EFFECT OF MANNAN OLIGOSACCHARIDE (MOS) SUPPLEMENTATION ON THE IMMUNE STATUS OF MARES AND THEIR FOALS

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This thesis is dedicated to my parents, Steve and Charlotte Robertson, who have continuously provided me with unconditional love and support for the past 26 years.

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Newborn foals are susceptible to many pathogens that can cause health problems such as diarrhea, sepsis, and even death. The foal obtains the antibodies necessary to combat the onslaught of these pathogens from the mare's colostrum when it is ingested within the first 24 hours of life.

Previous research in other species suggests that mannan oligosaccharide (MOS) supplementation to the diet has positive effects on immune function, including increased serum and colostrum immunoglobulin levels. An experiment was designed to identify the effects of MOS supplementation to the diet on colostrum and serum immunoglobulin concentrations in the pregnant mare and serum immunoglobulin concentration in her foal. Twenty-six pregnant Thoroughbred (n=21) and Quarter Horse (n=5) mares were paired by expected foaling dates and assigned at random to the treatment or control group. Treatment mares received 10 g of MOS mixed in 45 g of ground corn in the

morning ration. Control mares received 55 g of ground corn in the morning ration. All mares were fed a concentrate designed to provide NRC recommended or higher nutrient intake when fed with Coastal bermudagrass hay or bahiagrass pasture *ad libitum* in season. Both treatments began 56 days before the expected foaling date (Day -56) for each mare and continued through 28 days post-parturition (Day +28). The IgG, IgM, and IgA values were determined on mare serum at Days -56, 0, and +28. The IgG, IgM, and IgA values were determined on colostrum collected before the foal had nursed. IgG, IgM and IgA values were determined on foal serum collected at 0 hour (before foals had nursed), 6 to 10 hours post-parturition, and at Day +7, +14, +28, +56, and +112 of age.

The mares receiving MOS supplementation had higher colostrum IgA ($p=0.008$) and IgG ($p=0.033$); and tended to have higher IgM ($p=0.076$) concentrations when controlled for prelactation colostrum loss, age, and breed. Prelactation adversely affected colostrum IgG ($p=0.006$) and IgA ($p=0.008$) immunoglobulin concentration, but had no effect on IgM concentration. There were no significant differences between treatments for mare IgG, IgM, and IgA serum levels at any collection period. Foals from control mares tended to have higher serum IgA concentration at 6 to 10 hours post-parturition than did foals from mares fed MOS ($p=0.09$). There were no other significant differences in foal serum immunoglobulin concentrations at any collection period. This trial suggests that MOS supplementation to pregnant mares increases colostrum immunoglobulin content.

CHAPTER 1
INTRODUCTION

Suckling foals are susceptible to many pathogens that cause various health problems such as diarrhea, enteritis, septicemia, and even death. These problems can result in major veterinary expenses and financial loss for horse breeding operations. The diarrhea that is associated with foal heat occurs in foals 7 to 12 days after birth and is considered the most common cause of diarrhea in young foals (Cohen 1997). This generally causes minimal stress for the foal and can resolve itself with little to no medical treatment. The diarrhea that occurs just after birth or later during lactation is often pathological in nature, and is a major health concern, because it can result in severe dehydration, reduced growth, and even death. Many organisms have been indicated in the development of diarrhea, including *Clostridium perfringens* (East *et al.* 2000), *Clostridium difficile* (Jones *et al.* 1988)*, Salmonella typhimurium and other Salmonella spp.* (Spier 1993)*,* and *rotavirus* (Dwyer 1993).

The foal obtains the antibodies necessary to combat the onslaught of these pathogens from the mare's colostrum when it is ingested within the first 24 hours of life (Jeffcott 1974). The immunoglobulin found in the greatest quantity in mare colostrum is IgG, followed by IgA and IgM (McGuire *et al.* 1973). Studies have shown that colostral IgG concentration is highly correlated with foal serum IgG concentration 18 hours after birth (LeBlanc *et al.* 1992). Failure of the mare to provide the foal with adequate antibodies via the colostrum may necessitate

the administration of supplemental colostrum or plasma to the foal shortly after birth.

When included as a supplement to the diet, mannan oligosaccharides (MOS) have been shown to have a positive effect on immune response in several species. Mannan oligosaccharides are indigestible complex polysaccharide molecules derived from yeast cell walls. Mannan oligosaccharides are commercially available as BioMos®, a nutritional supplement manufactured by Alltech, Inc. (Nicholasville, KY). Supplemental MOS in poultry diets increased both plasma IgG and bile IgA (Savage *et al.* 1996). In dogs supplemented with MOS, total lymphocyte count was increased, and serum IgA concentrations tended to be greater (Swanson *et al.* 2002). Mannan oligosaccharide supplementation increased serum IgM and tended to increase colostral IgG levels in sows (Newman 2001). In addition to the positive immune response elicited from MOS, they also serve as alternate attachment sites in the gut for gram-negative pathogenic organisms with mannose-specific type-1 fimbriae that adhere to intestinal epithelial cells to initiate disease (Ferket *et al.* 2002). These pathogens will bind to MOS present in the intestinal tract and pass through the gut, instead of attaching to host epithelial cells. Previous studies have demonstrated that MOS reduces *in vitro* attachment of *Salmonella typhimurium* to cultured intestinal cells (Oyofo *et al.* 1989) and decreases fecal concentrations of *Clostridium perfringens* in poultry (Finuance *et al.* 1999). Other *in vitro* studies have demonstrated agglutination of *Escherichia coli, Salmonella typhimurium, and S. enteritidis* in the presence of MOS (Spring *et al.* 2000).

There has recently been an increased emphasis on the reduction of antibiotic use in production diets because of the associated potential negative environmental and health issues. The swine, poultry, and cattle industries are interested in supplemental MOS because they may serve as a viable alternative to antibiotic use in ration formulation. Most of the previous research involving MOS has investigated the positive performance benefits seen with MOS addition to production diets. Studies have demonstrated that the addition of MOS to the diet results in increased average daily gain (Hooge 2003), increased gain-to-feed ratio (Davis *et al.* 2002), and heavier litter birth and weaning weights (O'Quinn *et al.* 2001). The immune response elicited by MOS supplementation in swine, poultry, and cattle has only recently begun to be investigated. To the author's knowledge, there have been no previous equine studies involving MOS supplementation.

Results obtained in previous research with other species suggest that MOS supplementation to the diet of the pregnant mare may increase the immunoglobulin content in her colostrum and protect her from colonization of pathogenic organisms in the gut. Greater immunoglobulin content in the colostrum will result in more protection for the foal from disease initiated by pathogenic organisms. The reduced occurrence of diarrhea and other problems caused by these organisms in suckling foals would result in healthier foals and decreased financial loss due to veterinary expenses for horse breeding operations.

CHAPTER 2
REVIEW OF LITERATURE

The Immune System

The immune system of the horse is a versatile defense mechanism that provides protection from a daily onslaught of pathogenic organisms. The body must be prepared to combat this invasion with an arsenal of cells capable of recognizing and eliminating these foreign microbes. The immunoglobulins are a group of molecules exhibiting this property, through their ability to effectively recognize and bind foreign antigen. These large glycoprotein molecules are present on B-cell membranes, and are also secreted by plasma cells. They are found throughout the body in the blood, mucosal tissues, and external secretions. Immunoglobulins synthesized by the pregnant mare will affect the survivability of her foal, because the foal relies on passive transfer to provide the major source of antibodies for a period of at least 1 month after birth (McGuire and Crawford 1973). After that, the foal's own immune system is able to begin producing immunoglobulins in a quantity that can mount an immunologic response that will provide protection from pathogenic organisms.

Immunoglobulins. The immunoglobulins are a large group of glycoprotein molecules found in the serum of the blood and other body fluids. They are part of the fraction of serum proteins termed the "globulins" and play an integral role in the immune response (Peakman and Vergani 1997). An antibody is an immunoglobulin (Ig) that exhibits antigen-binding ability. Therefore all

antibodies are Igs but not all Igs are antibodies. However, the two terms are commonly used interchangeably. The functions of antibodies include targeting foreign molecules, recruitment of effector responses, neutralization of toxins, and binding and removal of foreign antigens. Antibodies also serve as useful diagnostic tools. For example, to determine whether successful passive transfer of maternal antibodies in newborn foals has occurred, the IgG concentration in the foal's serum can be measured. The four major equine Ig isotypes are IgG, IgM, IgA, and IgE (Nezlin 1998). The average Ig concentrations found in the serum of mature horses are presented in Table 2-1.

Table 2-1. Immunoglobulin concentration in serum of mature horses

	IgG	IgA	IgM
Concentration (mg/dL)	1000 to 1500	60 to 350	100 to 200

Adapted from Tizard 1996: *Veterinary Immunology: An Introduction*, p. 155 Table 13-2. W.B. Saunders Co., Philadelphia.

IgG. IgG is the most abundant Ig found in the serum and in the colostrum. It is made and secreted by plasma cells found in the spleen, lymph nodes, and bone marrow (Tizard 1996). Plasma cells are the antibody-secreting cells that are differentiated from B lymphocytes (B-cells). IgG is the smallest of the Ig classes, therefore it is easily able to migrate from the blood into other tissues. IgG readily binds foreign antigen it comes into contact with. This leads to agglutination and opsonization, the process that makes foreign particles susceptible to phagocytosis by neutrophils. IgG antibodies also play a role in activating the complement system, a complex enzymatic pathway resulting in the ultimate destruction of invading microorganisms. There are five subclasses of equine IgG, which are IgG2a, IgG2b, IgG2c, IgG(B), and IgG(T), which are also

divided into two subclasses, IgG(T)a and IgG(T)b (Tizard 1996). These IgG subclasses are distinguished by their different γ-chain sequences and slight differences in biological function (Goldsby *et al.* 2003).

IgM. IgM is the second most abundant Ig found in the serum and the third most abundant in colostrum. IgM is the first class of Ig detected in a primary immune response and the first Ig produced by the neonate (Goldsby *et al.* 2003). The secreted form of IgM is the largest of the Igs and also has more antigen binding sites than the other isotypes. Because of its high affinity for antigen, IgM is more efficient than IgG at causing agglutination, neutralizing virus particles, and activating complement. The larger size of IgM restricts its ability to diffuse from the blood to other tissues. Through specialized binding sites, secretory cells in the respiratory and gastrointestinal tract are able to transport IgM molecules across mucosal linings. Once released into the intestinal lumen, they play an important accessory role to IgA, the most prevalent antibody found in mucosal secretions.

IgA. IgA is the third most abundant serum Ig and second most abundant colostrum Ig. However, as production shifts from colostrum to milk production, IgA becomes the predominant antibody found milk. IgA present in colostrum, milk, and other external secretions, including gastrointestinal tract secretions, primarily exists in the form of secretory IgA. Secretory IgA is different from the circulating monomeric form of IgA in serum. It is a complex molecule made up of the dimeric form of IgA attached to a glycoprotein chain called secretory component. Secretory component mediates the transport of IgA across mucosal

epithelium surfaces and provides protection from degradation by proteases that are abundant in the mucosal environment. The primary function of IgA is to prevent attachment of antigens to body surfaces. IgA can also serve as an opsonin and activate the complement system, although not as efficiently as IgG.

Mucosal immunity. The majority of IgA is produced by plasma cells in mucosal lymphoid tissues, which are located underneath the respiratory and gastrointestinal epithelium. The daily production of secretory IgA is greater than that of any other Ig isotype, mainly because of the sheer size of the intestine (Abbas *et al.* 2000). Most invasions by pathogenic organisms occur through ingestion or inhalation. In the intestine, secretory IgA binds to pathogenic organisms and provides protection by preventing their attachment to mucosal cells. Secretory IgA has been shown to successfully prevent attachment of bacteria such as *Salmonella*, *Vibrio cholerae*, and *Neisseria gonorrhoeae* in the gastrointestinal tract (Goldsby *et al.* 2003).

IgE. IgE is a minor class of Ig found in very low concentrations in the serum of a healthy horse. IgE, like IgA, is primarily synthesized by plasma cells beneath epithelial surfaces (Tizard 1996). The primary function of IgE is to activate mast cells, which are responsible for the reactions characteristic of a hypersensitivity reaction, such as hives or anaphylactic shock. IgE is also responsible for immunity to parasitic worms.

Each class of Ig plays a unique role in the protection of both mare and foal from disease. An immune system functioning at optimum capacity is essential for the mare to produce a healthy and viable foal. Because the mare has a diffuse

epitheliochorial placenta, no significant transfer of Ig molecules across the placental barrier can occur during gestation (Jeffcott 1974). The main vehicle for transfer of immunologic protection from the mare to the foal is the colostrum. Colostrum rich with maternal antibodies will increase the chances for the foal to successfully deal with antigenic stimulation it faces soon after birth.

Passive Immunity

Foals are born with essentially no circulating Igs, although measurable quantities of IgG and IgM may be detected in the serum at birth (LeBlanc 1990, Vivrette 2001). The acquisition of maternal antibodies by the newborn foal within the first 24 hours of life is essential for the foal's survival. Prior to parturition, selective concentration of Igs from the blood occurs in the mare's mammary gland to form the antibody component of the colostrum (Jeffcott 1972). When the foal ingests colostrum, specialized epithelial cells of the small intestine absorb the large Ig molecules present in the colostrum through pinocytosis (Jeffcott 1974). Passive immunity obtained by the foal from the mare is dependant upon the colostrum Ig content, the quantity of colostrum ingested, and the successful absorption of Igs by the newborn foal's digestive tract (Tizard 1996). Failure of any of these processes is known as "failure of passive transfer" (FPT). Important factors associated with the colostrum that influence successful passive transfer of maternal antibodies include colostral Ig concentration, time of colostrum ingestion, and occurrence of prelactation colostrum loss (McGuire *et al.* 1977, LeBlanc *et al.* 1992).

If the foal is not able to nurse or the colostrum is of poor quality, administration of colostrum from a colostrum bank or a colostrum substitute is

important to insure that the foal receives the essential antibodies that provide protection against pathogens. After 24 hours, the mechanism for absorption of large immunoglobulin molecules in the small intestine is no longer functional. This cease in function is referred to as “gut closure.” There are therapies available for a foal that has not successfully ingested an adequate quantity of colostrum before gut closure occurs, including IV administration of equine plasma or commercially available Ig supplements. However, colostrum contains beneficial factors including leukocytes, hormones, growth factors, and constituents that inhibit bacterial colonization in the intestine, which makes it preferable to IV immunoglobulin therapy for the treatment of FPT (Vivrette 2001).

Colostrum

The mare secretes colostrum for only a relatively short period of time. It is manufactured in the mammary gland during the last two weeks of pregnancy and is secreted the first time the foal suckles (McCue 1993). The colostrum contains high concentrations of three classes of Igs. IgG concentration is high at birth, but rapidly declines within the first 24 hours post-parturition (Pearson *et al.* 1984). Colostral IgA and IgM are lower than IgG at birth (McCue 1993). As lactation shifts from colostrum to milk production during the first day of lactation, IgA becomes the predominant class of Ig found in mare’s milk (Norcross 1982). The average content of the three classes of Igs found in mare’s colostrum and milk are shown in Table 2-2.

Table 2-2. Immunoglobulin content of mare's colostrum and milk

Fluid	IgG	IgA	IgM
Colostrum (mg/dL)	1500 to 5000	500 to 1500	100 to 350
Milk (mg/dL)	20 to 50	50 to 100	5 to 10

Adapted from Tizard 1996: *Veterinary Immunology: An Introduction*, p. 242 Table 19-1. W.B. Saunders Co., Philadelphia.

Prelactation

Many factors can affect the Ig concentration of the colostrum. One of the main determinants of colostral Ig content at parturition is whether or not the mare experienced prelactation colostrum loss prior to parturition. Premature lactation, or "prelactation" is one of the most important causes of FPT due to colostrum loss (McCue 1993). It is relatively common for mares to lactate prior to parturition, with the cause presumably associated with hormonal changes or certain conditions such as impending abortion, twin pregnancy, placentitis, and premature placental separation (Jeffcott 1974, McCue 1993). Mares that prematurely lactate for longer than 24 hours before foaling tend have lower colostral IgG concentrations than in mares that do not prematurely lactate (Leblanc 1990). Morris *et al.* (1985) found a significant upward linear trend in the percentage of mares that prelactated as colostral IgG decreased.

Breed

There have been reports demonstrating that breed of the mare can affect colostrum Ig content. In a study including Thoroughbred, Arabian, and Standardbred mares, breed of mare significantly affected colostral IgG concentration (LeBlanc *et al.* 1992). In another study, the mean IgG

concentration in the colostrum was 9,691±1,639 mg/dL in 14 Arabian mares and 4,608±2,138 mg/dL in 22 Thoroughbred mares (Pearson *et al.* 1984). Kohn *et al.* (1989) reported a mean colostral IgG concentration of 8,329±6,206.8 mg/dL in 36 Standardbred mares. This value is within the range reported in a study of 136 Standardbred mares by Morris *et al.* (1985). In another study, the mean IgG colostral concentration in 21 QH mares was found to be 5,843±722 mg/dL (LeBlanc *et al.* 1986). More investigation is needed to conclusively determine the exact degree of influence that breed has on colostral Ig content.

Age

The age of the mare may also correlate to colostrum quality. Pearson *et al.* (1984) suggests that age of the dam is a possible factor that influences colostral Ig concentration. In a study that included 293 mares, mean colostral IgG concentration was highest in mares between 3 and 10 years old, and FPT was most prevalent in foals whose dams were >15 years old (LeBlanc *et al.* 1992). Clabough *et al.* (1991) reported a possible association of an age >12 years old with FPT. However, other studies suggest that mare age does not have a significant effect on colostrum Ig content. Morris *et al.* (1985) reported that mare age did not significantly affect colostrum IgG content in a study of 136 Standardbred mares aged 3 to 24. The discrepancies between these reports may be due to variations in the time of colostrum sample collection. Future studies with greater sample sizes and less variation may further elucidate the effect of age on colostrum Ig content.

Foal Diarrhea

Diarrhea is one of the most common health problems experienced by foals. It is characterized by an increase in the water content of the feces and/or an increase in the frequency of defecation. Enteritis is a similar condition characterized by diarrhea along with inflammation of the intestinal tract. If left untreated for more than a few days, other problems may arise such as dehydration, electrolyte imbalance, and even death. Identifying the cause can be a challenge because there is a myriad of pathogenic organisms that can cause diarrhea in sucking foals. The most common noninfectious cause of foal diarrhea is associated with foal heat of the mare, which occurs between 7 and 12 days of age (Cohen 1997). This is usually self-limiting and can resolve itself with minimal medical treatment. Other noninfectious causes of diarrhea in young foals include nutritional causes, gastric ulceration, and antibiotic administration (Cohen 1997).

Rotavirus

Diarrhea that is pathogenic in nature presents a major concern for horse operations, primarily because of the infectious nature of the organisms that cause diarrhea. One extremely contagious viral cause of diarrhea in young foals is *rotavirus*. *Rotavirus* is the most common cause of foal enteritis in central Kentucky, Ireland, and Great Britain (Dwyer 1993). Although the mortality rate of *rotavirus* infection is low, there is a significant cost involved for treatment with fluid and drug therapy, increased labor for the care of sick foals, and disinfection of facilities to contain the outbreak (Dwyer 1993).

Salmonella

Diarrhea can also be caused by many different species of bacteria. The most common cause of bacterial diarrhea and enteritis in foals is considered to be *Salmonella* (Cohen 1997, Spier 1993). The genus *Salmonella* is a diverse population of Gram-negative bacteria. *Salmonella typhimurium* is the most common strain that causes disease, although many other strains of *Salmonella* have been implicated in cases of salmonellosis. In the host, *Salmonella* is capable of colonizing in the intestinal tract where it can invade the mucosal epithelium and spread to other locations (Spier 1993). When bacterial invasion occurs in other parts of the body, this condition is termed septicemia. This is a serious condition with a survival rate of only 26% reported in a study of 38 cases of septicemic foals admitted to a veterinary hospital for treatment (Koterba *et al.* 1984). Septicemia can also occur from invasion by many other bacterial species besides *Salmonella*.

Clostridium

Clostridium perfringens and *Clostridium difficile* are two species of Gram-positive bacteria that have been associated with enteritis and diarrhea in foals (Traub-Dargatz and Jones 1993, Jones *et al.* 1988). Infection with *C. perfringens* in foals was associated with a high case-mortality risk of 68% in a retrospective case study investigating 125 foals admitted to a veterinary teaching hospital (East *et al.* 2000). Another study reported a mortality risk of 54% with this infection (East *et al.* 1998). The majority of foals reported to have *C. perfringens*-associated enteritis have been under 3 days of age (Traub-Dargatz and Jones 1993).

Other Bacteria

The Gram-negative bacteria *Escherichia coli* is rarely associated with diarrhea in foals (Cohen 1997). However, *E. coli* accounted for 56% of all bacteria cultured from the blood in a study that examined 38 septicemic foals (Koterba *et al.* 1984). Another study found that *E. coli* was one of the most frequent causes of death in septicemic foals less than one week old (Platt 1973). Other less common bacterial causes of foal diarrhea that have been reported are *Rhodococcus equi*, *Bacteroides fragilis*, and *Compylobacter jejuni*, but the clinical significance of these organisms is not notable (Cohen 1997).

Mannan Oligosaccharides

Carbohydrates play a unique role within living systems. The function of a carbohydrate will vary depending on its structure and location within a biological system. Carbohydrates are important structural components of the majority of cell-surface and secreted proteins of animal cells (Osborn and Khan 2000). Carbohydrates are also a major source of metabolizable energy in the diet. Oligosaccharides are formed when 2-10 monosaccharide molecules are joined together to form a larger molecule. More than 10 monosaccharide molecules joined together would constitute a polysaccharide. Mannose is a monosaccharide that forms the building block of MOS. The small intestine does not contain the digestive enzymes required to break down mannan oligosaccharide bonds, therefore they arrive at the large intestine intact after ingestion and passage through the small intestine (Strickling *et al.* 2000). Mannose-based oligosaccharides occur naturally in cell walls of the yeast *Saccharomyces cerevisiae* and are relatively easy to obtain by centrifugation from a lysed yeast

culture (Spring *et al.* 2000). The commercially available product Bio-Mos® (Alltech, Inc., Nicholasville, KY) is a source of MOS from *Saccharomyces cerevisiae* cell walls. This product was introduced in 1993 as a feed additive for broiler chickens (Hooge 2003).

Lectins are carbohydrate-binding proteins that mediate interactions of cells with their environment through their initial interactions with other cell surface carbohydrates (Osborn and Khan 2000). Mannose residues on the surface of intestinal epithelial cells serve as receptor binding sites for certain pathogens with type-1 fimbriae that contain mannose-specific lectins (Ofek and Beachey 1978, Oyofo *et al.* 1989b, Spring *et al.* 2000, Röckendorf *et al.* 2002). Adherence to the intestinal cell wall is a prerequisite for the initiation of colonization by pathogenic organisms in the gastrointestinal tract (Ferket *et al.* 2002). Once binding by the pathogenic organism occurs, translocation across the intestinal wall and subsequent enteric infection can occur (Iji *et al.* 2001, Ferket *et al.* 2002).

In vitro Agglutination Studies

Mannan oligosaccharide preparations have been shown to agglutinate pathogens with mannose-specific type-1 fimbriae *in vitro*. Spring *et al.* (2000), in an attempt to investigate the ability of different enteric pathogens and coliforms to trigger MOS agglutination, showed that MOS agglutinated 7 of 10 strains *S. typhimurium* and *S. enteritidis* and 5 of 7 strains of *E. coli in vitro*. Strains of *S. cholerasuis*, *S. pullorum*, and *Campylobacter* did not result in MOS agglutination. Another study using several human isolates of *E. coli* showed high mannose-binding activity of the bacterial cells with the addition of D-mannose (Ofek and

Beachey 1978). This same study also demonstrated that D-mannose could displace over 90% of *E. coli* that had already adhered to intestinal epithelial cells *in vitro*. In another study, *E. coli* with type-1 mannose-specific lectins did not attach to mammalian cells in the presence of supplemental mannose (Salit and Gotschileh 1977).

Intestinal Environment Studies

Efforts to demonstrate that MOS has the same effect on bacterial populations in the intestinal environment have proven successful. Oyofo *et al.* (1989b) investigated the adherence of *S. typhimurium* to the small intestine of one-day-old chicks and found that adherence was significantly inhibited in the presence of D-mannose. Droleskey *et al*. (1994) found that incubation of *S. typhimurium* with cultured chick intestinal segments resulted in the loss of mucosal epithelial integrity evidenced by the complete shedding of the epithelium. It was found in this study that the addition of 2.5% D-mannose to the incubation medium inhibited the loss of epithelial cells. When provided in the drinking water of chicks, mannose significantly reduced intestinal colonization of *S. typhimurium* (Oyofo *et al.* 1989a). When supplemented to the diet of hens, MOS affected the birds' intestinal microflora by increasing the *Bifidobacterium* spp. and *Lactobacillus* spp., while decreasing colonization of *S. enteritidis* (Fernandez *et al.* 2002). The addition of 4,000 ppm of MOS to the diet of three-day-old chicks that were orally challenged with *S. typhimurium* significantly reduced cecal *S. typhimurium* concentrations on day 10 when compared with controls (Spring *et al.* 2000). In a separate trial using *S. dublin* as the challenge organism, the number of chicks that tested positive for *Salmonella* in the cecum

at day 10 was less in chicks that were consuming the MOS supplemented diet (Spring *et al.* 2000). In growing turkeys younger than six weeks of age, MOS supplemented birds had a higher total anaerobe count and a lower level of *C. perfringens* in cecal cultures (Finuance *et al.* 1999). These studies demonstrate that pathogens with the mannose-specific type-1 fimbriae adsorb to MOS instead of attaching to intestinal epithelial cell walls and, therefore, move through the intestine with less probability of initiating disease.

There have also been investigations into the intestinal environment effects of MOS supplementation to the diet of a companion animal species. Strickling *et al.* (2000) found that in dogs, fecal *C. perfringens* tended to be lower when supplemented with 5g MOS/kg diet DM. The same study found no diet effects on fecal bifidobacteria numbers or ileal bacteria colony forming units. Dogs supplemented with 2 g MOS/day had significantly lower fecal total aerobe and tended to have greater *Lactobacillus* populations (Swanson *et al.* 2002). 1 g/kg BW/day of MOS supplementation to the diets of 4 female beagle dogs resulted in a lower fecal pH (Zentek *et al.* 2002).

Performance

The use of antibiotics in food animal diets is a common practice in the industry. Antibiotics have been shown to improve growth, feed efficiency, and overall herd health when used in poultry, swine, and cattle production diets. Due to consumer concerns and increasing regulatory restrictions, producers have begun searching for alternatives to the use of antibiotic growth promotants in production diets. Mannan oligosaccharide supplementation has been

investigated as an alternative to antibiotic supplementation to enhance performance characteristics.

Poultry. Numerous studies have been conducted in poultry, because MOS was first introduced in 1993 as a feed additive for broiler chicken diets (Hooge 2003). Over 150 broiler chicken pen trials were analyzed to collectively determine the effects of MOS-supplemented diets versus negative and/or positive control (antibiotic) diets. The conclusion was that MOS supplementation results in bodyweight and feed conversion ratios comparable to antibiotic supplementation while significantly lowering mortality rate (Hooge 2003). Fritts and Waldroup (2000) reported that turkey poults fed 0.10% MOS had the same feed conversion as poults fed 55 ppm of the antibiotic bacitracin methylene disalicyclate (BMD) and significantly better feed conversion than negative controls. In a study conducted to determine growth effects in turkey hens with diets supplemented with MOS or antibiotics (BMD and virginiamycin), investigators found that birds fed 0.5g/ kg MOS supplemented birds had improved feed efficiency over birds fed the control or antibiotic-supplemented diet (Hulet *et al.* 2000). Mannan oligosaccharide was shown to be a suitable alternative to terramycin as a growth enhancer in turkey diets when no difference in bodyweight was seen between control and treatment animals after 105 days of supplementation (Stanley *et al.* 2000).

Both MOS and antibiotic growth promoters enhance the efficiency of nutrient utilization by reducing the competition between the host and intestinal pathogens. Without microbial competition for energy and other nutrients, there

are more nutrients available for absorption and metabolism by the host (Ferket *et al.* 2002). It is well documented that antibiotic supplementation to poultry diets increases the utilization of dietary energy (Buresh *et al.* 1985, Harms *et al.* 1986, Ferket *et al.* 2002). Although MOS supplementation has proved to be as effective as antibiotics in improving utilization of dietary energy, the mechanism is unclear and likely different than that used by antibiotic growth promotants. Possibly it is related to the improvement of characteristics of the intestinal lining (Ferket *et al.* 2002) or changes in digestive enzyme activities that are stimulated by MOS (Iji *et al.* 2001).

Swine. Pregnant sows fed 0.20% MOS three weeks prior to farrowing and 0.10% MOS throughout the 21-day lactation period produced piglets with heavier litter birth and weaning weights (O'Quinn *et al.* 2001). In a factorial experiment conducted to determine the effects of two levels of MOS (0 and 0.10%) and three levels of protein (20, 23, and 26%) in piglet diets, MOS supplementation improved weight gain and feed consumption regardless of protein level (Kim *et al.* 2000). The addition of minerals such as Zn and Cu in excess of NRC recommendations to swine diets is a common practice to improve performance (NRC 1998). However, this may result in an undesirable effect on the bacteria responsible for waste degradation in lagoons (Gilley *et al.* 2000). The addition of 0.20% MOS to the diets of nursery pigs increased average daily gain and average daily feed intake in the absence of excess zinc but had no effect or a negative effect in the presence of excess zinc (LeMieux *et al.* 2003). In a separate trial of the same study, the interactive effects of antibiotics

(oxytetracycline and neomycin) and MOS and of Zn and MOS were evaluated. Mannan oligosaccharide improved pig performance only when fed in combination with an antibiotic and no excess Zn. There was no effect or a negative effect in the presence of excess Zn or in the absence of an antibiotic (LeMieux *et al.* 2003).

Mannan oligosaccharides have also been considered as an alternative to excess Cu supplementation in swine diets for performance enhancement. The effects of MOS fed at either basal or excess levels of Cu in the diets of weanling and growing-finishing pigs were determined in an experiment by Davis *et al.* (2002). From day 0 to day 10, average daily gain, average daily feed intake, and gain : feed increased when MOS was added to diets containing basal levels of Cu. From day 10 to day 38, pigs fed diets containing excess Cu had greater ADG and ADFI regardless of MOS addition (Davis *et al.* 2002). The researchers concluded that MOS addition to swine diets results in a moderate improvement in gain and feed efficiency, but the magnitude of response is not as great as that seen with the addition of excess levels of Cu (Davis *et al.* 2002). Should trace mineral supplementation restrictions on swine diets come into effect, MOS supplementation may provide a viable performance-enhancing alternative.

Cattle. The production-enhancement effects of MOS supplementation in cattle diets have received relatively less attention than supplementation of poultry or swine diets. Heinrichs *et al.* (2003) investigated the effects of MOS or antibiotics in dairy calf milk replacer diets, and found the addition of 4 g MOS/ day was as effective as antibiotic use to maintain normal fecal fluidity and

consistency and to decrease scours severity. Feed consumption increased when MOS was included in the diet, but this did not result in a difference in growth measures (Heinrichs *et al.* 2003).

Immune Function

After MOS supplementation to production diets proved to increase weight gain and feed efficiency, identifying the mechanism of the physiological response associated with the positive growth responses was the next logical step. To do this, studies focused on measuring the parameters that are representative of a functional immune system. These parameters include Ig content of the serum, lymphocyte proliferation, and response to antigenic stimulation. The main antigenic components of yeast cells are mannans present in the isolated cell wall (Ballou 1970). Mannans found in the cell walls of *S. cerevisiae* have been shown to induce an antigenic response in humans (Young *et at.* 1998) Therefore some MOS-immune system interaction would be expected (Ferket *et al.* 2002).

Poultry. Savage *et al.* (1996) fed 0.11% MOS to male turkeys for 53 days and obtained blood and bile samples at the end of the period. The samples were analyzed using both radial immunodiffusion (RID) and rocket immuno-electophoresis (RI). Using RID, no significant differences were found, but RI analysis showed that concentrations of both blood and bile IgG and IgA were significantly increased in turkeys fed MOS (Savage *et al.* 1996). In a trial investigating the effects on humoral immunity in commercial laying hens, investigators injected the hens with sheep red blood cells (SRBC) suspended in a solution of bovine serum albumin (BSA) and obtained serum samples one, two, and four weeks post-sensitization. Hens supplemented with 0.05% MOS had

higher SRBC titers than controls at one week post-sensitization (Malzone et al. 2000). The BSA titers of the MOS-fed hens were numerically greater at week one and week two, but the differences were not statistically significant (Malzone et al. 2000). In broiler breeder diets, the addition of MOS significantly increased the antibody response to infectious bursal disease virus and also increased maternal antibody titers in the breeders' progeny (Shashidhara and Devegowda 2003).

Swine. Positive immune response effects have also been observed with MOS supplementation to swine diets. Newman and Newman (2001) supplemented sow diets with 5g MOS/ day for approximately 14 days pre-farrowing and continued supplementation throughout lactation. At farrowing, MOS treated sows had significantly higher serum IgM and colostrum IgM levels and numerically higher colostrum IgG levels (Newman and Newman 2001). The piglets from the MOS treated sows also weighed more on day 7, 14, and 21 post-farrowing than those from unsupplemented sows (Newman and Newman 2001). In another study evaluating sow and litter performance, concentrations of IgA, IgG, and IgM in pre-suckle colostrum samples were increased by MOS addition to the diet. IgG showed the greatest response, followed by IgM and IgA respectively (O'Quinn *et al.* 2001). As found in the previous trial, the piglets from the MOS treated sows also had heavier litter birth and weaning weights (O'Quinn *et al.* 2001).

To determine whether MOS modulated the cell-mediated immune response of the weaned pig, Davis *et al.* (2002) obtained blood samples from MOS supplemented growing-finishing pigs and measured lymphocyte proliferation *in*

vitro. Lymphocyte proliferation did not differ significantly between the control and MOS supplemented pigs (Davis *et al.* 2002). Although not demonstrated in this study, other researchers have demonstrated that MOS may have an inhibitory effect on certain lymphocyte functions (Muchmore *et al.* 1990, Podzorski *et al.* 1990). It is conceivable that immune function suppression could also be a means by which MOS improves gain and efficiency, because of the shift in metabolic activity to support the body's defense against foreign antigens that occurs during immune response activation (Spurlock 1997). Another mechanism of growth enhancement in swine may be through the alteration of intestinal microflora, as is what happens with supplementation with pharmacological levels of Cu (Davis *et al.* 2002).

Cattle. The addition of 10 g MOS/ day to the diet of 40 dairy cows resulted in numerically greater serum Ig levels in calves 24-hours post-calving than in the calves of unsupplemented cows (Franklin *et al.* 2002). In the same study, antibody titers to *rotavirus* vaccination following calving were numerically greater in claves from MOS supplemented cows (Franklin *et al.* 2002).

Dogs. Adult female dogs were supplemented with 1 g MOS per day for a 14 day period, and serum IgA concentrations tended to be greater and the percent of white blood cells that were lymphocytes was greater in dogs supplemented with MOS. Total white blood cell count and neutrophil concentration were unaffected by treatment (Swanson *et al.* 2002). The authors hypothesized that because serum IgG and IgM were not affected, a systemic immune response may not have occurred and was not the cause of the

increased lymphocytes and serum IgA. The trends for increased serum IgA and lymphocyte concentration may be due to the increased proliferation of B-lymphocytes and secretory IgA in the intestinal tract (Swanson *et al.* 2002). A study performed in rats reported increased cecal IgA contents and an increase in the proportion of IgA-presenting lymphocytes present in the cecal mucosal of rats fed glucomannans at 5% for three weeks (Kudoh *et al.* 1999).

These studies have demonstrated the positive effects of MOS on Ig concentration in serum and colostrum and on immune response to antigen challenge. However, a mechanism for this action has yet to be demonstrated. Some studies suggest that MOS supplementation stimulates intestinal lymphoid tissue resulting in increased development or activation (Guigoz *et al.* 2002, Ferket *et at.* 2002). The stimulatory effect may occur through a healthy population of gut microflora or “drag effects” of the indigestible oligosaccharide molecules as they move along the length of the intestine (Cunningham-Rundles and Lin 1998). The activation of lymphoid tissue may result in greater plasma cell production by B-cells found in underlying lymphoid follicles. These plasma cells then would be able to secrete Igs that can either be secreted into the intestinal lumen when associated with secretory component or end up in the circulation via transport through the lymphatic system.

To determine if MOS supplementation to the diet of pregnant mares would result in a change in the total Ig concentration of the mare’s colostrum and the serum of the mare or foal, the current experiment was proposed. Previous results from work in other species suggest that MOS supplementation will increase

colostrum Ig content, and therefore translate into increased foal serum Ig content after absorption of maternal antibodies is complete. Growth measurements of both mares and foals will indicate any negative effects of MOS supplementation on physical development. Determination of Ig content in serum and presuckle colostrum samples will indicate any change of immune status in the mare. Serum Ig concentration in the foals will reflect any effect on absorption of colostral Igs and initial serum Ig concentration and any long-term effect on immune status of the foal due to MOS supplementation of the dam.

CHAPTER 3
MATERIALS AND METHODS

Animals

Twenty-six pregnant Thoroughbred (n=21) and Quarter Horse (n=5) mares and their subsequent foals were used in this trial. The mares ranged from 3 to 24 years of age with a mean age of 9 (STD=6.1). The pregnant mares were paired by expected foaling dates and assigned at random to one of two treatment groups 56 days prior (d-56) to expected date of parturition. They continued on the treatment diet until 56 days post-parturition (d+56). The foals remained on the trial until 112 days of age (d+112). One mare leaked milk for 3 weeks prior to foaling, and her foal acquired septicemia and was hospitalized for one week after birth. No data from this mare or foal were used.

Housing and Management

During the course of the trial, the mares and their foals were housed at the University of Florida's Horse Research Center in Ocala, Florida. Pregnant mares were kept at pasture until pre-foaling signs were evident. They were then moved to a dry lot where they remained until they foaled. After foaling, the mare and her foal were moved to a small paddock for approximately one week and then were returned to pasture. A routine vaccination and anthelmintic schedule for all animals on trial was followed by farm management. The University of Florida Institutional Animal Care and Use Committee approved the protocol for this trial.

Diets

Treatment group 1 (n=13) served as controls and received supplement A, which consisted of 55g of ground corn as a placebo. Treatment group 2 (n=12) received supplement B, which consisted of 10g of MOS (Bio-Mos, Alltech, Nicholasville, KY) mixed in 45g of ground corn. Supplements A and B were top dressed on the morning ration and fed to the mares from day -56 until day +56. Feeding time was at 0700 hours (AM feeding) and 1500 hours (PM feeding). Mares and foals were brought into stalls for individual feeding for both AM and PM feedings. Foals remained in the stalls with their dam and potentially could have consumed some of her feed, depending upon her temperament and willingness to allow the foal access to her feed bucket. Both treatment groups were fed the same concentrate, HR-136, which was formulated to meet or exceed requirements for late gestating and lactating mares based on NRC recommendations (NRC 1989) when fed with bahiagrass pasture (*Paspalum notatum*) or Coastal bermudagrass hay (*Cynodon dactylon*) (see table 3-1). The amount of concentrate fed was adjusted according to each mare's body condition score (BCS) to maintain a minimum BCS of 5 (see table 3-2). The mares were also fed *ad-libitum* Coastal bermudagrass hay and/or bahiagrass pasture in season. Trace mineralized salt blocks and fresh water were available at all times. A creep-feeder was introduced when the oldest foal was 2 months of age, and HR-136 was provided as the creep feed.

Body Measurements

The mares were weighed and assessed for body condition scores every 28 days. Foals were weighed at birth, d+7, d+14, d+28, d+56, and d+112. Foal

body measurements taken at the same time were withers height, hip height, body length, and heart girth. The scale used was a digital walk-on scale. Body measurements were made with a sliding stick made specifically for the purpose of taking accurate body length and height measurements.

Colostrum and Blood Samples

Colostrum samples were obtained from the mare after the foal was born but before it was allowed to nurse. Three 1 ml aliquots from each colostrum sample were placed in cryogenic tubes and frozen at -80°C until further analysis. Jugular blood samples were collected from the mares between 0700 and 0900 hours on d-56, d-28, and d+28. Jugular blood samples were collected from the foals at birth before the foal was allowed to nurse, 6 -10 hours post-parturition (referred to as 8 hour sample), and between 0700 and 0900 hours on d+7, d+14, d+28, d+56, and d+112. Precision Glide Vaccutainer brand blood collection needles (20G, 1½ in.) were used to collect blood into Beckton Dickinson Vaccutainers. Samples were allowed to clot for one to two hours and then centrifuged at 3000 x G for 10 minutes to allow for separation and collection of serum. Three 1 ml aliquots from each serum sample were placed in polypropylene cryogenic vials and frozen at -80°C until further analysis. Colostrum samples and serum samples from both the mares and foals were analyzed for IgG, IgA, and IgM content using a commercially available single radial immunodiffusion kit (SRID Kit, VMRD, Inc., Pullman, WA).

Feed Sample Analysis

Monthly samples were taken of HR-136 and the Coastal bermudagrass hay for the duration of the experiment. To determine dry matter content of the

samples, the concentrate and hay were first put through a Wiley mill fitted with a 1mm screen to assure uniform particle size. 1 to 2 grams of the sample were then weighed to 4 decimal places on a Mettler balance and placed into ceramic crucibles. They were dried in a 105°C drying oven overnight and equilibrated for 1 hr. in a dessicator before weighed again to 4 decimal places.

The samples were analyzed for calcium, copper, manganese, zinc, and iron content by atomic absorption spectrophotometry (Miles *et al.* 2001) using the Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., Norwalk, CO). Crude protein content was analyzed by first digesting the sample according to the procedure put forth by Gallaher *et al.* (1975) and then determining the nitrogen content of the sample using the Alpkem auto analyzer (Alpkem Corp., Clackemas, OR). Phosphorus content was determined by using a calorimetric procedure (Technicon Industrial Systems, Tarrytown, NY) on the automated Alpkem analyzer (Alpkem Corp., Clackemas, OR).

Neutral and acid detergent fiber content was determined using the Ankom fiber analyzer (Ankom Technology, Fairport New York).

Prior to fat content analysis, carbohydrates were first extracted from the sample (AOAC 1995). Fat content was then determined by ether extraction using a soxhlet apparatus.

Statistical Analysis

The treatment effect on Ig concentration in the serum of the mares was analyzed using PROC GLM procedures with repeated measures in SAS (SAS 1989). Ig content of the mare's colostrum was analyzed using PROC GLM in SAS controlling for age, breed, and prelactation. The treatment effect on Ig

concentration in the serum of the foals was analyzed using PROC GLM procedures with repeated measures in SAS (SAS 1989). Treatment, sex, and breed effects on foal growth measurements were analyzed using PROC GLM procedures with repeated measures in SAS. Significance was considered to be $p<0.05$, and $p<0.10$ was considered a trend.

Table 3-1. Composition of Concentrate (HR-136)

Ingredient	Amount (%)
Corn, cracked	34.25
Oats, crimped	26.50
Soybean meal (48% CP)	10.00
Wheat bran	10.00
Molasses, blackstrap	8.00
Alfalfa meal pellets (17% CP)	7.50
Limestone, ground	1.50
Monocalcium phosphate	0.80
Salt	0.75
Vitamin premix[a]	0.30
Vitamin E[b]	0.15
Lysine 98%	0.05
Luprosil (mold inhibitor)	0.10
Trace mineral premix[c]	0.10

[a]Provides 4,400,000IU Vit A, 440,000IU Vit D, and 35,200IU Vit E/ kg premix
[b]Provides 44,200IU Vit E/ kg premix
[c]Provides 7,200mg Cu, 28,000mg Zn, 28,000mg Fe, 28,000mg Mn, 80mg Co, 80mg I, and 80mg Se/ kg premix

Table 3-2. Concentrate feeding rates for mares

Stage	Rate
Late Gestation	
-56 d to -28 d	0.75% BW[a]
-28 d to parturition	1.0% BW[a]
Early Lactation	
Parturition to +84 d	1.5% BW[a]
Late Lactation	
+84 d to +112 d	1.0% BW[a]

[a]Adjust concentrate feeding for body condition score (-20% above BCS 5/ +20% below BCS 5)

CHAPTER 4
RESULTS AND DISCUSSION

Feed Analysis

The average nutrient composition of HR-136 (n=7) and the Coastal bermudagrass hay (n=3) from monthly samples taken throughout the trial period are presented in Table 4-1.

Table 4-1. Concentrate (HR-136) and Coastal bermudagrass hay nutrient composition analysis

Nutrient	HR-136	Hay
Dry Matter (%)	94.78±1.1	88.44±3.6
Crude protein (%)	15.07±1.0	5.15±1.1
Fat (%)	2.62±0.8	1.64±0.4
ADF (%)	9.46±1.0	39.21±1.5
NDF (%)	25.01±1.5	80.35±1.1
Calcium (%)	1.16±0.4	0.46±0.3
Phosphorus (%)	0.62±0.2	0.15±0.1
Cu (ppm)	48.17±2.3	2.29±0.9
Mn (ppm)	124.83±4.7	51.67±4.3
Zn (ppm)	137.67±4.3	23.00±1.7
Fe (ppm)	267.50±4.8	101.67±7.4

All values Mean±SE
Dry matter basis (except dry matter)

Growth Analysis

For the duration of the experiment, mares maintained good body condition and remained at a healthy body weight during both gestation and lactation (See Table 4-2). Mares from treatment 1 (control) foaled 6 fillies and 7 colts, and mares from treatment 2 (MOS) foaled 6 fillies and 6 colts. There were no statistically significant differences ($p > 0.05$) between control and foals from MOS supplemented mares for any of the growth parameters measured (see Table 4-

3). Control foals weighed 50.3±1.7 kg at birth and gained a total of 140.0±4.1 kg during the 112-day trial. Foals from MOS supplemented mares weighed 48.9±0.8 kg at birth and gained 142.6±4.4 kg over the trial period. Control foals grew 26.4±1.0 cm in height, 29.6±1.0 cm in hip height, 44.2±1.3 cm in length, and 47.1±1.1 cm in heart girth. Foals from MOS supplemented grew 25.4±1.0 cm in height, 28.5±1.1 cm in hip height, 46.0±1.4 cm in length, and 48.2±1.2 cm in heart girth. Average daily gain measurements for both treatments were consistent with previously published data (Kavazis and Ott 2003, Lawrence *et al.* 1991).

The influence of sex on foal growth was minimal during the 112-day trial period. Because there was no significant treatment effect on growth, the data from the two treatment groups were pooled (see Table 4-4) to determine any influence of sex on growth. Average height was the only growth parameter that showed any trend towards significant difference between males and females. At d+112, colts tended to be taller than fillies ($p=0.08$).

To determine the influence of breed on foal growth, data from the two treatment groups were pooled (see Table 4-5). There was a trend for TB foals to be taller than QH foals at d+112 ($p=0.08$). TB foals had greater birth body length than QH foals ($p=0.04$). The total gain in body length tended to be greater for QH foals than TB foals ($p=0.06$).

Table 4-2. Influence of treatment on mare weight and body condition scores

Day	Weight (kg)		Body Condition Score	
	Treatment 1 (Control)	Treatment 2 (MOS)	Treatment 1 (Control)	Treatment 2 (MOS)
d-56	567.6±11.7	577.1±9.3	4.8±0.1	4.8±0.1
d-28	580.4±10.3	586.9±9.7	4.6±0.2	5.0±0.1
d0	514.1±10.4	526.1±10.2	4.6±0.1	4.7±0.1
d+28	528.0±12.5	538.2±10.6	4.6±0.1	4.8±0.1
d+56	529.4±12.0	543.1±11.0	4.6±0.2	4.8±0.1
d+84	535.2±11.6	547.3±11.1	4.6±0.2	4.9±0.2
d+112	531.8±11.0	544.9±9.7	4.6±0.2	4.6±0.1

All values are Mean ± SE

Table 4-3. Influence of treatment on foal growth

Growth parameter	Treatment 1 (Control)	Treatment 2 (MOS)
Birth weight (kg)	50.3±1.7	48.9±1.8
d+112 weight (kg)	190.2±5.0	191.5±5.4
Total weight gain (kg)	140.0±4.1	142.6±4.4
Birth withers height (cm)	98.2±1.0	99.9±1.1
d+112 withers height (cm)	124.6±0.7	125.3±0.8
Total withers height gain (cm)	26.4±1.0	25.4±1.0
Birth hip height (cm)	100.4±1.1	102.4±1.2
d+112 hip height (cm)	130.0±0.9	131.0±0.9
Total hip height gain (cm)	29.6±1.0	28.5±1.1
Birth length (cm)	73.3±1.0	72.8±1.1
d+112 length (cm)	117.5±1.1	118.8±1.2
Total length gain (cm)	44.2±1.3	46.0±1.4
Birth heart girth (cm)	80.1±1.2	80.0±1.3
d+112 heart girth (cm)	127.2±1.5	128.2±1.6
Total heart girth gain (cm)	47.1±1.1	48.2±1.2

All values are LSMean ± SE

Table 4-4. Influence of sex on foal growth

Growth parameter	Colts	Fillies
Birth weight (kg)	49.9±1.7	49.2±1.8
d+112 weight (kg)	188.3±4.6	190.7±4.9
Total weight gain (kg)	140.8±4.1	191.0±5.4
Birth withers height (cm)	100.0±1.0	98.2±1.1
d+112 withers height (cm)	125.8±0.7	124.1±0.8
Total withers height gain (cm)	26.0±1.0	25.9±1.0
Birth hip height (cm)	102.0±1.1	100.8±1.2
d+112 hip height (cm)	130.7±0.9	130.3±0.9
Total hip height gain (cm)	28.6±1.0	29.5±1.1
Birth length (cm)	72.4±1.0	73.7±1.1
d+112 length (cm)	117.1±1.1	119.1±2
Total length gain (cm)	44.7±1.3	45.5±1.4
Birth heart girth (cm)	79.5±1.2	80.6±1.3
d+112 heart girth (cm)	127.4±1.5	128.0±1.6
Total heart girth gain (cm)	47.9±1.1	47.4±1.2

All values are LSMean ± SE

Table 4-5. Influence of breed on foal growth

Growth parameter	QH	TB
Birth weight (kg)	50.0±2.4	49.2±1.2
d112 weight (kg)	195.2±6.5	186.5±4.0
Total weight gain (kg)	145.2±5.3	137.3±3.0
Birth withers height (cm)	97.8±1.5	100.3±0.8
d112 withers height (cm)	124.0±1.0	126.0±0.5
Total withers height gain (cm)	26.0±1.0	25.8±1.0
Birth hip height (cm)	100.3±1.6	102.6±0.8
d112 hip height (cm)	129.9±1.2	131.1±0.6
Total hip height gain (cm)	30.0±1.5	28.6±0.7
Birth length (cm)	71.2±1.5*	74.9±0.7*
d112 length (cm)	118.4±1.6	117.8±8
Total length gain (cm)	47.2±1.9	42.9±1.0
Birth heart girth (cm)	80.1±1.7	80.0±0.9
d112 heart girth (cm)	129.1±2.2	126.4±1.1
Total heart girth gain (cm)	49.0±1.6	46.3±0.8

All values are LSMean ± SE *p=0.04

Mare Serum Immunoglobulins

Mare serum Ig content was analyzed with treatment as the only source of variation.

IgG

For IgG serum concentration, control mares averaged 1807.6±130.8 mg/dL on d–56, 1525.1±191.5 mg/dL on d 0, and 1929.1±163.6 mg/dL on d+28. Mares fed MOS had an average serum IgG concentration of 1789.8±125.8 mg/dL on d–56, 1405.5±108.2 mg/dL on d 0, and 1874.7±96.1 mg/dL on d+28 (see Table 4-6). Although control mares had numerically higher serum IgG concentration at d-56, d 0, and d+28, the differences were not significant. The control mares had a numerically higher IgG concentration at the start of the experiment, and this is the likely reason control mare IgG concentration remained slightly above IgG concentration in mares fed MOS for the duration of the trial.

Table 4-6. Influence of treatment on mare serum IgG concentration

Day	Treatment 1 (Control)	Treatment 2 (MOS)
d-56 (mg/dL)	1807.5±130.8	1789.8±125.8
d0 (mg/dL)	1525.0±191.5	1405.5±108.2
d+28 (mg/dL)	1929.1±163.6	1874.7±96.1

All values are Mean ± SE

IgA

Average serum IgA concentration for control mares was 349.2±38.7 mg/dL at d–56, 424.9±31.1 mg/dL at d 0, and 378.6±31.9 mg/dL at d+28. Mares fed MOS had an average serum IgA concentration of 360.1±40.4 mg/dL at d–56, 419.0±44.0 mg/dL at d 0, and 412.0±68.2 mg/dL at d+28 (see Table 4-7). Mares fed MOS had numerically higher serum IgA concentration than control mare

serum IgA concentration throughout the duration of the experiment. Because this difference was present at the start of the experiment, this effect was not likely due to MOS supplementation.

Table 4-7: Influence of treatment on mare serum IgA concentration

Day	Treatment 1 (Control)	Treatment 2 (MOS)
d-56 (mg/dL)	349.2±38.7	360.1±40.4
d0 (mg/dL)	424.9±31.1	419.0±44.0
d+28 (mg/dL)	378.6±31.9	412.0±68.2

All values are Mean ± SE

IgM

Serum IgM concentration for control mares averaged 109.2±13.8 mg/dL on d–56, 115.6±12.7 mg/dL on d 0, and 101.9±21.6 mg/dL on d+28. Mares fed MOS averaged 98.8±8.8 mg/dL on d–56, 113.1±10.3 mg/dL on d 0, and 89.1±15.8 mg/dL on d+28 (see Table 4-8). Control mare serum IgM concentration remained numerically above mares fed MOS for the duration of the experiment, and this was not likely due to the treatment.

Table 4-8. Influence of treatment on mare serum IgM concentration

Day	Treatment 1 (Control)	Treatment 2 (MOS)
d-56 (mg/dL)	109.2±13.8	98.8±8.8
d0 (mg/dL)	115.6±12.7	113.1±10.3
d+28 (mg/dL)	101.9±21.6	89.1±15.8

All values are Mean ± SE

Discussion

There were no significant differences for IgG, IgA, or IgM concentration in samples obtained from the mares at d-56, d0, or d+28. This result agrees with results obtained in a previous study performed in 40 pregnant dairy cows to evaluate the effect of MOS supplementation on the immune status of dairy cows

and their calves. No overt differences in serum Ig levels were observed between cows that were supplemented with 10 g/MOS/day and the control group (Franklin *et al.* 2002).

Savage *et al.* (1996) reported an increase in plasma IgG and bile IgA in male turkeys after 53 days of MOS supplementation at 0.11% of the total diet. These turkeys were started on the supplementation protocol immediately after birth at one day of age. The data was analyzed using two different assays, and only one assay, rocket immuno-electrophoresis, revealed any significant difference in plasma IgG and bile IgA levels between the two treatment groups. The assay that did not reveal any difference was radial immunodiffusion, the same assay that is used in the current experiment.

Mare Colostrum Immunoglobulins

Mare colostrum data were analyzed to determine the treatment, prelactation occurrence, age, breed, treatment*age interaction, and treatment*breed interaction effects. Previous research suggests that prelactation, age, and breed can affect the Ig concentration in mare colostrum (McCue 1993, Leblanc 1990, Morris *et al.* 1985, LeBlanc *et al.* 1992, Pearson *et al.* 1984, LeBlanc *et al.* 1986, and Clabough *et al.* 1991), therefore it is important to consider these factors when evaluating colostrum content.

IgG

Colostrum IgG concentration for mares fed MOS was significantly higher than in control mares ($p=0.05$) when all sources of variation were taken into consideration in the overall ANOVA model. Colostrum IgG concentration for mares fed MOS was significantly higher than control mares due to treatment

(p=0.03), prelactation (p=0.006), and treatment*age (p=0.02). All other sources of variation were not significantly different between treatments (see Tables 4-9 and 4-10).

Table 4-9. Influence of treatment, prelactation occurrence, age, and breed on colostrum IgG

Source of Variation	Mean ± s.e.
Treatment	
Control (n=13) (mg/dL)	10242.2±1181.1
MOS (n=12) (mg/dL)	12824.0±2245.6
Prelactation	
Y (n=4) (mg/dL)	6934.2±1174.0
N (n=21) (mg/dL)	12555.5±1429.0
Age	
<12 years (n=18) (mg/dL)	11663.8±985.7
>12 years (n=7) (mg/dL)	11253.0±3585.5
Breed	
TB (n=20) (mg/dL)	12388.2±1542.8
QH (n=5) (mg/dL)	8627.1±1461.0

Table 4-10. ANOVA generated P values for colostrum IgG from a statistical model which included treatment, prelactation, age, breed, with treatment*age and treatment*breed interactions

Model	p=0.05
Treatment	p=0.0334
Prelactation	p=0.0063
Age	p=0.1377
Breed	p=0.4803
Treatment*Age	p=0.0163
Treatment*Breed	p=0.7593

IgA

Colostrum IgA concentration for mares fed MOS was significantly higher than in control mares (p=0.05) when all sources of variation were taken into consideration in the overall ANOVA model. Colostrum IgA concentration for mares fed MOS was significantly higher than control mares due to treatment

(p=0.008), prelactation (p=0.008), age (p=0.02), and treatment*age (p=0.04). All other sources of variation were not significantly different between treatments (see Tables 4-11 and 4-12).

Table 4-11. Influence of treatment, prelactation occurrence, age, and breed on colostrum IgA

Source of Variation	Mean ± s.e.
Treatment	
Control (n=13) (mg/dL)	47.7±9.5
MOS (n=12) (mg/dL)	112.1±38.9
Prelactation	
Y (n=4) (mg/dL)	43.6±12.6
N (n=21) (mg/dL)	88.0±24.9
Age	
<12 years (n=18) (mg/dL)	67.5±15.9
>12 years (n=7) (mg/dL)	106.6±57.5
Breed	
TB (n=20) (mg/dL)	85.0±26.4
QH (n=5) (mg/dL)	62.7±19.8

Table 4-12. ANOVA generated P values for colostrum IgA from a statistical model which included treatment, prelactation, age, breed, with treatment*age and treatment breed*interactions

Model	p=0.05
Treatment	p=0.0080
Prelactation	p=0.0079
Age	p=0.0177
Breed	p=0.1796
Treatment*Age	p=0.0356
Treatment*Breed	p=0.7746

IgM

Colostrum IgM concentration for mares fed MOS tended to be higher than in control mares (p=0.06) when all sources of variation were taken into consideration in the overall ANOVA model. Colostrum IgM concentration for mares fed MOS tended to be higher than control mares due to treatment

(p=0.08). The treatment*age interaction was significantly higher for mares fed MOS (p=0.04). All other sources of variation were not significantly different between treatments (see Tables 4-13 and 4-14).

Table 4-13. Influence of treatment, prelactation occurrence, age, and breed on colostrum IgM

Source of Variation	Mean ± s.e.
Treatment	
Control (n=13) (mg/dL)	133.2±12.2
MOS (n=12) (mg/dL)	154.1±8.1
Prelactation	
Y (n=4) (mg/dL)	126.3±23.0
N (n=21) (mg/dL)	147.5±7.7
Age	
<12 years (n=18) (mg/dL)	154.7±6.9
>12 years (n=7) (mg/dL)	120.0±15.6
Breed	
TB (n=20) (mg/dL)	149.1±7.4
QH (n=5) (mg/dL)	125.0±20.6

Table 4-14. ANOVA generated P values for colostrum IgM from a statistical model which included treatment, prelactation, age, breed, with treatment*age and treatment*breed interactions

Model	p=0.06
Treatment	p=0.0764
Prelactation	p=0.2994
Age	p=0.2195
Breed	p=0.9598
Treatment*Age	p=0.0350
Treatment*Breed	p=0.9545

Discussion

The colostrum Ig concentration for all isotypes was highly variable. This could be due to many factors, some of which could not be accounted for in the statistical model. Ig content was determined by single radial immunodiffusion (SRID) using raw colostrum samples. This method has been used in previously

published reports (Zou *et al.* 1998, Turner *et al.* 2003). However, there have been other published reports that describe extracting the colostral whey (located between the superficial fat layer and the precipitate) to remove cellular debris and fat for use in the SRID assay (Waelchli *et al.* 1990, Pearson *et al.* 1984, LeBlanc *et al.* 1986, LeBlanc *et al.* 1992). It is possible that using colostral whey for the determination of Ig content could minimize the extreme variation in colostrum Ig values.

One of the QH mares from the control treatment was dropped from the statistical analysis because the Ig concentration in her colostrum was a significant outlier to the average distribution of expected Ig concentration in mare colostrum. The Ig content of her colostrum was 45,409.5 mg/dL for IgG, 278.5 mg/dL for IgA, and 220 mg/dL for IgM. These values were much higher than those from the other mares in the study and average values reported in the literature (Tizard 1996, LeBlanc *et al.* 1992, Morris *et al* 1985, Pearson *et al.* 1984, LeBlanc *et al.* 1986). In order to maintain a representative sample of the mare population, her data was not used for colostrum analysis.

When controlled for variation due to prelactation colostrum loss, age, and breed, IgG and IgA content of colostrum was significantly enhanced by MOS supplementation, and IgM content tended to be enhanced. This result agrees with previous findings of two other studies evaluating the effect of MOS supplementation on colostrum immunoglobulin content. Newman and Newman (2001) reported significantly increased presuckle colostrum IgM levels ($p=0.04$) in MOS supplemented sows and numerically greater IgM levels in colostrum 24-

hour post-farrowing. They also reported numerically increased presuckle and 24-hour post-farrowing colostrum IgG levels in MOS supplemented sows when compared to controls, but there was no effect on colostrum IgA concentration (Newman and Newman 2001). In another study involving sows, the addition of MOS resulted in significantly increased IgG (p=0.007) and IgM (p=0.03) concentration in presuckle colostrum (O'Quinn *et al.* 2001). Presuckle IgA levels tended to be greater in MOS supplemented sows (p=0.06) (O'Quinn *et al.* 2001).

There was a significant effect due to treatment (p=0.03), prelactation (p=0.006), and treatment*age interaction (p=0.02) for IgG colostrum content. The highly significant prelactation effect is expected, because lost colostrum cannot be replaced due to its limited production. The negative effect of prelactation on colostrum Ig content has previously been well documented (McCue 1993, Jeffcott 1974, Leblanc 1990, and Morris *et al.* 1985).

There was a significant effect due to treatment (p=0.008), prelactation (p=0.008), age (p=0.02), and treatment*age interaction (p=0.04) for IgA colostrum content. The highly significant prelactation effect is expected, for reasons stated previously. Age effect on colostrum content is not well defined, however some reports show that mean colostrum Ig concentration was highest in mares between 3 and 10 years old and lower in mares over 12 years old (LeBlanc *et al.* 1992, Clabough *et al.* 1991). Other reports show no effect of age on colostral Ig content (Morris *et al.* 1985, Kohn et al. 1989). In this experiment, mares that were >12 years old had higher mean colostrum IgA concentration. This may be due to the fact that many of the mares used in this study were

maiden mares. There was one maiden mare in the control treatment group and seven maiden mares in the MOS treatment group. It has been reported that primiparous (maiden) mares have lower colostrum Ig concentrations than multiparous mares, and this may explain the significant age effect on IgA content (Jeffcott 1972, Erhard *et al.* 2001). Although no significant age effect was seen for colostrum IgG or IgM, a significant treatment*age interaction was seen for all three isotype concentrations, and the unbalanced distribution of maiden mares in the treatment groups may have contributed to this effect.

The treatment effect approached significance (p=0.08) and there was a significant effect due to treatment*age interaction (p=0.02) for IgM colostrum content. The occurrence of prelactation did not significantly affect IgM concentration, possibly because the overall concentration of IgM in equine colostrum is relatively low (McCue 1993).

Foal Serum Immunoglobulins

Foal serum immunoglobulin concentration was analyzed with treatment as the only source of variation.

IgG

A detectable amount of IgG was present in foal serum at birth prior to colostrum ingestion. There were no significant differences in IgG concentration for any of the foal serum samples collected. The mean foal serum IgG concentration for each sample collection is presented in Table 4-15. Figure 4-1 presents this data in graphic format to illustrate the change in foal serum IgG concentration over time.

Table 4-15. Influence of treatment on foal serum IgG concentration

Day/hour	Treatment 1 (Control)	Treatment 2 (MOS)
0 hour (mg/dL)	82.6±11.4	88.2±11.4
8 hour (mg/dL)	1478.8±238.0	1420.0±227.6
d+7 (mg/dL)	1431.7±172.8	1322.5±159.4
d+14 (mg/dL)	1275.6±146.8	1229.7±121.6
d+28 (mg/dL)	1234.1±121.7	1322.7±129.7
d+56 (mg/dL)	930.1±65.0	907.3±58.1
d+112 (mg/dL)	653.9±27.6	648.7±15.2

All values are Mean ± SE

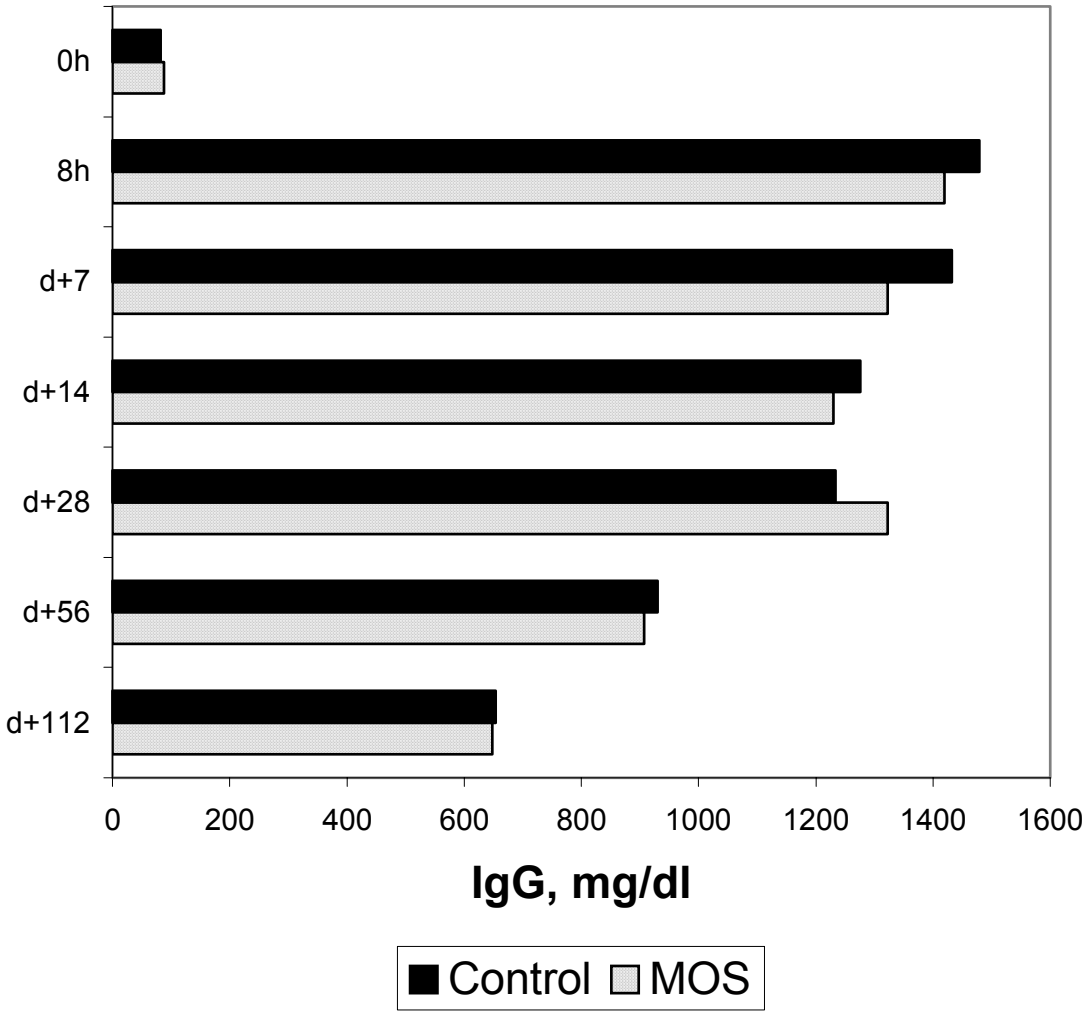

Figure 4-1: Mean foal serum IgG concentration

IgA

There was no detectable amount of IgA in foal serum at birth prior to colostrum ingestion. There were no statistically significant differences in foal serum IgA concentration, however, foals from control mares tended to have higher serum IgA concentration than foals from mares fed MOS at 6 -10 hours post-parturition ($p=0.09$). The mean foal serum IgA concentration for each sample collection is presented in Table 4-16. Figure 4-2 presents this data in graphic format to illustrate the change in foal serum IgA concentration over time.

Table 4-16. Influence of treatment on foal serum IgA concentration

Day/hour	Treatment 1 (Control)	Treatment 2 (MOS)
0 hour (mg/dL)	0	0
8 hour (mg/dL)	214.7±30.8	122.8±27.9
d+7 (mg/ dL)	81.3±7.6	84.7±21.7
d+14 (mg/ dL)	59.6±4.1	62.3±10.0
d+28 (mg/ dL)	67.5±7.5	61.4±3.5
d+56 (mg/dL)	98.9±8.5	93.9±9.4
d+112 (mg/dL)	140.4±11.2	119.1±7.3

All values are Mean ± SE

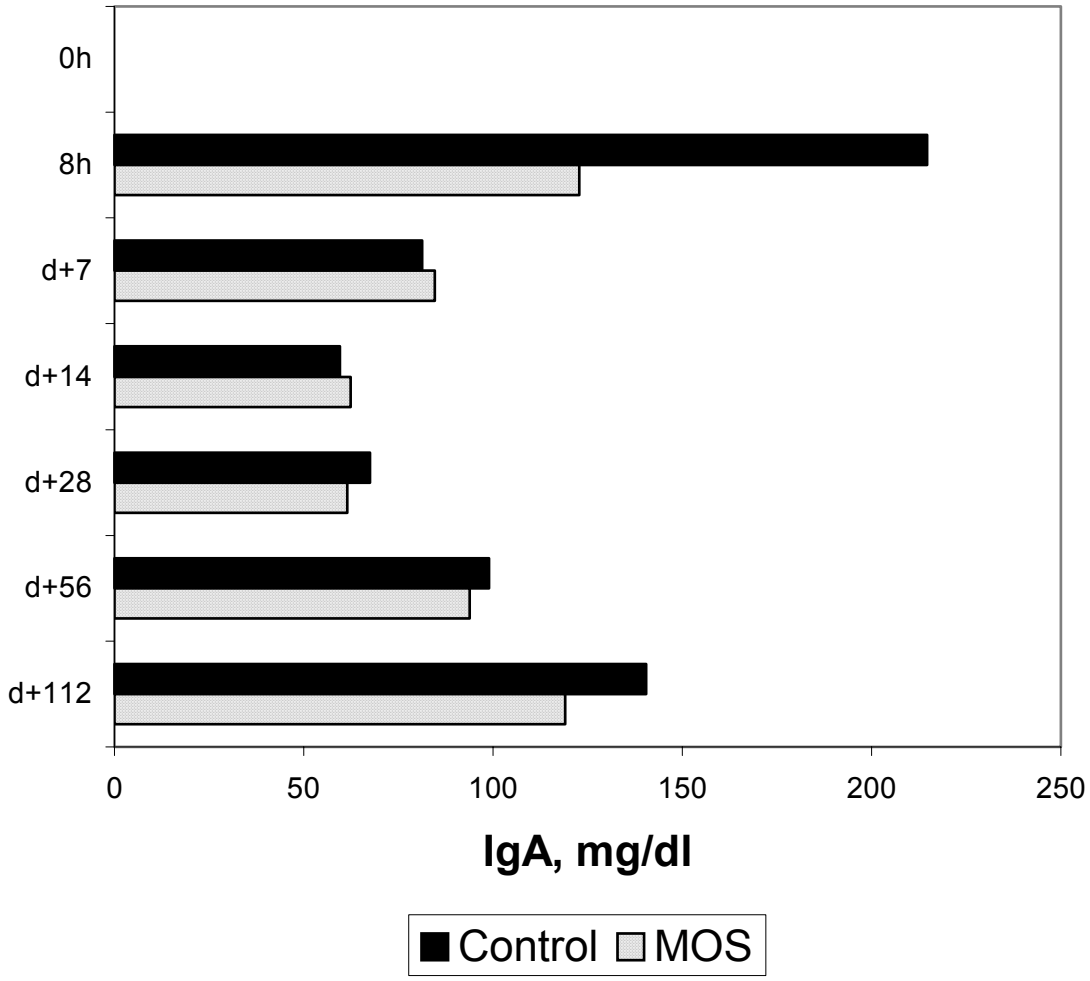

Figure 4-2: Mean foal serum IgA concentration

IgM

A detectable amount of IgM was present in foal serum at birth prior to colostrum ingestion. There were no significant differences in IgM concentration for any of the foal serum samples collected. The mean foal serum IgM concentration for each sample collection is presented in Table 4-17. Figure 4-3 presents this data in graphic format to illustrate the change in foal serum IgM concentration over time.

Table 4-17. Influence of treatment on foal serum IgM concentration

Day/hour	Treatment 1 (Control)	Treatment 2 (MOS)
0 hour (mg/dL)	17.0±2.0	17.5±1.5
8 hour (mg/dL)	40.2±6.8	41.0±5.0
d+7 (mg/dL)	33.9±3.9	35.5±4.1
d+14 (mg/dL)	37.1±4.0	40.9±3.3
d+28 (mg/dL)	46.7±6.9	41.3±3.3
d+56 (mg/dL)	77.8±8.3	67.3±8.6
d+112 (mg/dL)	109.2±9.0	119.5±7.6

All values are Mean ± SE

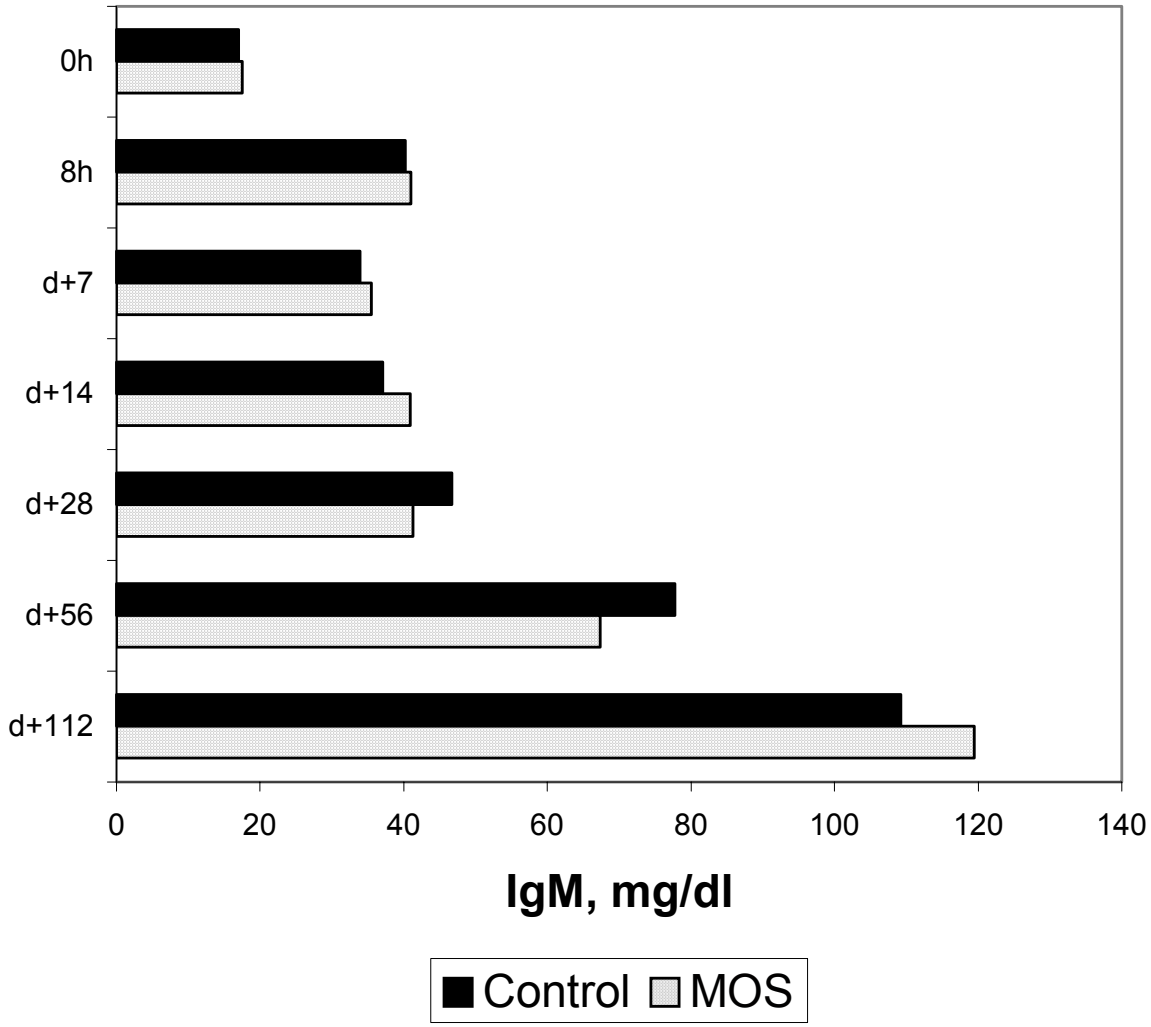

Figure 4-3: Mean foal serum IgM concentration

Discussion

There were no statistically significant differences for any serum concentration at any hour or day sampling period. Because foals were not fed the MOS directly, any immune response would be expected to come from the ingestion of colostrum with a higher concentration of immunoglobulins and predominantly be apparent in the first weeks of life. Franklin *et al.* (2002) reported numerically greater serum IgG and IgM concentration 24 hours post-calving in calves from cows supplemented with MOS, but the differences were not significant. LeBlanc *et al.* (1986) reported that mean foal serum IgG concentration increases concurrently with increasing colostral IgG concentration. Morris *et al.* (1985) reported similar results and showed a highly significant correlation between colostral IgG and foal serum IgG concentration ($r=0.584$, $p<0.001$). The positive association between colostrum Ig and foal serum Ig concentration after colostrum ingestion is well documented. However, there was no noticeable difference between the two treatment groups in this experiment, even with significantly higher Ig concentration in colostrum of mares fed MOS. This is most likely due to the fact that peak values of passively obtained maternal antibodies are reached around 18 hours after birth (Jeffcott 1972). The foal serum samples taken in this experiment to determine successful passive transfer were obtained between 6 and 10 hours post-parturition. At this time, full absorption of maternal antibodies is not yet complete (Kohn *et al.* 1989). Evaluation of foal serum from 6 – 12 hours post-parturition is appropriate to determine if proper absorption of maternal antibodies is occurring so that a treatment protocol for suspected FPT can be implemented if necessary (Erhard

et al. 2001, Vivrette 2001). However, obtaining a 24 to 36 hour post-parturition serum sample would have more accurately reflected the complete absorption of Igs from the mare's colostrum (Morris *et al.* 1985).

Serum IgA concentration in foals from control mares tended to be higher than in foals from mares fed MOS 6 - 10 hours post-parturition ($p=0.09$). The reason for this trend for foal serum IgA concentration to be higher in control foals is unclear. Intestinal permeability is selective in the horse, with IgG and IgM preferentially absorbed while IgA remains in the intestine (Tizard 1996). The IgA content of colostrum was most significantly increased by MOS supplementation, but this was not reflected in the 6 –10 hour foal serum samples. The principal form of IgA in human colostrum is secretory IgA, which is resistant to the proteolytic effects of enzymes present in the neonatal gut (Chapel *et al.* 1999). Perhaps the increased quantity of IgA in the colostrum of mares fed MOS was primarily in the form of secretory IgA, and significant amounts could not be immediately absorbed across the intestinal epithelium. There is evidently some absorption of colostrum IgA as shown by the initial increase in serum IgA concentration and subsequent decrease for both treatment groups over the first 7 days of life. A foal serum sample obtained 24 to 36 hours post-parturition may have reflected higher peak absorption of IgA in the foals from MOS supplemented mares due to the higher content of IgA in the colostrum of mares fed MOS.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Supplementing pregnant mares with 10 g/MOS/day 56 days prior to expected date of parturition through the first 56 days of lactation significantly increased IgG and IgA content and tended to increase IgM content in the colostrum. Supplementation had no effect on serum Ig content of mares or foals, except at 8 hours after birth when control foals had significantly higher serum IgA concentration than foals from mares fed MOS. Because the timing of foal serum sampling at 8 hours after birth was not ideal, this may have prevented an accurate portrayal of full absorption of maternal antibodies. However, no ill effects were seen as a result of MOS supplementation, and greater Ig content in the colostrum increases the chance for successful passive transfer to occur. Supplementation of pregnant mare diets with MOS may be a beneficial practice to help protect the mare from pathogenic organisms and to boost the Ig content of her colostrum.

Although not investigated in this experiment, MOS supplementation of sucking and weanling diets may be beneficial as well. Foals that are provided a source of MOS may be better protected from pathogenic organisms present in the environment and therefore may have a reduced incidence of illness caused by these organisms. This is a promising area for further research.

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BIOGRAPHICAL SKETCH

Kelly Robertson Spearman was born on August 28, 1977, in Tuscaloosa, Alabama. She lived there for 3 years until her family moved to Montevallo, AL, in 1980. In 1986, they moved to Anniston, AL, which is where Kelly's interest in horses began. Her 5th grade English teacher also taught riding lessons, and although her family knew nothing about horses, they agreed to bi-monthly riding lessons that would fit into an already busy schedule of piano lessons, choir practice, gymnastics, and church activities. Kelly and her family moved back to Tuscaloosa in 1989, and her interest in horses continued to grow, as she started taking dressage lessons at a local barn. This sparked an enduring fascination with the art of dressage and its training philosophies.

The family moved to Montgomery, AL, in 1994, just before her senior year, and she graduated with a 3.9 GPA from Jefferson Davis High School. While in high school, she also worked as a pharmacist assistant, attended a performing arts school for piano, and was the accompanist for the school's jazz choir. She received a freshman academic scholarship to Auburn University, and graduated in 1999 with a B.S. degree in animal and dairy sciences, with *cum laude* honors. While at Auburn, she was a member of the university honors program, a charter member of the Auburn Equestrian Team, and vice president of the horseman's club. For three summers during her college career, she was the wrangler for a working cattle ranch in northwest Colorado. She is a member of Alpha Zeta

honorary fraternity, and Gamma Sigma Delta honor society. After graduating from Auburn University, Kelly moved to Missouri for 1 year and worked as an assistant trainer, working with young horses. She moved back to Alabama, and became a North America Handicapped Riding Association certified instructor, and she was an instructor for Special Equestrians in Birmingham, AL.

In 2001, Kelly received a presidential fellowship to study Equine Nutrition at the University of Florida under Dr. Edgar A. Ott. While at the University of Florida, Kelly taught numerous undergraduate equine classes, and participated in many equine nutrition research projects. She will continue her education at the University of Florida, as she works toward a Ph.D. in equine nutrition.