EFFECT OF YEAST CULTURE SUPPLEMENTATION ON DIGESTIBILITY OF VARYING QUALITY FORAGE IN MATURE HORSES

by

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(Under the Direction of Josie A. Coverdale)

ABSTRACT

Supplementation of yeast culture has yielded variable results in many species. Improving the digestibility of lower quality forages could be advantageous both for the producer and horse health. The objective of this study was to evaluate the effect of non-viable *Saccaromyces cerevisiae* on digestibility of high and low quality forage in mature horses. Sixteen geldings of Quarter Horse (n = 14) and Thoroughbred (n = 2) breeding, were used in a 4 x 4 Latin Square design with 28-d treatment periods with total collection the last 3 days of each period. Diets consisted of a commercial grain and either high or low quality forage, with or without the addition of yeast culture (HY, HC, LY, and LC). Digestibility of CP, NDF, and hemicellulose was significantly increased when yeast culture was supplemented with low quality hay. Supplementation of yeast culture to mature horses improved digestibility of lower quality bermudagrass hay.

INDEX WORDS: Horse, *Saccharomyces cerevisiae,* Digestibility, Forage

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DEDICATION

This thesis is dedicated to my family, thank you for making this possible for me. The never ending love and support from a thousand miles away kept me going, I will never be able to express to you what you mean to me. You never stopped believing in me and were always understanding of the time it took to accomplish this, you accepted my absence at holidays and family functions and continued to encourage me. I truly could not have done this without each one of you.

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CHAPTER 1

INTRODUCTION

Because of their unique digestive systems, horses need fiber in their diet. The foregut of the mature horse, the primary site of non-structural carbohydrate digestion is dramatically smaller (< 50 L) when compared to the hindgut, the site for structural carbohydrate, or fiber degradation (> 100 L) (Argenzio et al., 1974). Fiber consists of cellulose, hemicellulose, pectin and lignin which are indigestible by mammalian enzymes and has relatively high hydration capacity that allows it to contribute to gut fill and motility (Ball et al., 2002). Additionally, because of the capacity of the hindgut and the ability of microorganisms to efficiently digest fiber, roughage should be the main component of the mature horse's diet.

Coastal bermudagrass, (*Cynodon dactylon)* is the most important summer pasture grass in the southern United States. It is a warm weather grass that adapts well to most soils, and as a hay it is commonly fed to horses of all ages and stages of production (Ball et al., 2002). Coastal bermudagrass hay can be an adequate source of protein, vitamins, and minerals for horses when grown in favorable conditions and harvested correctly (NRC, 1989). Different areas of the United States offer a wide variety of land and soil composition, some not suitable for growing high quality horse hay. Likewise, rainfall differs from year to year and a consistent quality or variety of forage cannot be made available for all horses. During poor environment conditions high quality hay may be scarce in some areas and may not be available. Periodically only a lesser quality hay will be available and may cause digestive problems for the horse such as colic.

Although the horse is able to utilize diets high in fibrous materials, their digestive system is not as efficient as a ruminant animal. Structural carbohydrates are digested by microorganisms in the hindgut, past the small intestine, the main site of nutrient absorption (Frape, 2004). Further, the bulky part of the digesta is subject to the s-shaped twists and turns of the large and small colon. With each curve of the large intestine, the opportunity for blockage and impaction heightens. Therefore, it is essential for the bulkier portion of the digesta to be broken down as completely as possible. Since digestion of fiber is limited prior to the cecum, it is necessary that microbes in the large intestine utilize fiber to the fullest extent.

It is thought that yeast culture, and other probiotics, favorably alter the microbial environment in the hindgut (Fuller, 1997). The exact mechanisms for this alteration is unknown, but has been hypothesized that the pH is maintained more desirably, and yeast culture provides additional nutrients for the microbes to thrive, thus increasing the total number of viable organisms (Fuller, 1997).

If yeast culture can provide enhanced microbial environment conditions and/or increase the total number of hindgut microorganisms, then digestibility of forages may be improved, since the hindgut and more specifically the activities of the hindgut microorganisms are responsible for fiber degradation. A possible added benefit for feeding probiotics is therapeutic and helps when an animal is diseased or in poor nutritional health. In these instances, the hindgut environment may be less favorable as compared to that of a healthy horse, consequently, total microbe numbers might be decreased, as more energy is being used by the horse to fight disease, gain weight, or

simply to survive. When lower quality forage is fed, even if total microbe numbers are ideal, the horse might benefit from an increase in total bacteria numbers, as more microbes will be able to interact with the hay, digesting it more rapidly and completely before it can become a problem and possibly lead to impaction. Further, yeast culture supplementation may decrease the speed of digesta flow through the GI tract. If mean retention time is increased, then the hindgut microbes will have more time to interact with the digesta, possibly improving fiber digestibility.

CHAPTER 2

REVIEW OF LITERATURE

Digestion begins with intake. The horses' lips, tongue and teeth acquire, ingest and physically alter the form of feed to a state that facilitates mixing with digestive enzymes (Frape, 2004). Food is ground by the teeth and mixed with saliva, starting the digestive process. Teeth are important in harvesting and chewing feed, as the structural carbohydrates occupy more space in the gut and require extensive chewing to reduce the particle size to facilitate passage to the lower digestive tract (Frape, 2004). Horses require healthy teeth to grind feed, and increase its surface area enhancing enzymatic and microbial action on plant cell walls for efficient fermentation (Frape, 2004).

Presence of feed in the mouth stimulates secretion of saliva, which is continually secreted in large quantities (Frape, 2004). The functions of saliva are threefold; lubrication for the ease of passage of the digesta, an important source of water for bacteria health and survival, and as a buffer for the proximal stomach (Frape, 2004). Sodium bicarbonate provides the buffering capacity that permits some microbial fermentation in the fundic region of the stomach (Frape, 2004).

Stomach

Food enters the stomach of the horse through the esophagus. There is a one way flow of digesta from the esophagus to the stomach regulated by the cardiac sphincter (Argenzio et al., 1974). The cardiac sphincter is a powerful, muscular valve located at the top of the stomach with a high angle of attachment that discourages two way flow of digesta (Argenzio et al., 1974). The stomach serves to store food, to mix it, and to

propel it into the duodenum. In a typical 500 kg horse, stomach capacity is approximately 7.5 to 15 L (Argenzio et al., 1974). Liquids pass rapidly to the small intestine; 75% disappears within 30 min. of ingestion (Argenzio et al., 1974). Solid particles are slower with only 25% disappearing within 30 min. (Argenzio et al., 1974), while only about 20% of the ingesta will remain in the stomach after 2 hours (Kern et al., 1974). Sodium bicarbonate from the saliva buffers the proximal stomach, maintaining pH around 4 in the fundic region where fermentative bacteria thrive.

The saccus caecus is prior to the fundic region and is the first site of fermentation, starch and sugar are converted to lactic acid here by lactic acid bacteria (Frape, 2004). The proximal stomach's mucosal surface is comprised of squamous cells which can be damaged by the constant secretion of HCl in the distal stomach because this area lacks the mucus-bicarbonate barrier possessed by the glandular mucosa. The margo plicatus is the main site of gastric ulcer occurrence because it separates the squamous mucosa from the glandular mucosa (esophageal from the fundic and pyloric regions) (Frape, 2004). Secretion of gastric juices is continual and is not influenced by the presence or absence of food, this presents a problem unique to the equine species, which is heightened during long fasts as the gastric juices can irritate the squamous mucosa and cause ulcers (Frape, 2004). The glandular mucosa is protected from the acidic conditions, thus the threat of ulcers in this area is low.

The fundic region contains parietal cells that secrete HCl which partially breaks down solid particles (Frape, 2004). Secretion of HCl then activates the chief cells in the pyloric (distal) region to secrete pepsin which begins protein digestion and stops

fermentation (Frape, 2004). The pyloric region has 15 – 20 times greater activity of pepsin than the fundic region, but the extent of protein digestion in this area is small because of the rapid passage of digesta (Frape 2004). Workers at the University of Maryland (Kern et al., 1974) observed that the predominant bacteria in the stomach of ponies were Gram-positive (rods and cocci) which they deemed consistent with a lactic acid type of fermentation. The microbial species present must be able to withstand moderate acidity (pH 5.4), as with common types of *lactobacilli* and *streptococci* (Frape, 2004). Few celluloytic bacteria (100 to 300 per gram of digesta) reside in the upper GI tract which limits the stomach's ability to contribute to forage digestion (Frape, 2004).

Small Intestine

Digesta enters the small intestine through the pyloric sphincter. The small intestine is divided into three sections with the duodenum marking the beginning, followed by the jejunum and the ileum at the terminal end (Argenzio et al., 1974). This is the primary site for the digestion and absorption of dietary fat and long-chain fatty acids, as well as proteins and non-structural carbohydrates (Hintz et al., 1971).

In a 500 kg horse the small intestine is 21 to 25 m in length and has a capacity of 40 to 50 L (Frape, 2004). Within 45 min. after a meal, transit of the digesta will begin to reach the cecum. Much of the digesta will disappear from the small intestine within 2 to 8 hours after ingestion. Ingesta pH in the small intestine is much higher, (approximately 7.4), after leaving the acidic conditions of the pyloric region of the stomach (Kern et al., 1974). The small intestine is the first site of nutrient absorption, with the highest absorption occurring in the duodenum and decreasing towards the ileum. The extent of

nutrient absorption is surprising when one considers the rapid passage of digesta through the foregut. Most of the starch, fat, protein, vitamins and minerals contained in feed are digested and absorbed prececally (Reitnour et al., 1970).

Digestion of long, branched chains of carbohydrates depends entirely on secretions of enzymes in the small intestine (Frape, 2004). When digesta enters the duodenum, the gastric hormones secretin and cholecystokinin are released, prompting the pancreas to secrete digestive enzymes. The pancreas secretes α-amylase, lipase and various proteases while the intestinal mucosa secretes α-glucosidases which disrupt the α-linked D-glucose units of non-structural carbohydrates which are then able to be actively absorbed by the blood stream (Kern et al., 1974). Because protein cannot be absorbed, amino-peptidases and carboxy-peptidases are secreted by the wall of the intestine and work in conjunction with pancreatic proteases to hydrolyze protein into amino acids, dipeptides and tripeptides for active absorption (Kern et al., 1974). Horses lack a functional gall bladder; therefore, bile continuously drains from the liver and emulsifies fats through bile salts. Pancreatic lipase then hydrolyzes neutral fats to fatty acids and glycerol (Frape, 2004).

Some bacteria are present here, the majority being cellulolytic, with nearly equal concentrations of amylolytic and proteolytic (Kern et al., 1974). Gram-positive cocci are prevalent in the ileum with both Gram-positive and negative rods being more prevalent in the middle and final portions of the small intestine (Kern et al., 1974). Maintaining and enhancing viability of bacterial populations in the small intestine is important since degradation prior to the large intestine means heightened absorption capabilities (Frape,

2004). The horse will be able to more efficiently utilize the dietary nutrients if degradation in the small intestine is enhanced.

The available carbohydrates or non-structural carbohydrates that Hintz et al. (1971), referred to as "starch" are digested in the anterior portion of the small intestine. In a high grain (80% concentrate) diet, 72% was digested duodenally and in an all forage diet, 51% of available carbohydrates were digested duodenally. Therefore, chemical composition of the diet largely influences the site of digestion. Furthermore, diets that are high in grain have created management difficulties for horse owners.

Because of the relatively low volume and rapid rate of passage through the foregut, it is easy to overwhelm the digestive capacity of the stomach and small intestine. The horse's digestive system is designed so that ideally soluble carbohydrates, proteins and fats from feed are digested in the foregut. Thus, it is important to feed relatively small amounts, two to four times each day for efficient digestion when large amounts of concentrate are needed (Frape, 2004). Due to the one-way passage of digesta and relatively small capacity of the stomach and small intestine, large meal sizes force soluble carbohydrates, protein and fat into the hindgut. This is not only an inefficient digestion strategy, but could possibly lead to digestive upset in the horse.

Conversely, nearly all structural and the remaining non-structural carbohydrates in the digesta pass to the cecum, though there is a small amount of disappearance of neutral detergent fiber (NDF) in the anterior small intestine (Hintz et al., 1971). Compared to ruminant animals, the material that enters the large intestine from the small intestine is typically more uniform than that entering the rumen. Fibrous feed residues,

microorganisms, intestinal secretions and cell debris are the typical composition of the material leaving the small intestine along with non-structural carbohydrates, or the non fiber portion of the ration (starch, sugar and pectin) that escaped digestion in the small intestine. Digesta then flows into the large intestine where it is subjected to microbial fermentation (Frape, 2004).

Large Intestine

Although the horse lacks the complex fore stomach of a ruminant, unique characteristics of the large intestine allow it to utilize structural carbohydrates in a manner similar to ruminants. At the distal end of the ileum there is a greatly enlarged blind sack known as the cecum which precedes the large and small colon (Argenzio et al., 1974). The large intestine, referred to as the hindgut of the horse, must serve essentially the same functions as the ruminant fore stomach. The large intestine accommodates the more fibrous and bulky portions of the diet. Considerable mixing also occurs in each compartment of the large intestine, with little retrograde flow (backwards movement of ingesta) between them (Argenzio et al., 1974). The cecum and colon are the major sites of neutral detergent fiber (NDF) disappearance (Hintz et al., 1971) which is comprised of cellulose, hemicellulose and lignin.

The stomach and small intestine of mammals do not produce enzymes capable of degrading structural carbohydrates, and in particular the beta 1-4 bonds between glucose molecules. Fiber is degraded by the activity of microbes that produce cellulases, hemicellulases, pectinases, and other enzymes (Lewis, 1995). These microbes and their enzymes are most numerous in the cecum and colon of the horse. The degree of

fermentative digestion depends primarily on the source of dietary fiber and the presence of nitrogen, minerals and vitamins that are essential for the microbial populations residing in the hindgut (Frappe, 2004).

In the large intestine of the horse reside protozoa and bacterial populations that are responsible for fiber degradation. Protozoa number from 0.5×10^5 to 1.5×10^5/ml of digesta (Frappe, 2004). This number is about .1 % the bacterial population (Frappe, 2004). Even thought the total numbers are dramatically less than bacteria, total mass is similar as individual protozoa are substantially larger (Frappe, 2004). Protozoa contribute little to total digestibility and when removed completely, fiber digestibility was unchanged (Moore and Dehority, 1993).

Intestinal bacteria are responsible for breaking down the complex molecules of cellulose, hemicellulose, and pectin, specifically the β-bonds. Structural carbohydrates are first broken down into their component parts, monosaccharides, then into volatile fatty acids (VFA's) such as acetic, proprionic, and butyric which are suitable for absorption. The majority of bacteria reside in the cecum and ventral colon, six to seven times more than in the terminal colon (Frappe, 2004). When compared to starch and protein, fermentative digestion requires more time (Lewis, 1995). Ideally, if the flow of digesta is delayed, greater absorption and utilization of the fibrous material could be attained.

Microorganisms convert fiber to VFA's that are absorbed via passive diffusion from the hindgut and utilized as an energy source for the horse. These acids are

efficiently used by the animal, though they are not as efficiently absorbed as they would be in the small intestine (Kellems and Church, 2002).

Cecum

Along with the colon, the cecum is the horse's primary fiber digesting region. This organ serves as a fermentation vat and the beginning of the large intestine. There are two muscular valves, one of which allows passage of digesta from the ileum into the cecum and another that propels contents from the cecum to the right ventral colon (Frape, 2004). The cecum is approximately 0.9 to 1.2 m in length and has a capacity of 25 to 30 L (Argenzio et al., 1974). Bacteria reside here that digest most of the fiber and non-structural carbohydrates not previously digested (Frape, 2004). Some bacterial protein is produced at this time but is not efficiently absorbed along with the nutrients ingested (Frape, 2004).

Specific types of bacteria are needed to continue the digestive process. Cellulose, hemicellulose, as well as starch and other soluble carbohydrates not previously digested in the small intestine flow into the cecum. The digestion of cellulose and other fibrous plant materials depend of the availability of microorganisms to digest the walls of plant cells and to ferment the carbohydrates. These undigested nutrients are fermented by bacteria and protozoa that reside there, producing enzymes, which contribute to the degradation of pectins and hemicellulose at an optimum pH of 5-6 (Bonhomme-Florentin, 1988). Volatile fatty acids (acetic, proprionic and butyric) are produced in large quantities from carbohydrate fermentation, are passively absorbed through the cecal and colonic epithelium, and distributed for use throughout the body. Other nutritional

components are released from the ingested feed via fermentation such as vitamin K and B-complex vitamins (Frape, 2004). Digestive enzymes in the stomach and small intestine work with the gastric juices to start the breakdown, and then bacteria in the large intestine digest the feed into usable, absorbable compounds.

Diet had no significant effects on the number of celluloytic bacteria in pony cecal ingesta in a study by Kern et al. (1973). These researchers also determined that 19.7% of the cecal bacteria are proteolytic. Rods, both gram-positive and negative predominated with *Streptococcus bovis* and *Bacteroides* species the most common. Total volatile fatty acid production in the cecum is 6 fold greater than in the small intestine and over 10 fold greater than production in the stomach (Elsden et al., 1946).

Intestinal contractions increase during feeding: digesta is forced out of the cecum into the ventral colon by large contractions (Frape, 2004). The sigmoid configuration (S-shape) of the junction largely prevents the reflux of digesta back into the cecum (Argenzio, 1974).

Large and Small Colon

The large colon is 3 to 3.7 m in length, 20 to 25 cm in diameter and has a capacity of 50 to 60 L (Argenzio et al., 1974). Four sections make up the large colon: the ventral right colon, the sternal flexure to left ventral colon, the pelvic flexure to the left dorsal colon, and the diaphragmatic flexure to the right dorsal colon which connects to the small colon (Argenzio et al., 1974). The pelvic flexure is an area where obstruction commonly occurs (Frape, 2004). The two segments of the ventral, as well as the two dorsal colon segments show uniform mixture of digesta and are therefore thought

of as two compartments, rather than four (Argenzio et al., 1974). The small colon is approximately the same length of the large colon, 3 m, but only 7.5 to 10 cm in diameter with a capacity of 18 to 19 L. When the small colon enters the pelvic inlet, it becomes the rectum, which then leads to the exterior or anus.

After cecal fermentation, digesta enters the colon for further digestion and absorption. The colon is the primary site of water absorption, indicated by the differences in water content between the digesta of the colon and cecum (Hintz et al., 1971). The expected number of viable cellulolytic bacteria per gram of ingesta is variable and the differences in the large and small colon are difficult to distinguish. According to Kern et al. (1974) total numbers of viable bacteria are expected to be similar in the cecum and colon even though the terminal colon contains very few (6 times as few) fiber digesting bacteria as compared to the cecum. Moore et al. (1993) reported a contradiction; the mean concentration of cellulolytic bacteria and fungi were ten times greater in the colon than cecum. This difference might be due to the increased weight of digesta in the colon, six times greater according to Kern et al., (1974). Total volatile fatty acid production in the colon (ventral and distal) is 25 fold greater than in the small intestine and 50 fold greater than production in the stomach (Elsden et al., 1946).

The colon is the primary site of net water absorption in the horse and the percent DM in the digesta of the colon is greater than that of the small intestine and cecum (Hintz et al., 1971). The importance of digestion in the colon increases as the proportion of forage in a diet increases. When greater amounts of forages are fed, more total carbohydrates are converted to VFA's by bacteria in the hindgut. When compared to

high grain diets, the non-structural carbohydrates are digested primarily in the small intestine and absorbed as glucose before reaching the hindgut.

Mean Retention Time (MRT)

Mean retention time (MRT) is the length of time the digesta takes to pass through each segment of the digestive tract. MRT can be used to describe time of digesta through individual parts such as the stomach, or large sections such as the large intestine. Ideal MRT would provide adequate time for enzymes and microbes to break down digesta, but not so long that digestive upsets occur from impaction or blockage colic. Maximum digestion and absorption of nutrients including fiber from forages is paramount for the well being of the horse. For this to occur the forage must be highly digestible and remain in the gastrointestinal tract the necessary length of time to ensure it is properly degradated and utilized. Therefore, mean retention time has a large impact on fiber fermentation, and thus digestion.

Increasing retention time is a desirable characteristic of feeding fiber in the equine diet. Hintz et al. (1971) observed that the percentage of DM in small colon-rectal samples was greater 4 hours after feeding for diets containing grain than for an all forage diet. This study shows that a high grain diet will quickly pass through the digestive tract, but when forage is added to the diet, MRT is increased. Likewise, de Fombelle et al. (2004) observed an increase in MRT when the diet contained increased proportions of fiber. A longer mean retention time would be desirable only if it results in increased fiber digestibility. If no increase in digestibility exists, then increasing MRT would not be a worthwhile goal.

Argenzio et al. (1974) and workers observed a decrease in MRT when an increase in particle size of diet was fed, regardless of the specific segment of the GI tract. They also showed that liquid, as well as smaller 2 mm particles, passed at similar rates through the ventral and dorsal colon, while the major retention of larger particles (greater than 2 mm) was at the dorsal colon. At 10 days, they recorded that 100% of the liquid and only 40% of the 2 cm particles were excreted in the feces.

Processing forages may improve digestibility which in turn could decrease the incidence of digestive problems in the horse. Possible processing options for forage include chopping (reduce particle size), grinding or pelleting (reduce particle size further as well as increase surface area in contact with digestive enzymes). According to Drogoul et al. (2000a), mean retention time as related to particle size of forage varies at specific areas of the gastrointestinal tract. Therefore, they evaluated the effect of physical form (ground, pelleted and chopped) of a total hay diet on total tract digestibility in ponies, with the main focus on fiber digestion and digesta passage rate. Ideally, increasing fiber digestion would lead to improved utilization of the forage and a decrease in digestive problems, especially colic, that may be caused from impaction of poor quality forages. Drogoul et al. (2000a) observed that OM, CP, NDF and ADF digestibility were not significantly different between the ground, pelleted, or the chopped hay diets. Conversely, particle and solute mean retention time were significantly longer in the ground and pelleted as compared to the chopped hay. Further study determined this was a consequence of an increased retention time in the colon (Drogoul et al. 2000b). To the contrary, while grinding and pelleting does increase retention time (specifically in

the colon), it also causes a reduction in the rate of fiber degradation. Drogoul et al. (2000 a and b) determined that rate of flow through the hindgut can be attributed to physical movement from one compartment to another rather than to gut motility. Resistance to flow is greater in the colon (80% of entire gut MRT) and is increased for larger food particles. Despite the significant increase in MRT observed in both studies, apparent digestibility of fiber was not altered. Even though this study observed no benefits of increasing MRT on fiber utilization, the potential still exists and further study is warranted.

When supplementing refaunated sheep with *Aspergillus oryzae*, total mean retention time of solid particles of digesta was increased for the entire digestive tract. No specific increase was seen in the rumen MRT, and no effect was seen on total protozoa numbers (Jouany et al., 1998). No investigations have tested the influence of yeast culture on MRT in monogastric animals. If supplementation of yeast culture is able to slow MRT, greater dietary digestibility may be achieved.

Probiotics

Probiotics, or direct fed microbials (DFM) consist of live or non-viable, naturally occurring microbial supplements (Fuller, 1997). Probiotics are microbial feed additives that improve animal performance, as they may show an improved establishment of beneficial gut microflora (Fuller, 1997). Categories include yeast, bacteria, phage or fungi, and can be used as a live preparation of micro-organisms or a non-viable microbial supplement (Fuller, 1997). Probiotics ideally favorably alter the intestinal microflora

balance, promote increased digestion, boost immune function and increase resistance to infection (Fuller, 1997).

Feeding live beneficial bacteria and digestive enzymes may optimize digestion and maximize the health and well being of the animal. Probiotics are becoming increasingly popular among equine producers as a need is felt for more efficient utilization of feeds and a desire for a more natural, healthier way to supplement the horse. That being said, variability in efficacy is inherent with any probiotic preparation, as the effectiveness of the supplement can also be altered by age, growth phase and stress level of the animal.

Yeasts are commonly added to bread dough as they produce carbon dioxide and leaven (raise) the bread before baking. They are also used for making ethanol in fermentation of fruit juices and grain extracts (Claus, 1989). Yeasts are single-celled fungi and facultative anaerobes that typically occur in clusters or chains (Doelle, 1975). Variation in size is great, ranging in width from 2- 50 μm to 1 – 10 μm, which is 2 to 10 times larger than the average bacteria (prokaryotic) cell (Claus, 1989).

Yeasts are approximately 50% protein, 40% carbohydrates, 8% minerals and 2% fat (Frape, 2004 and Kellems and Church, 2002). The largest component of the culture is protein, enzymes such as proteases and amylases similar to those secreted by the pancreas (Fuller, 1997). The increase in fiber digestion observed in studies of live yeast culture supplements may be due to these enzymes. Conversely, heat from pelleting kills the live organisms in the culture, thus discontinuing the activities of their enzymes. A benefit may be seen in horses of poor condition when non-viable yeast culture is fed in large

amounts, 30 – 50 g/day, but this cost may outweigh the benefit for horses of normal or good condition (Frappe, 2004). Yeast culture has no attachment or rapid growth in the animal gastrointestinal tract when fed as a supplement, therefore, the preparation must be continually administered for expected results to be seen. Two strains of yeast culture supplements have been investigated and used commercially, *S. carlsbergensis* and *Saccharomyces cerevisiae*, with the latter used more heavily.

Investigations of Yeast Culture in Other Species

There has been considerable interest recently in the use of fungal cultures to improve productivity in livestock practices. Many studies have been conducted to determine the usefulness of probiotic supplements in feed, and the results, though typically positive, are variable. Animal production benefits such as increased milk yield and weight gain, which are highly desirable to the producer have been reported (Piva et al., 1993; Shaver and Garrett, 1997; Robinson and Garrett, 1999; Erasmus et al., 1992; Williams et al., 1992 and Putnam et al., 1997). In addition, more subtle positive occurrences such as fewer days off feed and in the sick pen, decreased incidence of digestive upsets, improved body condition of animals, and others have been attributed to assorted fed probiotics (Fuller, 1997). Because of the growing concern over the use of antibiotics in animal production and the positive effects observed with yeast culture supplementation, a huge benefit for producers might be seen through more efficient feeding practices and improved production outcomes.

Bovine – Dairy

Investigations conducted to study the effect of yeast culture supplementation on dairy cattle have yielding variable results. Dairy producers would specifically benefit from increased milk yields, increased milk fat percentage and overall increased dry matter intake (DMI). These positive outcomes are beneficial to all dairy producers, as well as consumers and are the main focus of current research regarding yeast culture supplementation.

An increase in actual and adjusted milk yields was observed in several studies when cows were supplemented with yeast culture (Shaver and Garrett, 1997, Robinson and Garrett 1999, Piva et al., 1993, Erasmus et al., 1992,Putnam et al., 1997, and Williams et al., 1991). Increases in milk components such as milk fat and protein are also beneficial and were observed in several studies (Putnam et al., 1997, Piva et al., 1993, and Robinson and Garrett, 1999). Increased DMI is essential to increased milk yields and was observed in both Williams et al., (1991), and Robinson and Garrett, (1999). Though there was an enhanced postpartum DMI observed in both primiparous and multiparous cows, the primiparous cows tended to produce more milk and had numerically greater milk components when supplemented with yeast culture (Robinson and Garrett, 1999).

Parameters of rumen fermentation have also affected by yeast culture supplementation. Yoon and Stern (1996) observed the influence of yeast culture supplement on ruminal fermentation and microbial populations. The addition of the supplement increased ruminal OM and CP digestion and stimulated proteolytic bacterial

counts in vivo (Yoon and Stern, 1996). Similarly, Erasmus et al., (1992) observed an increased apparent digestibility of CP and ADF when yeast culture was supplemented to lactating Holstein cows.

It is interesting to note that when cows were fed a low CP diet and supplemented with yeast culture (ruminally degradable CP 9.1 vs. 11.4%) increased concentrations of fat, milk and milk protein were recorded (Putnam et al., 1997). Robinson et al. (1995) observed that cows supplemented with yeast culture lost less body condition prepartum and exhibited numerically higher weight gains than control (unsupplemented) cows. Yeast supplemented cows also exhibited a higher calculated energy density compared to the NRC estimated value than the non supplemented cows.

Along with *S. cerevisiae* the influence of *Aspergillus oryzae* has been studied for its effects on total tract digestibility of differing dietary components. Workers in Arizona (Gomez-Alarcon et al., 1990) observed increases in DM, CP and hemicellulose digestibility and an increase in milk production of cows in early lactation. An increase in ruminal bacteria counts coupled with a decrease in ruminal lactate concentration was also observed in cows supplemented with *A. oryzae.*

Bovine – Beef

Ruminant nutritionists and microbiologists are concerned with improving production efficiency of beef animals by manipulating the microbial ecosystem of the rumen. Producers in the animal feed industry are interested in the effects of microbial feed additives on animal performance as the public has growing concerns over the use of antibiotics and growth promoters in animals intended for consumption. Furthermore,

more efficient rumen fermentation is an economic benefit to the producer and has positive influence on animal health.

Steers receiving a yeast culture supplement containing *S. cerevisiae* (Yeast Culture Beef Research Report 1994-1) had improved feedlot performance. Although no difference was observed in feed intake, the cattle did exhibit higher rates of live weight gain and therefore, a positive trend for feed efficiency based on live weight gain. Carcass characteristics were also examined and fat deposition appeared to be positively influenced. Steers receiving the yeast supplement tended to have a reduced external body fat (fat depth) and had a more desirable marbling score indicating increased internal body fat (Yeast Culture Beef Research Report 1994-1). In another study at the University of Georgia, researchers found that a similar yeast culture product stimulated growth of cellulolytic bacteria in vitro, particularly *Fibrobactor succinogenes* and *Ruminococcus albus* (Nisbet and Martin, 1991 and Callaway and Martin, 1997). Calloway and Martin (1997) observed an initial rate increase in cellulose digestion by *Fibrobactor succinogenes* S85 and *Ruminococcus flavefaciens* FD1 but no increase in total cellulose digestibility. Concluding that yeast culture provided growth factors, organic acids, B vitamins, amino acids, and stimulated growth of rumen bacteria that utilize lactate and digest cellulose.

Cole et al. (1992) found that calves infected with an infectious bovine rhinotracheitis virus (IBRV) that were fed yeast culture had increased dry matter intake (DMI), especially evident on day 1 post inoculation. Calves fed the yeast culture supplement also tended to lose less weight after IBRV inoculation than control calves,

because of the higher DMI. Further, calves receiving yeast culture had a decreased number of sick days, requiring fewer days on antibiotics. Fewer sick days and days treated with antibiotics because of yeast culture supplementation could be of major economic importance to producers.

Beauchemin et al., (2003) observed beneficial effects of adding yeast culture (*Saccharomyces cerevisiae)* to a direct-fed microbial (DFM) preparation, compared to the undesirable metabolic changes that took place in steers fed the DFM alone. In the same study, DM digestion increased when yeast culture was added, however, no other influence on nutrient digestibility was observed.

Growth of cellulolytic microorganisms was stimulated with the addition of *S. cerevisiae* to the diet of steers fed a fescue hay-based ration (Dawson et al., 1990). Workers in Kentucky also saw increased concentrations of cellulolytic microorganisms in steers that received a supplement containing only yeast. This increase was 5 to 40 times greater than in the unsupplemented steers (Dawson et al., 1990). This major increase in cellulolytic microorganisms would ideally provide increased digestion of fiber from forages. Similar results from Newbold et al. (1995) outlines an increase in the number of ruminal total bacteria and celluloytic bacteria when yeast culture was used in vivo and in vitro. An increase in the proportion of propionate was also seen. These results were associated with a statistically significant increase in the rate of straw degradation in the rumen. Callaway et al. (1997) had conflicting results in that yeast culture did not seem to alter celluloytic bacteria concentrations. In Japan, workers discovered that medium pH, ammonia-N and numbers of protozoa were unaltered with the addition on yeast culture in

vitro. The addition of the yeast cells did however cause a linear increase in total VFA production in Tokyo (Lila et al., 2004).

Poultry

In the fast turnover poultry industry, time is of the essence. A product is only advantageous to a producer if it increases weight gain or improves mortality rates. Feeding a consistent diet to thousands of birds a day is a challenge, one that can be simplified with the addition of quality supplements.

Data reported from Teeter (1993) indicated that yeast culture has the potential to significantly counteract numerous environmental stresses resulting in improved performance. When broilers were exposed to stressful situations, such as the addition of indigestible litter to the grower ration and heat stress, survivability significantly increased when yeast culture was supplemented by up to 5 % when fed litter with no heat stress and 12 % when the litter fed birds were housed in the heat stressed environment compared to the non supplemented birds. Addition of yeast culture also numerically increased final bird weight by 2% in both the litter and heat stress environments. Yeast culture supplementation also resulted in a 5% improvement in feed efficiency in litter fortified rations and a 2.5% increase in rations without litter. Yeast culture produced the greatest response when litter was fed and stress increased.

Porcine

Increased concerns regarding antibiotic use in swine and the potential ban of some antibiotics may allow direct-fed microbials to take on a bigger role in the swine industry.

Improving nutrient digestion and microbial numbers in the large intestine of the pig are significant goals for producers that yeast culture supplementation may help improve.

Little research with pigs has shown improvement in digestion or nutrient utilization with the addition of yeast culture supplements. Even so, Kornegay et al. (1995) reported beneficial production responses in one trial (n = 54) of 3 week grower phase pigs. When fed yeast culture, ADG increased, however, there were no other improvements of apparent digestibility of DM, N, NDF, ADF or apparent absorption of P.

Workers in Tennessee (Mathew et al., 1998) saw improved growth performance as evidenced by increases in total intake and average daily gains (ADG) in weanling pigs fed a yeast culture supplement. Though these improvements were seen, no differences between the control and supplements groups were noted in relation to net concentrations of fermentation products or alterations in tested intestinal microflora.

Van Heugten at al. (2002) observed variable results between two experiments where yeast culture was supplemented to nursery pigs (exp. 1) and live yeast culture was supplemented to nursery pigs weaned at 17 d of age (exp. 2). ADG was significantly greater ($P < 0.01$) when pigs were supplemented with live yeast culture in experiment two. The growth performance of pigs in experiment one was unchanged by the addition of yeast culture to the diet. Yeast inclusion did however decrease total pathogenic bacteria counts in experiment two, but decreased the digestibility of DM, fat, and GE.

Veum et al. (1995) observed no positive results when yeast culture was fed to determine effects on nutrient digestibility and reproductive performance. Yeast culture

supplementation had no effect on apparent digestibility of DM, energy, CP, ADF, or NDF during gestation or lactation, and no decrease in days from weaning to first estrus when compared to control sows, further, no differences in live pigs/liter at birth or 21d litter weights were observed.

Ovine

Jouany et al., (1998) observed an increase in apparent digestibility of plant cell wall components when yeast culture was fed to refaunated sheep. Further, in situ digestion of ADF was increased when *S. cerevisiae* and *A. oryzae* were supplemented to refaunated sheep, but were not significant when supplemented to defaunated sheep. Growth of protozoa in the rumen was also enhanced with *S. cerevisiae* supplementation. Mean retention time increased when *A. oryzae* was supplemented, possibly contributing to the increased digestibility of plant cell wall components. This specific finding encourages more investigation to determine the effect of probiotics on MRT.

Investigation of Yeast Culture in Equines

Previous research has shown that a variety of fermentation products are stimulatory to hindgut digestion and can beneficially alter microflora parameters. However, the available data is variable to say the least and some reports are incomplete. While some research showed an increase in microbial populations and fiber digestibility, others indicate that feeding yeast culture in small amounts does not significantly alter total tract digestion of energy or protein (Webb et al., 1985).

While no effect of yeast culture on DM, NDF or ADF digestibility was observed, other small but positive effects were reported by Lattimer et al. (2005). Organic matter

(OM) was increased in vitro at 24 h but not 48 when compared to control, which could be increased MRT due to yeast culture. Lattimer et al. (2005) hypothesized that yeast culture improved the ability of the microflora to capture and convert ammonia into microbial cell protein in high forage diets, as a decrease in ammonia concentration was seen when yeast culture was supplemented. A significant increase in the in vitro concentration of acetate was also seen when yeast culture was supplemented. Since there was increased numbers of celluloytic bacteria, Lattimer et al. (2005), suggested that yeast culture supplementation resulted in an increased energetics of the microflora. There were no significant effects on pH and lactate concentrations when supplemented with yeast culture, which disagrees with other previous research (Medina et al., 2002).

A live yeast culture supplement, *Saccharomyces cerevisiae,* fed at a concentration of 10 g per day yielded the greatest effect when combined with a high fiber (HF) or high starch (HS) diet for researchers in France (Medina et al., 2002). The interaction with the high fiber diet had a positive effect on *Lactobacilli* concentrations in the cecum, but presented no overall influence on other bacterial counts. Mean cecal pH increased and the value was greater at 4, 6 and 8 hours post feeding when horses consumed the high starch diet supplemented with yeast culture. Further, mean cecal lactic acid concentration decreased in the high starch diet from 2h to 10h post feeding when yeast culture was added. On the same diet, cecal concentration of ammonia and the concentration during the first 5h post feeding decreased. Total volatile fatty acid concentration was not affected.

Medina et al. (2002), found the interaction of yeast culture supplementation and a high fiber diet resulted in decreased total anaerobic bacteria concentrations and *streptococcus* counts in the colon. However, an increase in the [(acetate + butyrate)/propionate] ratio was observed. Oppositely the interaction with a high starch diet produced an increase in the percentage of acetate and a decrease in lactic acid concentration in the colon, with similar effects seen in the cecum. Both diets had increases in viable yeast cells in both the cecum and colon, though the greatest effects were observed in the cecum. Yeast culture supplementation had positive effects on the intestinal ecosystem of the horse by limiting the extent of the undesirable changes usually seen from the intentional starch overload of the small intestine, tested by the high starch diet. Likewise, positive changes of the intestinal ecosystem were seen when yeast culture was added to the high fiber diet such as increases in molar percentages of acetate and butyrate, as well as a positive interaction for lactobacilli concentration.

Positive numerical effects were observed in a study at Clemson University with the addition of *S. cerevisiae* yeast culture to the diets of young (3 yr old) horses receiving 50% or 70% forage (Godbee, 1983). No statistical analysis was provided, however, Godbee observed large numerical increases in retained nitrogen. Digestibility was numerically greater for NDF, ADF, hemicellulose, CP, P, and Mg when *S. cerevisiae* yeast culture was supplemented. There was also a small increase in DM intake observed. This data contradicts data reported by Switzer et al. (2003) who found no effect of a similar yeast culture supplement on DM, P, or ADF digestibility in aged (14 – 18 yr) horses consuming 50% of their diet as forage.

Researchers at The University of Maryland, (Glade and Biesik, 1986), using a yeast culture supplement containing S. cerevisiae observed increased microbial production of ammonia and amino acids in yearling horses. The result of this enhancement was improvement in the quality of nitrogenous compounds absorbed from the large intestine. Another benefit seen in this study was an increase in net nitrogen retention in horses receiving yeast culture. This may result in increased feed efficiency and a decrease in feed-to-gain ratios because of increased utilization of absorbed nitrogen for tissue production. While Glade and Biesik (1986) found no significant changes in the digestibility of DM, NDF, or ADF, hemicellulose digestibility was significantly greater when horses were supplemented with live yeast culture. It is unknown whether the yeast triggered environmental changes in the large intestine that favored hemicellulose-fermenting organisms, or whether hemicellulose fermentation was directly enhanced by the yeast.

When research was conducted to determine the effects of yeast culture on cecal pH following a high starch meal, few significant differences were observed (Hall et al., 2005). There was a trend for an overall higher cecal pH in horses receiving the yeast culture. The pH was higher (although not statistically significant) at 0 h, 2 h, 6 h post feeding, and at 4 h when the most dramatic decrease in pH post feeding was seen, the yeast culture supplemented horses possessed a statistically higher pH. *S. cerevisiae* supplementation appeared to minimize the level of adverse changes in pH at 4 h post feeding in the cecum of the horse (Hall et al., 2005).

Addition of yeast culture to the diet of previously unconditioned adult horses (Glade and Campbell-Taylor, 1981) yielded positive effects on athletic fitness when horses participated in 6 weeks of gradually increasing exercise. Plasma lactate concentrations were significantly lower ($P < 0.01$) in horses receiving the yeast culture supplement. Changes in plasma glucose concentrations were not significantly effected by yeast supplementation. Glade and Campbell-Taylor (1981) suggested an enhanced state of athletic training (fitness) was achieved with yeast culture supplementation because of a decrease in heart rate during the first 5 and last 10 min. of a 35 min. workout.

Conclusions

In conclusion, improvements in production and reproduction have been seen in equines, dairy, beef, pigs, and poultry in multiple studies when yeast culture was supplemented with no clear mechanisms to explain the positive results. Effects of probiotic use vary, though typically positive, and are not usually dramatic. Beneficial effects have been seen when evaluating a number of performance parameters such as an increase in the number of ruminal bacteria (Dawson et al., 1990; Yoon and Stern, 1996; Erasmus et al., 1992; Nisbit and Martin, 1991; Calloway and Martin, 1997 and Newbold et al., 1995). Nutrient utilization and different aspects of metabolism with the use of fungal probiotics also have been increased (Beauchemin et al., 2003). Production parameters have also been enhanced with increases in total milk yield, milk components, and milk fat percentages (Shaver and Garrett 1997; Robinson and Garrett, 1999; Piva et al., 1993; Erasmus et al., 1992; Putnam at al., 1997 and Williams et al., 1991). Yeast

culture supplementation also increased DM intake and increased gains (Robinson and Garrett, 1999; Erasmus et al., 1992; Williams et al., 1992 and Putnam et al., 1997).

In the equine, a broad range of effect of yeast culture on digestibility exists, and more research is needed in this area to solidify previous results. In previous studies an increase in microorganisms in the cecum were observed by Medina et al. (2002). Further, increased nutrient utilization was seen when NDF, ADF, CP, P, Mg, and hemicellulose digestibility was enhanced (Godbee, 1983 and Glade and Biesik, 1986). In another study horses were able to maintain a more desirable pH in the cecum with yeast culture supplementation (Hall et al., 2005). Increasing the digestibility of feedstuffs for the equine is a worthwhile goal. If a more efficient method to feed horses exists, a huge benefit to producers and hobbyist alike will be seen. Aside from more efficient feeding practices, a safer way to feed the equine may exist if digestibility of poorer quality forages is enhanced or if mean retention time is increased allowing fiber digesting microbes have ample time to interact with fibrous digesta. Probiotics may improve the health, life, and productivity of the horse, by decreasing incidence of digestive disorders and increasing efficiency of feeding practices.

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CHAPTER 3

EFFECT OF YEAST CULTURE SUPPLEMENTATION ON DIGESTIBILITY OF VARYING QUALITY FORAGE IN MATURE HORSES

ABSTRACT

Supplementation of yeast culture has yielded variable results in many species, particularly when fed to horses. Improving the digestibility of lower quality forages could be advantageous both for the producer and horse health. The objective of this study was to evaluate the effect of non-viable *Saccaromyces cerevisiae* on digestibility of high and low quality forage in mature horses. Sixteen geldings (483.6 ± 25.5 kg and 6.8 ± 3.2 yr), of Quarter Horse (n = 14) and Thoroughbred (n = 2) breeding, were used in a 4 x 4 Latin Square design with 28-d treatment periods. Russell bermudagrass hay of either high (13.1% CP, 73.1% NDF, 35.3% ADF and 6.0% ash) or low (8.1% CP, 75.3% NDF, 37.6% ADF and 4.3% ash) quality was offered at 1.35% of BW (DM). Diets consisted of high quality forage with the addition of yeast culture (HY), high quality forage without yeast culture (HC), low quality forage with the addition of yeast culture (LY), and low quality forage without yeast culture (LC). All horses were fed a commercial grain mix (12.6% CP, 25.4% NDF, 12.1% ADF and 4.0% ash) offered at 0.45 % of BW (DM) daily. *Saccaromyces cerevisiae,* was added to grain during the morning feeding at a rate of 56 g per horse. BW was measured weekly and feed intake was adjusted accordingly. Fecal samples were obtained every 6 hr on the last 3 days of each treatment period. Horses receiving low quality hay (LY and LC) had greater intake expressed as a percentage of BW compared to horses receiving high quality hay (HY and HC) ($P < 0.01$). There was no influence of yeast culture supplementation on intake of grain or forage ($P < 0.23$ and $P < 0.62$ respectively). DM, OM, CP and NDF digestibilities were greater in the diets HC and HY compared to LC and LY ($P < 0.01$, $P < 0.01$, $P < 0.01$ and

P < 0.01 respectively). DM, CP and NDF digestibilities were greater for horses receiving LY compared to LC (P < 0.09, P < 0.03 and P < 0.05 respectively). Supplementation of yeast culture to mature horses improved digestibility of lower quality bermudagrass hay.

MATERIALS AND METHODS

The University of Georgia Animal Care and Use Committee approved all procedures used in this experiment.

Horses and Dietary Treatments

Quarter horse (n = 14) and Thoroughbred (n = 2) geldings from the University of Georgia Equine Unit were utilized in a 4 x 4 Latin square design with 28-d treatment periods. Days 1 – 25 were used for diet acclimation, with total fecal collections occurring on days 26 – 28. Geldings were blocked by age and BW and were randomized within blocks to each treatment group. Horses were assigned to one of two fecal collection groups, with two horses from each treatment group assigned to each total fecal collection time (Table 1). Total fecal collections occurred one week apart. Horses ranged in age from 2 to 16 yr with an average of 6.8 +/- 3.2 yr, and ranged in BW from 405 to 581 kg and averaged 483.6 +/- 25.5 kg.

Table 1. Latin square experimental design designating horses to treatment (high quality hay without yeast = HC, high quality hay with yeast = HY, low quality hay without yeast = LC, and low quality hay with yeast = LY) and fecal collection groups.

	R 1, 3	Y 1, 3	G 1, 3	B 1, 3	R 2, 4	Y 2, 4	G 2, 4	B 2, 4
WK 1-5	LY	LC	HY	HC	LY	LC	HY	HC
WK 6-9	LC	HY	HC	LY	LC	HY	HC	LY
WK 10-13	HY	HC	LY	LC	HY	HC	LY	LC
WK 14-17	HC	LY	LC	HY	HC	LY	LC	HY

All horses received a mixed sweet feed (Sweet Horse Feed, Godfrey's Warehouse, Madison, GA containing grain products, plant protein products, roughage products, cane molasses, calcium carbonate, and salt) fed at approximately 0.45 % of BW (DM) daily, and either a high quality Russell bermuda grass hay, or a low quality Russell bermuda grass hay fed at approximately 1.35 % of BW (DM) daily. Both the high and low quality hays were harvested from University of Georgia fields in May 2006 from adjacent fields. Treatments consisted of four diets: high quality forage with the addition of yeast culture (HY), high quality forage without yeast culture (HC), low quality forage with the addition of yeast culture (LY), and low quality forage without yeast culture (LC). *Saccaromyces cerevisiae* (Diamond V XP, Diamond V, Diamond V Mills Inc., Cedar Rapids, IA) was added to grain during the morning feeding at a rate of 56 g per

horse. Horses were fed twice daily in 2.4 m x 1 m individual feeding stalls and allowed access to the diets for approximately 2.5 hr for each feeding. During the remainder of the day, horses were allowed ad libitum access to water, a mineral source (12% Ca, 12% P, 12% NaCl, 0.05% Mg, 100 ppm I, 28 ppm Se, 515 ppm Zn, 125 ppm Cu, 500 ppm Mn, 220,000 IU/kg vitamin A, 11,000 IU/kg vitamin D, 44 IU/kg vitamin E) and free exercise. No additional vitamins were supplemented. Body weight was measured weekly at a consistent time, and diets were adjusted accordingly for the treatment period. Vaccinations and deworming practices were consistent with farm protocol. Horses were vaccinated annually for tetanus, Western equine encephalitis, Eastern equine encephalitis, West Nile, influenza, equine herpes virus (EHV-1, EHV-4), strangles and rabies. Horses were dewormed every 2 mo with non-boticide in the spring and summer and a boticide in fall and winter.

Sample Collection

Total fecal collections were obtained for the last 3 days of each treatment period. During the 3-d collection period, horses were fitted with fecal collection harnesses (Equisan Marketing Pty. Ltd., South Melbourne, Victoria, Australia) and housed in individual stalls (3.0 m x 3.0 m) with ad libitum access to water. A marker, 30 g Cr_2O_3 (Chromic Oxide Sesqui; Fisher Scientific, Fair Lawn, NJ) was administered to each horse by voluntary intake with their first meal of the total fecal collection period to measure mean retention time (MRT). Fecal samples were removed from the collection harnesses every 6 hr. For each 6 hr collection, a 10% subsample was collected, placed in individual bags, and frozen for further analysis. Consumption of grain, hay, and water was recorded

for each 24 hr. Samples of grain and hay were also obtained throughout the total collection period and stored for subsequent analysis.

Preparation of Samples for Analysis

Hay and grain sampled during the total collection period was ground through a 2-mm screen in a Wiley Mill (Thomas Scientific, Swedsboro, NJ). Fecal samples from the 6 hr collections were thawed and mixed for each 24 hr period. A 100 g subsample for each 24 hr period was taken, 25 g from each collection time, and dried in an oven at 55^{o}C to a constant weight for 72 hr. Fecal samples were then allowed to air-equilibrate for 12 hr and ground with the same 2-mm screen.

Chemical Analyses

Hay, grain, and fecal samples were analyzed for DM, OM, and Kjeldahl N according to AOAC (1995) protocols. Crude protein was calculated as the percentage of Kjeldahl N x 6.25. All samples were analyzed for NDF, ADF, lignin, cellulose, and hemicellulose according to procedures described by Van Soest et. al., (1991) and modified (Komarek et al., 1994) for use in an Ankom fiber apparatus (Ankom Technology, Fairport, NY). Gross energy content was determined by bomb calorimetry using a Parr isoperbal colorimeter (Patt Instrument Co., Moline, IL). For chromic oxide analysis the procedure from Fenton and Fenton (1979) was utilized for the fecal samples.

DM was determined by weighing a 1 g sample of fecal, grain and hay and placing the sample in the oven at the following times and temperatures: 135° C for 2 hr, 110° C for 8 hr, and 100° C for 8 hr. Samples were allowed to cool for approximately 4 hr and then placed in a dessicator for 10 min. Each sample was weighed and recorded.

Measurements of neutral detergent fiber were achieved by sealing a 0.5 g sample in a nylon bag placed in a suspender. 2000 ml of NDF solution, 20 g of Sodium Sulfite and 4.0 ml of alpha-amylase reagents were added to the digestion vessel and then the bags in the suspender were placed inside. The vessel was heated and agitated for 60 min. After 1 hr, heat and agitate were extinguished and the valve was opened to facilitate empting of NDF solution. Once the solution was drained, 2000 ml of hot water and 4.0 ml of alpha-amylase was added and agitated for 3 min. This was repeated 2 more times, excluding alpha-amylase for the 3rd rinse. Excess water was removed from the bags, then soaked in acetone for 3 min. Excess acetone was removed, and the bags were placed in a drying oven at 105^{0} C for a minimum of 2 hr. The bags were then placed in a desiccator for 15 min prior to being weighed and recorded. Bags were then stored for ADF analysis.

Measurements of acid detergent fiber were achieved by placing the nylon bags in a suspender and into the digestion vessel with 2000 ml of ADF reagent previously added. The vessel was agitated and heated for 60 min. After 1 hr, the valve was opened to facilitate empting of the ADF reagent. Once the vessel was empty, 2000 ml of hot water was added, bags were agitated for 3 min, then the vessel was emptied and the process was repeated two more times. Excess water was squeezed out of the bags, then they were soaked in acetone for 3 min. The excess acetone was then removed and the bags were placed in a drying oven at 105^{0}C for a minimum of 2 hr. The bags were then placed in a desiccator for 15 min prior to being weighed and recorded. Bags were then stored for lignin analysis.

Measurements of acid detergent lignin were achieved by using dried nylon bags containing a sample that had previously undergone NDF and ADF analysis. The bags were then placed in a 3 L beaker. Approximately 250 ml of 72 % H_2SO_4 was added to the beakers and a 2 L beaker was placed on top of the bags to keep them submerged in the liquid. Bags were agitated at start and at 30 min intervals by pushing and lifting the 2 L beaker up and down approximately 30 times. After 3 hr excess H_2SO_4 was poured off and the bags were rinsed with tap water to remove all acid. Rinses were repeated until the pH was neutral. The pH was determined using an Accumet AR 25 Duel Channel pH/ ion meter (Fisher Scientific, Hampton, NH).

Fecal, grain and hay samples were analyzed for ash content. An Erlenmeyer flask was weighed, recorded, then a 1 g sample was measured into each flask. Flasks were then placed in a Fisher Isotemp Vacuum Oven, model # 281 (Fisher Scientific, Hampton, NH). Samples were cooked at approximately 500 – 550° C for 3 hr. Samples were removed and placed in a drying oven at approximately 110° for 1 hr. Samples were then placed in a desiccator for 20 min removed, weighed and recorded.

Crude protein content of the grain, hay and feces was determined using the FP-528 protein/nitrogen analyzer (Leco Corporation, St. Joseph, MI). The FP-528 is a microprocessor based, software-controlled instrument that determines nitrogen content with three phases during each cycle. The sample is purged of any atmospheric gases that have entered during sample loading of the purge phase. During the burn phase, the sample is flushed with pure oxygen for very rapid combustion. Then the products of combustion are passed through the Furnace Filter and Thermoelectric Cooler. Finally, in

the analyses phase the combustion gases are allowed to become homogeneous by passive mixing. The sample is swept through hot copper to remove oxygen and convert NOx to N_2 and the carbon dioxide and water are removed. The remaining combustion product is nitrogen which was measured by the thermal conductivity cell.

Gross energy content was determined by bomb Calorimetry using a Parr isoperbial colorimeter (Patt Instrument Co., Moline, IL). The oxygen bomb calorimeter measures the heat of combustion or calorific value of a material. A small pellet from a sample of grain, hay and feces was made, weighed, and recorded. The pellet was placed in the calorimeter bomb. A piece of wire small in diameter was then cut, measured and recorded. The wire was fitted into the colorimeter bomb, careful to make sure it was touching the pellet. The bomb is loaded into the jacket, submerged in water. The water is kept at a constant temperature, while the temperature of the bomb rises as heat is released by the combustion of the sample. All temperatures are continuously measured and corrected for heat loss.

For chromic oxide analysis a procedure from Fenton and Fenton (1979) was utilized for the fecal samples. A 0.1 g sample of Cr_2O_3 was placed in a 50 ml Erlenmeyer flask to be used as a standard along with the previously ashed samples in individual Erlenmeyer flasks. 15 ml of molybdate reagent was added to each Erlenmeyer flask and then digested on a hot plate in a perchloric acid hood until a yellow-red color appeared. Samples were then allowed to digest for approximately 10 min. after the color change and removed from the hot plate. Samples were cooled for approximately 20 minutes and

then quantitatively transferred into 50 ml volumetric flasks. Each original flask was rinsed 2 times with water taking care to ensure no sample was retained on the sides of the flask and then the sample was diluted to volume. The Cr_2O_3 standard transferred to a 100 ml volumetric flask and diluted to volume. 5 ml was removed and placed on a centrifuge for 15 min. at 2000 rpm. Samples were then read at 440 mm on a spectrophotometer.

Apparent digestibility was then calculated using the values for each feed intake component, subtracting the fecal output for that component and dividing by feed intake again:

$$\frac{\text{(nutrient intake – nutrient output)}}{\text{nutrient intake}}$$

Statistical Analysis

All data was analyzed by ANOVA using the PROC GLM procedures of SAS (SAS Inst. Inc., Cary, NC) with treatment, period and horse in the model statement. Least square means and standard errors were obtained according to treatment effects on the parameters tested. Data were analyzed for 4 treatments (high quality hay without yeast = HC, high quality hay with yeast = HY, low quality hay without yeast = LC, and low quality hay with yeast = LY). Main treatment effects used horse within treatment as the error term. Three orthogonal contrasts were made comparing diets containing yeast (HY + LY) versus those without (HC + LC), high quality hay (HC + HY) versus low (LC + LY) and the interaction between yeast supplementation and forage quality. In all cases,

probabilities < 0.05 were considered statistically significant. Probability values between 0.05 and 0.15 were considered to be trends towards significance.

RESULTS AND DISCUSSION

Chemical composition of the grain and hays used in the dietary treatments is listed in Table 2. Percentages of DM, ADF, ash, and CP for the high quality Russell bermuda grass hay were within 5 % of reported values for sun-cured Coastal bermuda grass at 15 – 28 d growth (88.4, 30.0, 6.7, and 10.6 %, respectively; NRC, 1989). Values for NDF were greater than NRC (1989) values (73.08 compared to 64.5 % listed in NRC, 1989), which could be due to the difference in variety of Coastal and Russell Bermuda grass hays. Percentages of DM, NDF, ADF, ash, and CP for low quality Russell bermuda grass were within 5 % of reported values for sun-cured Coastal bermuda grass at > 40 d growth (93.0, 71.3, 35.7, 7.5, and 7.3 %, respectively, NRC, 1989). The two hay sources used in this study differed greatly in quality as evidenced by the chemical composition. The low quality hay possessed greater amounts of fiber, (NDF and ADF) and indigestible components (lignin and ash) when compared to high quality hay.

Table 2. Chemical composition of ingredients used in mature horse diets.

		Hay	
	Grain	High	Low
DM, %	90.49	89.59	89.43
NDF, %	21.82	73.08	75.35
ADF, %	10.62	35.22	37.59
Lignin, %	2.39	4.38	6.08
Ash, %	3.91	6.00	4.33
CP, %	12.56	13.13	8.08
GE, Mcal/kg	4.343	4.19	4.17

Voluntary Intake and Body Weight

Horses readily consumed all diets with few refusals of dietary treatments. Values for least square means of total DMI expressed as a percent of body weight increased when horses were fed the low quality hay (LC and LY). This increase in intake of low quality forage may be an attempt by the horses to maintain caloric intake by increasing DMI. There was no influence of yeast culture supplementation on grain or hay intake expressed as a percentage of BW. Least square mean values for BW were not affected by yeast culture supplementation or quality of hay. A search of the literature found no previously reported values for intake of Russell variety bermuda grass hay in horses.

Table 3. Dry matter intake and body weight of mature horses receiving diets of varying forage quality and yeast culture supplementation[a].

	Diet[b]					*P* values		
	LY	LC	HY	HC	S.E.M.	L vs H	Y vs C	Inter.[d]
Total Intake, % BW[c]	1.68	1.67	1.63	1.62	0.014	0.002	0.50	0.86
Grain Intake, % BW	0.45	0.45	0.45	0.45	0.002	0.42	0.23	0.28
Hay Intake, % BW	1.23	1.22	1.18	1.17	0.014	0.001	0.62	0.98
BW, kg	485.3	484.2	484.3	487.1	25.505	0.47	0.49	0.11

[a]Each mean represents sixteen individually fed horses.
[b] HY = high quality forage with the addition of yeast culture; HC = high quality forage without yeast culture; LY = low quality forage with the addition of yeast culture; LC = low quality forage without yeast culture.
[c]Diets were offered based on weekly BW measurements.
[d]Inter. is an abbreviation for interaction between hay quality and yeast supplementation

Digestibility

Apparent digestibilities of dietary treatments are listed in Table 4. DM, OM, CP, NDF, ADF, cellulose, and DE digestibility were greater ($P < 0.01$) for horses receiving high quality hay (HY and HC) opposed to those consuming low quality hay (LY and LC). Horses receiving HY and LY trended to have greater cellulose digestibility ($P < 0.11$) than horses not receiving yeast culture supplementation (HC and LC). Values for least square means also showed a trend for an interaction between quality of hay and yeast culture supplementation for DM, OM, CP, hemicellulose, and NDF digestibility ($P < 0.14$).

There was a tendency for DM ($P < 0.09$) digestibility to increase when yeast culture was supplemented in the diet containing low quality hay (Table 4). Similarly, apparent digestibility of CP, NDF, and hemicellulose ($P < 0.02$, $P < 0.05$, and $P < 0.008$

respectively) were greater when yeast culture was supplemented to the low quality hay diet. There were no differences in apparent digestibility of ADF or GE when yeast culture was supplemented with the low quality hay diet ($P > 0.78$). Other than cellulose, there were no differences observed in digestibility when yeast culture was supplemented with a high quality hay diet ($P > 0.38$). It is possible that yeast culture supplementation enhanced digestibility only when there is some sort of nutritional challenge. In a healthy horse consuming a quality diet that meets nutrient requirements, yeast has very little, if any effect. However with a challenge of a low quality (lowly digestible) hay, yeast supplementation positively influenced nutrient digestibility.

Digestibility values in Table 4 were lower in the high and low quality hay with yeast supplementation for DM, DE, and CP than previously reported values (58.78, 56.64, and 65.00 respectively) in Webb et al. (1985). CP digestibility values disagree with Switzer et al. (2003) who observed 77% digestibility when horses were supplemented with yeast culture. Values reported for NDF digestibility when yeast was supplemented (32.75) were lower then this study's values for higher quality Bermudagrass hay with or without yeast supplementation (Webb et al., 1985). DM and cellulose digestibilities were much lower, 46 vs. 63 % and 37 vs. 53 %, in this study compared to Moore et al. (1993) where a high forage diet (90/10, forage/concentrate) was fed to ponies. These differences could be due to laboratory differences or variety of hay (Webb utilized Coastal bermuda grass hay while Moore fed alfalfa hay). Apparent digestibility of CP and ADF in this study are higher (56 vs. 47 and 28 vs. 24,

respectively) than Godbee (1983) observed, however DM, NDF and hemicellulose are similar (45 vs. 48, 36 vs. 35, and 47 vs. 47, respectively).

Table 4. Apparent digestibility (%) of diets containing varying forage quality and yeast culture supplementation offered to mature horses[a].

	Diets[b]					*P*-values		
Item	LY	LC	HY	HC	SEM	H vs. L	C vs. Y	Inter.[c]
DM, %	40.9	37.7	44.6	46.2	1.3	<0.0001	0.5509	0.0737
OM, %	42.8	40.0	46.4	47.7	1.3	0.0001	0.5789	0.1449
NDF, %	30.4	25.1	36.6	37.8	1.8	<0.0001	0.2610	0.0810
ADF, %	19.4	18.5	28.0	29.6	2.3	0.0001	0.8807	0.5775
Cellulose, %	31.1	25.7	38.9	37.1	2.2	0.0001	0.1130	0.4119
Hemicellulose	41.1	35.8	46.0	48.6	1.3	<0.0001	0.2987	0.0039
DE, %	46.5	46.0	49.6	50.9	1.8	0.0104	0.7755	0.5441
CP, %	52.3	47.5	56.3	57.6	1.5	<0.0001	0.2403	0.0535

[a] Each mean represents sixteen individually fed horses.
[b] HY = high quality forage with the addition of yeast culture; HC = high quality forage without yeast culture; LY = low quality forage with the addition of yeast culture; LC = low quality forage without yeast culture.
[c]Inter. is an abbreviation for interaction between hay quality and yeast supplementation

Data collected on acid detergent lignin and ash were not suitable as several of the least squares means for digestibility estimates were negative. Negative numbers observed for lignin and ash can not be correct and can only be explained by laboratory error or contamination of samples with inorganic material. No useful measurements of MRT were recorded because the six hr samples were erroneously combined into one 24

hr sample and then analyzed for various contents. The rate at which the chromic oxide passed through the digestive tract could not be determined because the feces were not measured as precisely as planned (every 6 hours).

Previous studies using yeast culture supplementation observed increased microbial populations (Yoon and Astern, 1996, Erasmus et al., 1992, Nisbet and Martin, 1991, and Calloway and Martin, 1997). Previous work with extreme conditions, such as malnourished or sick animals, and indigestible feed components showed improvements in production, such as decreased sick days or decreased mortality rates when yeast was supplemented in cattle and chickens (Cole et al., 1992 and Teeter, 1993). These observations promote the theory that yeast culture supplementation has the ability to enhance gastrointestinal tract conditions in a positive manner particularly when the animal is faced with stressful conditions.

Results from previous studies supplementing yeast culture in the equine diet are sparse and in some cases, not definitive. Improvements in the hind gut ecosystem have been observed when yeast culture was supplemented with an intentional starch overload of the small intestine by modifying cecal pH, concentrations of lactic acid and ammonia, and molar percentages of acetate and butyrate (Medina et al., 2002). Increased digestibility was observed in this study when yeast was supplemented with the low quality hay, and is probably related to the yeast culture enhancing a negative condition in the hindgut created from a poor quality diet. Further, studies have shown numerical increases in digestibility of CP (Switzer et al., 2003 and Webb et al., 1985) and ADF, NDF, and hemicellulose (Godbee, 1983) when yeast culture was supplemented in the diet

of mature horses. More research is needed to expand these observations. In this study, a numerical increase in cellulose digestibility was seen when yeast was supplemented to high quality hay. However when yeast was supplemented to low quality hay, DM, OM, NDF, ADF, cellulose, hemicellulose, DE, and CP all showed numerical increases, with all except OM and DE significant ($P < 0.15$). The previous studies mentioned probably did not observe much yeast culture influence on digestibility because the horses were not exposed to a stressful situation such as a low quality hay diet.

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CHAPTER 4

CONCLUSIONS

These results indicate that yeast culture supplementation could benefit mature horses consuming a less than desirable forage. Results from investigations with other species also support these findings and indicate that probiotic supplements may favorably alter the intestinal ecosystem of an animal that is faced with an extreme condition. Yeast supplementation has increased ADG and decreased mortality rates when animals were diseased, malnourished, or whose diet consisted of indigestible or poorly digestible components (Cole et al., 1992 and Teeter, 1993).

The results of this investigation indicated that probiotic supplementation did not alter the digestibility of high quality forage in mature horses. Yeast was supplemented at the recommended dosage, but perhaps this was not enough to make a difference in a healthy horse on a quality diet. One study determined that for yeast culture to be effective when fed to mature horses, it needed to be fed at 10 times the recommended dosage McDaniel et al. (1993).

In conclusion, it appears that yeast culture supplementation does favorably enhance digestion when coupled with an extreme condition. Further research needs to be conducted to solidify these results and to examine the effect of larger doses on a mature, healthy horse under favorable conditions.

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