EFFECTS OF MATERNAL NUTRITION MANIPULATION ON MARES AND THEIR FOALS

A Dissertation

by

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ABSTRACT

Previous research documented the fetus is sensitive to nutrition of the dam, but this has not been thoroughly investigated in horses. Objectives of the current studies were to determine effect of manipulation of maternal nutrition during the last third of pregnancy on mare performance, intake, hormones, foaling parameters, colostrum, and foal passive transfer of immunity and growth, and effects of supplemental arginine.

Plane of nutrition influenced mare performance, and DMI was influenced by time with the first trial finding all mares consumed less in the 10th month of pregnancy compared to the 11th month, and the second trial finding all mares consumed less during the 11th month. Additionally, the second study determined arginine supplementation has no detrimental effects on DMI. Both studies indicated the dual marker system was sufficient at estimating DMI. Neither trial found an influence of treatment on foaling parameters or physical measurements obtained following parturition, and the second study determined arginine supplementation also did not affect foaling or measurements. The first study determined maternal nutrition did not affect foal growth or ADG.

When colostrum quality was evaluated, the first study determined mares consuming only hay had increased specific gravity and Brix% indicating higher quality. This was confirmed by IgG analysis finding a tendency for increased IgG concentration. However, colostrum volume was not affected by nutrition, nor was total g IgG. The second study found contrasting results with greater specific gravity in mares on a high plane of nutrition, and a tendency for moderate plane of nutrition mares to have greater

volume. Additionally, the second study determined that arginine supplementation does not influence colostrum volume or quality (measured by specific gravity or Brix %).

In the first trial, maternal diets affected glucose and insulin AUC in mares, which altered insulin dynamics in the resulting foals. Foal insulin AUC and peak insulin concentration were greater in foals from mares supplemented with concentrate compared to foals from mares fed hay alone. These studies have provided a wealth of information to help elucidate the impact of maternal nutrition in late gestation on mares and their foals.

DEDICATION

This dissertation is dedicated to my family. Cristina, thank you for introducing me to my lifelong passion and for being the first to teach me what to do with a horse. I might not have gotten started with all this if it wasn't for you. Mom, thank you for your never-ending support: from my first elementary school project to turning in my dissertation, it was your constant support and encouragement that made this possible. Dad, thank you for helping me believe I could do anything I put my mind to, and always keeping things in perspective. I could not have done this without each one of you.

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INTRODUCTION AND LITERATURE REVIEW

Introduction

Previous research in many species has documented that maternal nutrition influences fetal development, and that the fetus is sensitive to the nutrition of the dam during pregnancy (Wu et al., 2006). The fetus is able to adapt to its environment during critical periods of growth, where one single genotype may give rise to a range of physiological states in response to environmental conditions during development (Barker, 2004). Developmental programming can alter fetal growth with external stimuli such as reduced nutrient and blood supply to the fetus. This reduced nutrient supply can have lasting effects on development with consequences such as reduced neonatal health, skeletal muscle growth, feed efficiency, and athletic performance (Rossdale and Ousey, 2002). Intrauterine growth retardation (IUGR) is defined as impaired growth and development of the conceptus or its organs during pregnancy (Wu et al., 2006).

Both under and overnutrition have negative effects on uterine environment and fetal growth, and may be a cause of IUGR. While the impact of maternal nutrition in the horse has not been thoroughly investigated, overnutrition is a common occurrence in the industry with obesity rates ranging from 45 to 50% (Stephenson et al., 2011). Obesity in mares as a result of overfeeding causes a reduction in fetal growth and occasional fetal death (Pugh, 1993). In other species, overnutrition has negative effects on uterine

environment and fetal growth (Wu et al., 2006). In sheep, maternal overnutrition resulted in increased incidences of abortion or still births and decreased gestational length, which may be due to alterations in placental secretion of hormones such as progesterone (Wallace et al., 2001). Overfeeding has also been shown to reduce the initial prenatal colostrum volume in sheep (Davidson et al., 2000) and lower concentrations of IgG and crude protein (Wallace et al., 2001).

Arginine is a conditionally essential amino acid with an unknown requirement in the horse at any stage of development. Supplemental arginine has great potential in fetal programming due to its role in nitric oxide (NO) synthesis, which enhances blood flow and increases nutrient delivery from maternal to fetal circulation (Reynolds and Redmer, 2001). To date there is little research concerning the influence of maternal nutrition in horses, and the role of maternal dietary arginine in fetal development in horses is also unknown. Therefore the possible interaction of overnutrition of the mare along with arginine supplementation is unknown and holds great potential for future research.

Equine Pregnancy

There are many unique aspects of pregnancy in the mare, especially in late gestation, compared to other livestock species. In most species, estrogens increase and progestagens decrease nearing the end of gestation; however, in mares estrogens decline and progestagens rise throughout the last weeks then rapidly decrease in concentration during the last few days prior to parturition (Ousey, 2006; Fowden et al., 2008; Samper, 2009). In other livestock species such as cattle, sheep, and pigs, progesterone (P4) plays

the most important role of all progestagens in maintaining quiescence of the female reproductive tract during pregnancy, and the decline in progesterone over the last 2 weeks of gestation is associated with a gradual reduction in the progesterone block of pregnancy which helps to initiate contractions of the uterine myometrium (Challis et al., 2000). In horses however, P4 is only at high concentrations during the first 150 d of pregnancy when it is produced solely by the corpus luteum, and there is very little detectable P4 in maternal plasma during the last third of gestation (Pashen, 1984). Instead, uterine quiescence is maintained by metabolites of pregnenolone (P5) and P4 produced by uteroplacental tissues, and are commonly referenced as total progestagens (Short, 1959; Holtan et al., 1991; Silver, 1994; Fowden et al., 2008). In mares, this increase in progestagens during the last few weeks prior to parturition is associated with mammary gland development (Ousey et al., 2005; Ousey, 2004). Total progestagen concentrations vary widely between breeds, individuals, and based on the antibody used in the assay (Ousey, 2006). A previous study evaluated progestagen concentrations of mares during the last trimester using either ELISA or RIA specific for P4 but cross-reacting with other progestagens, and found total progestagen concentrations ranged from approximately 3 ng/mL to 7 ng/mL analyzed using an ELISA or from 5 ng/mL to 22 ng/mL when analyzed using RIA (Ousey, 2006). Haluska and Currie (1988) found progestagen concentrations in pregnant pony mares during the last trimester ranged from approximately 4 to 8 ng/mL. Total progestagen concentrations increase throughout the last 30 days of gestation, peak and then decline rapidly during the last 24 to 48 h prior to parturition (Fowden et al., 2008).

Also in contrast to other livestock species, total estrogen concentration in pregnant mares declines gradually throughout late gestation, especially during the last 3 to 4 weeks of pregnancy (Fowden et al., 2008; Ousey, 2006). The role of estrogens in pregnant mares has not been evaluated to the same extent as progestagens with few studies reporting actual concentrations. Haluska and Currie (1988) measured mean concentrations of estradiol ranging from 230 to 244 pg/mL in pony mares on days 198 to 279 of gestation which then decreased steadily until parturition. In Thoroughbred mares, estradiol concentrations rose through day 240 of gestation then steadily declined until parturition, with concentrations ranging from 64 to 199 pg/mL (Nett et al., 1973). Estradiol concentrations in both studies were highly variable between individual mares. Although the total concentration of estrogen decreases, in the final 24 to 48 hours prior to parturition, the concentration of estradiol doubles which plays an important role in the onset of parturition (O'Donnell et al., 2003).

Additionally, there is a pre-partum surge in fetal cortisol concentration in the horse, however this surge occurs relatively late in gestation in comparison to other livestock species (Silver and Fowden, 1994; Fowden and Silver, 1995). The pre-partum surge of fetal cortisol is necessary for final maturation of the fetus, and is critical to trigger onset of labor and myometrial contractions in the mare (Silver, 1990; Challis et al., 2000; Ousey, 2004). Previous studies have conflicting results concerning maternal cortisol increase prior to parturition with some studies finding no increases and others observed increased cortisol days before parturition (Silver and Fowden, 1994; Hoffman et al., 1996; Cudd et al., 1995). The placenta converts cortisol to inactive cortisone,

therefore fluctuations of maternal cortisol are unlikely to influence the fetus (Chavatte et al., 1995). However, cases of sustained maternal cortisol due to exogenous challenges of ACTH caused premature delivery of foals (Ousey et al., 2000).

One source of sustained maternal stress may be undernutrition, which has been shown to increase plasma cortisol concentrations in sheep (Edwards and McMillen, 2001), however cortisol concentration in lambs from undernourished ewes did not differ from lambs from ewes fed at NRC nutrient recommendations for pregnancy (Bloomfield et al., 2003). Additionally, foals from mares restricted from receiving concentrate during late gestation (fed at approximately 100% NRC DE recommendations) did not have altered cortisol concentrations immediately after birth or at a year of age compared to foals from mares supplemented with concentrate (fed at approximately 120% NRC DE recommendations; Cavinder et al., 2012).

Maternal insulin resistance gradually develops as pregnancy progresses in most species, including the horse (Kemp et al., 1996; Bell et al., 2000; Hoffman et al., 2003b). During the last third of pregnancy mares are insulin resistant and have decreased glucose effectiveness, and diet can further alter glucose and insulin dynamics (George et al., 2011). This insulin resistance promotes increased glucose and amino acid availability to the fetus, however may actually decrease total placental blood flow (Bird et al., 2003). This alteration in nutrient delivery is due to increased proteolysis caused by insulin, which inhibits synthesis of nitric oxide (NO) which is a major regulator of placental blood flow (Bird et al., 2003; Marliss et al., 2006). Therefore, insulin resistance occurring in late gestation may alter nutrient availability to the developing fetus.

Beyond just endocrine changes, pregnancy causes additional nutritional factors to change such as DMI. In ruminants, DMI is affected by gut fill and significant decreases are observed with advancing pregnancy (Forbes, 1971; Forbes, 1986; Weston, 1982; Allen, 1996). Uterine growth in late gestation may limit capacity of the rumen, and therefore DMI is reduced. Previous research in dairy cattle observed a 30 to 35% reduction in DMI during the 3 weeks prior to parturition (Grummer, 1995). However, more recently in Holstein dairy cows, no alterations in ruminal capacity were measured; indicating factors other than capacity may cause reduction of DMI during gestation (Park et al., 2011). Mechanisms controlling DMI in horses, especially during pregnancy, remain unclear.

Frape et al. (1982) and Aiken et al. (1989) indicated that intake in horses was regulated by energy requirements rather than gastrointestinal volume. However, in horses more so than other livestock species, diet composition can impact digestion end-products, and likely effect DMI (Vermorel et al., 1997). Aiken et al. (1989) determined that yearlings consumed 2.5% BW and mature geldings voluntarily consumed 2% BW of Coastal bermudagrass hay, when forage was the sole source of nutrients. The increased forage intake per unit BW in yearlings could be related to increased energy requirements for growth (Aiken et al., 1989). Additionally, ponies fed diets with diluted energy content due to addition of sawdust increased intake as caloric density decreased; however, when the diet was diluted to 50 % by weight with sawdust the ponies increased intake but were unable to consume sufficient amounts to maintain constant caloric

intake. This was likely due to limitations in capacity of the gastrointestinal tract (Laut et al., 1985).

Research regarding DMI in pregnant mares is extremely limited, but if DMI in horses is linked solely to energy requirements, the increased DE requirement of late gestation would increase DMI of mares as pregnancy progresses if diet nutrient content is held constant. However, based on research in ruminants the reduction in capacity of the gastrointestinal tract due to advanced gestation may result in decreased DMI (Forbes, 1971; Forbes, 1986; Weston, 1982; Allen, 1996). Laut et al. (1985) suggests a combination of both factors may occur in horses, where energy requirements may increase intake until capacity becomes limiting. This presents a need for future research to evaluate DMI in pregnant mares.

There are many difficulties associated with determining voluntary DMI, and when utilizing large numbers of horses over a long time period there are often limitations that prohibit individual feeding and total collections (Sales, 2012). Thus, alternative methods can be utilized to estimate intake indirectly by dividing total fecal excretion by indigestible nutrients from the diet (Dove and Mayes, 1991). Chromic oxide is a commonly used external marker in equine and ruminant studies (Patterson et al., 2001; Frape et al., 1982). However, chromic oxide is a carcinogen, and it is not approved by the FDA as a feedstuff. A suggested alternative to chromic oxide is TiO_2, which is not a hazardous substance and can be legally added to feedstuffs up to 1% of the finished product (AAFCO, 1996). Titanium dioxide has been used as an alternative digestibility marker in cattle and sheep (Titgemeyer et al., 2001; Myers et al., 2006;

Glindemann et al., 2009), pigs (Jagger et al., 1992) and chickens (Short et al., 1996). Hafez et al. (1988) concluded TiO_2 has a 99% fecal recovery rate when fed to dairy cows, and Titgemeyer et al. (2001) found that fecal recovery averaged 93% when fed with forage and grain. Therefore the use of TiO_2 to evaluate DMI in pregnant mares holds great potential for future research.

Gestation length is extremely variable in horses compared to other livestock species, with a reported range of delivery of normal and healthy foals from 305 to 405 days gestation (Rossdale et al., 1984; Samper, 2009; Davies Morel et al., 2002). Gestational length in horses may be influenced by numerous factors such as breed, age, and parity of the mare as well as breed, size, and sex of the foal (Davies Morel et al., 2002). External factors such as maternal nutrition, climate, and season at the time of foaling have also been demonstrated to effect length of gestation (Silver, 1990; Rossdale and Silver, 1982). Research in sheep has shown maternal undernutrtion during pregnancy can decrease gestational length (Fowden et al., 1994; Edwards and McMillen, 2002). Undernutrition in mares as a result of fasting after 300 days gestation decreased gestational length due to increased prostaglandin production (Fowden and Silver, 1987). However, when mares were fed to achieve various body condition scores (low, moderate, and obese) throughout late gestation there was no influence on gestational length (Henneke et al., 1984; Kubiak et al., 1988). Although previous research indicates there are numerous factors that influence gestational length, precise mechanisms to explain these effects are not well understood and most of the research was conducted using small numbers and various breeds (Davies Morel et al., 2002).

Equine Parturition

Parturition marks the end of gestation and the beginning of extra-uterine life for the foal. Research evaluating normal foaling variables and factors that affect parturition are limited. Studies in other species, such as humans and cattle, have determined that increased fatness causes difficulties during labor and parturition. For example, obese women are 2 to 3 times more likely to require a cesarean delivery compared to leaner women due to an inability to progress through labor normally (Vahratian et al., 2004; Chu et al., 2007). In cattle, obesity results in prolonged parturition, increased incidence of cows requiring assistance, and even resulted in death of cows due to complications while calving (Arnett et al., 1971).

Previous research in mares evaluated effects of increasing BCS and observed no effect on foaling parameters (Kubiak et al., 1988). Kubiak et al. (1988) reported average durations for several intervals throughout parturition and found the following values: length from rupture of the allantochorionic membrane/release of fetal fluid to passage of the hips of the foal was 14.81 ± 3.43 min in moderate BCS mares and 19.54 ± 3.43 min in obese mares; length from birth to foal standing for ≥ 10 seconds was 62.05 ± 5.62 min in foals from moderate BCS mares and 41.92 ± 5.62 min in foals from obese BCS mares; length from birth to passage of placenta was 66.67 ± 14.92 min in moderate BCS mares and 65.92 ± 14.92 min in obese mares (however, 2 mares were not included in the data due to retaining their placentas for greater than 180 min). An additional study observed parturition in mares supplemented with arginine and found no effects of

arginine supplementation, but reported combined means for the following time intervals: rupture of the allantochorionic membrane to complete delivery of foal was 16.5 ± 1.5 min; time from rupture of the allantochorionic membrane to expulsion of placenta was 34.8 ± 12.2 min; time from birth to first nursing was 95.1 ± 9.5 min (Mortensen et al., 2011). These previous studies provide basic estimates of normal characteristics of parturition, and also illustrate that parturition in mares is not drastically altered in response to body condition or arginine supplementation.

Developmental Programming and the Uterine Environment

Growth and development of the fetus can be influenced by numerous factors, such as genetics, epigenetics, age of dam, and alterations in uterine environment (Redmer et al., 2004; Wu et al., 2006). These influencing factors, summarized in Figure 1, then affect function of the placenta, altering transfer of nutrients to the fetus (Bell and Ehrhardt, 2002; Wu et al., 2006). This results in developmental programming, where one single genotype may give rise to a range of physiological states in response to conditions during uterine development (Barker, 2004). During fetal growth, there are periods of rapid cell division, referred to as critical periods, where rate of cell division may be impacted by alterations in the uterine environment (Widdowson and McCance, 1975; Barker, 1995; Barker, 2004). Tissues and organs have varied hierarchies of priority during fetal development, therefore undergo these critical periods of growth at different times (Barker et al., 2012). Reduced transfer of nutrients to the fetus will cause cell division to slow, which may reduce the numbers of cells in particular organs and is one

of the mechanisms of uterine conditions permanently programming the adult body (Barker, 1995; Barker, 1997).

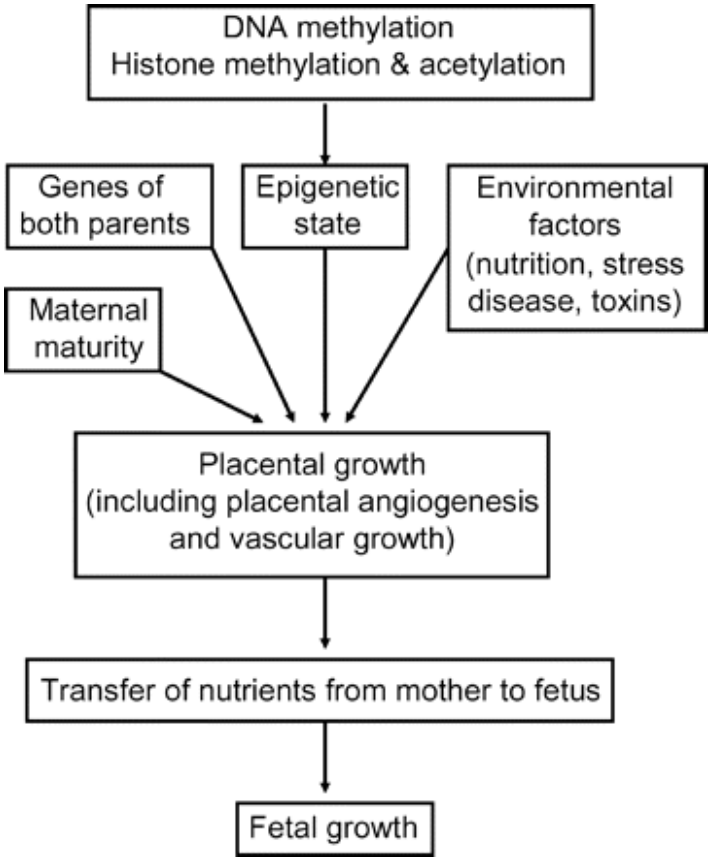

Figure 1. Regulation of mammalian fetal growth. Several factors regulate intrauterine growth by affecting placental nutrient transfer, therefore impacting nutrient availability to the fetus (adapted from Wu et al., 2006).

One illustration of how factors other than genetic potential for growth can alter fetal development is the ability of uterine capacity to create physiological and biochemical limitations on the developing fetus (Bazer et al., 1969). Effects of altering uterine capacity have been demonstrated utilizing embryo transfer to place an embryo into a genetically larger or smaller uterine environment (Dickinson et al., 1962; Ferrell,

1991; Allen et al., 2002). The effect uterine capacity has on fetal growth can be seen in equine studies that compared restricted and enhanced uterine environments by transferring Thoroughbred or pony embryos into Thoroughbred or pony mares. They observed greater birth weights in foals from enhanced uterine environments (pony embryos in Thoroughbred mares) and decreased birth weights in foals from restricted uterine environments (Thoroughbred embryos in pony mares; Allen et al., 2002; Ousey et al., 2004).

Limitations of uterine environment can also be seen in the immature dam. Common practices in production animal management include breeding females at an immature body weight and body size to increase productivity (Wu et al., 2006). Furthermore, during the first pregnancy, the fetus and dam may compete for nutrients which results in lower birth weights compared to subsequent pregnancies (Wu et al., 2004). Parity also effects birth weight, with offspring being significantly heavier in multiparous dams compared to maiden (Hintz et al., 1979; Wilsher and Allen, 2003), which is due to a reduced ability of the uterus to expand in early pregnancies resulting in less intrauterine space (Bhuvanakumar and Satchidanandam 1989). Another method of increasing productivity is twin pregnancies, however this also illustrates a limiting uterine environment as offspring from twin pregnancies have decreased birth weights compared to singleton pregnancies (Guerra-Martinez et al., 1990; Gootwine, 2005). Further evidence of the ability of uterine environment to impact fetal growth and birth weight is the similarity of birth weights observed in half-siblings that share a mother and

lack of similarity of birth weights observed in half-siblings sharing a father (Carr-Hill et al., 1987).

Effects of Maternal Nutrition

Previous research indicates two major causes of impaired fetal growth are insufficient uterine capacity and improper maternal nutrition (Wu et al., 2006), and that prenatal growth is sensitive to alterations in maternal nutrition at all stages of development (Robinson et al., 1999). Nutrition programs vary widely across animal production operations. Extensive production systems rely heavily on grazing of forages with little or no supplemental concentrate, which results in inadequate nutrient supply for the demands of gestation and lactation (Fontaneli et al., 2005; Wu et al., 2006). Conversely, intensive management systems provide supplemental grain meals to increase nutrient intake, which increases ovulation rate in certain livestock species (Cole, 1990; Wu et al., 2006).

An excellent example of the impact of maternal undernutrition throughout gestation can be seen when evaluating the Dutch famine of 1944 through 1945 where daily rations for the adult population ranged from 400 to 800 calories (Roseboom et al., 2001). This study evaluated the varied effects of undernutrition based on timing of the nutrient restriction. Infants exposed to undernutrition during mid to late gestation had lowered birth weight, body length, and head circumference compared to infants born prior to the famine. Infants exposed to the famine only during early gestation did not differ in birth characteristics compared to infants not exposed to the famine. However,

when asked to rate their adult health, those exposed to famine in early gestation more often rated their health as poor which indicated maternal malnutrition during early gestation may alter adult health without affecting birth weight (Roseboom et al., 2001). Furthermore, numerous animal experiments have induced maternal undernutrition during gestation by restricting intake of complete rations or by offering diets deficient in energy or protein, all of which resulted in reduced fetal growth and reduced birth weights (if pregnancies allowed to continue to term; Pond et al., 1969; Tudor, 1972; Osgerby et al., 2002; Vonnahme et al., 2003).

Overnutrition, resulting from increased intake of energy, protein, or both, is also common in animal production as increasing energy intake increases ovulation rate in many species (Wu et al., 2006). Flushing is the term used to designate increasing plane of nutrition during a short period of time prior to conception to improve reproductive efficiency in pigs, sheep, and cattle (Cole, 1990; Wu et al., 2006). Briefly increasing nutrient supply, or flushing, in broodmares does not have the same effect, and current recommendations are to maintain broodmares at a moderate to fleshy body condition (BCS 6 on a 1 to 9 scale) in order to have earlier ovulations and shorter initial estrus after foaling (Henneke et al., 1984; Kubiak et al., 1988). Overnutrition throughout gestation, like undernutrition, decreases fetal growth in pigs, horses, and sheep (Cole, 1990; Pugh, 1993; Wallace et al., 2004). Overnutrition in adolescent sheep decreased placental mass by 33% and birth weight by 29% compared to moderate-intake dams (Wallace et al., 2001). Overnutrition also resulted in increased abortion or stillbirth during late gestation, as well as reduced gestation length (Wallace et al., 2001). Obesity

in mares as a result of overfeeding causes a reduction in fetal growth and occasional fetal death (Pugh, 1993). During growth, when lambs from overfed (150% NRC) and control (100% NRC) dams were placed in an ad-libitum feeding situation, offspring from over-fed dams had altered appetite and consumed approximately 10% more feed compared to offspring from control dams (Long et al., 2010).

Overnutrition in the dam can also have profound effects on glucose and insulin metabolism of the offspring. Research in sheep determined that obesity decreased insulin sensitivity and amount of insulin receptors in peripheral tissue in mature sheep (Bergman et al., 1989). The effects of decreased insulin sensitivity of the dam may impact glucose and insulin metabolism in the fetus. Maternal obesity in sheep resulted in maternal and fetal hyperglycemia and hyperinsulinemia, as well as a 236% increase of fetal pancreas weight along with a 50 % increase in number of insulin positive cells per unit area of fetal pancreas (Ford et al., 2009). Lambs from mothers fed at 150% of NRC recommendations had decreased acute insulin response to glucose, and tendency for reduced insulin sensitivity compared to lambs from mothers fed at 100% NRC (Long et al., 2010). Similar effects of maternal obesity are reported in human studies, where offspring born to obese mothers were insulin resistant, and children exposed to maternal obesity are twice as likely to develop obesity, hypertension, and glucose intolerance (Boney et al., 2005; Hull et al., 2008; Catalano et al., 2009).

Timing of alterations in maternal nutrition during pregnancy creates different effects on the developing fetus. When nutrition is manipulated in the very early stages of embryonic development, proliferation of cells is impacted and may result in decreased

number of cells and smaller organ size; however, altered maternal nutrition during mid- to late gestation impacts differentiation of cells and has altered function and type of cells rather than number or overall organ size (Barker, 1995; Barker, 1997; Langley-Evans, 2009). Furthermore, maternal nutrient restriction during early to mid-gestation decreased number and proportions of skeletal muscle fibers (Zhu et al., 2006). This is an important factor to consider because muscle fiber numbers are fixed at birth, and therefore early developmental programming may impact athletic performance of horses in their adult life (Rossdale and Ousey, 2002). Altered maternal nutrition (over- and under) during late gestation has more of a metabolic effect on offspring, altering glucose transporters and responsiveness of cells (Roseboom et al., 2001; Gardner et al., 2005). Compared to other livestock species, the horse matures later in gestation, making fetal development more susceptible to alterations in maternal nutrition when changes occur during the last third of pregnancy (Rossdale and Silver, 1982).

Colostrum and Passive Transfer of Immunity

The mare's epitheliochorial placenta prohibits transfer of antibodies to the fetus prior to parturition; therefore the foal relies on passive transfer of maternal antibodies from colostrum to resist infection before it develops its own antibodies (McGuire and Crawford, 1977; McKinnon and Voss, 2005). Colostrum is the first milk secreted by animals following parturition (Tyler and Ensminger, 2006), and mares begin concentrating immunoglobulins from blood into the mammary glands during the two weeks prior to parturition (McKinnon and Voss, 2005). There are five classes of

antibodies: IgA, IgD, IgE, IgM, and IgG found in colostrum. Immunoglobulin G antibodies provide neonatal immunity and transfer of maternal antibodies across the small intestine (Abbas et al., 2010). In the horse industry, producers are mainly concerned with IgG's role in the transfer of maternal antibodies through colostrum.

Failure of passive transfer of immunity occurs somewhat frequently in foals with research indicating failure of passive transfer rates range from 10 to 24% (McGuire and Crawford, 1977; Tyler-McGowan et al., 1997). Failure of passive transfer of immunity in dairy calves results in mortality rates of approximately 50% and can cause long-term consequences on the health and productivity for the survivors (Tyler and Ensminger, 2006). Foals require approximately 2 g of IgG per kg of body weight to achieve a healthy plasma concentration of 2000 mg IgG/dL (Radostits et al., 2007). Average colostrum contains approximately 10,000 mg IgG/dL, therefore, an average sized 45 kg foal would need to consume at least 1 L of colostrum in order to obtain a sufficient amount of IgG (Radostitis et al., 2007). Failure of passive transfer is clinically defined as a plasma IgG concentration of less than 400 mg IgG/dL, but concentrations less than 800 mg IgG/dL have also been reported as increased likelihood of the foal failing to protect itself against infection (Radostitis et al., 2007). Therefore, plasma concentrations of > 800 mg IgG/dL are desirable.

Maternal nutrition influences the quality and quantity of milk and colostrum. Overfeeding has been found to reduce the initial volume of colostrum that accumulates prenatally in sheep (Davidson et al., 2000). Colostrum from ewes on a higher plane of nutrition had reduced concentrations of available IgG compared to dams on a more

moderate plane of nutrition (Wallace et al., 2001). Ewes fed 140% of nutrient requirements had decreased colostrum volume and weight compared to ewes fed 100% of requirements (Meyer et al., 2011). In contrast, extremely malnourished mothers have decreased yield and quality, and typically have milk that is deficient in protein, calcium, and fat (Jelliffe and Jelliffe, 1978). Ewes fed 60% of requirements had decreased colostrum volume and weight compared to ewes fed at 100% requirements (Meyer et al., 2011). In horses, mares fed increased DE had decreased quality as indicated by lower IgG concentrations (Thorson et al., 2010). Research in sheep fed increased DE throughout late gestation had no difference in IgG concentration; however, colostrum volume and total g colostral IgG were lower in dams consuming greater DE (Swanson et al., 2008). Maternal nutrition not only alters colostrum quality of the dam, it also affects the offspring's ability to absorb IgG. Lambs from undernourished ewes (60 % nutrient requirements) had increased serum IgG at 24 h compared to lambs from ewes fed 100% or 140% nutrient requirements (Hammer et al., 2011). The poor quality of colostrum from obese animals is likely related to problems with mammary gland development. Research in heifers has shown that over feeding before and during puberty of the dam results in greater adipose tissue within the mammary gland compared to epithelial tissue which was likely related to the decrease in volume produced, yet no negative effects were caused when over feeding occurred in post-puberty heifers (Sejrsen et al., 2000). However, in mature rats, diet induced obesity caused inadequate alveolar development and abnormal side-branching during pregnancy (Flint et al., 2005).

Research in other species indicated overnutrition has numerous negative effects on fetal development, and while impact of overnutrition in the horse has not been thoroughly investigated, overnutrition is a common occurrence in the industry with obesity rates ranging from 45 to 50% (Stephenson et al., 2011). Research in other species has progressed to investigating possible treatment options to combat the negative effects of maternal obesity, including supplemental arginine. Supplemental arginine has great potential to impact fetal programming due to its role in nitric oxide (NO) synthesis, which enhances blood flow and increases nutrient delivery from maternal to fetal circulation (Reynolds and Redmer, 2001). The role of maternal dietary arginine supplementation in fetal development of horses is unknown and there is an unknown requirement for arginine in the horse at any stage of development. Therefore, the possible interaction of overnutrition of the mare along with arginine supplementation holds great potential for future research.

Arginine

Arginine is a conditionally essential amino acid that has many crucial roles in nutrition and metabolism, such as serving as a precursor for NO, ornithine, urea, polyamines, proline, creatine, and glutamate (Wu and Morris, 1998; Summarized in Figure 2). Because of its many versatile roles, the interest in arginine is growing and there is increasing evidence to support that dietary supplementation of arginine is beneficial (Wu et al., 2009). As a precursor for NO, arginine holds great promise as a therapeutic agent for IUGR due to the role of NO in placental growth and angiogenesis.

Nitric oxide is a vasodilator which allows for more efficient uterine and placental blood flow (Wu et al., 2009). Arginine is formed in the liver through the urea cycle, however there is no net synthesis of arginine due to its rapid hydrolysis by arginase (Wu et al., 2009). Dietary supplementation of arginine prevented fetal growth retardation in rats, and increased birth weight in rats and pigs (Mateo et al., 2007). Supplementation of L-arginine to obese rats reduced white fat gain, increased skeletal muscle, decreased serum triglycerides, improved insulin sensitivity, and increased brown fat tissue (Jobgen et al., 2009).

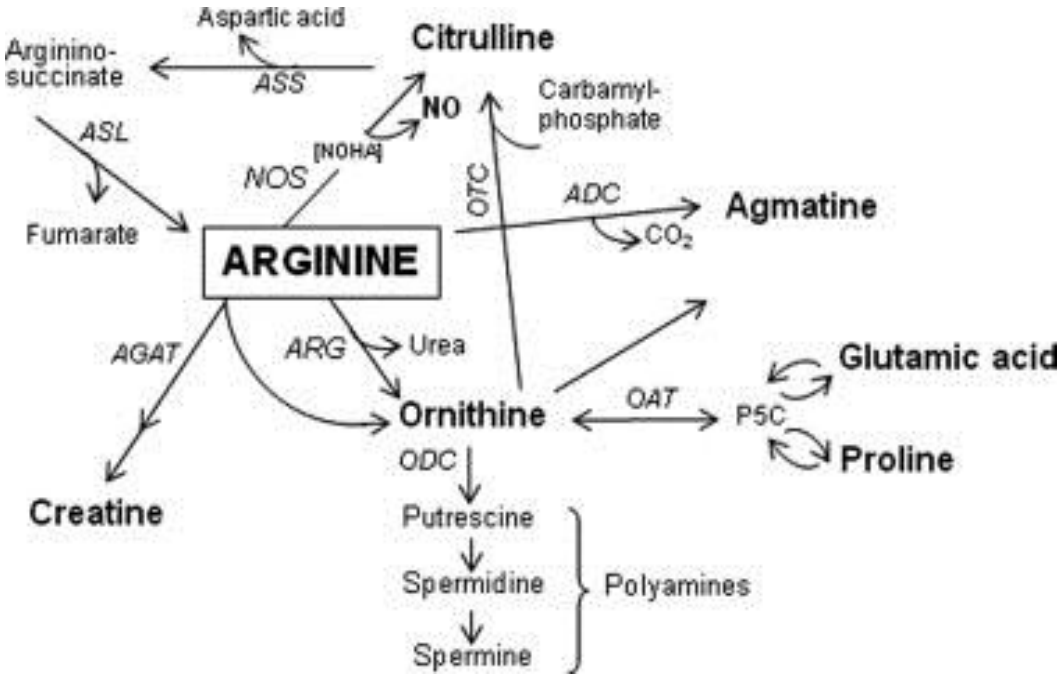

Figure 2. Arginine metabolic pathways. The enzymes involved in the main arginine metabolic pathways within endothelial cells are as follows: ADC = arginine decarboxylase; AGAT = arginine–glycine amidinotransferase; ARG = arginase; ASL= argininosuccinate lyase; ASS = argininosuccinate synthetase; NOS = nitric oxide synthase; OAT = ornithine aminotransferase; ODC = ornithine decarboxylase; OTC = ornithine transcarbamilase; P5C = Δ^1-pyrroline-5-carboxylate (adapted from Barilli et al., 2012).

Furthermore, arginine plays an important role in the immune system during pregnancy and in neonates. Arginine is necessary for adequate lymphocyte maturation and supplemental arginine has been found to improve immune response and decrease morbidity and mortality in neonatal pigs (Li et al., 2007). Along with the numerous other metabolic advantages of supplemental arginine that could benefit overnutrition mares, enhancing immune function could help to combat the compromised immune system in foals from overfed mares that have lower quality colostrum. Additionally, mare's milk has an extremely high concentration of arginine compared to other mammals, second only to feline milk in arginine, with concentration increasing in the transition from colostrum to milk while most other species decrease. This may suggest an increased requirement for arginine in horses compared to other species (Davis et al., 1994).

To date there is little research concerning the influence of maternal nutrition in horses, and the role of maternal dietary arginine supplementation in fetal development is also unknown. Only one study has investigated effect of arginine on foaling variables and gestation length in horses. Mortensen et al. (2011) supplemented Arg once daily at 1% of the diet beginning 21 d prior to expected foaling date and observed a shorter gestation length in Arg supplemented mares. However, the authors acknowledged that differences in gestation length may have been due to factors other than Arg supplementation, such as low number of animals per treatment (n = 8), or as a result of variables that have been shown to influence gestation length in previous studies, such as: season, breed, gender of fetus, parity, and age of mare (Mortensen et al., 2011).

Additionally, there was a lack of an isonitrogenous control diet which makes results difficult to attribute to arginine supplementation rather than simply variations in dietary nitrogen content. Therefore, the possible interaction of overnutrition of the mare along with arginine supplementation remains unknown and holds great potential for future research.

Implications

Over feeding is a common occurrence in the horse industry with obesity rates ranging from 45 to 50% (Stephenson et al., 2011). Common management practices in horses include feeding starch-rich cereal grains in large meals, which may promote insulin resistance (Hoffman et al., 2003a), and have been linked to obesity (Jeffcott et al., 1986). Furthermore, previous research has determined that reproductive efficiency, quantified as decreased time to onset of estrus and decreased number of cycles until conception, was enhanced beginning at a BCS 5 or greater (Henneke et al., 1984). Additionally, there were no observed detrimental effects to a higher BCS, and maintaining mares at a BCS 6 was recommended (Kubiak et al., 1987; Henneke et al., 1984). This encourages equine producers to maintain broodmares at a fleshier condition.

Furthermore, longer lifespans and production goals that encourage greater longevity in comparison to other livestock species result in increased incidence of metabolic disorders in horses comparable to metabolic diseases in humans (Fulop et al., 2006; Frank et al., 2010). Data from the National Health and Nutrition Examination Survey 2009-2010 showed that 35.7% of the total United States population and 31.9% of

women aged 20 to 39 are obese (defined as a body mass index ≥ 30; Ogden et al., 2012). Research in sheep indicates they may serve as an effective model for human disease (Ford et al., 2009), however the horse may be a more appropriate animal model for the aforementioned reasons. The increasing obesity epidemic in the human and horse populations, as well as the potential for arginine to mitigate negative effects of obesity allows for great opportunity for the horse to be used as an appropriate animal model for further investigation into the effects of maternal obesity.

CHAPTER II

INFLUENCE OF MATERNAL PLANE OF NUTRITION ON MARES AND THEIR FOALS: DETERMINATION OF MARE PERFORMANCE AND VOLUNTARY DRY MATTER INTAKE DURING LATE PREGNANCY USING A DUAL MARKER SYSTEM

Introduction

Little information exists concerning DMI in pregnant mares. Previous research in equines involving intake as a percent of BW indicated DMI was proportionate to energy requirements and not volume of the gastrointestinal tract (Frape et al., 1982). When fed a forage only diet, mature horses consumed 2.0% BW while yearlings consumed 2.5% BW in response to increased energy demands for growth (Aiken et al., 1989). Therefore, increased energy requirements in the last third of gestation could alter DMI of mares. Research in ruminants suggests DMI decreases as pregnancy progresses due to decreased rumen capacity with increased size of the uterus (Forbes, 1971; Forbes, 1986; Weston, 1982). However, research in Holsteins found no changes in capacity with advancing pregnancy (Park et al., 2011). Further research is necessary to determine what relationship exists between intake and pregnancy in the mare, particularly when fed high forage diets.

Direct measurement of voluntary DMI of hay is difficult when using large numbers of animals and total fecal collection. This method cannot be used in group housed animals, and warrants use of indigestible markers as an alternative strategy

(Sales, 2012). Several studies in other species have used dual marker systems to estimate digestibility and forage intake (Cochran et al., 1986). In previous equine studies, chromic oxide (Cr_2O_3) has been commonly used as an external marker, but is a carcinogen and not approved by the Food and Drug Administration as a dietary additive. Titanium dioxide (TiO_2) has been shown to be an acceptable digestibility marker in many species and is a safe alternative to Cr_2O_3 (Titgemeyer et al., 2001).

The objective of this study was to determine the effect of altered plane of nutrition on DMI of hay during the third trimester using a dual marker system and by evaluating mare performance.

Materials and Methods

Care, handling, and sampling of animals were approved by the Texas A & M University Animal Care and Use Committee.

Horses and Treatments

Thirty pregnant Quarter horse mares (538 to 695 kg BW; 4 to 19 yr of age) were blocked by expected foaling date and randomly assigned within block to one of two treatments: hay (H), or concentrate + hay (CH; concentrate fed at 0.75% BW as-fed; Vitality, Cargill Animal Nutrition, Elk River, MN; Table 1). All mares were allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay and water and were group housed by block. Treatments began 110 d prior to expected foaling date and terminated at parturition. All mares were brought in from the group housing twice daily (at 0545 and 1645) and placed in individual feeding stalls (3.0 × 3.0 m) in order to offer grain to CH mares. Mares receiving CH were given 45 min to consume grain per

feeding, and refusals were weighed, recorded, and used to determine calculations of DMI. Mares fed hay only were also placed in individual feeding stalls throughout this time although no concentrate was offered. This ensured access to hay in group housing was similar for both treatment groups. Grain offered to CH mares was adjusted according to change in BW every 14 d.

Table 1. Nutrient composition of texturized concentrate and coastal bermudagrass (*Cynodon dactylon*) hay fed to pregnant Quarter horses

Item	Concentrate[1]	Hay[2]
DM, %	89.9	91.9
	% DM basis	
CP	18.7	9.6
ADF	5.9	36.2
NDF	14.3	70.1
Fat	7.0	1.8
Ca	1.1	0.4
P	0.9	0.2
Mg	0.4	0.2
K	1.1	1.2
Na	0.6	0.1
DE,[3] Mcal/kg DM	3.5	2.4

[1]Concentrate consisted of a commercially available mare & foal texturized concentrate (grain products, plant protein products, processed grain by-products, roughage products) provided by Cargill Animal Nutrition (Elk River, MN).

[2]Hay consisted of coastal bermudagrass (*Cynodon dactylon*).

[3]DE calculated from equations in NRC (2007).

Mare Performance

Mare performance parameters (BW, BCS, and rump fat (RF)) were recorded every 14 d until parturition. Body weights were taken using a digital platform scale (CAS Corp., Seoul, Rep. of Korea), and BCS was determined by 3 individuals on a scale of 1 to 9 as described by Henneke et al.(1983) with 1 = poor and 9 = extremely fat. Rump fat measurements were obtained via ultrasound images (Aloka SSD-500V, Aloka

Inc., Tokyo, Japan) on the left hip at a point 5 cm dorsal of halfway between the first coccygeal vertebrae and the ischium (Westervelt et al., 1976). The last measurements obtained prior to foaling were considered a pre-partum measurement, and amount change over the feeding period calculated as the difference between the pre-partum and d 0 measurements. Additionally, mare BW was obtained 12 h post-parturition (post-partum BW), and amount change due to parturition was calculated as the difference between post-partum and pre-partum measurements.

Determination of Voluntary DMI

A dual marker system was utilized to estimate DMI using titanium dioxide (TiO_2) as the external marker and acid detergent insoluble ash (ADIA) as the internal marker (Titgemeyer et al., 2001). Intake was evaluated during 9, 10, and 11 months of pregnancy. Titanium dioxide was provided in 5 g boluses twice daily for 14 d. In previous studies external markers have been top-dressed on concentrate grain rations; however, this presented limitations for the current study's H treatment which received no concentrate. Previous studies in horses under pasture grazing conditions utilized boluses to administer Cr_2O_3, but reported some horses ejected or bit into the capsules, which resulted in an non-quantifiable loss of Cr_2O_3 (Parkins et al., 1982). Therefore, we offered TiO_2 in grain-based dosing devices (TDD) which were made in bulk quantities by the same group of individuals to maintain consistency throughout each TDD. Each TDD contained approximately 28 to 35 g DM of concentrate (Vitality, Cargill Animal Nutrition, Elk River, MN), 10 to 15 mL liquid molasses, and 2.5 g TiO_2. All mares were individually hand-fed 2 TDD prior to each meal to ensure complete consumption of the

marker. The TDD were readily accepted by mares in both treatment groups resulting in no refused TDD.

Fecal samples were obtained during the last 4 d of TiO_2 supplementation at 9 (d 26 to 28 of trial), 10 (d 52 to 56), and 11 (d 80 to 84) mo of gestation. Samples were collected twice daily via rectal palpation at 12 h intervals with times advancing 3 h each d to account for diurnal variation. A 200 to 400 g sample of feces was stored at -20°C for subsequent analysis. Samples of hay, grain, and TDD were collected at feeding times during each day of fecal collections and stored at -20°C for subsequent analysis.

All fecal, grain, hay, and TDD samples were analyzed for ADIA and TiO_2 concentrations. Samples were thawed and dried in a forced air oven (Lindberg/Blue M, Asheville, NC) at 60°C for 72 h then ground through a 1-mm screen (Thomas Scientific, Swedesboro, NJ). Samples were composited for each collection prior to analysis. The DM of grain, hay, and fecal samples was determined by drying for 24 h at 105°C in a forced air oven (Lindberg/Blue M). Concentrations of TiO_2 were determined by colorimetric assay using previously described methods (Titgemeyer et al., 2001; Short et al., 1996). Concentrations of ADIA were determined using an ANKOM-Fiber Analyzer (ANKOM-Technology, Fairport, NY) using techniques described by Llewellyn et al. (2006). Titanium dioxide concentration was used to estimate daily fecal production and ADIA was used to estimate DMI. Both values determined estimated DMI of hay.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Main effects tested were block, treatment, time, and treatment by time

interaction. Block was found to be insignificant and was removed from the model. A paired t-test was used to compare CH to H at individual time points. Means are reported as LSMeans ± SD. Probabilities less than or equal to 0.05 were considered statistically significant and less than or equal to 0.10 considered a trend towards significance.

Results

Mare Performance

Over the 110 d trial, mare BW tended to be greater ($P \leq 0.09$) for CH mares and BCS and RF were greater ($P \leq 0.01$, Table 2) than H. When evaluating change (last measurement prior to foaling minus d 0) BW and BCS were significantly affected by dietary treatment ($P \leq 0.01$) with CH mares gaining more BW, BCS, and RF than H mares (Table 2). However, there was no effect of treatment when mare BW was evaluated 12 h post parturition ($P \geq 0.19$), and no difference in BW change due to parturition ($P \geq 0.17$).

Table 2. Effect of dietary DE manipulation on mare performance (BW, BCS, and Rump Fat) during the last third of pregnancy (represented as LSMeans)

	Treatment[1]			*P*-value
Measurement	CH	H	SEM	Trt[2]
BW, kg				
d 0[3]	611.84	617.37	11.16	0.72
Pre-Partum[4]	637.20	609.30	11.74	0.09
Pregnancy change[5]	25.36	-8.07	4.04	< 0.01
Post-Partum[6]	557.94	538.97	10.21	0.19
Parturition change[7]	-79.27	-70.34	4.65	0.17
BCS[8]				
d 0[3]	7.01	7.19	0.12	0.26
Pre-Partum[4]	7.32	6.50	0.15	< 0.01
Pregnancy Change[5]	0.31	-0.69	0.10	< 0.01
Rump Fat, cm[9]				
d 0[3]	0.29	0.28	< 0.01	0.62
Pre-Partum[4]	0.31	0.29	< 0.01	0.01
Pregnancy Change[5]	0.03	0.01	< 0.01	0.12

[1]Treatments were applied beginning 110 d prior to expected foaling date and terminated upon parturition. CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.

[2]Trt = effect of treatment (CH or H).

[3]Measurement obtained on trial d 0, 110 d prior to expected foaling date.

[4] Last measurement obtained prior to parturition.

[5]Change represented as the difference between Pre-Partum and d 0 measurements.

[6]Measurement obtained 12 h post parturition.

[7]Change represented as the difference between Post-Partum and Pre-Partum measurements.

[8]Score represents average score of 3 evaluators using a 1 to 9 scoring system (Henneke et al., 1983).

[9]Rump fat measurements obtained on the left hip at a point 5 cm dorsal of halfway between the first coccygeal vertebrae and the ischium (Westervelt et al., 1976) using an ultrasound instrument (Aloka SSD-500V, Aloka Inc., Tokyo, Japan).

Intake

Hay DMI was affected by treatment, with H mares consuming a greater ($P < 0.01$) percentage of BW than CH mares (2.3% vs. 1.8% BW; Table 3). However, with

respect to total DMI, there was no influence of treatment with H mares consuming 2.3% BW compared to 2.4% BW in CH mares. Regardless of treatment, month of gestation influenced hay DMI (kg; $P \leq 0.05$) and total DMI (kg; $P \leq 0.05$) with all mares consuming less during the 10^{th} mo of gestation and a greater amount in the 11^{th} mo.

Using measure of nutritive value and calculations described in the NRC (2007), the concentrate was calculated to contain 3.45 Mcal/kg DE and hay contained 2.36 Mcal/kg. Using hay intakes estimated with the dual marker system, H mares consumed an average of 32.03 Mcal DE/d while CH mares consumed 38.95 Mcal DE/d. According to NRC (2007) values, all mares exceeded DE requirements for 9, 10, and 11 months gestation (23.1 to 25.7 Mcal) for horses with a mature BW of 600 kg.

Discussion

Mares supplemented with concentrate tended to be heavier and had greater pre-partum BCS and RF compared to mares fed only hay. Mares fed hay only began the trial at a BW of 617.37 kg ± 11.16 and a BCS of 7.19 ± 0.12, and had a final BW of 609.30 kg ± 11.74 and BCS of 6.50 ± 0.15. Although BCS decreased in H mares, all mares remained above a BCS of 6 which is considered appropriate for pregnant mares (Henneke et al., 1983; Henneke et al., 1984). Mares that conceive in a moderate body condition are expected to increase in BW by 12 to 15% during gestation (NRC, 2007). In the current study, H mares had a 1.3% decrease in BW. According to the NRC (2007), because fetal growth is greatest during the last 60 days of gestation, an increase in mare BW is expected and failure to gain weight indicates DE consumption was insufficient for tissue deposition.

Table 3. Effect of dietary DE manipulation on intake of concentrate, hay, and total DMI of pregnant Quarter horses during month 9, 10, and 11 of gestation (represented as LSMeans).

	Treatment[1]		SEM	*P*-values[2]		
Item	CH	H		Trt	Month	Trt×Month
Mare BW[3], kg	623.9	603.5	10.1	0.16	< 0.01	0.02
9 mo[4]	612.5^{A}	600.5^{A}	9.7	-	-	-
10 mo	624.7^{B}	602.5^{A}	10.2	-	-	-
11 mo	634.5^{C}	607.7^{B}	10.8	-	-	-
Concentrate DMI, kg	3.7	0.0	0.1	< 0.01	0.97	0.97
9 mo	3.6	0.0	0.1	-	-	-
10 mo	3.7	0.0	0.1	-	-	-
11 mo	3.7	0.0	0.1	-	-	-
Concentrate DMI, % BW	0.6	0.0	0.01	< 0.01	0.86	0.86
9 mo	0.6	0.0	0.02	-	-	-
10 mo	0.6	0.0	0.02	-	-	-
11 mo	0.6	0.0	0.02	-	-	-
Hay DMI, kg[5]	11.2	13.6	0.5	< 0.01	0.04	0.98
9 mo	11.2^{A,B}	13.2^{A,B}	0.8	-	-	-
10 mo	10.0^{A}	12.5^{A}	0.8	-	-	-
11 mo	12.3^{B}	14.8^{B}	1.1	-	-	-
Hay DMI, % BW	1.8	2.3	0.1	< 0.01	0.06	0.96
9 mo	1.8^{A,B}	2.2^{A,B}	0.2	-	-	-
10 mo	1.6^{A}	2.1^{A}	0.1	-	-	-
11 mo	1.9^{B}	2.4^{B}	0.2	-	-	-
Total DMI, kg	14.8	13.6	0.5	0.11	0.04	0.99
9 mo	14.8^{A,B}	13.4^{A,B}	0.8	-	-	-
10 mo	13.7^{A}	12.5^{A}	0.8	-	-	-
11 mo	16.0^{B}	14.8^{B}	1.1	-	-	-
Total DMI, % BW	2.4	2.3	0.1	0.32	0.06	0.95
9 mo	2.4^{A,B}	2.2^{A,B}	0.2	-	-	-

Table 3 Continued

Item	Treatment[1]		SEM	*P*-values[2]		
	CH	H		Trt	Month	Trt×Month
10 mo	2.2[A]	2.1[A]	0.1	-	-	-
11 mo	2.5[B]	2.4[B]	0.2	-	-	-
Dry Matter Digestibility, %	61.5	56.4	1.9	0.07	0.70	0.37

[1]CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.

[2]Trt = effect of treatment (CH or H). Month = month of gestation (9,10,11). Trt×Month = interaction.

[3]BW recorded immediately following fecal collections during each month of gestation.

[4]Fecal collections performed at the start of the 9th, 10th, and 11th month of gestation based on the average of the block. (CH: 9 mo: n = 15, 10 mo: n = 15, 11 mo: n = 13) (H: 9 mo: n = 15, 10 mo: n = 15, 11 mo: n = 14).

[5] Forage intake determined using a dual marker system with titanium dioxide as external marker and ADIA as internal marker (Titgemeyer et al., 2001).

[A-C]Values within column lacking a common superscript differ by $P \leq 0.10$.

Accordingly, mares will mobilize body stores to meet the needs of fetal development. We observed a decrease in BW and BCS in H mares from d 0 until parturition. However, there was no difference in post-partum BW, obtained 12 h post parturition, or in BW change due to parturition. Additional measurements obtained 12 h post parturition determined no effect of maternal diet on foal birth weight in kg or as a percentage of mare BW ($P \geq 0.34$; Winsco et al., 2010). Foals from CH mares weighed 49.71 ± 1.46 kg and were 8.8% of mare BW while foals from H mares weighed 48.90 ± 1.46 kg and were 9.2% of mare BW (Winsco et al., 2010). The decrease in BW and BCS in H mares during pregnancy indicates DE consumption was insufficient for late gestation and mares mobilized body stores to allow for normal fetal development.

Using the dual-marker estimated forage intake, H mares consumed an average of 32.03 Mcal DE/d while CH mares consumed 38.95 Mcal DE/d. According to NRC (2007) values, all mares exceeded DE requirements for the last 3 mo gestation (average of 24.3 Mcal) for horses with a mature BW of 600 kg. Accordingly, mares consumed approximately 130% and 160% of recommended DE for H and CH, respectively. However, H mares decreased BW and BCS as they approached parturition, indicating the diet was unable to support the requirements of late pregnancy. A previous study from our laboratory reported similar results, where mares received the recommended amounts of DE for gestation yet BCS and RF decreased (Thorson et al., 2010). The study by Thorson et al. (2010) was the first trial of this kind performed at our facility, and it was hypothesized that the apparently elevated DE requirement of the mares may be due to environmental conditions. Cold and heat stress occur at temperatures outside of the

thermoneutral zone (TNZ), which previous research has suggested is between 5 to 25^{o}C for mature horses (NRC, 2007). Temperatures outside of the TNZ may alter the resting metabolic rate and increase maintenance DE requirements. Temperature range throughout the duration of this trial (4^{o}C to36^{o}C) did fall below and above the TNZ depending on time of foaling; however, the average monthly temperatures for all months of the trial were within the TNZ. Additionally, a more recent trial at our facility also evaluated pregnant mare performance during late gestation on similar diets. We observed mares that received above NRC DE recommendations failed to gain the expected amount of weight during late gestation (Winsco et al., 2012). Based on these three trials, it appears that the NRC may underestimate DE requirements. The current study also indicates that coastal bermudagrass (*Cynodon dactylon*) hay alone may not support nutrient demands of late pregnancy. Currently, the NRC (2007) states that DE recommendations for pregnancy were based on calculated energy requirements for estimated tissue accretion during gestation, and DE recommendations for maintenance may be impacted by type of diet (NRC, 2007). High-forage diets alter heat of fermentation, and increase gastrointestinal tissue mass impacting energy requirements (Vermorel et al., 1997; NRC, 2007). Body weight of horses can be maintained at a lower DE when fed diets high in fat (Potter et al., 1989). Therefore it may be beneficial for future studies to more precisely evaluate DE requirements for mares in late gestation.

In ruminants, DMI is affected by gut fill and significant decreases in DMI are observed with advancing pregnancy (Forbes, 1971; Forbes, 1986; Weston, 1982; Allen, 1996). Uterine growth in late gestation may limit capacity of the rumen and therefore

DMI is reduced. However in Holstein dairy cattle, no alterations in ruminal capacity were observed, indicating factors other than capacity may reduce DMI during gestation (Park et al., 2011). Mechanisms controlling DMI in horses remains unclear. Frape et al. (1982) and Aiken et al. (1989) indicated that intake in horses was regulated by energy requirements rather than gut volume. Aiken et al. (1989) determined that yearlings consumed 2.5% BW and mature geldings voluntarily consumed 2% BW of coastal bermudagrass hay, when hay was the sole source of nutrients. Increased forage intake per unit BW in yearlings could be related to increased energy requirements for growth (Aiken et al., 1989). Additionally, ponies fed diets with diluted energy content due to addition of sawdust increased intake as caloric content decreased (Laut et al, 1985). Research regarding DMI in pregnant mares is extremely limited. If DMI in horses is linked solely to energy requirements, the increased DE requirement of late gestation would increase DMI of mares as pregnancy progresses when diet nutrient content is held constant. This does not agree with the results of the current study. Month of gestation altered forage intake (kg DM) and total intake (kg DM) with all mares consuming less during the 10^{th} mo of gestation and a greater amount in the 11^{th} mo (Table 3). Therefore based on results of the current study, it appears factors other than energy requirements dictate DMI. Mares fed only hay decreased BW and BCS as they approached parturition, and still decreased DMI in mo 10, therefore it appears energy requirements were not driving DMI.

Results from the current study indicate that DMI of hay decreased with the addition of concentrate to the diet, which agrees with previous research which

determined that feeding a mixed diet of forage and concentrate decreased forage DMI compared to a forage only diet (Vermorel et al., 1997). Vermerel et al. (1997) performed a series of 4 experiments feeding a forage only diet consisting of medium quality grassland hay (8.6 to 11.9% CP, 53.2 to 71.5% NDF, 27.8 to 37.6% ADF) or a diet consisting of 60 to 70% of the same grassland hay in addition to 30 to 40% of pelleted concentrate (10.5 to 12.7% CP, 10.9 to 13.6% NDF, 3.0 to 5.4% ADF). Our study fed coastal bermudagrass hay of similar nutritive value, and supplemented CH mares with concentrate of slightly higher values (Table 1).

The dual marker system allowed for calculation of DMI of hay in group housed horses, and our estimated intakes are comparable with previous data. The NRC (2007) estimates DMI for 500 to 600 kg pregnant broodmares to be 2.0% BW, and the previous NRC (1989) recommended pregnant mares consume 1.0 to1.5% BW in forage and 0.5 to 1.0% BW in concentrate. A previous study directly measured individual voluntary intake of timothy and alfalfa hay in pregnant mares at 5 and 10 mo gestation and found that mares consumed an average of 2.02% BW at 5 mo and 1.9% BW at 10 mo gestation (McCown et al., 2011). These values are comparable to intakes observed in the current study during 10 mo gestation where CH mares consumed 1.61% BW and H mares consumed 2.08% BW in forage (Table 3). McCown et al. (2011) provided a balancer pellet at 0.90 kg per day. This supplementation is greater than the H treatment of our study, and less than the CH treatment, which may explain why the hay intake measured by McCown et al. (2011) falls between the range calculated in our study.

Direct measurement of voluntary DMI of hay cannot be used in group housed animals, and warrants use of indigestible markers as an alternative strategy (Sales, 2012). Thus, alternative methods must be utilized to estimate intake indirectly by dividing total fecal excretion by indigestible nutrients from the diet (Dove and Mayes, 1991). Markers may underestimate intake due to external markers overestimating fecal output and internal markers underestimating digestibility. In the current study, estimated intakes are comparable to previous studies that measured actual intakes, therefore it appears that markers did not underestimate intake. Chromic oxide is a commonly used external marker in equine and ruminant studies (Patterson et al., 2001; Frape et al., 1982). However, chromic oxide is a carcinogen, and it is not approved by the FDA as a feedstuff. A suggested alternative to chromic oxide is TiO_2, which is not a hazardous substance and can be legally added to feedstuffs up to 1% of the finished product (AAFCO, 1996). Titanium dioxide has been used as an alternative digestibility marker in cattle and sheep (Titgemeyer et al., 2001; Myers et al., 2006; Glindemann et al., 2009), pigs (Jagger et al., 1992) and chickens (Short et al., 1996). Hafez et al. (1988) concluded TiO_2 has a 99% fecal recovery rate when fed to dairy cows, and Titgemeyer et al. (2001) found that fecal recovery averaged 93% when fed with forage and grain. The actual intake and fecal output were not determined in the current study, so it cannot be determined whether or not TiO_2 over or underestimated intake or fecal output.

Although more research is needed to validate the use of TiO_2 and ADIA for estimating forage intake in group-housed horses, the total intake values and forage intake amounts calculated in the current study are within reasonable physiologic values and are

in agreement with previous studies. Furthermore, mares decreased forage intake as concentrate was added to the diet, which is also in agreement with previous research (Vermorel et al., 1997). This indicates that the dual marker system utilized in the current study was sufficient at estimating forage intake in group- housed mares.

Furthermore, these data indicate that alteration of maternal plane of nutrition of mares in late gestation influenced mare performance and altered DMI. Based on calculations, all mares exceeded NRC (2007) DE recommendations but decreased BW and BCS during gestation, underscoring a need for future work investigating DE requirements during pregnancy on a forage based diet. Additionally, DMI was influenced by time, with all mares consuming less in the 10^{th} month of pregnancy compared to the 11^{th} month which suggests DMI in pregnant mares was not driven solely by the increased energy demands of late gestation. Continued research investigating manipulation of maternal nutrition and its effects on voluntary intake would be beneficial to more completely understand the relationships of these observations.

CHAPTER III

INFLUENCE OF MATERNAL PLANE OF NUTRITION ON MARES AND THEIR FOALS: EVALUATING MARE HORMONES DURING LATE GESTATION, FOALING PARAMETERS, COLOSTRUM QUALITY, AND FOAL PASSIVE TRANSFER

Introduction

Previous research has documented that the fetus is sensitive to nutrition of the dam during pregnancy (Wu et al., 2006). Both over- and undernutrition may alter fetal development. Reduced maternal nutrient supply can have lasting effects on development with consequences such as reduced neonatal health, growth, and athletic performance (Rossdale and Ousey, 2002). While the impact of maternal overnutrition in the horse has not been thoroughly investigated, over feeding is a common occurrence with obesity rates from 45 to 50% (Stephenson et al., 2011). Obesity in mares as a result of overfeeding causes a reduction in fetal growth and occasional fetal death (Pugh, 1993). In other species, overnutrition has negative effects on uterine environment and fetal growth (Wu et al., 2006). In sheep, maternal overnutrition resulted in increased incidences of abortion or still births and decreased gestational length, which may be due to alterations in placental secretion of hormones such as progesterone (Wallace et al., 2001). Overfeeding has also been shown to reduce the initial prenatal colostrum volume in sheep (Davidson et al., 2000) and lower the concentrations of IgG and crude protein (Wallace et al., 2001). Ewes fed 140% of nutrient requirements had decreased colostrum

volume and weight compared to ewes fed 100% of requirements (Meyer et al., 2011). A similar study in horses reported reduced IgG concentration in colostrum of mares fed increased DE during late gestation (Thorson et al., 2010). The negative consequences of maternal overnutrition encouraged further investigation into the effects of dietary energy manipulation on mares and their foals. Therefore, the objective of this study was to determine the effect of maternal nutrition during the last third of pregnancy on mares and their foals by evaluating mare performance parameters and hormones, foaling parameters, colostrum volume and quality, foal passive transfer of immunity, and foal physical measurements.

Materials and Methods

Care, handling, and sampling of animals were approved by the Texas A & M University Animal Care and Use Committee.

Horses and Treatments

Thirty pregnant Quarter horse mares (538 to 695 kg BW; 4 to 19 yrs of age) were blocked by expected foaling date and randomly assigned within block to one of two treatments: hay (H), or concentrate + hay (CH; concentrate fed at 0.75% BW as-fed; Vitality, Cargill Animal Nutrition, Elk River, MN; Table 4). All mares were allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay and water and were group housed by block. A dual marker system utilizing titanium dioxide and acid detergent insoluble ash was used to calculate voluntary forage intake at 9, 10, and 11 mo gestation (See Chapter II). Treatments began 110 d prior to expected foaling date and

terminated at parturition. All mares were brought into individual feeding stalls (3.0 × 3.0 m) twice daily (at 0545 and 1645) in order to offer grain to CH mares. Mares receiving CH treatment were given 45 min to consume grain per feeding, and refusals were weighed and recorded. Grain intake for CH mares was adjusted according to change in BW every 14 d.

Table 4. Nutrient composition of texturized concentrate and coastal bermudagrass (*Cynodon dactylon*) hay (DM basis) fed to pregnant Quarter horses

Item	Concentrate[1]	Hay[2]
DM, %	89.9	91.9
CP, %	18.7	9.6
ADF, %	5.9	36.3
NDF, %	14.3	70.1
Fat, %	7.0	1.8
Ca, %	1.1	0.4
P, %	0.9	0.2
Mg, %	0.4	0.2
K, %	1.1	1.2
Na, %	0.6	0.1
DE,[3] Mcal/kg DM	3.5	2.4

[1]Concentrate consisted of a commercially available mare & foal texturized concentrate (grain products, plant protein products, processed grain by-products, roughage products) provided by Cargill Animal Nutrition, Elk River, MN).

[2]Hay consisted of coastal bermudagrass (*Cynodon dactylon*).

[3]DE calculated from equations in NRC (2007).

Mare Hormones and Performance

Mare performance parameters (BW, BCS, and rump fat (RF)) were recorded and blood samples were collected every 14 d until parturition. Body weights were taken using a digital platform scale (CAS Corp., Seoul, Rep. of Korea), and BCS was determined by 3 individuals on a scale of 1 to 9 as described by Henneke et al. (1983)

with 1 = poor and 9 = extremely fat. Rump fat measurements were obtained via ultrasound images (Aloka SSD-500V, Aloka Inc., Tokyo, Japan) on the left hip at a point 5 cm dorsal of halfway between the first coccygeal vertebrae and the ischium (Westervelt et al., 1976).

Blood samples were obtained prior to morning feeding via jugular venipuncture into evacuated tubes containing either EDTA or sterile non-additive tubes (Becton-Dickinson, Franklin Lakes, NJ). Blood samples collected into tubes containing EDTA were immediately placed on ice until centrifugation at 2,700 × *g* for 20 min at room temperature. Blood samples collected into sterile non-additive tubes were allowed to coagulate for 1 h at room temperature prior to centrifugation at 2,700 × *g* for 20 min at room temperature. Plasma was harvested from tubes containing EDTA and stored at -20°C for subsequent determination of progesterone (P4). Serum was harvested from sterile non-additive tubes, and samples were stored at -20°C for subsequent determination of estradiol (E2), and cortisol (CORT). All hormone concentrations were determined by chemiluminescence immunoassay (Immulite 1000, Siemens). The coefficients of variation within assays were 8.09%, 6.56%, and 5.76% for P4, E2, and CORT, respectively.

Foaling Parameters, Colostrum Quality, and Passive Transfer

All mares were monitored for signs of impending parturition and brought into individual stalls when foaling appeared to be imminent. Parturition was observed and the following foaling parameters were recorded: time of water break to birth, time from birth to stand, and time of birth to placenta expulsion. Total length of gestation was calculated

and placenta weight was recorded. Immediately after parturition mares were stripped of their initial volume of colostrum by hand-milking. Colostrum volume (CV), specific gravity (SG), and Brix% of colostrum were measured immediately after milking, and samples were stored at -20°C for subsequent determination of IgG concentration. Within 1 h after birth, foals were administered 250 mL of their dam's colostrum via nasogastric tube and then allowed to nurse ad libitum. To evaluate passive transfer of immunity, blood samples were obtained via jugular venipuncture into sterile non-additive tubes (Becton-Dickinson, Franklin Lakes, NJ.) immediately prior to colostrum dose (0 h), and at 6, 12, 18, and 24 h post. Blood samples were allowed to coagulate for 1 h at room temperature prior to centrifugation at 2,700 × *g* for 20 min at room temperature. Serum was harvested, and samples were stored at -20°C for subsequent determination of IgG concentration. Colostrum and serum samples were analyzed for IgG concentration using a portable clinical laboratory analyzer (Quick Test II, Midland Bioproducts, Boone, IA). The coefficients of variation within assays were ≤ 10%. Physical measurements of mares and foals were obtained 12 h post parturition and included mare BW, foal BW, foal wither and hip height, and foal body length.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Main effects tested were block, treatment, time, and treatment by time interaction. Block was found to be insignificant and was removed from the model. A paired t-test was used to compare CH to H at individual time points. Means are reported

as LSMeans ± SD. Probabilities less than or equal to 0.05 were considered statistically significant and less than or equal to 0.10 considered a trend towards significance.

Results

Mare Intake, Performance, and Hormones

A dual marker system utilizing titanium dioxide and acid detergent insoluble ash was used to calculate voluntary forage intake at 9, 10, and 11 mo gestation and determined intake of hay was influenced by treatment ($P < 0.01$), with CH mares consuming 1.79% BW while H mares consumed 2.25% BW. The total dietary intake was not different between treatments ($P = 0.32$) with CH mares consuming 2.38% BW while H mares consumed 2.25% BW (See Chapter II).

Mare BW tended to be greater ($P \leq 0.09$) and BCS and RF were greater ($P \leq 0.01$) for CH mares (See Chapter II). Mares on CH gained BW, BCS, and RF throughout gestation (evaluated as last measurement prior to foaling minus d 0) while H mares lost BW and BCS; however, there was no effect of treatment when mare BW was evaluated 12 h post parturition ($P \geq 0.19$), and no difference in BW change due to parturition ($P \geq 0.17$ See Chapter II).

There was a tendency for a treatment by time interaction ($P < 0.10$) with plasma P4 concentrations rising sharply in CH mares beginning at d 70 and being greater ($P < 0.05$) than H mares at d 84 (Table 5; Figure 3). Regardless of diet, plasma P4 concentrations increased steadily over time ($P < 0.01$) from d 70 to d 112 (2.26 to 8.61 mg/ml; Figure 3) in all mares. There was a treatment by time interaction in serum E2

concentrations ($P < 0.05$) with CH mares having lower ($P < 0.05$) E2 concentrations than H mares at days 56 and 70 (Table 5; Figure 3). In addition, serum E2 concentrations decreased sharply over time in all mares, regardless of diet ($P < 0.01$), from d 42 to d 70 (311.23 to 223.98 pg/ml; Figure 4). Serum CORT was also influenced by time ($P < 0.01$) with concentrations rising sharply prior to parturition (Table 3; Figure 5), and there was a treatment by time interaction ($P < 0.05$) with H mares having a rapid decline in plasma CORT until d 42 (Table 5; Figure 5).

Table 5. Effect of dietary DE manipulation on hormone (progesterone, estrogen, cortisol) concentrations of pregnant Quarter horses during the last third of pregnancy (represented as LSMeans)[1].

	Treatment[2]		SEM	*P*-values[3]		
Item[4]	CH	H		Trt	Time	Trt×Time
Progesterone, ng/mL	3.98	2.71	0.60	0.14	< 0.01	0.08
Estrogen, pg/mL	205.90	270.36	27.21	0.11	< 0.01	0.03
Cortisol, ug/dL	4.71	4.58	0.50	0.85	< 0.01	< 0.01

[1]Blood samples harvested every 14 d beginning 110 d prior to expected foaling date and terminating at parturition

[2]Treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.

[3]Trt = effect of treatment (CH or H). Time = effect of time. Trt×Time = interaction.

[4]Hormone concentrations determined by chemiluminescence immunoassay (Immulite 1000, Siemens).

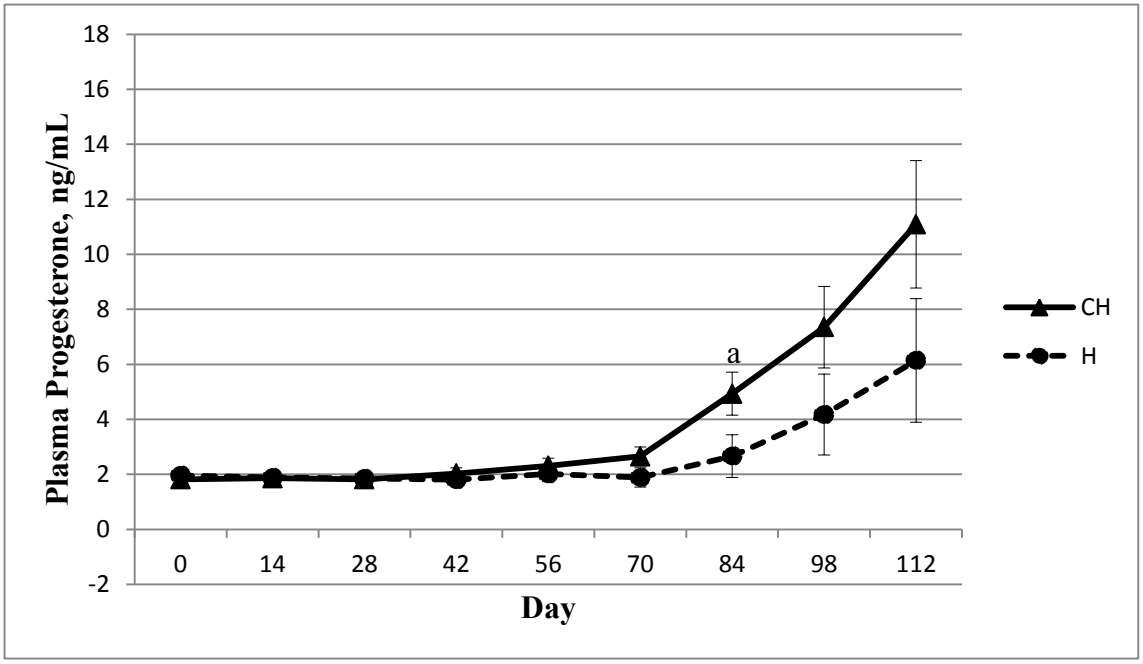

Figure 3. Effect of dietary DE manipulation on mare plasma progesterone concentrations during the last third of pregnancy. Treatments began 110 d prior to expected foaling date (d 0) and terminating at parturition. Treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Within day, the letter "a" indicates a treatment difference ($P < 0.05$) for CH vs. H.

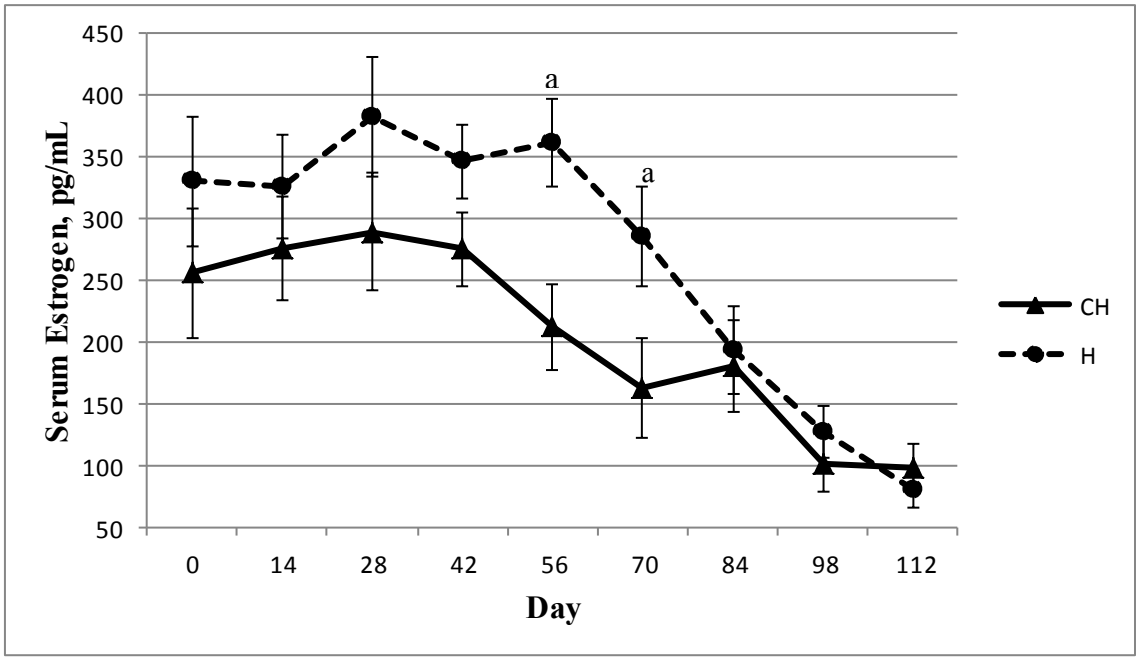

Figure 4. Effect of dietary DE manipulation on mare serum estrogen concentrations during the last third of pregnancy. Treatments began 110 d prior to expected foaling date (d 0) and consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Within day, the letter "a" indicates a treatment difference ($P < 0.05$) for CH vs. H.

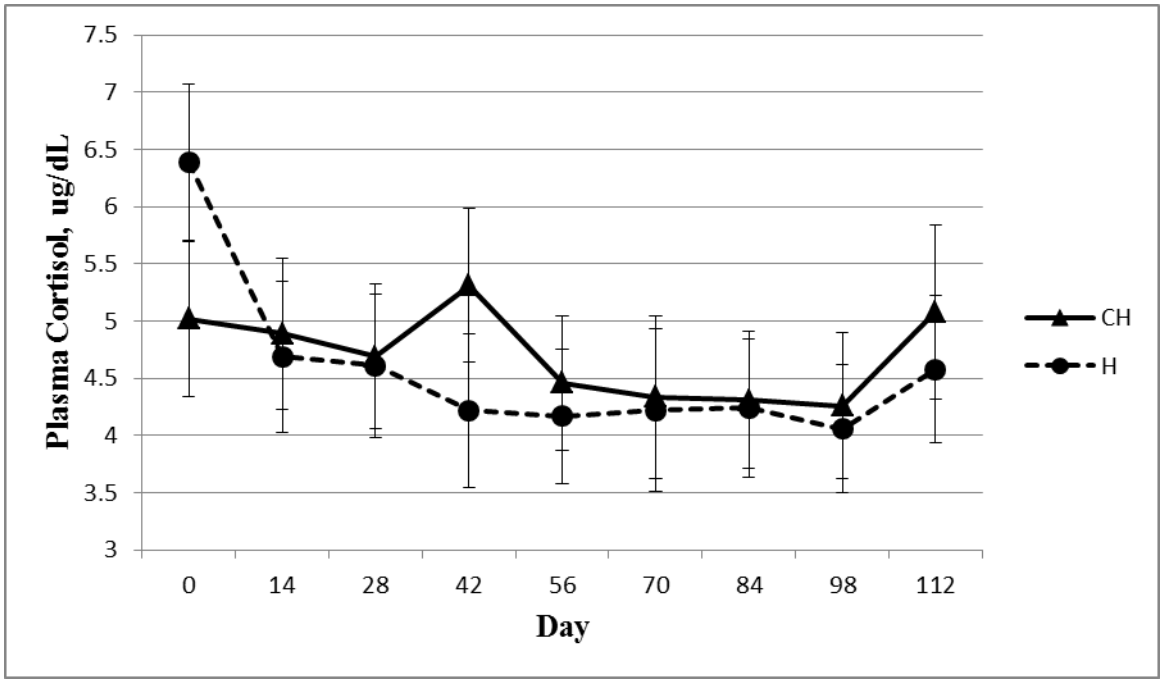

Figure 5. Effect of dietary DE manipulation on mare plasma cortisol concentrations during the last third of pregnancy. Treatments began 110 d prior to expected foaling date (d 0) and consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.

Foaling Parameters

There was no influence of dietary treatment on foaling parameters; however, time from birth to placenta expulsion tended ($P = 0.06$) to be longer in H mares (Table 6). There was also no effect of treatment ($P \geq 0.46$) on physical measurements obtained following parturition, although foals from H mares tended ($P = 0.06$) to exhibit greater hip height compared to foals from CH mares (Table 7). Ratio of placenta to mare BW, placenta to foal BW, and the ratio of foal to mare BW were not affected by treatment ($P \geq 0.16$; Table 6).

Table 6. Effect of dietary DE manipulation on gestation length and foaling parameters of pregnant Quarter horses (represented as LSMeans)

	Treatment[1]		SEM	*P*-value[2]
Measurement	CH	H		Trt
Gestation length[3], d	326.47	332.33	3.25	0.68
Time from:				
WB-Birth[4], min	12.88	13.38	2.21	0.87
Birth-PE[5], min	66.51	173.83	40.31	0.06
Birth-Stand[6], min	52.19	45.85	6.26	0.51
Weight Ratios[7]				
Placenta: Mare,%	0.83	0.99	0.01	0.16
Placenta: Foal,%	8.50	8.60	0.01	0.96
Foal: Mare,%	8.80	9.20	0.01	0.34

[1] CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.

[2]Trt = effect of treatment (CH or H).

[3]Calculated from last breeding date to foaling date. (CH n = 15; H n = 12).

[4]WB-Birth: time from water breaking to complete passage of foal through birth canal. (CH n = 12; H n = 10).

[5]Birth-PE: time from complete passage of foal through birth canal to the complete expulsion of the placenta. (CH n = 13; H n = 10).

[6]Birth-Stand: time from complete passage of foal through birth canal to foal standing for ≥ 10 seconds. (CH n = 12; H n = 10).

[7] Placenta weight recorded immediately following expulsion. Mare and foal BW measured 12 h after parturition. (CH n = 14; H n = 12). Placenta: Mare = Ratio of the placenta to mare BW. Placenta: Foal = Ratio of the placenta to foal BW. Foal: Mare = Ratio foal to mare BW.

Table 7. Effect of maternal dietary DE manipulation on Quarter horse foal physical measurements obtained 12 h post parturition (represented as LSMeans)

	Treatment[1]		SEM	*P*-value[2]
Measurement	CH (n = 15)	H (n = 12)		Trt
Body Weight, kg	49.71	48.90	1.46	0.68
Body Length, cm	75.78	76.46	0.90	0.59
Wither Height, cm	95.30	96.15	0.84	0.46
Hip Height, cm	96.17	98.21	0.77	0.06

[1] CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.
[2]Trt = effect of treatment (CH or H).

Colostrum Characteristics and Foal Passive Transfer

There was an influence of dietary treatment on colostrum quality indicated by greater ($P \leq 0.05$) specific gravity and refractometer values (Brix %) and a tendency for increased IgG concentrations ($P = 0.09$) in H mares compared to CH (Table 8). Although colostral IgG concentration tended to be different between treatment groups, colostrum volume and total colostral IgG (calculated from volume and concentration) were not different ($P \geq 0.27$; Table 8). Average colostral IgG concentrations were 162.55 g IgG/L for H mares and 123.59 g IgG/L for CH mares (Table 8). Foal serum IgG concentrations were not different ($P \geq 0.63$) among foals from H and CH mares (Figure 6). Foals from both groups achieved IgG concentrations indicative of passive transfer of immunity by 6 hr of age (H = 11.7 g IgG/L, CH = 9.3 g IgG/L; Figure 4).

Table 8. Effect of dietary DE manipulation in pregnant Quarter horses during the last third of pregnancy on colostrum volume and quality (represented as LSMeans)

	Treatment[1]		SEM	*P*-value[2]
Measurement	CH (n = 13)	H (n = 10)		Trt
Total Volume, mL	1314.60	1215.50	157.00	0.66
Brix, %[3]	25.38	29.40	1.09	0.02
Specific Gravity[4]	1.03	1.05	<0.01	0.03
IgG, g/L[5]	123.59	162.55	15.60	0.09
Total IgG, g[6]	156.50	201.60	27.50	0.27

[1] CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.

[2]Trt = effect of treatment (CH or H).

[3]Measure of dissolved sugars in a liquid. Equine Colostrum Refractometer (Animal Reproduction Systems, Chino, CA).

[4]Measured by Equine Colostrometer (Lane Manufacturing, Denver, CO).

[5]Colostrum samples analyzed for IgG concentration via clinical laboratory analyzer (Midland Bioproducts, Boone, IA).

[6]Total IgG (g) calculated by volume (L) × concentration (g/L).

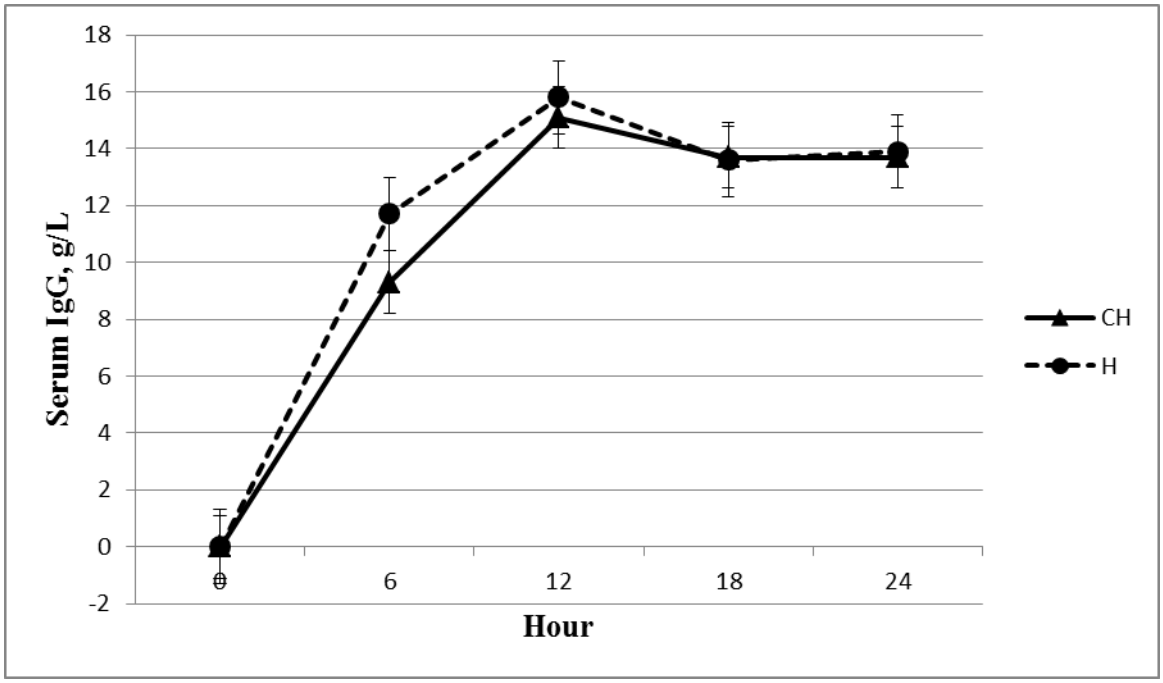

Figure 6. Effect of maternal dietary DE manipulation on foal serum IgG concentrations. Treatments were applied to the dams during last trimester of pregnancy, and consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Effect of treatment: $P \geq 0.63$. Effect of time: $P < 0.01$.

Discussion

Mares supplemented with concentrate tended to be heavier and had greater pre-partum BCS and RF compared to mares fed only hay. Mares fed hay only began the trial at a BW of 617.37 kg ± 11.16 and a BCS of 7.19 ± 0.12, and had a final BW of 609.30 kg ± 11.74 and BCS of 6.50 ± 0.15 (See Chapter II). Mares that conceive in a moderate BCS are expected to increase in BW by 12 to 15% during gestation (NRC, 2007). Mares fed only hay decreased by 1.3 % BW, which indicates that although there was no difference in mare BW between treatment groups, the growth of the foal during the last

third of pregnancy affected the BW of the mare. According to the NRC (2007), because fetal growth is greatest during the last 60 d gestation, an increase in mare BW is expected. Failure to gain weight results in mares mobilizing body stores to meet the needs of fetal development. We observed a decrease in BW and BCS in H mares from d 0 until parturition, supporting the idea that they were mobilizing body stores to meet fetal growth. Although BCS decreased in H mares, all mares remained above a BCS of 6 which is considered appropriate for pregnant mares (Henneke et al., 1984). For more information on mare performance, see Chapter II.

Previous research has documented that alterations in maternal nutrition may affect the endocrine system during pregnancy (Fowden et al., 2005). In situations of maternal overnutrition, hormone concentrations are usually below normal levels (Dziuk, 1992). Research in ovariectomized females determined decreases in progesterone in overfed states (ewes fed twice maintenance requirements; gilts fed 3 kg versus 1 kg per day) are caused by an increase in metabolic clearance rate (Parr et al., 1993; Prime and Symonds, 1993). In over fed pregnant pigs, administration of exogenous progesterone enhances embryo survival rates (Jindal et al., 1997). Furthermore, overnutrition in sheep has been found to result in a decreased gestational length, which is likely caused by the decreased progesterone concentrations (Wallace et al., 2001). In the current study, there was no effect of maternal nutrition on progesterone concentration or gestation length.

In this study, dietary treatment did not affect mare hormone concentrations prior to parturition, and all remained within physiologically normal ranges for mares in late gestation. Using the calculated forage intake, H mares consumed an average of 32.03

Mcal DE/d while CH mares consumed 38.95 Mcal DE/d (See Chapter II). According to NRC (2007) values, all mares exceeded DE requirements for the last 3 mo gestation (average of 24.3 Mcal) for horses with a mature BW of 600 kg. This results in approximately 130% and 160% of recommended DE in H and CH mares, respectively. Although all mares were exceeding DE recommendations, H mares decreased BCS and BW. Similar nutritional planes have caused the same response in BCS in previous studies at this facility (Thorson et al., 2010). Based on the changes in BCS and BW observed in the mares in this study, it is unlikely that they were significantly exceeding maintenance requirements to create an effect on hormone concentrations.

In mares, total progestagen concentrations increase during the last few weeks prior to parturition, which is associated with mammary gland development (Ousey et al., 2005; Ousey, 2004). There are numerous progestagens in pregnant mare plasma, therefore assays using an antibody raised against P4 usually cross-react with other progestagens and measure total progestagens rather than P4 specifically (Ousey, 2006). The authors acknowledge the values reported in this study more likely reflect total progestagens although the assay utilized an antibody raised against P4. Previous studies have measured progesterone concentrations ranging from 3 to 22 ng/mL (Haluska and Currie, 1988; Ousey, 2006). In the current study, progesterone concentrations ranged from 1.82 ng/mL to 11.09 ng/mL from trial d 0 to d 112, which corresponds to the last third of gestation. Samples were taken every 14 d until parturition so the last sample obtained from each mare ranged from 1 to 13 d prior to parturition. Progesterone concentration peaks during the last few days, or even hours, prior to parturition (Fowden

et al., 2008; Ousey, 2004). Therefore, the lower concentrations of progesterone measured in the current study are likely due to the sampling interval and the likelihood of missing peak concentration prior to foaling.

Total estrogen concentration in pregnant mares declines gradually throughout the end of gestation (Fowden et al., 2008; Ousey, 2006). Mares in the current study exhibited normal pattern and concentration of estrogen concentration compared to previously reported values (Haluska and Currie, 1988; Ousey, 2006; Fowden et al., 2008). There was no influence of dietary treatment on mare cortisol concentrations. Cortisol concentration was influenced by time, with H mares decreasing from d 0 to d 14, and all mares increasing prior to parturition. There is a pre-partum surge in fetal cortisol concentration; however previous studies have conflicting results on maternal cortisol prior to parturition with some studies finding no increases in maternal cortisol and others having increased cortisol days before parturition (Silver and Fowden, 1994; Hoffman et al., 1996; Cudd et al., 1995).

Foaling variables and foal and mare physical measurements did not differ between treatments; although the time from birth to placenta expulsion tended to be longer in H mares. Similar results have been observed in dairy cattle, with cows of a lower BCS having increased risk of retained placenta (Markusfeld et al., 1997; Hoedemaker et al., 2009). Although H mares tended to have a longer time to placenta expulsion, all mares expelled their placentas within 180 min, the time cut-off for classification as a retained placenta (Samper, 2009).

Previous research in mares evaluating effects of increasing BCS as well as diets of restricted DE intake also observed no effect on foaling parameters or foal birth weight in kg or as a percentage of mare BW (Banach and Evans, 1981; Kubiak et al., 1988; Guay et al., 2002; Heidler et al., 2004). The NRC (2007) estimates foal birth weight at 9.7% of nonpregnant mare weight. This study found comparable foal birth weight as a percent of nonpregnant mare weight (weights taken 12 h post parturition) with 8.8% for CH mares and 9.2% for H mares. These results further support the idea that when maternal nutrition is altered, nutrients may be preferentially partitioned to the foal.

Overfeeding can have negative consequences on colostrum quality and has been found to reduce the initial volume of colostrum that accumulates prenatally in sheep (Davidson et al., 2000). Colostrum from ewes on a higher plane of nutrition had reduced concentrations of IgG compared to dams on a more moderate plane of nutrition (Wallace et al., 2001). In the current study, plane of nutrition influenced colostrum quality indicated by H mares having greater Brix % values and specific gravity and a trend for increased IgG concentration in H mares compared to CH. A recent study conducted in our laboratory utilizing a similar herd and dietary treatments also determined mares fed hay only had a greater Brix % compared to mares supplemented with concentrate (Thorson et al., 2010). However, unlike the current study, specific gravity did not differ across dietary treatments (Thorson et al., 2010). These results indicate mares on a decreased plane of nutrition may partition nutrients preferentially toward milk synthesis. This is also supported by results of a study by Guay et al. (2002) who reported that mares receiving a diet with less crude protein had greater milk crude protein

concentrations at parturition compared to mares receiving higher levels of dietary crude protein. Also, it should be noted that while there were dietary treatment effects on colostrum quality estimates, all values were considered to be adequate for Brix % and specific gravity. Previous research evaluating effects of maternal nutrition on mare colostrum volume is limited. Research in sheep fed increased DE throughout late gestation found colostrum volume and total g IgG were lower in dams consuming a greater amount of DE (Swanson et al., 2008). Previous research in cattle and sheep suggest differences in colostrum volume and quality as a result of overnutrition may be related to inadequate alveolar development or a greater deposition of adipose tissue compared to epithelial tissue within the mammary gland (Sejrsen et al., 2000; Flint et al., 2005).

Although concentration of IgG tended to be lower in colostrum of mares fed CH, calculated total g of IgG was not different. Previous research in sheep indicates maternal nutrition throughout gestation can affect the offspring's ability to absorb IgG. Lambs from undernourished ewes (60 % nutrient requirements) had increased serum IgG at 24 h compared to lambs from ewes fed 100% or 140% nutrient requirements (Hammer et al., 2011). In our study, foal serum IgG concentrations were not different among foals from H and CH mares. To minimize morbidity and mortality, it is recommended that plasma IgG concentrations reach a minimum of 4.0 to 8.0 g IgG/L by 24 h of age (LeBlanc et al., 1992). Colostrum with a specific gravity greater than or equal to 1.06 has been found to contain an adequate concentration of IgG resulting in foals with serum IgG concentrations of 5.2 g/L or higher (LeBlanc et al., 1986). Mares fed hay only had

specific gravity of 1.05 while mares supplemented with concentrate had specific gravity of 1.03, however foals from these mares were still able to achieve adequate serum concentration of IgG by 6 hr of age (H = 11.7 g IgG/L, CH = 9.3 g IgG/L). All foals were provided with 250 mL of their dam's colostrum via nasogastric tube within 1 h of birth, and this standardization of initial feeding may have contributed to the high passive transfer level even though colostrum specific gravity was lower than the recommended level of 1.06.

In summary, these data indicate that alteration of maternal plane of nutrition of mares in late gestation influenced mare performance. Maternal plane of nutrition did not influence foaling parameters or foal physical characteristics. Dietary treatment of the mare affected colostrum quality estimates and mares fed CH tended to have lower colostral IgG concentration, but maternal nutrition did not affect volume of colostrum or total g IgG, and as a result did not influence foal passive immunity. Continued research investigating manipulation of maternal nutrition and its effects on mares in late gestation and any influences on characteristics of parturition and colostrum would be beneficial to more completely understand the relationships of these observations.

CHAPTER IV

INFLUENCE OF MATERNAL PLANE OF NUTRITION ON MARES AND THEIR FOALS: GLUCOSE AND INSULIN DYNAMICS

Introduction

Research indicates maternal nutrition influences fetal development, and the fetus is sensitive to nutrition of the dam during pregnancy (Wu et al., 2006). Developmental programming can alter fetal growth with external stimuli such as reduced nutrient and blood supply to the fetus. Overnutrition has negative effects on uterine environment and fetal growth (Wu et al., 2006). Research in this area in horses is extremely limited. However, over feeding is a common occurrence in the horse industry with obesity rates ranging from 45 to 50% (Stephenson et al., 2011). Common management practices in horses include feeding starch-rich cereal grains in large meals, which may promote insulin resistance (Hoffman et al., 2003a), and has been linked to obesity (Jeffcott et al., 1986).

Furthermore, during the last third of pregnancy mares are insulin resistant and have decreased glucose effectiveness, but diet can further alter glucose and insulin dynamics (George et al., 2011). Research in sheep determined that obesity decreased insulin sensitivity and concentration of insulin receptors in peripheral tissue (Bergman et al., 1989). Maternal obesity in sheep resulted in maternal and fetal hyperglycemia and hyperinsulinemia, as well as increasing weight of the fetal pancreas and number of insulin positive cells per unit area of fetal pancreas (Ford et al., 2009). Research in

horses in this area is extremely limited. A study evaluated effects of maternal diet on insulin and glucose metabolism of foals, and determined that a high starch maternal diet resulted in greater baseline glucose and insulin concentrations and a tendency for decreased insulin sensitivity of foals at 160 days of age (George et al., 2009). Therefore, the objective of the current study was to further investigate impact of maternal plane of nutrition on glucose and insulin dynamics of mares during late gestation and in their foals, as well as determine effects of maternal plane of nutrition on foal physical growth.

Materials and Methods

Care, handling, and sampling of animals were approved by the Texas A & M University Animal Care and Use Committee.

Horses and Treatments

Thirty pregnant Quarter horse mares (538 to 695 kg BW; 4 to 19 yrs of age) were blocked by expected foaling date and randomly assigned within block to one of two treatments: hay (H), or concentrate + hay (CH; concentrate fed at 0.75% BW as-fed; Vitality, Cargill Animal Nutrition, Elk River, MN; Table 9). All mares were allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay and water and were group housed by block. A dual marker system utilizing titanium dioxide and acid detergent insoluble ash was used to calculate voluntary forage intake at 9, 10, and 11 mo gestation (See Chapter II). Treatments began 110 d prior to expected foaling date and terminated at parturition. All mares were brought in from group housing twice daily (at 0545 and 1645) into individual feeding stalls (3.0 × 3.0 m) to offer grain to CH mares.

Mares on treatment CH were given 45 min to consume concentrate per feeding, and refusals were weighed, recorded, and used to determine calculations of DMI. Grain intake for CH mares was adjusted according to BW every 14 d. Mare performance parameters (BW, BCS, and rump fat (RF)) were recorded every 14 d until parturition. Body weights were taken using a digital platform scale (CAS Corp., Seoul, Rep. of Korea), and BCS was determined by 3 individuals on a scale of 1 to 9 as described by Henneke et al. (1983) with 1 = poor and 9 = extremely fat. Rump fat measurements were obtained via ultrasound images on the left hip at a point 5 cm dorsal of halfway between the first coccygeal vertebrae and the ischium (Westervelt et al., 1976) using an ultrasound instrument (Aloka SSD-500V, Aloka Inc., Tokyo, Japan).

Table 9. Nutrient composition of texturized concentrate and coastal bermudagrass (*Cynodon dactylon*) hay (DM basis) fed to pregnant Quarter horses

Item	Concentrate[1]	Hay[2]
DM, %	89.9	91.9
CP, %	18.7	9.6
ADF, %	5.9	36.3
NDF, %	14.3	70.1
Fat, %	7.0	1.8
Ca, %	1.1	0.4
P, %	0.9	0.2
Mg, %	0.4	0.2
K, %	1.1	1.2
Na, %	0.6	0.1
DE,[3] Mcal/kg DM	3.5	2.4

[1]Concentrate consisted of a commercially available mare & foal texturized concentrate (grain products, plant protein products, processed grain by-products, roughage products) provided by Cargill Animal Nutrition (Elk River, MN).

[2]Hay consisted of coastal bermudagrass (*Cynodon dactylon*).

[3]DE calculated from equations in NRC (2007).

After parturition, all mares returned to a similar plane of nutrition. All mares and foals were group housed by original block and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay and water. All mares were group fed twice daily with the same concentrate fed to CH mares during pregnancy at approximately 1.0% BW as-fed (Vitality, Cargill Animal Nutrition, Elk River, MN). Feed was provided in individual feeders spaced approximately 6 m apart to minimize feeding behavior effects of intake (Hoffman et al., 2003a).

Modified Frequent Sampling IV Glucose Tolerance Test

A modified frequent sampling intravenous glucose tolerance test (FSIGT) was performed on mares 20 d prior to expected foaling date and on foals at 80 and 160 d of age using methods previously described (Caumo et al., 2000; Hoffman et al., 2003a). Number of animals included in FSIGT data varies as some mares foaled prior to the FSIGT and death loss for reasons unrelated to treatment resulted in loss of foals prior to FSIGT. On the day of the FSIGT, BW was taken using a digital platform scale (CAS Corp., Seoul, Rep. of Korea) at 0500. Jugular intravenous (IV) catheters (Abbocath Catheters, Abbott Park, IL; mares: 14 g, foals: 16 g) were placed between 0530 and 0600 after aseptic preparation and local analgesia of the overlying skin and secured in place with adhesive bandaging tape. After catheter placement, horses were given 1 h of rest prior to starting the FSIGT. Horses were not offered the morning concentrate meal but all were allowed ad libitum access to water and the same hay offered in group housing (Table 1) throughout the FSIGT as fasting may affect insulin action in horses (Forhead and Dobson, 1997). Horses were housed in the same individual stalls used for daily

feeding. When FSIGT was performed on foals, mares and foals were kept in stalls as a pair and a handler restrained the foals using familiar handling techniques.

A baseline plasma sample was harvested immediately prior to starting the FSIGT, and then a glucose bolus of 0.3 g/kg BW (dextrose solution 50%, Agripharm Products, Westlake, TX) was administered IV (Hoffman et al., 2003a). Blood samples were harvested at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 min after glucose bolus administration. At 20 min after initial glucose bolus, an insulin bolus of 30 mU/kg BW for mares (Vetsulin, Intervet Inc., Millsboro, DE; Hoffman et al., 2003a) and a 10 mU/kg BW for foals was administered IV, and additional blood samples were harvested at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min after initial glucose injection. The lower dose of insulin was chosen for foals based on procedures described by George et al. (2009) to avoid inducing excessive hypoglycemia. Catheter patency was maintained by administering heparinized saline (0.9% sodium chloride containing 10 units of heparin per mL) after harvesting blood. Blood samples were harvested into evacuated tubes containing sodium heparin (158 U.S.P. Units, Becton-Dickinson, Franklin Lakes, NJ) and immediately placed on ice until centrifugation at 2,700 × *g* for 20 min at room temperature. All samples were centrifuged within 15 min of harvesting. Plasma was harvested and samples were stored at -20°C for subsequent determination of glucose and insulin.

Glucose concentrations were analyzed using a colorimetric assay (Glucose Procedure No. 510, Sigma Diagnostics) and insulin concentrations determined using a commercial RIA kit (Coat-A-Count Insulin, Siemens, Los Angeles, CA) which has been

validated for equine use (Tinworth et al., 2011). The coefficients of variation within assays were ≤ 5.0%, and ≤ 8.0% for glucose and insulin, respectively. The coefficients of variation between assays were ≤ 5.0%, and ≤ 10.0% for glucose and insulin, respectively.

Foal Physical Growth Measurements

To evaluate the influence of maternal plane of nutrition on foal physical growth characteristics over time, several measurements were obtained on foals beginning at 12 h post parturition and continuing every 30 d until 150 d of age. Measurements were obtained on a level concrete surface with foals standing square while being restrained by a handler. Measurements consisted of wither and hip height at their highest points, body length (from the point of the shoulder to the ischial tuberosity), and BW recorded using a digital platform scale (CAS Corp., Seoul, Rep. of Korea).

Statistical Analysis

Area under the curve (AUC) for glucose and insulin concentrations was determined using PROC EXPAND procedure of SAS (SAS Inst. Inc., Cary, NC). All AUC data and peak concentrations of glucose and insulin, as well as foal physical growth measurements were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Main effects tested were block, treatment, time, and treatment by time interaction. Block was found to be insignificant and was removed from the model. A paired t-test was used to compare CH to H at individual time points. Means are reported as LSMeans ± SD. Probabilities less than or equal to 0.05 were considered

statistically significant and less than or equal to 0.10 considered a trend towards significance.

Results

A dual marker system utilizing titanium dioxide and acid detergent insoluble ash was used to calculate voluntary forage intake at 9, 10, and 11 mo gestation and determined intake of hay was influenced by treatment ($P < 0.01$), with CH mares consuming 1.79% BW while H mares consumed 2.25% BW. The total dietary intake was not different between treatments ($P = 0.32$) with CH mares consuming 2.38% BW while H mares consumed 2.25% BW (See Chapter II).

Mare BW tended to be greater ($P \leq 0.09$) and BCS and RF were greater ($P \leq 0.01$) for CH mares (See Chapter II). Mares on CH gained BW, BCS, and RF throughout gestation (evaluated as last measurement prior to foaling minus d 0) while H mares lost BW and BCS; however, there was no effect of treatment when mare BW was evaluated 12 h post parturition ($P \geq 0.19$), and no difference in BW change due to parturition ($P \geq 0.17$). Mare BW was not different at the time of FSIGT test (CH= 644.08 kg ± 42.8; H = 608.82 ± 36.16; $P > 0.05$).

Mare glucose AUC was greater for CH compared to H ($P < 0.01$; Table 10). Mares fed CH had greater glucose concentration from 30 to 150 min after glucose bolus administration (Figure 7). There was no effect of treatment on mare peak glucose concentrations ($P \geq 0.23$; Table 10). Mare insulin AUC and peak insulin concentration were greater for CH mares compared to H ($P \leq 0.05$; Table 10). Mares fed CH had

higher plasma insulin concentrations at 27 to 35 min and 70 to 120 min after glucose bolus administration (Figure 8).

Table 10. Effect of dietary DE manipulation on mare glucose and insulin area under the curve (AUC) at 11 mo gestation (represented as LSMeans)[1]

	Treatment[2]		SEM	*P*-value
Item	CH	H		Trt
Mare Glucose AUC	289.86	244.68	8.44	<0.01
Mare Peak Glucose, mg/dL	4.01	4.13	0.07	0.23
Mare Insulin AUC	18,252.93	8,030.82	3,588.08	0.05
Mare Peak Insulin, mU/mL	586.95	371.88	69.53	0.04

[1] Frequent sampling IV glucose tolerance test used previously described methods and dosages (Hoffman et al., 2003a).

[2]Treatments applied to mares beginning 110 d prior to expected foaling date and terminated upon parturition. Treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. CH: n = 12; H: n = 14. Mare FSIGT performed 20d prior to expected foaling date of block, and some mares foaled prior to this time.

[3]Trt = effect of treatment (CH or H).

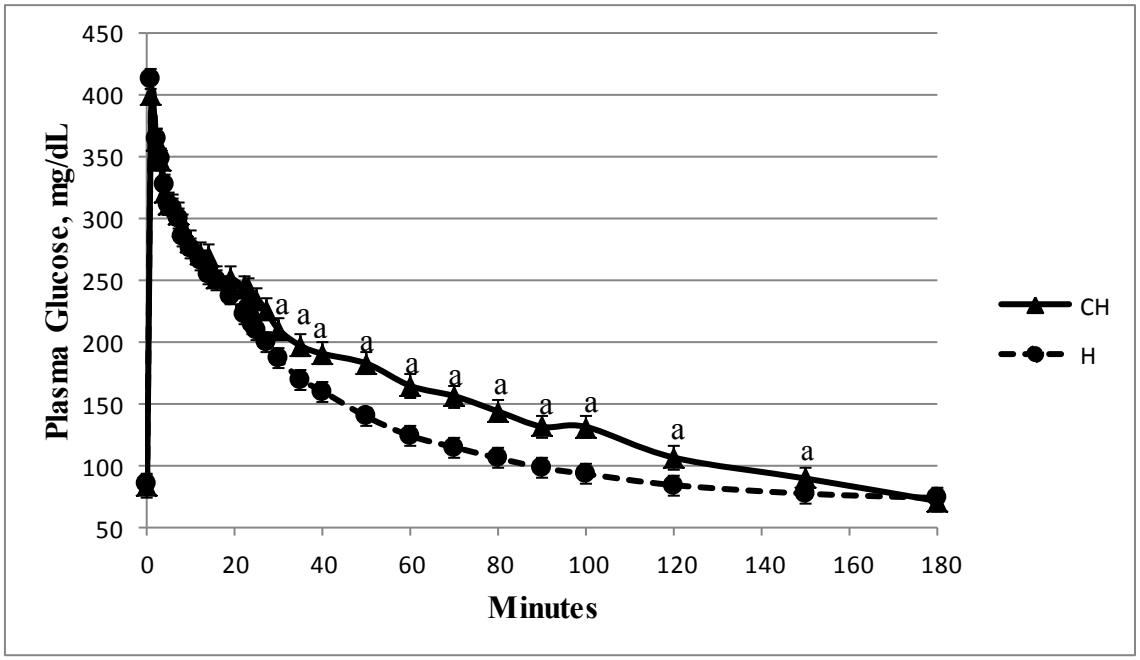

Figure 7. Effect of dietary DE manipulation on plasma glucose concentrations of mares during a frequent sampling IV glucose tolerance test performed 20 d prior to expected foaling date. Dietary treatments began 110 d prior to expected foaling date and consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. CH: n = 12; H: n = 13. P-values for effect of treatment, time, and treatment×time < 0.01. Within minute, the letter "a" indicates a treatment difference ($P < 0.05$) for CH vs. H.

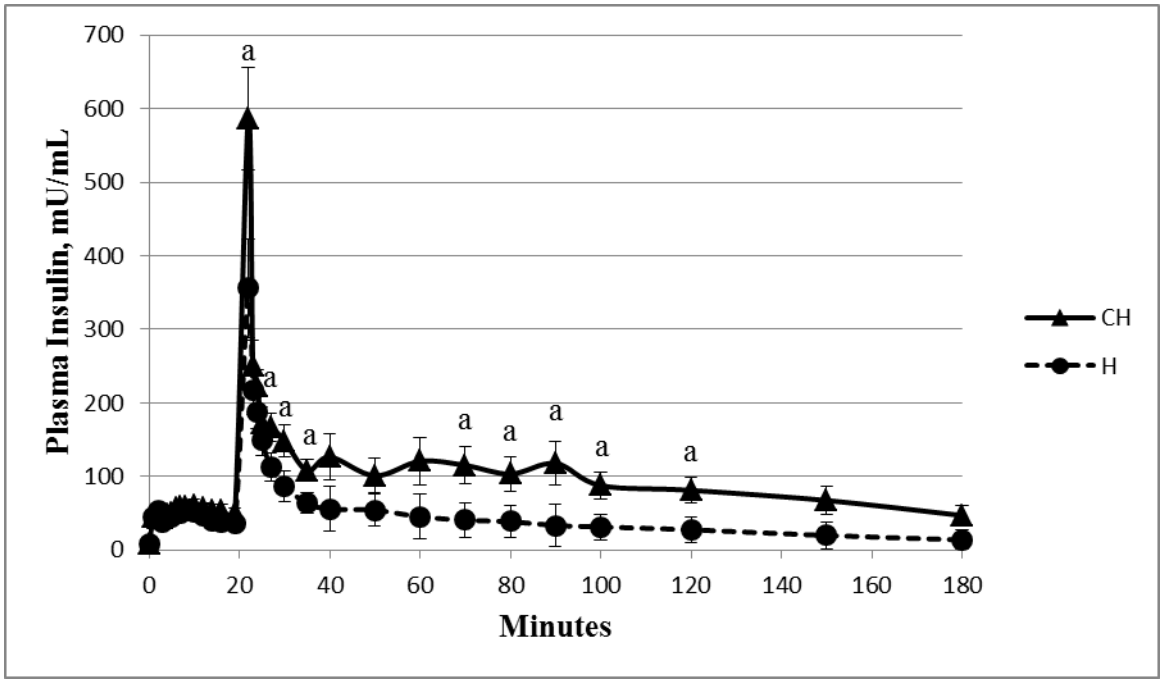

Figure 8. Effect of dietary DE manipulation on plasma insulin concentrations of mares during a frequent sampling IV glucose tolerance test performed 20 d prior to expected foaling date. Dietary treatments began 110 d prior to expected foaling date and consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. CH: n = 12; H: n = 13. P-values for effect of treatment, time, and treatment×time ≤ 0.01. Within minute, the letter "a" indicates a treatment difference ($P < 0.05$) for CH vs. H.

Foal glucose AUC and peak glucose were not influenced by maternal treatment ($P \geq 0.82$; Table 11), but both increased with age ($P \leq 0.05$; Table 11; Figure 9; Figure 10). Foal insulin AUC tended to be greater in foals from CH mares compared to foals from H mares ($P \leq 0.07$; Table 11) and decreased with foal age ($P \leq 0.02$; Table 11). Foal peak insulin concentration was influenced by maternal treatment with foals from CH mares having greater peak insulin compared to foals from H mares ($P \leq 0.04$; Table 11). At 80 d of age, foals from CH fed mares had greater insulin concentrations from 1

to 19 min following glucose bolus administration (Figure 11). However at 160 d of age, foals from CH fed mares had greater insulin concentrations from 22 to 25 min following glucose bolus administration, which was immediately following the insulin bolus (Figure 12). There was no effect of maternal nutrition on foal BW, ADG, wither height, hip height, or body length over 150 d ($P \geq 0.57$; Table 12). All measurements increased over time as expected with growth.

Discussion

Mares supplemented with concentrate tended to be heavier and had greater pre-partum BCS and RF compared to mares fed only hay. Mares fed hay only began the trial at a BW of 617.37 kg ± 11.16 and a BCS of 7.19 ± 0.12, and had a final BW of 609.30 kg ± 11.74 and BCS of 6.50 ± 0.15 (See Chapter II). Mares that conceive in a moderate BCS are expected to increase in BW by 12 to 15% during gestation (NRC, 2007). Mares fed only hay decreased by 1.3 % BW, and we observed a decrease in BCS in H mares from d 0 until parturition, which indicates CH mares mobilized body stores to meet the needs of fetal development. Although BCS decreased in H mares, all mares remained above a BCS of 6 which is considered appropriate for pregnant mares (Henneke et al., 1984).

Table 11. Effect of maternal dietary DE manipulation on foal glucose and insulin area under the curve (AUC) and peak values at 80 and 160 d of age (represented as LSMeans)[1]

Item[4]	Treatment[2]		SEM	*P*-values[3]		
	CH	H		Trt	Age	Trt×Age
Pooled Foal Glucose AUC	222.38	220.25	6.88	0.82	0.03	0.41
d 80 Glucose AUC	215.30	205.21	10.06	-	-	-
d 160 Glucose AUC	229.47	235.29	9.40	-	-	-
Pooled Foal Peak Glucose, mg/dL	3.10	3.07	0.11	0.85	<0.01	0.78
d 80 Peak Glucose, mg/dL	2.72	2.74	0.12	-	-	-
d160 Peak Glucose, mg/dL	3.47	3.39	0.20	-	-	-
Pooled Foal Insulin AUC	9,017.34	4,645.96	1,660.71	0.07	0.02	0.21
d 80 Insulin AUC	13,360.00	6,100.50	3,255.04	-	-	-
d 160 Insulin AUC	4,674.45	3,191.41	504.91	-	-	-
Pooled Foal Peak Insulin, mU/mL	678.41	401.86	92.10	0.04	0.63	0.10
d 80 Peak Insulin, mU/mL	615.58	515.11	151.18	-	-	-
d 160 Peak Insulin, mU/mL	741.24	288.62	75.29	-	-	-

[1] Frequent sampling IV glucose tolerance test used previously described methods and dosages (Hoffman et al., 2003a; George et al., 2009).

[2]Treatments applied to mares beginning 110 d prior to expected foaling date and terminated upon parturition. Treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Treaments terminated upon parturition, and all mares and foals placed on same plane of nutrition throughout lactation, which consisted of group feeding approximately 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. For FSIGT at both d80 and d160, CH: n = 14; H: n = 12.

[3]Trt = effect of treatment (CH or H). Age = effect of age (80 or 160 days). Trt×Age = interaction.

[4]Pooled items include data for foals at both d80 and d160.

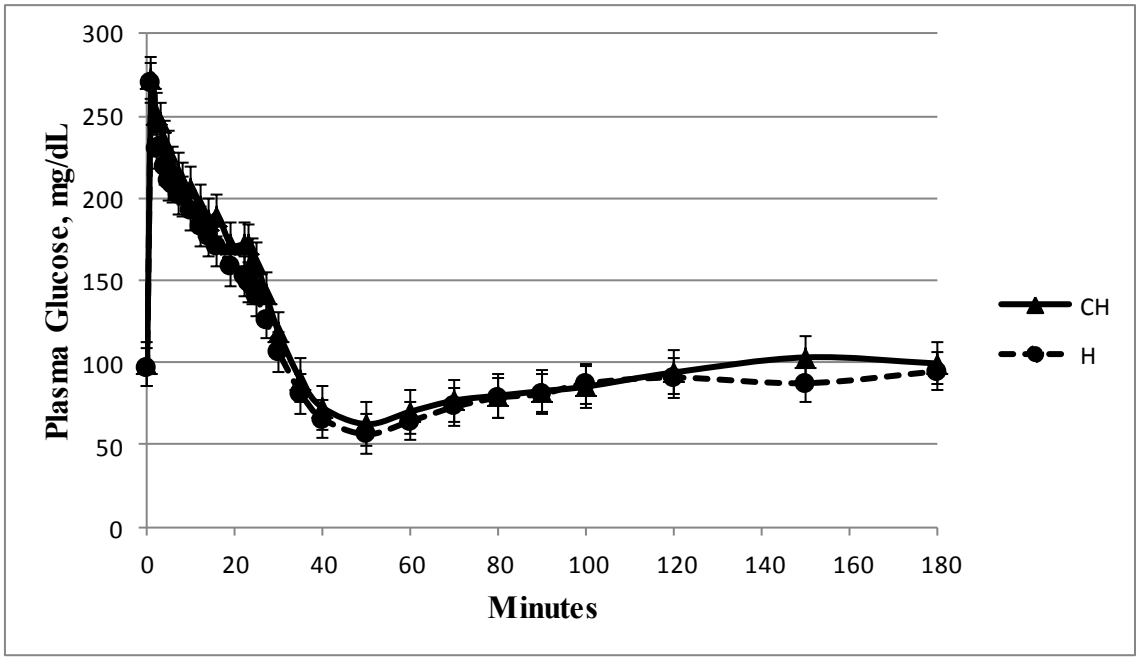

Figure 9. Effect of maternal dietary DE manipulation on plasma glucose concentrations of foals during a frequent sampling IV glucose tolerance test performed at 80 d of age. Maternal dietary treatments began 110 d prior to expected foaling date and terminated upon parturition. Maternal dietary treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Treaments terminated upon parturition, and all mares and foals placed on same plane of nutrition throughout lactation, which consisted of group feeding approximately 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. CH: n = 14; H: n = 12. P-values: effect of treatment: $P = 0.13$, effect of time: $P < 0.01$, and effect of treatment×time: $P = 0.31$.

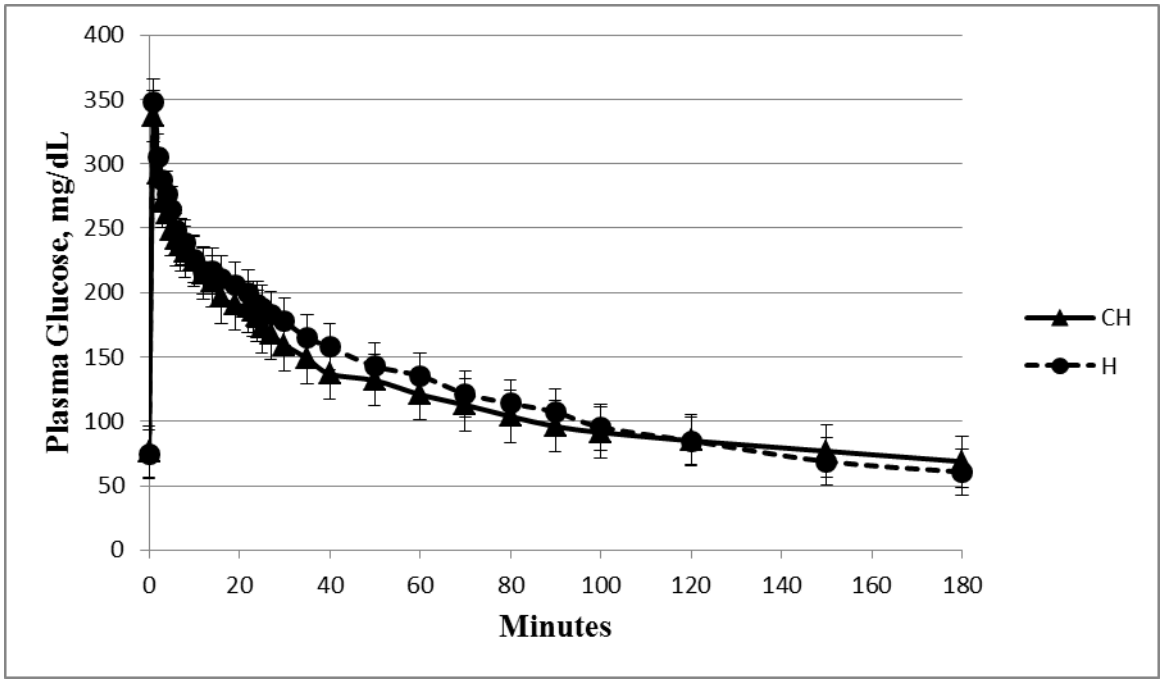

Figure 10. Effect of maternal dietary DE manipulation on plasma glucose concentrations of foals during a frequent sampling IV glucose tolerance test performed at 160 d of age. Maternal dietary treatments began 110 d prior to expected foaling date and terminated upon parturition. Maternal dietary treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Treaments terminated upon parturition, and all mares and foals placed on same plane of nutrition throughout lactation, which consisted of group feeding approximately 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. CH: n = 14; H: n = 12. P-values: effect of treatment: $P = 0.23$, effect of time: $P < 0.01$, and effect of treatment×time: $P < 0.01$.

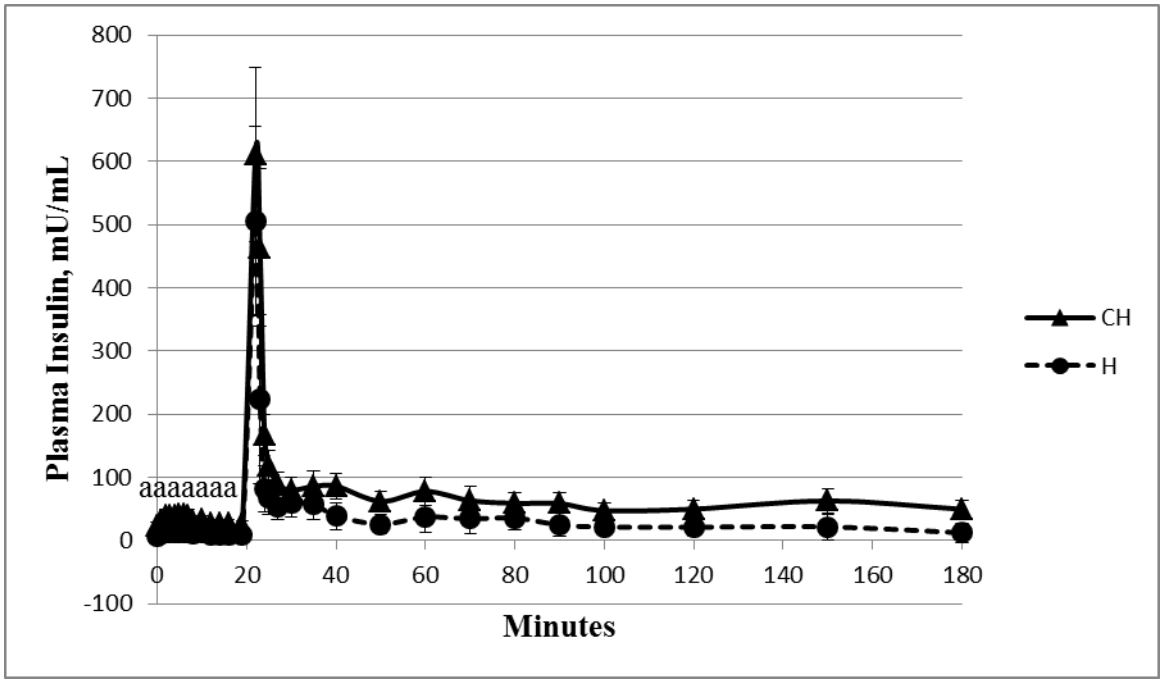

Figure 11. Effect of maternal dietary DE manipulation on plasma insulin concentrations of foals during a frequent sampling IV glucose tolerance test performed at 80 d of age. Maternal dietary treatments began 110 d prior to expected foaling date and terminated upon parturition. Maternal dietary treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Treaments terminated upon parturition, and all mares and foals placed on same plane of nutrition throughout lactation, which consisted of group feeding approximately 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. CH: n = 14; H: n = 12. P-values: effect of treatment: $P = 0.07$, effect of time: $P < 0.01$, and effect of treatment×time: $P = 0.98$. The minutes between the "a" letters indicate a treatment difference ($P < 0.05$) for CH vs. H during samples harvested at 1,2,3,4,5,6,7,8,10,12,14,16,and 19 minutes post glucose bolus administration.

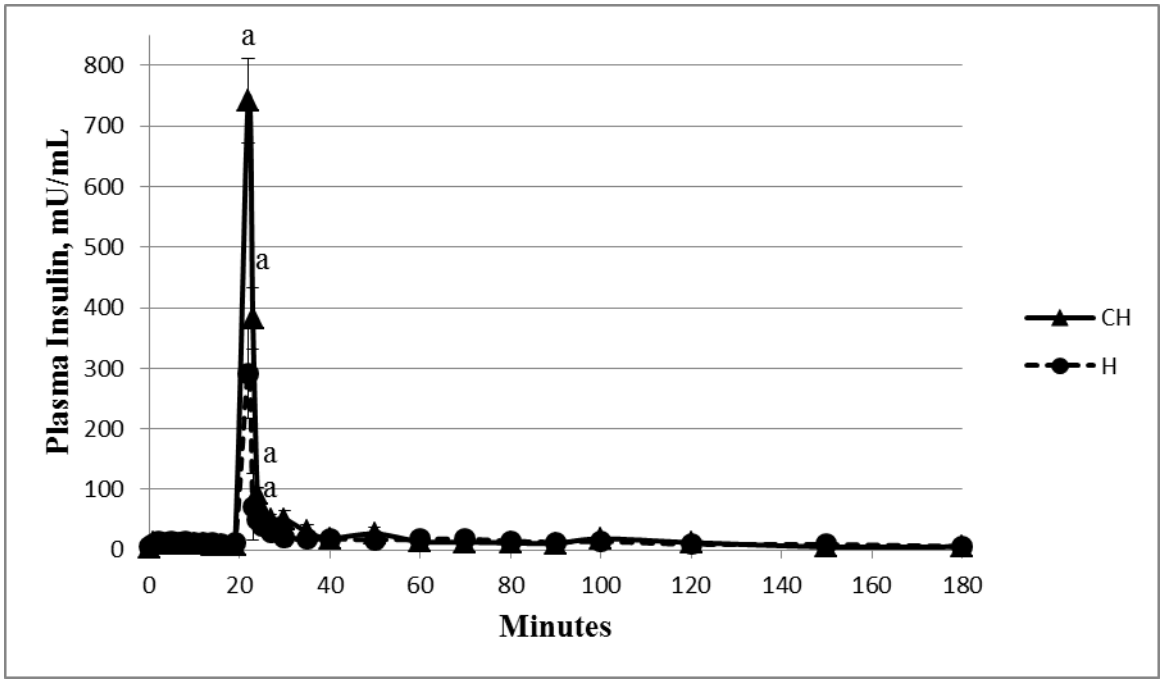

Figure 12. Effect of maternal dietary DE manipulation on plasma insulin concentrations of foals during a frequent sampling IV glucose tolerance test performed at 160 d of age. Maternal dietary treatments began 110 d prior to expected foaling date and terminated upon parturition. Maternal dietary treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay; H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Treaments terminated upon parturition, and all mares and foals placed on same plane of nutrition throughout lactation, which consisted of group feeding approximately 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. CH: n = 14; H: n = 12. P-values for effect of treatment, time, and treatment×time < 0.01. Within minute, the letter "a" indicates a treatment difference ($P < 0.05$) for CH vs. H, at samples harvested at 22, 23, 24, and 25 minutes post glucose bolus administration (2, 3, 3, and 5 minutes post insulin bolus administration).

Table 12. Effect of maternal dietary energy manipulation on foal physical measurements obtained 12 h post parturition and every 30 d through 150 d of age (represented as LSMeans)

	Treatment[1]		SEM	*P*-value[2]		
Measurement[3]	CH (n = 15)	H (n = 12)		Trt	Time	Trt×Time
BW, kg	143.86	140.51	2.92	0.68	<0.01	<0.01
d0 BW, kg	49.79	48.46	1.46	-	-	-
d150 BW, kg	215.32	210.58	4.17	-	-	-
BL, cm	106.40	106.56	0.60	0.84	<0.01	0.48
d0 BL, cm	75.74	76.46	0.92	-	-	-
d150 BL, cm	125.47	125.75	1.07	-	-	-
WH, cm	111.76	111.80	0.71	0.97	<0.01	0.09
d0 WH, cm	95.59	96.15	0.84	-	-	-
d150 WH, cm	122.43	123.14	0.75	-	-	-
HH, cm	115.94	115.88	0.64	0.94	<0.01	0.01
d0 HH, cm	96.41	98.21	0.75	-	-	-
d150 HH, cm	128.69	128.64	0.55	-	-	-
ADG, kg	1.10	1.08	0.03	0.57	-	-

[1]Treatments applied to mares beginning 110 d prior to expected foaling date and terminated upon parturition. Treatments consisted of: CH = Mares supplemented with texturized concentrate (provided by Cargill Animal Nutrition) offered at 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. H = Mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay. Treaments terminated upon parturition, and all mares and foals placed on same plane of nutrition throughout lactation, which consisted of group feeding approximately 0.75% BW (as-fed) and allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.

[2]Trt = effect of maternal treatment (CH or H). Time = effect of time. Trt×Time = interaction.

[3]BW = Body Weight; BL = Body Length; WH = Wither Height; HH = Hip Height; ADG calculated as change in BW over 150 days.

Hay intake calculations resulted in H mares consumed 13.6 kg DM and CH mares consumed 11.2 kg DM, therefore the calculated DE intake of H mares was 130% of NRC DE recommendations and 160% in CH mares. This data was discussed in further detail in Chapter II.

There are two common approaches used in equine studies to evaluate glucose and insulin metabolism and obesity. The first method, commonly used in other species such as sheep, is to over feed the dam based on percentage NRC nutrient requirements and observe any alterations in BCS that may occur as a result of the diet (Ford et al., 2009; Long et al., 2010). The second method, more commonly used in equine studies, is to evaluate horses relative to BSC alone rather than diet. Studies have fed the dam to achieve a target BCS, (Ousey et al., 2008), or simply grouped horses by their existing BCS and evaluated insulin and glucose response to BCS (Jeffcott and Field, 1986; Hoffman et al., 2003a). Jeffcott and Field (1986) linked insulin resistance in ponies to obesity by comparing obese and non-obese groups although they did not report the BCS range of each group. Mares fed to achieve an obese BCS of 8 to 9 had greater serum insulin concentrations compared to mares fed to achieve a moderate BCS of 4 to 5 (Ousey et al., 2008). However, currently there is no indication of a specific BCS at which altered glucose and insulin metabolism occur.

In the current study, CH mares had greater glucose and insulin AUC and also had greater BCS compared to H mares ($P \leq 0.01$). However, although there was a difference in BCS, the two treatments were only 0.37 of a BCS different, which suggests that diet composition had a greater influence on glucose and insulin AUC than BCS. This idea is confirmed by results of a similar study performed by George et al. (2009) where mares fed increased starch exhibited greater glucose and insulin concentrations and also had a greater BCS compared to low starch mares, but were only 0.60 of a BCS different, therefore the differences observed in glucose and insulin metabolism were

more likely attributed to differences in diet. Therefore, it would be beneficial for future equine studies to feed based on dietary requirements or percent BW rather than for a target BCS as the response of glucose and insulin concentration seems to be more sensitive to alterations in diet, therefore having a more detailed description of nutrient intake would be useful when comparing across studies.

Evaluation of mare glucose tolerance confirmed that maternal diet influences insulin and glucose dynamics of the dam and may allow for metabolic programming of the foals as a response to maternal nutrition. Mare glucose and insulin AUC indicates an effect of dietary treatment with CH mares having greater concentration of glucose and insulin compared to H, in addition to increased peak insulin in CH mares. A previous study evaluating a high starch feed (14.5% CP, 3.4% crude fat, 10% ADF, 17.6% NDF, 39.2% starch) compared to a high fat and fiber feed (15.5% CP, 13.9 % crude fat, 28.2% ADF, 42.2% NDF, 4.4% starch) in Thoroughbred mares determined the high starch fed mares had greater glucose and insulin concentrations compared to mares fed low starch (George et al., 2011). These results are similar to the current study, where mares fed concentrate had greater glucose and insulin AUC compared to mares fed hay only.

It appears mares have the ability to adapt to increased dietary starch over time. Hoffman et al. (2003b) performed oral glucose challenges on thoroughbred mares fed diets high in sugar and starch or fiber and fat and found mares consuming a greater amount of starch actually cleared glucose more rapidly, but observed no differences in insulin concentrations. Hoffman et al. (2003b) attributed the faster glucose clearance rate of the mares on the high sugar and starch treatment to their adaptation to consuming

meals rich in hydrolysable carbohydrates. Furthermore, Hoffman et al. (2003b) utilized a lower glucose bolus of 0.2 g/kg of BW administered orally compared to the current study's bolus of 0.3 g/kg of BW administered intravenously. The lower dose was chosen to provide a similar glucose load as that provided by a 0.5 kg meal of heavy oat grain and adaptation diets were provided beginning in mid-gestation. Mares fed concentrate in our study had greater glucose and insulin AUC compared to mares fed hay only, therefore did not appear to adapt to the higher starch content of the diet. The difference in dose, administration method, and duration of time on dietary treatments may explain the difference in mare response.

In the current study, this alteration in mare glucose dynamics relative to diet corresponded to changes in the foals. This is similar to previous research which has demonstrated increasing concentration of maternal glucose increases its delivery to the fetus by altering the maternal-fetal glucose gradient (Aldoretta and Hay, 1999). In the current study, foal insulin AUC tended to be greater and peak insulin was greater in foals from CH mares. Furthermore, at 80 d of age, foals from CH mares showed increased endogenous insulin response to the administration of the glucose bolus (Figure 14). Although there was no effect of maternal treatment on foal glucose AUC or peak concentrations, this altered response of endogenous insulin following glucose bolus administration indicates foals from CH mares experienced metabolic programming due to maternal nutrition during the last third of gestation. At 160 d of age, foals from CH mares had greater insulin concentrations from 22 to 25 min indicating a difference in the ability to clear exogenous insulin. George et al. (2009) did not report AUC or actual

glucose concentrations over time, but used MinMod Millenium software to calculate indexes. George et al. (2009) reported insulin sensitivity as the capacity of insulin to promote glucose disposal as well as the index of overall insulin action potential and observed a trend for lower insulin sensitivity at 160 d of age in foals from mares fed a higher starch diet. In our study, foal glucose AUC and peak glucose increased with age and foal insulin AUC decreased with age. George et al. (2009) observed a decrease in baseline plasma glucose and insulin concentrations with increasing foal age from FSIGT tests completed at 5, 40, 80, and 160 d of age. When comparing baseline plasma glucose and insulin these values declined greatly from 5 to 80 d of age and there was little change in baseline values from 80 to 160 d which were the sampling ages in the current trial.

Research in sheep determined that obesity, as a result of feeding 150% NRC recommendations throughout gestation, decreased insulin sensitivity using the euglycemic clamp procedure (Bergman et al., 1989). Maternal obesity in sheep resulted in increased maternal and fetal AUC for glucose and insulin, as well as a 236% increased weight of fetal pancreas along with a 50 % increase in number of insulin positive cells per unit area of fetal pancreas (Ford et al., 2009). Lambs from ewes fed 150% of NRC recommendations had decreased insulin response to glucose, and a tendency for reduced insulin sensitivity compared to lambs from mothers fed 100% NRC recommendations (Long et al., 2010). These results are similar to that of the current study, where CH mares had greater glucose and insulin AUC indicating reduced glucose clearance and insulin resistance, which impacted the resulting foals by

increasing insulin concentrations. Lambs from over-fed dams (150% NRC recommendations) and control dams (100% NRC recommendations) were placed in an ad-libitum feeding situation post weaning at 19.5 ± 0.5 mo and found offspring from over-fed dams had altered appetite and consumed approximately 10% more feed compared to offspring from control dams (Long et al., 2010). In our study, concentrate during lactation was offered in a group housed setting in feed pans placed on the ground, therefore foals had access to their dam's grain and intake could have differed between individuals. A difference in foal intake could explain the differences in insulin with increasing foal age. Further research investigating effects of maternal nutrition on foal appetite and voluntary intake would be beneficial.

Previous research found no effect of maternal nutrition and condition on resulting foal BW but did not evaluate foal growth over time (Ousey et al, 2008; George et al., 2009). Our study also found no effect of maternal nutrition on foal BW, and additionally found no effect on wither height, hip height, or body length over 150 d ($P \geq 0.68$; Table 12). All measurements were influenced by time as expected with normal growth.

Research in humans has found that offspring born to obese mothers were similar in BW to those born to lean mothers, but were insulin resistant, and children exposed to maternal obesity are twice as likely to develop obesity, hypertension, and glucose intolerance (Boney et al., 2005; Hull et al., 2008; Catalano et al., 2009). Data from the National Health and Nutrition Examination Survey 2009-2010 show that 35.7% of the total United States population and 31.9% of women aged 20-39 are obese (obesity defined as a body mass index ≥ 30; Ogden et al., 2012). While data from other livestock

species, including sheep, indicates they may serve as an effective model for human disease (Ford et al., 2009), the horse may be a more appropriate animal model. Our study demonstrated alterations in maternal plane of nutrition effects insulin dynamics in mares and foals. Additionally, common management practices in horses such as feeding starch-rich cereal grains in large meals, and following current recommendations to maintain broodmares at a fleshy condition score for improved reproductive efficiency have contributed to obesity rates ranging from 45-50% (Kubiak et al., 1987; Henneke et al., 1984; Hoffman et al., 2003a; Stephenson et al., 2011). Furthermore, longer lifespans and a production setting that allows for greater longevity result in increased incidence of metabolic disorders in horses comparable to metabolic diseases in humans (Fulop et al., 2006; Frank et al., 2010). This allows for great opportunity for the horse to be used as an ideal animal model for further investigation to better understand the effects of maternal obesity.

CHAPTER V

INFLUENCE OF MATERNAL PLANE OF NUTRITION AND ARGININE SUPPLEMENTATION DURING LATE PREGNANCY ON VOLUNTARY DRY MATTER INTAKE AND FOALING PARAMETERS OF QUARTER HORSE MARES

Introduction

Maternal nutrition influences fetal development, and the fetus is sensitive to nutrition of the dam during pregnancy (Wu et al., 2006). Overnutrition has negative and lasting effects on fetal growth and development (Wu et al., 2006; Rossdale and Ousey, 2002). Overfeeding is a common occurrence in the horse industry with obesity rates ranging from 45 to 50% (Stephenson et al., 2011). Obesity in mares causes reduced fetal growth and occasional fetal death (Pugh, 1993). Overnutrition of pregnant ewes resulted in decreased gestational length and reduced prenatal colostrum accumulation (Davidson et al., 2000; Wallace et al., 2001). Research in horses found reduced IgG concentration, and reduced on-farm estimates of quality but no difference in colostrum volume of mares fed increased DE during late gestation (Thorson et al., 2010; Winsco et al., 2010).

As a precursor for nitric oxide (NO), arginine (Arg) holds great promise as a therapeutic agent for compromised fetal development. Nitric oxide is a vasodilator and increases placental growth due to angiogenesis, improving efficiency of uterine and placental blood flow (Wu et al., 2009). Dietary supplementation of Arg prevents fetal growth retardation and increases birth weight in rats (Mateo et al., 2007). There is little

research concerning the influence of maternal overnutrition on equine offspring, and potential role of maternal dietary Arg supplementation in fetal development is also unknown.

Furthermore, information regarding DMI of pregnant mares is limited, and it is often difficult to measure because mares are group-housed. The use of dual marker systems to estimate DMI warrants further investigation in the horse. Research in mares fed increased DE during late gestation found concentrate supplementation decreased DMI and mares consumed less during month 10 when measured with a dual marker system (See Chapter II). Further research is necessary to fully understand how gestation affects DMI in horses and if Arg supplementation alters DMI. Therefore, the objective of this study was to determine effect of altered maternal nutrition and Arg supplementation during the last third of pregnancy on DMI, foaling, and colostrum characteristics.

Materials and Methods

Care, handling, and sampling of animals were approved by the Texas A & M University Animal Care and Use Committee.

Horses and Treatments

Thirty-two mares (468 to 668 kg of BW; 3 to 19 yrs of age) were blocked into 4 groups by expected foaling date (n = 8/block) and randomly assigned within block to treatments. The average expected foaling dates for each block were: block 1 = mid-January, block 2 = late-February, block 3 = mid-April, and block 4 = late-May. Treatments were arranged as a 2 × 2 factorial with two planes of nutrition (Nutr),

moderate (Mod; 0.5% BW AF concentrate/d) or high (High; 1% BW AF concentrate/d) and two levels of amino acid supplementation (AA), 0.21 g/kg/d arginine (Arg, L-Arginie-HCl ≥ 98.5% purity, powdered crystalline form) or no supplemental Arg (Con; L-alanine ≥ 98.5% purity, crystalline form, to maintain isonitrogenous diets). Concentrate supplementation consisted of a commercially available feed (Vitality Mare & Foal, Cargill Animal Nutrition, Elk River, MN; Table 13). Amino acids were top-dressed and thoroughly mixed into grain meals (Ajinomoto AminoScience LLC, Raleigh, NC, USA). Treatments began 110 d prior to expected foaling date and terminated at parturition. Mares were housed by block and allowed ad libitum access to water and coastal bermudagrass *(C. dactylon)* hay (Table 13). Mares were brought in from group housing twice daily (at 0615 and 1615) into feeding stalls (3.0 × 3.0 m) in order to offer concentrate individually. Mares were given 50 min per feeding, and refusals were weighed, recorded, and used to determine calculations of DMI. Concentrate intake was adjusted according to change in BW every 14 d.

Table 13. Nutrient composition of texturized concentrate and coastal bermudagrass (*Cynodon dactylon*) hay (DM basis) fed to pregnant Quarter horses

Item	Concentrate[1]	Hay[2]
DM, %	91.83	92.81
CP, %	17.76	8.72
ADF, %	8.30	39.70
NDF, %	14.07	68.97
Fat, %	6.96	1.78
Ca, %	0.94	0.44
P, %	0.83	0.24
Mg, %	0.43	0.24
K, %	1.07	1.16
Na, %	0.53	0.22
DE,[3] Mcal/kg DM	3.54	2.42

[1]Concentrate consisted of a commercially available mare & foal texturized concentrate (grain products, plant protein products, processed grain by-products, roughage products) provided by Cargill Animal Nutrition (Elk River, MN).

[2]Hay consisted of coastal bermudagrass (*Cynodon dactylon*).

[3]DE calculated from equations in NRC (2007).

Mare Performance

Mare performance parameters (BW, BCS, and rump fat (RF)) were recorded every 14 d until parturition. Body weights were taken using a digital platform scale (CAS Corp., Seoul, Rep. of Korea), and BCS was determined by 3 individuals on a scale of 1 to 9 as described by Henneke et al.(1983) with 1 = poor and 9 = extremely fat. Rump fat measurements were obtained via ultrasound images on the left hip at a point 5 cm dorsal of halfway between the first coccygeal vertebrae and the ischium (Westervelt et al., 1976) using an ultrasound instrument (Aloka SSD-500V, Aloka Inc., Tokyo, Japan).

Determination of DMI

A dual marker system was utilized for estimation of DMI using titanium dioxide (TiO_2) as the external marker and acid detergent insoluble ash (ADIA) as the internal marker. Intake was evaluated for a 24 h period during 9, 10, and 11 months of pregnancy. Titanium dioxide was provided at 5 g twice daily for 14 d. Mares were supplemented with TiO_2 immediately prior to each feeding by top-dressing 5 g TiO_2 onto approximately 200 g of the concentrate. Mares were observed to ensure complete consumption of the TiO_2 and were then provided with the remainder of the concentrate meal. Fecal samples were obtained on the last 4 d of TiO_2 supplementation at 9 (d 26 to 28 of trial), 10 (d 52 to 56), and 11 (d 80 to 84) mo of gestation. Samples were collected twice daily via rectal palpation at 12 h intervals with times advancing 3 h each d to account for diurnal variation and to ultimately represent a 24 h period. A 200 to 400 g sample of feces was stored at -20°C for subsequent analysis. Concentrate and hay samples were collected at feeding times during each day of fecal collections and stored at -20°C for subsequent analysis.

All fecal, concentrate, and hay samples were analyzed for ADIA and TiO_2 concentrations. Samples were thawed and then dried in a forced air oven (Lindberg/Blue M, Asheville, NC) at 60°C for 72 h then ground through a 1-mm screen (Thomas Scientific, Swedesboro, NJ). Samples were composited for each collection prior to analysis. The DM of grain, hay, and fecal samples was determined by drying for 24 h at 105°C in a forced air oven (Lindberg/Blue M). Concentrations of TiO_2 were determined by colorimetric assay using previously described methods (Titgemeyer et al., 2001;

Short et al., 1996) modified for use on a digestion block (SCP Science DigiPrep HT, Champlain, NY). Concentrations of ADIA were determined using an ANKOM-Fiber Analyzer (ANKOM-Technology, Fairport, NY) using techniques described by Llewellyn et al. (2006). Titanium dioxide concentration was used to estimate daily fecal production and ADIA was used to estimate DMI. Both values determined estimated DMI. Values were determined using the following equations:

1. Feces per d (kg) = [(10 g TiO_2 per d)/((x% TiO_2)/1000)]/1000
2. ADIA in feces (kg) = [(kg feces per d) × (x% ADIA in feces/100)]
3. ADIA in grain (kg) = [(kg grain intake per d) × (x% ADIA in grain/100)]
4. Forage intake per d (kg) = [(kg ADIA in feces – kg ADIA in grain)/ (x% ADIA in forage/100)]

Parturition

All mares were monitored for signs of impending parturition during each meal and brought into stalls when foaling appeared to be imminent. Parturition was observed and the following foaling parameters were recorded: time of water break to birth, time from birth to stand, and time of birth to placenta expulsion. Total length of gestation was calculated and placenta weight was recorded. Immediately after parturition mares were stripped of their initial volume of colostrum by hand-milking. Colostrum volume (CV), specific gravity (SG; Equine Colostrometer, Lane Manufacturing, Denver, CO), and Brix% (Equine Colostrum Refractometer, Animal Reproduction Systems, Chino, CA) of colostrum were measured immediately after milking, prior to nursing. To ensure adequate passive transfer of immunity, foals were administered 250 mL of their dam's

colostrum within 1 h after parturition via nasogastric tube and then allowed to nurse ad libitum. Physical measurements of mares and foals were obtained 12 h post parturition, which included: mare BW, foal BW, foal wither and hip height at their highest points, and foal body length (point of shoulder to ischial tuberosity).

Statistical Analysis

Intake data were analyzed using the PROC MIXED procedure of SAS to account for repeated measures, and foaling data were analyzed using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). Main effects tested included plane of nutrition (Nutr), amino acid (AA), block, time, and their interactions. Foaling parameter data were included only when parturition was observed. Means are reported as LSMeans ± SD. A paired t-test was used to compare individual time points. Probabilities less than or equal to 0.05 were considered statistically significant and less than or equal to 0.10 considered a trend towards significance.

Results

Mare Performance

There was no influence of Nutr or AA on LSMean BW over the 110 trial ($P \geq 0.19$; Table 14). However, when expressed as change in BW from d 0 to parturition, Nutr influenced mare BW ($P \leq 0.03$; Table 14) as High mares gained more compared to Mod. There was no effect of AA on BCS of mares ($P \geq 0.35$; Table 14), however, Nutr influenced mean BCS and change in BCS ($P \leq 0.06$; $P \leq 0.02$, respectively; Table 14) as High mares increased while Mod mares decreased. There was a tendency for High mares

to have a greater RF compared to Mod ($P \leq 0.08$; Table 15), however Nutr did not affect RF change ($P \geq 0.19$; Table 14). Supplementation of Arg influenced RF change, as Arg mares decreased and Con mares increased ($P \leq 0.04$; Table 14).

Block tended to influence BW change BCS change over the 110 d experiment. Blocks 1 and 2 tended to gain greater BW compared to blocks 3 and 4 ($P \leq 0.06$). However, blocks 3 and 4 tended to increase in BCS while blocks 1 and 2 maintained similar ($P \leq 0.06$). Additionally, block influenced mean RF and RF change, with block 1 mares having the greatest amount of RF and block 1 and 2 increasing RF while block 3 and 4 decreased ($P \leq 0.03$ and $P \leq 0.01$, respectively).

Intake

There was no effect of Arg on total DMI or hay DMI expressed as kg or percent BW ($P \geq 0.27$; Table 15). However, Arg supplementation tended to decrease concentrate intake ($P \leq 0.09$; Table 15). Mare Nutr influenced hay DMI ($P \leq 0.04$) with Mod mares consuming a greater percentage of BW compared to High (Table 15), however Nutr did not affect total DMI as percent BW ($P \geq 0.24$). Block influenced hay DMI and total DMI expressed in kg and as percent BW with blocks 3 and 4 consuming a greater amount ($P \leq 0.01$). Regardless of dietary treatment, month of gestation influenced hay DMI as a percent BW ($P \leq 0.04$) and total DMI kg ($P \leq 0.02$) with all mares consuming less during the 11^{th} mo (Table 15). Upon calculation, grain contained an estimated 3.54 Mcal/kg DE and hay 2.42 Mcal/kg (Table 13). Based on calculated DE and DMI, all mares exceeded NRC (2007) requirements for 9, 10, and 11 mo gestation for mares with a mature BW of 600 kg.

Table 14. Influence of plane of nutrition and arginine supplementation on physical characteristics of pregnant Quarter horses (represented as LSMeans)[1]

Measurement	Treatment[2]				SEM	*P*-values[3]			
	High Con (n = 8)	High Arg (n = 8)	Mod Con (n = 7)	Mod Arg (n = 8)		Nutr	AA	Nutr×AA	Block
BW, kg	578.6	587.0	544.5	567.2	20.9	0.19	0.44	0.72	0.21
BW change, kg	36.4	25.8	3.1	23.5	7.9	0.03	0.52	0.05	0.06
BCS[4]	6.4	7.0	6.0	6.0	0.4	0.06	0.41	0.44	0.61
BCS change	0.3	0.7	-0.1	-0.1	0.2	0.02	0.35	0.51	0.06
Rump Fat, cm[5]	0.35	0.30	0.29	0.29	0.02	0.08	0.12	0.23	0.03
Rump Fat change, cm	0.05	-0.01	<0.01	-0.02	0.02	0.19	0.04	0.24	0.01

[1]Treatments were applied beginning 110 d prior to expected foaling date. Measurements were obtained every 14 d, and change is represented as the difference from d 0 to last measurement obtained prior to foaling

[2]Mare dietary treatments were arranged as a 2 × 2 factorial with two planes of nutrition, moderate (Mod; 0.5% BW AF grain/d) or high (High; 1% BW AF grain/d) and two levels of L-arginine supplementation, 0.21 g/kg/d (Arg) or no supplemental Arg (Con; L-alanine to maintain isonitrogenous diets). LS means are reported.

[3]Nutr = effect of nutrition: Mod (0.5% BW AF grain/d) or High (1% BW AF grain/d); AA = effect of amino acid supplementation: Arg (0.21 g/kg/d L-Argine) of Con (L-alanine to maintain isonitrogenous diets). Nutr×AA = interaction of nutrition and amino acid supplementation. Block = effect of blocking of mares by expected foaling date.

[4]Score represents average score of 3 evaluators using Henneke scoring techniques (Henneke et al., 1983).

[5]Rump fat measurements obtained on the left hip at a point 5 cm dorsal of halfway between the first coccygeal vertebrae and the ischium (Westervelt et al., 1976) using an ultrasound instrument (Aloka SSD-500V, Aloka Inc., Tokyo, Japan).

Table 15. Influence of plane of nutrition and arginine supplementation on DMI of pregnant Quarter horses during month 9, 10, and 11 of gestation (represented as LSMeans)[1]

	Treatment[2]					*P*-values[3]				
Item	High Con	High Arg	Mod Con	Mod Arg	SEM	Nutr	AA	Nutr×AA	Month	Block
Mare BW[4], kg	572.0	584.0	544.0	557.4	11.3	0.01	0.24	0.94	0.26	0.01
9 mo[5]	559.4	568.8	537.7	549.6	19.0	-	-	-	-	-
10 mo	571.5	579.8	542.6	559.0	19.0	-	-	-	-	-
11 mo	585.3	603.3	551.6	563.7	20.6	-	-	-	-	-
Concentrate DMI, kg	4.7	4.3	2.4	2.4	0.1	<0.01	0.09	0.08	0.90	0.71
9 mo	4.8	4.2	2.4	2.3	0.2	-	-	-	-	-
10 mo	4.8	4.1	2.4	2.5	0.2	-	-	-	-	-
11 mo	4.6	4.5	2.5	2.5	0.3	-	-	-	-	-
Concentrate DMI, % BW	0.8	0.7	0.4	0.4	0.02	<0.01	0.01	0.02	0.81	0.30
9 mo	0.9	0.8	0.4	0.4	0.03	-	-	-	-	-
10 mo	0.8	0.7	0.5	0.4	0.03	-	-	-	-	-
11 mo	0.8	0.7	0.4	0.5	0.03	-	-	-	-	-
Hay DMI, kg	9.0	8.6	8.9	10.4	0.6	0.12	0.27	0.07	0.05	0.01
9 mo	$9.3^{A,B}$	7.9^{A}	10.1^{A}	9.9^{A}	0.9	-	-	-	-	-
10 mo	10.2^{A}	9.6^{A}	8.3^{A}	12.1^{B}	0.9	-	-	-	-	-
11 mo	7.6^{B}	8.3^{A}	8.3^{A}	9.4^{A}	1.0	-	-	-	-	-
Hay DMI, % BW	1.6	1.5	1.6	1.9	0.1	0.04	0.42	0.12	0.04	0.01
9 mo	$1.7^{A,B}$	1.4^{A}	1.9^{A}	$1.8^{A,B}$	0.2	-	-	-	-	-
10 mo	1.8^{A}	1.7^{A}	1.6^{A}	2.2^{A}	0.2	-	-	-	-	-
11 mo	1.3^{B}	1.4^{A}	1.5^{A}	1.7^{B}	0.2	-	-	-	-	-
Total DMI, kg	13.7	12.9	11.3	12.9	0.5	0.02	0.48	0.02	0.05	0.01
9 mo	$14.0^{A,B}$	12.2^{A}	12.4^{A}	12.1^{A}	0.9	-	-	-	-	-
10 mo	14.9^{A}	13.7^{A}	10.8^{A}	14.5^{B}	0.9	-	-	-	-	-
11 mo	12.2^{B}	12.8^{A}	10.7^{A}	11.9^{A}	1.0	-	-	-	-	-
Total DMI, % BW	2.4	2.2	2.1	2.3	0.1	0.24	0.74	0.05	0.03	0.01
9 mo	2.5^{A}	2.2^{A}	2.3^{A}	2.2^{A}	0.2	-	-	-	-	-

Table 15 Continued

	Treatment[2]					*P*-values[3]				
Item	High Con	High Arg	Mod Con	Mod Arg	SEM	Nutr	AA	Nutr×AA	Month	Block
10 mo	2.6[A]	2.4[A]	2.0[A]	2.6[B]	0.2	-	-	-	-	-
11 mo	2.1[B]	2.2[A]	2.0[A]	2.1[A]	0.2	-	-	-	-	-

[1]Treatments were applied beginning 110 d prior to expected foaling date. Dual marker system utilized titanium dioxide as external marker and ADIA as internal marker.

[2]Treatments were arranged as a 2 × 2 factorial with two planes of nutrition, moderate (Mod; 0.5% BW AF grain/d) or high (High; 1% BW AF grain/d) and two levels of L-arginine supplementation, 0.21 g/kg/d (Arg) or no supplemental Arg (Con; L-alanine to maintain isonitrogenous diets). All mares allowed ad libitum access to coastal bermudagrass (*Cynodon dactylon*) hay.

[3]Nutr = effect of nutrition: Mod (0.5% BW AF grain/d) or High (1% BW AF grain/d); AA = effect of amino acid supplementation: Arg (0.21 g/kg/d L-Argine) of Con (L-alanine to maintain isonitrogenous diets). Nutr×AA= interaction of plane of nutrition and amino acid supplementation. Month = month of gestation (9,10,11). Block = effect of blocking of mares by expected foaling date. Nutr×AA×Month *P*-values ≥ 0.23.

[4]Body weight recorded immediately following fecal collections during each month of gestation.

[5]Fecal collections performed during the 9th, 10th, and 11th month of gestation based on the average of the block. High Con: 9 mo: n = 8, 10 mo: n = 8, 11 mo: n = 7. High Arg: 9 mo: n = 8, 10 mo: n = 8, 11 mo: n = 7. Mod Con: 9 mo: n = 8, 10 mo: n = 7, 11 mo: n = 7. Mod Arg: 9 mo: n = 8, 10 mo: n = 8, 11 mo: n = 7.

[A-C]Values within column lacking a common superscript differ by $P \leq 0.10$.

Foaling and Colostrum Characteristics

There was no influence of Nutr or AA on gestation length, foaling parameters, ratio of placenta to mare BW, placenta to foal BW, and ratio of foal BW to mare BW ($P \geq 0.15$; Table 16). Block influenced the ratio of placenta to mare BW and foal to mare BW ($P \leq 0.01$) and tended to influence placenta to foal BW with blocks 3 and 4 being greater than blocks 1 and 2 for all measurements ($P \leq 0.10$). There was no effect of AA on CV, SG, or Brix% ($P \geq 0.32$; Table 17). Mare Nutr tended to influence CV ($P \leq 0.08$) with Mod mares having greater volume compared to High (Table 17). There was an influence of Nutr ($P \leq 0.03$) on colostrum quality indicated by greater SG in High mares compared to Mod (Table 17). Block influenced CV and SG, with blocks 3 and 4 having greater values compared to 1 and 2 ($P \leq 0.01$). Maternal Nutr and AA supplementation did not affect any foal physical measurements obtained 12 h post parturition ($P \geq 0.30$; Table 18). Block influenced foal body length ($P \leq 0.02$) and tended to influence foal BW with foals from mares in blocks 3 and 4 having greater measurements compared to foals from mares in blocks 1 and 2 ($P \leq 0.09$).

Table 16. Influence of plane of nutrition and arginine supplementation on gestation length and foaling parameters in Quarter horse mares (represented as LSMeans).[1]

Parameter	Treatment[2]				SEM	*P*-values[3]		
	High Con	High Arg	Mod Con	Mod Arg		Nutr	AA	Block
Gestation length[4], d	339.3	340.5	343.0	338.1	4.9	0.87	0.67	0.20
Time from[5]								
WB-Birth, min	16.2	23.8	16.3	12.9	5.8	0.20	0.60	0.16
Birth-PE, min	188.3	155.9	240.2	239.6	94.5	0.43	0.85	0.61
Birth-Stand, min	44.6	43.9	46.2	35.4	9.2	0.66	0.47	0.57
Weight Ratios								
Placenta: Mare[6],%	0.8	0.7	0.7	0.8	0.1	0.93	0.79	0.01
Placenta: Foal[6],%	9.4	8.2	8.0	8.7	0.8	0.48	0.71	0.10
Foal: Mare[7],%	8.5	8.7	8.9	9.7	0.5	0.15	0.30	0.01

[1]Treatments were applied beginning 110 d prior to expected foaling date.

[2]Mare dietary treatments were arranged as a 2 × 2 factorial with two planes of nutrition, moderate (Mod; 0.5% BW AF grain/d) or high (High; 1% BW AF grain/d) and two levels of L-arginine supplementation, 0.21 g/kg/d (Arg) or no supplemental Arg (Con; L-alanine to maintain isonitrogenous diets). LS means are reported.

[3]Nutr = effect of nutrition: Mod (0.5% BW AF grain/d) or High (1% BW AF grain/d); AA = effect of amino acid supplementation: Arg (0.21 g/kg/d L-Argine) of Con (L-alanine to maintain isonitrogenous diets). Block = effect of blocking of mares by expected foaling date. Nutr × AA *P*-values > 0.10.

[4]Calculated from last breeding date to foaling date. (High Con n = 7; High Arg n = 8; Mod Con n = 5; Mod Arg n = 8).

[5]WB-Birth: time from water breaking to complete passage of foal through birth canal. Birth-PE: time from complete passage of foal through birth canal to the complete expulsion of the placenta. Birth-Stand: time from complete passage of foal through birth canal to foal standing for ≥ 10 seconds. (High Con n = 7; High Arg n = 7; Mod Con n = 5; Mod Arg n = 7).

[6]Placenta: Mare: Ratio of the placenta to mare BW. Placenta: Foal: Ratio of the placenta to foal BW. Mare and foal body weights measured 12 h after parturition. (High Con n = 7; High Arg n = 7; Mod Con n = 5; Mod Arg n = 8).

[7]Foal: Mare: Ratio foal to mare BW. Mare and foal body weights measured 12 h after parturition. (High Con n = 7; High Arg n = 8; Mod Con n = 5; Mod Arg n = 8).

Table 17. Influence of plane of nutrition and arginine supplementation on colostrum volume and quality in Quarter horse mares (represented as LSMeans).[1]

Measurement[4]	Treatment[2]				SEM	*P*-values[3]		
	High Con (n = 7)	High Arg (n = 7)	Mod Con (n = 5)	Mod Arg (n = 7)		Nutr	AA	Block
Total Volume, mL	988.4	723.8	1120.3	1111.0	156.8	0.08	0.34	0.01
Brix, %[5]	24.3	27.3	27.8	27.9	1.7	0.18	0.32	0.38
Specific Gravity[6]	1.07	1.09	1.06	1.06	0.01	0.03	0.37	0.01

[1]Treatments applied beginning 110 d prior to expected foaling date.

[2]Mare dietary treatments were arranged as a 2 × 2 factorial with two planes of nutrition, moderate (Mod; 0.5% BW AF grain/d) or high (High; 1% BW AF grain/d) and two levels of L-arginine supplementation, 0.21 g/kg/d (Arg) or no supplemental Arg (Con; L-alanine to maintain isonitrogenous diets).

[3]Nutr = effect of nutrition: Mod (0.5% BW AF grain/d) or High (1% BW AF grain/d); AA = effect of amino acid supplementation: Arg (0.21 g/kg/d L-Argine) of Con (L-alanine to maintain isonitrogenous diets). Block = effect of blocking of mares by expected foaling date. Nutr×AA *P*-values > 0.10.

[4] Measurements gathered immediately following parturition prior to nursing.

[5]Measure of dissolved sugars in a liquid. Equine Colostrum Refractometer (Animal Reproduction Systems, Chino, CA).

[6]Measured by Equine Colostrometer (Lane Manufacturing, Denver, CO).

Table 18. Influence of maternal plane of nutrition and arginine supplementation on physical measurements of Quarter horse foals (represented as LSMeans).[1]

Measurement[2]	Treatment[3]				SEM	*P*-values[4]		
	High Con (n = 7)	High Arg (n = 8)	Mod Con (n = 5)	Mod Arg (n = 8)		Nutr	AA	Block
BW, kg	43.8	45.2	43.9	47.8	2.9	0.61	0.30	0.09
Body Length, cm	71.4	73.4	72.5	73.9	2.0	0.64	0.34	0.02
Wither Height, cm	92.2	93.0	94.0	93.3	1.9	0.53	0.96	0.86
Hip Height, cm	94.4	94.8	94.6	95.9	2.1	0.72	0.63	0.38

[1]Treatments applied beginning 110 d prior to expected foaling date.

[2]Measurements obtained 12 h post parturition, Body Length measured from point of the shoulder to the ischial tuberosity, Wither and Hip Height measured at their highest point.

[3]Mare dietary treatments were arranged as a 2 × 2 factorial with two planes of nutrition, moderate (Mod; 0.5% BW AF grain/d) or high (High; 1% BW AF grain/d) and two levels of L-arginine supplementation, 0.21 g/kg/d (Arg) or no supplemental Arg (Con; L-alanine to maintain isonitrogenous diets). LS means are reported.

[4]Nutr = effect of nutrition: Mod (0.5% BW AF grain/d) or High (1% BW AF grain/d); AA = effect of amino acid supplementation: Arg (0.21 g/kg/d L-Arginine) of Con (L-alanine to maintain isonitrogenous diets). Block = effect of blocking of mares by expected foaling date. Nutr×AA *P*-values > 0.10.

Discussion

As expected, High mares gained a greater amount of BW and tended to have greater RF over the 110 d trial compared to Mod, and Nutr also affected change in BCS as High mares increased BCS while Mod mares decreased. Similar effects on BW, RF, and BCS gain as a result of increased grain supplementation have been observed in previous mare trials at this facility (See Ch II). There was no effect of AA on mean BCS of mares, however supplementation of Arg influenced RF change, with Arg mares decreasing in RF and Con mares increasing. Similar effects have been observed in other species (rats, pigs, humans) where arginine supplementation increased lipolysis and inhibited lipogenesis (Jobgen et all 2009b), resulting in a decreased white adipose tissue mass coupled with increased skeletal muscle mass (Fu et al., 2005; Lucotti et al., 2006; Jobgen et al., 2009a; Jobgen et al., 2009b; Tan et al., 2009).

There was no effect of Arg on total DMI or hay DMI expressed in kg/d or percent BW. Previous research in rats indicated that Arg supplementation may alter intake due to its role as a NO precursor, which acts as a signal mechanism in triggering appetite evidenced by injection of a NO inhibitor resulting in decreased voluntary intake while injection of L-Arg significantly increased voluntary intake (Morley and Flood, 1991). However, contrasting evidence has been demonstrated in other studies where Arg supplementation had no effect on intake (Fu et al., 2005; Lassala et al., 2010). These studies administered Arg via intravenous infusion (sheep) or orally (rats) by offering Arg supplemented drinking water (Fu et al., 2005; Lassala et al., 2010). The administration method of Arg may impact its effects on intake. Our use of oral Arg supplementation

may have presented a palatability challenge with Arg mares tending to consume less concentrate due to addition of powdered Arg. Further differences in administration method exist due to catabolism of arginine by the gastrointestinal tract causing a lowered bioavailability of oral arg compared to arg administered intravenously (Wu et al., 2007).

Nutrition influenced hay DMI with Mod mares consuming a greater percentage of their BW in hay compared to High, however Nutr did not affect total DMI expressed as percent BW. This is similar to results found in previous trials that determined feeding a mixed diet of hay and concentrate decreased hay intake compared to a diet of hay only, but total intake as a percent BW remained unchanged (See Chapter II). Regardless of dietary treatment, month of gestation influenced hay and total DMI with mares consuming less during the 11^{th} mo compared to the 10^{th} mo. This is in contrast to a previous study in our laboratory, where mares consumed less in hay as well as total intake in the 10^{th} mo gestation and increased intake into the 11^{th} mo (Winsco et al., 2011). However, results of the current trial agree with research in ruminants that suggests DMI is affected by gut fill and significant decreases in DMI are observed with advancing pregnancy (Forbes, 1971; Forbes, 1986; Weston, 1982; Allen, 1996). Uterine expansion throughout gestation as a result of fetal growth limits capacity of the rumen and DMI is reduced. The contrasting results found by Winsco et al. (2011) may have been a result of different dosage techniques to provide TiO_2, or alterations in analysis techniques in the current study which utilized a digestion block providing more consistent temperature during acid digestion. In contrast to previous equine studies which indicated intake in horses was regulated by energy requirements rather than gut

volume (Frape et al., 1982; Aiken et al., 1989), results from both the previous trial in our laboratory (See Chapter II) and the current study both agree that factors other than energy requirements influence DMI in pregnant mares.

Based on calculations, mares DMI of hay was 1.5% to 1.9% BW which resulted in 2.1% to 2.4% BW total DMI. These results are comparable to our previous study that utilized TiO_2 in a dual marker system and estimated that mares offered hay only consumed 2.3% BW, while mares offered concentrate at 0.75% BW (as-fed) consumed 1.8% BW in hay for 2.4% BW total (See Chapter II). The NRC (2007) estimates DMI for 500 to 600 kg pregnant broodmares to be 2.0% BW and the previous NRC (1989) recommended pregnant mares consume 1.0 to 1.5% BW in forage and 0.5 to1.0% BW in concentrate. A previous study directly measured individual voluntary intake of timothy and alfalfa hay in pregnant mares at 5 and 10 mo gestation and found that both hay type and mo gestation did not affect DMI, and that mares consumed an average of 2.02% BW at 5 mo and 1.9% BW at 10 mo gestation (McCown et al., 2011). Furthermore, intake of similar coastal bermudagrass hay (11.3% CP, 78.3% NDF, 40% ADF) was directly measured in yearling Quarter horses at 2.1% BW (LaCasha et al., 1999). Although more research is needed to validate the use of TiO_2 and ADIA for estimating forage intake in group-housed horses, the total DMI and hay DMI calculated in the current study are within reasonable physiologic values and are in agreement with previous studies. Furthermore, mares decreased hay DMI as concentrate was added to the diet and as gestation progressed, which is also in agreement with previous research (Vermorel et al.,

1997). This indicates that the dual marker system utilized in the current study was sufficient at estimating DMI in group- housed mares.

Upon analysis and calculations described in the NRC (2007), the concentrate contained 3.54 Mcal/kg DE and hay contained 2.42 Mcal/kg. Using the average intake of concentrate and calculated hay intake in month 9, 10, and 11, High Con mares consumed an average of 38.42 Mcal DE/d, High Arg mares consumed an average of 36.03 Mcal DE/d, Mod Con mares consumed an average of 30.04 Mcal DE/d, and Mod Arg mares consumed an average of 33.57 Mcal DE/d. According to NRC (2007) values, all mares exceeded DE requirements for the last 3 mo gestation (average 24.3 Mcal DE/d) for horses with a mature BW of 600 kg. Therefore, our mares consumed approximately 158%, 148%, 124%, and 138% of recommended DE for High Con, High Arg, Mod Con, and Mod Arg, respectively. However, despite estimated DE intake exceeding NRC (2007) requirements, Mod mares decreased BCS, indicating the diet was unable to support the needs of late pregnancy. Previous studies from our laboratory reported similar results, where mares exceeded NRC DE recommendations for late gestation yet decreased in BCS and RF (Thorson et al., 2010, See Chapter II). The results of these three trials indicate a need for future studies to more precisely evaluate DE requirements for mares in late gestation. Furthermore, the BCS decline of Mod mares while consuming approximately ≥ 124% DE recommendations suggests the High diets used in the current study were not actually exceeding NRC DE recommendations by 150% which is the level of overnutrition utilized in similar trials in other species. This may explain the lack of effect of Nutr in some variables within the current study compared to

effects observed in other species as a result of overnutrition at 150% NRC recommendations.

Foaling variables did not differ with maternal Nutr. These results are similar to previous research in our laboratory which found no influence of maternal DE manipulation on similar parameters (Winsco et al., 2010a). Kubiak et al. (1988) reported similar results, finding no effect of DE intake on duration of stages of parturition in mares that were fed to obesity (estimated DE intake of 150% NRC (1978) recommendations) or fed to maintain a moderate BCS (estimated DE intake of 100% NRC (1978) recommendations). These results suggest that overfeeding mares in late gestation will not alter parameters associated with parturition.

Arginine supplementation did not affect any foaling time intervals, gestation length, or weight ratios. Previous research in pigs, sheep, and rats also found no difference in gestation length as a result of Arg supplementation (Mateo et al., 2007; Zeng et al., 2008; Lassala et al., 2010). Only one other study investigated effect of Arg on foaling variables and gestation length in horses. Mortensen et al. (2011) supplemented Arg at 1% of the diet beginning 21 d prior to expected foaling date and observed a shorter gestation length in Arg supplemented mares however, the authors acknowledged difference in gestation length may have been due to low number of animals per treatment (treatment n = 8), or as a result of a number of variables that may also influence gestation length such as season, breed, gender of fetus, parity, and age of mare (Mortensen et al., 2011). Additionally, there was a lack of an isonitrogenous control diet, and a difference in Arg administration, which was provided once daily

compared to twice daily in our study. A large single dose of arginine could alter results as arginine shares a transport system with lysine and histidine, therefore excess arginine may result in amino acid imbalance or alterations in transport (Edmonds et al., 1987; Wu et al., 2007). However, the study by Mortensen et al. (2011) provides a comparison for foaling parameters, placenta weights, birth weights, and physical characteristics, which in both studies were unaffected by Arg.

Previous research in other species (sheep, rats, pigs) has found increased birth weights as a result of maternal supplementation of Arg, in both under and over-fed models (Mateo et al., 2007; Zeng et al., 2008; Lassala et al., 2010). However, it is difficult to make direct comparisons across studies due to varying levels of over and under nutrition. Furthermore there is difficulty comparing arginine supplementation across species because of differences between monotocous and polytocous species, as well as a varying ability to synthesize endogenous arginine (Ball et al., 2007). The lack of influence of Nutr and Arg supplementation on foal physical characteristics observed in this study, in addition to a previous study in our laboratory evaluating Nutr alone, suggests nutrients may already be preferentially partitioned to the foal making it difficult to create any observable differences without a more extreme variation in nutrient plane.

Maternal Nutr tended to influence CV with Mod mares having greater volume compared to High. Research in sheep has demonstrated overfeeding reduced the initial volume and IgG concentration of colostrum (Davidson et al., 2000; Wallace et al., 2001). Previous research in horses found no influence of maternal DE manipulation on total colostrum volume (Winsco et al., 2010a). There was an influence of Nutr on

colostrum quality indicated by greater SG in High mares compared to Mod. This is in contrast to results from a previous study which observed a lower SG in colostrum from mares consuming increased DE (Winsco et al., 2010a). It should be noted that in the current study, although dietary treatment influenced colostrum quality, all SG values obtained were within ranges considered adequate for passive transfer (1.06 to 1.09). Colostrum with a specific gravity of ≥ 1.06 has been found to contain an adequate concentration of IgG to result in foals with serum IgG concentrations exceeding minimum concentrations necessary for passive transfer of immunity (LeBlanc et al., 1986). There was no effect of Nutr on Brix%, however previous research in horses found mares on a lower plane of nutrition, receiving forage only, had greater Brix % values and SG than mares on a higher plane of nutrition and supplemented with concentrate (Thorson et al., 2010; Winsco et al., 2010a). These increased quality estimates from the previous trials were confirmed by laboratory analysis of IgG, which was also greater in mares that received forage only (Winsco et al., 2010a). However, previous studies calculated total g IgG as a function of concentration and volume and determined that the total IgG available was not different between nutritional planes (See Chapter III). Therefore, although there are not consistent trends observed in SG and Brix% among trials within our laboratory, these are simply on-farm estimates and colostrum quality should be confirmed by analysis of IgG concentration to more accurately compare across studies.

An effect of block was inconsistently observed in many variables. The cause of this effect is not known, however it is likely due to variation within mares due to a

relatively low number of mares per treatment or a seasonal effect due to mares foaling over a 6 mo period. The average expected foaling dates for each block were: block 1 = mid-January, block 2 = late-February, block 3 = mid-April, and block 4 = late-May. Blocks 1 and 2 gained a greater amount of BW and increased RF, but maintained BCS. Blocks 3 and 4 increased in BCS but gained less BW and RF compared to blocks 1 and 2. However, blocks 3 and 4 had greater DMI (hay and total DMI, expressed in kg and as percent BW). Along with the later foaling date, this increased intake may have contributed to the increased ratio of placenta to mare BW, foal to mare BW, placenta to foal BW, foal BW, foal body length, CV, and SG in mares from blocks 3 and 4. In addition to blocking mares by expected foaling dates, mare age, BW, BCS, and RF were also accounted for and were not different between blocks at the beginning of the trial. Although average mare age was not different, mare parity was unknown and could also have contributed to these block effects.

In summary, as expected, a higher plane of maternal nutrition resulted in greater BW gain and BCS. Arginine supplementation resulted in a loss of RF whereas unsupplemented mares increased RF. Plane of nutrition influenced DMI, with moderate plane of nutrition mares receving a lower percentage of concentrate in their diet consuming a greater percentage BW in hay although total DMI was unaffected. All mares, regardless of diet, consumed less during the 11th mo gestation. Supplemental arginine had no effect on DMI. Foaling was not affected by maternal plane of nutrition or arginine supplementation. Colostrum volume decreased with increased plane of nutrition, but colostrum quality as indicated by specific gravity increased. Continued

research investigating manipulation of maternal nutrition and its effects on DMI, foaling, and colostrum would be beneficial as well as additional work to determine the interaction of maternal supplementation of arginine to mitigate these effects.

CHAPTER VI

SUMMARY AND IMPLICATIONS

Overnutrition is a common occurrence in the horse industry with obesity rates ranging from 45 to 50% (Stephenson et al., 2011). Common management practices in horses include feeding starch-rich diets in large meals, which may promote insulin resistance (Hoffman et al., 2003a), and have been linked to obesity (Jeffcott et al., 1986). Furthermore, previous research has determined that reproductive efficiency, quantified as decreased time to onset of estrus and decreased number of cycles until conception, was enhanced beginning at a BCS 5 or greater (Henneke et al., 1984). Additionally, there were no observed detrimental effects to a higher BCS, and therefore maintaining mares at a BCS 6 was recommended (Kubiak et al., 1987; Henneke et al., 1984). This encourages equine producers to maintain broodmares at a fleshier condition and the consequences of this overnutrition have not been fully explored.

These studies have provided a wealth of information to help elucidate the impact of maternal nutrition on mares and their foals. These data indicate that alteration of maternal plane of nutrition of mares during late gestation influenced mare performance and altered DMI. Based on calculations, all mares exceeded NRC (2007) DE recommendations but failed to increase BW and BCS as expected, underscoring a need for future work investigating DE requirements during pregnancy on forage based diets. Additionally, data provided insight to DMI during pregnancy, which was influenced by month of gestation, with the first trial finding all mares consumed less in the 10^{th} month

of pregnancy compared to the 11th, and the second trial finding all mares consumed less during the 11th month. This indicates DMI in pregnant mares was not driven solely by the increased energy demands of late gestation, and that mare intake behaves similarly to ruminants, which decrease intake with progressing gestation. Additionally, the second study determined that arginine supplementation has no detrimental effects on DMI which will be beneficial for future research with this amino acid.

Although more research is needed to validate the use of TiO_2 and ADIA for estimating forage intake in group-housed horses, total intake values and forage intake amounts calculated in the current study are within reported physiologic values in previous studies. Furthermore, mares decreased forage intake as concentrate was added to the diet, which is also in agreement with previous research and indicates that the dual marker system was sufficient at estimating forage intake in group- housed mares with and without grain supplementation.

Both studies illustrated that foaling characteristics are not largely influenced by maternal nutrition, as each study found no influence of dietary treatment (including arginine supplementation) on foaling parameters or physical measurements obtained following parturition. The first study also determined that maternal nutrition had no effect on foal growth or ADG over the first 150 days.

When colostrum quality was evaluated, the initial study determined mares consuming only hay had increased specific gravity and Brix% of colostrum indicating a higher quality, which was confirmed by IgG analysis finding a tendency for increased IgG concentration. However, colostrum volume was not affected by maternal nutrition,

nor was total g IgG (calculated from volume and concentration). The second study found contrasting results with greater specific gravity in mares on a high plane of nutrition compared to moderate, and a tendency for moderate plane of nutrition mares to have greater volume compared to high plane of nutrition mares. Based on these results, it is likely that on-farm estimates of colostrum quality such as specific gravity and Brix % may have too much variability to be useful in a research setting and require laboratory analysis of IgG concentration to more accurately compare across studies. Additionally, the second study determined that arginine supplementation does not influence colostrum volume or quality (measured by specific gravity or Brix %). These projects are also only the second to evaluate the impact of maternal nutrition on glucose and insulin metabolism in foals. Maternal diets affected glucose and insulin AUC in mares, which altered insulin dynamics in the resulting foals. Foal insulin AUC and peak insulin concentration were greater in foals from mares supplemented with concentrate compared to foals from hay fed mares.

Data from the National Health and Nutrition Examination Survey 2009-2010 showed that 35.7% of the total United States population and 31.9% of women aged 20-39 are obese (obesity defined as a body mass index ≥ 30; Ogden et al., 2012). While data from other livestock species, including sheep, indicates they may serve as an effective model for human disease (Ford et al., 2009), the horse may be a more appropriate animal model. Our study demonstrated alterations in maternal plane of nutrition effects insulin dynamics in mares and foals. Additionally, common management practices in horses such as feeding starch-rich cereal grains in large meals, and following current

recommendations to maintain broodmares at a fleshy condition score for improved reproductive efficiency have contributed to very high obesity rates (Kubiak et al., 1987; Henneke et al., 1984; Hoffman et al., 2003a; Stephenson et al., 2011). Furthermore, longer lifespans and a production setting that encourages greater longevity result in increased incidence of metabolic disorders in horses comparable to metabolic diseases in humans (Fulop et al., 2006; Frank et al., 2010). This allows for great opportunity for the horse to be used as an ideal animal model for further investigation to better understand the effects of maternal obesity.

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