EFFECTS OF *SACCHAROMYCES CEREVISIAE* ON NUTRIENT DIGESTIBILITY IN MATURE HORSES FED DIETS WITH HIGH AND LOW CONCENTRATE TO HAY RATIOS

By

AMY MICHELLE BROWN

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Kansas State University

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Thesis Approved:

Dr. Steven Cooper
Thesis Advisor

Dr. David Freeman

Dr. Robert Teeter

Dr. A. Gordon Emslie
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Beginning in the 1960's, ruminant nutritionists began several studies examining the effects of dietary enzyme supplements on utilization and efficiency of feedstuffs. Since this time, abundant research has been completed in both ruminant and equine nutrition in an effort to increase digestive efficiency within these animals. Not only have nutritionists hoped to improve feed efficiency and therefore reduce required intake, but more importantly, they hoped to avoid digestive upset. Specifically in equine, research has been focused on increasing carbohydrate digestion within the small intestine, thus reducing the incidence of starch overload in the hindgut. Consequently, this would reduce the incidence of laminitis and colic caused by excessive fermentation of glucose that bypasses the small intestine. Therefore, current research has focused on the feeding of direct-fed microbials such as *Saccharomyces cerevisiae* (yeast culture) and *Aspergillus oryzae* (fungal culture) to enhance gastrointestinal digestion by altering cecal and ruminal fermentation parameters.

Horses are nonruminant herbivores that naturally receive a majority of their diet from grazing or from longstem forages. However, because of the vast weight range and ever-changing work and exercise regimens, total ration nutrient requirements can vary greatly across classes of horses. It is not uncommon in the horse industry to replace 30-60% of the fibrous feeds with concentrates. In the working horse, energy requirements

are often met by increasing the amount of concentrate in the diet and thereby increasing the starch or soluble carbohydrate levels. Increasing these levels, while decreasing forage intake, predisposes the horse to starch overload in the hindgut which could result in digestive upset.

Although, there have been numerous theories proposed, the mode of action of yeast culture has not yet been defined. Amongst the variation, however, it has been found that these supplements modify the microbial population of the digestive system. One theory is that yeast culture provides various growth factors and pro-vitamins that help stimulate the growth of ruminal and cecal bacteria and may increase the lactic acid utilizing bacteria. Horses on a high concentrate diet supplemented with yeast culture could have lower cecal lactate levels, a higher pH, and possibly increased starch digestion in the small intestine. In the cecum, this decrease in lactate accumulation and a higher pH would also provide a more desirable environment for cellulolytic bacteria, thus enhancing fiber digestion and increasing production responses.

Numerous factors such as animal-to-animal variation, strain of yeast, and experimental procedures have contributed to the variation in results of yeast culture studies. However, the digestive advantages of enhanced nutrient digestibility, cecal fermentation and subsequent production parameters provide justification for nutritionists to continue to research yeast culture supplementation.

CHAPTER II

REVIEW OF LITERATURE

Dietary effects on equine digestion

Horses are nonruminant herbivores that contain a large cecum (16% of GI tract) and colon (45% of GI tract) designed to allow for the breakdown and utilization of roughages due to the fiber digesting bacteria. Microbial inhabitants such as bacteria, protozoa, and fungi are found within the hindgut (Julliand, 1992) and are similar to the microbes found within the rumen (Argenzio, 1990). However, bacteria and fungi seem to play a much bigger role in fiber digestion than do protozoa (Julliand, 1999). Results of this hindgut fermentation are most commonly carbon dioxide, methane, and volatile fatty acids that can be readily absorbed and utilized for as much as 30% of the energy source in horses (Bergman, 1991).

It is commonplace now, however, to substitute a large percent of the equine diet with cereal grains that are comprised of much higher starch levels than that of an all forage diet. Carbohydrate sources can be divided into two main groups: those hydrolyzed into simple sugars in the small intestine and those that undergo bacterial fermentation into volatile fatty acids (VFA's) in the hindgut (Hoffman, 2003). Hydrolyzable carbohydrates within the small intestine are acted upon by specific enzymes and yield free sugars such as galactose, fructose, and most importantly, glucose that are absorbed. However, a diet containing 0.2 % to 0.4% of their bodyweight per meal in starch exceeds the capacity of the small intestine to digest starch and results in the undegraded starch

spilling over into the hindgut (Kienzle, et al., 1992 and Potter et al., 1992). This can change the fermentative environment of the hindgut and result in decreased fiber digestion and an increased susceptibility to colic and laminitis.

In grazing horses, hindgut pH remains around 6.4 to 6.7 which provides a desirable environment for cellulolytic bacteria, but pH as low as 5.8 was recorded in the cecum of ponies being fed an all-concentrate diet (Goodson, 1988). Readily fermentable carbohydrates in the hindgut are anaerobically fermented by lactobacillus and streptococci and produce increased levels of lactate. This decreases the pH within the cecum and provides an unstable and undesirable environment for certain microbes, especially those involved in fiber digestion. Julliand et al. (2001) reported a ten-fold increase in lactate concentrations when comparing a 50:50 and 0:100 concentrate:hay diet containing grass hay and barley when collecting cecal and colon contents five hours after a meal. Researchers, Julliand et al., (2001) and Drougal et al. (2001), both feeding varying levels of barley in the diet found a decrease in neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility as well as decreased concentrations of cellulolytic bacteria within the cecum. These results were all contributed to the decrease in pH and the subsequent change in the cecal bacterial populations.

Additionally, an increase in the concentrate to forage ratio has been shown to change concentrations of volatile fatty acids. High starch intakes can decrease the relative amount of acetate and increase the relative amount of propionate produced in the hindgut. Julliand et al. (2001) found a decrease in the acetate:propionate ratio when feeding high concentrations of barley to horses. Findings have shown that in an anaerobic environment, fermentation of carbohydrates produce propionate while cellulose and

hemicellulose predominantly produce acetate (Church, 1988). Therefore, when feeding high concentrate diets, VFA concentrations will be significantly higher in propionate.

Another important consideration for equine nutrition is the rate of passage of feedstuffs through the equine digestive system. Argenzio (1974) found that the size and digestibility of feed particles influenced the rate of passage through the total tract when 2cm X 2mm particles were retained within the gastrointestinal tract longer than 2mm X 2mm particles. Furthermore, Drougal et al. (2001) examined the effect of three hay:grain ratios (100:0, 70:30, and 50:50) on digesta passage rate. This trial observed that feeding the 50:50 hay:grain diet reduced NDF digestibility (46.1% on 0:100 and 39.3% on 50:50). Researchers concluded from this and two companion studies (Julliand et al., 2001; de Fombelle et al., 2001) that increased levels of concentrate increased the rate of passage through the small intestine resulting in more carbohydrate overload in the hindgut and subsequent changes in microbial profiles. Supporting this, Varloud et al. (2004) found that time for digesta in the prececal digestive tract was approximately four hours and 42 minutes within the stomach, and estimated to be from 3.9 to 4.7 hours through the small intestine. This proves to be important if small grains are passed too rapidly into the cecum and are not completely utilized within the small intestine before being passed into the hindgut. Not only does the increased rate of passage increase the chance for digestive upsets, but as discussed in the previous studies, it may very well decrease other nutrient digestibilities.

Mode of Action of Yeast Culture

Adding direct-fed microbials to ruminant and equine diets such as *Saccharomyces cervisiae* and *Aspergillus oryzae* have become two of the most common feed additives

used to enhance gastrointestinal digestion by altering cecal and ruminal fermentative parameters. However, despite years of research the true mode of action is still unclear.

One theory is that the yeast or fungal culture enhances ruminal or cecal pH, thereby stimulating the growth of the microflora and increasing the VFA concentrations within the gastrointestinal tract (Carro et al., 1992, Martin and Nisbet, 1990, Martin et al., 1989). As previously discussed, large intestinal bacteria are most efficient within a stable environment. In ruminants, Dawson et al. (1990) studied the effects of feed supplements on the microbial activities within both rumen-simulating cultures and in the rumen of steers fed a fescue hay-based roughage diet. Two microbial supplements were fed; one contained *Saccharomyces cerevisiae* and the other contained a mixture of yeast, lactobacilli and enterococci. Concentrations of viable yeast cells were increased consistently both in cultures and in the rumen of steers receiving the supplements. Although there were no significant differences in ammonia concentrations, pH, or volatile fatty acid concentrations, they did observe a 5 to 40 times greater concentration of cellulolytic microorganisms both in the cultures and rumens of the yeast supplemented steers when compared to the unsupplemented. This increase in cellulolytic bacteria would support a greater amount of fiber digestibility.

Other studies have indicated however, that the viability of yeast can determine its effect on gastrointestinal parameters. In experiment two of the previous ruminant study, Dawson et al. (1990) evaluated both live yeast culture and inactivated supplements that had been heat treated at 121°C for 15 minutes. Live yeast cultures increased concentrations of both cellulolytic and anaerobic bacteria while also increasing the concentration of propionate, thus decreasing the acetate:propionate ratio within the

rumen. The inactivated yeast culture, however, elicited no change in fermentation parameters and resulted in similar pH and bacterial levels as those of the unsupplemented cultures and rumens. Even though the actual mechanism cannot be explained, these data sugget that live yeast cells were responsible for the increased cellulolytic bacteria.

In support of this, Koul et al. (1998) observed that autoclaved yeast cells produced no effect and that γ-irradiation treated yeast culture produced very marginal effects in fermentation parameters within the rumen of buffalo calves. These marginal results support Girard and Dawson (1995) who have identified a heat labile molecular weight component from the supernatant of *S. cerevisiae* strain 1026 culture and cell cytosol which is responsible for stimulation of growth of certain rumen microbes. The results from Koul et al. (1998) indicate that γ-irradiated yeast cells while still on the medium can no longer reproduce, but are still approximately 50% metabolically active. El Hassan et al. (1993) found similar results using a rumen simulation technique in which live and unautoclaved yeast culture stimulated rumen bacterial numbers and the γ-irradiated yeast had intermediate effects.

Others (Koul et al., 1998 and Dawson and Girard, 1997) agree that the effects of yeast culture may be short-lived as changes in lactate concentration and pH were only present two to four hours after feeding. These data would suggest that the stimulatory components present in the yeast cells are exhausted shortly after being added to the rumen. This may be explained by the hypothesis that small molecular weight components within the yeast culture are most likely short chain peptides. These are believed to be responsible for bacterial stimulation and have very short residence time in the rumen. The

peptides are most likely destroyed by the rumen bacterial population, and therefore, the yeast culture needs to be replenished by actively metabolizing yeast.

In other efforts to determine the mode of action, Newbold et al. (1996) examined the ability of the yeast respiratory activity to protect anaerobic rumen bacteria from damage by O_2. This was tested using different strains of yeast that had previously been shown to vary in their ability to increase the viable count of rumen bacteria when *Saccharomyces cerevisiae* NCYC 240, NCYC 1026, and Yea-Sacc were added to rumen fluid. In vitro an increased rate of O_2 disappearance (46 to 89%) and increased bacterial numbers were observed. Therefore, it was concluded that the ability of *S. cerevisiae* to stimulate rumen bacteria is at least partially dependent on its respiratory activity.

It was determined by the 1990 American Type Culture Collection that there are over 1,000 different strains of *S. cerevisiae*, however, it is not known how effective each strain is as a probiotic (Newbold et al., 1995). In an in vitro study utilizing ruminal fluid, Newbold et al. (1995) compared five different strains of *S. cerevisiae* with all of them tending to stimulate total and cellulolytic bacterial numbers. One strain was significant in increasing total bacteria while a different strain significantly increased cellulolytic bacteria when compared to the others. This provides evidence that the effectiveness of yeast culture on digestive parameters may be at least partially dependent upon the strain.

Yet another theory for the mode of action of yeast culture is the ability to remove toxic factors in cecal and ruminal fluid such as lactic acid. Although previous findings are very conflicting, numerous studies do agree that the supplementation of yeast culture can significantly lower cecal and ruminal lactate concentrations in vivo (Koul et al., 1998; Kumar et al., 1994; Medina et al., 2002; Williams et al., 1990) and in vitro (Newbold et

al., 1998; Nisbet and Martin, 1991; Waldrip and Martin, 1993). Since increased lactate concentrations are known to lower the pH of the cecum and rumen and thus decrease fiber digestion, removal of excess lactate is very beneficial.

Other studies, however, propose that *S. cerevisiae* does not utilize lactic acid as a substrate, but reduces concentrations and increases pH by increasing lactate utilizing bacterial numbers within the rumen (Williams et al. 1991). Callaway and Martin (1997) used two strains of the most common ruminal lactate utilizing bacteria, *Selenomonas ruminantium* and *Megasphaera elsdenii*, and incubated them with a basal medium plus DL-lactate for 24 hours both with and without yeast culture supplementation. This resulted in a 55% increase in the growth of *S. ruminantium* and a thirteen fold increase in *M. elsdenii*. Additionally, the yeast culture added to the *S. ruminantium* increased the acetate and total VFA concentrations and numerically increased propionate. In a similar study by Nisbet and Martin (1991), the same increases in *S. ruminantium* were observed and lactate uptake was significantly increased within the rumen by the addition of yeast culture. Similar increases in bacterial numbers were also found with the addition of *Aspergillus oryzae* to ruminal in vitro mediums (Beharka and Nagaraja, 1998; Waldrip and Martin, 1993).

According to these findings, it seems that yeast culture must provide growth factors for these lactate utilizing bacteria. Nisbet and Martin (1991) found varying levels of common growth factors for ruminal bacteria, such as aspartate, fumarate, and especially malate. When *Saccharomyces cerevisiae* was analyzed, it was found to contain 4.9mM of malate which may provide a growth factor for these bacteria, and therefore increase their concentrations and increase lactate uptake. In contrast, Newbold et al.

(1996) found that growth of these ruminal bacteria was not mediated by malic acid as no differences were observed in total viable bacteria when L-malic acid was either mixed with the diet or continuously infused into the rumen as an aqueous solution.

Another possible explanation for the mode of action is provided by Williams et al. (1991) who found significant decreases in oligosaccharides in the ruminal fluid of steers that had been supplemented with yeast culture. These data suggest that oligosaccharides such as maltose and maltriose may enter the cell of *S. cerevisiae* by the action of a permease. Here, they are converted to glucose and serve as substrates for yeast growth (Panchal et al., 1984). This is of great importance, especially in equine nutrition. If yeast culture can convert these oligosaccharides prececally, then starch overload in the hindgut may be minimized and therefore, reducing the incidences of laminitis and colic.

One of the most common explanations for the variability in yeast culture efficacy is a combination of both type of diet and rate of passage. As previously discussed, the rate of passage through the equine gastrointestinal tract is believed to be longer for high roughage diets and faster for diets high in concentrates. Therefore, when yeast is supplemented to diets high in cereal grains, yeast may not be in the gastrointestinal tract long enough to be fully effective.

Another response to variablility may be the level of supplement included in the diet. Martin and Nisbet (1990) examined the effects of different levels of *A. oryzae* on coastal bermudagrass, soluble starch, and amino acids. They found that this fungal culture, added at 0, 0.4, or 1.0g/liter, stimulated the ruminal microorganism fermentation of starch, bermudagrass, and amino acids to varying degrees. However, when it was added at 1.0g/liter, NDF and ADF digestibility were decreased. Therefore, it was

concluded that supplementation at high levels may actually prove to be detrimental to digestion coefficients.

Effects of Yeast Culture Supplementation on Fermentation Parameters

Supplementing yeast culture to equine diets is believed to enhance nutrient digestibility but has met with varying results. One of the most common findings associated with yeast inclusion is increased nitrogen retention. Glade and Biesik (1986) demonstrated an increase in nitrogen retention ($P<0.01$) from 7 to 13g when Thoroughbred yearlings were supplemented with 112 g of dried live yeast culture (*S.cerevisiae*) per day. Additionally, the proportion of fecal nitrogen that was water-soluble increased 47%, suggesting that the microbial production of ammonia and amino acids was enhanced.

Furthermore, Glade (1990) reported that nursling and weanling foals exhibited increased plasma concentrations of arginine, isoleucine, leucine, lysine, glutamine, methionine, and valine when consuming yeast culture (*S. cerevisiae)*. Additionally, feed efficiency improved 8% in these foals suggesting that yeast culture may influence amino acid balance and nitrogen metabolism, thus resulting in enhanced growth. Within the yearlings horses being fed mixed hay-grain diets, nitrogen retention was significantly increased and the portion of fecal nitrogen appearing as absorbable water soluble compounds also increased ($P<0.01$) suggesting that yeast supplementation decreases endogenous fecal nitrogen (Glade & Sist, 1988). Switzer et al. (2003) also observed an increase in apparent crude protein when fed 4.54 kg of yeast culture per 909 kg of body weight. Furthermore, both *S. cerevisiae* and *A.* oryzae have resulted in significant

nitrogen retention both in vivo and in vitro within the ruminant (Wiedmeier et al., 1987; and Arambel et al., 1987).

In contrast Hall et al. (1990), feeding horses a corn/rice hull based concentrate and alfalfa cubes found no differences in dry matter, NDF, ADF, crude protein or nitrogen balance when feeding 0, 10, 20, or 40 g/hd/d of yeast culture. Additionally utilizing in vitro ruminal fluid, Carro et al. (1992) observed a significant nitrogen retention determined by a reduction of ammonia output when adding yeast culture to a 30:70 forage:concentrate diet. However, when the previous study supplemented yeast culture to the 70:30 or 50:50 forage:concentrate diets, there was no difference in ammonia output or microbial nitrogen syntheis.

Yeast culture supplementation has also been shown to cause an increase in dry matter (DM) and organic matter (OM) digestibility due to an increase in diet fermentation. In pregnant mares, horses supplemented with 20g of yeast culture (*S.cerevisiae*) per day had significant increases in apparent digestibilities of DM, energy, NDF, ADF, hemicellulose, and cellulose (Glade, 1991). In support of these findings, Glade & Sist (1988) found that yearlings being fed mixed hay-grain diets supplemented with 4g of a live yeast culture had an increase of 9% for NDF, 4.8% for DM, 14.7% for hemicellulose, and 8.2% for cellulose digestibility. These findings further support the theory of yeast cultures enhancing fiber digestion. Furthermore, Hall et al. (1990), Switzer et al. (2003), and Godbee (1983) all observed a trend for increased disappearances of DM, OM, gross energy, NDF, ADF, and lignin when mature geldings were supplemented with yeast culture (*S.cerevisiae*).

Additionally, Carro et al.(1992) utilized a 70:30 forage:concentrate, 50:50, or 30:70 forage:concentrate diet to study the effects of yeast supplementation on digestibility. Results from this trial found no significant effects of yeast culture on dry matter or NDF digestibility on the low- or medium-concentrate diets. However, when supplemented to the high concentrate diet, there was a significant increase in these parameters. Further ruminant studies, both in vivo and in vitro, have shown a tendency for dry matter, NDF, and ADF digestibilities to increase with the supplementation of *Aspergillus oryzae* (Wiedmeier et al., 1987; Beharka and Nagaraja, 1993; Arambel et al., 1987).

The resulting increases in digestibility found in these studies are believed to be due to a change in the microbial profile of the cecum and colon. Again however, there have been conflicting results. Moore (1994) observed that mature ponies fed a 70:30 concentrate to roughage diet had short term significant increases in protozoa, total bacteria, cellulolytic bacteria, lactobacilli, and lactate utilizers in the hindgut when supplemented with *S. cerevisiae*. Medina et al. (2002) fed both a high fiber and high starch diet with and without the inclusion of 10 g of *S. cerevisiae* to cecum and colon fistulated horses. When fed the high starch diet, total anaerobic and lactic-acid utilizing bacteria increased significantly, whereas the concentrations of cellulolytic bacteria decreased within the cecum. However, within the colon, concentrations of lactobacilli and streptococci increased and the pH decreased along with the [(acetate+ butyrate) /propionate] ratio. Therefore, this trial concluded that the changes in bacterial concentrations within the cecum coincided with the abundance of yeast cells and may have limited the incidence of digestive upset within the cecum.

Further equine studies have shown contrasting changes in both pH and fermentation parameters. McDaniel et al. (1993) supplemented *A. oryzae* in vitro to equine cecal fluid from mature geldings being fed a 70:30 diet of coastal bermudagrass and grain, respectively. Initial fermentation of soluble starches with fungal supplementation increased the concentrations of acetate, propionate, NH_3, and L-lactate, however it decreased final pH. Also within this study, *A. oryzae* was added to Trypticase, alfalfa hay and bermudagrass, with a decrease in pH and decrease in NDF and ADF digestibilities. However, when cecal fluid was adapted to *A. oryzae,* there was no significant effect on any parameter measured, suggesting that microorganisms within the cecum may have adapted to the product. Similarly, Hall and Miller-Auwerda (2005) found that adding *Saccharomyces cerevisiae* to a pelleted corn ration had no effect on cecal pH at 0, 2, 6, 8, or 10 hours after feeding. Nevertheless, the yeast did result in an increased pH at 4 hours after feeding which may have aided in avoiding digestive upset.

Numerous ruminant studies have also seen similar effects in ruminal pH, volatile fatty acid concentrations, and bacterial populations. During an in vivo study using dairy cows, Yoon and Stern (1996) observed no difference in ruminal pH, ammonia N, or total VFA concentrations but did discover a significant increase in proteolytic and cellulolytic bacterial counts when supplementing a fungal culture.

Furthermore, when buffalo calves were fed a high roughage diet supplemented with *Saccharomyces cerevisiae*, higher pH levels were recorded up to 6 hours post-feeding when compared with the control group. Additionally, concentrations of total viable and cellulolytic bacteria along with acetate and the acetate:propionate ratio were higher in the group supplemented with yeast culture (Kumar et al., 1997). Wiedmeier et

al. (1987) also found an increased acetate:propionate ratio with the addition of fungal supplements in Holstein cows. On the other hand, when Holstein cows were fed a 60:40 concentrate:corn silage ration with and without yeast culture, ruminal pH, ammonia, molar proportions of acetate and isovalerate, and the acetate:propionate ratio were all decreased while anaerobic and cellulolytic bacterial counts were increased (Harrison et al., 1988). Furthermore, Martin et al. (1989) had similar findings in vitro in which an increase in propionate and a corresponding decrease in the acetate:propionate ratio were observed when live yeast culture was supplemented to coastal bermudagrass, soluble starch, and Trypticase.

Energy metabolism in equine nutrition

Energy Partitioning. When the feedstuffs are consumed by the animal, the organic molecules undergo oxidation to produce energy within the body. This energy is then released from the body as heat or retained within the animal for use in powering the animal's metabolic processes. These feedstuffs can contain varying levels of utilizable energy based upon their chemical composition and how it is utilized within the animal. Determining energy requirements within the horse, however, can prove to be a difficult process.

Energy is defined as the potential or capacity to do work which makes partitioning energy requirements in horses extremely unique since they have over a 10-fold range of mature body weights and a vast range of work and exercise levels for each range of body weight. Currently, the most common system for evaluating energy content in feedstuffs is by the energy portioning system:

For this system, energy content is most commonly expressed as calories (cal), kilocalories (kcal), or megacalories (Mcal) of gross energy (GE), digestible energy (DE), metabolizable energy (ME), or net energy (NE). Another common unit of measure that is used to express energy, mainly in European countries, is joules (J), kilojoules (kJ), or megajoules (MJ) (1 Mcal=4.184 MJ; 1 MJ=0.239 Mcal; 1 MJ=239 kcal). More classical research may also refer to energy in total digestible nutrients (TDN) where 1 kg of TDN is equivalent to 4.4 Mcal of digestible energy (NRC, 1989).

Determined by combustion within a bomb calorimeter, gross energy is the energy released from a substance when combusted and is determined by the proportions of carbohydrate, protein, and fat within the substance since these are the main sources of energy to the body. Following the energy partitioning system, dietary GE intake minus the energy found in feces results in digestible energy. Metabolizable energy accounts for the energy lost within urine and gas, while net energy takes the heat increment of the animal into account. Heat increment is the inefficient loss of energy as heat which is produced by digestion and metabolism of nutrients within the body. Net energy is further split into that portion of energy that must be used for maintenance within the body and that which can be utilized for production or growth.

Currently, in equine nutrition, DE is used to express and calculate energy requirements for numerous reasons. First, there are few ME values which have been

calculated for equine feedstuffs within the United States. Furthermore, using these feedstuff values from cattle requirements may be an overestimation due to the differences in digestive anatomy and physiology. Additionally, within the horse, it is very difficult to obtain a realistic estimation of gas losses. In a study by Pagan and Hintz (1986), they estimated the methane loss to be 2.9% $\pm$ 0.10% of GE and 4.6 $\pm$ 0.10% of DE which is much lower than the common 8% of GE loss for ruminants. Additionally, numerous factors such as animal-to-animal variation, body composition, environmental temperature and humidity, all effect energy requirements. Relative to exercise, the intensity and duration of work, weight of tack and rider, running surface conditions, and degree of fatigue can all influence the required energy for horses. Therefore, numerous DE equations have been developed to meet the weight and work level differences when calculating energy requirements (NRC, 1989).

Energy Sources and Utilization. Carbohydrates are the primary energy source in equine diets and are broken down by pancreatic and intestinal enzymes within the small intestine before they experience fermentation within the hindgut. Within the small intestine, nonstructural carbohydrates are hydrolyzed into sucrose, fructose, galactose, and most commonly, glucose which can be absorbed and then utilized for energy within the body. Hintz et al. (1971), when feeding mature ponies, reported that 71% of carbohydrates from a high-corn diet and 46% from a high-alfalfa diet were digested prececally. Nonstructural carbohydrates that are not digested in the small intestine and structural carbohydrates such as hemicellulose and cellulose are passed into the cecum where they undergo anaerobic microbial fermentation. Within the cecum and colon, carbohydrate fermentation produces volatile fatty acids such as acetate, propionate,

butyrate, valerate, and isovalerate. A diet higher in these structural carbohydrates (high forage diet) will produce more volatile fatty acids to be utilized for energy (NRC, 1989). When fed a 1:0 forage:concentrate ratio diet, acetate was found to be 73% of the VFA's produced, while propionate and butyrate were 17 and 8%, respectfully. On a 1:4 forage:concentrate diet, however, the percent of VFA's changed to 59, 25, and 11% of acetate, propionate, and butyrate, respectfully (Hintz, 1971). This supports the findings that the acetate:propionate ratio, while on a high forage diet, will be higher than when feeding a high concentrate diet.

Lipids are a concentrated source of energy that can be readily utilized by the animal for energy production. Dietary fats and fat solubles from intestinal chyme are formed into biliary micelles and then intestinal micelles. They are primarily digested within the duodenum of the small intestine and require pancreatic lipase and colipase. (Breazile, 2005). Lipids in the form of triglycerides are broken into fatty acids and a glycerol. This glycerol can be converted into glucose and then utilized through glycolysis. The resulting fatty acids undergo beta-oxidation to produce two-carbon units and the resulting acetyl-CoA. Providing oxygen is available, this acetyl-CoA goes through the citric acid cycle and results in a net production of 12 units of ATP (Murray, et al. 2000). Therefore, if horses are performing at a low-intensity level of work, allowing for aerobic exercise, fats can yield 9 kcal/g of energy compared to that of 4 kcal/g provided by carbohydrates.

Additionally, added fat diets have become increasingly popular in the equine industry due to the high digestibility of the lipids. In a study by Bush et al. (2001), four mature horses were fed a 60:40 forage:concentrate diet containing 0, 5, 10, or 15% corn

oil. Although not statistically significant, this trial observed that dry matter digestibility was highest for the 10% corn oil, but fat digestibility was highest for horses fed 15% corn oil (78.5%), further suggesting that the increased level of fat provides more substrate for lypolysis to occur and, therefore, higher levels of fat being digested. Additionally, this study reported no differences in crude protein, gross energy, or fiber digestibility at any level of added-fat. However, increased fat diets can have problems rancidity (NRC, 1989) and can depress fiber digestion. Jansen et al. (2000) reported that horses consuming 37% of net energy of their diet in the form of soybean oil experienced depressed crude fiber, NDF, and ADF digestibilities, suggesting that more fat may be entering the cecum and decreasing fermentation of cellulolytic bacteria.

Protein is the third source of energy available in feedstuffs. However, the requirements have to be adjusted for the needs of the animal, the quality of the protein available, and the digestibility of that protein. Mature horses are much less sensitive to protein quality than are growing horses. Protein digestibility also changes with the source of protein and type of diet. It has been reported that protein digestibilities may be as low as 43% on a grass hay diet and as high as 69% on an alfalfa:concentrate diet. Protein is digested within the small intestine and absorbed as amino acids. Protein, bypassing the small intestine, is digested in the hindgut to release ammonia which serves as a source of nitrogen and energy (NRC, 1989).

It is the objective of the current study to determine if *Saccaromyces cerevisiae* will affect the in vivo nutrient digestibility of mature horses on high concentrate or high forage diets. Additionally, energy values will be determined to see the correlation between DE and ME when providing differing levels of a carbohydrate source in the diet.

CHAPTER III

MATERIALS AND METHODS

Animals and Management. Eight mature, stock-type geldings were paired by weight and each assigned to a sequence using 4x4 Latin squares to form a crossover experimental design (Table 1). The objective of this study was to determine the effects of supplemental yeast on digestion coefficients in horses fed either a 70:30 or 30:70 concentrate:forage diet Horses ranged from 5 to 17 y ears of age with a mean age of 9 and with a mean weight of 506.2 kg. Thirty days prior to the start of the trial, horses were vaccinated, dewormed and had their feet trimmed and teeth floated. Horses were assembled at the OSU Equine Center and housed individually in 12'x12' box stalls while receiving a minimum of four hours of free exercise per day in a dry-lot arena. Free, unlimited access to water was provided and horses continued to receive routine health care, as needed, while on trial. All animals were housed and maintained according to the Oklahoma State University Institutional Animal Care and Use Committee Guidelines.

TABLE 1. Latin Square Experimental Design

Sequence (Horse)	**Treatments**			
seq=1 (3 and 4)	HC*	HCY**	HFY±	HF++
seq=2 (1 and 7)	HCY	HC	HF	HFY
seq=3 (5 and 8)	HF	HFY	HC	HCY
seq=4 (2 and 6)	HFY	HF	HCY	HC

seq = sequence

*HC = high concentrate, no yeast; **HCY = high concentrate, with yeast; ±HF = high forage, no yeast; ++HFY = high forage, with yeast

Diet. The experiment utilized two diets with a concentrate:forage ratio of 70:30 (high concentrate, HC) and 30:70 (high forage, HF), both with and without the inclusion of a yeast culture preparation containing *Saccharomyces cerevisiae* (Diamond V "XP" Cedar Rapids, IA). Experimental treatments were: a) high concentrate with no supplemental yeast culture (HC); b) high concentrate with yeast culture supplemented (HCY); c) high forage with no supplemental yeast culture (HF); and d) high forage with yeast culture supplemented (HFY). Supplemental yeast culture was added to the concentrate as a top-dressing at each feeding at the recommended feeding level of 37.5 g/hd/d (assuming a 500 kg horse consuming 1.5% of BW/d) within the total ration.

Diets were formulated to meet or exceed NRC (1989) recommended requirements for DE, CP, Ca and P and fed at levels to maintain body weight. The concentrate ration was composed primarily of rolled corn, whole oats, and molasses while the forage component was Bermudagrass hay (Table 2). Prior to feeding treatment diets, all horses were allowed a 30 day acclimation period and fed the concentrate and forage in a 50:50 ratio.

Once the horses were receiving treatments, the HC and HCY sequences were fed at 1.3% of their body weight and the HF and HFY sequences were fed at 1.5% to maintain isocaloric intakes. Horses were fed in two equal feedings at 7 a.m. and 5 p.m. and were weighed every three days at 7 a.m. prior to the morning feeding to monitor weight gain or loss. Grain and hay samples were taken periodically throughout each acclimation period and samples were taken during each day of the collection periods for subsequent nutrient analysis (Table 2). All feed refusals were collected and recorded before each feeding throughout both the acclimation and collection period, however, no

TABLE 2. Diet composition for treatment diets, % as fed

	Diet	
Ingredient	**HC***	**HF****
Bermuda Grass Hay	30.00	70.00
Shelled Corn, Cracked	44.41	19.03
Oat Grain	21.00	9.00
Liquid Cane Molasses	3.50	1.50
Trace Mineralized Salt	0.35	0.15
Limestone	0.53	0.23
Dicalcium Phosphate	0.21	0.09
Analyzed Nutrient Composition, DM basis		
Determined DE (Mcal/kg)†	2.68	2.26
CP, %	9.29	8.96
NDF, %	32.36	53.34
ADF, %	15.31	26.62
Lignin, %	4.09	5.87
Starch, %	41.97	20.73
Fat, %	4.50	3.13
Ca, %	0.28	0.35
P, %	0.29	0.22
K, %	0.79	0.87
Na, %	0.14	0.14
Mg, %	0.16	0.19

*HC = high concentrate, no yeast; **HF = high forage, no yeast
† Determined DE was obtained by an average of the energy provided by HC and HF diets divided by the average intakes of horses during those treatments.

intake adjustments were required. Water intake was also monitored and recorded during collection periods.

Collection Period. The experiment included four, 21-day acclimation periods, each followed by a 72 hour total fecal and urine collection. Animals were bedded on wood shavings and prior to each collection period, stalls were thoroughly cleaned and ¾" thick, rubber mats were placed in stalls. Each animal stood tied for the 72 hour period

with enough room to eat, drink, and lay down. Additionally, horses were hand-walked for 15 minutes/day to provide exercise.

Urine Collections. Total urine output was collected via harness every 2 hours during each 72 hour collection period. Total volume was recorded and a representative sample (2% of the total volume) was taken from each collection and composited during each period. Additionally, 6N HCL was added to a 20 mL subsample of urine in varying amounts until a pH of <2.5 was reached. Both acidified and non-acidified samples were frozen until subsequent mineral and nitrogen analysis could be performed.

Fecal Collections. Feces were collected every 2 hours during each 72 hour collection period. After each 24 hour period, total fecal output was weighed and multiple grab samples were taken and frozen for later analysis. Any contaminated feces were collected and weighed but not added to the total collection for subsampling.

Feed and Fecal Analysis. Fecal samples from each horse were thawed at room temperature for 24 hours. A 50 g subsample of the feces, feed, and hay were then placed into a drying oven at 50℃ for 96 hours to determine dry matter. Dried samples were weighed and ground. Initial dry matter weights were used to convert wet weights to a dry matter basis. All feed, hay and fecal samples were then sent to Dairy One (Ithaca, NY) for analysis of ADF, NDF, starch, fat, calcium, phosphorus, sodium, potassium, and magnesium.

Samples were analyzed for ADF and NDF using the ANKOM A200 Filter Bag Technique (A200-Ankom Technology, Macedon, NY). The ADF samples were individually weighed into filter bags and digested as a group of 24 in 2 L of ADF solution in an ANKOM A200 Digestion Unit. Samples were then rinsed three times with boiling

water, followed by an acetone rinse and dried at 100℃ for two hours. Samples were then weighed again for ADF determination. Similarly, NDF samples were weighed individually into filter bags and digested as a group of 24 in NDF solution, 4 mL α-amylase and 20 g of sodium sulfite in an ANKOM A200 Digestion Unit. Samples were then rinsed three times in boiling water, with α-amylase added to the first two rinses. Following this, samples were rinsed in acetone and dried at 100℃ for two hours. Samples were weighed again for NDF determination.

Starch was determined using the YSI 2700 SELECT Biochemistry Analyzer (YSI Incorporated, Yellow Springs, OH). Samples were pre-extracted for sugar by incubation in a water bath and by filtration on Whatman 41 filter paper. Residues were thermally solubilized using an autoclave and then incubated with a glucoamylase enzyme to hydrolyze starch and produce dextrose. Samples were then injected into the sample chamber of the YSI Analyzer where dextrose diffuses into a membrane containing glucose oxidase. The dextrose is then oxidized to hydrogen peroxide and D-glucono-4-lactone. This hydrogen peroxide is detected amperometrically at the platinum electrode surface. Current flow at the electrode surface is directly proportional to the hydrogen peroxide concentration, and therefore, the dextrose concentration. Starch content is then determined by multiplying dextrose by 0.9. Fat was analyzed through ether extraction by the Tecator Soxtec System HT6 (FOSS Tecator, Eden Prairie, MN).

Minerals were determined by ashing samples in a muffle furnace at 500℃ for 4 hours, then 3 mL of 6N HCl were added to the ash residue and evaporated to dryness on a 100° - 120℃ hot plate. Minerals were extracted with an acid solution (1.5N HNO_3 + 0.5 N

HCl) and determined using inductively coupled plasma spectroscopy (Thermo Jarrell Ash IRIS Advantage, Franklin, MA).

Urine Mineral Analysis. Individual urine samples were thawed at room temperature for 24 hours and then transported to the OSU Soils Testing Lab (Stillwater, OK) for mineral analysis. All samples were analyzed for Ca, P, Na, K, and Mg using inductively coupled plasma spectroscopy (Thermo Jarrell Ash IRIS Advantage, Franklin, MA).

Nitrogen Analysis. All feed, fecal and urine samples were sent to the OSU Soils Testing Lab (Stillwater, OK) for total nitrogen analysis by combustion according to the AOAC guideline 990.3 using the LECO analyzer (St. Joseph, MI). Both acidified and non-acidified urine samples were analyzed for nitrogen with no difference in nitrogen values between samples.

Gross Energy Analysis. Gross energy values for feed, fecal and urine samples were determined using the bomb calorimeter (Parr Institute, Series 1261, Moline, IL). Feed and fecal samples were formed into approximately 1 g pellets and exact weights were recorded. After complete combustion within the bomb, samples were removed and the remaining wire was measured and recorded. Additionally, the crucible and bomb were rinsed with distilled H_2O, two drops of methyl orange solution was added and the solution was then titrated with Na_2CO_3 to correct for any S or N oxides formed from the sample.

Urine was analyzed using 2x3 inch flat, plastic bags (Jeb Plastics, Inc, Wilmington, DE). Bags were cut in half to approximately 1.5 inches and exact weights were recorded. Urine, 3 to 4 mls, was then added to the bags and weight was recorded

again. Urine and bags were dried at 55°C in a circulating air oven and allowed to dry (approximately 96 hours). Samples were then analyzed in the bomb calorimeter, entering 1 g as the weight. Ten empty bags, cut to 1.5 inches, were bombed as a correction value to find the average cal/g value of the bags. Urine energy was then calculated as: (total calories released-(bag weight, g * bag average cal/g))/urine weight, g.

Statistical Analyisis. Data were analyzed using the mixed procedure of SAS (2001) with sequence, period, and treatment as main effects and horse(sequence) as a random effect. Least squares means were calculated for each parameter and the Kenward-Rogers p-diff procedure was used to test for differences between treatment means with significance declared at $P < 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

DM, NDF, and ADF Digestibility. Data for intake, fecal output, and apparent DM digestibility is shown in Table 3, while intake, fecal output, and true digestibility values for NDF and ADF are shown in Table 4. There were no significant interactions or main effects as a result of yeast supplementation. These data coincide with Hall et al. (1990) who observed that Quarter Horse geldings consuming a diet consisting of corn/rice hulls and alfalfa cubes experienced no effect of yeast culture on apparent digestibility of dry matter, neutral detergent fiber, acid detergent fiber, hemicellulose or lignin. Additionally, McDaniel et al. (1993) observed that *A. oryzae* added to incubation vessels containing alfalfa or Bermudagrass hay and inoculated with cecal fluid from an unadapted, mature horses actually decreased in vitro DM, NDF and ADF digestibility and resulted in no effect from adapted cecal fluid. In ruminants, Carro et al. (1992) found no significant differences on the previously mentioned parameters when *S. cerevisiae* was added to a rumen inoculum from steers on a 70:30 or 50:50 forage:concentrate diet. Other in vitro studies (Martin and Nisbet, 1989 and Newbold et al. 1993) also concluded that yeast supplementation had no effect on digestive parameters when incubated in ruminal fluid. Additionally, Harrison et al. (1988) added *S. cerevisiae* to ruminally fistulated Holstein cows being fed a 60:40 concentrate to hay ratio and found no differences in DM, ADF, NDF, hemicellulose, or starch digestion even though the number of cellulolytic bacteria increased. In contrast, others (Glade and Sist, 1988 and Glade 1991) observed that

Table 3. Effect of Yeast Culture on Apparent Dry Matter Digestibility[a]

	Treatment Diets				
Item	HC[*]	HCY[**]	HF[†]	HFY[±]	SEM[d]
Dry Matter					
Intake, g/d	6196[b]	6233[b]	7168[c]	7126[c]	245
Fecal, g/d	2285[b]	2334[b]	3312[c]	3234[c]	177
Digestibility, %	62.9[b]	62.4[b]	54.1[c]	54.7[c]	1.9

[a] Values are least squares means

[b,c] Means within a row with different superscripts differ (P<0.05).

[d] Values are average standard error.

[*]HC = high concentrate, no yeast; [**]HCY = high concentrate, with yeast; [†] HF = high forage, no yeast; [±]HFY = high forage, with yeast

Table 4. Effect of Yeast Culture on True NDF and ADF Digestibility[a]

	Treatment Diets				
Item	HC[*]	HCY[**]	HF[†]	HFY[±]	SEM[d]
NDF					
Intake, g/d	2107[b]	2115[b]	3853[c]	3824[c]	114
Fecal, g/d	1447[b]	1471[b]	2813[c]	2775[c]	95
Digestibility, %	31.2[b]	30.4[b]	27.1[c]	27.3[c]	1.1
ADF					
Intake, g/d	1045[b]	1048[b]	1944[c]	1929[c]	56
Fecal, g/d	953[b]	970[b]	1375[c]	1341[c]	78
Digestibility, %	8.8[b]	7.4[b]	29.9[c]	30.6[c]	6.1

[a] Values are least squares means

[b,c] Means within a row with different superscripts differ (P<0.05).

[d] Values are average standard error.

[*]HC = high concentrate, no yeast; [**]HCY = high concentrate, with yeast; [†] HF = high forage, no yeast; [±]HFY = high forage, with yeast

yearling and mature horses experienced a significant increase in DM, NDF, and ADF digestibility when supplemented with yeast culture. Additionally, in an in vitro ruminant study, Carro et al. (1992) reported an increased DM and NDF digestibility in incubation vessels containing a 30:70 forage:concentrate diet.

Apparent DM digestibility and true NDF digestibility was higher for horses receiving the HC diets than the HF, however true ADF digestibility was higher on the HF diets ($P<0.05$). This is to be expected since the high concentrate diets were higher in nonstructural polysaccharides such as starch (37.61% on HC compared to 18.69% on HF) which are degraded almost completely within the small intestine.

Digestibility is greatly influenced by both the amount and chemical composition of the fiber contained in the diet. Therefore, it is within reason that the apparent digestibility of the high forage diet should be much lower than that of the high concentrate. The HF diets contain a much higher concentration of structural carbohydrates such as hemicellulose, cellulose and lignin. Van Soest (1982) found that lignin is the most significant limiting factor in plant cell wall digestion and shows high resistance to chemical degradation. Therefore, lignin is almost completely indigestible. Additionally, there is much evidence that strong chemical bonds exist between lignin and plant polysaccharides and cell wall proteins which result in these compounds being unavailable to digestion (McDonald et al., 1995). Mature hays are rich in lignin and therefore, low in digestibility unless chemically treated to break the bonds between lignin and other carbohydrates. Because horses receiving the HF diet received significantly higher amounts of lignin (420 g/d on HF; 255 g/d on HC) this can explain the significantly lower apparent digestibility between the high concentrate (62.86%) and high

forage diets (54.11%). True NDF digestibility was higher (P<0.01) in the HC (31.25%) when compared to HF (27.05%) diets as a result of intake. The extreme difference of intake on the high forage versus the high concentrate diets resulted in a lower digestibility due to the high levels of lignin that decreases the extent of digestion in a high forage diet. True ADF digestibility, however, was higher for HF (29.9%) when compared to HC (8.8%).

Crude Fat. Data for intake, fecal output and apparent digestibility of crude fat is shown in Table 5. Yeast culture supplementation had no effect (P>0.05) on any of the previously mentioned parameters. However, there was a significant effect due to the type of diet. High concentrate diets yielded apparent digestibilities of 67 to 68% while the high forage diets were only 54 to 55% digestible. Fat digestibilities from the current research relate with previous findings in other studies (Fonnesbeck et al, 1967; Bowman et al., 1979; Sturgeon et al., 2000) in which mean estimates for apparent digestibilities of ether extract in horses and ponies were found to be 42-49% in forages and 55-76% for grains. Fat-added diets consisting mainly of triglycerides were found to have apparent digestibility values ranging from 88-94%, nearing that of the estimated 100% (Kane et al., 1979; McCann et al., 1987). Using the endogenous fat estimation of 0.22 g/d/kgBW (Kronfeld, 2004), true fat digestibility of the current diet was calculated (Table 5). These values exceeded the 100% digestibility that is expected from fats (Kronfeld, 2004).

Variation in fat digestibility between grains and forages has previously been explained by either the presence of nonhydrolyzable substances in the ether extract or from the dilution of endogenous fecal fat. The low digestibility values presented in the current data may also be explained by an extensive study by Kronfeld et al. (2004) who

Table 5. Effect of Yeast Culture Apparent Crude Fat Digestibility[a]

	Treatment Diets				
Item	HC[*]	HCY[**]	HF[†]	HFY[±]	SEM[d]
Crude Fat					
Intake, g/d	277[b]	279[b]	225[c]	224[c]	9.4
Fecal, g/d	91[b]	89[b]	104[c]	101[c]	8.8
Digestibility, %	67.2[b]	68.3[b]	54.1[c]	55.2[c]	2.5
True Digestiblity, %	107	107	103	104	

[a] Values are least squares means

[b,c] Means within a row with different superscripts differ (P<0.05).

[d] Values are average standard error.

[*]HC = high concentrate, no yeast; [**]HCY = high concentrate, with yeast; [†] HF = high forage, no yeast; [±]HFY = high forage, with yeast

compiled 188 equine digestion balance observations on five basal feeds and 18 test feeds with added fats. Using an endogenous fecal fat estimate of 0.22g/d/kgBW found from linear regression of data (Kronfeld, 2004), a majority of the observations neared a 100% true digestibility. However, a third theory was also proposed from examining these observations. This was a first-order relationship between the observed digestibilities and the amount of fat within the diet. It was observed that when the fat content was 20 to 24g/kg, the efficiency of absorption was half of the maximal rate. This low content of fat from the diet leads to a low substrate concentration within the small intestine. This is believed to slow the rate of lipolysis which is especially detrimental to forages since they are believed to pass from the mouth to the cecum as rapidly as within 2 to 3 hours after consumption. Therefore, the rate of passage does not allow for complete digestion and because the low substrate inhibits lipolytic activity, there is a decreased rate of fat digestion from forages.

In fat-added diets, the substrate concentration is increased in the upper small intestine which slows gastric-emptying and prolongs the amount of time the feedstuff experiences prececal digestion. Therefore, it is concluded that fat digestibility is a function of the rate of lypolysis. This results in low fat digestibility when substrate concentration in the small intestine is low due to a low fat content in the food which coincides with the present data.

Nitrogen Metabolism. Data for intake, fecal output, urinary output, balance, and digestibility of nitrogen is given in Table 6. There was no significant interaction of yeast culture supplementation on nitrogen balance or digestibility. This coincides with Hall et al. (1989) who saw no effect of supplementing yeast culture at 10, 20, or 40 g/hd/d to a

corn/rice hull based basal diet with alfalfa cubes. In this study, all horses were maintained in positive nitrogen balance throughout the study. Nitrogen intake was highly correlated with fecal and urinary nitrogen excretions, nitrogen absorbed, and nitrogen balance. Using nitrogen balance as a measure of nitrogen metabolism, comparisons of intake, excretion, and retention showed no significant effects from the yeast culture supplementation. In relation to those findings, Switzer et al. (2003) saw no significant difference in crude protein digestiblity when yeast culture was supplemented to aged horses at a level of 9.09 kg/909 kg.

In contrast to the current study, Switzer et al. (2003) found a significant difference in apparent crude protein digestibility when yeast was added at a level of 4.54 kg/909 kg. Supporting these findings, Glade and Sist (1990) observed a significant increase in nitrogen retention of nursling foals supplemented with a dried, live yeast culture preparation. Additionally, Carro et al. (1992) observed a trend ($P<0.10$) for increased nitrogen retention and reduced ammonia output when yeast culture was supplemented to ruminal fluid from a high concentrate (30:70) forage:concentrate diet. Also from Glade and Sist (1988), yearlings fed a mixed hay-grain diet supplemented with live yeast culture experienced a significant increase in nitrogen retention. From reviewing the previous studies, it may be concluded that yeast culture may have a greater effect on nitrogen retention in growing horses than in mature, sedentary equids.

Table 6. Effects of Yeast Culture on Nitrogen Balance and Apparent Digestibility [a]

	Treatment Diets				
Item	HC[*]	HCY[**]	HF[±]	HFY[++]	SEM[e]
NRC Req. g/d [†]	105.35	106.12	106.40	106.71	
Intake, g/d	87.67	88.32	87.25	86.80	3.28
Fecal, g/d	36.17[b]	33.79[b]	47.58[c]	48.28[c]	2.88
Urine, g/d	51.43	47.22	46.62	46.92	2.47
Balance, g/d	0.065[bc]	7.31[b]	-6.96[cd]	-8.39[d]	3.05
Digestibility, %	58.26[b]	61.45[b]	45.78[c]	44.23[c]	2.72

[a] Values are least squares means.
[bcd] Means within a row with different superscripts differ (P<0.05).
[e] Values are average standard errors.
[†] NRC, 1989 recommended calculation for crude protein. CP g/d = 40* Mcal of DE/day divided by 6.25 to obtain nitrogen requirement.
[*]HC = high concentrate, no yeast; [**]HCY = high concentrate, with yeast; [±]HF = high forage, no yeast; [++]HFY = high forage, with yeast

When analyzing nitrogen balance and digestibility there was a significant effect of diet (Table 6). Due to unexpected nutrient composition of the diets, all animals experienced a deficient nitrogen intake. Nitrogen digestibility was observed to be from 58-62% for high concentrate diets while digestibility was decreased to 44-46% on the high forage diets. Fecal output of nitrogen from animals receiving the high forage diet was also significantly greater, thus proving that less nitrogen was retained from these diets due to the differences in digestibility. The increased nitrogen excreted in the feces also led to the negative nitrogen balance. This may also be partially explained by the physiology of protein digestion. Diets high in loose hay have been shown to pass from the mouth to the cecum very rapidly after consumption, and therefore, less protein may be digested as compared to a high concentrate diet that has a longer lag time within the small intestine.

Additionally, these data can be explained by the type of diet and digestibility of protein consumed. In a study by Gibbs et al. (1988) it was concluded that forage protein digestion may be much greater in hindgut than previously thought. Numerous studies have concluded that protein digestibility can vary greatly with the source of protein (Slade et al., 1970; Reitnour and Treece, 1971; Gibbs et al., 1988) and also from the concentration within the diet (Slade et al., 1970; Prior et al., 1974; Freeman et al., 1985; Gill et al., 1985). Although mature horses are thought to be less sensitive to these factors than growing horses, they are still a major determinant in the level of protein digested. The NRC (1989) has reported that apparent protein digestibility may be as low as 43% on a low protein (8% CP) grass hay diet. This coincides with the current study while animals were receiving the 30:70 concentrate:hay diet. The low digestibility of the high

concentrate diet can be explained by the high ratio of concentrate to hay. The NRC (1989) also reported that when the concentrate:hay ratio is increased above 1:1, protein digestibility may be as high as 70-75%. In contrast, Meyers et al. (1987) found that when the concentrate:hay ratio was 3:1 there may be a reduction in protein digestibility. This theory can be used to explain the low nitrogen digestibility of the high concentrate diet.

Protein digestibility of the current treatment diet can be used to explain the results in nitrogen balance. When horses were receiving the high forage diet, both with (-8.39 g/d) and without (-6.96 g/d) yeast culture, they were in a negative nitrogen balance. Higher quality forages, such as alfalfa are more digestible and higher in protein and energy. The NRC (1989) suggests that sun-cured, full bloom alfalfa may contain around 15.5% crude protein, whereas sun-cured bermudagrass will only provide around 7.3% crude protein. Horses in the current trial received only mature, bermudagrass hay that contained 8.2% crude protein, thus providing only a minimal source of nitrogen for the mature horse. Supporting this, Reitnour and Treece (1971) saw a negative nitrogen balance in mature horses receiving a basal diet and when supplemented with urea. When the same animals were supplemented with soybean meal or fishmeal, both high-quality protein sources, the animals had a significantly higher nitrogen retention. Additionally, Meyer et al. (1985) concluded that ponies receiving a low N diet resulted in a negative N balance. Therefore, from these data, it can be concluded that a combination of both low nitrogen intake and a low-digestible protein sources are responsible for the negative N balance of the HF diet and for the low amount N retained on the HC diet.

Mineral Balance. Intake, fecal output, urinary output and the resulting balance of calcium, phosphorus, potassium, sodium, and magnesium are shown in Table 7. There

Table 7. Effect of Yeast Culture on Mineral Balance[a]

	Treatment Diets				
Item	HC[*]	HCY[**]	HF[±]	HFY[++]	SEM[e]
Calcium					
NRC Req., g/d	20.08	20.24	20.30	20.36	
Intake, g/d	17.26[b]	17.36[b]	25.13[c]	24.97[c]	0.79
Fecal, g/d	17.67[b]	16.76[bc]	15.56[cd]	14.33[d]	0.86
Urine, g/d	8.72[b]	7.52[b]	10.95[c]	11.72[c]	0.89
Balance, g/d	-9.12[b]	-6.91[b]	-1.38[c]	-1.08[c]	1.15
Phosphorus					
NRC Req., g/d	14.06	14.17	14.21	14.25	
Intake, g/d	17.89[b]	18.04[b]	15.78[c]	15.71[c]	0.62
Fecal, g/d	17.33	16.56	15.89	15.77	1.05
Urine, g/d	1.10[b]	1.26[b]	0.12[c]	0.18[c]	0.26
Balance, g/d	-0.54	0.21	-0.24	-0.24	0.77
Potassium					
NRC Req., g/d	25.10	25.30	25.38	25.45	
Intake, g/d	50.49[b]	50.78[b]	62.71[c]	62.32[c]	2.12
Fecal, g/d	14.61[b]	13.22[b]	22.83[c]	19.94[c]	1.90
Urine, g/d	40.29[b]	39.68[b]	44.51[bc]	47.25[c]	2.13
Balance, g/d	-4.41	-2.11	-4.63	-4.87	2.16
Sodium					
Intake, g/d	8.97[b]	9.03[b]	10.03[c]	9.97[c]	0.36
Fecal, g/d	11.96[bc]	13.99[b]	10.62[c]	11.78[bc]	1.53
Urine, g/d	5.99[b]	7.92[b]	2.63[c]	2.29[c]	0.86
Balance, g/d	-8.97[d]	-12.88[c]	-3.23[b]	-4.09[b]	1.84
Magnesium					
NRC Req., g/d	7.53	7.59	7.61	7.64	
Intake, g/d	10.08[b]	10.14[b]	14.11[c]	14.02[c]	0.45
Fecal, g/d	6.12[b]	5.89[b]	8.34[c]	7.99[c]	0.45
Urine, g/d	3.43[b]	3.21[b]	4.08[c]	4.35[c]	0.54
Balance, g/d	0.53[b]	1.05[bc]	1.69[c]	1.67[c]	0.25

[a] Values are least square means.
[bcd] Means within a row with different superscripts differ (P<0.05)
[e] Values are average standard errors.
[*]HC = high concentrate, no yeast; [**]HCY = high concentrate, with yeast; [±]HF = high forage, no yeast; [++]HFY = high forage, with yeast.
NRC (1989) requirements calculated as recommended: Ca = 0.04BW; P = 0.028BW; K = 0.05BW; Mg = 0.015.

were no significant differences in these parameters as a result of yeast culture supplementation. Intakes of some minerals were lower than expected due to differences in the actual concentrate compared to what was formulated.

Calcium. Due to unexpected nutrient composition within the diet, actual daily intakes of calcium were lower than formulated, and resulted in the animals being calcium deficient while receiving the high concentrate diet. By utilizing the NRC (1989) calcium requirement equation, horses should have consumed 20.08 g/d, 20.24 g/d, 20.30 g/d, and 20.36 g/d while receiving the HC, HCY, HF, and HFY diets, respectively. Intakes and urinary output were significantly lower but fecal outputs were significantly higher while receiving the high concentrate diet. Therefore, due to the high fecal output, horses were in a negative calcium balance across all diets, but significantly more negative while receiving the high concentrate diet This negative balance may be attributed to endogenous losses which have been estimated to be 20 mg of calcium/kg of body weight/day (NRC, 1989). Another theory for the negative calcium balance is the calcium:phosphorus ratio. Because of the unexpected deficiency, while on the high concentrate diet, this ratio fell slightly below 1:1. Research has proven that excess phosphorus reduces the rate of calcium absorption from the intestine, which may explain the high levels of calcium excreted in the feces (NRC, 1989).

Apparent calcium digestibility is shown in Table 8. Apparent digestibilities were -2.34%, 3.34%, 38.23%, and 42.67% when receiving the HC, HCY, HF, and HFY diets, respectively. Yeast culture tended to increase calcium digestibility (P=0.08). These results suggest that, although the mode of action is not understood, yeast culture supplementation may increase the absorption of calcium within the small intestine,

Table 8. Effect of Yeast Culture on Apparent Mineral Digestibility[a]

	Treatment Diets				
Item	HC[*]	HCY[**]	HF[±]	HFY[++]	SEM[e]
Calcium, %	-2.34[b]	3.34[b]	38.23[c]	42.67[c]	2.79
Phosphorus, %	3.20	8.77	-0.53	-0.089	4.28
Potassium, %	70.76[bc]	73.68[b]	63.72[d]	68.09[cd]	2.32
Sodium, %	-34.29[bc]	-54.18[b]	-5.16[c]	-17.65[c]	14.67
Magnesium, %	38.76	41.57	40.94	42.89	4.26

[a] Values are least square means.
[bcd] Means within a row with different superscripts differ (P<0.05)
[e] Values are average standard errors.
[*]HC = high concentrate, no yeast; [**]HCY = high concentrate, with yeast; [±]HF = high forage, no yeast; [++]HFY = high forage, with yeast

therefore increasing apparent digestibility. This is in contrast to the findings of Switzer et al. (2003) who found no significant differences in calcium digestibility when supplementing aged geldings with three levels of yeast culture. There was also a main effect ($P<0.01$) of diet on apparent calcium digestibility. This is mainly in response to the significant difference in calcium intakes, due to the unexpected calcium deficiency of the high concentrate diet.

Phosphorus. Intake, fecal and urinary output and the resulting balance are found within Table 7. There was no significant difference in any parameter as a result of yeast culture supplementation. However, there was a main effect of diet upon phosphorus intake and urine excretions. These data can be attributed to the significantly higher percent of phosphorus within the 70:30 diet since the concentrate contained approximately twice the amount of phosphorus as the forage component. Feces are the primary pathway for phosphorus excretion, however the kidneys also excrete some phosphorus when large amounts of phosphorus are fed (NRC, 1989). This explains the significant difference of diet on urine output. While receiving the high concentrate diet, the horses' phosphorus intake was much higher than calculated requirements. However, when fed the high forage diet, horses were at or only slightly above maintenance. This difference resulted in significantly more phosphorus excreted within the urine. Negative phosphorus balances were observed in the HC (-0.54 g/d), HF (-0.24 g/d), and the HFY (-0.24 g/d) diets. This may be due to endogenous phosphorus excretions that have been estimated at 10 mg/kg of body weight/day (NRC, 1989).

Apparent phosphorus digestibility results are shown in Table 8. There was no significant effect of yeast culture supplementation. This coincides with Switzer et al.

(2003) who saw no differences in phosphorus digestibilitywhen supplementing yeast culture to aged geldings. While on the high concentrate diet, apparent digestibilities were positive at 3.2% for HC and 8.77% for HCY. While on the high forage diet, there were negative digestibilties of -0.53% and -0.089% for HF and HFY, respectively. Again, this is most likely a result of intake. Because the high forage diet was only at or slightly above the NRC requirement, this resulted in a digestibility near 0%.

Potassium. Intake, fecal and urinary output and the resulting balance are found within Table 7. There was no significant difference as a result of yeast culture supplementation across parameters. There was a significant effect of diet on potassium intake, fecal output, and urinary output, however. Forages generally contain 1-2% potassium, whereas cereal grains usually contain only 0.3 to 0.4% on a dry matter basis (NRC, 1989). This explains the higher intake values and differences in excretions of the animals while receiving the high forage diet. Horses were in a negative potassium balance across treatment diets. Potassium is the major intracellular cation and is highly involved in maintenance of the acid-base balance and osmotic pressure within cells in the body. When horses are exercising, requirements greatly increase for potassium to maintain the osmotic pressure that may be manipulated due to sweating. Since the horses in the present study were on voluntary exercise and each had fans while in the stalls, sweating was at a minimum. Additionally, urine is the primary pathway for excretion of potassium. Considering these physiological mechanisms, the low requirement for potassium and the intakes being well over maintenance levels, may be the explanation for the high levels of potassium excreted in the urine.

Apparent potassium digestibility results are presented in Table 8. Digestibilities were 70.76% and 73.68% for the HC and HCY diets and 63.72% and 68.09% for the HF and HFY diets, respectfully. Yeast culture had no effect (P>0.05) on potassium digestibility. There was a main effect of diet upon apparent potassium digestibility, most likely due to the increased amount of potassium in the feces on the HF diets. However, it was observed that the HFY digestibility was not significantly different from the HC digestibility.

Sodium. Intake, fecal and urinary output and the resulting balance are found within Table 7. There was no significant difference as a result of yeast culture supplementation across parameters. There was a significant effect of diet on sodium intake, fecal output, urinary output and the resulting balance. Because the sodium requirement is extremely dependent upon sweat, environmental temperature and exercise, there is no set requirement for sodium chloride, which is the common form of sodium for horses. However, because natural feedstuffs only contain less than 0.1 percent of sodium, sodium chloride is often supplemented at 0.5 to 1.0 percent of the diet (NRC, 1989). Due to differences in nutrient composition, actual percent of sodium within the diet was much less than what was originally formulated. Additionally, the NRC (1989) states that the endogenous sodium lost in the idle horse is approximately 15 to 20 mg/kg of body weight/day. Therefore, if sodium is 100% absorbed, a 500-kg horse would need to receive 19 to 25 g of sodium chloride per day. Horses in the current study only received 7 to 12 g of trace mineralized salt per day, and therefore, it is reasonable to assume that the horses were sodium deficient while on trial. The endogenous excretion of sodium may also help explain the negative sodium balance observed across treatments. Another theory

is that of the sodium-potassium interaction. Because potassium levels were greatly exceeding the maintenance requirements, the serum levels may have resulted in a decreased serum concentration of sodium, and therefore, a higher level of sodium excretion (NRC, 1989).

Apparent sodium digestibility results are shown in Table 8. Digestibilities were negative across alltreatment diets (-34.29% for HC, -54.18% for HCY, -5.16% for HF, and -17.65% for HFY), with no significant effect of yeast culture supplementation. There was a main effect of diet on apparent sodium digestibility. This is a result of slightly higher intakes and decreased sodium levels found in the feces on the high forage diet.

Magnesium. Intake, fecal and urinary output and the resulting balance are found within Table 7. There was no significant difference as a result of yeast culture supplementation across parameters. However, there was a significant effect of diet on magnesium intake, fecal output, urinary output and the resulting balance. The ratio of magnesium within the hay and grain approaches 2:1 and therefore, horses received a significantly greater level of magnesium while consuming the high forage diet. Additionally, the higher level of intake resulted in higher levels of fecal and urine output when comparing the high forage to the high concentrate diet Magnesium balance was positive across all treatment diets, although animals on HF diets were significantly higher in their balance than the HC diets.

Apparent magnesium digestibility results are shown in Table 8. There were no significant effects of yeast culture or diet on magnesium digestibility. Apparent digestibilities were 38.76% for HC, 41.57% for HCY, 40.94% for HF, and 42.89% for HFY. These data are supported by the NRC (1989) which states that true absorption of

magnesium is 40 to 60 percent. Additionally, the current study coincides with Switzer et al. (2003) who observed no effect of yeast supplementation when added at a level of 9.09 kg/909 kg. However, Switzer et al. (2003) did find a significant increase in magnesium digestibility when aged geldings were fed 4.54 kg/909 kg of yeast culture .

Energy Partitioning. Energy values, gross (GE), digestible (DE) and metabolizable, (ME) are shown in Table 9. There were no significant effects of yeast culture supplementation on intake, fecal or urinary output of energy. This coincides with Hall et al. (2003) who observed no significant difference in apparent digestibility of gross energy as a result of yeast supplementation. There was a main effect of diet upon gross, fecal, and urinary energy due to the horses being fed at 1.3% of their body weight while on the HC and 1.5% while receiving the HF diets. Feeding at these intake levels allowed horses to maintain similar average bodyweights across periods. (Individual weights across periods are found in Appendix A and individual intakes for each horse are found in Appendix B).

Coinciding with apparent dry matter digestibility and the increased fecal output while on the HF diets, there was more fecal energy excreted on the 30:70 concentrate:forage diet. However, there was significantly more energy excreted in the urine from the high concentrate diets due to an increased volume of urine output while horses were receiving the HC diets. Available energy was calculated by subtracting this urine energy from digestible energy. Utilizing the results obtained by Pagan and Hintz (1986), a methane estimate was used to calculate metabolizable energy. This value, 0.774 Mcal/d was obtained from a 500 kg horse being fed 27.73 Mcal/d of gross energy intake from a pelleted diet of 75% alfalfa and 25% oats.

Table 9. Effects of Yeast Culture on Energy Metabolism (Mcal/d)[a]

Item	HC[+]	HCY[++]	HF[±]	HFY[±+]	SEM[d]
Intake	26.0[b]	26.2[b]	29.9[c]	29.8[c]	1.05
Fecal	9.8[b]	10.1[b]	14.4[c]	14.2[c]	0.77
NRC Req.,[†]	16.5	16.6	16.6	16.7	
DE	16.3	16.1	15.6	15.6	0.70
Urine	5.4[b]	5.5[b]	3.6[c]	3.9[c]	0.72
AE[*]	10.9	10.6	11.9	11.7	1.20
Methane[††]	0.77	0.77	0.77	0.77	
ME	10.2	9.8	11.2	10.9	

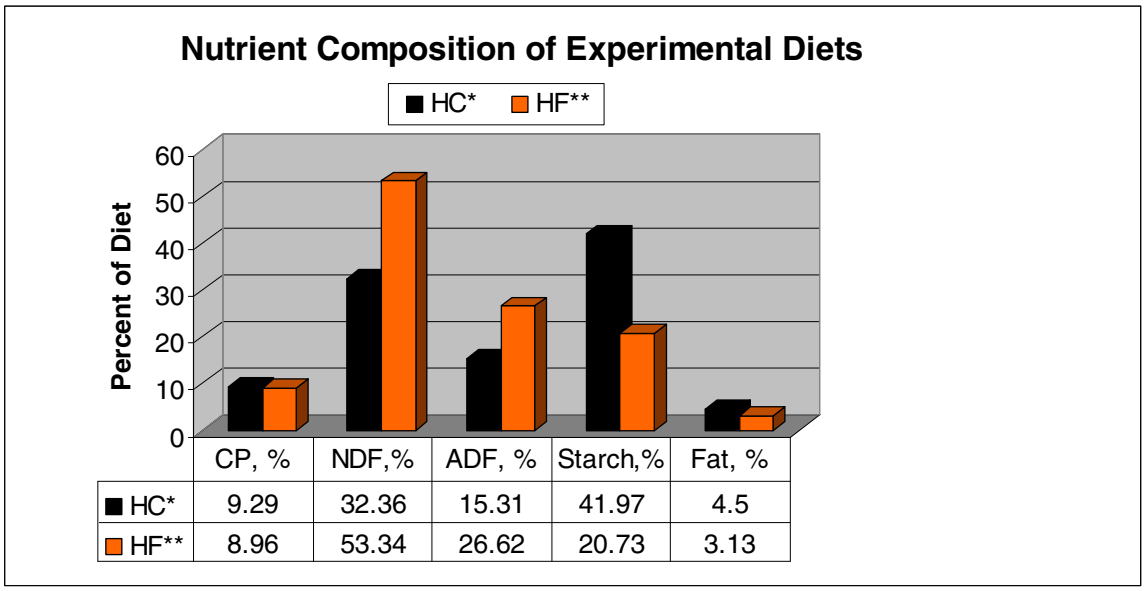
[a] Values are least squares means
[bc] Means within a row with different superscripts differ (P<0.05)
[d] Values are average standard errors
[†] DE Requirement calculated from NRC, 1989 recommended equation:
DE = 1.4 + 0.03BW
[††] Methane estimates were taken from Pagan and Hintz (1986) for a 500 kg horse consuming 27.73 Mcal/d
*Available energy (AE) is defined as DE minus urine energy.
[+]HC = high concentrate, no yeast; [++] HCY = high concentrate, with yeast; [±]HF = high forage, no yeast; [±+]HFY = high forage, with yeast

The primary determinant of gross energy is the degree of oxidation of its organic substances, expressed in the ratio of carbon plus hydrogen to oxygen. All carbohydrates, whether structural or nonstructural, have similar ratios, and hence, all have approximately the same gross energy content (McDonald et al., 1995). Therefore, because the diets consisted mainly of different types of carbohydrates the gross energy values of the diets were similar (4235.11 cal/g on HC and 4157.90 cal/g on HF). These values, across GE, fecal output, DE, urinary output, and ME were at or slightly lower than those found previously by Pagan and Hintz (1986). In this study, four horses of different weight ranges were fed at three levels of energy intake utitilizing a pelleted ration of 75% alfalfa meal and 25% oats. For the 500 kg horse, similar to the weight range of all animals in the current study, Pagan and Hintz (1986) fed gross energy levels ranging from 23.759 to 27.730 Mcal/d. Digestible energy was calculated from 15.256 to 17.401 Mcal/d and urine energy released ranged from 1.598 Mcal/d to 1.467 Mcal/d. Consequently, after accounting for methane energy loss, their ME values ranged from 12.983 to 15.160 Mcal/d. When consuming the high forage diet, gross energy levels and fecal energy losses for the current study were higher than the values previously reported (Pagan and Hintz, 1986). However, the DE estimates were extremely similar ranging from 16.23 Mcal/d on the HF diet to 16.58 Mcal/d on the HC. Urine energy losses were much higher in the current study as compared to the values obtained by Pagan and Hintz (1986). These data are a result of urinary output (Appendix D) as there was an extremely high correlation between energy excreted in the urine and volume of urinary output (R=0.993). Additionally, the differences may be due to differences in experimental procedures and/or laboratory analysis between studies. In the current study, urine was dried at 55°C in a

circulating air oven, whereas the previous study used lyophilized urine. There has not been extensive research to determine the most precise method of urine collection or subsequent energy analysis of the samples. Due to the high energy losses in urine and the low energy calculated as lost in methane gas, ME values from Pagan and Hintz (1986) were higher than those obtained in the current study when horses were on the high concentrate diet. Because there are few studies examining all of these parameters, raw data for each horse is found in Appendix C.

Although diets contained similar energy values, and some energy was provided by the lipids and proteins present within the diet, the primary source of energy was from the metabolism of carbohydrates (Figure 1). Feeding a high concentrate versus a high forage diet, results in differences in energy metabolism with varying end products depending on the site of digestion. The high concentrate diet yields a large portion of energy prececally, primarily in the form of glucose. Within the small intestine, these nonstructural polysaccharides are acted upon by enzymes such as amylase, maltase, maltriase, and oligosaccharidase. These enzymes break down starch to glucose. Additionally, lactose is acted upon by lactase to produce glucose and galactose, and sucrose is broken down by sucrase to produce glucose and fructose. Glucose and galactose are picked up by Glut 1 Na-linked transporters and fructose is picked up by Glut 5. Another carbohydrate transporter, Glut 2, then allows for transportation of these substrates across the basilateral membrane and into the portal blood. Glut 1 and Glut 5 then continue transportation into the liver where glucose can then be made available throughout the body and within the cytosol of the cell to be utilized for glycolysis (Breazile, 2005). The metabolism of

Figure 1. Nutrient Composition of Treatment Diets



	CP, %	NDF,%	ADF, %	Starch,%	Fat, %
HC*	9.29	32.36	15.31	41.97	4.5
HF**	8.96	53.34	26.62	20.73	3.13

*HC = high concentrate, no yeast; **HF = high forage, no yeast

glucose produces pyruvate and subsequently, production of energy in the form of ATP.From the structural carbohydrates in the high forage diet and those nonstructural carbohydrates that escaped prececal digestion, the main source of energy production in the hindgut is in the form of volatile fatty acids (VFA's). Primarily, these are acetate, propionate, and butyrate, with some levels of isovalerate and valerate. These are produced by anaerobic fermentation within the cecum and are absorbed across the cecum wall to constitute a significant portion of energy for horses on a high forage diet. Volatile fatty acids travel to the liver where each is converted to an energy source. Propionate undergoes conversion to malate, phosphoenolpyruvate, and eventually glucose through gluconeogenesis (McDonald et al., 1995). Butyrate is converted to D-3-hydroxybutyrate which is utilized as an energy source primarily by skeletal and heart muscle (McDonald, et al., 1995). Acetate is the major product of carbohydrate digestion and is found in the peripheral blood and used as an energy source by the tissues. It is converted to acetyl-CoA which is a vital component of the TCA cycle to yield large numbers of ATP for energy (McDonald et al., 1995).

CHAPTER V

CONCLUSIONS AND IMPLICATIONS

Yeast culture supplementation. The supplementation of *Saccharomyces cerevisiae* to ruminant and equine diets has shown varying efficacy on digestion coefficients through both in vivo and in vitro trials. According to the current data, yeast culture supplementation resulted in no significant differences on DM, NDF, ADF, crude fat, nitrogen retention, or gross energy. This coincides with numerous studies, including Hall et al. (1989) who also observed no significant difference in DM, CP, NDF, ADF, GE, or nitrogen retention when horses were supplemented with different levels of yeast culture. Additionally, it had no effect on phosphorus, sodium, or magnesium balance or digestibility. However, yeast supplementation did show a tendency to increase apparent calcium digestibility (P=0.08).

Results of this study both coincide and contrast numerous other ruminant and equine studies. Since the mode of action of yeast culture is still not understood, it is difficult to determine why there were little results from this trial. Speculation can be made, however, that more significant effects have been seen in growing horse trials (Glade and Sist, 1988), with mares in gestation or lactation (Glade, 1991), and geriatric horses (Switzer et al., 2003). Therefore, yeast culture may be more beneficial when supplemented to horses other than those that are mature and sedentary.

Additionally, more research may need to focus on both the strain and viability of the yeast culture used. From reviewing the literature, it can be concluded that more

positive results were also observed when horses were supplemented with a dried, live yeast culture (Girard and Dawson, 1995; Koul et. al., 1998), rather than when supplemented with a killed yeast culture.

Other parameters such as animal-to-animal variation and truly controlling intake of all nutrients are parameters that may affect results and are extremely hard to quantify. However, according to the current study and others supporting the present results, it can be concluded that yeast culture supplementation has very minimal effects on improving digestion coefficients or levels of mineral retention.

High concentrate versus high forage diets. The present study provides very useful data to compare digestibility parameters when varying the concentrate:hay ratios in equine diets. Values from the high forage or high concentrate diets obtained for DM, NDF, and ADF coincide with previous studies and are reasonable due to the variability in starch and fiber content. The HC diets were expected to be more highly digestible due to the presence of soluble polysaccharides that can be digested prececally versus the fibrous content of the 30:70 high forage diet. Additionally, crude fat values support the findings of Kronfeld et al. (2004) with low apparent digestibility values being a result of the extremely low fat content of the diet. This results in a low substrate concentration in the small intestine and inhibits the amount of time and ability for lypolysis to fully digest the fat content of the diet. In addition, the nitrogen retention and digestibility values further support numerous findings that forages have a digestibility as low as 43% when being fed a low, 8% crude protein diet (NRC, 1989). The current study resulted in supporting data that coincides with numerous in vivo studies for varying the concentrate:forage ratio in

equine diets, and can be very useful in analyzing intake, output and digestibility data due to different carbohydrate sources.

Energy partitioning. Most species in animal nutrition formulate nutrient requirements using either metabolizable (ME) or net energy (NE) values. However, because there is a lack of data providing these values for the common feedstuffs within equine nutrition, diets are still formulated using digestible energy (DE) values. Utilizing the current calculations, energy requirements only account for what is lost in feces. The present study provides energy data for both a high concentrate diet consisting mainly of corn, oats, and molasses, and a high forage diet of coastal bermudagrass. Additionally, gross energy samples were obtained on all fecal and urine samples, in effort to estimate both digestible and metabolizable energy values. A methane loss estimate was utilized for this study from a similar study by Pagan and Hintz (1986) for a 500 kg horse being fed 27.73 Mcal/d in gross energy. The obtained data from the current study showed a lower conversion of DE to ME of an average 63% as compared to the expected 80-90% (Appendix C). Urine energy values were higher while on the HC diet and were a result of differences in urine output (Appendix D). DE values were extremely similar to those found in the study by Pagan and Hintz (1986) however ME values were lower due to the high levels of urine energy. Because metabolizable energy accounts for more energetic losses from the animal, it should be used as a more precise means of calculating energy requirements. Further research should be conducted to obtain more ME values for common feedstuffs and to better the collection and analysis means for urine energy. This will allow for more precise calculations of nutrient requirements across the numerous classes of horses.

CHAPTER VI

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CHAPTER VII

APPENDIX

APPENDIX A

Average Weight (kg) of Individual Horses per Period

	Period 1 (TRT)	Period 2 (TRT)	Period 3 (TRT)	Period 4 (TRT)
Horse 1	530.25 HCY [**]	530.25 HC[*]	536.60 HF[±]	536.15 HFY[±±]
Horse 2	513.92 HFY	508.48 HF	503.94 HCY	510.75 HC
Horse 3	494.87 HC	493.96 HCY	497.59 HFY	497.14 HF
Horse 4	508.02 HC	512.11 HCY	513.92 HFY	514.37 HF
Horse 5	419.12 HF	419.12 HFY	420.03 HC	432.27 HCY
Horse 6	517.10 HFY	511.20 HF	508.48 HCY	510.75 HC
Horse 7	525.26 HCY	523.90 HC	531.61 HF	534.79 HFY
Horse 8	542.04 HF	540.23 HFY	532.52 HC	541.59 HCY

[*]HC = high concentrate, no yeast; [**]HCY = high concentrate, with yeast; [±]HF = high forage, no yeast; [±±]HFY = high forage, with yeast

APPENDIX B

Actual Intakes for Individual Horses per Period

HC = high concentrate, no yeast; HCY = high concentrate, with yeast;
HF = high forage, no yeast; HFY = high forage, with yeast

Period	Horse #	Trt	Intake (kg/d) DMB Grain	Hay	Total
1	1	HCY	4.43	2.04	6.47
	2	HFY	2.13	4.64	6.77
	3	HC	4.10	1.87	5.97
	4	HC	4.26	1.87	6.13
	5	HF	1.80	3.99	5.79
	6	HFY	2.13	5.09	7.23
	7	HCY	4.43	2.01	6.44
	8	HF	2.30	5.41	7.70

Period	Horse #	Trt	Intake (kg/d) DMB Grain	Hay	Total
2	1	HC	4.428	2.038	6.465
	2	HF	2.132	5.094	7.226
	3	HCY	4.100	1.868	5.967
	4	HCY	4.264	1.868	6.131
	5	HFY	1.804	4.075	5.879
	6	HF	2.132	5.094	7.226
	7	HC	4.428	2.038	6.465
	8	HFY	2.296	5.433	7.729

Period	Horse #	Trt	Intake (kg/d, DMB) Grain	Hay	Total
3	1	HF	2.296	5.264	7.559
	2	HCY	4.264	2.038	6.301
	3	HFY	2.132	4.924	7.056
	4	HFY	2.132	5.094	7.226
	5	HC	3.444	1.528	4.972
	6	HCY	4.428	2.038	6.465
	7	HF	2.296	5.264	7.559
	8	HC	4.592	2.207	6.799

Period	Horse #	Trt	Intake (kg/d, DMB) Grain	Hay	Total
4	1	HFY	2.296	5.264	7.559
	2	HC	4.264	2.038	6.301
	3	HF	2.132	4.924	7.056
	4	HF	2.132	5.094	7.226
	5	HCY	3.444	1.528	4.972
	6	HC	4.428	2.038	6.465
	7	HFY	2.296	5.264	7.559
	8	HCY	4.920	2.207	7.127

APPENDIX C

HC = high concentrate, no yeast; HCY = high concentrate, with yeast;
HF = high forage, no yeast; HFY = high forage, with yeast

HORSE 1

Period	Treatment	GE	Fecal	DE	Urine	ME	ME:DE
		(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	%
1	HCY	27.9	8.8	19.1	8.8	10.3	53.8
2	HC	27.2	8.9	18.4	7.9	10.5	57.1
3	HFY	32.3	15.1	17.2	4.8	12.4	72.0
4	HF	32.3	14.9	17.3	6.4	10.9	62.8

HORSE 2

Period	Treatment	GE	Fecal	DE	Urine	ME	ME:DE
		(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	%
1	HFY	29.1	13.5	15.7	3.2	12.4	79.4
2	HF	30.7	13.3	17.4	3.3	14.1	81.0
3	HCY	26.7	8.8	17.9	3.5	14.4	80.4
4	HC	26.7	8.4	18.3	3.3	15.1	82.2

HORSE 3

Period	Treatment	GE	Fecal	DE	Urine	ME	ME:DE
		(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	%
1	HC	25.8	9.3	16.4	3.1	13.3	81.3
2	HCY	25.1	8.1	17.0	2.7	14.3	83.9
3	HFY	30.1	14.3	15.8	3.1	12.7	80.3
4	HF	30.1	13.7	16.5	3.7	12.7	77.4

HORSE 4

Period	Treatment	GE	Fecal	DE	Urine	ME	ME:DE
		(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	%
1	HC	26.5	9.5	17.0	5.7	11.3	66.6
2	HCY	25.8	11.6	14.3	8.6	5.6	39.6
3	HFY	30.8	15.6	15.2	5.7	9.5	62.5
4	HF	30.8	14.6	16.2	4.0	12.3	75.6

HORSE 5

Period	Treatment	GE	Fecal	DE	Urine	ME	ME:DE
		(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	%
1	HF	24.9	9.6	15.3	3.3	12.0	78.5
2	HFY	25.0	10.1	14.9	6.3	8.6	57.5
3	HC	21.1	9.6	11.5	9.3	2.1	18.6
4	HCY	21.1	9.6	11.5	6.7	4.7	41.2

HORSE 6

Period	Treatment	GE	Fecal	DE	Urine	ME	ME:DE
		(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	%
1	HFY	31.1	13.7	17.3	2.2	15.1	87.5
2	HF	30.7	12.5	18.3	2.7	15.6	85.2
3	HCY	27.4	9.6	17.8	4.7	13.1	73.6
4	HC	27.4	10.1	17.4	3.4	14.0	80.6

HORSE 7

Period	Treatment	GE	Fecal	DE	Urine	ME	ME:DE
		(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	%
1	HCY	27.8	11.4	16.4	5.4	10.9	66.7
2	HC	27.2	11.8	15.4	6.4	9.0	58.5
3	HF	32.3	18.2	14.1	3.8	10.3	73.2
4	HFY	32.3	15.6	16.7	3.7	13.0	77.8

HORSE 8

Period	Treatment	GE	Fecal	DE	Urine	ME	ME:DE
		(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	(Mcal/d)	%
1	HF	33.1	18.3	14.8	1.8	13.0	87.6
2	HFY	32.9	15.5	17.4	2.1	15.3	87.9
3	HC	28.9	10.5	18.3	3.8	14.5	79.0
4	HCY	30.2	12.7	17.6	3.6	13.9	79.2

APPENDIX D

Urine Output (L) of Individual Horses per Period

HC = high concentrate, no yeast; HCY = high concentrate, with yeast;
HF = high forage, no yeast; HFY = high forage, with yeast

	Period 1 (TRT)	**Period 2 (TRT)**	**Period 3 (TRT)**	**Period 4 (TRT)**
Horse 1	18.0 HCY	16.8 HC	13.5 HF	10.4 HFY
Horse 2	5.6 HFY	5.7 HF	6.9 HCY	6.5 HC
Horse 3	5.7 HC	5.0 HCY	5.6 HFY	7.4 HF
Horse 4	11.9 HC	18.6 HCY	11.4 HFY	8.6 HF
Horse 5	6.3 HF	13.1 HFY	20.9 HC	16.1 HCY
Horse 6	3.6 HFY	4.9 HF	10.9 HCY	6.6 HC
Horse 7	11.1 HCY	13.8 HC	7.5 HF	7.9 HFY
Horse 8	2.6 HF	3.4 HFY	8.0 HC	7.9 HCY

VITA

Amy Michelle Brown

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF *SACCHAROMYCES CEREVISIAE* ON NUTRIENT DIGESTIBILITY IN MATURE HORSES FED DIETS WITH HIGH AND LOW CONCENTRATE TO HAY RATIOS

Major Field: Animal Science

Biographical:

Education: Graduated from Girard High School, Girard, Kansas in May 2000; received Bachelor of Science degree in Animal Science from Kansas State University, Manhattan, Kansas in May of 2004. Completed the requirements for the Master of Science degree with a major in Animal Science (equine nutrition emphasis) at Oklahoma State University in July 2006.

Experience: Raised on a farm in rural Girard, Kansas; employed as a laboratory assistant by Kansas State University, Department of Animal Science; employed as an assistant trainer for Cofrancesco Farms in New Jersey during the summers; employed as a graduate research and teaching assistant at Oklahoma State University from 2004 to 2006.

Professional Memberships: American Quarter Horse Association, American Society of Animal Science.

Name: Amy M Brown Date of Degree: July, 2006

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EFFECTS OF *SACCHAROMYCES CEREVISIAE* ON NUTRIENT DIGESTIBILITY IN MATURE HORSES FED DIETS WITH HIGH AND LOW CONCENTRATE TO HAY RATIOS

Pages in Study: 68 Candidate for the Degree of Master of Science

Major Field: Animal Science

Scope and Method of Study: The objective of this study was to determine the effects of *Saccharomyces cerevisiae* on nutrient digestibility in mature geldings being fed either a high concentrate (70:30) or high forage (30:70) diet. Eight mature, sedentary geldings were fed four treatment diets consisting of a) high concentrate with no supplemental yeast culture (HC); b) high concentrate with yeast culture supplemented (HCY); c) high forage with no supplemental yeast culture (HF); and d) high forage with yeast culture supplemented (HFY). Periods consisted of 21-days of acclimation followed by a 72 hour total fecal and urine collection period. Two simultaneous 4X4 Latin squares were utilized to form a crossover experimental design and the Kenward-Rogers p-diff procedure was used to test for differences between treatment means. Samples were analyzed for dry matter, NDF, ADF, fat, calcium, phosphorus, sodium, potassium, and magnesium and the resulting digestibilities and balances were calculated. Additionally, gross energy values were obtained from all samples by use of a bomb calorimeter.

Findings and Conclusions: There was no significant effect of yeast culture on the intake, fecal output, urinary output, balance or digestibility of the analyzed parameters. However, the lower digestibility of the HF diets resulted in a significant diet effect. Energy metabolism of the various diets was evaluated by partitioning gross energy into digestible (DE) and metabolizable energy (ME). Gross energy intakes were higher for the HF diets due to a higher level of feed intake. Energy excreted from the feces was higher on the HF diets, however, significantly more energy was excreted in the urine from the HC diets. Therefore, DE and ME values were not significantly different across treatments. It can be concluded from this study that yeast culture supplementation is not beneficial to horses being fed for maintenance.

ADVISOR'S APPROVAL: Dr. Steven Cooper