Effects of Maternal Dietary Yeast Supplementation on Foal Growth and Microbial Diversity of the Hindgut in Quarter Horse Mares and Their Offspring

Thesis

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ABSTRACT

Microbial populations in the hindgut of horses can be influenced by dietary yeast supplementation. Influencing the microbiome of the mares may have an effect on the gastrointestinal microflora of their foals and contribute to the colonization and development of their naïve gastrointestinal microbial ecosystem which is important to the overall health and well-being of the foal. The objective of this study was to evaluate the effect of a maternal dietary live yeast supplement on the diversity of the gastrointestinal microflora in the hindgut of Quarter Horse mares and the growth and development of their offspring. In this study, eight Quarter Horse mares (14.5 ± 7.5 yr) were randomly assigned to one of two treatment groups: yeast or control. All mares received a basal diet consisting of 0.5% BW of a 16% CP pelleted concentrate, with water and mixed grass hay *ad libitum*. Mares in the yeast treatment group were fed a targeted dose of 1 g (4.5 x 10^9 cfu)/45.4 kg of BW per day of a live culture of *Saccharomyces cerevisiae* from d 250 of gestation to 90 d post-parturition. Mare fecal samples were collected monthly from d 250 of gestation to d 180 post-parturition. Foal fecal samples were collected within 2 hr of birth, at 12 and 24 hr after birth and monthly post-parturition until d 240. Fecal samples were pooled by production status, treatment and day. Pooled fecal samples were subjected to PCR to ascertain the presence or absence of *Saccharomyces cerevisiae* using
primers specific to the ITS2 rRNA gene sequences of *Saccharomyces cerevisiae*. 

*Saccharomyces cerevisiae* was not detected in any of the fecal samples. Pooled fecal samples were also subjected to PCR with universal, *Firmicutes*, and *Streptococcus* primers specific to 16S rRNA bacterial gene sequences, and subsequent denaturing gradient gel electrophoresis (DGGE) analyses were performed so that any changes in bacterial diversity could be observed and analyzed. Images were captured and analyzed with Bionumerics software and subjected to Multi-Dimensional Scaling (MDS) to compare microbial profiles. DGGE and MDS analysis using all primers revealed clusters due to yeast supplementation in the microbial profiles of the mares in this study.

Statistical analysis of band counts observed from the microbial profiles of the mares revealed a significant difference due to treatment using universal primers on d 30 to d120 ($P < 0.05$). There were no statistical differences in mare band counts due to treatment when *Firmicutes* or *Streptococcus* primers were used. Differences between foal treatment groups were found within the first 24 h after birth based on the qualitative analyses of dendrograms and MDS using all of the bacterial primers. Statistical analysis of band counts from foal microbial profiles revealed a significant difference due to treatment on d 90 ($P = 0.048$) using universal primers. Significant differences in band counts between foals due to treatment with live yeast were also observed using *Firmicutes* primers on d 0.5 and d 1 ($P < 0.05$). Bacterial DNA was detected in the meconium samples using universal and *Streptococcus* primers. The microbial profiles of the foal fecal samples appeared to proliferate and diversify with the age of the foal based on DGGE-PCR results. There were no differences in foal growth due to live yeast.
supplementation of the maternal diet. Overall, supplementation of the maternal diet with live yeast did not impact foal growth but did influence the microbial profiles in the hindgut of mares and their offspring.
DEDICATION

Dedicated to my Granny, Judy Lee Bryant
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I would like to first thank my family and friends who have supported me throughout this process. My parents, Doug and Becky Share, and my two sisters, Katie and Victoria, have been there when I need to have a complete meltdown and just vent my frustrations for a bit. My friends Penny, Masa and Corey have been irreplaceable during this time. Thanks for listening and giving me good advice on how to handle the stress that comes along with graduate school! I would also like to thank Devon. Thanks for the incredible pep talks and always taking the time to listen when I needed to talk things out. Thank you to my advisor, Dr. Kimberly Cole, for having patience with me as she realized my lack of writing ability and the countless hours that she spent preparing me both in written corrections and defense practice. Dr. John Mark Reddish, thank you for your endless support as things always seemed to go wrong in the lab. I appreciate all of your help and the occasional kick in the backside when I needed it! Thank you to Dr. St-Pierre for not just giving me the answers to my statistics but for taking the time to teach me how to answer them on my own. Finally, I would like to give my biggest thank you to my fellow graduate student, Katie Barnhart. Thank you for dealing with my crazy mood swings, talking me off my mental cliffs and never doubting me. I would not have been able to make it through my graduate program without you!
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Chapter 1

Literature Review
1.1 Introduction

In horses, the establishment of microflora in the hindgut helps to maintain the health of the hindgut ecosystem which, in turn, assists in the prevention of intestinal disorders and forms a barrier against pathogens (Rolfe, 1996; Salimen et al., 1996; Tuoloma et al., 1999; Weese et al., 2003; Ward et al., 2004; Weese et al., 2004; Tanabe et al., 2008; Miyauchi et al., 2009; Fukada et al., 2011; Ogita et al., 2011a; Ogita et al., 2011b; Tanabe, 2013; Tanabe et al., 2014). Therefore, the development of the gastrointestinal (GI) tract microbiome is crucial to the health of an animal. The neonatal gastrointestinal tract is thought to be sterile until it is exposed to bacteria from the mare vaginal secretions, feces, saliva and/or milk as well as the environment (Sakaitani et al., 1999; Biasucci et al., 2010; Dominguez-Belloa et al., 2010; Kuhl et al., 2011; Earing et al., 2012). Diverse maternal and environmental bacterial populations quickly establish in the gastrointestinal tract of the foal early after birth and are introduced through the ingestion of milk (Eadie et al., 1970; Mackie et al., 1999; Sakaitani et al., 1999; Favier et al., 2002; Yuyama et al., 2004). The microbes in the hindgut of the foal changes as it matures, largely due to a change in feeding habits as the foal switches from consuming solely milk to consuming forage (Boy and Duncan, 1979). The microbiome in the gastrointestinal tract of horses is sensitive to many different factors, including changes in diet (Goodson et al., 1988; de Fombelle et al., 2001; Medina et al., 2002). The prophylactic use of supplements such as probiotics, prebiotics and synbiotics, in order to prevent gastrointestinal upset or reduce the severity that can result from gastrointestinal
upset has been suggested (Collins and Gibson, 1999; Kopp-Hoolihan, 2001; Tanabe et al., 2014).

Prebiotics and probiotics have been added to horses’ diets in an effort to re-establish homeostasis after gastrointestinal upset due to physiological or environmental stress (Pellegrini et al., 1999; Ward et al., 2004; Weese et al., 2004; Berg et al., 2005; Tanabe et al., 2014). Yeast, considered both a pre- and probiotic, has been shown to positively affect the hindgut by improving NDF and ADF digestibility and reducing the changes in pH and lactic acid levels in the large intestine after feeding (Goodson et al., 1988; de Fombelle et al., 2001; Medina et al., 2000; Medina et al., 2002; Jouany et al., 2008). Proper development and maintenance of microbial populations in the hindgut of the horse can reduce or prevent undesirable changes to the gastrointestinal microflora and contribute to the overall health and well-being of the horse (Medina et al., 2002; Julliand, 2005; Jouany et al., 2008; Tanabe et al., 2014). One proposed method of optimizing development of the foal’s gastrointestinal microbiome is through supplementation of the maternal diet.

Maternal diet has been shown to affect the growth and development of offspring in many monogastric species, including humans, mice and horses (Nathanielsz, 2006; Armitage et al., 2004; Ford et al., 2007; Clapp, 2002; Thum et al., 2012; Faubladier et al., 2013). Recent research shows that probiotic supplementation of pregnant mares during late gestation and early lactation can affect their offspring’s gastrointestinal microflora during the first few days of life (Faubladier et al., 2013). Foals of the mares supplemented with a fermented feed product had a greater establishment of total
anaerobes and lactate utilizers at an earlier time than the foals whose dams were not supplemented. Foals whose dams received a probiotic during late gestation and early lactations had higher concentrations of total anaerobes and lactate utilizers on d 1 of age (Faubladier et al., 2013). However, the effects of supplementing the maternal diet with live yeast in horses on maternal or foal GI microflora has not yet been studied.

1.2 Development of the Foal Gastrointestinal Tract and the Microbial Environment

The neonatal gastrointestinal tract is thought to be sterile until it is exposed to bacteria from the mare’s vagina, feces, saliva and/or milk (Kuhl et al., 2011; Earing et al., 2012). The foal’s meconium has been shown to not contain any bacteria when using traditional plating techniques as well as PCR-DGGE (Denaturing Gradient Gel Electrophoresis) and TGGE (Temperature Gradient Gel Electrophoresis; Sakaitani et al., 1999; Biasucci et al., 2010; Dominguez-Belloa et al., 2010; Kuhl et al., 2011; Earing et al., 2012). Diverse maternal and environmental bacterial populations, including Lactobacillus and Streptococcus, have been shown to quickly establish early after the birth of the foal and are introduced through the ingestion of milk (Eadie et al., 1970; Mackie et al., 1999; Sakaitani et al., 1999; Yuyama et al., 2004). The development of the hindgut in horses occurs with the development of the microbial environment (Lawrence and Lawrence, 2009). Microbial colonization occurs quickly after birth and is diet dependent (Favier et al., 2002). As the foal matures, the microbes in the hindgut change, in large part due to a change in diet as the foal slowly switches from consuming solely milk to grazing more and consuming forage (Boy and Duncan, 1979). From the
time the foal is born to the time it is weaned, large shifts from enzymatic digestion to anaerobic fermentation are associated with the change in diet and the amount ingested by the foal (Mackie et al., 1999). As the foal consumes more feed that is made up of complex carbohydrates, such as cellulose and hemicellulose, bacteria that can digest such compounds, such as *Fibrobacter succinogenes*, establish in larger numbers than when milk was the primary source of energy so that the foal can utilize these complex carbohydrates (Boy and Duncan, 1979; Mackie et al., 1999). Microbial colonization of the foal’s gastrointestinal tract is crucial for the fermentation of dietary components which allows the foal to utilize nutrients gained from microbial digestion.

1.3 Intestinal Microbial Ecosystem of the Horse

Horses have a plethora of different types of microorganisms that are present in their gastrointestinal ecosystem. These organisms have complex relations both with the host and other microorganisms in the gastrointestinal environment and are important for the health of the gut (Julliand, 2005). The nutrients received by the horse play a key role in determining the balance and establishment of microorganisms in the gastrointestinal tract. Depending on the region of the gastrointestinal tract, the microbial profile and action of those microbes differ. Most work that has been done with concern to the gastrointestinal environment in horses has been in the hindgut which includes the cecum and colon (Julliand, 2005).
The hindgut of the horse has a nearly neutral pH and a slower passage rate than that of the foregut, making it a beneficial environment for microbes. Research has shown that the microbial populations of the large intestine are very diverse and can be found in high concentrations (Kern et al., 1973; Kern et al., 1974; Goodson et al., 1988; Mackie and Wilkins, 1988; Julliand, 1996; Julliand et al., 2001; Medina et al., 2002; de Fombelle et al., 2003). As many as $10^9$ colony forming units per milliliter (cfu/mL) in the cecum and $10^8$ cfu/mL in the colon have been observed (Mackie and Wilkins, 1988).

One of the main roles of the bacterial environment in the hindgut is to break down plant cell walls and to then ferment them into volatile fatty acids (VFAs) so that they can be utilized by the horse for energy (Argenzio et al., 1974). The hindgut of the horse has a dense population of strict anaerobes, which are shown to be more concentrated in the cecum than the colon (Kern et al., 1973; Kern et al., 1974; Bellet, 1982; Baruc et al., 1983; Maczulak et al., 1985; Goodson et al., 1988; Mackie and Wilkins, 1988; Moore and Dehority, 1993; de Vaux and Julliand, 1994; Julliand et al., 1999; Julliand et al., 2001; Medina et al., 2002; de Fombelle et al., 2003). The phylum *Firmicutes* accounts for the majority of identified sequences found in the feces of horses (46-70%) which includes many differing types of bacteria, including fiber-utilizing, starch-utilizing and protein-utilizing bacteria (Kern et al., 1973; Shepherd et al., 2012; Dougal et al., 2013).
1.3.1 Fiber-utilizing Bacteria

Microorganisms adhering to particles, and thought to be involved in the degradation of cell-wall polysaccharides, are known as fibrolytic bacteria. Fibrolytic bacteria include cellulolytic bacteria, which primarily degrade the cellulose portion of the cell wall (Mackie and Wilkins, 1988). Common cellulolytic bacteria found in the hindgut of horses are *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* (Bonhomme, 1986; Lin and Stahl, 1995; Julliand et al., 1999; Drogoul et al., 2000). The concentration of cellulolytic bacteria varies in the intestine of the horse and makes up a small percentage of the total anaerobic microflora found in the hindgut of the horse but the number of cellulolytic bacteria has been found in higher numbers in the cecum than the colon, numbers vary from $10^4$ to $10^7$ cfu/mL, suggesting that the cecum is the main site of fiber digestion (Julliand, 1996; de Fombelle et al., 2003; Medina et al., 2002). The overall microbial density has been found to be higher in the colon than in the cecum (Julliand et al., 2001; Medina et al., 2002). The proportion of cellulolytic bacteria was higher in the cecum than the colon (de Fombelle et al., 2003) suggesting that the cecum was the main site of hindgut fiber fermentation. In a study done by de Fombelle and others (2003), seven horses were fed diets high in fiber or starch and samples were taken from different segments of the gastrointestinal tract. Cellulolytic bacterial counts in the segments of the gastrointestinal tract before the cecum were not above $3.0 \times 10^3$ cfu/mL while the cellulolytic counts in the hindgut averaged $5.3 \times 10^5$ cfu/mL (de Fombelle et al., 2003). However, a more recent study conducted by Hastie and others (2008) found that the predominant fiber-utilizing bacteria in the hindgut of horses, *Fibrobacter*
succinigenes and Ruminococcus flavefaciens had lower concentrations in the cecum when compared to the colon of horses.

1.3.2 Starch-utilizing Bacteria

Along with fibrolytic bacteria, starch-utilizing, including both lactate-producing and lactate-utilizing bacteria, are other main groups of bacteria found in the hindgut of horses (de Fombelle et al., 2001; Medina et al., 2002; de Fombelle et al., 2003; Jassim et al., 2005; Sadet-Bourgeteau and Julliand, 2010). These bacterial groups are involved in the break-down of soluble carbohydrates, such as starch, that elude enzymatic digestion in the small intestine. Starch digestion can occur in the hindgut of the horse by microbes, which leads to the production of lactate by lactic acid-producing microbes, such as Lactobacillus and Streptococcus species, which grow under acidic conditions and are sustained by fermentable carbohydrates (Mungal et al., 2001; Bailey et al., 2003; Jassim et al., 2005; Julliand, 2005). Research performed by Julliand and colleagues (2001) studied the effects of varying hay:grain ratios on the microbial profiles in mature male ponies and found that Lactobacillus spp. counts were lower in the cecum, $4.17 \times 10^5 - 7.34 \times 10^6$ cfu/mL, when compared to counts in the colon at $4.29 \times 10^6 - 3.79 \times 10^8$ cfu/mL. Streptococcus spp. were also lower in the cecum, $5.0 \times 10^5 - 8.94 \times 10^6$ cfu/mL when compared to the colon, $3.23 \times 10^6 - 4.38 \times 10^7$ cfu/mL (Julliand et al., 2001). Both Lactobacillus and Streptococcus species have been seen to increase in concentration in the hindguts of horses when starch was added to the diet (Garner et al., 1978; Medina et al., 2002). Jouany and colleagues (2009) also observed an increase in Lactobacillus spp.
concentrations in the cecum when horses were fed a high starch diet compared to a high fiber diet. However, no differences due to diet were observed, in either the colon or cecum, in *Streptococcus* spp. concentrations. A carbohydrate overload can cause a drop in the pH of the hindgut of horses and allow for a sudden increase of *Streptococci*, which has been theorized to cause laminitis through toxin production (Mungal *et al.*, 2001; Bailey *et al.*, 2003; Milinovich *et al.*, 2005; Endo *et al.*, 2007). The main amylolytic and glycolytic lactate-utilizers tend to be *Veillonella* spp. and *Megasphaera* spp. (Baruc *et al.*, 1983; Maczulak *et al.*, 1985; Julliand, 2005). Concentrations of lactic acid-utilizing bacteria tended to be higher in the colon than in the cecum (Julliand *et al.*, 2001; de Fombelle *et al.*, 2001; Medina *et al.*, 2002; de Fombelle *et al.*, 2003). A study done by Jouany and others (2008) saw no significant differences in lactate-utilizing bacterial concentrations in the cecum when horses fed high starch diets compared to horses fed high fiber diets. However, Medina and colleagues (2002) found that lactate-utilizing bacteria increased when horses were fed high starch diets compared to high fiber diets.

### 1.3.3 Proteolytic Bacteria

Microorganisms thought to be responsible for breaking down protein are known as proteolytic bacteria. Proteolytic bacteria are found throughout the gastrointestinal tract of horses but there are conflicting reports on where they are most predominant (Reitnour and Salsbury, 1972; Gibbs *et al.*, 1988; Mackie and Wilkins, 1988). Research done by Kern and others (1974), showed that there was a 30, or higher, fold increase in proteolytic activity in the small intestine when compared to the colon or cecum of ponies, but a large
portion of that activity may have been due to enzymatic breakdown. Other research shows that 40-70% of nitrogen digestion occurs after the small intestine (Reitnour and Salsbury, 1972; Gibbs et al., 1988). There are no protein digestive enzymes known to secrete into the hindgut of horses, indicating that bacteria are responsible for any protein digestion in the hindgut (Reitnour and Mitchell, 1979; Baruc et al., 1983; Maczulak et al., 1985). Approximately 20% of all bacteria found in the hindgut of horses are thought to be proteolytic and include *Butyrivibrio fibrosolvens* and *Streptococcus* spp. (Kern et al., 1973; Reitnour and Mitchell, 1979; Mackie and Wilkins, 1988; Daly et al., 2001; Julliand, 2005; Frape, 2010). Mackie and Wilkins (1988) observed higher concentrations of proteolytic bacteria in the cecum than in the colon. Bacteria utilize protein as carbon, nitrogen and ammonia sources which are needed for the survival and growth of microflora found in the hindgut of horses (Baruc et al., 1983; Maczulak et al., 1985; Wallace, 1996).

1.3.4 Yeast

Limited research has been done to ascertain the type or amount of yeast indigenous to the gastrointestinal tracts of horses. In a previous study, yeast was only found in the gastrointestinal tract of 52.4% of the 252 horses and mules sampled (Van Uden et al., 1958). Those yeasts were then separated into three categories: obligate saprophytes, facultative saprophytes, and passers-by. Obligate anaerobes are yeasts that have their natural habitat in the digestive tract. *Candida albicans* was the most predominant obligate anaerobic yeast found in horses in this study with 4.4% of horses
having *C. albicans* in their gastrointestinal tract (Van Uden *et al*., 1958). Yeasts that
grow naturally inside and outside the horse are called facultative saprophytes. The most
predominant one found in the horse’s gastrointestinal tract is *Trichosporon cutaneum,*
being found in 21.8% of the 252 horses studied (Van Uden *et al*., 1958). Passer-bys are
yeasts that aren’t naturally found in the intestinal tract and are passing through.

*Saccharomyces* spp. were the most prevalent yeast found in horses belonging to this
category with 14 horses, out of 252 studied, having isolates of various species (van Uden
*et al*., 1958). In the horse, yeast species have been theorized to be involved in the
digestion of cellulose and hemicellulose as well as soluble carbohydrates (Orpin, 1981;
Moore and Dehority, 1993). Overall, the microbiome of the horse is very diverse and the
organisms in the hindgut can be affected by many factors (Goodson *et al*., 1988; Hintz
and Cymbaluk, 1994; de Fombelle *et al*., 2001; Medina *et al*., 2002).

### 1.4 Factors Affecting the Intestinal Microbial Ecosystem of the Horse

The microbiome in the gastrointestinal tract of horses is sensitive to many
different factors, including physiological stress such as illness, environmental stress such
as weaning and changes in diet (Goodson *et al*., 1988; Hintz and Cymbaluk, 1994; de
Fombelle *et al*., 2001; Medina *et al*., 2002). Different aspects of the diet can influence
the gastrointestinal microflora of the horse. The type of microbes and their activity in the
hindgut of horses are very dependent on the concentrate to forage (fiber: starch) ratio
(Goodson *et al*., 1988; de Fombelle *et al*., 2001; Medina *et al*., 2002). There are also
studies indicating that the level of fat in the diet can affect the hindgut microflora of the
horse (Bush et al., 2000; Jansen et al., 2000; Jansen et al., 2002; Jansen et al., 2007).
Stress can influence the relationship of microbes in the gut, making the gastrointestinal
ecosystem unstable and increasing pathogenesis (Hintz and Cymbaluk, 1994).

1.4.1 Stress

Stress is defined as the reaction of the body to stimuli that disrupts normal
physiological equilibrium (Rostagno, 2009). Typical stressors in horses can be illness,
transportation, a change in their environment (such as weaning) or diet (Swanson, 2002).
If the horse is stressed continuously and hormones, such as cortisol, are not limited by
normal feedback mechanisms, there can be a change in the microbial ecosystem of the
horse (Blum et al., 2002). Increased levels of endogenous corticosteroids, a product of
stress, have the potential to decrease the secretion of mucin which can result in extensive
growth of coliform bacteria, such as Escherichia coli (Montes and Pugh, 1993).

1.4.1.1 Illness

Changes in the horse’s microbiome, due to stress or as a response to certain
factors, can be associated with the onset of illness (Costa and Weese, 2012). Significant
increases in lactic-acid producing bacteria after a rapid ingestion of starch is often
followed by the development of laminitis (Garner et al., 1978; Milinovich et al., 2006;
Crawford et al., 2007; Milinovich et al., 2007; Milinovich et al., 2008). Studies
performed by Milinovich and colleagues (2006, 2007, 2008) studied the microbial ecology of the horse’s hindgut during oligo-fructose induced laminitis in order to observe possible changes in the microbiome that could contribute to the incidence of laminitis. They found that *Streptococcus* proliferated quickly prior to the onset of laminitis and also observed increases in lactate concentrations in the cecum of the five Standardbred horses used in that study. The impact of a large increase in lactic-acid producing bacteria is detrimental to cecal pH and multiple responses can occur (i.e. lactic acidosis, mucosal damage; Milinovich *et al*., 2007). Treatment of illness with medications, such as oxytetracycline, has also been shown to alter the gastrointestinal microbiome. The study conducted by White and Prior (1982) found large increases in *Streptococcus* counts following oral administration of oxytetracycline in horses while observing a decrease in lactate-utilizing *Veillonella* spp.

### 1.4.1.2 Transport

The transport of horses is very common in the horse industry. It has been shown that the amount of time that a horse is transported, whether short duration or long, can stress the animal. Just being in a trailer or getting into one can be a stressor (Bradshaw *et al*., 1996). This stress can increase with vibrations, changes in temperature, and water deprivation (Waran *et al*., 1995; Broom, 2005). Research has shown detrimental health effects due to transportation stress, including respiratory infections (Okiawa *et al*., 1995). Age of the horse and exposure to the stressor can also have affect how the horse responds to the stressor. Younger, more naïve horses, seem to respond more severely than the
more experienced animals behaviorally and they also had greater hormonal indicators of stress (Fazio et al., 2003; Waring, 2003; White et al., 1991).

1.4.1.3 Weaning

Weaning can be a source of mental and physical stress in many species (McCall et al., 1987). Weaning in other monogastrics, rabbits and piglets, has been shown to have an impact on the gastrointestinal microflora (Gouet and Fonty, 1979; Konstantinov et al., 2003; Konstantinov et al., 2006). The changes in environment and social interactions are large as the foals no longer have access to their mares (Waran et al., 2008). In one study, ten Thoroughbred foals were given 10 g per day of a commercial probiotic, containing multiple Lactobacillus species, from one month prior to weaning to 4 days post-weaning. Foals showed an increase in cortisol post-weaning, indicating that weaning was a stressful event (Swanson, 2002). These foals showed successful colonization of lactic acid bacteria in the gastrointestinal tract but there were no significant differences in the stress indicators, plasma cortisol, lactate, IgA and IgG, due to treatment (Swanson, 2002).

1.4.2 Diet

A change in diet composition can change the diversity and ratio of microbes in the gastrointestinal tract (Clarke et al., 1990). The rapid ingestion of starch has been shown to lead to increased counts of total anaerobes in the hindgut (Goodson et al., 1988; de
Fombelle et al., 2001). Other shifts of the normal bacterial profile can be caused as well, especially concerning the ingestion of concentrate and starch.

Fiber is usually represented by forage in the diet of horses. Short-chain volatile fatty acids (VFAs) are the product of fiber fermentation. These products, mainly acetate, propionate and butyrate, are then absorbed into the blood and used as a source of energy for ATP production (Mackie and Wilkins, 1988). Carbohydrates are often added to a horse’s diet in the form of concentrates, which are high in starch. The digestion of starch yields VFAs and lactic acid (Frape, 2004).

Small additions of starch, in the form of concentrate, (0-30%) can lead to an increase in the amount of cellulolytic bacteria, like Fibrobacter and Ruminococcus, in the hindgut. However, when the amount of concentrate exceeds the amount of forage in the diet and the amount of starch in the diet, or meal, surpasses the amount that the foregut can digest and absorb, the overflow of non-degraded starch is allowed to reach the hindgut (Kern et al., 1973; Bellet, 1982; de Vaux and Julliand, 1992; Potter et al., 1992; Kienzle, 1994; Julliand, 1996; Julliand et al., 2001; Biddle et al., 2013). When the microflora in the hindgut then have access to that starch, changes in the gastrointestinal environment and microbial profiles can occur. In the hindgut, particularly the cecum and right ventral colon, total anaerobes are observed to increase in response to an overload of starch in the diet (Moore and Dehority, 1993; Julliand et al., 2001; Medina et al. 2002; de Fombelle et al., 2003). Research has shown that the cellulolytic bacteria drastically decrease due to a proliferation of starch-utilizing bacteria, an increase in lactic acid production and a drop in luminal pH (Kern et al., 1973; Bellet, 1982; de Vaux and
Julliand, 1992; Julliand, 1996; Julliand et al., 2001; Biddle et al., 2013). When non-degraded starch overflows into the hindgut, lactic acid-producing bacteria have been shown to increase while lactic-acid utilizers do not increase as much (Garner et al., 1978; Goodson et al., 1988; Julliand et al., 2001; Medina et al., 2002). This increased ratio of lactate-producers to lactate utilizers causes an increase of lactic acid in the hindgut, an increase in volatile fatty acids and greatly reduces the pH (Garner et al., 1978; Julliand et al., 2001; Medina et al., 2002; Biddle et al., 2013). Changes in pH and the increase in lactic acid can also irritate the lining of the gut and then affect other bacteria through apoptosis as well as possibly change the permeability of the gut lining to allow toxins and larger molecules to pass through the membrane and cause harm to the host (Pagan, 1998; Biddle et al., 2013). When the pH decreases and goes below 6.0, there is a suppression of cellulolytic bacteria in the cecum which points to a decrease in fibrolytic activity and favors lactic-acid producing bacteria thus further lowering the pH due to lactic acid production (Milinovich et al., 2008; Biddle et al., 2013).

Fat is a common supplement in the diet of horses and is often used to increase the energy content of the feed (Bush et al., 2000). The research studying the effect of fat addition to the horse’s diet on the hindgut’s environment and ability to digest fiber has produced conflicting results. Some studies observed a decrease in either apparent neutral detergent fiber or acid detergent fiber digestibility after dietary fat supplementation (Rich et al., 1981; Worth et al., 1987; Jansen et al., 2000). Other studies state that there was an increase in digestibility of either acid detergent fiber or neutral detergent fiber (Rich et al., 1981; Scott et al., 1987; Webb et al., 1987; Hughes et al., 1995; Julen et al., 1995).
Studies also found no effect of dietary fat supplementation on apparent crude fiber digestibility (Kane and Baker, 1977; Kane et al., 1979; Davidson et al., 1987; McCann et al., 1987; Meyer et al., 1987). Jansen and colleagues (2000) observed that much of the research, observing the effect of fat supplementation on horse diets, differed in many ways, including the amount of crude fiber used in each study. Studies by Jansen and others (2000, 2002, 2007) consistently observed a decrease in fiber digestibility in response to an iso-energetic replacement of non-structural carbohydrates with soybean meal. However, when extra fat was added to a basal diet, that effect was not observed (Jansen et al., 2000). There are several proposed microbial-associated causes for the reduced fiber digestibility. Fat consumption stimulates bile secretion (Meyer et al., 1997). An increase in bile acids entering the hindgut could inhibit bacterial activity and decrease fiber digestibility (Floch et al., 1972). Fatty acids, from undigested fat reaching the hindgut, could also have a toxic effect on certain microbes as well as a negative coating effect on the microbes (Palmquist, 1984). In order to combat the detrimental effects of stressors, physiological or environmental, on the hindgut microbiome in horses, various dietary supplements have been suggested (Medina et al., 2002; Weese et al., 2004; Berg et al., 2005; Jouany et al., 2008; Tanabe et al., 2014).

1.5 Dietary Supplements

1.5.1 Prebiotics

Gibson and colleagues (2004) defined prebiotics as “…selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the
gastrointestinal microflora that confers benefits upon host well-being and health.”
Prebiotics are non-digestible for the host animal but rather are fermentable by particular gastrointestinal microflora. They are designed to create a favorable environment for the proliferation and metabolic activity of lactic acid bacteria (Gibson et al., 2004). Certain bacteria, such as *Lactobacillus* and *Bifidobacteria*, ferment prebiotics in order to produce energy and carbon for growth. Increases in the number of certain bacteria, such as *Lactobacillus*, can inhibit the growth of potential pathogenic bacteria through the production of bacteriocins and other substances (Fujiwasa et al., 1993; Bogovic-Matijasic et al., 1998; Savadogo et al., 2004). *Lactobacillus acidophilus*, a specie of *Lactobacillus* found in horses, has been shown to produce multiple bacteriocins that inhibit pathogenic bacteria (Fujiwasa et al., 1993; Bogovic-Matijasic et al., 1998). Lactic acid bacteria concentrations, such as species of *Lactobacillus* and *Streptococci*, increase with the use of prebiotics while bacteria that are potentially pathogenic are left with fewer nutrients and binding sites in the gastrointestinal tract (Yuki et al., 2000; Respondek et al., 2008). However, if concentrations of certain LAB, such as *Streptococcus*, are too high, they can be detrimental to the animal and can lead to disorders such as acidosis (Vermorel and Martin-Rosset, 1997; Milinovich et al., 2008). This often occurs in response to the rapid ingestion of high-starch meal when there is a severe decrease in luminal pH that favors lactic-acid producing bacteria and the death of cellulolytic bacteria. The action of prebiotics in horses is not well documented or studied. Studies have observed changes in the microflora, when analyzing equine feces, due to the supplementation of prebiotics (Pellegrini et al., 1999; Berg et al., 2005). The amount of *Lactobacilli* didn’t change
while *E. coli* concentrations and pH levels decreased in the fecal content of Quarter Horse yearlings that were treated with fructo-oligofructose over three separate 10-day feeding trials (Berg *et al.*, 2005). Other research has shown an increase in total bacteria counts, *Streptococci*, and lactate-utilizing bacteria in the gastric juice when horses were treated with oligofructose in the diet (Nadeau *et al.*, 2000). Other common dietary supplements used in horses are probiotics.

### 1.5.2 Probiotics

Probiotics, including direct-fed microbials (DFM) and some species of yeast, are living microorganisms that have beneficial effects on the host, such as improving intestinal symbiosis (Lu and Walker, 2001). Probiotics are referred to as “generally regarded as safe” (GRAS) (Schoster *et al.*, 2014). Probiotics have been found to improve nutrient utilization (Fuller, 1989; Yoon and Stern, 1995; Hooper *et al.*, 2000; Swyers *et al.*, 2008). They have also been promoted and used as a strategy to combat the effects of stress to the host (Teitlbaum and Walker, 2002). Detrimental effects to the microflora, whether diet change or stress of some kind, are thought to be reduced by the addition of probiotics to the diet all in an effort to decrease the chance of the host suffering from a reduction in health and overall well-being (Teitlbaum and Walker, 2002; Weese *et al.*, 2003; Ward *et al.*, 2004; Weese *et al.*, 2004; Tanabe *et al.*, 2014).

Lactic acid bacteria (LAB), as a group, contain a large amount of microbes that are considered probiotic. *Streptococcus* and *Lactobacillus* are a few bacteria that belong
in the LAB group. Strains of *Lactobacillus* have been investigated as potential probiotics. Some LAB strains, specifically different strains of *Lactobacillus*, have shown little to no colonization after subjects were given doses of different concentrations over a 5 day period (Weese *et al*., 2003). However, others LAB strains have been detected in fecal material after horses were given an oral dose and were shown to inhibit some potentially pathogenic bacteria, such as *E. coli*, *in vitro* (Weese *et al*., 2003; Weese *et al*., 2004). A study conducted by Tanabe and colleagues (2014) administered a probiotic to 101 Thoroughbred foals from birth to 20 weeks of age to ascertain if the probiotic had a positive effect on the incidence of diarrhea. The researchers concluded that adding a probiotic to the young Thoroughbreds’ diets both prevented and reduced the severity of diarrhea (Tanabe *et al*., 2014). Research conducted by Ward and others (2004) observed that probiotics administered to horses reduced *Salmonella* infection by 65% in horses that were hospitalized. The species and dosages of probiotics may play an important role in whether it is considered effective or not (Gibson and Fuller, 2000; Yuki *et al*., 2000; Laukova *et al*., 2008). Some yeast strains, primarily those of *Saccharomyces cerevisiae*, are also classified as probiotics.

1.5.3 Yeast

Some yeast species, mostly those of *Saccharomyces*, are included in the probiotics category but depending on the substrate it is grown on, it can be both a pre- and probiotic, also known as a synbiotic. Synbiotics are used as a way to improve survival and attachment of live microbial dietary supplements in the gastrointestinal tract.
by mixing pro- and prebiotics in order to beneficially affect the host (Gibson and Roberfroid, 1995). A synergistic effect due to coupling probiotics and prebiotics has been observed in vivo in many monogastric species, suggesting that combined, pre- and probiotics could have a greater effect than either alone (Pool-Zobel et al., 1996; Burns and Rowland, 2000; Bomba et al., 2002; Fernia et al., 2002). The particular strain of yeast found in most of the published literature is *Saccharomyces cerevisiae* CBS 493.94 (Glade, 1991; Medina et al., 2002; Jouany et al., 2008). *Saccharomyces cerevisiae* CBS 493.94 is used as a live preparation of Brewer’s yeast and grown on a media of ground yellow corn, diastatic malt and cane molasses (Yea-Sacc, Alltech Inc., Lexington, KY; EFSA, 2009). *Saccharomyces cerevisiae* CBS 493.94 is then dried to preserve its fermenting action. In studies described by the European Food Safety Authority, live yeast supplementation seemed to increase dry matter digestibility (EFSA, 2009). A variety of mature horses, including stallions and geldings, were fed 10 g per day of a commercial yeast supplement. Fiber digestion was significantly increased which was contributed to a significant increase in total anaerobes and lactate-utilizing bacteria observed in the cecum of horses fed *Saccharomyces cerevisiae* when compared to horses that were not given the yeast supplement (EFSA, 2009). In another study conducted by the European Food Safety Authority (2009), pregnant mares were fed 20 g per day of a live yeast supplement four weeks pre-parturition to four weeks post-parturition. Pregnant mares that were supplemented with yeast had higher abilities to digest fiber and crude protein than the pregnant mares that did not receive the supplement. Mares that received the yeast also had significantly greater milk production as well an increased nutrient
composition of the milk produced. The foals of the mares supplemented with yeast had a greater average daily gain than the foals of the mares that did not receive yeast. Nine young horses, yearlings, were used in a quasi cross-over design study and the effects of dietary yeast supplement at 8 g per head per day observed (EFSA, 2009). The young horses that received the yeast supplement had improved digestibility of neutral detergent fiber and acid detergent fiber.

Yeast culture supplementation ideally transfers viable populations of yeast to the targeted gastrointestinal site in order to stimulate microbial populations (Nisbet and Martin, 1991; Dawson, 1992; El Hassan et al., 1993). *Saccharomyces cerevisiae* is classified as a probiotic organism (Schoster et al., 2014). Research has shown that the healthy, stable gastrointestinal environment, specifically the microbes through complex, interacting mechanisms, can resist colonization by enteric pathogens which could cause harm to the host (Rolfe, 1996). The theorized mechanisms include competition for essential nutrients or epithelial attachment sites, the production of antimicrobial compounds by indigenous microflora, and metabolizing nutrients which can then create an environment that is not conducive for enteric pathogens, such as a reduction of the luminal pH via the production of short-chain fatty acids (Steer et al., 2000; Fooks and Gibson, 2002). Some probiotics have been shown to attach to the intestinal mucosa indicating that those probiotics could prevent the attachment of pathogens to those sites through competitive exclusion (Salminen et al., 1996; Tuomala et al., 1999). The metabolic end products of certain probiotics have been shown to inhibit adhesion or invasion by pathogenic bacteria (Bernet et al., 1994). Besides preventing pathogen
colonization, it has been suggested that probiotics may strengthen the epithelial barrier by promoting epithelial repair, therefore contributing to the prevention of pathogens moving across the epithelium (Blomberg et al., 1993; Kaila et al., 1995).

A weakened intestinal epithelial tight junction can lead to increased intestinal permeability, which can result in not only pathogenic infection, but also inflammation. Studies have shown that probiotics, through enforcement of the tight junction barrier in the intestines and maintenance of the intestinal strength, can help prevent inflammation and diarrhea (Tanabe et al., 2008; Miyauchi et al., 2009; Fukada et al., 2011; Ogita et al., 2011a; Ogita et al., 2011b; Tanabe, 2013; Tanabe et al., 2014). The use of probiotics has also been suggested in order to cause anti-inflammatory effects on the GI tract thus helping to stabilize the microbial environment (Carol et al., 2006).

The effect of yeast supplementation has not been studied as extensively in horses as it has in ruminants and other monogastric animals. Yeast cultures are thought to stimulate the microbial ecosystem of the gastrointestinal tract, as evidenced by increased growth of anaerobic microorganisms and increased activity of cellulolytic bacteria. It is proposed, based on research done in vitro, that metabolites produced by the yeast cultures could be involved in the stabilization of the environment within the rumen, causing higher growth rates of bacteria and fungi (Chaudeyras et al., 1996). Increased growth of anaerobic fungi may be significant to plant cell wall breakdown which could lead to an increase of bacteria in the rumen. It is also thought that supplementing yeast could help remove trace oxygen from the environment of the rumen, thus enhancing growth of microbes (Wallace et al., 1996). There have been increases observed in the number of
CFUs of cellulolytic bacteria (Dawson et al., 1990; Newbold et al., 1996), proteolytic bacteria, total anaerobes (Wiedmeier et al., 1987; El Hassan et al., 1993) and lactic-acid utilizing bacteria (Girard et al., 1993) when yeast cultures were supplemented to ruminants. The effects on pH are also worth mentioning as rapid changes in pH can lead to an increase in microbes that do not utilize fiber as well having detrimental effects also observed in horses, such as acidosis as luminal pH decreases (Vermorel and Martin-Rosset, 1997; Milinovich et al., 2008; Biddle et al., 2013). In dairy goats, live yeast supplementation was found to significantly reduce E. coli levels found in fecal samples while increase the amount of Lactobacillus (Stella et al., 2005). Research has also been conducted in other monogastric species, concerning the effect of Saccharomyces cerevisiae on gastrointestinal microflora. Adding Saccharomyces cerevisiae to laying hens’ diets was observed to lower E. coli and increase Lactobacilli counts (Park et al., 2002; Hassanein and Soliman, 2010). The mechanisms by which E. coli counts were reduced are thought to be competitive exclusion by Lactobacillus as well as maintaining a pH that was unfavorable to E. coli, caused by changes in the microbial environment due to the supplementation of yeast favoring Lactobacillus (Chaucheryas-Durand and Fonty, 2002). Horses supplemented with yeast had slighter changes in pH and lactic acid levels, in the large intestine, after feeding than horses not fed yeast in the diet, demonstrating that yeast may improve microbial balance by stimulating the populations of cellulolytic bacteria and their activity (Goodson et al., 1988; de Fombelle et al., 2001; Medina et al., 2002). This points to yeast supplementation reducing undesirable changes in the hindgut.
ecosystem in response to feeding by reducing a shift in the proportion of lactic acid-producing to lactic acid utilizing bacteria (Medina et al., 2002).

Horses are hindgut fermenters and their digestive tract reflects an animal that is used to ingesting feed over a long period of time (Frape, 1998; Russell and Gahr, 1999). Dietary yeast supplementation has been shown to influence nutrient digestibility, by improving cellulose digestibility, and microbial populations such as Lactobacillus spp. and Streptococcus ssp. in the hindgut (Medina et al., 2002; Jouany et al., 2008). Reported increases of dry matter (DM) digestibility are common when yeast is supplemented in the diet which suggests an increase in digestible energy (Glade and Sist, 1988; Hall et al., 1990; Glade, 1991; Hill and Gutsell, 1997; Medina et al., 2000). An increase in digestibility of acid detergent fiber (ADF) and neutral detergent fiber (NDF) has also been observed when the equine diet was supplemented with yeast (Godbee, 1983; Glade and Biesik, 1986; Glade and Sist, 1988; Glade 1991; Hill and Gutsell, 1997; Medina et al., 2000; Morgan et al., 2007). A study by Morgan and colleagues (2007) investigated the effect of dietary yeast supplementation on the digestibility of forages that were of different quality. Sixteen horses were used in a 4x4 Latin square design and fed either a high-quality or low-quality grass hay and either received 56 g per day of a live yeast supplement or did not. Neutral detergent fiber and hemicellulose digestibility increased in horses that were fed a low-quality hay and yeast over horses that were fed low-quality forage and did not receive yeast. These results are representative of a change in hindgut microflora. In order to improve the digestibility of ADF and NDF
digestion, the microbes that are responsible for the digestion of ADF and NDF, primarily cellulolytic bacteria, must be favored.

Horses supplemented with yeast have also been shown to have an increased crude protein digestibility (Glade and Biesik, 1986; Glade and Sist, 1991; Morgan et al., 2007). A study by Morgan and others (2007) observed that crude protein digestibility was increased when horses that were fed low-quality forage, received a live yeast supplement as compared to horses that received low-quality forage and did not receive yeast. This can be important in young horses, especially in periods of growth (Glade and Cambell-Taylor, 1990; Bennet-Wimbush et al., 1991; Glade, 1991). In young, growing horses, the need for amino acids is increased (Bennet-Wimbush et al., 1991). However, the mechanisms by which protein digestion is increased by the supplementation of yeast has not been studied extensively (Hill et al., 2006). There was a utilization of muscle glycogen more quickly to fat oxidation seen after yeast supplementation which has been theorized to enhance the utilization of long-term energy supplies (Kolterman et al., 1993; Miller-Graber et al., 1994). This has been shown to be useful to horses under long term, heavy exercise (Biels et al., 1990; Harris, 1997).

*Saccharomyces cerevisiae* has been reported to survive, but not colonize, in parts of the hindgut in horses when a daily dose is given (Medina et al., 2002; Jouany et al., 2008; Jouany et al., 2009). Jouany and others (2009) observed concentrations of *Saccharomyces cerevisiae* averaging $4.4 \times 10^6$ cfu/mL and $5.6 \times 10^4$ cfu/mL in the cecum and right-ventral colon, respectively, when horses were supplemented with 10 g per day of a commercial dietary yeast supplement. The concentrations of *Saccharomyces*
*Saccharomyces cerevisiae* were higher in the cecum than the colon. When *Saccharomyces cerevisiae* is supplemented in the diet, especially when there is concentrate as opposed to a strictly fiber diet, the pH values stabilized in the hindgut and do not change as drastically following a meal (Moore *et al*., 1994; Medina *et al*., 2002). When eight mature horses were given 10 g of a commercial *Saccharomyces cerevisiae* supplementation, the ratio of lactic acid-producing: lactic acid-utilizing bacteria was reduced in the hindgut, coupled with a decrease in lactic acid concentrations (Medina *et al*., 2002). When horses were on a high fiber diet, there was a significant increase in *Lactobacillus* spp in the cecum and a significant decrease of *Streptococcus* spp in the colon (Medina *et al*., 2002). An improvement in fibrolytic activity that correlated with a molar percentage increase of acetate when the horse was supplemented with *Saccharomyces cerevisiae* was also reported (Medina *et al*., 2002). The study concluded that yeast supplementation lessened undesirable changes in the hindgut and reduced changes in lactic acid concentrations as well as pH in high-starch diets.

1.6 Effect of Maternal Diet on Offspring’s Growth and Development

Maternal nutrition has been shown to affect insulin-glucose regulation, predispose offspring to metabolic disorders and affect fetal growth, as well as lead to a more rapid establishment of certain microflora (Nathanielsz, 2006; Armitage *et al*., 2004; Ford *et al*., 2007; Clapp, 2002; Thum *et al*., 2012; Faubladier *et al*., 2013). Studies in swine and sheep suggest that the establishment of the intestinal microbial environment in
young animals could have an effect on the bacterial ecosystem of the adult animal (Thompson et al., 2008; Yanez-Ruiz et al., 2010). The main microbial environments that the foal is exposed to in early life are from the mare (Mackie et al., 1999; Biasucci et al., 2010; Dominguez-Belloa et al., 2010; Kuhl et al., 2011). When a foal is born, it is exposed to bacteria from the mare’s vagina, feces, saliva and/or milk (Biasucci et al., 2010; Dominguez-Belloa et al., 2010; Kuhl et al., 2011). Milk can contain up to $10^9$ microbes/L with the most abundant microbes including Streptococci and Lactobacilli (Moughan et al., 1992; Mackie et al., 1999). Research has shown that supplementing the maternal diet with yeast culture increased milk production and nutrient content (Glade, 1991). A study by Glade (1991) fed eight, pregnant mares 20 g per head per day of a commercial yeast supplement four weeks pre-parturition until 4 weeks post-parturition. Dry matter and crude protein digestibility significantly increased when the lactating mares were supplemented with yeast. Increases in energy content, sugars, total lipids, total nitrogen and total amino acids were also observed in the milk of the mares that were supplemented with yeast. This study also demonstrated an increased growth rate of the foals whose dams were treated with yeast culture. It has been suggested that the foal’s GI microflora could be optimized by modifying the mare’s microbial ecosystem (Thum et al., 2012). In mice, supplementation of the maternal diet during gestation and lactation with prebiotics may have influenced the maternal intestinal microflora as well as the microflora of the offspring in a positive manner (Fujiwara et al., 2008; Fujiwara et al., 2010). It was observed in mice, that adding 50 g per kg of fructo-oligosaccharides (FOS) to the maternal diet affected the offsprings’ intestinal microflora for up to two weeks.
post-parturition, as shown through distinct clustering in a dendrogram analysis (Fujiwara et al., 2010). Recent research by Faubladier and colleagues (2013) showed that probiotic supplementation of pregnant mares can affect their offspring’s gastrointestinal microflora during the first few days of life. Foals of the mares supplemented with probiotics had a greater establishment of total anaerobes and lactate utilizers at an earlier time than the foals whose dams were not supplemented, which may be caused by the shift in the bacterial ecosystem of the mare and/or the change in lactation. The researchers proposed that the effect on early establishment of total anaerobes and lactate utilizers could be caused by the modification of the bacterial ecosystem of the mare and/or the alteration in milk production. Research has also shown an increased body weight observed in foals of mares supplemented with probiotics from late gestation to early lactation (Faubladier et al., 2013). When mares were supplemented from d 300 of gestation to 60 d post-parturition, their offspring were heavier than those foals of non-supplemented mares from 19 d to 60 d post-parturition.

1.7 Summary

The physical development of the gastrointestinal tract occurs with microbial colonization and is largely dependent on the diet changes of the foal as it matures. In order to lessen the detrimental effects of stress on microflora caused by diet change, transport or weaning, probiotics and prebiotics have been added to the horse’s diet in an attempt to re-establish homeostasis in the gastrointestinal tract. Many feed additives that contain yeast, particularly live yeast supplements, are considered synbiotics, which
combine pro- and prebiotics to beneficially affect the host. Research has shown that microbial populations in the hindgut of horses can be influenced by dietary yeast supplementation. Maternal diet has been shown to have an effect on the growth and development of their young and it has been suggested that the foal’s GI microflora could be optimized by altering the mare’s microbial ecosystem since the maternal gastrointestinal microbiome is one of the primary microbial environments that the foal is exposed to in early life. The objective of this study was to evaluate the effect of a maternal dietary live yeast supplement on the diversity of the gastrointestinal microflora in the hindgut of Quarter Horse mares and the growth and development of their offspring.
Chapter 2

Effects of Maternal Dietary Yeast Supplementation on Foal Growth and Microbial Diversity of the Hindgut in Quarter Horse Mares and Their Offspring

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INTRODUCTION

It is widely recognized that the development and maintenance of microbial populations in the equine hindgut are crucial for the overall health and well-being of the horse (Tuoloma et al., 1999; Ward et al., 2004; Weese et al., 2004; Julliand, 2005; Miyauchi et al., 2009; Tanabe et al., 2014). Diverse maternal and environmental bacterial populations have been shown to establish quickly in the gastrointestinal tract of the foal after the ingestion of milk which can contain up to $10^9$ microbes/L (Moughan et al., 1992; Sakaitani et al., 1999; Yuyama et al., 2004). The colonization and development of the microbial ecosystem is largely dependent on the diet that the foal receives as it matures (Boy and Duncan, 1979; Favier et al., 2002). As the foal ages, shifts from enzymatic digestion to anaerobic fermentation occur as the foal consumes more feed that is made up of complex carbohydrates (Boy and Duncan, 1979; Mackie et al., 1999). Bacteria that can digest such compounds, such as *Fibrobacter succinogenes*, establish in larger numbers than when milk was the primary source of energy so that the foal can utilize these complex carbohydrates (Boy and Duncan, 1979; Mackie et al., 1999).

Changes in the type of microbes and their activity in the hindgut of horses, caused by diet or stress, can induce undesirable effects and cause harm to the host. These
undesirable effects may include pathogenic bacteria colonizing the hindgut or a decrease in luminal pH leading to illness (Pagan, 1998; de Fombelle et al., 2001; Jansen et al., 2002; Medina et al., 2002; Milinovich et al., 2008; Biddle et al., 2013). Rapid ingestion of a high starch meal can cause a drastic decrease in cellulolytic bacteria due to proliferation of starch-utilizing bacteria, increased lactic acid production and a drop in luminal pH. The drop in the concentration of cellulolytic bacteria results in a decrease in fibrolytic activity and favors lactic-acid producing bacteria thus further lowering the luminal pH due to lactic acid production (Kern et al., 1973; Bellet, 1982; de Vaux and Julliand, 1992; Julliand, 1996; Julliand et al., 2001; Biddle et al., 2013). Changes in pH and the increase in lactic acid can irritate the lining of the gut and then affect other bacteria through apoptosis as well as possibly change the permeability of the gut lining to allow toxins to cause harm to the host (Pagan, 1998; Biddle et al., 2013). The prophylactic use of beneficial supplements, such as probiotics, prebiotics and synbiotics, to prevent gastrointestinal upset or reduce the severity that can result from gastrointestinal upset has been documented in several species including horses (Kopp-Hooliham, 2001; Medina et al., 2002; Ward et al., 2004; Tanabe et al., 2014).

Prebiotics and probiotics have been added to equine diets in an effort to re-establish homeostasis after gastrointestinal upset due to physiological or environmental stress (Pellegrini et al., 1999; Ward et al., 2004; Weese et al., 2004; Berg et al., 2005; Tanabe et al., 2014). Yeast has been shown to positively affect the hindgut by reducing changes in pH and lactic acid levels in the large intestine after feeding (Medina et al., 2002). The addition of yeast to a horse’s diet has also been shown to improve cellulose
digestibility and reduce a shift in the proportion of lactic acid-producing to lactic acid utilizing bacteria after the ingestion of a meal (Medina et al., 2002; Jouany et al., 2008). Although yeast has been shown to influence the microbial ecosystem of the equine gastrointestinal tract, the ability of a maternal live yeast dietary supplement to influence the development of the foal’s microbial populations has not been studied (Medina et al., 2002; Julliand, 2005; Jouany et al., 2008; Tanabe et al., 2014).

Maternal diet has been shown to affect the growth and development of offspring in many monogastric species, including humans, mice and horses (Glade, 1991; Nathanielsz, 2006; Ford et al., 2007; Clapp, 2002; Thum et al., 2012; Faubladier et al., 2013). Foals born from mares fed a yeast supplement during late gestation and early lactation had greater average daily gains (ADG) than foals born from mares that did not receive the yeast supplement (Glade, 1991). In a more recent study, foals born from mares supplemented with a probiotic during late gestation and early lactation had greater concentrations of total anaerobes and lactate utilizers by 1 d of age compared to the foals whose dams were not supplemented (Faubladier et al., 2013). If probiotics added to the maternal diet during gestation and early lactation can influence foal growth and the development of the gastrointestinal microbial populations during early life, it may be possible that a yeast supplement may also influence foal growth and the development of gastrointestinal microbial populations and diversity (Medina et al., 2002; Jouany et al., 2008; Faubladier et al., 2013). Therefore, the objective of this study was to evaluate the effect of a maternal dietary live yeast supplement on the diversity of the gastrointestinal
microflora in the hindgut of Quarter Horse mares and the growth and development of their offspring.
MATERIALS AND METHODS

Experimental Design

Eight pregnant Quarter Horse mares (14.5 ± 7.5 yr) were used in a completely randomized design to evaluate the effect of dietary live yeast supplementation on total growth of foals and microbial diversity in the gastrointestinal tracts of the mares and their offspring. Each mare received a basal diet consisting of 0.5% BW/d of a 12% CP pelleted concentrate until parturition with water and mixed grass hay *ad libitum*. After parturition, the basal diet consisted of approximately 1% BW/d of a 16% CP pelleted concentrate with water and mixed grass hay *ad libitum*. Mares were randomly assigned to one of two treatments from d 250 of gestation to 90 d (+/- 15d) post-parturition: the basal diet (CTL) or the basal diet supplemented with a targeted dose of 1g (4.5 x 10^9 cfu)/45.4 kg of BW per day of a live culture of *Saccharomyces cerevisiae* (Alltech; Nicholasville, KY). Concentrate samples were taken monthly and pooled to obtain a composite sample for nutrient analysis. Forage samples were collected by random sampling from different parts of the hay bales while samples from the pasture were collected bi-weekly by mixing eight 6 in x 6 in grass samples cut at random throughout the pasture. Forage samples were pooled to obtain a composite sample for nutrient analysis. Composite concentrate and forage samples were analyzed (Dairy One...
Cooperative Inc.; Ithaca, New York) for crude protein, acid detergent fiber, neutral detergent fiber, starch, digestible energy, crude fat and ash (concentrate only; Table 1). Prior to parturition, and within 7 d after parturition, mares were housed outdoors in paddocks with access to shelter. For parturition, mares were housed in 3.7 x 7.3 m box stalls. By 28 d (+/- 14d) post-parturition, mares were acclimated to grass pasture. Throughout the study, foals did not have access to the mares’ concentrate. Starting at approximately 14 d of age, foals were creep fed with a 16 % CP pelleted concentrate at 1% of BW and increased to 3% of BW by weaning. Fresh fecal samples were collected from the mares on d 250, 280 and 310 of gestation, d of foaling (d0), 12 h (d0.5), d1 and d14 post-foaling as well as every 28 d (+/- 3d) until the foals were weaned (d180). Fecal samples were collected from the foals within 2 h of birth (meconium) (d0), 12 h after birth (d0.5), d1 and 14, as well as every 28 d (+/- 3d) until 2 months after weaning (d240). The growth measurements body weight (BW; kg), wither height (WH; cm) and hip height (HH; cm) were taken on foals at 12 h after birth (d0.5) and d14, as well as every 28 d (+/- 3d) until 2 months after weaning (d240).

**Microbial Analysis of Fecal Samples**

After collection, fecal samples were stored at -20º C until further analysis. One gram of feces from each horse was pooled by production status (mare/foal), treatment, and day. DNA was extracted using a Repeated Bead Beating Plus Column RB++C method (Yu and Morrison, 2004) with a modified protocol for elution: 30 μL instead of 200 μL of AE. The subsequent DNA was purified using a Qiagen mini DNA kit (Qiagen
Inc.; Valencia, CA). The protocol from the Q33130 kit was used with the following modifications: 50 μL of working solution was used instead of 200 μL, 2.5 μL of standards or DNA samples were used in each well rather than 10 μL. Purified DNA was subjected to electrophoresis on a 1% agarose gel at 80 v for 1 h. Quantification of DNA was determined using Quant-iT PicoGreen (Molecular Probes Inc.; Eugene, OR). DNA was analyzed using PCR-DGGE. The primers used were specific to the V2-V3 region of 16S rDNA of all bacterial species (universal), bacteria belonging to the phylum *Firmicutes*, and bacteria of the *Streptococcus* genera. The genera included in the *Streptococcus* group were *Streptococcus, Enterococcus, Lactococcus, Vagococcus* and *Tetragenococcus*. Primers specific to ITS2 rDNA of *Saccharomyces cerevisiae* were also used. All primers used to obtain PCR product suitable for use in DGGE are shown in Table 2. The reaction mixture for PCR using universal primers (HDA1, HDA2) (50 μL) contained 0.25 μL of each 100 μM primer and Taq polymerase, 50 ng of the DNA template, 5.00 μL of PCR reaction buffer (Invitrogen; Life Technologies Corp., Eugene, OR), 1.02 μL of BSA, and 3.57 μL of 50 mM MgCl₂, and 0.408 μL 100 mM dNTP. The reaction mixture for PCR using the primers specific for the phylum *Firmicutes* (FirmFGC, FirmR) (50 μL) contained 0.25 μL of each 100 μM primer and Taq polymerase, 0.5 ng of the DNA template, 5 μL of PCR reaction buffer (Invitrogen; Life Technologies Corp., Eugene, OR), and 2.5 μL of 50 mM MgCl₂, and 0.408 μL dNTP. Distilled water (Life Technologies Corp., Eugene, OR) was added to each reaction for a final total volume of 50 μL. The PCR reaction mixture using the *Streptococcus* primers (LAC3F, LAC2RGC) (50 μL) contained 0.25 μL of each 100 μM primer and Taq
polymerase, 50 ng of the DNA template, 5 μL of PCR reaction buffer (Invitrogen; Life Technologies Corp., Eugene, OR), 1.02 μL of BSA, and 3.57 μL of 50 mM MgCl₂, and 0.408 μL dNTP. The reaction mixture for PCR (50 μL) with *Saccharomyces cerevisiae* (ShafGC, Schar) contained 0.1 μL of each primer, 0.5 μL of Taq polymerase, 50 ng of the DNA template, 5 μL of Mg-free PCR reaction buffer, 5 μL of 25 mM MgCl₂ and 5 μL 2 mM dNTP.

**DGGE Analysis**

Before samples were used for DGGE, 3.0 μL of each PCR product was subjected to 1.0 % agarose gel electrophoresis to confirm successful amplification of the V2-V3 region. Then, 8.0 μL aliquots of PCR product were resolved in a 7.5% polyacrylamide gel containing a 40%-60% gradient of denaturants (formamide and urea) for total bacterial products or a 30%-70% gradient for all other PCR products. The DGGE gel was run in 1.0 % Tris-acetate-EDTA (TAE) buffer at 60° C and 82 v for 16 h using INGENY phorU-2 (Ingeny; Leiden, The Netherlands). The subsequent images were captured using an AlphaImager HP® (ProteinSimple; Santa Clara, CA).

**Qualitative and Statistical Analysis of Microbial Profiles**

Captured images were uploaded into BioNumerics (Applied Maths NV; Sint-Martens, Latem, Belgium) and analyzed for banding patterns and positions to depict diversity in the form of a dendrogram. The program used a band-searching algorithm to detect bands in the DGGE gels and bands were manually selected. The lanes were
converted to densitometric curves and normalized using bands on the standard sample to make sure that the location of bands was consistent across all gels. Clusters were determined by comparing patterns and a dendrogram was used to show relative similarities which is shown by the length of the lines.

Similarity coefficients were derived by comparing pooled samples DGGE profiles (banding patterns, fingerprints) according to time point in BioNumerics. The fingerprints of each of the pooled samples were then depicted with Multi-Dimensional Scaling (MDS) in BioNumerics, which evaluated the similarity and dis-similarity of the bacterial profiles and assigned each sample a single point in space. This analysis is used to show the samples relation to each other in space and compare within and between treatments without complex banding patterns or dendrograms.

Bands for each sample were counted after positions were analyzed in BioNumerics. Band counts were compared by treatment group (Mare CTL: Mare SC, Foal CTL: Foal SC) and by production status (Mare CTL: Foal CTL, Mare SC: Foal SC) and then analyzed using PROC LOESS in SAS v. 9.3 (SAS Institute; Cary, NC.).

**Foal Growth Measurements and Statistical Analysis**

Body weight (BW) was measured in kg using a scale and was used to calculate average daily gain (ADG; kg/d). Wither height (WH) and hip height (HH) measurements were taken on foals standing square on a flat surface using a level and a tape measure
made of non-stretch material that was marked in centimeters (cm). Growth data were analyzed with PROC MIXED in SAS v. 9.3 (SAS Institute; Cary, NC.).
RESULTS

DNA Extraction and Primer Amplification

DNA was successfully extracted from all pooled fecal samples. PCR product specific to each bacterial primer set were successfully obtained using the extracted DNA for all pooled samples. *Saccharomyces cerevisiae* was not detected in any of the mare and foal fecal samples in either treatment group. An appropriate PCR product (amplicon) was obtained for the live yeast supplement with the SchafGC and Schar primers and was subsequently used as a positive control for the PCR reactions with the fecal samples.

Microbial Diversity in Mares

Total Bacteria

There were no differences in band counts representing total bacteria between mare treatment groups during late gestation and the first two weeks of lactation (Figure 1a). Mares that were supplemented with live yeast (SC) had an increased number of bands compared to CTL mares on d 30, 60, 90 and 120 (P < 0.05) of lactation suggesting increased bacterial diversity in the hindgut microflora of SC mares. Similarity coefficients averaged 55% throughout the study (Table 3). At d 120 of lactation, the similarity coefficient was 30%, indicating that the mares that received the live yeast
supplement (SC) had banding patterns that were approximately 70% different than the mares who did not receive the live yeast supplement (CTL).

The dendrogram and Multi-Dimensional Scaling (MDS) analyses of the total bacterial profiles showed clustering by treatment group (Figures 2a and 2b). The clusters of mares in the SC group were more closely related than those in the CTL group as shown by shorter connecting branches in the dendrogram and less space between points in MDS. This clustering indicates that the addition of live yeast to the mare’s diet appeared to reduce the variation of overall bacteria in the hindgut of Quarter Horse mares.

**Firmicutes Bacteria**

There were no differences in the number of bands observed in DGGE profiles between the mares in the SC group compared to CTL when using *Firmicutes* primers (Figure 1b). Similarity coefficients comparing the relatedness of bacterial profiles representing *Firmicutes*, which includes many fiber and starch-utilizing bacteria commonly found in horses, averaged 48% throughout the study (Table 4).

The dendrogram and MDS analyses of profiles representing bacteria found in the phylum *Firmicutes* revealed distinct clusters between treatment groups (Figures 3a and 3b). The clusters of the SC mares were more closely related as shown by shorter connecting branches in the dendrogram and less space between points in MDS, indicating that the addition of live yeast appeared to reduce the variation of bacteria in the hindgut of Quarter Horse mares.
*Streptococcus* Genera Bacteria

There were no differences observed in band counts between the mares in the CTL group compared to SC mares using *Streptococcus* primers (Figure 1c). Similarity coefficients of DGGE profiles of the *Streptococcus* genera bacteria, which includes predominant starch-utilizing bacteria found in the hindgut of horses, averaged 36% throughout the study (Table 5). At d -60, 1, 120 and 180, the similarity coefficients were 25%, 30%, 0% and 26%, respectively, meaning that the mares in the SC group had a banding pattern that was approximately 75%, 70%, 100% and 74% different than the mares in the CTL group.

The dendrogram and MDS of *Streptococcus* genera bacterial profiles, comparing the mares in the SC group and the mares in the CTL group showed clusters by treatment group (Figures 4a and 4b). Shorter connecting branches in the dendrogram and less space between points in MDS show that the clusters of SC mares were more closely related during late gestation and early lactation than CTL mares. This clustering indicates that supplementation of live yeast appeared to decrease the variation in the bacterial profiles during late gestation and early lactation.

**Microbial Diversity in Foals**

**Total Bacteria**

Foals whose dams were not supplemented (CTL) had an increased number of bands, representing total bacteria, compared to foals whose dams received a live yeast
supplement (SC) at d 90 post-parturition indicating increased bacterial diversity in the hindgut of foals (P = 0.048; Figure 5a). Similarity coefficients of DGGE profiles representing total bacteria averaged 50% throughout the study (Table 3). On d 0.5 and 240 of age, similarity coefficients were 23% and 36%, respectively. This suggests that on d 0.5, foals in the SC group had a 77% difference in bacterial diversity when compared to foals in the CTL group while there was a 64% difference in bacterial diversity between CTL and SC foals at d 240 of age.

The dendrogram and Multi-Dimensional Scaling (MDS) showed decreased variation in total bacteria within the first day of life in SC foals (Figures 6a and 6b). Clusters representing the first days of life in SC foals are more closely related than those of CTL foals as shown by shorter connecting branches in the dendrogram and less space between points in MDS. Differences between treatment groups within the early days of life were observed, indicating that maternal dietary yeast supplementation decreased variation within the total bacterial profile in the hindgut of Quarter Horse foals in the first days of life.

**Firmicutes Bacteria**

Foals in the CTL group had an increased number of bands compared to foals in the SC group on d 0.5 (P = 0.006) and d 1 (P = 0.049) indicating that SC foals had a decreased bacterial diversity within *Firmicutes* as compared to CTL foals (Fig. 5b). Similarity coefficients depicting the relatedness of profiles representing *Firmicutes* bacteria, which includes fiber and starch-utilizing bacteria found in the hindgut of horses, averaged 51% throughout the study (Table 4). On d 0.5 there was a similarity coefficient
of 7.4%, suggesting that on d 0.5, foals in the SC group had a 92.6% difference in bacterial diversity when compared to foals in the CTL group.

The dendrogram and MDS of *Firmicutes* bacterial profiles comparing the banding patterns of SC and CTL foals show differences between the first days of life compared to later time points (Figures 7a and 7b). MDS analysis of bacteria belonging to the phylum *Firmicutes* shows that the microbial profiles of SC foals are more closely related to each other than those of CTL foals within the first day of life. The relative distance of points in MDS indicates that maternal dietary yeast supplementation reduced the variation within many common fiber- and starch-utilizing bacteria in the hindgut of Quarter Horse foals (Figure 7b).

**Streptococcus Genera Bacteria**

There were no differences in the number of bands observed in DGGE profiles using *Streptococcus* genera primers between foals in the CTL group compared to SC foals (Figure 5c). Similarity coefficients of DGGE profiles of bacteria found in the *Streptococcus* genera, which includes many common starch-utilizing bacteria found in horses, averaged 28% throughout the study. On d 0, 0.5, 1, 14, 90, 180, 210 and 240 of age, the similarity coefficients were 0%, 30%, 16%, 29%, 27%, 26% and 0%, respectively, suggesting that on these days, foals in the SC group had a 100%, 70%, 84%, 71%, 73%, 74% and 100% difference in bacterial diversity when compared to foals in the CTL group.
The dendrogram and Multi-Dimensional Scaling (MDS) comparing the banding patterns representing starch-utilizing bacteria species commonly found in the hindgut of horses showed decreased variation within the first day of life for SC foals compared to CTL foals (Figures 8a and 8b). Shorter connecting branches in the dendrogram and less space between points in MDS support the observation that the microbial profiles of the first days of life in SC foals are more similar than those of CTL foals. Differences between treatment groups within the early days of life were also observed indicating that maternal dietary yeast supplementation altered the bacterial profile of the *Streptococcus* genera in the hindgut of Quarter Horse foals.

**Comparison of Microbial Diversity in Mares and Their Foals**

**Total Bacteria**

The number of bands representing total bacteria from pooled mare fecal samples remained relatively constant throughout the study, regardless of treatment. Mares had increased band counts compared to foals within the first weeks of life ($P < 0.05$; Figure 9a and 13a). The differences observed in band counts lessened with the age of the foal regardless of treatment, indicating that the microbial profile of the hindgut diversifies as the foal matures.

The dendrogram and Multi-Dimensional Scaling (MDS) comparing the banding patterns representing total bacteria of the mares and their offspring showed greater variation within overall bacterial profiles of foals early in life but decreased in variation
as the foal aged (Figures 10a, 10b, 14a and 14b). Clusters within the foals were less closely related, represented by longer connecting branches in the dendrogram and more space between points in MDS, at early time points but became shorter and closer as the foal aged indicating that the total bacterial profile in the foal establishes rapidly after birth and the variation within the microbial populations decrease as the foal ages.

**Firmicutes Bacteria**

The number of bands representing bacteria from the phylum *Firmicutes* from pooled mare fecal samples remained relatively constant throughout the study, regardless of treatment. Microbial diversity of bacterial profiles of *Firmicutes* detected in fecal samples, as represented through band counts, increased with the age of the foal until d 0.5 (P < 0.01; Figures 9b and 13b). Similarity coefficients values increased with the age of the foal regardless of treatment, starting at d 0 with a 0% similarity (Table 4).

The dendrogram and MDS comparing bacterial profiles within the phylum *Firmicutes* for the mares and their foals showed greater variation in foals within the first 24 h after birth (Figures 11a, 11b, 15a and 15b). Clusters representing foal microbial profiles within the first day of life were less closely related, represented by longer connecting branches in the dendrogram and more space between points in MDS, but became shorter and closer as the foal aged. This indicates that the bacteria found in the phylum *Firmicutes* decrease in variation as the foal matures.
**Streptococcus Genera Bacteria**

Mares had increased band counts, representing bacteria in the *Streptococcus* genera, compared to their foals within the first 24 h of life, regardless of treatment (P < 0.01; Figures 9b and 13b). Similarity coefficients values increased with the age of the foal regardless of treatment, starting at d 0 with a 5.3% similarity coefficient (Table 5).

The dendrogram and Multi-Dimensional Scaling (MDS) comparing the banding patterns representing the bacterial profiles of the *Streptococcus* genera of mares and their offspring showed greater variation within foal bacterial profiles early in life but decreased in variation as the foal aged regardless of treatment (Figures 12a, 12b, 16a and 16b). Clusters within foals were less closely related, represented by longer connecting branches in the dendrogram and more space between points in MDS, at early time points but became shorter and closer as the foal matured, indicating that the bacterial populations of the *Streptococcus* genera establish rapidly in the foal after birth and the variation within the microbial populations decrease as the foal ages.

**Foal Growth**

Maternal dietary yeast supplementation did not influence body weight (Figure 17a), average daily gain (Figure 17b), wither height (Figure 18a) or hip height (Figure 18b) at any time point throughout the study. Body weight of foals in the CTL group averaged 45.7 ± 7.9 kg at birth compared to 48.5 ± 7.9 kg for foals in the SC group. Yeast supplementation of the mares ceased at d 90 at which time body weight of CTL
foals averaged 156.6 ± 7.7 kg compared to an average of 156.8 ± 7.7 kg for SC foals. At weaning, the body weight of CTL foals averaged 257.5 ± 10.0 kg while SC foals averaged 249.9 ± 10.0 kg. At birth, wither heights of CTL foals averaged 94.2 ± 3.1 cm whereas wither heights of the SC foals averaged 98.7 ± 3.1 cm. When the yeast supplementation of the mares was stopped at d 90, CTL foals’ wither height averaged 114.4 ± 2.2 cm while SC foals averaged 117.8 ± 2.2 cm. At weaning, wither heights of CTL foals averaged 127.6 ± 2.2 cm compared to an average of 131.9 ± 2.2 cm for SC foals. CTL foals’ hip heights averaged 97.2 ± 3.1 cm at birth while SC foals averaged 101.4 ± 3.1 cm. At d 90, CTL foals averaged 120.0 ± 2.5 cm in hip height compared to an average of 123.4 ± 2.5 cm in SC foals. At weaning, the hip heights of CTL foals averaged 133.3 ± 1.8 cm while SC foals averaged 135.5 ± 1.8 cm.
DISCUSSION

Maternal diet has been shown to influence foal growth and development (Glade, 1991; Faubladier et al., 2013). However, the addition of a live yeast supplement to the diet of mares during late gestation and early lactation did not affect foal growth in the present study. Although Glade (1991) observed an increase in ADG within the first month of life in foals born from mares fed Saccharomyces cerevisiae, the dosage used in the study was approximately double that which was used in the current study. Previous research evaluating the use of probiotics in horses has suggested a dose dependent response which may explain the increase in ADG observed in the Glade (1991) study (Weese et al., 2003).

Supplementation of the mares’ diet with live yeast appeared to decrease the variation in microbial profiles in pooled fecal samples representative of total bacteria, including fiber- and starch-utilizing bacteria, in the hindgut of Quarter Horse mares. Dendrogram and MDS clusters suggest that yeast added to a horse’s diet may buffer changes in the microbial ecosystem of the hindgut. Medina and colleagues (2002) reported that yeast supplementation can alter concentrations of Lactobacillus spp. and Streptococcus ssp. in the hindgut of horses. In addition, yeast has been shown to
positively affect the hindgut by reducing the changes in pH and lactic acid levels in the hindgut after feeding (Goodson et al., 1988; de Fombelle et al., 2001; Medina et al., 2002). Lactic acid levels and pH were not measured in the present study.

Starting on d 14, mares were turned out to pasture in increasing increments of time until d 28 when all mares were allowed on pasture ad libitum. Fecal sampling on d 30 to d 120 revealed that mares in the SC group had increased band counts, representative of total bacteria, while a decrease in band counts was observed for mares in the CTL group. These changes in band counts suggest shifts in hindgut microbial populations due to the change in forage type from hay to pasture. The laboratory feed analysis (Table 1) showed increased crude protein and decreased crude fat, ADF and NDF in pasture samples compared to hay. This change in diet could have influenced the diversity and ratio of microbes in the gastrointestinal tract (Clarke et al., 1990; Medina et al., 2002).

PCR/DGGE of meconium samples revealed multiple bands which indicate that bacteria were present in the hindgut of foals in utero. These findings are supported by previous studies in which bacteria were detected in meconium samples of foals when PCR-DGGE and ARISA fingerprinting methods were used (Kuhl et al., 2011; Earing et al., 2012; Faubladier et al., 2014). This contradicts other research that did not detect bacteria in meconium samples leading to the conclusion that the foal’s gastrointestinal tract is sterile at birth (Faubladier et al., 2013). A possible explanation for the conflicting data may be the method used to collect the fecal samples. In the current study, samples were carefully collected immediately after defecation but may have been exposed to environmental bacteria before being placed into sterile tubes.
An increase in band counts representing bacteria in the phylum *Firmicutes* obtained from pooled fecal samples was observed in the first 14 d after birth for foals in both treatment groups. This suggests a rapid establishment of fiber- and starch-utilizing bacteria in the hindgut of foals. SC foals showed decreased band counts compared to CTL foals within the first 24 h, indicating that supplementation of the maternal diet with live yeast decreased the diversity observed in the microbial profiles in the hindgut of their offspring. In a similar study in which mares were supplemented with probiotics during late gestation and early lactation, foals born from supplemented mares had greater establishment of total anaerobes and lactate utilizers at an earlier time than the foals whose dams were not supplemented (Faubladier *et al.*, 2013).

Foals had fewer band counts compared to the mares for the first 24 h after birth. Depending on the type of bacteria analyzed, the number of bands observed in foal fecal samples increased to numbers comparable to that of the mares by 30 d after birth. This increase in diversity of hindgut microflora as the foal ages has also been reported in other studies (Favier *et al.*, 2002; Kuhl *et al.*, 2011; Earing *et al.*, 2012; Faubladier *et al.*, 2013; Faubladier *et al.*, 2014).

Overall, the addition of live yeast to the mare’s diet appeared to limit the variation in microbial profiles of their hindgut suggesting that *Saccharomyces cerevisiae* may buffer shifts in the microbial ecosystem. The addition of *Saccharomyces cerevisiae* to the mare’s diet during late gestation and early lactation also appeared to limit the variation within the microbial profiles in the hindgut of their offspring within the first 24 h post-partum. Therefore, it may be possible to optimize the colonization of microflora
in the hindgut of foals during the first days of life in order to reduce or prevent undesirable changes to the gastrointestinal microflora and contribute to the overall health of the foal.
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Appendix A

Tables
**Table 1.** Chemical composition of dietary components received by mares and foals on a dry matter (DM) basis obtained from laboratory analyses performed at a commercial feed testing facility (Dairy One, Ithaca, NY).

<table>
<thead>
<tr>
<th></th>
<th>Concentrate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Concentrate&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Hay – parturition&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Hay (pre-weaning)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Hay (post-weaning)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Pasture&lt;sup&gt;f&lt;/sup&gt;</th>
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<tr>
<td><strong>DE (Mcal/kg)</strong></td>
<td>3.22</td>
<td>3.09</td>
<td>2.01</td>
<td>1.93</td>
<td>1.84</td>
<td>1.93</td>
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<tr>
<td><strong>Nutrients (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>19.3</td>
<td>18.6</td>
<td>11.4</td>
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<td>15.7</td>
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<tr>
<td>ADF</td>
<td>13.8</td>
<td>13</td>
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<td>43.2</td>
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</tr>
<tr>
<td>NDF</td>
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<td>Starch</td>
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<td>0.2</td>
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<td>0.2</td>
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<tr>
<td>Crude Fat</td>
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<tr>
<td>Ash</td>
<td>7.53</td>
<td>7.55</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Concentrate consisted of a commercially available horse pelleted concentrate fed to mares and foals until approximately d 140 post-parturition (Buckeye Nutrition, Dalton, OH).

<sup>b</sup> Concentrate consisted of a commercially available horse pelleted concentrate fed to mares and foals after approximately d 140 post-parturition (Nutrena, Minneapolis, MN).

<sup>c</sup> Hay consisted of mixed grasses that was fed while the mares were housed inside for foaling.

<sup>d</sup> Hay consisted of mixed grasses that was available *ad libitum* to mares and foals prior to weaning along with access to pasture.

<sup>e</sup> Hay consisted of mixed grasses that was available *ad libitum* to mares and foals after weaning along with access to pasture.

<sup>f</sup> Pasture consisted of mixed grasses that was available *ad libitum* to mares and foals after approximately d 28 along with access to mixed grass hay.
Table 2. Primer sequences used for PCR-DGGE. GC clamp utilized for DGGE is included in bold along with references for each primer.

<table>
<thead>
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<th>Primer</th>
<th>Primer Sequence</th>
<th>Target</th>
<th>Reference Paper</th>
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<td>HDA1</td>
<td>(5'-3'): CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG -- GAC TCC TAC GGG AGG CAG CAG T</td>
<td>Universal</td>
<td>Walter et al., 2000</td>
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<tr>
<td>HDA2</td>
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<td>Vanhoutte et al., 2006; Manzano et al., 2004</td>
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<td>FirmFGC</td>
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<td>FibRGC</td>
<td>(5'-3'): ACC ATG CAC CAC CTG TC</td>
<td>Firmicutes</td>
<td>Bacchetti De Gregoris et al., 2011</td>
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Table 3. Similarity coefficients (%) calculated based on the similarity of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with universal primers (HDA1, HDA2), comparing within treatment and within mare-foal pairs. Mares were fed a control diet (CTL) or a diet supplemented with a live yeast (SC) in a randomized design. Fecal samples were taken on mares from d 250 of gestation to weaning and from foals at birth to 60d post-weaning. Day 0 represents parturition.

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Table 4. Similarity coefficients (%) calculated based on the similarity of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with *Firmicutes* primers (FIRMFGC, FIRMR), comparing within treatment and within mare-foal pairs. Mares were fed a control diet (CTL) or a diet supplemented with a live yeast (SC) in a randomized design. Fecal samples were taken on mares from d 250 of gestation to weaning and from foals at birth to 60d post-weaning. Day 0 represents parturition.

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<table>
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Appendix B

Figures
Figure 1. Influence of live yeast supplementation on diversity of gastrointestinal microflora of eight Quarter Horse mares as shown through band counts. Mares were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. DGGE images, depicting microbial profiles from pooled fecal samples, were analyzed with BioNumerics software (Applied Maths, Austin, TX) to generate band counts. Band counts obtained with a) universal primers (HDA1, HDA2), b) *Firmicutes* primers (FIRMFGC, FIRMR) and c) *Streptococcus* primers (LAC3F, LAC2RGC). Day 0 represents parturition. Band counts were analyzed with PROC LOESS of SAS v. 9.3. Significant differences in the number of bands are represented by * (P < 0.05) and ** (P < 0.01).
Figure 2. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with universal primers (HDA1, HDA2), of the gastrointestinal microflora of eight Quarter Horse mares. Mares were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Fecal samples were collected on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition for mares. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of total bacteria. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. CTL mares are represented by lighter spheres (●) and SC mares are represented by darker spheres (◆).
Figure 3. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with *Firmicutes* primers (FIRMFGC, FIRMR), of the gastrointestinal microflora of eight Quarter Horse mares. Mares were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Fecal samples were collected on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition for mares. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of bacteria found in the phylum *Firmicutes*. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. CTL mares are represented by lighter spheres (●) and SC mares are represented by darker spheres (●).
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Figure 5. Influence of maternal dietary live yeast supplementation on diversity of gastrointestinal microflora of eight Quarter Horse foals as shown through band counts. Mares were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. DGGE images, depicting microbial profiles from pooled fecal samples, were analyzed with BioNumerics software (Applied Maths, Austin, TX) to generate band counts. Band counts obtained with a) universal primers (HDA1, HDA2), b) Firmicutes primers (FIRMFGC, FIRMR) and c) Streptococcus primers (LAC3F, LAC2RGC). Day 0 represents parturition. Band counts were analyzed with PROC LOESS of SAS v. 9.3. Significant differences in the number of bands are represented by * (P < 0.05) and ** (P < 0.01).
Figure 6. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with universal primers (HDA1, HDA2), of the gastrointestinal microflora of eight Quarter Horse foals whose dams were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Samples were collected on day of birth (d 0), 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 post-parturition for foals. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of total bacteria. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. CTL foals are represented by lighter spheres ( ) and SC foals are represented by darker spheres ( ).
Figure 7. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with *Firmicutes* primers (FIRMFGC, FIRMR), of the gastrointestinal microflora of eight Quarter Horse foals whose dams were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Samples were collected on day of birth (d 0), 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 post-parturition for foals. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of bacteria found in the phylum *Firmicutes*. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. CTL foals are represented by lighter spheres ( ) and SC foals are represented by darker spheres ( ).
Figure 8. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with *Streptococcus* primers (LAC3F, LAC2RGC), of the gastrointestinal microflora of eight Quarter Horse foals whose dams were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Samples were collected on day of birth (d 0), 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 post-parturition for foals. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of bacteria found in the *Streptococcus* genera. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. CTL foals are represented by lighter spheres (○) and SC foals are represented by darker spheres (●).
Figure 9. Influence of production status on diversity of gastrointestinal microflora of four Quarter Horse mares and their offspring as shown through band counts. Mares were fed a basal diet without the addition of a live yeast supplement (CTL). DGGE images, depicting microbial profiles from pooled fecal samples, were analyzed with BioNumerics software (Applied Maths, Austin, TX) to generate band counts. Band counts obtained with a) universal primers (HDA1, HDA2), b) Firmicutes primers (FIRMFGC, FIRMR) and c) Streptococcus primers (LAC3F, LAC2RGC). Day 0 represents parturition. Band counts were analyzed with PROC LOESS of SAS v. 9.3. Significant differences in the number of bands are represented by * (P < 0.05) and ** (P < 0.01).
Figure 10. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with universal primers (HDA1, HDA2), of the gastrointestinal microflora of four Quarter Horse mares, that were fed a basal diet without the addition of a live yeast supplement (CTL), and their offspring. Mare fecal samples were collected on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition for mares. Foal fecal samples were collected on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of total bacteria. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. Mares (M) are represented by lighter spheres (○) and foals (F) are represented by darker spheres (●).
Figure 11. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with *Firmicutes* primers (FIRMFGC, FIRMR), of the gastrointestinal microflora of four Quarter Horse mares, that were fed a basal diet without the addition of a live yeast supplement (CTL), and their offspring. Mare fecal samples were collected on day -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition for mares. Foul fecal samples were collected on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of bacteria in the phylum *Firmicutes*. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. Mares (M) are represented by lighter spheres (●) and foals (F) are represented by darker spheres (●).
Figure 12. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with *Streptococcus* primers (LAC3F, LAC2RGC), of the gastrointestinal microflora of four Quarter Horse mares, that were fed a basal diet without the addition of a live yeast supplement (CTL), and their offspring. Mare fecal samples were collected on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition for mares. Foal fecal samples were collected on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of bacteria found in the *Streptococcus* genera. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. Mares (M) are represented by lighter spheres ( ) and foals (F) are represented by darker spheres ( ).
Figure 13. Influence of production status on diversity of gastrointestinal microflora of four Quarter Horse mares and their offspring as shown through band counts. Mares received a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. DGGE images, depicting microbial profiles from pooled fecal samples, were analyzed with BioNumerics software (Applied Maths, Austin, TX) to generate band counts. Band counts obtained with a) universal primers (HDA1, HDA2), b) Firmicutes primers (FIRMFGC, FIRMR) and c) Streptococcus primers (LAC3F, LAC2RG). Day 0 represents parturition. Band counts were analyzed with PROC LOESS of SAS v. 9.3. Significant differences in the number of bands are represented by * (P < 0.05) and ** (P < 0.01).
Figure 14. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with universal primers (HDA1, HDA2), of the gastrointestinal microflora of four Quarter Horse mares, that received a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition, and their offspring. Mare fecal samples were collected on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition for mares. Foal fecal samples were collected on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of total bacteria. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. Mares (M) are represented by lighter spheres (■) and foals (F) are represented by darker spheres (●).
Figure 15. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with *Firmicutes* primers (FIRMFGC, FIRMR), of the gastrointestinal microflora of four Quarter Horse mares, that received a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition, and their offspring. Mare fecal samples were collected on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition for mares. Foal fecal samples were collected on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of bacteria found in the phylum *Firmicutes*. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. Mares (M) are represented by lighter spheres ( ) and foals (F) are represented by darker spheres ( ).
Figure 16. Relatedness of PCR-denaturing gradient gel electrophoresis (DGGE) profiles of pooled fecal samples, generated with *Streptococcus* primers (LAC3F, LAC2RGC), of the gastrointestinal microflora of four Quarter Horse mares, that received a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition, and their offspring. Mare fecal samples were collected on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition for mares. Foal fecal samples were collected on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. The dendrogram (a) was generated based on a distance matrix calculated by the Jaccard coefficient and pooled (n = 2) PCR product for each treatment and day. The number and positions of the bands representing each sample (lane) visually depict the diversity of bacteria in the *Streptococcus* genera. MDS (b) was generated from the DGGE cluster analysis similarity matrix and reduces the complex DGGE profiles into one point in space. Mares (M) are represented by lighter spheres ( ) and foals (F) are represented by darker spheres ( )
Figure 17. Influence of maternal dietary live yeast supplementation on (a) body weight and (b) average daily gain (ADG) of eight Quarter Horse foals. Mares were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Body weight and ADG were analyzed in PROC MIXED of SAS v. 9.3. Measurements were taken at 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. Significant differences in the number of bands are represented by * (P < 0.05).
Figure 18. Influence of maternal dietary live yeast supplementation on (a) wither height and (b) hip height of eight Quarter Horse foals. Mares were fed a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Wither height and hip height were analyzed in PROC MIXED of SAS v. 9.3. Measurements were taken at 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. Significant differences in the number of bands are represented by * (P < 0.05).
Appendix C

Supporting Figures
Figure 19. PCR (Polymerase Chain Reaction) analysis of the gastrointestinal microflora of Quarter Horse mares that received a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition and their offspring. Analysis of the 18S rRNA gene of fecal samples is represented with SchafGC and Schar Saccharomyces cerevisiae primer amplification on a 1% agarose gel. Lane 1 is a 100 base pair ladder. Lanes 2 through 4 represent SC mares d -90, 60 and 1 with Lanes 5 through 7 representing SC foals, d 0.5, 1, and 60. Lanes 9 and 10 represent CTL mares d -30 and 0.5 while Lanes 11 to 13 represent CTL foals d 0, 1, and 30. Lanes 8, 14, and 16 have no samples and Lane 15 represents the dietary yeast supplement fed to the mares. Day 0 represents parturition and d -90, -60 and -30 represent d 250, 280 and 310 of gestation, respectively.
Figure 20. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of eight Quarter Horse mares that received a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Fecal samples were taken on mares from d 250 of gestation to weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with HDA1 and HDA2 universal primer amplification on an 8% polyacrylamide gel. Lane 1, 16 and 30 are 100 base pair ladders. Lanes 2 has no sample while Lanes 3 through 15 represent CTL mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition. Lanes 17 through 29 represent SC mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition.
Figure 21. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of eight Quarter Horse mares that received a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Fecal samples were taken on mares from d 250 of gestation to weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with *Firmicutes* primer (FIRMFGC, FIRMR) amplification on an 8% polyacrylamide gel. Lane 1, 15 and 30 are 100 base pair ladders. Lane 29 has no sample. Lanes 2 has no sample while Lanes 2 through 14 represent CTL mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition. Lanes 16 through 28 represent SC mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition.
Figure 22. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of eight Quarter Horse mares that received a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Fecal samples were taken on mares from d 250 of gestation to weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with LAC3F and LAC2RGC Streptococcus primer amplification on an 8% polyacrylamide gel. Lane 1, 2 and 16 are 100 base pair ladders. Lanes 3 through 15 represent CTL mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition. Lanes 17 through 29 represent SC mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition.
Figure 23. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of eight Quarter Horse foals whose dams received a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Fecal samples were taken from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with HDA1 and HDA2 universal primer amplification on an 8% polyacrylamide gel. Lane 1, 15 and 28 are 100 base pair ladders. Lanes 2 has no sample while Lanes 3 through 14 represent CTL foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. Lanes 16 through 27 represent SC foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.
Figure 24. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of eight Quarter Horse foals whose dams received a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Fecal samples were taken from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with *Firmicutes* primer (FIRMFGC, FIRMR) amplification on an 8% polyacrylamide gel. Lane 1, 14 and 28 are 100 base pair ladders. Lanes 27 has no sample while Lanes 2 through 13 represent CTL foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. Lanes 15 through 26 represent SC foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.
Figure 25. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of eight Quarter Horse foals whose dams received a basal diet (CTL) or a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition. Fecal samples were taken from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with LAC3F and LAC2RGC *Streptococcus* primer amplification on an 8% polyacrylamide gel. Lane 1, 15 and 28 are 100 base pair ladders. Lanes 2 has no sample while Lanes 3 through 14 represent CTL foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition. Lanes 16 through 27 represent SC foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.
Figure 26. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of four Quarter Horse mares that received a basal diet (CTL) and their offspring. Fecal samples were taken on mares from d 250 of gestation to weaning and from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with HDA1 and HDA2 universal primer amplification on an 8% polyacrylamide gel. Lane 1, 17 and 30 are 100 base pair ladders. Lanes 2 through 16 represent CTL mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition with Lane 14 and 15 having no samples. Lanes 18 to 29 represent CTL foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.
Figure 27. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of four Quarter Horse mares that received a basal diet (CTL) and their offspring. Fecal samples were taken on mares from d 250 of gestation to weaning and from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with *Firmicutes* primer (FIRMFGC, FIRMR) amplification on an 8% polyacrylamide gel. Lane 1, 15 and 28 are 100 base pair ladders. Lanes 2 through 14 represent CTL mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition. Lanes 16 to 27 represent CTL foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.
Figure 28. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of four Quarter Horse mares that received a basal diet (CTL) and their offspring. Fecal samples were taken on mares from d 250 of gestation to weaning and from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with LAC3F and LAC2RGC Streptococcus primer amplification on an 8% polyacrylamide gel. Lane 1, 15 and 28 are 100 base pair ladders. Lanes 2 through 14 represent CTL mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition. Lanes 16 to 27 represent CTL foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.
Figure 29. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of four Quarter Horse mares that received a basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition and their offspring. Fecal samples were taken on mares from d 250 of gestation to weaning and from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with HDA1 and HDA2 universal primer amplification on an 8% polyacrylamide gel. Lane 1, 15 and 28 are 100 base pair ladders. Lanes 2 through 14 represent SC mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition. Lanes 16 to 27 represent SC foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.
Figure 30. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of four Quarter Horse mares that received basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition and their offspring. Fecal samples were taken on mares from d 250 of gestation to weaning and from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with *Firmicutes* primer (FIRMFGC, FIRMR) amplification on an 8% polyacrylamide gel. Lane 1, 15 and 28 are 100 base pair ladders. Lanes 2 through 14 represent SC mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition. Lanes 16 to 27 represent SC foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.
Figure 31. DGGE (Denaturing Gradient Gel Electrophoresis) banding pattern of the gastrointestinal microflora of four Quarter Horse mares that received basal diet supplemented with a live yeast (SC) from d 250 of gestation to 90 d (+/- 15 d) post-parturition and their offspring. Fecal samples were taken on mares from d 250 of gestation to weaning and from foals at birth to 60d post-weaning. Analysis of V2-V3 region of the 16S rRNA gene of pooled fecal samples is represented with LAC3F and LAC2RGC *Streptococcus* primer amplification on an 8% polyacrylamide gel. Lane 1, 16 and 29 are 100 base pair ladders while lane 2 has no sample in it. Lanes 3 through 15 represent SC mares on d -90, -60 and -30 of gestation, day of foaling (d 0), d 0.5, 1, 14, 30, 60, 90, 120, 150 and 180 post-parturition. Lanes 17 to 28 represent SC foals on day of birth (d 0) and 0.5, 1, 14, 30, 60, 90, 120, 150, 180, 210 and 240 d post-parturition.