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Evaluation Of A Novel Endophyte-Infected Tall Fescue Cultivar As A Safe Forage For Pregnant Mares

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EVALUATION OF A NOVEL ENDOPHYTE-INFECTED TALL FESCUE

CULTIVAR AS A SAFE FORAGE FOR PREGNANT MARES

By

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Fescue toxicosis is a condition that afflicts livestock grazing endophyte-infected tall fescue and is particularly detrimental to pregnant mares. A two year evaluation study of a novel endophtye-infected cultivar, PDF 584, did not induce fescue toxicosis in late-term pregnant mares. All mares delivered viable foals except in E+ group which had two viable foals, one stillborn-dystocia and one compromised foal which was euthanized at 72 h pp. Serum P4 concentrations were similar among PDF 584, NE+, and E- mares ($p>0.05$). Foal BW and foal/placental weight ratios were similar for PDF 584, NE+, and E- ($p>0.05$). Foal serum P4 was similar on 1 d and 2 d in all groups, but was lower ($p = 0.049$) in the PDF 584group than the E- foals on day 0. Neutrophil/lymphocyte ratios were similar (~5:1) in all foals on d 0 and 2. IgG values were similar ($p>0.05$) among PDF 584, NE+, and E-.

Key words: novel endophyte-infected fescue, mares, fescue toxicosis

DEDICATION

This thesis is dedicated to my family.

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CHAPTER I

INTRODUCTION

Tall fescue (*Festuca arundinacea,* Syn *Lolium arundinaceum* (Schreb.) Darbysh.) is a perennial, common cool-season grass grown widely in the United States (Roberts and Andrae, 2004; Gunter and Beck, 2004) and it occupies approximately 14 million hectares (Oliver et al., 2000). In the United States, over 700,000 horses and 8.5 million cattle (Ball et al., 2007) are grazing on tall fescue pastures, 63% of these pastures are infected by endophyte (McCluskey et al., 1999). Tall fescue was brought from Europe to the United States in late 1800s. However, the official discovery of tall fescue was in 1931 by the University of Kentucky. In 1943, a fescue cultivar known as "Kentucky 31" was released from the University of Kentucky. Since then, "Kentucky 31" has become widely accepted by farmers due to advantages such as resistance to overgrazing, drought resistance, and high nutrient quality for grazing animals (Bacon, 1995). While endophyte-infected tall fescue has many advantages over the non-infected fescue, it is heavily contaminated with compounds (i.e. ergot alkaloids), some of which are now known to be toxic to livestock.

'Endo' means within and 'phyte' means plant which explaining that this fungus grows between tall fescue cells. The principle cause of fescue toxicosis in livestock and horses are ergot alkaloids, which are the toxic compounds that are produced by the endophytic fungus called *Neotyphodium coenophialum* previously described as

Acremonium coenophialum. The ergot alkaloids that are produced by some endophyte are ergovaline, ergovine, lysergamine, and ergotamine. Ergovaline is the most toxic alkaloid found in infected tall fescue (Roberts and Andrae, 2004; Gadberry et al., 2003; Porter, 1995). Ergot alkaloid concentrated in the seed heads (Roberts and Andrae, 2004; Kemp et al., 2007). However, alkaloid can be found in stems and leaves with highest concentrations in late spring and reduced in the summer (Roberts and Andrae, 2004). In addition, concentration of 50 ng of ergovaline/g infected tall fescue is thought to be sufficient to induce signs of fescue toxicosis in cattle consuming contaminated forage (Porter, 1995). Finally, *Neotyphodium coenophialum* protects plant from environmental stressors such as nematodes, insects, and drought (Porter and Thompson, 1992).

The *Neotyphodium coenophialum* endophyte is a member of the family Clavicipitaceae (Bacon and DeBattista, 1991). The family of ergot alkaloid compounds isolated from *Neotyphodium coenophialum*-infected tall fescue include; ergopeptines, clavines, and simple lysergic acid amides (Yates et al., 1985). Ergovaline is the major ergopeptine alkaloids found in tall fescue infected by the endophyte *Neotyphodium coenophialum* (Yates et al., 1985). In addition, Yates and Powell (1988) reported that ergovaline is the primary ergot alkaloid associated with the induction of fescue toxicosis in animals that consume tall fescue infected with *Neotyphodium coenophialum.* In cattle and sheep, it has been reported that ergot alkaloid exposurue induced vasoconstriction of blood vessels supplying the hoof which makes it the primary cause of fescue foot (Abney et al., 1993) and heat intolerance (Rhodes et al., 1991). Ergot alkaloids, also bind to the D2 dopamine receptors in the hypothalamus thus inhibiting prolactin release from the anterior pituitary gland (Porter and Thompson, 1992) which leads to agalactia in cattle

(Paterson et al., 1995) and horses (Cross et al., 1995). Moreover, the vasoconstriction activities of ergot alkaloids is thought to be involved in the placental thickening in mares that consume endophyte-infected fescue which leads to decreased placental function and feto-maternal hormonal exchange resulting in prolonged gestation length and increased incidence of dyxtocia in these animals. (Porter and Thompson, 1992).

CHAPTER II

LITERATURE REVIEW

Fescue Toxicosis in Cattle

Fescue toxicosis is a serious problem in cattle. It is manifested by a number of symptomatic problems that, in serious cases, may lead to fatality. These symptoms include fescue foot, increased body temperature, increased respiratory rate, fat necrosis, decreased feed intake, reduction in average daily gain, decreased serum prolactin which can lead to agalactia (low in milk production) in lactating cows, and low conception rates.

<u>Fescue foot:</u>

The first description of fescue foot in the United State was in 1950. It thought to be associated with cold temperature that link with endophyte-infected fescue observation (Bacon, 1995). Grazing for few days in endophyte – infected tall fescue thought to be sufficient period to show the clinical signs of fescue foot in 20% of the herd and this percentage increases when the herd is kept longer in infected pastures. The signs of fescue foot are swelling and skin discoloration around the hooves, which will lead to lameness in severe cases. The ergot alkaloid induced vasoconstriction of blood vessels supplying the hoof is the primary cause of fescue foot (Abney et al., 1993).

Body temperature and respiratory rate:

Increased body temperature and high respiratory rate were observed in cattle consuming endophyte-infected tall fescue (Merrill et al., 2007; Roberts and Andrae, 2004; Porter and Thompson, 1992; Rhodes et al., 1991). Affected cattle seek out for shedding and water pools to aid thermoregulate their body heat. The ergot induced vasoconstriction is also the primary cause of heat intolerance in cattle. This activity leads to decreased blood flow to peripheral tissues, body core and brain areas, which affect the regulation of body temperature (Rhodes et al., 1991). Respiratory rate increases in order to dissipate body heat as the core body temperature increases.

Fat necrosis:

Is the condition that is caused by infection of fat tissues, which effected by lipolytic enzymes. This syndrome is thought to be associated with fertilizing tall fescue with high level of nitrogen (Bacon, 1995). Fat necrosis lesion is characterized by dark yellow color compared with regular color of fat and causes digestive and reproductive problems, and kidney failure. This problem is further complicated since ergot alkaloid compounds are lipophilic and are deposited in fat tissues. These fat stores of ergot alkaloids are continuously released and may take months to deplete. Therefore, fescue toxicosis symptoms may be observed for extended periods after animals are removed from the toxic pasture (Roberts and Andrae, 2004).

Decreased forage intake:

Cows and steers grazing endophyte- infected tall fescue had decreased body weights compared with cows and steers that consumed endophyte-free tall fescue (Paterson et al., 1995). Similar studies reported a decrease in weight gain in bulls (Schuenemann et al., 2005) and heifers (Burke et al. 2007). Paterson et al. (1995) reported that average daily gain decreased from 30% up to 100% in steers grazed on endophyte-infected fescue compared with steers grazed on endophyte-free fescue. That some study reported a 0.045 kg/day decrease in steer gain weight for each 10% increase in endophyte infection (Paterson et al. 1995). During the hours between 12 noon and 6 pm, steers on endophyte –free pasture spend 60% of the time grazing while steers in endophyte - infected pasture spend 6% of the same time grazing. The authors concluded that this reduced intake accounted for the decrease in average daily gain (Stuedemann et al. 1985). When environmental temperature reduced greater than 32 ºC, cows in toxic pasture consumed less forage than cows in endophyte-free pasture (Paterson et al. 1995).

Milk production:

Cattle grazing endophyte – infected tall fescue have been shown to exhibit a decrease in milk production (Paterson et al., 1995). This decline in milk production is a result of the negative effects of ergot alkaloid on lactogenesis (milk production). Ergots bind D2 dopamine receptors in the hypothalamus and inhibiting prolactin release from the anterior pituitary gland (Porter and Thompson, 1992). Paterson et al. (1995) reported that milk yield was decreased by 25% in cattle grazing endophyte-infected fescue compared to those who were grazing on endophyte-free pasture. The correlation between milk production and fungus infection is 0.15 kg decrease in milk yield per day for each 10% increase of endophyte infection in the pasture (Paterson et al., 1995). Cows also have another source of prolactin, which produces from the placenta. Prior to calving, the placental prolactin helps milk production and mammary gland development. The involvement of prolactin is primarily in mammogenesis (development of mammary gland) and lactogenesis, but has no effects in galactopoiesis (continued milk production) (Porter and Thompson, 1992). Because cows have placental lactogen and prolactin in last stage of gestation, they do not usually have complete agalactia (Forsyth, 1986).

Puberty, conception rate, and bull fertility:

Puberty in heifers is marked by an increase of progesterone level in the serum (Porter and Thompson, 1992). In a three- year study, shows that heifers grazing on high endophyte-infected pasture had low concentrations of progesterone in the serum at 15 months of age, indicating delayed puberty compared to heifers on low endophyte-infected pasture (Porter and Thompson, 1992). Also, heifer fertility is decreased when heat stress increased (Burke et al., 2001). A reduction in conception rate has been reported in cows grazing endophyte-infected tall fescue (Paterson et al., 1995; Porter and Thompson, 1992). In one study, reported by Paterson et al. (1995), that conception rates for cows grazing on low endophyte-infected pasture were 86% vs 67% in high endophyte-infected tall fescue. In another study, the conception rate was 96% for heifers consuming low endophyte-infected tall fescue compared with 55% for heifers consuming high endophyte-infected (Schmidt et al. 1986). The correlation between conception rate and the percentage infected grass was estimated to be a 3.5% decrease in conception rate for each 10% increase in endophyte infection of pasture (Porter and Thompson, 1992). A three –year study on pregnant heifers grazing endophyte-infected fescue reported that heifers carring viable calves in the first year of the study was 11% on high endophyte-infected compared with 58% on low endophyte-infected pasture. In the second year, the percentage of viable calves increased to 63% on high endophyte-infected and 84% for heifers on low endophyte-infected pasture. In the third year, the percent of calves viability decreased to 42% for heifers in high endophyte-infected versus 53% on low endophyte-infected pasture (Washburn and Green, 1991). Schuenemann et al. (2005) reported a decreased cleavage rate for embryos fertilized with sperm collected from bulls grazing infected fescue compared with sperm that collected from bulls grazing non-infected fescue. However, there was no difference in sperms morphology or motility (Schuenemann et al., 2005).

Fescue Toxicosis in Sheep

Sheep show signs of fescue toxicosis similar to that of cattle when they consume endophyte-infected tall fescue. However, sheep had lower reproductive consequences compared with cattle and horses. Sheep, when exposed to infected tall fescue pasture, also exhibit fescue foot, increased body temperature, and decreased milk yield (Forsyth, 1986). Decreasing blood flow in peripheral tissues leads to an increase in body temperature (Rhodes et al., 1991). The reason of their hyperthermia is because sheep are not capable of cooling themselves efficiently (Rhodes et al., 1991). Sheep grazing on endophyte-infected fescue pasture show a reduction in milk production because of the effects of ergot alkaloid on prolactin secretion. However, having placental lactogen reduces the frequency of having a complete agalactia (Forsyth, 1986). Ewes show low conception rates as a negative consequence of fescue toxicity in reproduction, (Porter and Thompson, 1992; Brendemuehl et al., 1994). Porter and Thompson (1992) reported that this reduction in conception rate might be because of delayed the onset of estrus.

Fescue Toxicosis Models in Rats and Mice

Rats and mice have been used as a model of fescue toxicosis studies. Spiers et al. (2005) examined the responses of rats to fescue toxicosis by feeding them endophyte-infected tall fescue seeds. They reported an increase in body core temperature, decreased average daily gain, and low activity compared with rats that consumed endophyte-free tall fescue seeds (Spiers et al., 2005). Mice fertility is also affected; however, female mice seem to be more sensitive to the effects of consuming endophyte-infected seeds than males (Porter and Thompson, 1992). Male rats showed a reduction in epididymal weight and daily sperm production (Porter and Thompson, 1992). Female rats showed prolonged estrus and decreased implantation (Porter and Thompson, 1992). Mice have placental lactogen during pregnancy similar to cattle and sheep (Forsyth, 1986). Thus, they do not exhibit complete agalactia (Forsyth, 1986). Filipov et al. (1999) reported a decrease in hypothalamic dihydroxyphenylacetic acid-dopamine metabolites (DOPAC) when they treated mice with different doses of ergotamine.

Fescue Toxicosis in Horses

Fescue toxicosis causes serious reproductive problems in horses. In pregnant mares, these consequences are characterized by abortion, increased gestation length, dystocia, placental problems, agalactia, stillborn foals, and in severe cases mare and foal mortality (Cross et al., 1995). Mares grazing endophyte-infected tall fescue pasture had prolonged luteal phase. It was suggested that this increase in length of the luteal phase could be related to a failure of the endometrium to synthesize and release prostaglandin F2α (PGF2α) at the appropriate time (Brendemuehl et al., 1994).

Body temperature:

Due to decrease of blood flow to the peripheral tissues, body temperature in most animals consuming endophyte-infected fescue increases (Rhodes et al., 1991). However, no increase in body temperature was reported in horses grazing endophyte-infected tall fescue. The reason for not observing an increase in horse's body temperature is likely because they are more efficient in cooling themselves (by sweating) than other animals such as sheep and cattle (Cross et al., 1995). For that reason, a distinct increase in sweating was observed in pregnant mares grazed endophyte-infected pastures (Cross et al., 1995).

Early embryonic loss and abortion:

Brendemuehl et al. (1994) reported a higher percentage of embryonic death in mares grazed on endophyte-infected fescue (30%) compared with mares that consumed endophyte-free fescue (7.7%). Thus, early embryonic death might be related to the toxic effects of ergot alkaloid on the embryo (Brendemuehl et al., 1994). Cross (1997) reported that the abortion rate after 30 days of gestation is very rare. Abortion rate in mares grazing infected fescue is 18% more than those grazing different grasses (Porter and Thompson, 1992).

Prolonged gestation length and dystocia:

Pregnant mares that grazed on infected tall fescue exhibited prolonged gestation and dystocia (Cross, 1997; Cross et al., 1995; Porter and Thompson, 1992). This increase in gestation length is between 20 (Porter and Thompson, 1992) to 25 days (Monroe et al., 1988) more than the regular range of gestation, which is 335-345 days. Porter and Thompson (1992) reported a 38% increase in gestation length in mares that consumed endophyte-infected fescue. A consequence of prolonged gestation is often an increase in the incidence of dystocia in mares grazing endophyte-infected tall fescue pastures. Fetal malpresentation, large foal, and non preparation of the reproductive tract are the main causes of dystocia in mares that consumed endophyte-infected fescue (Cross, 1997).

Thickened/retained placenta:

The placentas of mares consuming endophyte-infected tall fescue are thickened and/or retained in most cases (Roberts and Andrae, 2004; Cross, 1997; Monroe et al., 1988). The vasoactivity (vasoconstrictive effects) of ergot alkaloid is thought to be the primary cause of thickened placentas in mares grazing endophyte-infected fescue (Porter and Thompson, 1992). This activity reduces blood flow to the internal organs and leads to thickening of the placentas (Porter and Thompson, 1992).

Agalactia:

In most cases, mares grazing on infected fescue pastures had complete agalactia (Cross, 1997; Cross et al., 1995; Porter and Thompson, 1992). Sheep, cattle, and mice have a placental lactogen during pregnancy; mares, in contrast, rely on the pituitary prolactin to stimulate the prepartum lactogenesis, which their placentas do not produce prolactin (Cross et al., 1995). Since ergot alkaloids mimic dopamine by binding D2 dopamine receptors (responsible for stimulating pituitary prolactin), which inhibit the releasing of prolactin from the pituitary gland and lead to complete agalactia (Cross et al., 1995; Porter and Thompson, 1992). Agalactia occurs in 88% of mares grazing on toxic fescue pasture and, if the agalactic mares produced milk, it will be oily-brownish color instead of white milk (Cross, 1997).

Foal viability:

According to Earle et al. (1990), 50% of mares that grazed on endophyte-infected fescue had stillborn foals and 86% of the foals that survive the delivery died. Those foals that survived delivery were dysmature, emaciated, and had long hair and overgrown hooves (Cross, 1997; Porter and Thompson, 1992). Most foals that survived delivery also died due to low colostrum in the mares milk (Cross, 1997).

Endocrine Effects

Progesterone:

Progesterone is the primary hormone for pregnancy maintenance. It plays an essential role for embryo implantation in the uterus (Cross, 1997; Brendemuehl et al., 1994). In the early pregnancy, progesterone is secreted from the primary corpus luteum and any decrease in progesterone concentration will lead to early embryonic loss (Brendemuehl et al., 1994). Later, the endometrial cups release equine chorionic gonadotrophin (ECG), which stimulates the accessory corpus lutea to produce progesterone (Brendemuehl et al., 1994). In late gestation, the placenta is the main pregnenolone (P5) source in gravid mares (Fashen, 1984). Mares grazing endophyte-infected tall fescue had decreased serum progesterone concentrations (Cross et al., 1995; Brendemuehl et al., 1994; McCann et al., 1992). McCann et al. (1992) concluded that "placental progesterone secretion may be altered by some function of vasoconstriction." They also concluded that the decrease in progesterone concentrations maybe involved in prolonged gestation length in mares consuming infected fescue since the progesterone concentrations increase in the last two weeks of pregnancy (McCann et al., 1992).

Dopamine and prolactin secretions:

Decreased serum prolactin concentration is one of the most significant signs of fescue toxicosis in animals grazing endophyte-infected tall fescue pastures (Arns et al., 1997; Cross, 1997; Browning et al., 1997; Paterson et al., 1995; Porter and Thompson, 1992; McCann et al., 1992). Prolactin is secreted from the anterior pituitary lactotrophs and it is regulated by dopamine secretion (Filipov et at., 1999; Cross, 1997; Porter and Thompson, 1992). Dopamine is secreted from the hypothalamus and transported to the anterior pituitary through the hypothalamic/hypophysial portal system (Ben-Jonathan and Hansko, 2001; Cross, 1997). Dopamine inhibits prolactin secretion via interaction with D2 dopamine receptors located on lactotrophs in the anterior pituitary (Ben-Jonathan and Hansko, 2001; Cross, 1997; Cross et al., 1995). Binding these receptors by dopamine agonists factors (i.e., ergot alkaloids) will reduce or eliminate prolactin secretion (Cross, 1997; Cross et al., 1995; Paterson et al., 1995; Porter and Thompson, 1992). In addition, Filipov et at., (1999) reported a decrease in dopamine synthesis in mice that consumed ergotamine (an ergot alkaloid compound). Prolactin is involved in mammary gland development and in milk production (Porter and Thompson, 1992). Therefore, negative effects on prolactin secretion will affect milk yield negatively. Initiation of the mare's mammary gland development starts two to six weeks prior to parturition and any effects on prolactin secretion can delay udder development (Cross, 1997). In males, Schuenemann et al. (2005) reported a decrease in serum prolactin concentration in bulls on infected fescue pastures. Even though prolactin is important for lactation, it is also involved in osmoregulation, immune responses, and in growth (Schuenemann et al., 2005).

Management, Prevention, and Treatment of Fescue Toxicosis

Management of infected pastures depends on the presence of ergot alkaloid. Therefore, the first step to managing pasture is to determine the presence of endophyte in the pasture itself. This determination is accomplished by testing a sample from the expected pasture. According to Roberts and Andrae (2004) there are two methods to test the sample; chemical or microscopic procedure. The chemical procedure is faster and more accurate than the microscopic one (Roberts and Andrae, 2004). However, both methods are measuring presence or absence of endophyte in the plant without measuring the actual concentration of ergot alkaloids (Roberts and Andrae, 2004).

Pasture management:

If the percentage of endophyte in the pasture ranges between 20 to 40 percent, the pasture is classified as moderate infected, while over 50 percent is considered to be a highly infection pasture (Roberts and Andrae, 2004). Two methods are suggested to manage the high infected pasture. These methods include replacing the entire infected pasture with endophyte-free or novel endophyte-infected tall fescue (Roberts and Andrae, 2004), or by diluting infected pasture with legumes such as red and white clover, and annual lespedeza (Roberts and Andrae, 2004). The reason for diluting endophyte-infected pasture is to minimize the concentration of ergot alkaloid that is consumed which will minimize symptoms associate with fescue toxicity. Zhuang et al. (2005) reported that if the percentage of infection in the pasture is greater than 70%, then replacing the infected pasture with endophyte-free is economically worthy and if the pasture is less than 70% infected, then the dilution approach is more economically beneficial.

"Kentucky 31" pasture:

Cross (1997) highly recommend the removal of pregnant mares from endophyte-infected pasture from 30 to 90 days prior to the expected foaling date. This removed prevent mares from developing the clinical signs of fescue toxicosis (Cross, 1997). In similar study, McCann et al. (1992) reported a positive development of pregnancy outcome in the pregnant mares that were grazing in endophyte-infected fescue when they removed them to endophyte-free fescue pasture in a period of 305 to 370 days of gestation. Boosinger et al. (1995) observed that short term removal of pregnant mares from endophyte-infected pastures at 300 days of gestation, minimized the signs of fescue toxicity and lead to increase the mammary gland development, decrease the occurrence of dystocia, and increase foals viability.

Energy supplementation:

Feeding pregnant mares cracked corn for 90 days prior to the expecting foaling day as an energy supplementation had no beneficial effects on fescue toxicity signs (Earle et al., 1990). Earle et al. (1990) reported a high mortality rate in both mares and foals that used corn as a supplementation to reduce the severity of fescue toxicosis. In addition, more than 50% of the mares experienced dystocia and prolonged gestation length (Earle et al., 1990). Monroe et al. (1988) injected mares that were grazing (140 days of gestation) on endophyte- free and endophyte-infected pastures with 2.5 mg/45 kg body weight selenium intramuscularly at 140 of gestation but did not prevent fescue toxicosis symptoms.

Therapeutic treatment:

Redmond et al. (1991) examined the effects of Phenothiazine (blocks dopaminergic transmission) on pregnant mares grazing on endophyte-infected tall fescue pastures. They started administration of Phenothiazine (2 gm/head/day in 454 gm of a corn-dried molasses carrier) 40 days prior to expected foaling day (Redmond et al., 1991). Their conclusion was that there were no beneficial effects of Phenothiazine on gravid mares consuming endophyte-infected fescue even though there was an increase in serum prolactin concentrations. Wimbush and Loch (1998) injected pregnant mares that grazed infected fescue with a single i.m injection of fluphenazine deconoate (D2-dopamine receptor antagonist) on day 320 of gestation. They observed shorter gestation length and normal delivery in treated mares compared with non treated mares. However, they suggested further research is needed to determine effective time and dose of administrations. In another therapeutic study, Paterson et al. (1995) reported that the use of piquindone (dopamine antagonist) in sheep and steers effectively increased serum prolactin concentrations.

Domperidone:

A study by Cross et al. (1999) examining the clinical effectiveness of domperidone showed positive outcomes when they used it as a preventative treatment for pregnant mares grazing endophyte-infected pastures. The recommended usage is a single oral dose administered daily [1.1 mg/kg body weight] started 10 or more days prior the expected foaling day (Cross et al., 1999). According to Cross et al. (1999), when domperidone was used 16-30 days before the expected foaling day, mares foaled by an average of 2.97 days prior to their expected foaling day (335 day). When the treatment was initiated 10-15or 1-9 days before the expected foaling day, mares foaled after their expected foaling date by an average of 3.26 and 6.59 days, respectively. When gravid mares were treated with domperidone, the percentage of live foals reached 98% and retained placentas decreased to 7% (Cross et al., 1999). Therefore, with all these improvements in the clinical signs of fescue toxicosis, domperidone has been demonstrated to be an appropriate therapy to treat fescue toxicosis in pregnant mares.

Novel endophyte-infected tall fescue:

Watson et al. (2004) compared the productivity of cows grazing on endophyte-infected fescue pasture with non toxic endophyte-infected tall fescue (MaxQ™, AR542). This strain of endophyte was developed because to have nil ergot alkaloid (Realini et al., 2005) and was initially discovered and isolated by Ag Research in New Zealand (Bouton et al., 2002). Cows that consumed AR542 infected pasture had heavier calves at birth, calves had higher average daily gain and weaning weight, and higher serum prolactin concentrations than that for those cows that grazed on wild-type endophyte-infected pastures (Watson et al., 2004). Parish et al. (2003) evaluated the effect of non-ergot alkaloid AR542 exposure to lambs. They found that lambs grazed on AR542 had higher serum prolactin concentrations compared with those lambs grazing on the wild-type endophyte pastures. Average daily gain was higher in AR542 lambs and the signs of heat stress were lower in lambs consuming AR542 compared with those that grazed on wild-type endophyte infected pasture (Gunter and Beck, 2004; Parish et al. 2003). Andrae (2003) reported that 20% of the pregnant mares grazing endophyte-infected tall fescue pastures aborted, 60% retained their placenta, and 80% were agalactic while no fescue toxicity signs were reported on the mares that grazed in MaxQ™ (AR542) pastures.

CHAPTER III

MATERIALS AND METHODS

Experiment 1 (Year I, 11 March to 5 June, 2008):

This Study was performed at the Livestock Animal Research Center of Mississippi State University, Mississippi State and funded by Noble Foundation, Ardmore, OK and Mississippi Agriculture and Forestry Experiment Station, Mississippi State.

Animals:

On March 11, 2008 twenty pregnant mares (6-20 years old, with an average age of 13 years), matched by stage of gestation (~ 280 day of gestation) and blocked by age were randomly assigned to one of four tall fescue pasture treatments (Table 3.1).

Pasture treatment groups:

The test pasture for these studies was the novel, endophyte-infected tall fescue cultivar PDF 584 (PDF 584, Noble Foundation, Ardmore, OK), MaxQ (NE+ve, Pennington Seeds, Macon, GA) served as a non-toxic endophyte-infected tall fescue cultivar previously shown to be safe for pregnant mares (negative control), while toxic, endophyte-infected tall fescue (E+ve) and enodphyte-free tall fescue (E-ve) served as positive and naïve controls. Late term gestation mares (approximately 280 days) were assigned to one of four pastures, group A mares were placed on PDF 584 fescue pasture (n = 6), group B mares were placed on NE+ve fescue pasture (n = 6), group C mares were placed on E-ve fescue pasture (n = 4), while group D mares were placed on E+ve fescue pastures (n= 4; Table 3.1). Tiller samples were found to be > 88% endophyte-infected in pastures PDF 584 and NE+ve, <8% infected in E-ve and 100% infected in E+ve pastures. Figure 3.1 shows stands of the tall fescue pastures at peak growing stages in late April 2008. Mares on E+ ve pasture were sampled to obtain pro-inflammatory cytokine data since the outcome of pregnant mares grazing E+ ve pasture is well documented (Ryan et al., 1998; Cross et al., 1995; Porter and Thompson, 1992). This group was only maintained for three weeks. Mares were supplemented with 14% crude protein; 1.4 kg/h/d of a daily ration. Protein supplement tubs were maintained in the pastures at all times, and mares had free access to fresh water. Mares were assigned to graze on ryegrass pasture for two weeks before the initiation of the study in order to synchronize them to the same type of grass and evacuate their digestive tract of the different grasses they had been consuming.

Sample collection:

Blood samples were collected by jugular venipuncture on the starting day and then 3× per week for serum progesterone (P4) concentrations, prolactin concentrations, dopamine, and pro-inflammatory cytokine analysis. Body weight was recorded and urine samples collected for assessment of ergot alkaloid from each mare on the starting day, 1× per 2 weeks, and 48 hour (h) post-partum (pp). Urine was collected using sterile gloves, lubricant, and urethral catheterization to avoid introduction of opportunistic pathogens to the vagina or the urethra. Ultrasonography for fetal well-being was performed on the starting day and the every 14 days until mares delivered. Recovered placentas and foal birth weights were recorded at foaling day to compute birth weight/placental ratio. Blood samples were collected from foals by jugular venipuncture between zero-12 h and at 48 h (pp) for P4, complete blood count which included red and white blood cells, lymphocyte, and neutrophil concentrations (for foals maturity), and IgG values for sufficient colostrum consumption (Table 3.2). Reference ranges for analysis were based on The Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University. All mares on E+ pasture were removed from the toxic fescue pasture on day 335 of gestation and kept on ryegrass pasture in order to avoid the negative consequences since the outcome for pregnant mares grazing on toxic fescue is well documented (Ryan, 1998; Cross et al., 1995; Porter and Thompson, 1992). After 24 hours of removing the mares, mares were treated with Equidone™ (domperidone 11%; Equi-Tox Pharmaceutical Research and Development, Pendleton, SC) administrated orally (0.5 mg/kg body weight) and daily up to parturition day to induce lactation (Cross et al., 1999). All data from E+ ve treatment group include mare and foal observations, mare and foal progesterone, mare and foal body weight, placental weight, foal IgGs and CBCs were reported in this paper. Additional parameters evaluated include length of gestation, signs of agalactia, dystocia, and retained placental. Placentas were called retained in a period between two to three hours postpartum (pp) and they were treated with 0.4 cc oxytocin. Aborted fetuses and neonatal deaths were submitted to the Diagnostic Laboratory at Mississippi State University, College of Veterinary Medicine for necropsy and histopathology. In addition, placental specimens were submitted for histopathology analysis. Serum and urine samples were stored at -20 ºC while dopamine samples were stored at – 62.22 ºC.

Table 3.1

Treatment Groups and Forage Cultivar Description for YEAR I (2008)

Treatment Group Year one	Type of Endophyte	Cultivar	Race of Tall Fescue
Group A (PDF 584) (n = 6)	AR584: Novel, non-toxic (experimental)	PDF 584 (PDF)	Northern European continental
Group B (NE+ ve) (n = 6)	AR542: Novel, non-toxic (MaxQ, non-toxic control)	Jesup	Northern European continental
Group C (E- ve) (n = 4)	Nil: Endophyte-free (naive control)	Jesup	Northern European continental
Group D (E+ ve) (n = 4)	"Kentucky-31": Toxic (positive control)*	Jesup	Northern European continental

* Need mares to obtain pro-inflammatory cytokine data.

Figure 3.1 Examples of stands of fescue in experimental pastures in Year I (Experiment 1); A) PDF 584 pasture, B) MaxQ pasture, C) E+ve pasture, and D) E+ ve pasture.

Experiment 2 (Year II, 4 March to 16 May, 2009):

A similar procedure to that described in experiment one (Year I, 2008) was used in experiment two (Year II, 2009). In year two, sixteen pregnant mares (6-20 years old, with an average age of 13 years), matched by stage of gestation (~ 280 day of gestation) and blocked by age were randomly assigned to one of three treatment groups (Table 3.3).

Pasture treatment groups:

In Year II, late term gestation mares (approximately 280 days) were assigned to one of three pastures, group A mares were placed on PDF 584 fescue pasture (n = 6), group B mares were placed on NE+ve fescue pasture (n = 5), while group C mares were placed on E-ve fescue pasture (n = 5; Table 3.2). The level endophyte infection of tiller samples was similar to that reported in Year I. Examples of tall fescue stands in pastures in early March 2009 are shown in Figure 3.2.

Sample collection (Year I and Year II):

Samples were collected as described in experiment one (year I, 2008). However, foal blood samples were collected between zero – two h and at 48 h (pp) for serum progesterone clearance, CBCs (to detect foals maturity). Foal blood also was collected at 24 h (pp) for IgG values. Sampling times were adjusted in this experiment especially foal sampling times (weight at birth, serum P4, CBCs, and IgG) to have more accurate data with measurement of foal samples as close to delivery as possible.

Progesterone concentration analysis:

Blood serum was collected by jugular venipuncture and spun down for 30 minutes at 3000 RPM and 4º C. Serum aliquots were placed into 1.5 ml plastic tubes then stored at -20º C. Progesterone concentration was analyzed by a commercially available solid-phase I^{125}-progesterone radioimmunoassay (RIA) kits (Coat-A-Count Progesterone PITKG-7; Siemens Medical Solution Diagnostics, Los Angeles, CA). The assay was designed for the direct measurement of progesterone in serum or plasma. Assay sensitivity for progesterone was 0.02 ng/ml and the intra- and inter –assay coefficient of variation (CV) were 6.19% and 8.64%, respectively and the acceptable CV for each sample was <10%. Detailed description of the analysis procedure can be found in Appendix A.

Complete blood count analysis:

Whole blood was collected by jugular venipuncture and kept in anti-coagulated tube. Complete blood count (CBC) was determined in The Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University using a Cell-Dyne 3700 system (Abbott, Ramsey, Minnesota, USA), while white blood cells (WBC) and red blood cells (RBC) were counted by Electrical Impedance. Detailed description of the analysis procedures can be found in Appendix B.

Immunoglobulin G (IgG) analysis:

Blood was collected by jugular venipuncture and submitted to Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University where samples were analyzed for the IgG content using a commercially available kit (Equine Serum IgG, DVM stat; VDX Veterinary Diagnostics, CA). Detailed description of the analysis procedure can be found in Appendix C.

Table 3.2

Summary of Treatment Groups and Sample Collections

Treatment Groups	Parameters
Novel non toxic (Experimental) (PDF 584) (n = 12) Non-toxic, endophyte-infected tall fescue pasture (NE+ ve) (n = 11) Endophyte-free tall fescue pasture (naive control) (E- ve) (n = 9) Toxic endophyte-infected tall fescue (E+ ve) (n = 4)*	Mare weight: 1×/2 weeks and 48 h (pp) to obtain mare gain weight. Urine: 1×/2 weeks and 48 h (pp) for ergot alkaloids assessment. Mare blood collection: 3×/week and 48 h (pp) for Progesterone (P4), Cytokine, prolactin, dopamine concentrations. Foal blood collection: At 0 d, 1 d, and 2 d (pp) for complete blood counts for foal maturity detection, which included red and white blood cells, lymphocyte, and neutrophil concentrations. IgG value for sufficient colostrums consumption. Ultrasonography: 1×/2 weeks to determined foal viability. Pregnancy outcome: placental/foal weights, length of gestation and delivery complications. Pathology: Gross and histopathology analyses for dead foals and placenta.

*For pro-inflammatory cytokines first year only.

Table 3.3

Treatment Groups and Forage Description for YEAR II (2009)

Treatment Group Year II	Type of Endophyte	Cultivar	Race of Tall Fescue
Group A (PDF 584) (n = 6)	AR584: Novel, non-toxic (Experimental)	PDF 584 (PDF)	Northern European continental
Group B (NE+ ve) (n = 5)	AR542: Novel, non-toxic (MaxQ, positive control)	Jesup	Northern European continental
Group C (E- ve) (n = 5)	Nil: Endophyte-free (naive control)	Jesup	Northern European continental

Figure 3.2 Examples of stands of fescue in experimental pastures in Year II (Experiment 2); A) PDF 584 pasture, B) MaxQ pasture, and C) E-ve pasture.

CHAPTER IV

RESULTS

Results of Experiment 1:

Pregnancy observation outcomes:

All mares on E- ve fescue and on the NE+ ve (MaxQ) pastures had normal deliveries, produced health viable foals, and had excellent udder development on the parturition day. However, one of the six mares on NE+ ve pasture had a retained placenta (16.67%). All mares on PDF 584 pasture had normal deliveries, health viable foals, and excellent udder development (Figure 4.1). However, one mare was removed eight days before delivery due to an eye injury that required the mare to be confined to a stall. On E+ ve pasture, all mares developed serious signs of fescue toxicosis such as agalactia (Figure 4.2). Three mares delivered live foals and all of them required plasma due to the low of colostrum consumptions. One of the three foals euthanized due to poor viability indices at 72 hours of age. The fourth mare had severe case of dystocia (25%) and delivered stillborn foal, and by this, (50%) of the foals in E+ve group died. The mare went through severe shock postpartum and was immediately placed on fluids and shipped to the Morgan Freeman Equine Center, College of Veterinary Medicine at Mississippi State University. After recovery, mare was bred, but failed to conceive on subsequent inseminations. Three of four mares on E- ve that were inseminated conceived, and all mares on E + ve were bred but they all remained open. All six mares on PDF 584 pasture were exposed and they had been confirmed bred. Five mares on NE+ ve pasture were exposed and three were confirmed bred (Table 4.1).

Statistical analysis:

Data were analyzed as a complete randomized design utilizing four treatment groups (PDF 584, NE+ve, E-ve, and E+ve). Data were checked for normality and were analyzed using the GLM procedures of SAS (SAS Institute Inc., Cary, NC). Data were analyzed to examine differences between treatment groups, not across time. The mean and standard error of the mean (Mean ± SE) are presented for all data sets. Data with a P value less than 0.05 were considered to be significant and data with P value less than 0.1 were consider to be trend.

Figure 4.1 Normal udder development in mare that was grazing in PDF 584 pasture in Year I (Day 326 of gestation, picture was taken two hours after parturition).

Figure 4.2 Typical signs of an undeveloped udder in mare that was grazing in E+ ve pasture in Year I (Day 331 of gestation, picture was taken two hours after parturition).

Table 4.1

Summary of Pregnancy Outcome and Subsequent Re-breeding

Treatment Group	Pregnancy Outcome	Retained Placenta	Stillborn Foal	Breeding/ Conception
Group A (PDF 584) (n = 6)	6 viable foals	0	0	6 mares exposed, 6 mares confirmed bred
Group B (NE+ ve) (n = 6)	6 viable foals	1		5 mares exposed, 3 confirmed bred
Group C (E- ve) (n = 4)	4 viable foals	0	0	3 exposed, 3 confirmed bred
Group D (E+ ve) (n = 4)	Two viable foals, required plasma, one euthanized 3 days of age, one dystocia/ stillborn	0	1	4 exposed, 4 returned open

Mare body weights:

There was no significant difference (P > 0.05) among the four pasture treatment groups on the mare body weight gain during gestation period and up to Day 2 postpartum. The body weights mean and standard errors on day -42 for PDF 584, E+ve, E-ve, and NE+ve were 567.3 ± 18.9, 549.5 ± 31.6, 563.8 ± 24, and 583.7 ± 9.7kg, respectively. The weights on day -28 were 577.7 ± 16.7, 588 ± 18.8, 584.6 ± 20.6, and 597 ± 11.2, respectively while for day -14 were 582.8 ± 18.1, 571.5 ± 26.7, 589.5 ± 20, and 606.7 ± 13.5 kg (PDF 584, E+ve, E-ve, and NE+ve, respectively). Means for PDF 584, E+ve, E-ve, and NE+ve on day 0 were 509.2 ± 19.8, 486.9 ± 26.8, 517.7 ± 18.8, and 538 ± 19.8kg, respectively. Mare weights for the four pasture treatment groups PDF 584, E+ve, E-ve, and NE+ve on 2 d pp were 510.2 ± 20.5, 508.6 ± 26.1, 515.7 ± 18.8, and 533.2 ± 16.8kg, respectively (Table 4.2).

Gestation length:

All mares on PDF 584, E+ ve, E-ve, and NE+ve foaled close to their foaling expected date (335d) with no significant differences among the groups and their means were 345.7 ± 2.4, 346 ± 6.5, 340 ± 4.3, and 338 ± 2.7, respectively (Figure 4.3). The reason of not seeing significant differences in gestation length between the all three groups and E+ve group, as what was expected, thought to be because of the removal of the mares on E+ve pasture on their 335d of gestation to non- toxic pasture and treated them with domperidone (0.5 mg/kg BW) which works to induce lactation and helps on shortening gestation period (Figure 4.4 and 4.5) (Cross et al., 1999).

Mare progesterone consentration:

Serum progesterone concentrations in the E+ ve group was lower ($P<0.05$) than PDF 584 on day -33 (3.3 ± 0.09, 6.25 ± 0.68 ng/ml, respectively). Progesterone concentration in E+ ve group was lower ($P<0.05$) than all pasture treatment groups on day -9 (5.29 ± 2.01 ng/ml) for E+ ve compared to 14.68 ± 1.88, 16.45 ± 2.07, and 25.87 ± 9.73 ng/ml for PDF 584, E- ve , and NE+ve, respectively. In addition, E+ ve was lower ($P < 0.05$) than all pasture treatment groups on day 0. On the parturition day (day 0), E-ve and NE+ve progesterone concentrations (2.0 ± 0.14, 2.05 ± 0.23 ng/ml, respectively) were greater ($P<0.05$) than PDF 584 and E+ ve (1.42 ± 0.16, 1.0 ± 0.35 ng/ml, respectively). On day 2 pp, E- ve was significantly difference ($P<0.05$) than E+ ve (0.4 ± 0.04 and 0.22 ± 0.1 ng/ml, respectively), and NE+ ve was greater ($P<0.05$) than PDF 584 and E+ ve; Figure 4.6 shows the differences change in progesterone concentrations.

Foal weight and placental weight:

Foal birth weight was not significantly different among pasture treatment groups; Figure 4.7. Placental weights were greater (P □ 0.05) in E+ ve group (6.08 ± 1.05kg) compared with PDF 584 and NE+ ve (3.83 ± 0.58 and 3.96 ± 0.43kg, respectively); Figure 4.8 shows the differences in placental weights. These increased in placental weights were confirmed by placenta/ foal weight ratio, which was tendency to have greater ratio in E+ ve treatment group (P □ 0.1) compared with PDF 584 and NE+ ve; Figure 4.9.

Foal complete blood counts (CBCs) and immunoglobulin-G (IgG):

For Foal WBC, there was no significant difference ($P > 0.05$) among pasture treatment groups in 0-12 h or in 48 h of age (Figure 4.10). In RBC, there was an increased ($P<0.05$) in E+ ve group in 48h of age compared between E- ve and NE+ ve, while there was tendency to have greater ($P<0.1$) value in E+ ve treatment group compared with PDF 584 in 48 h; Figure 4.11. In foal neutrophil/lymphocyte ratio, there was a decreased ($P<0.05$) in E+ ve pasture treatment group compared between NE+ ve at 48 h of age while there was no significant differences ($P<0.05$) among groups in 0-12 h of age; Figure 4.12. The three foals that survive delivery in E+ve group required plasma; therefore, no IgG value is reported for these foals. In addition, one of the three foals of E+ve group was euthanize at 72 h of age. The IgG values for PDF 584, NE+ve, and E-ve are reported in the combined results of experiment 1 and experiment 2.

Table 4.2

Changes in Mare Weight Gain (%) Prior to Parturition through 2 d Postpartum (YEAR I)

Treatment groups	-42 d (Kg)	-28 d (%)	-14 d (%)	0 d (%)	2 d pp (%)
PDF 584	567.3 ± 18.9	1.8	0.9	-0.1	0.2
E+ve	549.5 ± 31.6	7	-2.8	-14.4	4.5
E-ve	563.8 ± 2	3.7	0.8	-12.2	-0.4
NE+ve	583.7 ± 9.7	2.3	1.6	-11.3	-0.9

No differences in mares weights (P > 0.05)

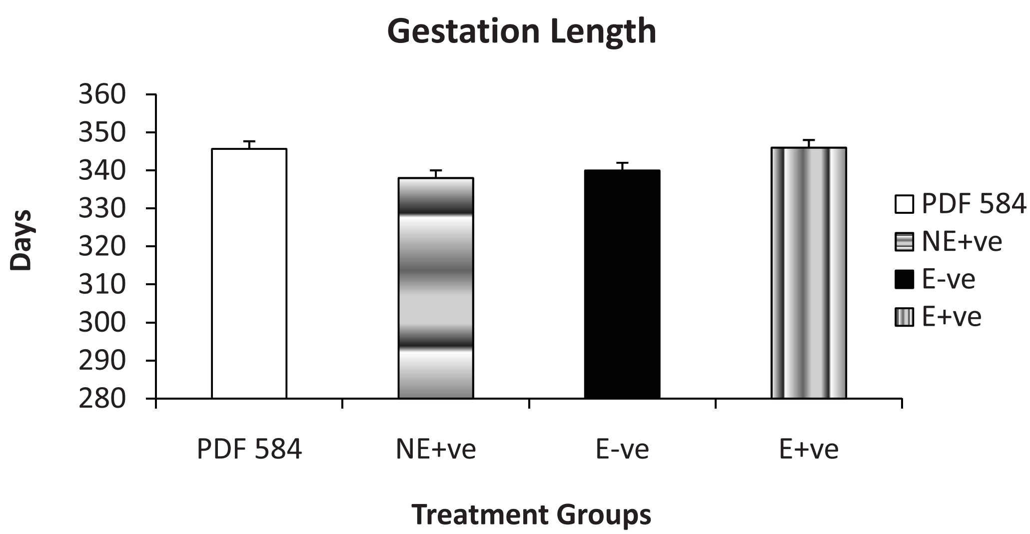

Figure 4.3 Gestation Length: Mares gestation length was similar (P>0.05) among pasture treatment groups PDF 584, NE+ve, E-ve, and E+ve (345.7 ± 2.4, 338 ± 2.7, 340 ± 4.3, and 346 ± 6.5, respectively)

Figure 4.4 An example of the absence of normal udder development of mare on Day 335 of gestation that was grazing in E+ve pasture.

Figure 4.5 An example of induced udder development of the same mare shown in Figure 4.4 after eight days of domperidone (0.5 mg/kg BW, per os twice daily) administration. The mare foaled on the ninth day of treatment corresponding to Day 344 of gestation.

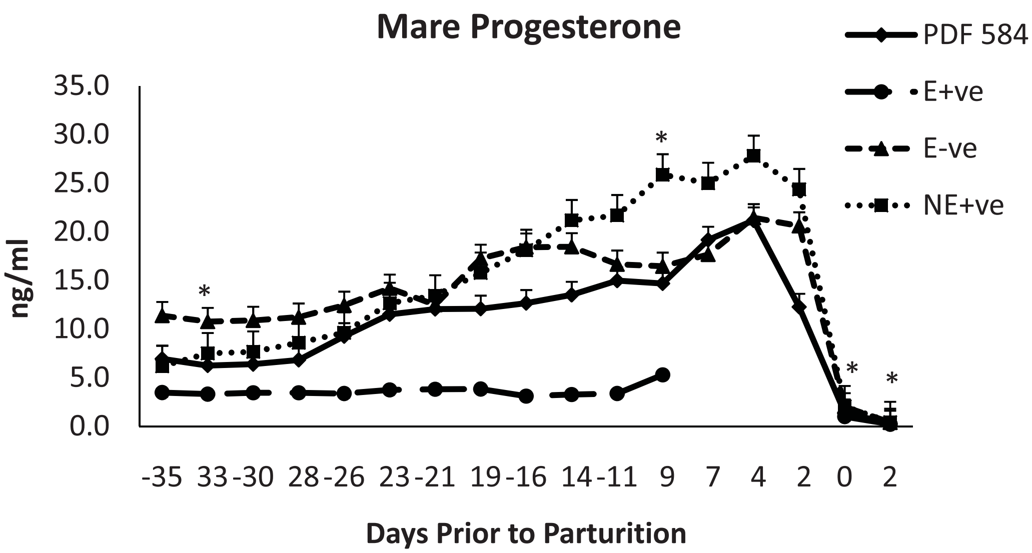

Figure 4.6 Mare Serum Progesterone Concentrations: *Differences in progesterone concentrations which E-ve was greater (P< 0.05) than PDF 584 and E+ ve in Day -33. E+ve group had lower (P<0.05) progesterone concentration than all treatment groups (PDF 584, NE+ve, and E-ve) in Day -9 and Day 0 (parturition). Progesterone concentration for E-ve and NE+ve was greater (P<0.05) than PDF 584 and E+ve on Day 0. On Day 2 pp, E+ve was lower than E-ve while NE+ve was greater than PDF 584 and E+ve (P<0.05).

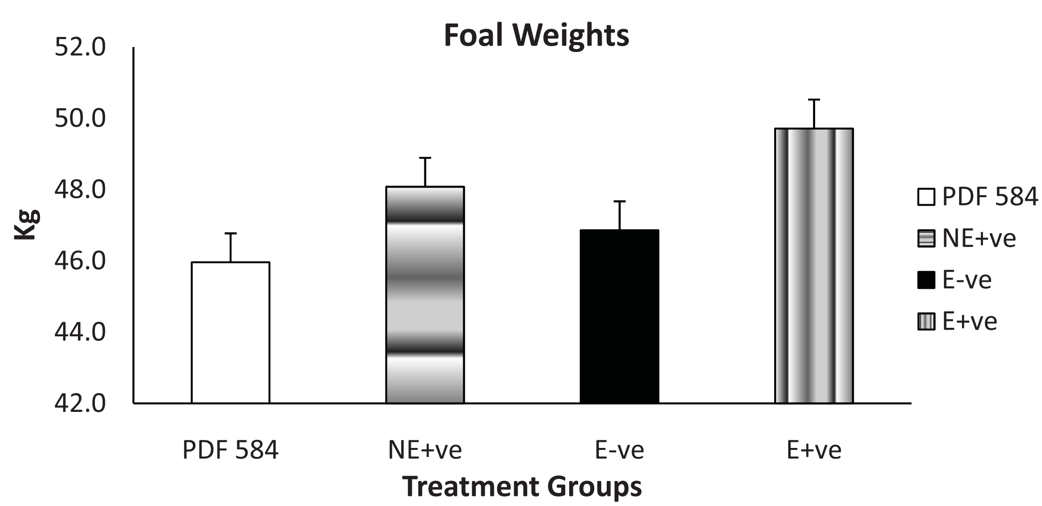

Figure 4.7 Foal birth weight was not significantly different among pasture treatment groups (PDF 584, NE+ve, E-ve, and E+ve)

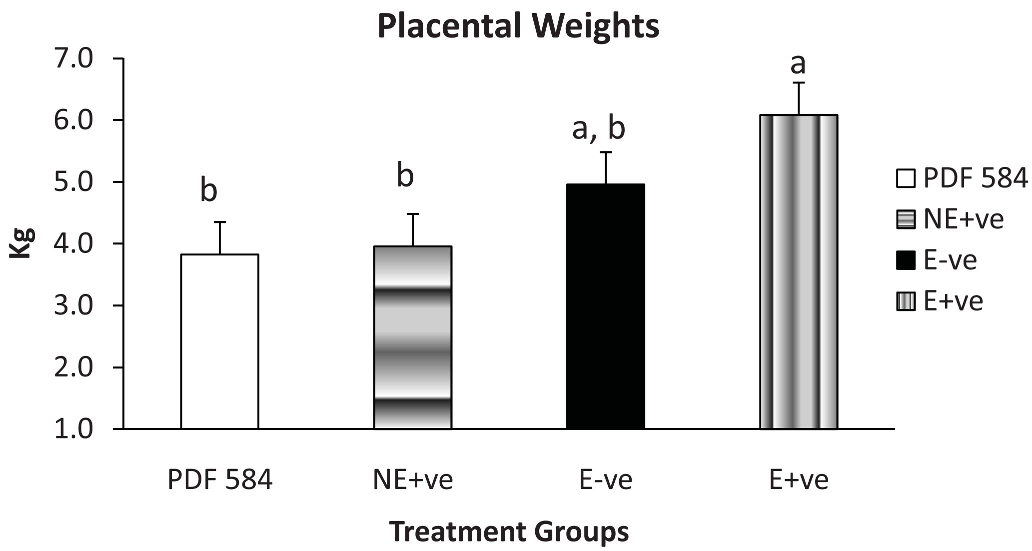

Figure 4.8 Placental Weights for E+ ve group were greater than PDF 584 and NE+ ve ($P < 0.05$) while there was no significant difference between E-ve and all pasture treatment groups ($P > 0.05$). a= different than b. while a,b= similar to both columns a and b.

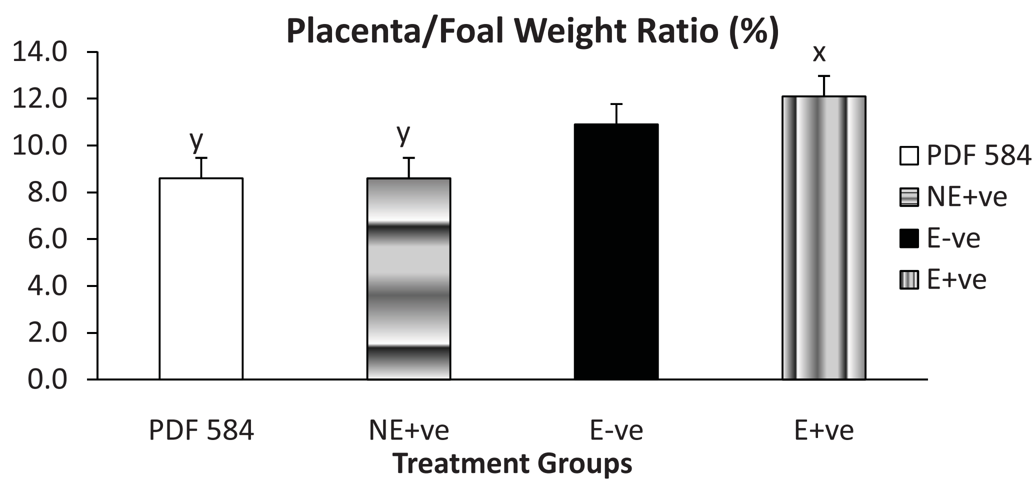

Figure 4.9 Placental/Foal Ratio (%): E+ ve pasture treatment group had tendency to have grater ratio compared with PDF 584 and NE+ ve ($P < 0.1$). This tendency indicated that E+ve group had trend to have a heavier placental weight than PDF 584 and NE+ ve treatment groups. x= tendency to have greater in ratio than y.

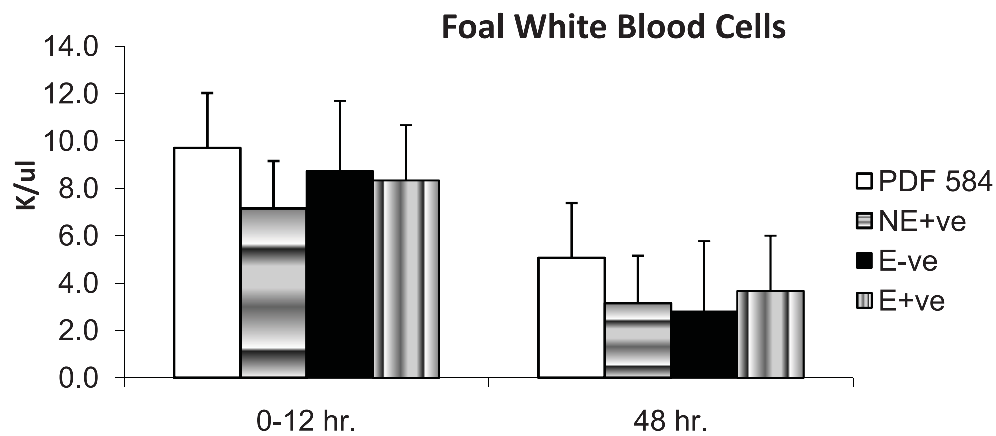

Figure 4.10 Foals white blood cells were not significantly differences in all pasture treatment groups in the both time of collections (0-12 h and 48 h).

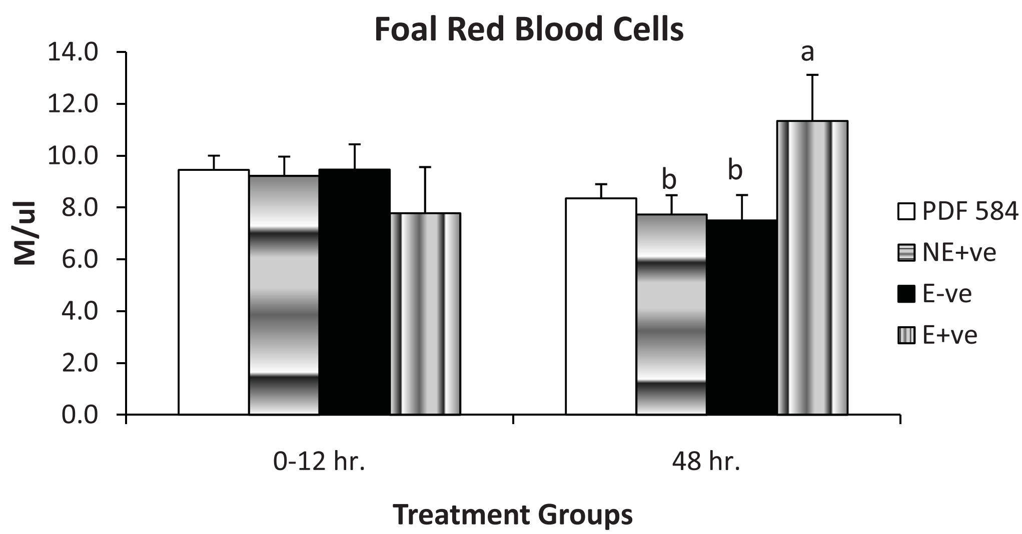

Figure 4.11 Foals red blood cells (RBC) were greater ($P<0.05$) in E+ve pasture treatment group at 48 h of age compared between E-ve and NE+ve (11.34±0.9, 7.5±1.4, and 7.7±0.9 M/ul, respectively). E+ve treatment group also, had tendency ($P < 0.1$) to have greater red blood cells counts than PDF 584 at 48 h (11.3±0.9, 8.4±0.7 M/ul, respectively). The increased in RBC might indicate to immaturity of the foals that delivered by mares that were grazing in E+ve pasture treatment group. a= an increased in foal RBC than b.

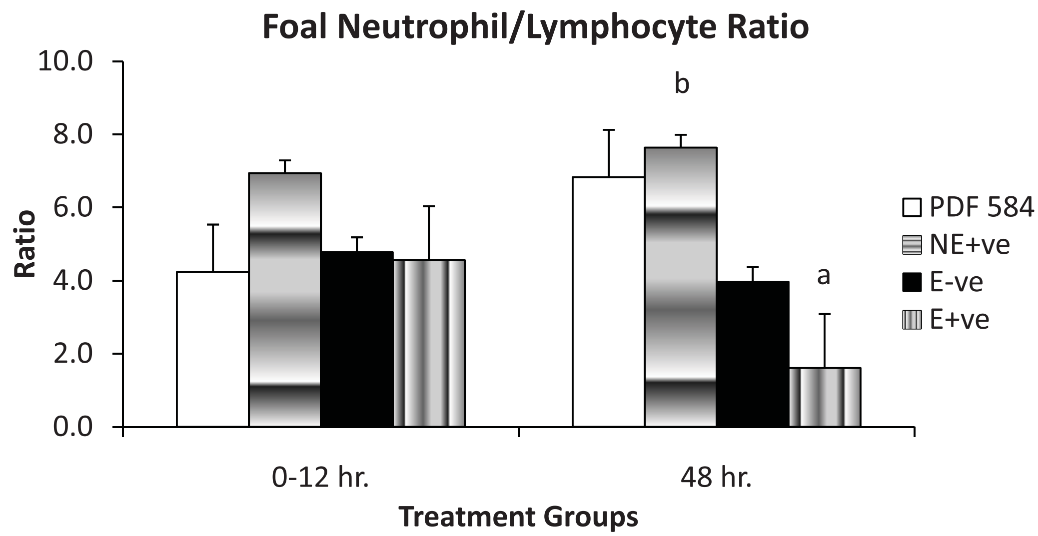

Figure 4.12 Foal neutrophil/lymphocyte ratio was not significantly difference (P>0.05) among pasture treatment groups in 0-12 h. However, foal neutrophil/lymphocyte ratio was lower (P < 0.05) in E+ve pasture treatment group (1.61±0.52) at 48 h compared between NE+ ve treatment group (7.64±0.9). Low neutrophil/lymphocyte ratio (particularly, lower than 2) indicates that these foals that delivered by mares grazed in E+ve pasture were premature. a= lower ratio than b.

Result for Experiment 1 and Experiment 2 combined together with an exception of E+ ve group:

Pregnancy observation outcomes:

All mares in PDF 584, NE+ve, and E-ve pastures treatment groups delivered healthy viable foals. One mare in the PDF 584 group was removed eight days before delivery due to an eye injury that required the mare to be confined to a stall. Two days prior parturition, one mare in E-ve group had vaginal discharge and cloudy fluid was detected by ultrasound. That mare was removed from E-ve pasture on the same day to a ryegrass pasture for close observation over that night and returned to the E- ve pasture on the next day after the no sign of discharge and no cloudy fluid was found on ultrasonograph. Another mare in E-ve group had poor udder development, for which, her foal was given 250 ml of colostrum. Retained placenta occurred in 16.67% of PDF 584 group (2/12), 18.18% in NE+ve (2/11), and 11.11% in E-ve group (1/9). Placenta was called retained when mare failed to drop it 2-3 h pp. One mare in NE+ve had premature placental separation (9.09%) (Table 4.3). In the ultrasonograph, all foals in the three treatment groups were viable and active since the initiation of the study until delivery.

Statistical analysis:

Data were analyzed as a complete randomized design utilizing three of the four treatment groups (PDF 584, NE+ve, and E-ve). The E+ve treatment group was omitted from statistical analysis since the objective of the study was to evaluate the novel PDF 584 and this group was included primarily to obtain pro-inflammatory cytokine data. Data were checked for normality and were analyzed using the MIXED procedures of SAS (SAS Institute Inc., Cary, NC). The following data sets were found to be non-normal and were log transformed to fit assumptions of normality; Mare Progesterone, Foal Progesterone, and Foal neutrophil/lymphocyte ratio. Non-normal data was back transformed in order to present physiologic data. Year was considered to be a random effect in the model and a repeated statement for day of study was used for the following data sets; mare and foal body weight, mare and foal progesterone, foal CBC's, and foal IgG. Data were analyzed to examine differences between treatment groups, not across time. The mean and standard error of the mean (Mean ± SE) are presented for all data sets. Data with a P value less than 0.05 were considered to be significant.

Table 4.3

Pregnancy Outcome

Treatment Groups	n	Pregnancy Outcome	Retained Placenta	Premature Placental Separation
PDF 584	12	12 viable foals	2 (16.67%)	
NE+ve	11	11 viable foals	2 (18.18%)	1 (9.09%)
E-ve	9	9 viable foals	1 (11.11%)	

Mare body weight:

There was no significant difference (P > 0.05) between the three pasture treatment groups (PDF 584, NE+ve, and E-ve) on mare body weight gain during gestation or on d 0 or d 2 pp; Figure 4.13 (Table 4.4).

Foal/mare weight ratio:

The ratio was similar in all pasture treatment groups PDF 584, NE+ve, and E-ve (means 9.4 ± 0.4, 9.2 ± 0.4, and 9.5 ± 1.1, respectively); Figure 4.14. This ratio is measured to determine the foal weight out of the mare weight and if that ratio is over 10, foal will called heavier than normal.

Gestation length:

All mares on the treatment pastures PDF 584, NE+ve, and E-ve foaled close to their foaling expected date (335 d) with no significant differences (P>0.05) among the groups and their means were (341.75 ± 2.3, 341.64 ± 2.4, and 341 ± 2.7 days, respectively); Figure 4.15.

Mares progesterone concentration (P4):

Mares in all pastures treatment groups were not significantly different ($P>0.05$) in progesterone concentrations; Figure 4.16 shows the changes in progesterone concentration.

Foals birth weight, placental weight, and foal/placental weight ratios:

All weights were similar ($P>0.05$) among pastures treatment groups (PDF 584, NE+ve, and E-ve); Figures 4.17, 4.18, and 4.19.

Foal progesterone concentration (P4):

Foal serum P4 concentration was similar on day 1 and day 2 in all pastures treatment groups, but was lower ($P< 0.05$) in the PDF 584 group (4.76 ± 1.01 ng/ml) than the E-ve (9.45 ± 1.1 ng/ml) in day 0; Figure 4.20 shows the progesterone concentration for the three pasture treatment groups in Day 0, Day 1, and Day 2.

Foal complete blood count (CBC):

WBC, RBC, and neutrophil/lymphocyte ratio were not significantly different ($P > 0.05$) across all pasture treatment groups; Figures 4.21, 4.22, and 4.23, respectively.

Foal immunoglobulin-G (IgG):

There was no significant difference ($P> 0.05$) among the three pastures treatment groups PDF 584, NE+ve, and E-ve in IgG concentration; Figure 4.24 shows the representative IgG concentration in Year I and Year II combined together.

Table 4.4

Changes in Mare Weight Gain (%) Prior to Parturition through 2 d Postpartum (YEAR II)

Treatment Groups	-42 d (Kg)	-28 d (%)	-14 d (%)	0 d (%)	2 d pp (%)
PDF 584	583.06 ± 13.3	2	1.3	-13.6	0.9
NE+ve	591.74 ± 13.8	1.6	1.6	-12.3	1.1
E-ve	556.66 ± 15.3	3.2	1.5	-13.2	0.8

No differences in mares weights ($P > 0.05$)

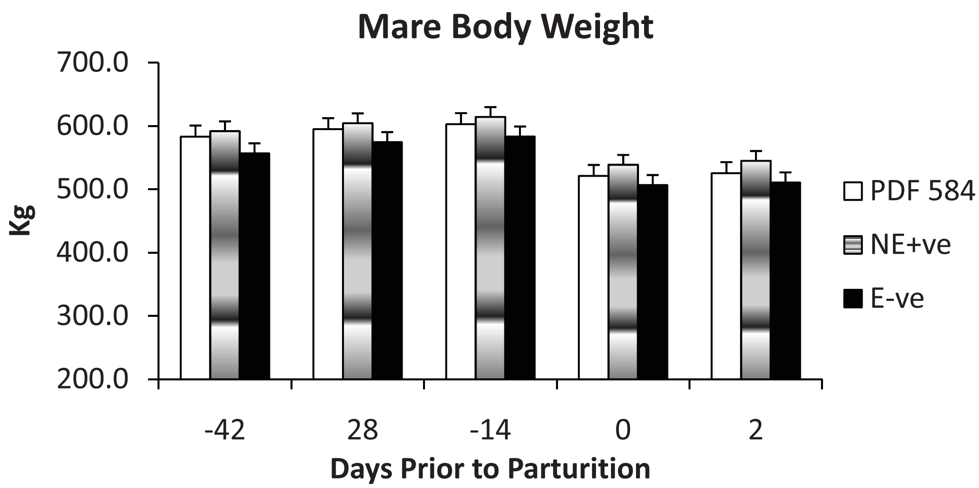

Figure 4.13 Mare body weight was not significantly difference (P> 0.05) in the all pastures treatment groups PDF 584, NE+ve, and E-ve.

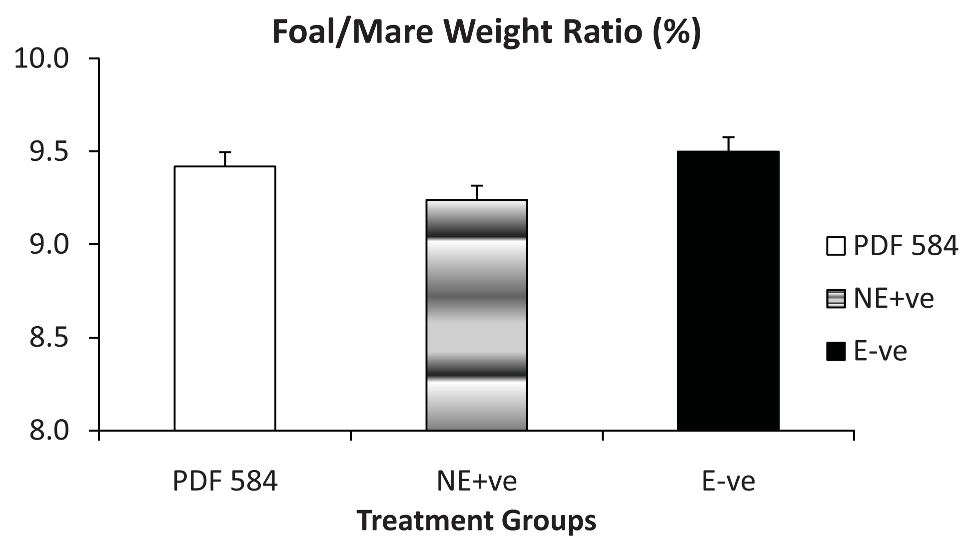

Figure 4.14 Foal/mare weight ratio was not significantly difference (P > 0.05) between pastures treatment groups PDF 584, NE+ve, and E-ve. If this ratio was greater than 10%, that indicated that these foals are heavier than normal.

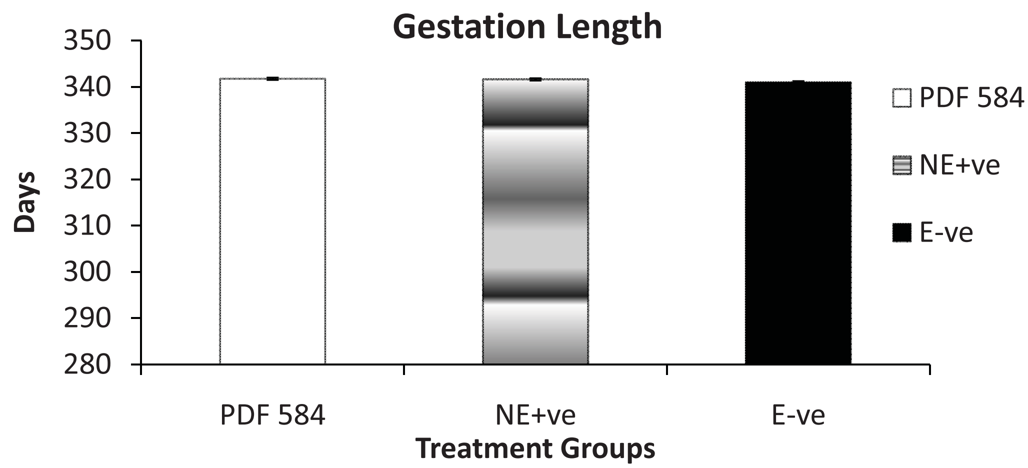

Figure 4.15 Mare gestation length was similar (P>0.05) among pasture treatment groups PDF 584, NE+ve, and E-ve (means 341.75 ± 2.3, 341.64 ± 2.4, and 341 ± 2.7 d, respectively).

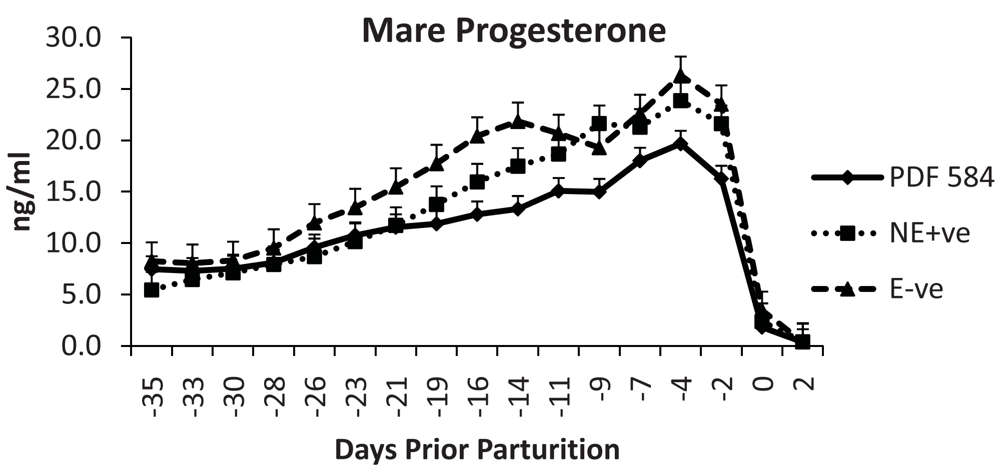

Figure 4.16 Mare progesterone concentration (P4) was not significantly difference (P > 0.05) among pastures treatment groups PDF 584, NE+ve, and E-ve. Data was log transformed and the transformed back to use physiological concentrations.

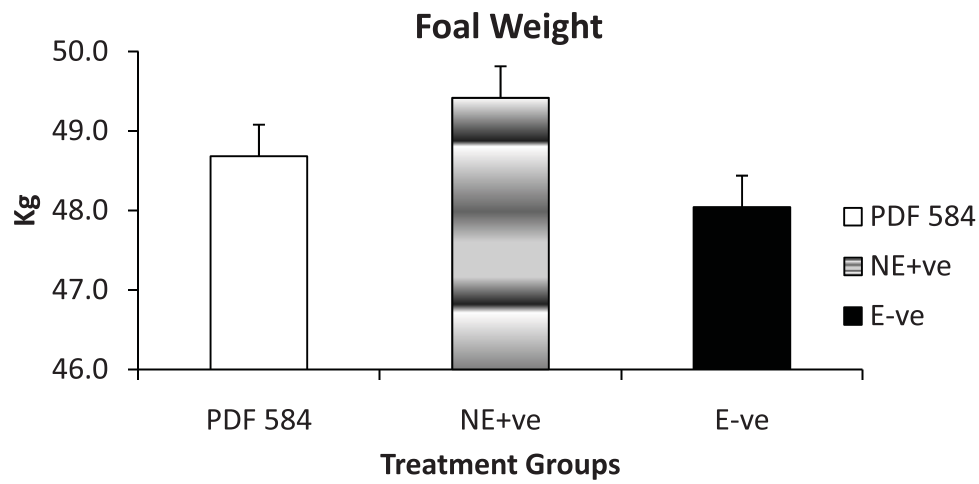

Figure 4.17 Foal weight was not significantly different (P>0.05) among pasture treatment groups PDF 584, NE+ve, and E-ve (48.68 ± 1.5, 49.42 ± 1.6, and 48.04 ± 1.8 kg, respectively).

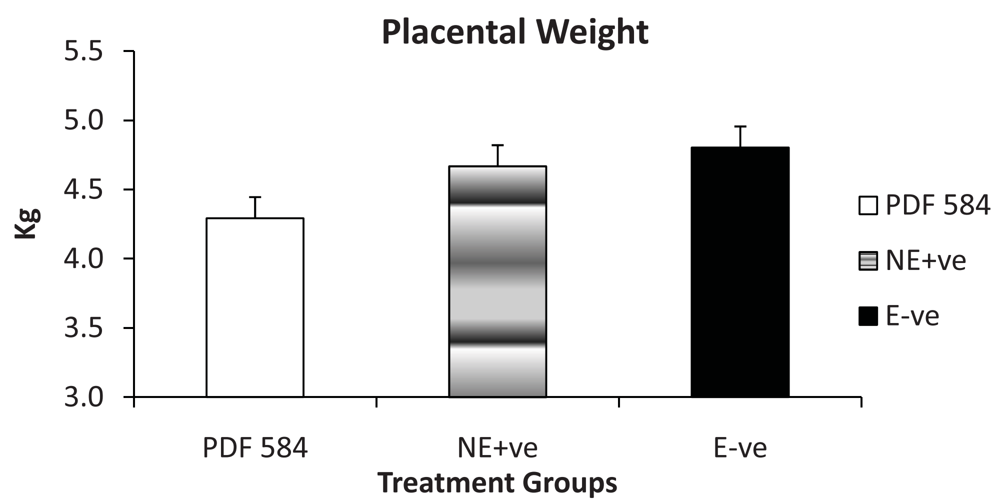

Figure 4.18 Placental weight was similar (P>0.05) in all pasture treatment groups PDF 584, NE+ve, and E-ve (4.29 ± 0.29, 4.67 ± 0.29, and 4.8 ± 0.31 Kg, respectively).

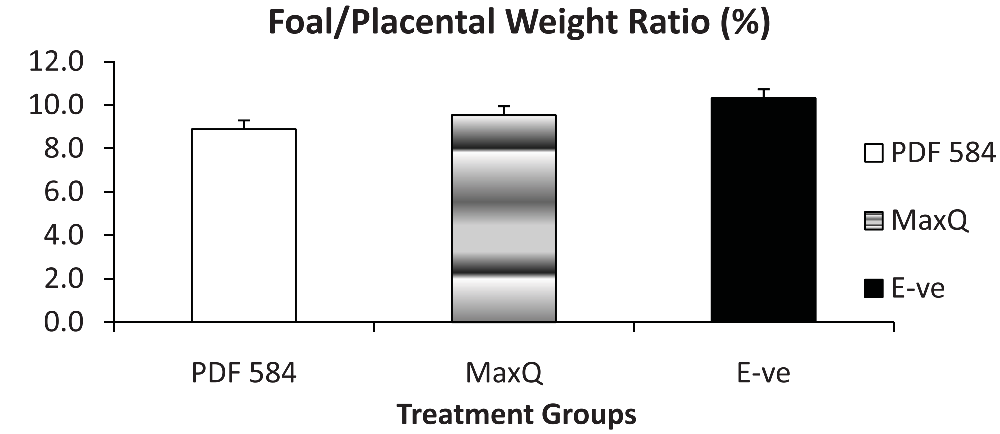

Figure 4.19 Foal/Placental weight ratio was not significantly difference (P>0.05) among pasture treatment groups PDF 584, NE+ve, and E-ve (8.88 ± 0.61, 9.53 ± 0.61, and 10.31 ± 0.65 %, respectively). When this ratio is greater than 11 that indicate that particular placenta is heavier than normal.

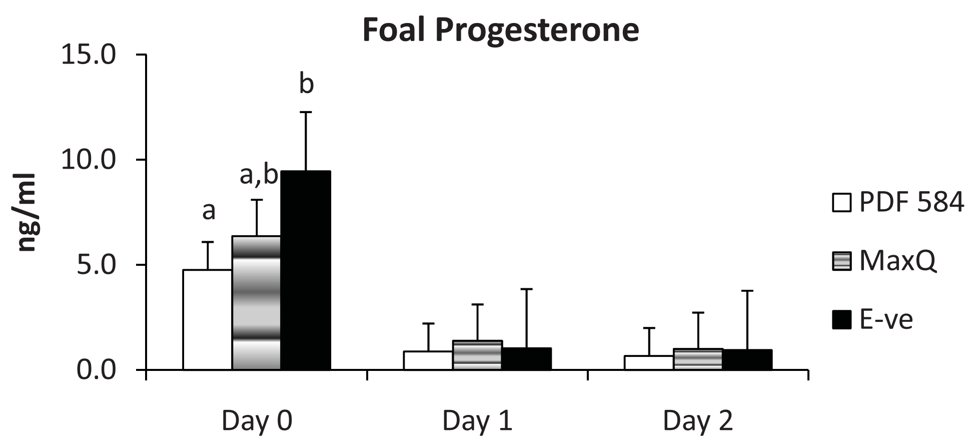

Figure 4.20 Foal P4 concentrations were similar on Day 1 and Day 2 in all groups (P > 0.05), but was lower (P<0.05) in the PDF 584 treatment group (4.76 ± 1.01 ng/ml) than the E-ve (9.45 ± 1.1 ng/ml) on Day 0. PDF 584 group and E-ve were similar to NE+ve group on Day 0. Data was log transformed and transformed back to use physiological concentrations.

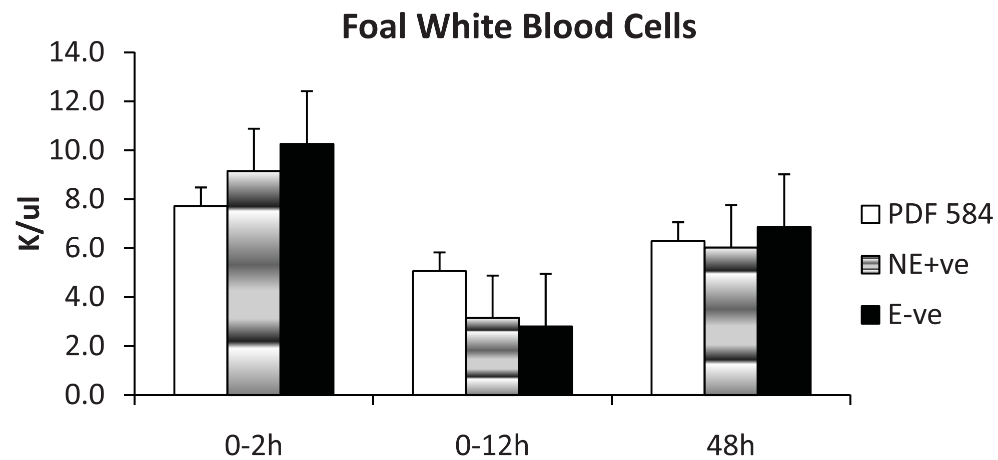

Figure 4.21 Foal white blood cell counts were not significantly different (P>0.05) among pasture treatment groups PDF 584, NE+ve, and E-ve at 0-2, 0-12, and 48 h. The period between 0-2 h was the 2nd year collection time and 0-12 h was the 1st year of collection time while 48 h was the both years combined.

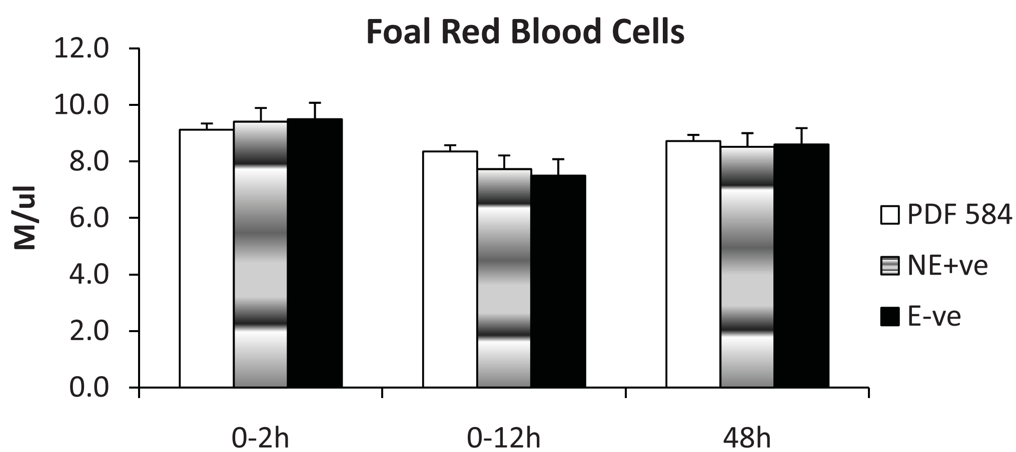

Figure 4.22 Red blood cell counts were similar (P>0.05) in all pasture treatment groups at 0-2 h, 0-12 h, and 48 h.

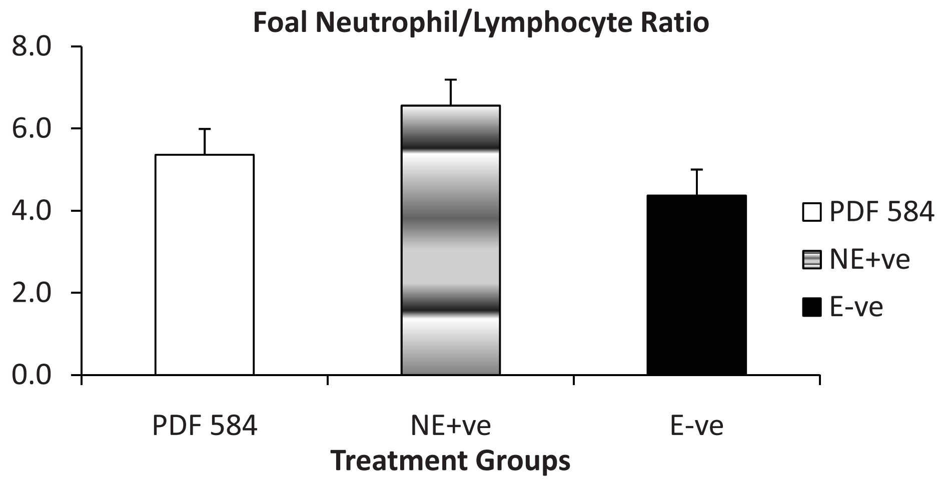

Figure 4.23 Foal neutrophil/lymphocyte ratio was similar (P>0.05) in all pasture treatment groups PDF 584, NE+ve, and E-ve (5.36 ± 0.78, 6.56 ± 0.87, and 4.37 ± 0.96%, respectively). Data was log transformed and then transformed back to use the physiological concentration.

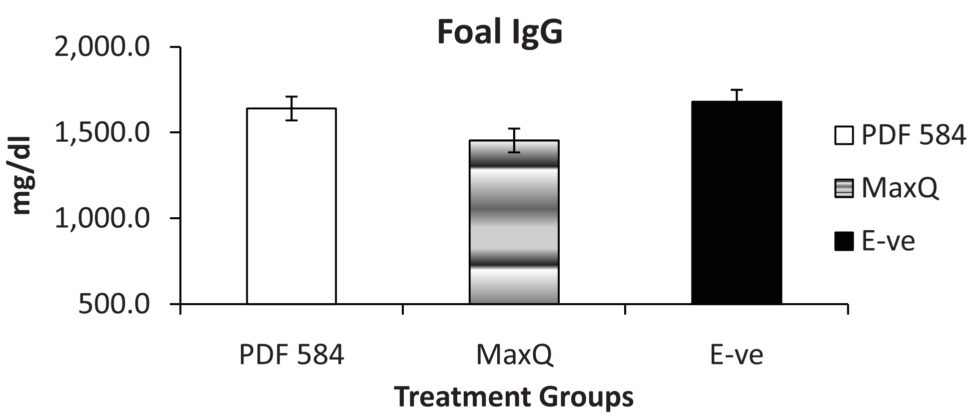

Figure 4.24 Foal IgG concentrations were similar (P>0.05) in all pasture treatment groups PDF 584, NE+ve, and E-ve (1640.67 ± 163.54, 1454.4 ± 179.15, and 1680 ± 179.15 mg/dl, respectively). This figure is represented the IgG concentrations in Year I and Year II.

CHAPTER V

DISCUSSION

Toxic endophyte-infected tall fescue has been documented to have negative effects on pregnant mares (Porter and Thompson, 1992; McCann et al., 1992; Brendemuehl et al., 1994; Cross et al., 1995). However, no information is available for the effects of a novel, non-toxic endophyte-infected tall fescue, cultivar, PDF 584 in pregnant mares. Therefore, this study aimed to evaluate if a novel, non-toxic endophyte-infected tall fescue, cultivar, PDF 584 is a safe forage for pregnant mares.

Ultrasonography was used to detect the viability and activity of the foals in the initial time of the study and then (1×/2 weeks) in all mares grazing on tall fescue treatment pastures (PDF 584, NE+ve, and E-ve). In this study, all foals were active prior to parturition.

Monroe et al. (1988) reported that mares grazing endophyte-infected pastures had lower weight gain compared to mares consuming endophyte-free fescue (Monroe et al., 1988). In other species, such as cattle, a decrease in average daily gain was reported in cows, steers (Roberts and Andrae, 2004; Paterson et al., 1995), bulls (Schuenemann et al., 2005), and heifers (Burke et al. 2007) consuming endophyte-infected fescue compared with the others that consumed endophyte-free fescue. In this study, mare body weight was not difference among groups (PDF 584, NE+ve, and E-ve).

Increased gestation length is one of the characteristic symptoms of fescue toxicosis in pregnant mares consuming endophyte-infected fescue (Porter and Thompson, 1992). This increase ranges between 20 (Porter and Thompson, 1992) to 25 days (Monroe et al., 1988) more than the regular range of gestation, which is 335-340 days. McCann et al. (1992) concluded that the decrease in progesterone concentrations may be involved in prolonged gestation length in mares consuming infected fescue since the progesterone concentrations increases in the last two weeks of pregnancy (McCann et al., 1992). In this study, no differences were found in gestation length among the three fescue treatment groups PDF 584, NE+ve, and E-ve (341.75 ± 2.3, 341.64 ± 2.4, and 341 ± 2.7 kg, respectively). Prolonged gestation length lead to increase the size and the weight of foal (Cross, 1997). For that regards, foal/mare weight ratio was measured in this study and significant differences were observed among groups PDF 584, NE+ve, and E-ve (9.4 ± 0.4, 9.2 ± 0.4, and 9.5 ± 1.1, respectively).

Progesterone is an essential hormone for pregnancy regulation (Cross, 1997; Brendemuehl et al., 1994). In late stage of gestation, progesterone is produced by the placenta (Fashen, 1984). In mares consuming toxic endophyte-infected fescue, progesterone concentration is significantly lower than mares grazed on endophyte-free fescue pastures (Cross, 1997; Cross et al., 1995; McCann et al., 1992). McCann et al. (1992) concluded that "placental progesterone secretion may be altered by some function of vasoconstriction." Differences in progesterone concentrations were not observed among groups (PDF 584, NE+ve, and E-ve) in this study.

Mares consuming endophyte-infected fescue had a high instance of foal loss due to severe dystocia which can also lead to losing both mares and foals. If foals survive delivery, they are unlikely live because of the poor colostrum in mares' milk (Cross, 1997). Earle et al. (1990) reported that 50% of the mares that were grazing infected fescue had stillborn foals and 86% of the foals that survived the delivery died. They also reported that 57% of the mares in toxic endophyte-infected pasture died due to severe dystocia (Earle et al., 1990). Those foals that survived delivery were dysmature, emaciated, and had long hair and overgrown hooves (Cross, 1997; Porter and Thompson, 1992). Mares grazing endophyte-infected fescue pastures mostly had a complete agalactia (Cross, 1997; Cross et al., 1995; Porter and Thompson, 1992). Monroe et al. (1988) reported that 87.5% of the mares that grazed on endophyte-infected pasture were agalactic. Negative outcome at parturition such as agalactia, dystocia, stillborn foals, dysmature foals, and mare death were not observed in this study in PDF 584, NE+ve, and E-ve groups. However, mares in E+ve had two viable foals required plasma, one stillborn-dystocia and one compromised foal, which was euthanized at 72 h pp. Also, one mare in E-ve group had poor udder development. For that reason, her foal was given 250 ml of colostrums and her gestation length was 356 days.

Retained placenta is more common in mares grazing endophyte-infected fescue and the occurrence is about 71.4% while it is 12.5% in mares that grazing endophyte-free fescue (Monroe et al., 1988). Retained placenta occurred in 10.6% of the regular deliveries and it frequently occurs after dystocia (Provencher et al., 1988). According to Sevinga et al. 2004, disturbance of uterine contractions and feto-maternal hormonal exchange may lead to retained placenta. Therefore, the decrease in blood flow from ergot alkaloids (Abney et al., 1993) is thought to be the cause of retained placentas in mares grazing endophyte-infected tall fescue. In this study, 16.67%, 18.18%, and 11.11% of the mares had retained placenta on PDF 584, NE+ve, and E-ve groups, respectively. In addition, NE+ve and E+ve groups had premature placental separation (9.09% and 25%, respectively), NE+ve foal lived because the membrane was brooked while E+ve was still-born foal.

CHAPTER VI

CONCLUSION

Progesterone is an essential hormone for pregnancy and the decrease in progesterone concentration is one of the fescue toxicosis symptoms. In this study PDF 584 did not show any significant decrease in progesterone concentration. Negative outcomes at parturition such as dystocia, thickened placenta, and agalactia are also common symptoms of fescue toxicosis and none of these signs were observed in mares grazing on PDF 584 pasture. Severe dystocia may lead to foals and mares mortality as negative consequences of fescue toxicosis, however, none of the mares died and all foals in PDF 584 pasture were viable and healthy in this study. Therefore, PDF 584 is a safe forage for late-term pregnant mares.

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APPENDIX A

RADIOIMMUNOASSAY PROCEDURES FOR PROGESTERONE

Blood serum is collected by jucular venipuncture and spun down for 30 minutes at 3000 RPM and 4º C. Serum was either pipette and put into 1.5 ml plastic tubes or was poured directly into the tubes and then stored at -20º C.

Steps of the procedure:

1- Allow the serum to thaw and reach room temperature and then the reagent was mixed.
2- Six plain test tubes were labeled for total count and for nonspecific binding in triplicate, and then label the anti-progesterone coated tubes in duplicate, but the standard tubes were triplicate.
3- Pipette 100 μL from each standard reagent (0, 0.1, 0.5, 1, 2, 10, 20, and 40 ng/mL) into the plain and the coated standard tubes.
4- Added 100 μL of Progesterone reagent to every tube.
5- Tubes were mixed gently. Tubes were incubated at 10º C for 18 hours.
6- Decant all tubes except the total count tubes.
7- Count all tubes for one minute in a gamma counter.

APPENDIX B

PROCEDURES FOR COMPLETE BLOOD COUNT

Whole blood was collected by jucular venipuncture and kept in anticoagulated tube. Complete blood count (CBC) was determined in The Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University using Cell-Dyne 3700 by Abbott and the WBC and RBC were counted by Electrical Impedance.

Steps of the procedure:

1. Use 0.5 ml to perform a CBC. Mix the sample for 15 minutes.
2. Label two blood smears and then fill a capillary tube with the blood sample for packed cell volume (PCV) and protein determinations. Stain both slides manually or using automatic slid stainer.
3. Place the capillary tube in the hematocrit centrifuge and spin the tube for four minutes.
4. Analyze the specimen on the Cell Dyne and then compare the hematocrit from the Cell Dyne to the PCV from the capillary tube.
5. On the stained slides, count the differential WBC and RBC.

APPENDIX C

PROCEDURES FOR IGG

Blood was collected by jucular venipuncture. IgG value was determined in The Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University using (DVM stat) VDX Veterinary Diagnostics.

Steps of the procedure:

1. Kit should be removed 30 minutes before testing the sample.
2. Label the pre-filled reagent tube with the serum sample I.D.
3. Add 5µl of the serum and control sample to the reagent tubes.
4. Mix the samples by 5 times inversion and incubate samples for 10 minutes.

Place the sample on the DVM stat to measuring the IgG value.