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Impact of supplementing rumen-protected arginine on blood flow parameters and luteinizing hormone concentration in cyclic beef cows consuming endophyte-infected tall fescue seed

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I am submitting herewith a thesis written by Melissa Ann Edwards entitled "Impact of supplementing rumen-protected arginine on blood flow parameters and luteinizing hormone concentration in cyclic beef cows consuming endophyte-infected tall fescue seed." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Abstract

Livestock grazing endophyte-infected tall fescue can have decreased performance due to vasoconstriction and reduced reproductive performance. Therefore, the objective of this study was to determine the effect of supplementing rumen-protected arginine to cows consuming endophyte-infected tall fescue seed on caudal blood flow and LH dynamics. Four ruminally cannulated, open beef cows (539 ± 30 kg) were used in a 2 x 2 factorial arrangement of treatments utilizing a Latin Square design with 4 periods of 31 d each. Each cow was assigned to individual pens and fed orchardgrass hay (10.3% CP and 85% NDF; OM basis) during a 10-d adaptation period, followed by a 21-d collection period in which each cow was assigned one of 4 treatments: (1) rumen-protected ARG (180 mg/kg of BW) and 1.0 kg/d of endophyte-infected fescue seed (**AE+**); (2) rumen-protected ARG (180 mg/kg of BW) and 1.0 kg/d of non-infected fescue seed (**AE-**); (3) 1.0 kg/d of endophyte-infected fescue seed (**E+**) alone; or (4) 1.0 kg/d of non-infected fescue seed (**E-**) alone. In each period, doppler ultrasound measurements for blood flow parameters were quantified on d 1, 5, 10, 15, and 19. On d 20 of each period, blood samples were collected every 10 min for 6 h and then once every hour for 12 h. Caudal blood flow exhibited an interaction (P = 0.05) of ARG × fescue seed type, resulting from an increase in blood flow in cows fed rumen-protected ARG. In addition, mean velocity was greater (P = 0.01) with the inclusion of rumen-protected ARG in the diet. Caudal artery area (P = 0.03) and diameter (P = 0.01) were decreased in cows consuming E+ compared to E- with no effect ($P \geq$ 0.38) by ARG supplementation. Mean serum LH concentration exhibited (P = 0.02) an ARG × fescue seed type interaction. Cows consuming E+ had decreased LH concentrations compared to all other treatments. However, cows consuming AE+ had similar LH concentrations compared to cows consuming AE- or E-. Thus, supplementing rumen-protected ARG to cows grazing

endophyte-infected fescue seed has the potential to increase reproductive performance and peripheral blood flow.

Key Words: arginine supplementation, beef cows, caudal blood flow, endophyte-infected fescue seed

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Chapter 1

Literature Review

Introduction

For beef producers, maintaining an efficient herd in order to maximize profitability and sustainability is dependent upon optimizing cattle and forage balance. In the mid-South, a majority of livestock producers have endophyte-infected tall fescue (*Lolium* arundinaceum) as their primary forage base for livestock. Endophyte-infected tall fescue is excellent grazing forage due to its high-quality nutritive value, high forage production, and long growing season. Tall fescue is a cool season, perennial grass, which is widely adaptable to a large range of environments and is mainly found in the central, eastern United States (Williams et al., 1984). However, the majority of tall fescue is infected with an endophytic fungus, *Neotyphodium coenophialum*. When consumed by cattle, this endophytic fungus may detrimentally alter reproduction and nutrition, which are the two important factors that contribute to profitability of livestock producers. For example, endophyte-infected tall fescue has been known to decreased peripheral blood flow (Rhodes et al., 1991; Aiken et al., 2007; Aiken et al., 2009; Foote et al., 2012) and reduce hormone release, including prolactin and LH (Burke et al., 2001a). Therefore, incorporating innovative management practices that can reduce these negative effects on cattle reproduction and nutrition may improve economic returns and sustainability in endophyte-infected tall fescue regions.

Supplementing livestock grazing endophyte-infected tall fescue with rumen-protected arginine (ARG) may provide livestock producers the opportunity to counteract the detrimental effects on reproductive performance and blood flow. Arginine infused post-ruminally has been reported to increase the release of LH in peripubertal lambs (Recabarren et al., 1996).

Furthermore, ARG may increase blood flow parameters (Saevre et al., 2010) via increased nitric oxide production, which may improve nutrient uptake and reproductive performance. However, the impacts of supplementing rumen-protected ARG in female cattle consuming endophyte-infected tall fescue, has not been determined.

Endophyte-infected tall fescue

Tall fescue is one of the most dominant forages found in the US, which cover over 15 million hectares and is grazed by approximately 8.5 million cattle in the US (Hoveland, 1993). However, 90% of tall fescue pastures in the US contain an endophytic fungus found in the plant (Stuedemann and Hoveland, 1988). The endophytic fungus can reside in the intercellular spaces in the sheaths of the leaves (Bacon et al., 1977; Porter and Thompson, 1992); yet, predominate concentrations are found in the interior of the seed head of the plant with concentrations peaking when tall fescue is in its reproductive stage during the spring and fall (Belesky et al., 1988). Even with the presence of the endophyte in tall fescue, there are some positive aspects to the endophytic fungus. Research has shown that endophyte-infected tall fescue is more persistent under grazing and environmental pressures than endophyte-free fescue (Hill et al., 1998). The greater persistence of endophyte-infected tall fescue is due to its enhanced ability to tolerate stress. Due to the beneficial, symbiotic relationship between the endophytic fungus and tall fescue (Bacon, 1993), endophyte-infected tall fescue has increased resistance to pests, disease, and persistence to various extreme environmental conditions such as drought, and allows for a higher tolerance to mismanagement of grazing (Stuedemann and Hoveland, 1988). Therefore, these positive attributes of endophyte-infected tall fescue make it excellent forage for grazing livestock.

Grazing endophyte-infected tall fescue, especially during spring and summer months when concentrations of ergot alkaloids are high, can have a negative impact on livestock performance. During these times, livestock grazing endophyte-infected tall fescue pastures can be subjected to a chronic health-related issue called fescue toxicosis (Hoveland et al., 1983). This condition can lead to an unthrifty appearance, especially during summer months due to high ambient temperatures. One particularly noticeable symptom of the presence of fescue toxicosis is a rough, retained hair coat (Stuedemann and Hoveland, 1988; Wagner, 2008). Additional symptoms may include increased core body temperature (Hannah et al., 1990; Rhodes et al., 1991; Browning and Leite-Browning, 1997a; Al-Haidary et al., 2001), increased respiration rates, reduced BW gains, and reduced feed intake. Furthermore, consumption of endophyte-infected tall fescue may lead to hormonal imbalances and therefore, negatively impact reproductive efficiency. These symptoms are a few examples of how fescue toxicosis can disrupt several physiological systems including the reproductive, nervous, and cardiovascular system (Strickland et al., 2011). It can also cause behavioral changes, in which cattle seek shade and graze less during the hottest periods of the day (Bond et al., 1984). Cattle grazing E+ prefer to stand in water more frequently (Stuedemann et al., 1985), to help to reduce elevated body temperatures caused by the endophyte, which will lead to decreased grazing time.

Symptoms of fescue toxicosis can occur year round; however, these symptoms are exacerbated when temperatures are elevated (Thompson and Stuedemann, 1993). Symptoms can vary in severity and may go undetected by producers (Strickland et al., 2011), which may lead to higher production costs or losses than necessary. Today, losses in cattle production due to fescue toxicosis has been estimated to cost the United States approximately $600 million annually, making fescue toxicosis one of the leading animal health-related issue for the beef industry (Strickland et al., 2011).

Body heat is regulated by altering respiration rates and skin vaporization through dissipation from the blood. Endophyte-infected tall fescue reduces dissipation of body heat by decreasing peripheral blood flow (Walls and Jacobson, 1970). This condition is more obvious when ambient temperatures are above 32^{o}C (Hemken et al., 1981). Aldrich et al. (1993) reported that steers fed endophyte-infected tall fescue with temperatures at 32^{o}C did not increase skin vaporization; whereas, steers fed non-infected tall fescue diets did increase skin vaporization. In addition, Holstein calves fed an endophyte-infected tall fescue diet with temperatures at 31^{o}C, resulted in elevated rectal temperatures and increased respiration rates compared to calves consuming a fescue-orchardgrass diet with lower endophyte concentrations (Hemken et al., 1981).

In metabolism studies, ground endophyte-infected tall fescue seed has been used as a model to mimic the effect of grazing endophyte-infected tall fescue pastures. Aiken et al. (2007) studied the effects of supplementing endophyte-infected tall fescue and non-infected tall fescue seed in beef heifers on hemodynamics of the caudal artery. This study reports a decrease in caudal artery area in heifers receiving endophyte-infected tall fescue in 4 h after the administration of fescue seed and remained lower for the remainder of the period. In addition, heart rates were lower for heifers receiving endophyte-infected tall fescue for the majority of the treatment period compared to heifers receiving non-infected tall fescue, resulting in decreased blood flow rates in endophyte-infected tall fescue fed heifers. Furthermore, Rhodes et al. (1991) concluded that endophyte-infected tall fescue intake results in a decrease in both peripheral, core body and blood flow to areas of the brain, which control body temperature.

In addition to altering blood flow dynamics, a decrease in circulating prolactin (PRL) in the blood is commonly used to determine the presence of fescue toxicosis (Elsasser and Bolt, 1987; Schillo et al., 1988). Although PRL secretion from the anterior pituitary can be directly reduced by several hormones and substances (Lamberts and MacLeod, 1978), PRL secretion is primarily mediated by dopamine (Gibbs and Neill, 1978). Dopamine is released from the hypothalamus and travels via the hypophyseal portal system to act on the anterior pituitary lactotrophs (Lamberts and Macleod, 1990). Ergot alkaloids have similarities in structure to dopamine (Sibley and Creese, 1983) and can act as dopamine agonist, which may be responsible for the inhibition of PRL secretion from the anterior pituitary (Elsasser and Bolt, 1987; Schillo et al., 1988).

While PRL concentration is a reliable indicator of fescue toxicosis, it is also involved in the induction of lactation and the development of the mammary glands (Karg and Schams, 1974). First-calf heifers consuming endophyte-infected tall fescue hay had a 50% decrease in milk yield at 100 d postpartum (Schmidt et al., 1986). For each 10% increase in ergot alkaloid concentration in the diet, a 1.05 kg/d decrease in milk production is expected. Furthermore, dairy cows fed endophyte-infected tall fescue hay had both a decrease in milk yield and a decrease in DMI (Hemken et al., 1979). This decrease in lactation may be due to the combination of the inhibitory effect endophyte-infected tall fescue has on PRL secretion and the reduced feed intake.

Cattle exposed to either endophyte-infected tall fescue or the combination of endophyte-infected tall fescue and heat stress, may cause decreased pregnancy and conception rates (Badinga et al., 1985; Schmidt et al., 1986; Wolfenson et al., 1988). Burke et al. (2001b) concluded that the endophyte-infected tall fescue and heat stress treatment led to reduced serum cholesterol concentrations, which is the precursor for both progesterone and estradiol production. Serum progesterone concentrations were reduced in horses (Monroe et al., 1988), ewes (Burke et al., 2006), and heifers consuming endophyte-infected tall fescue or dosed with ergotamine tartrate (Burke et al., 2001b; Jones et al., 2003). However, age of the animal may be a factor when determining the extent to which ergot alkaloids affect progesterone concentrations (Mahmood et al., 1994). Mahmood et al. (1994) reported cyclic yearling heifers were less sensitive to the effects of endophyte-infected tall fescue compared to cyclic weaned heifers, which had reduced progesterone concentrations due to grazing endophyte-infected tall fescue. There are many mechanisms which may explain reduced concentrations of progesterone. Ergot alkaloids can reduce serum progesterone concentration by stimulating uterine smooth muscle by the release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and stimulating uterine smooth muscle and comprising the function of the corpus luteum (Browning et al., 1998). In addition, vasoconstriction of blood flow to the ovary or corpus luteum due to ergot alkaloids may also play a large role in reducing progesterone release into systemic circulation (Jones et al., 2003). Therefore, any mechanisms leading to a reduced circulating progesterone could lead to embryonic loss if progesterone concentrations are reduced after recognition of pregnancy (Remsen et al., 1982).

Under thermoneutral conditions, consumption of endophyte-infected tall fescue has led to a decrease in estradiol concentrations before ovulation (Burke et al., 2001b). Prior to ovulation, the preovulatory surge of GnRH is controlled by the combination of high estradiol and low progesterone. However, during periods when estradiol is low, there is a negative feedback on the preovulatory center, inhibiting high amplitude pulses of GnRH (McDowell et al., 1998). Thus, a reduction of estradiol caused by consuming endophyte-infected tall fescue may contribute to a decrease in reproductive efficiency.

It has been widely accepted that glutamate plays an important role in controlling GnRH neuron excitability (Clarkson and Herbison, 2006; Iremonger et al., 2010). Injections of glutamate agonists or antagonists into the brain can stimulate or inhibit LH secretion, respectively (López et al., 1990; Urbanski and Ojeda, 1990; Brann and Mahesh, 1991; Ping et al., 1997). Thus, glutamate signaling at GnRH neurons is important in controlling their excitability, and therefore, GnRH secretion (Iremonger et al., 2010). However, the rate of glutamate uptake in the brain is decreased when the ergot alkaloid, ergovaline (50, 100, and 200 μM) is present (Xue et al., 2011). This suggests that livestock grazing or consuming endophyte-infected tall fescue will have inhibited glutamate transporter activity, which will ultimately decrease glutamate uptake in the brain. Inhibiting glutamate transporter activity may essentially decrease GnRH secretion into the anterior pituitary, decrease LH release, delay preovulatory LH surge, and therefore, delay ovulation. In fact, prepubertal beef heifers consuming endophyte-infected tall fescue hay had a decrease in circulating LH and FSH concentrations (McKenzie and Erickson, 1991). This effect on LH release may occur fairly rapidly. Ergotamine tartrate, a synthetic chemical that mimics the actions of endophyte-infected tall fescue, decreased LH concentration in Holstein cows within 4 h after a single intravenous infusion (Browning et al., 1998).

A decrease in forage intake (Hemken et al., 1979; Peters et al., 1992; Parish et al., 2003) while consuming endophyte-infected tall fescue can reduce BW gains and adipose stores (Brown et al., 1992). Strickland et al. (2011) suggested this reduction in BW gains may result in decreased calving rates. Furthermore, a decrease in BW gains may also result in a decrease in pregnancy rates due to production of smaller dominant follicles (Rhodes et al., 1995; Bossis et al., 1999). A decrease in the diameter of the dominant follicle (Burke et al., 2001b; Burke and

Rorie, 2002) and a decrease in the number of large follicles present (McKenzie and Erickson, 1991; Burke et al., 2001b; Burke and Rorie, 2002) has been found in heifers consuming endophyte-infected tall fescue. Therefore, a decreased nutrient intake and BW loss may impair development and growth of follicles and therefore, reduce reproductive efficiency.

The decrease in reproductive performance in livestock grazing endophyte-infected tall fescue may also occur after fertilization (Schrick et al., 2012). Rahe et al. (1991) reported that beef heifers fed endophyte-infected tall fescue hay had decreased embryo survivability. Similarly, Schuenemann et al. (2005) reported that beef cows treated with ergotamine tartrate had a significantly lower percentage of embryos that developed to compacted morula or greater (49%) compared to cows that did not receive ergotamine tartrate (88%). Collectively, the literature illustrates the vast effects that endophyte-infected fescue can have on reproduction.

Arginine Supplementation

Arginine is considered a non-essential, basic amino acid (AA) for healthy adult mammals (Rose et al., 1954), but is considered an essential AA (EAA) for young, growing mammals (Mertz et al., 1952; Heird et al., 1972; Ha et al., 1978). It is synthesized from glutamate, glutamine, and proline in various mammals including pigs, sheep, and rats via the intestinal-renal axis (Wu and Morris, 1998). The catabolism of ARG occurs via many pathways to produce either nitric oxide (NO), creatine, agmatine, polyamines, proline, or glutamate (Wu and Morris, 1998; Wu et al., 2009).

Arginine has a wide range of physiological functions. It is essential for maintaining inter-organ metabolism as well as ammonia detoxification. Supplementation of ARG can hasten wound healing, promote growth, improve lymphocyte immune responsiveness, reduce posttraumatic BW loss, and enhance hormone secretion (Sitren and Fisher, 1977; Seifter et al., 1978; Pui and Fisher, 1979; Barbul et al., 1981; Barbul et al., 1983; Barbul et al., 1984; Wu et al., 2009). Therefore, ARG plays an important role in various physiological functions throughout the body.

Arginine supplementation in the diet of ruminants has been less extensively studied due to the catabolism of ARG by microbes in the rumen. Technology for capsulation or protection of ARG from microbial degradation has been slow to progress due to the structure of ARG. Therefore, post-ruminal infusions or multiple intravenous injections of ARG are typically used in scientific studies; however, this method is not practical for livestock production. As newer methods of protecting ARG are discovered, the use of rumen-protected ARG supplementation has become increasingly popular among studies and will become more applicable at a production setting.

The classification of ARG as an EAA or nonessential amino acid (NEAA) is dependent upon the species being examined and the nutritional status of that species (Visek, 1986). Previous research has demonstrated that adult mammals can produce sufficient amounts of endogenous ARG to operate all physiological functions (Scull and Rose, 1930; Easter et al., 1974); however, other studies have indicated that endogenous ARG production may be insufficient in adult mammals (Egan et al., 1970; Davenport et al., 1990) and especially in the diets of young, growing mammals (Rose et al., 1948; Mertz et al., 1952; Heird et al., 1972; Ha et al., 1978). Furthermore, ARG levels in milk are considered sufficient for maintaining homeostasis; yet, pre-ruminant, milk-fed calves met maximal growth rates when ARG was added in their diets (Fligger et al., 1997). In ruminants with a fully developed, functional rumen, ARG is generally considered a NEAA since it can be synthesized endogenously (Black et al., 1957; Downes, 1961). However, Egan et al. (1970) suggests that while rumen microbes are efficient in production and synthesis of NEAA and EAA, they may lack sufficient production of ARG. Thus, ARG requirements may exceed the ruminant's synthetic capabilities, depending on the phase of physiological status (lactation and/or growth) of the animal (Egan et al., 1970).

Since rumen microbial protein is the key supplier of AA to the small intestine, proper balance of post-ruminal AA supply is needed for increased AA utilization. Total AA, NEAA, and EAA utilization were increased in beef heifers by abomasal ARG infusion compared to infusion (Davenport et al., 1990). The reduction of serum AA concentrations in ARG-infused heifers suggest that ARG may cause an increase in endogenous protein synthesis, which indicates more of these AA are being incorporated into peripheral tissues (Bergen, 1979). Furthermore, Koenig et al. (1982) reported a reduction in total AA in conjunction with an increase in N retention when ARG was continuously infused in the abomasum of heifers. In addition, N retention as a percent of total N intake has been increased when heifers were infused abomasally with either a low ARG (0.33 g ARG/kg BW) or high ARG (0.50 g ARG/kg BW) compared to saline with no difference between low or high ARG concentrations, suggesting that N requirements may have been met with the low ARG treatment (Davenport et al., 1990). Therefore, Davenport et al. (1990) suggested that the lower quantities of N retention in the heifers receiving the saline indicated that available AA from the microbial CP and RUP fractions may be inadequate to meet tissue demands. Furthermore, continuous abomasal ARG infusions led to elevated blood urea N by 24-h and remained elevated throughout the experiment compared to the saline infusions, indicating that a portion of the infused ARG treatment may be hydrolyzed by arginase into urea. Therefore, elevated serum ARG concentrations in ARG infused heifers may have escaped arginase hydrolysis (Davenport et al., 1990), making it available for endogenous protein synthesis.

Due to constriction of blood vessels while grazing endophyte-infected tall fescue, ARG supplementation may have the potential to be beneficial in cattle grazing endophyte-infected fescue because of its role in nitric oxide (NO) production. Arginine is a precursor for NO production, which is produced by the enzyme NO synthase. Nitric oxide synthase oxidizes ARG to citrulline for various tissue utilization (Gouge et al., 1998; Saevre et al., 2010). Nitric oxide is produced in many tissues and has multiple functions, which include a role in immune responses, smooth muscle relaxation, neuronal signaling, and vasodilation (Moncada et al., 1991; Garthwaite and Boulton, 1995; Griffith and Stuehr, 1995; Gouge et al., 1998). Nitric oxide increases blood flow by causing vasodilation and stimulating the development of blood vessels (Wu and Morris, 1998) in which guanylate cyclase is activated (Gouge et al., 1998) in the smooth muscles of blood vessels, resulting in an increase in cyclic guanosine monophosphate.

Nitric oxide regulates blood flow in tissues including the uterus and placenta, which may alter the concentration of nutrients made available to tissues (Reynolds et al., 2006). Furthermore, NO stimulates angiogenesis and the development of vasculature (Wu et al., 2013) and inhibits a vasoconstrictor, endothelin-1, by endothelial cells (Wu et al., 2009), resulting in increased blood flow in both systemic and reproductive blood vessels. Thus, increasing NO synthesis by providing additional dietary arginine may increase blood flow and nutrient uptake.

Ewes supplemented with 360 mg/kg of BW rumen-protected ARG had increased ovarian blood flow time compared to ewes receiving no additional ARG (Saevre et al., 2010). This increase in blood flow time may be attributed to increased NO production, increasing capillary permeability. In addition, intravenous administration of ARG has been reported to improve BW at birth and embryonic survivability in sheep (Luther et al., 2008; Lassala et al., 2010, 2011). Lassala et al. (2010) reported that ARG injections in underfed, gestating ewes increased BW at birth of lambs by 21% compared to saline injections. Lassala et al. (2011) intravenously injected ARG between d 100 and 121 of gestation in ewes and reported a decrease in lambs born dead by 23%, an increase in live-born lambs by 59%, and an increase in BW at birth of quadruplet lambs by 23%. Results from all of these studies suggest that additional dietary ARG may play a major role in embryo development and survival and is essential to the reproductive female (Wu et al., 2009).

In addition to the positive impacts ARG has on blood flow via NO, ARG is a precursor for the excitatory AA, glutamate. Arginine readily crosses the blood-brain barrier (Pardridge, 1983), where it is metabolized to ornithine and then ornithine is converted to glutamate (Wroblewski et al., 1985). Glutamate is one of the principle AA neurotransmitters in the brain (Moriyama and Yamamoto, 2004; Clarkson and Herbison, 2006; Krnjević, 2010). Excitatory AA are neurotransmitters that transmit signals from a neuron to a target cell across a synapse. Vesicular glutamate transporters such as VGLUT1 and VGLUT2 will concentrate glutamate into synaptic vesicles for release into the synaptic cleft of glutamatergic neurons (Xue et al., 2011). Glutamate binds and activates the non-N-methyl-d-aspartic acid receptors (NMDA) on the postsynaptic side of the synapse, resulting in GnRH secretion from the hypothalamus (Donoso et al., 1990). Gonadotropin releasing hormone stimulates the synthesis and release of LH in the anterior pituitary, which is secreted in a pulsatile manner.

Arginine has been previously found to play a role in reproductive competence in young animals. In immature rats, puberty was delayed when rats were fed ARG-deficient diets (Pau and Milner, 1982). Intravenous infusions of ARG in prepubertal lambs has resulted in a greater mean LH concentration and number of LH pulses than ewes receiving infusions of saline or ornithine (Recabarren et al., 1996). Therefore, increasing the availability of the post-ruminal supply of ARG may increase GnRH and LH secretion in livestock. This may be even more beneficial for livestock grazing ARG deficient forages or endophyte-infected tall fescue pastures that may inhibit glutamate uptake in the brain.

Summary

Grazing endophyte-infected tall fescue pastures may reduce reproductive efficiency in beef cattle by causing vasoconstriction of peripheral hemodynamics and/or reducing the secretion of LH and prolactin. A reduction in LH release may negatively impact follicular growth by interrupting the preovulatory rise in estradiol and thus, delay the LH surge and/or eventually delay ovulation. Therefore, it is important that efforts take place to improve cattle reproductive performance in regions where endophyte-infected tall fescue is prevalent. Supplementing rumen-protected ARG has the potential to improve overall nutrition and reproductive efficiency in cattle by counteracting the negative effects due to grazing endophyte-infected tall fescue. Arginine may stimulate an increase in LH release; therefore, reducing the probability of delayed ovulation and hence, improve reproductive performance. Furthermore, rumen-protected ARG may also alter systemic blood flow. Therefore, increasing the availability of ARG in cows grazing endophte-infected tall fescue may improve cattle reproductive performance and ultimately, improve profitability and sustainability.

Chapter 2

Impact of supplementing rumen-protected arginine on blood flow parameters and luteinizing hormone concentration in cyclic beef cows consuming endophyte-infected tall fescue seed

Introduction

Tall fescue (*Lolium* arundinaceum) is a cool-season perennial grass which predominately grows in the southeastern U.S. (Thompson et al., 2001) and is grazed by over 8.5 million cattle as reviewed by Hoveland (1993). However, grazing endophyte-infected tall fescue can negatively impact production in beef cattle due to the endophytic fungus, *Neotyphodium coenophialum*, which infects 90% of tall fescue pastures (Sleper and West, 1996). Multiple studies have indicated a decrease in pregnancy rates (Gay et al., 1988; Peters et al., 1992; Coblentz et al., 2006), follicular development (McKenzie and Erickson, 1991; Rhodes et al., 1995; Bossis et al., 1999) and systemic (Aiken et al., 2007) and reproductive (Dyer, 1993) vasoconstriction due to cows consuming endophyte-infected tall fescue. Vasoconstriction may lead to decreased nutrient and hormone uptake in target tissues. In total, these losses have been estimated to cost the U.S. cattle industry over $600 million dollars annually (Hoveland, 1993).

Arginine (ARG) supplementation may have the potential to be beneficial in animals grazing endophyte-infected tall fescue because of its role in nitric oxide (NO) and glutamate production (Wu and Morris, 1998). Nitric oxide increases blood flow by causing vasodilation and stimulating the development of blood vessels (Wu and Morris, 1998). In addition, ARG is a precursor for the excitatory AA, glutamate. Glutamate promotes gonadotropin-releasing hormone (GnRH) secretion via binding and activating the non-N-methyl-D-aspartic acid receptors (Donoso et al., 1990). Therefore, the objective of this study is to determine the effect of supplementing rumen-protected ARG with endophyte-infected or non-infected tall fescue seed on peripheral blood flow, circulating LH, and serum metabolites in beef heifers. Our hypothesis is that rumen-protected ARG will increase LH release and peripheral blood flow in cows consuming endophyte-infected fescue seed.

Materials and Methods

All animal handling and experimental procedures were in accordance with guidelines set by the University of Tennessee Institutional Animal Care and Use Committee. This experiment was conducted at the Joe Johnson Animal Research and Teaching Unit (JARTU) at the University of Tennessee in Knoxville, TN.

Animals

Four ruminally cannulated, non-pregnant, cyclic cows (approximately 2.5 yr of age) were used in a 2 x 2 factorial arrangement of treatments utilizing a Latin Square design with 4 periods of 31 d each. Cows were fed only the basal diet of orchardgrass for a 10-d adaptation period (d -9 to 0), followed by a 20-d collection period (d 1 to 20) during which cows received their respective treatments. Cows were housed in individual pens (2.4 m x 4.8 m) at JARTU with automatic individual waterers. A 42 d washout period occurred between treatment periods in which cows grazed Eastern Gamagrass (*Tripsacum dactyloides*) pastures to eliminate any potential carry-over effect of the fescue treatments. Once the washout period was over, the cows were taken back to JARTU and reassigned to treatments.

Treatments

Cows received a basal diet of orchardgrass hay (10.3% CP and 85% NDF; OM basis) fed ad libitum during the adaptation and experimental periods at 0700 and 1700 h each day. During each 20-d treatment period (Figure 1), cows assigned one of the 4 treatments: (1) rumen-protected ARG (180 mg/kg of BW) and endophyte-infected fescue seed (**AE+**); (2) rumen-protected ARG (180 mg/kg of BW) and non-infected fescue seed (**AE-**); (3) endophyte-infected fescue seed (**E+**) alone; and (4) non-infected fescue seed (**E-**) alone. Supplements were given intra-ruminally to each cow to reduce variation in supplement intake. Endophyte-infected (15.5% CP and 59% NDF; OM basis) and noninfected (15.9% CP and 59% NDF; OM basis) tall fescue seed were fed at 0.5 kg of ground seed twice daily. Endophyte-infected seed was purchased from Turner Seed Inc. ('Falcon IV'; 2.24 mg/kg ergovaline; 1.71 mg/kg ergovalinine; Winchester, KY) and the E- seed was purchased from Tennessee Farmer's Cooperative ('Kentucky 32'; 0.02 ppm ergovaline; 0.00 mg/kg ergovalinine; Monticello, KY). Fescue seed samples were ground through a 1-mm screen, and assayed for ergovaline and ergovalinine by HPLC florescence using a modification of a procedure established by Yates and Powell (1988). Fescue seed treatments were ground through a 2 mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) before being dosed ruminally. Treatments containing ARG were hand mixed into the ground fescue seed before administration into the rumen.

Prior to the initiation of the study, 8 ruminally-cannulated beef cows were provided ad libitum access to orchardgrass hay for 65 d to washout any potential carryover effects to grazing endophyte-infected fescue pastures. Cows were then brought to JARTU on d -9 which began the first day of the 10 d (d -9 to 0) adaptation period. At that time, cows were estrus synchronized utilizing the 7 d CO-Synch + controlled internal drug-releasing device (Eazi-Breed CIDR, Pfizer Animal Health, New York, NY) synchronization protocol. On d -9, cows were treated with a 2-mL i.m. injection of GnRH (Cystorelin, Merial, Iselin, NJ) and a CIDR which was inserted. On d -2, the CIDR was removed and the cows each received a single 5-mL i.m. injection of PGF (Lutalyse, Pfizer Animal Health, New York, NY). On d 0 cows each received another 2-mL i.m.

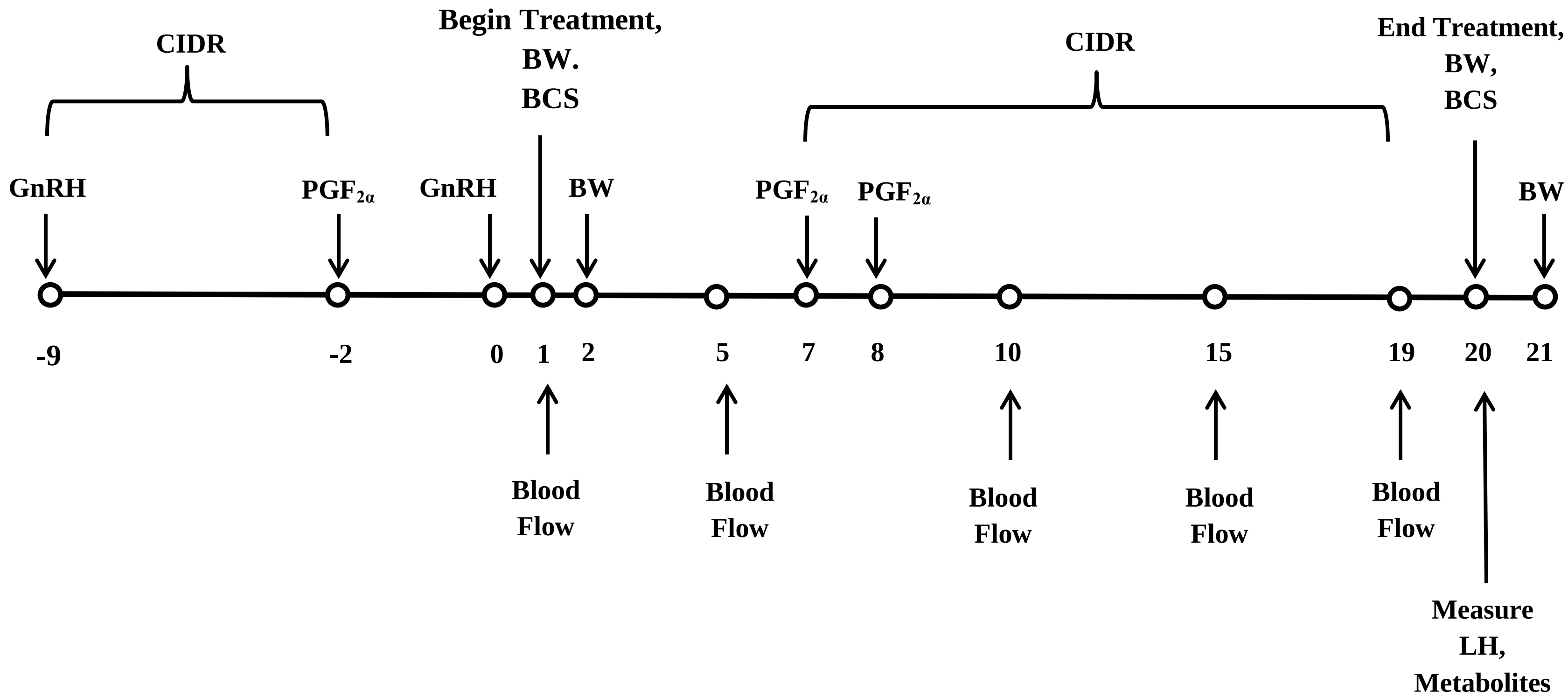

Figure 1. Schematic diagram for each treatment period.

injection of GnRH (Cystorelin, Merial, Iselin, NJ) to stimulate ovulation. On d 0 and 1, transrectal ultrasonography was used by a trained technician to select the 4 cows that were most responsive to the estrus synchronization protocol. The four heifers that possessed the largest dominant follicles were selected to remain in the experiment while the remaining four cows were discontinued from the study and returned to the experiment station.

Sampling

Color Doppler ultrasonography was used on d 1, 5, 10, 15, and 19 by a trained technician. Each scanning period began at approximately 1400 and ended at approximately 1600. Color Doppler scans of the caudal artery under the fourth coccygeal vertebra (Aiken et al., 2007; Aiken et al., 2009) of the tail were analyzed using a GE Healthcare LOGIQ e Vet with a 12L-RS linear array transducer set to 2.5-cm depth. To determine the mean artery lumen area, five cross-sectional scans were taken for each cow, using Brightness mode (B-Mode). Each individual scan was frozen during maximal flow or peak systolic phase and then frames were saved within the memory of the ultrasound. The caudal artery diameter 1 (cm), diameter 2 (cm), area (cm^2), and circumference (cm) were traced utilizing the ellipse method. Brightness mode pulse frequency was set at 8 MHz, and gain was set at 66. Five color Doppler images were also scanned in a longitudinal transducer orientation. Color Doppler pulse frequency was set at 5 MHz and gain was set at 13.5. Doppler insonation angle was set to 60^{o} to retrieve a good color signal. Peak systolic velocity, end diastolic velocity, mean velocity, resistance index, and pulsatility index were measured over a cardiac cycle within each of the five scans and then averaged. Pulsatility index and resistance index were both used as measures of resistance within the blood vessel (Petersen et al., 1997).

On d 7, a CIDR was reinserted in each cow to manipulate the estrous cycle to provide a sustained source of progesterone. A 5-mL i.m. injection of PGF (Lutalyse, Pfizer Animal Health) was administered on d 7 and 8 to ensure regression of the CL. The inserted CIDR was removed on d 19. On d 20, an intravenous catheter was inserted in the jugular vein of each cow 1 h prior to intensive blood sampling. A 12-gauge hypodermic needle (Ideal Instruments, Schiller Park, IL) was used to puncture the jugular vein. Approximately 0.45 m of Tygon tubing (0.10 cm i.d., 0.18 cm o.d., Cole-Parmer Instrument Company, Vernon Hills, IL) was threaded through the needle and into the jugular vein. The remaining tubing was secured with adhesive tape to the neck of the cow and down the middle of the back. A blunt end 18-gauge needle (Salvin Dental Specialties, Charlotte, NC) was inserted into the end of the catheter, and a 10-mL syringe was used as a tubing end cap. Eighteen hours after removal of CIDR on d 19, 9-mL blood samples were collected every 10 min for 6 h and then once every hour for 12 h. For the intensive bleeding period, cows were housed in individual bleeding stanchions (0.91 m × 2.4 m). Catheters were flushed with 5 mL of a 0.9% sterile saline immediately before and after each collection time. After collection, 9-mL blood samples were placed in Corvac serum separator tubes (Corvac, Sherwood Medical, St. Louis, MO). Blood samples were cooled and subsequently centrifuged for 20 min at 2,000 x *g* at 4^{o}C. Serum was collected and stored at -20^{o}C in plastic vials for later analysis. Serum samples were assayed for LH concentrations by RIA using the procedures of Moura and Erickson (1997). Inter- and intraassay CV were less than 13%, and sensitivity of the assay was 31.3 pg/mL. The number of LH pulses during the 18-h blood sampling period was determined by taking the overall LH mean concentration and then adding 1 SD. Any value above this was considered a pulse. In addition, LH amplitude was determined by taking the concentration of LH from the baseline to the top of the peak (Goodman and Karsch, 1980).

On d 20, additional blood samples were collected via the jugular catheter, at 0 (prior to supplemental treatments), 0.5 (post supplemental treatments), 1, 2, 4, 6, 8, 10, and 12 h for nutrient status measurements. After collection, two 9-mL blood samples were collected and placed in Vacutainer tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ) and Corvac serum separator tubes (Corvac, Sherwood Medical, St. Louis, MO). Blood samples were cooled and subsequently centrifuged for 20 min at 2,000 x *g* at 4^{o}C. Plasma and serum samples were harvested and stored at -20ºC in plastic vials for later analysis. Plasma samples were analyzed for circulating concentrations of glucose (Cayman Chemical Company, Ann Arbor, MI), and NEFA (Wako Pure Chemical Industries, Ltd., Mountain View, CA) using commercial kits. Serum samples were analyzed for nitric oxide (NO; Cayman Chemical Company, Ann Arbor, MI), urea N (SUN; BioAssay Systems, Hayward, CA) using commercial kits. Serum prolactin was assayed by RIA using the procedures of Bernard et al. (1993). Insulin was analyzed by solid-phase RIA (Count-A-Coat, Siemens Medical Solutions Diagnostics, Los Angeles, CA) as reported by Camacho et al. (2012).

Body condition scores (1 = emaciated, 9 = obese; Wagner et al. (1988)) were assigned to each cow by visual observation and palpation on d 1 and 20 of each treatment period by two trained technicians. In addition, cows were weighed on consecutive days at the beginning and end of every period.

Statistical Analysis

Normality of data distribution was evaluated using PROC UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Circulating blood metabolite concentrations and peripheral blood flow measurements were analyzed with sample time as the repeated factor with compound symmetry as the covariance structure using the MIXED procedures of SAS. The covariance structure (CS) was determined to be the most desirable covariance structure according to the Akaike's information criterion. The effects model included treatment, period, sample time and their interactions. The LSMEANS option was used to calculate treatment means. Separation of main effects and any interactions were accomplished using the PDIFF statement.

Results and Discussion

Bodyweight and Body Condition Score

Cow BW and BCS at the initiation and end of each period were not influenced ($P > 0.10$; Table 1) among the treatments. Supplementation of ARG in gilts did not impact differences in BW (Mateo et al., 2007). In contrast, Fligger et al. (1997) reported an increase in BW gain when newborn calves were orally dosed with ARG; however, calves were supplemented with approximately 1.5-fold the amount of ARG (500 $mg \cdot kg^{-1} \cdot d^{-1}$ of BW) as compared to cows in the current study.

Serum Luteinizing Hormone

Mean serum LH concentration exhibited ($P = 0.02$; Table 2) an ARG × fescue seed type interaction. Cows consuming endophyte-infected fescue seed without additional ARG had decreased LH concentrations. However, when cows were fed endophyte-infected fescue seed with ARG, serum LH concentrations were similar to cows consuming non-infected with and without ARG. Post-ruminal infusion of ARG has been reported to increase mean plasma LH concentration in prepubertal ewes (Recabarren et al., 1996). In the current study, mean LH was reduced in cows fed endophyte-infected tall fescue seed compared to non-infected tall fescue seed. Similarly, McKenzie and Erickson (1991) observed a 23% decrease in mean LH

Table 1. Effects of supplementing rumen-protected arginine and fescue seed type on bodyweight and body condition score in young beef cows.

	RP-ARG[1]							
	0		180					
	Fescue Seed[2]					*P*-value		
Measurement	Non-infected	Infected	Non-infected	Infected	SEM	ARG	Fescue	ARG × Fescue
Initial BW[3], kg	545	520	543	548	30	0.67	0.75	0.63
Final BW[4], kg	543	522	540	545	32	0.87	0.86	0.69
Initial BCS[5]	6.00	5.75	6.00	6.25	0.35	0.49	1.00	0.49
Final BCS[6]	5.88	5.75	5.88	6.38	0.38	0.45	0.64	0.45

[1]Rumen-protected ARG supplement fed at 0 mg/kg or 180 mg/kg of BW.

[2]Non-infected tall fescue seed supplement fed at 0.5 kg twice daily; endophyte-infected tall fescue seed supplement fed at 0.5 kg twice daily.

[3]Initial BW for each cow, averaged between d 1 and 2 of treatment period.

[4]Final BW for each cow, averaged between d 20 and 21 of treatment period.

[5]Initial BCS for each cow on d 1 of treatment period.

[6]Final BCS for each cow on d 20 of treatment period.

Table 2. Effects of rumen-protected arginine and fescue seed type on serum LH dynamics in young beef cows.

	RP-ARG[1]							
	0		180					
	Fescue Seed[2]					*P*-value		
Measurement	Non-infected	Infected	Non-infected	Infected	SEM	ARG	Fescue	ARG× Fescue
LH mean, ng/mL	0.49	0.13	0.53	0.45	0.07	0.04	0.05	0.02
LH peaks, no.	8.3	5.8	7.8	7.9	0.80	0.48	0.09	0.16
LH amplitude, ng/mL	1.37	0.78	1.41	1.21	0.09	0.05	0.02	0.10

[1]Rumen-protected ARG supplement fed at 0 mg/kg or 180 mg/kg of BW.
[2]Non-infected tall fescue seed supplement fed at 0.5 kg twice daily; endophyte-infected tall fescue seed supplement fed at 0.5 kg twice daily.

concentration in 3-mo-old heifers consuming endophyte-infected tall fescue hay. Furthermore, LH concentrations were reduced after ergotamine treatment in steers (Browning et al., 1997b), Holstein cows (Browning et al., 1998), and rats (Raj and Greep, 1973). Christopher et al. (1990) reported that LH producing gonadotroph cells in the anterior pituitary were adversely affected when heifers consumed endophyte-infected tall fescue. In contrast, Browning et al. (2001) suggested that a decrease in circulating LH concentrations in heifers during the follicular phase of the estrous cycle treated with ergotamine did not involve inhibitory effects on the anterior pituitary. Therefore, reduced circulating LH concentrations in cows consuming endophyte-infected tall fescue may hinder endocrine function and reproductive efficiency.

The number of LH peaks did not exhibit (P = 0.16; Table 2) an ARG × Fescue seed type interaction; however, fescue treatment tended (P = 0.09) to have an effect on mean number of LH peaks. Cows receiving endophyte-infected fescue seed tended to have lower number of LH peaks compared to those receiving non-infected fescue seed. In addition, number of LH peaks was not influenced (P = 0.48) by inclusion of ARG in the diet. In contrast, ewes infused with ARG had a greater percentage of LH pulses that were > 1 ng/mL compared to heifers not receiving ARG (Recabarren et al., 1996).

Luteinizing hormone amplitude did tend (P = 0.10; Table 2) to be influenced by ARG × fescue seed type interaction. The tendency for the interaction was due to the effect that rumen-protected ARG had on cows fed endophyte-infected fescue seed. Rumen-protected ARG increased LH amplitude in cows fed endophyte-infected fescue seed compared to cows receiving no rumen-protected ARG with endophyte-infected fescue seed. Mean LH amplitude was reduced (P = 0.02) for cows receiving endophyte-infected fescue seed compared to non-infected fescue seed. Similarly, Browning et al. (1997b) reported a decrease in LH amplitude in steers after intravenous ergotamine tartrate infusions. This decrease in LH amplitude due to cows grazing endophyte-infected fescue could lead to reduced folliculogenesis due to a reduction in estradiol production from LH and FSH. Burke and Rorie (2002) reported a decrease in the diameter of follicles when cows grazed endophyte-infected tall fescue pastures during the early postpartum period. However, length of estrous cycle, days open, and pregnancy rates were not altered indicating that follicular function was not affected. In contrast, several studies have found a decrease in pregnancy rates in cows grazing endophyte-infected tall fescue (Gay et al., 1988; Peters et al., 1992; Coblentz et al., 2006).

An increase in mean LH amplitude occurred ($P = 0.05$) when cows received rumen-protected ARG compared to receiving no additional ARG. In contrast, Recabarren et al. (1996) reported no difference in mean LH pulse amplitude when prepubertal ewes received ARG infusions. Amplitude of LH will increase throughout the follicular phase of the estrous cycle. Without this increase in amplitude of LH, estradiol levels will remain low, thus preventing an LH surge. The rapid increase in LH during this surge allows for oocyte maturation to begin which plays an important role in oocyte development. Therefore, these results indicate that ARG supplementation in young cows grazing endophyte-infected fescue may stimulate LH secretion and LH amplitude and thus, potentially affect ovarian function, reproductive cyclicity, oocyte maturation, and subsequently affect embryo development.

Serum Prolactin

Circulating prolactin (PRL) concentration was not influenced ($P = 0.73$; Table 3) by ARG × fescue seed type interaction. Furthermore, serum PRL concentrations were unaffected ($P = 0.35$) when cows received rumen-protected ARG compared to those not receiving rumen-protected ARG. In contrast, several studies reported an increase in PRL concentrations when

Table 3. Effects of rumen-protected arginine and fescue seed type on circulating serum metabolites in young beef cows.

	RP-ARG[1]							
	0		180					
	Fescue Seed[2]					*P*-value		
Measurement	Non-infected	Infected	Non-infected	Infected	SEM	ARG	Fescue	ARG × Fescue
Prolactin, pg/mL	42.9	30.7	51.3	34.8	12.8	0.35	0.05	0.73
NEFA, umol/L	159	143	234	201	34	< 0.01	0.10	0.51
Glucose, mg/dL	62.76	65.31	69.08	67.33	4.05	0.01	0.83	0.17
Insulin, ng/mL	0.60	0.37	0.88	0.62	0.11	0.01	0.03	0.85
Urea N, mg/dL	10.41	10.85	18.59	17.96	0.74	< 0.01	0.88	0.39
Nitric oxide, µM	4.88	4.26	4.68	4.77	0.16	0.48	0.24	0.09

[1]Rumen-protected ARG supplement fed at 0 mg/kg or 180 mg/kg of BW.

[2]Non-infected tall fescue seed supplement fed at 0.5 kg twice daily; endophyte-infected tall fescue seed supplement fed at 0.5 kg twice daily.

Holstein cows (Chew et al., 1984) and heifers (McAtee and Trenkle, 1971a) received ARG infusions. Fescue seed type influenced ($P = 0.05$) circulating PRL concentrations. Cows that received endophyte-infected fescue seed had lower PRL concentrations compared to cows receiving non-infected fescue seed. Likewise, serum PRL concentrations in beef heifers receiving endophyte-infected fescue seed has been reported to decrease compared to heifers receiving non-infected fescue seed (Aiken et al., 2009). In contrast, Harmon et al. (1991) reported no change in circulating PRL concentrations when steers received endophyte-infected fescue hay compared to those receiving non-infected fescue hay. Circulating PRL concentrations are a good indication of fescue toxicosis considering PRL is regularly decreased in the presence of endophyte-infected tall fescue (Lipham et al., 1989; Paterson et al., 1995; Aiken et al., 2007).

Blood Metabolites

Plasma NEFA concentration was not influenced ($P = 0.51$; Table 3) by an ARG × fescue seed type interaction. However, NEFA concentrations were greater ($P < 0.01$) in heifers receiving rumen-protected ARG compared to heifers that did not receive ARG. In addition, plasma NEFA concentration tended ($P = 0.10$) to be greater when heifers received non-infected fescue seed compared to endophyte-infected fescue seed. However, NEFA concentrations were relatively low and within the normal range for all treatments (Drackley, 2000).

Plasma glucose concentrations did not exhibit ($P = 0.17$; Table 3) an ARG × fescue interaction. In addition, circulating glucose concentrations were unaffected ($P = 0.83$) by fescue seed types. Similarly, Harmon et al. (1991) reported no differences in circulating glucose concentrations in steers consuming either endophyte-infected fescue or non-infected fescue. In this study, rumen-protected ARG had greater ($P = 0.01$) plasma glucose concentration compared to cows not receiving ARG. Likewise, McAtee and Trenkle (1971b) reported an increase in plasma glucose concentrations when ARG was infused in Holstein heifers. However, Davenport et al. (1990) did not find a difference in serum glucose concentrations in heifers receiving abomasal ARG infusions compared to heifers not receiving ARG. Since ARG is a glucogenic AA, it may be providing additional glucogenic precursors, which may be contributing to an increase in circulating glucose concentrations.

Serum insulin concentrations did not exhibit ($P = 0.85$; Table 3) an ARG × fescue interaction. However, circulating insulin concentrations were lower ($P < 0.03$) for cows receiving endophyte-infected fescue seed compared to those receiving non-infected fescue seed. In contrast, Harmon et al. (1991) reported no change in insulin when steers consumed either endophyte-infected fescue or non-infected fescue. Furthermore, Browning et al. (2000) reported a decrease in plasma insulin concentrations during the first 2 h after Holstein heifers were intravenously infused with an ergotamine bolus compared to saline treated heifers. In the current study, cows receiving rumen-protected ARG had a significantly higher ($P < 0.01$) concentration of circulating insulin than cows that did not receive rumen-protected ARG. In contrast, Davenport et al. (1990) did not reveal a difference in serum insulin concentrations when heifers were abomasally infused with ARG. However, multiple studies have reported an increase in serum or plasma insulin concentrations in Holstein cows (Hertelendy et al., 1970; Chew et al., 1984), Holstein heifers (McAtee and Trenkle, 1971b), sheep (Hertelendy et al., 1970; Sano et al., 1995), rats (Kohli et al., 2004) and pigs (Hertelendy et al., 1970) when supplement with additional ARG.

Serum urea N was greater ($P < 0.01$; Table 3) in cows that were supplemented with rumen-protected ARG compared to cows receiving no ARG. Similarly, studies have reported an increase in circulating SUN concentrations when beef heifers (Davenport et al., 1990) and dairy cows (Chew et al., 1984) were infused with ARG. The greater quantity of circulating SUN indicates that supplementing rumen-protected ARG may provide a greater level of dietary protein intake, which may increase the availability of N for tissue use. Serum urea N did not differ ($P = 0.88$) between fescue seed types. Similarly, Harmon et al. (1991) reported no difference in urea N in steers consuming either endophyte-infected fescue or non-infected fescue.

Circulating NO concentrations tended to be influenced ($P = 0.09$; Table 3) by the interaction of ARG × fescue seed type. Circulating NO concentrations were unaffected by rumen-protected ARG ($P = 0.48$). In contrast, Morris et al. (2000) reported a significant increase in circulating NO concentrations in healthy adult humans orally receiving ARG supplementation. Furthermore, ARG supplementation in rats resulted in increased endothelial NO production (Kohli et al., 2004). However, Liu et al. (2009) reported no difference in NO production in adult, male humans supplemented with ARG but, subjects received ARG for only three days prior to NO metabolite analysis. Furthermore, circulating NO concentrations were unaffected ($P = 0.24$) by fescue seed type. In contrast, Al-Tamimi et al. (2007) reported a decrease in nitrate/nitrite concentrations in rats receiving endophyte-infected fescue diets, indicating reduced endogenous NO production.

Color Doppler Ultrasonography

Caudal artery area ($P = 0.03$; Table 4) and diameter ($P = 0.01$) were reduced in cows receiving endophyte-infected fescue seed compared to non-infected fescue seed. Likewise, mean caudal artery area has been reported to decrease for beef heifers receiving endophyte-infected fescue seed compared to heifers receiving non-infected fescue seed (Aiken et al., 2007; Aiken et al., 2009). Furthermore, Aiken et al. (2013) reported a decrease in caudal artery area when steers

Table 4. Effects of rumen-protected arginine and fescue seed type on caudal blood flow parameters in young beef cows.

	RP-ARG[1]							
	0		180					
	Fescue Seed[2]					*P*-value		
Measurement	Non-infected	Infected	Non-infected	Infected	SEM	ARG	Fescue	ARG × Fescue
Area, mm^2	5.65	5.20	5.89	5.09	0.25	0.84	0.03	0.39
Diameter, mm	3.60	3.31	3.75	3.24	0.20	0.89	0.01	0.38
PSV[3], cm/s	54.09	52.70	57.99	52.64	2.92	0.59	0.35	0.58
EDV[4], cm/s	3.47	7.66	3.33	3.92	1.22	0.09	0.05	0.14
MNV[5], cm/s	35.84	38.57	43.31	42.26	1.99	0.01	0.68	0.39
Resistance index[6]	0.94	0.87	0.95	0.93	0.02	0.05	0.03	0.21
Pulsatility index[7]	1.44	1.23	1.28	1.21	0.07	0.15	0.03	0.28
Blood flow[8], mL/min	572.00	629.00	793.00	670.00	58.00	0.01	0.46	0.05

[1]Rumen-protected ARG supplement fed at 0 mg/kg or 180 mg/kg of BW.

[2]Non-infected tall fescue seed supplement fed at 0.5 kg twice daily; endophyte-infected tall fescue seed supplement fed at 0.5 kg twice daily.

[3]PSV = peak systolic velocity.

[4]EDV = end diastolic velocity.

[5]MNV = mean velocity.

[6]Resistance index was calculated as RI = (PSV – EDV)/PSV.

[7]Pulsatility index was calculated as PI = (PSV – EDV)/MNV.

[8]Blood flow was calculated as BF = MNV × cross-sectional diameter × 60 s.

grazed endophyte-infected fescue compared to steers grazing non-infected fescue pastures. Osborn et al. (1992) reported a decrease in blood flow to various peripheral tissues in steers consuming an endophyte-infected tall fescue diet during thermoneutral and elevated environment temperatures. This reduction in blood flow may lead to a reduced ability for the animal to dissipate heat; therefore, lead to elevated peripheral and core body temperatures (Rhodes et al., 1991) and increased respiration rates.

Peak systolic velocity (PSV) did not differ (*P* = 0.35; Table 4) between tall fescue seed treatments. Likewise, Aiken et al. (2009) did not report a difference in PSV for beef heifers treated with endophyte-infected tall fescue seed. In contrast, Aiken et al. (2007) reported an increase in PSV from baseline at 76 h, in beef heifers after consumption of endophyte-infected tall fescue seed compared to those consuming non-infected tall fescue seed. Furthermore, PSV did not differ (*P* = 0.59) when cows received rumen-protected ARG compared to cows not receiving ARG. In contrast, PSV in the corpus luteum increased when ewes were treated with 90 and 360 mg/kg BW of rumen-protected ARG compared to ewes not receiving rumen-protected ARG (Saevre et al., 2010). However, end diastolic velocity (EDV) was lower (*P* = 0.05) for cows receiving non-infected tall fescue seed compared to endophyte-infected tall fescue seed. In contrast, Aiken et al. (2007) reported no difference in EDV in beef heifers receiving either endophyte-infected or non-infected tall fescue seed. Mean velocity was greater (*P* = 0.01) for cows receiving rumen-protected ARG compared to those that did not receive ARG. Mean velocity did not differ (*P* = 0.68) for cows receiving endophyte-infected tall fescue seed compared to cows receiving non-infected tall fescue seed. Likewise, other studies reported that endophyte-infected tall fescue seed did not influence mean velocity in beef heifers compared to those receiving non-infected tall fescue seed (Aiken et al., 2007; Aiken et al., 2009).

Cow receiving endophyte-infected tall fescue seed decreased (*P* = 0.03) resistance index (RI) compared to cows receiving non-infected tall fescue seed. Furthermore, resistance index was increased (*P* = 0.05) in cows receiving ARG compared to cows that did not receive ARG. In addition, pulsatility index (PI) was greater (*P* = 0.03) for heifers receiving non-infected fescue seed compared to endophyte-infected fescue seed. In contrast, Aiken et al. (2007) reported no difference in PI in heifers receiving endophyte-infected fescue seed versus non-infected fescue seed, except for an increase in PI at h 4 and h 172 post infected fescue seed treatment. Furthermore, PI did not differ (*P* = 0.15) for cows receiving ARG compared to cows not receiving ARG. In contrast, rumen-protected ARG did not influence RI and PI in the CL or ovarian hilus in ewes (Saevre et al., 2010). Blood flow was influenced (*P* = 0.05) by the interaction of ARG × fescue seed type. Blood flow was unaffected by fescue seed type. However, blood flow was increased in cows receiving ARG compared to cows that did not receive ARG. Overall, supplementing ARG improved the rate of blood flow when cows received endophyte-infected tall fescue seed or non-infected tall fescue seed.

Implications

In conclusion, supplementing young cows grazing endophyte-infected tall fescue with rumen-protected arginine may offset negative effects of endophyte-infected tall fescue. The inclusion of rumen-protected ARG with endophyte-infected tall fescue seed, led to an increase in caudal blood flow and LH release. This increase in peripheral blood flow may lead to increased nutrient and hormone uptake. The increase in LH during the follicular phase may provide an optimal environment for oocyte quality and subsequent embryonic development competence. Thus, this study indicates that supplying additional post-ruminal arginine in livestock grazing endophyte-infected tall fescue may be beneficial and potentially mitigate some negative effects of fescue toxicosis in regards to blood flow and cow fertility.

Research Summary and Implications

Endophyte-infected tall fescue is the most prevalent forage source for cattle in temperate regions of the U.S. This forage is prevalent due to its high tolerance to environmental stressors such as drought, pests, and grazing. However, its adverse effects on cattle production due to its endophytic fungus, can negatively impact beef cattle reproductive efficiency and thus, reduce profitability. Supplementing rumen-protected arginine (ARG) may offset some of these negative effects on reproduction caused by endophyte-infected tall fescue.

Arginine is a basic amino acid that may be deficient in the diets of ruminants. It is a precursor for the production of glutamate, a major excitatory amino acid that is responsible for neurotransmission in the central nervous system. Glutamate is responsible for stimulating the release of gonadotropin releasing hormone which stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone. Luteinizing hormone is the main hormone that stimulates the release of estradiol in the follicle and thus, the preovulatory surge that results in ovulation of the dominant follicle. Luteinizing hormone secretion is reduced when in the presence of ergovaline, the main ergot alkaloid present in endophyte-infected tall fescue. This may result in delayed ovulation due to insufficient release of estradiol to stimulate the preovulatory LH surge. Therefore, supplementing ARG in diets of ruminants may be beneficial due to its ability to increase the availability of glutamate in endophyte-infected tall fescue diets.

Arginine is required for the synthesis of nitric oxide (NO). Nitric oxide increases capillary permeability in both systemic and reproductive vasculature which is decreased in endophyte-infected tall fescue diets in cows. This resulting vasoconstriction due to the consumption of endophyte-infected tall fescue may reduce nutrient and hormone uptake in target tissues. Supplementing 180 mg/kg of BW of rumen-protected ARG in beef cows resulted in increased caudal blood flow and mean circulating LH concentrations and LH amplitudes. Therefore, supplementing rumen-protected ARG in cows may counteract these negative effects due to endophyte-infected tall fescue. However, it is unknown whether supplementing ARG will stimulate sufficient preovulatory LH release to stimulate ovulation. Thus, further research is needed to determine optimal ARG levels to supplement to acquire adequate circulating LH concentrations needed to reduce the time period until ovulation.

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Vita

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