal DM may increase when forage fiber digestibility decreases. This relationship occurred; a trend to increase NDF digestibility in the Low Con resulted in a decrease in fecal DM from the horses fed the Low Con diet. Horses in the High Con treatment tended to digest a higher percentage of energy than those in the Low Con treatment. This has been found by many researchers, including more recently Drogoul et al. (2001).

The research protocol could be improved by eliminating the effects of location, temperature, and diets fed between digestibility studies. The number of horses was adequate especially if they could all be housed at the same facility to enable the comparison of breed without confounding effects. Since the 4-d digestibility study was done with horses in stalls, it may have been beneficial if the horses were individually stalled during the 10-d adjustment period. Pellets were excellent to assure consumption of exact ratios of concentrate to roughage while feeding ad libitum, but the pellets may have had an effect on digestion.

In conclusion, horses fed any of the three treatment diets consumed enough nutrients to meet the current nutritional standards for weanlings based on the amount consumed and the digestibility of nutrients in the treatments (Table 11; NRC, 1989). Cymbaluk (1989) found that horses fed free choice will consume 20 to 50% more energy than suggested by the NRC for moderate growth. In the current study, all three treatments consumed energy values suggested by the NRC (1989) but a long-term study with the diets may find different results. Ott et al. (2002) suggests feeding weanlings 1.54 to 1.70 kg of 90% DM concentrate for each 100 kg BW daily and providing ad libitum access to forage to consume adequate energy for maximum growth. The weanlings in the present study, in all three treatment groups, consumed about 2.9% of their BW daily which is equivalent to consuming 2.7 kg feed /100 kg BW/ d total feed for a 230 kg horse (average size of all weanlings) during the digestibility studies. Thus, only the High Con group consumed greater than 1.54 kg/100 kg BW/d of concentrate as suggested by Ott et al. (2002).

All weanlings consumed the NRC recommended amount per day of protein. Amino acid profiles were not explored beyond lysine and the amount of digested protein needed by weanlings is still unknown. Under usual feeding conditions with conventional feeds, if the lysine is adequate, then the other amino acids are usually considered adequate (Hintz, 1986). It has been suggested that the NRC (1989) requirements for dry matter intake, digestible energy, and protein are excessive for weanling horses (Ott et al., 2002). Furthermore, there are currently no recommendations for fiber. Although fiber should be fed to maintain health and welfare of the horse, too much fiber could dilute the energy and nutrients of the total diet. Thus, the complete diet for weanlings should be formulated to contain balanced nutrients for feeding ad libitum, but more importantly, must contain the digestible nutrients needed for maintenance and growth.

Table 11: Requirements for weanlings at 6 mo expected to reach 500 kg mature BW, but currently weighing 215 kg and consuming 2.9 % BW/d; NRC recommended diets compared to High Con, Equal, and Low Con treatment diets.

	NRC	*High Con*	*Equal*	*Low Con*
Concentrate: Roughage	70:30	70:30	50:50	30:70
Adjusted BW	215	215	215	215
% BW consumed	2 - 3.5	2.9	2.9	2.9
Kg/d consumed	4.3 - 7.5	6.2	6.2	6.2
GE Mcal energy	-	24.7	24.3	24.4
DE digested (Mcal)	15.0 - 17.2	17.8	17.1	16.3
CP consumed(g)	750 - 860	870	936	796
CP digested (g)	-	643	644	509
ADF consumed (g)	-	1023	1091	1428
ADF digested (g)	-	333	363	567
NDF consumed (g)	-	1858	1821	2207
NDF digested(g)	-	647	632	914

CHAPTER 5

IMPLICATIONS

Results of this study showed a difference in the ability of weanlings to digest protein and fiber in diets with varying concentrate to roughage ratios. Future research is needed to determine the amount of nutrients absorbed and utilized from the diets, not just the amount consumed and digested of the treatment diets.

The current requirements for equine diets are guided by feed values, not digestibility of the feeds in each class of horse. Given that the current study only documented differences during two short-term periods, a long-term feeding trial with the diets would be instrumental in determining how the diets would be absorbed and utilized, and if any of the diets would result in negative outcomes such as increased cost, behavior problems, decreased growth, increase incidence of developmental orthopedic disorders, or gastro-intestinal problems.

The current study indicates that any of the three diets would provide the weanlings with the nutrients at the levels currently recommended by the NRC (1989). Currently in the industry, many managers feed weanlings less than the NRC recommended 70% concentrate presumably to avoid possible problems associated with feeding high concentrate – low fiber diets (Gibbs and Cohen, 2001; Jelan et al., 1996). Furthermore, feeding diets high in fiber to weanlings prone to developmental orthopedic disorders may be beneficial. Feeding a high-fiber diet dilutes dietary energy concentrations and enables the diets to be fed free-choice which may reduce future behavioral problems and promote equine welfare. There is limited available research conclusively supporting either feeding high concentrate or high fiber diets to weanlings.

Horse managers can formulate feed to provide weanlings nutrition to suit immediate goals and future expectations of soundness and digestive health. Although the current study shows that the weanlings digested the needed nutrients, feeding high fiber diets to young horses should be done with caution. Long-term studies of the diets used in the study are not available, and such diets may not be beneficial to the weanling as it matures. A future multiple-year study to explore the cumulative effects of high concentrate or high fiber diets on growth, development, and performance would be extremely useful to horse managers. The optimum growth rate and nutrient intake for productivity and longevity has not been determined; however an optimal nutrient to energy ratio in the diet is most important (NRC, 1989).

CHAPTER 6

REFERENCES

Cherney, D.J.R., J.A. Patterson, and J.A. Cherney. 1989. Use of 2-ethoxyethanol and a-amylase in the neutral detergent fiber method of feed analysis. J. Dairy Sci. 72(11):3079-3084.

Crowell-Davis, S.L., K. Houpt, and J. Carnevale. 1985. Feeding and drinking behavior of mares and foals with free access to pasture and water. J. Anim. Sci. 60:883-889.

Cymbaluk, N. 1989. Effects of dietary energy source and level of feed intake on growth of weanling horses. Equine Pract. 11(9):29-33.

Dawson, W.M, R.W. Phillips, and S.R. Speelman. 1945. Effects of feeding only Western range forage to horses from birth through three years. J. Anim. Sci. 4:47-51.

De Fombelle, A., V. Julliand, C. Drogoul, and E. Jacotot. 2001. Feeding and microbial disorders in horses: 1 - Effects of an abrupt indorporation of two levels of barley in a hay diet on microbial profile and activities. J. Equine Vet. Sci. 21(9):439-445.

Drogoul, C., A. de Fombelle, and V. Julliand. 2001. Feeding and microbial disorders in horses: 2 - Effect of three hay:grain ratios on digesta passage rate and digestibility in ponies. J. Equine Vet. Sci. 21(10):487-491.

Dulphy, J.P, W. Martin-Rosset, H. Dubroeucq, and M. Jailler. 1997. Evaluation of voluntary intake of forage trough-fed to light horses. Comparison with sheep. Factors of variation and prediction. Liv. Prod. Sci. 52:97-104.

Earle, I.P. 1950. Comparison of complete diets for horses. J. Anim. Sci. 9:251-254.

Ellis, J.M., T. Hollands, and D.E. Allen. 2002. Effects of forage intake on bodyweight and performance. Equine Vet. J., Suppl. 34:66-70.

Ellis, R.N. and T.L.J. Lawrence. 1979. Energy and protein under-nutrition in the weanling filly foal. Br. Vet. J. 135:331-337.

Evans J.W., A. Borton, H. Hintz, and L.D. Van Vleck. 1999. The horse, 2nd ed. W. H. Freeman and Company. New York.

Faverdin, P., R. Baumont, K.L. Ingvartsen. 1995. Control and prediction of feed intake in ruminants. Recent developments in the nutrition of herbivores. Proc. 4th Int. Symp. Paris.

Fonnesbeck, P.V. 1968. Consumption and excretion of water by horses receiving all hay and hay-grain diets. J. Anim. Sci. 27:1350-1356.

Francis-Smith, K. and Wood-Gush, D.G.M. 1977. Coprophagy as seen in Thoroughbred foals. Equine Vet. J. 9:155-157.

Frape, D.L. 1986. Growth of the horse. Equine Nutrition and Feeding. Longman Scientific and Technical. England.

Frape, D.L. 1989. Nutrition and growth and racing performance of thoroughbred horses. Proc. of the nutrition society, 48:141-152.

Frape, D.L. 1998. Equine nutrition and feeding, Second edition. Blackwell Science Ltd, Oxford, UK

Galef, B.G. 1979. Investigation of the functions of coprophagy in juvenile rats. J. Comp. Physiol. Psychol. 93:295-305.

Gibbs, P. G., G. D. Potter, and G. T. Schelling. 1988. Digestion of hay protein in different segments of the equine digestive tract. J. Anim. Sci. 66:400-406.

Gibbs, P., and N. Cohen. 2001. Early management of race-bred weanlings and yearlings on farms. J. Equine Vet. Sci. 21:279-283.

Gibbs, P.G. and G.D. Potter. 2002. Review: Concepts in protein digestion and amino acid requirements of young horses. Prof. Anim. Sci. 18:295-301.

Glade, M.J. and T.H. Belling. 1984. Growth plate cartilage metabolism, morphology, and biochemical composition in over and underfed horses. Growth. 48:473-479.

Glade, M.J. and N.K. Luba. 1987. Serum triiodothyronine and thyroxine concentrations in weanling horses fed carbohydrate by direct gastric infusion. Am. J. Vet. Res. 48:578-561.

Goering, H.K. and P.J. VanSoest. 1970. Forage and Fiber Analysis. Agriculture Handbook no. 379. U.S. Dept. Agriculture.

Grenet, E., W. Martin-Rosset, and M. Chenost. 1984. Compared size and structure of plant particles in the horse and the sheep feces. Can. J. Anim. Sci. 64 (suppl.): 345-346.

Gunter, S.A., F.T. McCollum III, R.L. Gillen, and L.J. Krysl. 1993. Forage intake and digestion by cattle grazing midgrass prairie rangeland or sideoats grama/sweetclover pasture. J. Anim. Sci. 71:3432-3441.

Haenlein, G.F.W. 1969. Nutritive value of a pelleted horse ration. Feedstuffs. 39:19.

Hawks, J., M. Hedges, P. Daniluk, H.F. Hintz, and H.F. Schryver. 1985. Feed preferences of ponies. Equine Vet. J. 17(1):20-22.

Heusner, G. L. 1992. Effect of month of birth on the foals. J. Equine Vet. Sci. 12(5):297-300.

Hintz, H. F., and R.G. Loy. 1966. Effects of pelleting on the nutritive value of horse rations. J. Anim. Sci. 25:1059-1062.

Hintz, H.F., J.E. Lowe, A.J. Clifford, and W.J. Viseck. 1970. Ammonia intoxication resulting from urea ingestion by ponies. J. Amer. Vet. Med. Assoc. 157:963-966.

Hintz, H.F., H.F. Schryver, and J.E. Lowe. 1971. Comparison of a blend of mild products and linseed meal as protein supplements for young growing horses. J. Anim. Sci. 33:1274.

Hintz, H.F. H.F. Schryner, and J.E. Lowe. 1972. Digestion in the horse. A review of available information on anatomy, site of digestion, feed intake, and other factore. Feedstuffs, July 2, 1972.

Hintz, H. F., R. L. Hintz, and L. D. Van Vleck. 1979. Growth rate of Thoroughbreds. Effect of age of dam, year and month of birth, and sex of foal. J. Anim. Sci. 48(3):480-487.

Hintz, H.F. and Kallfelz, F.A. 1981. Some nutritional problems of horses. Equine Vet. J. 13:183-186.

Hintz, H.F. 1983. Horse Nutrition – A practical guide. New York: Arco Publ.

Hintz, H.F. 1986. Protein requirements of the weanling. Equine Pract. 8(7):5-7.

Hintz, H.F. 1996. Mineral requirements of growing horses. Pferdeheilkunde. 12(3):303-306.

Jelan, Z. A., L. B. Jeffcott, N. Lundeheim, and M. Osborne. 1996. Growth rates in Thoroughbred foals. Pferdeheilkunde. 12(3):291-295.

Julliard, V., A. de Fombelle, C. Drogoul, and E. Jacotot. 2001. Feeding and microbial disorders in horses: Part 3- Effects of three hay: grain ratios on microbial profile and activities. J. Equine Vet. Sci. 11:543-546.

Laut, J.E., K.A. Houpt, H.F. Hintz, and T.R. Houpt. 1985. The effects of caloric dilution on meal patterns and food intake of ponies. Physiol. Behav. 35:549-554.

Martin-Rosset, W. and J.P. Dulphy. 1987. Digestibility interactions between forages and concentrates in horses: Influence of feeding level – comparison with sheep. Livestock Production Sci. 17:263-276.

Maynard, A.B., J.K. Loosli, H.F. Hintz, R.G. Warner. 1979. Animal nutrition, 7th ed. McGraw-Hill Book Company. New York.

McGreevy, P. C.J. Nicol, P. Cripps, L. Green, and N. French. 1995. Management factors associated with stereotypic and redirected behavior in the Thoroughbred horse. Equine Vet. J. 27:86-91.

Murphy, D., S.W. Reid, and S. Love. 1997. The effects of age and diet on the oral glucose tolerance test in ponies. Equine Vet. J. 29(6): 467-470.

Murray, M.J., C. Grodinsky, C.W. Anderson, P.F. Radue, and G.R. Schmidt. 1989. Gastric ulcers in horses: a comparison of endoscopic findings in horses with and without clinical signs. Equine Vet. J. (suppl.) 7:68-72.

Nicol, C.J., H.P.B. Davidson, P.A. Harris, A.J. Waters, and A.D. Wilson. 2002. Study of crib-biting and gastric inflammation and ulceration in young horses. Vet. Rec. 151:658-662.

N.R.C.: Nutrient Requirements of Horses (5th ed.). 1998. National Research Council, National Academy Press, Washington, D.C.

Olsson, N. and A. Ruudvere. 1955. Nutrition of the horse. Nutr. Abst. Rev. 25:1.

Ott, E.A., R.L. Asquith, J.P. Feaster,a nd F.G. Martin. 1981. Lysine supplementation of diets for yearling horses. J. Anim. Sci. 53:1496-1500.

Ott, E.A., Pas, and J. Kivipelto. 2002. Influence of energy and protein content of the concentrate and restricting concentrate intake on growth and development of weanling horses. Prof. Anim. Sci. 18:302-311.

Pagan, J.D., S.G. Jackson, and S. Caddel. 1996. A summary of growth rates of Thoroughbreds in Kentucky. Pferdeheilkunde. 12(3) 285-289.

Pond, W.G., D.C. Church, and K.R. Pond. 1995. Basic animal nutrition and feeding, fourth edition. John Wiley and Sons: New York.

Ralston, S.L., G. VandenBroek, and C.A. Baile. 1979. Feed intake patterns and associated blood glucose, free fatty acids, and insulin changes in ponies. J. Anim. Sci. 49:838-841.

Ralston, S.L. 1984. Controls of feeding in horses. J. Anim. Sci. 59(5):1354-1361.

Ralston, S.L. and C.A. Baile. 1982. Gastrointestinal stimuli in the control of feed intake in ponies. J. Anim. Sci. 55:243-253.

Rezende, A.S.C., I.B.M. Sampaio, G.L. Legorreta, and D.C.A. Moreira. 2000. Effects of two different nutritional programs on orthopedic alterations in mangalarga marchador foals. J. Equine Vet. Sci. 20(10):651-656.

Robertson, J.B. and P.J. Van Soest. 1977. Dietary fiber estimation in concentrate feedstuffs. J. Anim. Sci. 45(Suppl. 1):254.

Saastamoinen, M. 1990. Factors affecting growth and development of foals and young horses. Acta Agric. Scand. 40:387-396.

SAS. 1997. SAS's System for Mixed Models. Cary, NC: SAS Institute Inc.

Scheibe, K.M., K. Eichhorn, B. Kalz, W.J. Streich, and A. Scheibe. 1998. Water consumption and watering behavior of Prezwalski Horses (Equus ferus prewalskii) in a semireserve. Zoo Biology. 17:181-192.

Schryver, H.F., D.W. Meakim, J.E. Lowe, J. Williams, L.V. Soderholm, and H.F. Hintz. 1986. Growth and calcium metabolism in horses fed various level of protein. Equine Vet J. 19:280-287.

Schurg, W.A., R.E. Pulse, D.W. Holtan, and J.E. Oldfield. 1978. Use of various quantities and forms of rye grass straw in horse diets. J. Anim. Sci. 47:1287-1291.

Slade, L.M., D.W. Robinson, and K.E. Casey. 1971. Nitrogen metabolism in Nonruminant herbivores. I. The influence of nonprotein nitrogen and protein quality on the nitrogen retention of adult mares. J. Anim. Sci. 30:753-760.

Smith, G.K, D.E. Kealy, D.N. Biery, and D.F. Lawler. 2001. Influence of body condition score on canine osteoarthritis. Ralston Purina Nutrition Forum (proceedings).

Staniar, W.B., J.A. Wilson, L.H. Lawrence, W.L. Cooper, D.S. Kronfeld, and P.A. Harris. 1999. Growth of Thoroughbreds fed different levels of protein and supplemented with lysine and threonine. J. Anim. Sci. (suppl.) 79:2143-2151.

Thatcher, C.D. 1991. Nutritional aspects of developmental orthopedic disease in growing horses. Vet. Med. 7:743-747.

Thompson, K.N., S.G. Jackson, and J.R. Rooney. 1987. The effects of above average weight gains on the incidence of radiographic bone alterations and epiphysitis in growing horses. Proc. 10th Equine Nutr. Physiol. Soc. Symp. Fort Collins, Colo: Colorodo State University.

Willard, J.G., J.C. Willard, S.A. Wolfram, and J.P. Baker. 1977. Effects of diet on decal pH and feeding behavior of horses. J. Anim.Sci. 45:87-92.

Wright, I.M. and A.C. Pickles. 1991. Osteochondritis dissecans (OCD) of the femoro-patellar joint. Equine Vet. Educ. 3:86-93.

APPENDIX

Table 1A: Mean ± SE wither height, hip height, and cannon bone circumference (Cannon C) of Quarter Horses and Arabians at 5 and 8 mo.

	QH		*Arab*	
	5 mo	*8 mo*	*5 mo*	*8 mo*
Wither Height (cm)*	125.0±1.3	132.4±1.3	125.4±1.3	134.4±1.3
Hip Height (cm)	132.0±1.4[b]	139.3±1.4[a]	128.5±1.4[b]	138.8±1.4[a]
Cannon C (cm)*	24.5±1.2	31.9±1.2	28.5±1.2	38.8±1.6

[ab] Values within a measurement lacking common superscripts differ ($P<0.05$)
* Overall 5 mo less than 8 mo, regardless of breed: $P<0.05$

Table 2A: Average individual feed consumption (g/kg BW/d) of High Con, Equal, and Low Con by weanlings at 5 and 8 mo during the 10-d adjustment period.

Treatment	*5 mo*	*8 mo**
High Con	27.2±1.4	35.0±2.0
Equal	27.1±1.5	38.4±2.2
Low Con	25.3±1.4	36.7±2.5

* 8 mo greater than 5 mo, regardless of treatment ($P<0.05$)
*Overall QH consumed less than Arabs, regardless of age or treatment: $P<0.05$

Table 3A: Average as-fed calculated values for energy, CP, Ca, and P for whole oats, protein pellets, Purina Strategy feed, grass pasture, grass hay, and mixed hay.

	DM (%)	*Energy (Mcal/kg)*	*CP (%)*	*Fiber (%)*	*Ca (%)*	*P (%)*
Whole oats	89	2.85	11.8	10.7	0.08	0.34
Protein pellets*	90	-	32.0	8.0	1.3	0.8
Purina Strategy	90	-	14.0	8.0	0.80	0.60
Grass pasture	29	0.58	2.7	9.8	0.11	0.09
Grass hay	90	1.74	8.5	30.0	0.38	0.18
Alfalfa/grass hay	90	1.90	12.8	27.8	0.81	0.20

* Calf protein pellets (company name)

APPENDIX:
EQUINE CAECAL CONTENTS, EXTIMATION OF TOTAL CELLULOLYTIC NUMBERS IN ADULT HORSES

INTRODUCTION/ LITERATURE REVIEW

There is sizeable variation in management and feeding practices of domestic horses across the world and throughout history. This is reflective of the many uses for horses and the availability and costs of suitable environments and feedstuffs. The horse has developed specific digestive physiology, anatomy, and feeding behaviors associated with a high fiber diet consumed through grazing and browsing. Prior to domestication, horses survived on a diet consisting solely of available forages containing relatively large amounts of water, soluble proteins, lipids, sugars, and structural carbohydrates, but very little starch (Frape, 1998). Additionally, wild horses consume multiple meals throughout the day and night during short, frequent feedings. The art of feeding domestic horses of all classes is to provide the needed nutrients for service and performance while avoiding digestive and metabolic upsets resulting from feeding methods and ingredients.

The gastrointestinal tract of the horse is comprised of a small, simple stomach, a long small intestine containing relatively little volume, a large caecum, and a sacculated hind gut. This anatomy allows the horse, a nonruminant herbivore, to digest fibrous diets.

At birth the foal is completely dependent on milk and the digestive system grows, develops, and becomes inoculated with microbes in the first year to enable the horse to digest starches and eventually fibers. The ability to digest fiber is important to both domestic and wild horses. In the wild, once the horse is weaned from its dam, high fiber diets will be the basis of its diet. Although in domestic horses the amount of fiber in the horse's diet varies considerably with its intended use, adult horses are typically fed significantly more fiber than young horses; often receiving diets of 100% hay or grass. The age when the digestive system is fully developed and able to utilize the nutrients available in high fiber diets and the factors that initiate these changes have not been definitively determined.

The equine digestive tract continues to develop from birth through maturity. During the time after weaning, the large intestine grows faster than the remainder of the gastro-intestinal tract to accommodate the increased consumption of fiber in the diet (Frape, 1998). Although the proximal regions (stomach and small intestine) grow very little after birth, the distal regions (caecum and large intestine) continue to grow and develop until the horse is nearly 20 years old (Frape, 1998). However, digestion in the caecum and ventral colon depends almost entirely on the activity and vitality of the microflora of this region (Fonnesbeck, 1968; Vander Noot and Gilbreath, 1970). Inoculation of the hindgut is primarily done through coprophagy and ingestion of microbes during play, social, and exploratory behaviors (Fransis-Smith and Wood-Gush, 1977; Crowell-Davis and Houpt, 1985; Galef, 1979)

Horse feeds can generally be divided into two primary classes of feeds: roughages and concentrates. Roughages generally contain at least 20 % crude fiber (air-dry basis). Typically, roughages are fed to provide nutrients, reduce dry matter intake when mixed with other feeds, decrease the net energy intake of the total diet, maintain an active microbial population in the large intestine and caecum, and promote equine welfare through decreasing possible diet-associated behavior problems. Nutrient quality of roughages can vary and depends on type of roughage, climate effects, soil fertility, and stage of maturity when harvested (Hintz, 1983).

Concentrates are the portion of the horse's diet comprised of feeds rich in starch, protein, or both, and typically contain less than 15 to 17 % crude fiber (Frape, 1998). They are most often fed to increase the energy and nutrient concentration of the total diet in growing, breeding, or working horses. Nutrient content and formulation must be thoroughly investigated when feeding mixed concentrate diets because the concentrate ingredients in the concentrate can drastically vary in nutrient concentrations (Pond, 1995). Furthermore, high concentrate diets have been associated with colic, founder, and other physiological and digestive upsets in horses (Garner et al., 1978; Goodson et al., 1988). Therefore, there is increasing interest in determining which roughages are digested best to enable feeding higher levels of roughage while still maximizing growth, reproductive, and athletic performance.

Crude fiber describes the fiber portion of feed, but does not separate the carbohydrates into highly digestible and less digestible portions. Fiber is often described as acid detergent fiber (ADF) and neutral detergent fiber (NDF) to describe components that are mostly available, incompletely available, and mostly unavailable (Van Soest, 1987). The NDF portion contains the plant cell wall: hemicellulose, cellulose, and lignin, while ADF is representative of only cellulose and lignin. The soluble portion of a fiber sample is composed of cell contents and is made up of soluble carbohydrates, such as starch, organic acids, protein, and pectin (Maynard et al., 1979). Thus, the soluble portions are the most available, while the insoluble portions are partially available.

Horses digest relatively the same amount of fiber as mules, donkeys, zebras, and elephants (monogastrics); yet they digest less fiber than kangaroo (monogastric herbivore), chinchillas (monogastric herbivore), and ruminants (Evans, 1999). Koller et al. (1978) reported that ruminal microbes in cows fermented all timothy, orchard grass, and wheat straw at a faster rate than horses accustomed to an all hay diet. However, alfalfa was fermented at the same rate, presumably because the alfalfa used was of high quality and contained minimal indigestible fibers such as the cell wall and lignin. The fermentability of fibrous feedstuffs is dependent on the animal species, microflora present, microflora activity, and time ingesta is exposed to microbes (Sunvold et al., 1995). Fiber digestion in the horse is correlated to three main factors: the composition of the diet, especially the carbohydrate portion, the fibrolytic activity of the microbial ecosystem, and the rate of passage of the digesta through the digestive tract, especially in the hindgut (Drogoul et al., 2001).

Although, bacteria, protozoa, and fungi are all active in the hindgut of the rumen, bacteria have been the most studied symbiotic organisms present in the hindgut of the horse (Lee et al., 2000; Roderick and Wilkins, 1988). Different types of bacteria are present throughout the digestive tract of the horse. Equid require a nitrogen sources other than ammonia or urea, however energy derived from microbial activity is a vital part source of energy for the horse (Maczulak et al., 1985). Microbial action in the hindgut forms primarily acetate, propionate, butyrate, and small amounts of other acids. The amount of acids produced per unit dry-matter intake, and their relative proportions, depends directly on the nature of the feed fed to the horses (Fombelle et al., 2001).

An abrupt dietary change may be especially harmful to the hindgut microflora. A sudden change from a high roughage diet to a high concentrate diet increases the numbers of total viable anaerobic bacteria and lactobacilli in the caecum, while bacilli, xylanolytic, and pectinolytic bacteria decrease (Garner et al., 1978; Goodson et al., 1988). These changes have been associated with a decrease in pH and an increase in lactate (Garner et al., 1978; Goodson et al., 1988). More recently, Fomelle et al. (2001) reported changes in numbers and activities of microbial communities in the hindgut of horses in only 5 h after an abrupt incorporation of barley into a diet that was previously comprised of 100% meadow hay.

The microbial profile and the biochemical composition of the hindgut are correlated to the roughage to concentrate ratio fed (Moore et al., 1993). Drogoul et al. (2001) report that fiber digestibility is reduced as the amount of grain in the diet is increased. They explained this by the premise that the bacteria attack the simpler carbohydrates by preference, and the rate of passage is increased which decreases the available time for digestion to occur in the hindgut. In sheep, those fed a 60% concentrate diet had higher percentages of starch digesters and lower percentages of cellobiose-fermenting bacteria in the rumen as compared to sheep consuming a 100% hay diet (Dehority and Grubb, 1976). High concentrate diets increase the total bacteria, mostly due to the increase in amylolytic bacteria concentrations (Moore, 1993). Julliand et al. (2001) reported a ten-fold increase in lactate, the main end product of amylolytic population's fermentation, in the caecum and colon of horses fed 50:50 diet as compared to those fed a 100:0 (all hay) diet. Furthermore, when high starch diets decrease the pH in the hindgut, there is an increase in population and activity of lactobacilli and streptococci, normal inhabitants and major starch utilizers (Hungate, 1966; Garner et al., 1978).

Interestingly, the equine hindgut ecosystem responds similarly to ruminants when concentrates are introduced to 100% roughage diets (Fomelle et al., 2001). When starch is included in the diet, it increases the soluble carbohydrates in the diet which causes an increase in the rate of micro-organism multiplication followed by a chain of reactions (Dawson et al., 1997). Most importantly, a saturation of the digestive ecosystem's buffering system occurs which decreases the pH and thus, decreases cellulolytic bacteria and increases acidophilic flora such as lactobacilli and streptococci (Dawson et al., 1997; Goodson et al., 1988). This in turn causes a decrease in acetate, and an increase in lactate and propionate (Hintz et al., 1971; Willard et al., 1977). If these changes are drastic, they can result in laminitis, colic, and even death (Garner et al., 1978).

Changes in the gut mircoflora can be devastating in horses that over-eat concentrates and suffer from colic or acute equine laminitis, which is often deadly or severely handicapping. Garner et al. (1978) quantified caecal bacteria flora before and after horses overate concentrates. The onset of laminitis is characterized by intracaecal proliferation of lactobacilli and thus increased lactic acid production and increased amine production (Garner et al., 1978; Bailey et al., 2002). An increased intracaecal acidity reduced caecal *Enterobacteriaceae* and anaerobic *Streptococci* numbers 8 h after carbohydrate overload. While *Streptococci* tended to re-establish its population after 24 h, the *Enterobacteriaceae* continued to decrease.

Fiber utilization is extremely important to the health and survival of the horse. However, specific information regarding the enumeration and characterization of the microbes in the equine caecum is still limited. Three main ruminal cellulolytic bacterial species have been identified in the adult horse: *Fibrobacter succinogenes, Ruminococcus flavefaciens, and Ruminococcus albus* (Bonhomme, 1986; de Vaux, 1998). Furthermore, *Bacteroides* sp., *Bacillus cellulosae dissolvens, Clostridium* sp., *Eubacterium* sp., and *Butyrivibrio fibrisolvens* have also been observed (Julliand et al., 1999). However, the entire diversity of the hindgut microbes and whether their structures depend primarily on the diet or individual animal remain unclear (Julliand et al., 1999).

Determination of the number of total anaerobes and cellulolytic bacteria is important to enable the study of normal and diseased states in horses at different ages fed different diets. Although it is know that the plant cell wall is digested by bacteria, protozoa, and fungi in the cow rumen, the degree to which each microorganism participates is still not clearly defined (Lee et al., 2000). Although there are many similarities in the microbial populations of the horse and ruminants, the microbes of the equine caecum have been investigated much less than the rumen. The amount of total anaerobic microbes and cellulolytic degrading microbes is important to determine for every class and type of horse to enable future characterization of the microbes and a clearer understanding of fiber digestion.

The objective of this study was to determine the total anaerobic and cellulose degrading bacteria numbers in the caecal contents of adult horses.

MATERIALS AND METHODS

Caecal contents were obtained within an hour of death from euthanized horses (n=6) via a midline abdominal incision to exteriorize the caecum; an enterotomy incision of the the apex of the caecum allowed for collection of ingesta. Horses were euthanized at the Michigan State University Veterinary Diagnostic Laboratory and were either Michigan State University owned research horses or donated by their owners for research (approved by R. Fulton, DVM). Horses had clinically normal gastrointestinal systems and were euthanized for reasons not related to the gastrointestinal tract (Table 1).

Samples were collected into sterile 120 cc specimen containers with minimal head space air (to minimize oxygen exposure) and transported to the laboratory on ice. In the laboratory, contents were shaken to dislodge cellulolytic bacteria from particulate matter, and 60 cc of the fluid phase of the ingesta was centrifuged at 1000 rpm for 10 min to separate fluid from particulate matter.

Serial dilutions were used to estimate total and cellulolytic anaerobic bacteria numbers. Traditional serial dilutions were performed by adding 1 ml of the centrifuged ingesta to 9 ml of anaerobic cellulose degrading media (Table 2) and general anaerobic media (Table 3). One ml of each new solution was drawn out of each Hungue tube or Wheaton jar and added to another 9 ml of either the cellulose selective media or standard anaerobic media. This process was continued to make dilutions of 1:10, 1:100, 1:1000, 1:10,000, 1:100,000, and through to a dilution of $1:10^{-10}$. Serial dilutions were replicated three to five times and preformed for cellulolytic and general anaerobe enumeration. Anaerobic and cellulolytic media was prepared and stored at room temperature until used in the laboratory (Dehority et al., 1989).

After the inoculated cultures were serially diluted into the anaerobic medium and incubated at 37°C for 2 weeks, the numbers of cellulose degrading bacteria and total anaerobes were estimated by observing the vials for the degradation of cellulose disks in the cellulolytic medium and the cloudy appearance in the anaerobic media (Cochran, 1950; Dehority et al., 1989). Total anaerobic numbers and cellulolytic bacteria degradation was checked every 24 h and monitored for 14 d.

RESULTS

The concentrations of total viable bacteria in the caecal fluid from the caecal contents of the adult horses ranged from 10^3 – 10^7 microbes/ ml caecal fluid (Table 4). Thus, there was an average of 10^8 anaerobic microbes/ ml caecal fluid. The concentration of cellulolytics within the samples resulted in a range of 10^6 - 10^9 microbes/ ml with an average concentration of 10^5 microbes/ ml (Table 5).

Because of the limited number of horses and unknown nutritional background of some of the horses (Table 1), we were unable to compare the effects of age, diet, and gender on the anaerobic microbes in the caecum at this time. However, during the degradation of the cellulolytic disks in the cellulolytic media, the disks turned from white to yellow. Cellulolytic bacteria comprised about 64% of the total anaerobes (Table 6).

DISCUSSION

When the population of bacteria from the caecum samples were introduced to the nutrient rich medium, the organisms showed four major growth phases: the lag phase (metabolically active, adjusting to new media), the logarithmic phase (population growth), the stationary phase (number of live cells stays constant), and the decline phase (decrease in cell number at a logarithmic rate) to form the standard bacterial growth curve (Black, 1999). All caecal samples resulted in some growth of both anaerobic and cellulolytic bacteria. Mackie and Wilkins (1988) examined the duodenum, jejunum, ileum, caecum, and colon for total culturable and proteolytic bacteria in mature (3 to 5 yr) horses. As would be expected, they found a 100 fold increase in the number of both groups of bacteria in the caecum and colon as compared to the samples retrieved from the forgut of horses. Kern et al. (1974) reported that cellulolytic bacteria numbers were greater in the caecum than the terminal colon and stomach.

In the current study, the approximate number of cellulolytic bacteria, total anaerobes, and the resulting ratio was higher than has previously been reported in the literature. Interestingly, the horses used in the current study were all older than the mature horses usually used which are 3 to 10 years of age when age is reported.
The cellulolytic bacteria counts in the current study (average 10^5) were higher than those reported by Jullian et al. (1999), Goodson et al. (1988), and Kern et al. (1974). Additionally, the percentage of cellulolytic bacteria of the total anaerobes was much higher than 3.8% and 9% previously reported (Julliand et al., 1999; Kern et al., 1974).

Their counts were estimated with probes. In the current study, the lowest value possible to obtain was 10% (10^1 divided by 10^{10}); however, the lowest count actually found was 43%.

Interestingly, as the cellulolytic discs dissolved, they turned from white to yellow in color. This yellow color is a unique and well known trait of *Ruminococcus Flavefaciens,* one of the three main ruminal cellulolytic bacterial species that has been identified in ruminants, horses, and rats (Julliand et al., 1999; Macy et al., 1982). Further investigation under a microscope and DNA probe will be useful in positive identification of all the cellulolytic and total anaerobic bacteria present in the collected caecal contents.

The most probabley numbers, MPN, method will be helpful in future studies used simultaneously with the serial dilutions (Dehority et al., 1989; Cochran, 1950). Dilutions were made based on the estimated bacterial numbers within the sample. Over time, as the dilutions increased, a point was reached when some tubes contained organisms, while others did not show any growth.

Continued enumeration and classification of equine hindgut microbes will aid in the understanding of the microbe's structures and whether these structures depend on the diet or on other factors linked to the animal's unique characteristics. Further understanding of both the normal and diseased hindgut activity could lead to a decrease in deaths and disease related to gastrointestinal disorders and an economic gain to the horse industry. Nonbacterial direct-fed microbials are commonly used in the livestock industry, but their use in horses is only beginning to be explored (McDaniel et al., 1993; Weese, 2002). In the future, inoculation of horses with poor fiber digestion and young equines may increase animal welfare by allowing incorporation of a high fiber diet as well as promote health and longevity of the equine through increased feed and nutrient utilization.

ACKNOWLEDGMENTS

Thank you Dr. Nielsen and Dr. Yokoyama for facilitating this independent study project as part of my master's degree program. Thank you to the microbiology lab in Animal Science for the use of their equipment and materials.

LITERATURE CITED

Black, J.G. 1999. Microbiology, principles and explorations. 4th ed. Upper Saddle River, New Jersey: Prentice Hall.

Baily, S.R., A. Rycoft, and J. Elliott. 2002. Production of amines in equine cecal contents in an in vitro model of carbohydrate overload. J. Anim. Sci. 80:2656-2662.

Bonhomme, A. 1986. Attachment of horse cecal bacteria to forage cell walls. J. Vet. Sci. 48:313-322.

Cochran, W.G. 1950. Estimation of bacterial densities by means of the "most probable number." Biometrics 6:105-116.

Crowell-Davis, S.L. and K.A. Houpt. 1985. Coprophagy by foals: effects of age and possible functions. Equine Vet. J. 17(1):17-19.

Dawson, K.A., M.A. Rasmussen, and M.J. Allison. 1997. Digestive disorders and nutritional toxicity, the rumen microbial ecosystem, 2nd. Ed. Blackie Acedemic and Professional. London, England.

De Vaux, A. 1998. Ph.D. thesis. Universite de Bourgogne, Dijon, France.

Dehority, B.A. and J.A. Grubb. 1976. Basal medium for the selective enumeration of rumen bacteria utilizing specific energy sources. Appl. Microbiol. 32(5):703-710.

Dehority, B. A., P. A. Tirabasso, and A. P. Grifo, Jr. 1989. Most-probable-number procedures for enumerating ruminal bacteria, including the simultaneous estimation of total and cellulolytic numbers in one medium. Appl. Environ. Microbiol. 55:2789-2792.

Drogoul, C., A. de Fombelle, and V. Julliand. 2001. Feeding and microbial disorders in horses: 2 - Effect of three hay:grain ratios on digesta passage rate and digestibility in ponies. J. of Equine Vet. Sci. 21(10):487-491.

Evans J.W., A. Borton, H. Hintz, and L.D. Van Vleck. 1999. The horse, 2nd ed. W. H. Freeman and Company. New York.

Fombelle, A. de, V. Julliand, C. Drogoul, and E. Jacotot. 2001. Feeding and microbial disorders in horses: 1 - Effects of an abrupt indorporation of two levels of barley in a hay diet on microbial profile and activities. J. of Equine Vet. Sci. 21(9):439-445.

Fonnesbeck, P.V. 1968. Digestion of soluble and fibrous carbohydrate of forage by horses. J. Anim. Sci. 27:1336-1344.

Francis-Smith, K. and Wood-Gush, D.G.M. 1977. Coprophagy as seen in Thoroughbred foals. Equine Vet. J. 9:155-157.

Frape, D.L. 1998. Equine nutrition and feeding, Second edition. Blackwell Science Ltd, Oxford, UK

Galef, B.G. 1979. Investigation of the functions of coprophagy in juvenile rats. J. Comp. Physiol. Psychol. 93:295-305.

Garner, H.E., J.N. Moore, J.H. Johnson, L. Clark, J.F. Amend, L.G. Tritschler and J.R. Coffmann. 1978. Changes in the caecal flora associated with the onset of laminitis. Equine Vet. J. 10(4):249-252.

Goodson, J., W.J. Tyznik, J.H. Cline, and B.A. Dehority. 1988. Effects of an abrupt diet change from hay to concentrate on microbial numbers and physical environment in the cecum of the pony. Appl. Environ. Microbiol. 54:1946-1950.

Hintz, H.F., R.A. Argenzio, and H.F. Schryver. 1971. Digestion coefficients, blood glucose levels, and molar percentage of volatile acids in intestinal fluid of ponies fed varying forage:grain ratios. J. Anim. Sci. 33:992-995.

Hungate, R.E. 1966. The rumen and its microbes. New York: Academic Press.

Julliand, V., A. de Vaux, L. Millet, and G. Fonty. 1999. Identification of *Ruminococcus flavefaciens* as the predominant cellulolytic bacterial species of the equine cecum. Appl. Environ. Microbiol. 65:3738-3741.

Julliand, V., A. de Fombelle, C. Drogoul, and E. Jacotot. 2001. Feeding and microbial disorders in horses: 3 - Effects of three hay:grain ratios on microbial profile and activities. J. of Equine Vet. Sci. 21(11):543-546.

Kern, D.L., L.L. Slyter, E.C. Leffel, J.M. Weaver and R.R. Oltjen. 1974. Ponies vs. steers: Microbial and chemical characteristics of intestinal ingesta. J. Anim. Sci. 38(3):559-564.

Koller, B.L., H.F. Hintz, J.B. Robertson, and P.J. Van Soest. 1978. Comparative cell wall and dry matter digestion in the cecum of the pony and the rumen of the cow using in vitro and nylon bag techniques. J. Anim. Sci. 47(1):209-215.

Lee, S. S., J. K. Ha, and K. J. Cheng. 2000. Relative contributions of bacteria, protozoa, and fungi to invitro degradation of orchard grass cell walls and their interactions. Appl. Environ. Microbiol. 66:3897-3813.

Mackie, R.I. and C.I. Wilkins. 1988. Enumeration of anaerobic bacteria microflora of the equine gastrointestinal tract. Appl. Environ. Microbiol. 54(9):2155-2160.

Macy, J.M., J.R. Farrand, and L. Montgomery. 1982. Cellulolytic and non-cellulolytic bacteria in rat gastrointestinal tracts. Appl. Microbiol. 44(6):1428-1434.

Maczulak, A. E., K. A. Dawson, and J. P. Baker. 1985. Nitrogen utilization in bacterial isolates from the equine cecum. 50:1439-1443.

Maynard, A.B., J.K. Loosli, H.F. Hintz, R.G. Warner. 1979. Animal nutrition, 7th ed. McGraw-Hill Book Company. New York.

McDaniel, A. L., S. A. Martin, J. S. McCann, and A. H. Parks. 1993. Effects of *Aspergillus oryzae* fermentation extract on in vitro equine cecal fermentation. J. Anim. Sci. 71:2164-2172.

Moore, B. E. and B. A. Dehority. 1993. Effects of diet and hindgut defaunation on diet digestibility and microbial concentrations in the cecum and colon of the horse. J. Anim. Sci. 71:3350-3358.

Pond, W.G., D.C. Church, and K.R. Pond. 1995. Basic animal nutrition and feeding, fourth edition. John Wiley and Sons: New York.

Roderick, I. M. and C. A. Wilkins. 1988. Enumeration of anaerobic bacterial microflora of the equine gastrointestinal tract. Appl. Environ. Microbiol. 54:2155-2160.

Sunvold, G. D., H. S. Hussein, G. C. Fahey, Jr., N. R. Merchen, and G. A. Reinhart. 1995. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid in cattle. J. Anim. Sci. 73:3639-3648.

Vander Noot, G.W. and E.B. Gilbreath. 1970. Comparative digestibility of components of forage by geldings and steers. J. Anim. Sci. 31:351-355.

VanSoest, P.J. 1987. Nutritional ecology of the ruminant: ruminant metabolism, nutritional strategies, the cellulolytic fermentation, and the chemistry of forages and plant fibers. Cornell University Press. Ithaca, NY.

Weese, J.S. 2002. Probiotics, prepiotics, and synbiotics. Current Therapy in Equine Medicine. Elsevier Science. Philedalphia, USA.

Willard J.G., J.C. Willard, S.A. Wolfram, and J.P. Baker. 1977. Effect of diet on cecal pH and feeding behavior of horses. J. Anim. Sci. 45:87-93.

Table 1: Horse's used in random collection: Sex, age, breed, and nutritional history

Horse Number	Sex	Age (yr)	Horse history
1	Gelding	15	Standardbred. Pasture supplemented with mixed hay diet, chronic obstructive pulmonary disorder, COPD.
2	Mare	20	Grade horse. Mixed grass hay and pasture diet. Chronic COPD
3	Mare	20	Grade horse. Diet unknown.
4	Gelding	20	Grade horse. Diet unknown, presumed to be hay and grass.
5	Mare	11	Grade/Appaloosa. Blind in both eyes. Presumed to be hay and grass.
6	Gelding	14	Standardbred. Mixed grass and hay diet. Last 10 d: 5 d moldy hay followed by 5 d Purina Equine Senior complete diet

Table 2: Procedures and recipe for equine caecal cellulose anaerobic media

1. Prepared 40 Hungute tubes by cleaning and placing four Whatman No. 1 flilter paper discs into each tube. Discs were formed with a standard hole punch.

2. Prepare media. Mix ingredients in the order listed, while stirring with a mechanical stirrer.
   To make 600 ml mix:
   1200 mg Trypticase peptone
   300 mg Yeast extract
   45 ml Mineral 1: 0.6% K_2HPO_4
   45 ml Mineral 2:
      0.6 g KH_2PO_4
      0.6 g $(NH_4)_2SO_4$
      1.2 g NaCl
      0.25 g $MgSO_4 \cdot 7H_2O$
      0.16 g $CaCl_2 \cdot 2H_20$
   0.6 ml Resazurin solution:
      25 mg resazurin dissolved in 100 ml distilled water
   6 ml Hemin solution:
      50 mg hemin dissolved in 1 ml 1N NaOH. Make to 100 ml with distilled water.
      Autoclave at 121 C for 15 min.
   120 ml Rumen fluid:
      Spin stored rumen fluid at 10,000 RPM for 10 min.
   300 mg Cysteine HCl
   30 ml 8% Na_2CO_3 solution
   353.4 ml distilled water

3. pH solution to 6.8

4. Bring to a full boil over Bunsen burner with CO_2 blowing into flask
   Let cool, assure CO_2 continuing to blow over solution

5. Add 9 ml media to each Hengue tube and seal tightly assuring anaerobic conditions

6. Autoclave
   20 minutes at 120°C

Table 3: Procedures and recipe for equine caecal general anaerobic media

1. Prepared 40 Wheaton bottles by cleaning.

2. Prepare media.

   Mix ingredients in the order listed, while stirring with a mechanical stirrer.
   To make 500 ml mix:
   250 mg Celulose: Dextrose Anhydrous Powder
   250 mg Cellobiose
   250 mg Soluble starch
   250 mg Xylose
   1.0 g Trypticase peptone
   250 mg Yeast extract
   37.5 ml Mineral 1: 0.6% K_2HPO_4
   37.5 ml Mineral 2:
      0.6 g KH_2PO_4
      0.6 g $(NH_4)_2SO_4$
      1.2 g NaCl
      0.25 g $MgSO_4 \cdot 7H_2O$
      0.16 g $CaCl_2 \cdot 2H_20$
   0.5 ml Resazurin solution:
      25 mg resazurin dissolved in 100 ml distilled water
   5 ml Hemin solution:
      50 mg hemin dissolved in 1 ml 1N NaOH. Make to 100 ml with distilled water.
      Autoclave at 121 C for 15 min.
   100 ml Rumen fluid:
      Spin stored rumen fluid at 10,000 RPM for 10 min.
   250 mg Cysteine HCl
   25 ml 8% Na_2CO_3 solution
   294.5 ml distilled water

3. pH solution to 6.8

4. Bring to a full boil over Bunsen burner with CO_2 blowing into flask

   Let cool, assure CO_2 continuing to blow over solution

5. Add 9 ml media to each Wheaton bottle and seal tightly assuring anaerobic conditions

6. Autoclave

   20 minutes at 120°C

Table 4: Cellulolytic bacteria activity

Horse	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
1	+	+	+	+	+	+	+	+[a]	-	-
2	+	+	+[b]	+[a]	-	-	-	-	-	-
3	+	+	+	+	+	+[a]	+[a]	-	-	-
4	+	+	+	+	+[b]	+[a]	-	-	-	-
5	+	+	+	+[c]	+[d]	-	-	-	-	-
6	+	+[c]	+[d]	+[e]	+[e]	-	-	-	-	-

+ Degradation of cellulose discs
- No cellulose degradation visible
[a] Degradation visible in one out of three replications
[b] Degradation visible in two out of three replications
[c] Degradation visible in two out of four replications
[d] Degradation visible in one out of four replications

Table 5: Total anaerobe activity

Horse	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
1	+	+	+	+	+	+	+	+[a]	+[c]	-
2	+	+	+	+	+	+	+	+[b]	-	-
3	+	+	+	+	+	+	+	+[c]	-	-
4	+	+	+	+	+	+	+[c]	-	-	-
5	+	+	+	+	+	+	+	+	+	-
6	+	+	+	+	+	+	+	+[d]	+[e]	-

+ Cloudy media, visible anaerobe activity

- Clear media, no anaerobe activity

[a] Degradation visible in four out of five replications

[b] Degradation visible in two out of five replications

[c] Degradation visible in one out of five replications

[d] Degradation visible in three out of four replications

[e] Degradation visible in two out of four replications

Table 6: Estimation of individual, average, and range of cellulolytic bacteria, total anaerobes, and percent cellulolytic bacteria of the total anaerobes

Horse number	Cellulolytic bacteria (number/ 1 ml)	Total anaerobes (number / 1 ml)	Cellulolytic bacteria (% of total anaerobes)
1	10^7	10^8	88%
2	10^3	10^7	43%
3	10^5	10^7	71%
4	10^5	10^6	83%
5	10^4	10^9	44%
6	10^5	10^9	56%
Average	10^5	10^8	64%
Range	$10^3 – 10^7$	10^6 - 10^9	

VITA

Susan Kathleen Turcott is the daughter of Riley and Mary Lou Turcott and was born on February 19, 1978 in Petoskey, Michigan.

Following graduation from Petoskey High School, Susan attended Michigan State University. She received her bachelor of science in Animal Science in May, 2001. She began graduate school in August 2001 at Michigan State University were she continued to foster her interest in animal science with research in equine nutrition. In addition to her course work, Susan continued to be an active participant in the equine industry through riding and showing. While in graduate school, Susan coached two successful years of hunt seat and stock seat intercollegiate horse show association riding teams, taught horsemanship at Michigan State University, and volunteered to help with many youth, adult, and community extension programs. She enjoys community service, outdoor activities, and spending time with her friends and family (dogs included).

Susan hopes to continue her active involvement in the equine industry through helping others to flourish through learning to appreciate and understand the horse.