THE EFFECT OF SUPPLEMENTATION STRATEGY, STRESS LEVEL, AND TALL FESCUE TYPE ON PERFORMANCE OF FALL-WEANED BEEF CALVES

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THE EFFECT OF SUPPLEMENTATION STRATEGY, STRESS LEVEL, AND TALL FESCUE TYPE ON PERFORMANCE OF FALL-WEANED BEEF CALVES

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ABSTRACT

The beef cattle marketing structure imposes stress on calves due to weaning, transport, commingling, and adaptation to new diets, resulting in a weakened immune systems at the height of disease risk, frequently causing bovine respiratory disease. Backgrounding programs facilitate opportunities for calves to overcome stressors by building immunity, and adapting the rumen to high concentrate diets for improved feedlot performance. Four experiments were conducted to compare backgrounding strategies and effects of supplementation frequency performance and the effects of the ruminal environment. In Exp. 1, 48 weaned steers were used to investigate the effects of transportation and supplementation frequency, while in Exp. 2, 36 heifers were used to investigate only supplementation frequency. No differences in gains were observed due to transportation stress or supplementation frequency. Weaning stress resulted in elevated ($P < 0.05$) creatine kinase and neutrophil:lymphocyte ratios during the first week. In Exp. 3, 48 calves were used to compare the effect of tall fescue type on performance and health. Calves on novel endophyte fescue had higher ADG ($P = 0.07$) than on endophyte-infected fescue. Experiment 4 investigated the changes in ruminal environment due to supplementation frequency. No differences were observed between supplementation frequencies for ruminal pH, ammonia, or VFA concentration, and DM, or CP digestibility. Therefore, the rumen maintained a hospitable environment to promote bacterial protein synthesis and fiber digestion with every 48

h supplementation. Backgrounding calves with high fiber co-product supplements or on novel endophyte fescue can enhance calf performance.

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INTRODUCTION

The current structure of the beef industry involves a highly stressful period of time as calves are weaned, transported, commingled, withheld from feed and water, and finally relocated in a feedlot. This production strategy results in elevated risk of respiratory diseases. According to the United States Department of Agriculture Animal and Plant Health Inspection Service, the leading cause of morbidity and mortality of feedlot cattle is a result of Bovine Respiratory Disease (BRD) Complex, commonly referred to as "Shipping Fever" (USDA, 2004). Marketing stress induces BRD, costing the feeder calf industry $ 750,000,000 annually (Chirase et al., 2004). Nearly 64.3 % of all calves sold are not vaccinated for respiratory disease prior to weaning and shipping, with producers in the Southeast region being least likely to vaccinate (USDA, 1997).

Management systems, including preconditioning and backgrounding, can aid in lowering the stress level of the calves, reduce illness, and improve animal performance. Backgrounding systems help familiarize cattle with feed bunks and automatic waters, condition the digestive system from a forage-based to concentrate-based diets, and build a healthy immune system. Backgrounding systems can take place in drylot or on pasture with or without the use of concentrate supplementation. Only 25.8 % of beef cattle operations currently take part in backgrounding and preconditioning prior to sale into a feedlot (USDA, 1997). A backgrounding system can be economically viable for cattle producers by using low input management strategies, co-product feed supplementation, and increased calf performance upon entering a feedlot or pasture based finishing system. Backgrounding programs can result in $ 0.23 to $ 0.30 /kg of gain profit for producers, with an average gain of 136 kg in four months (Peel, 2003). A

well-balanced nutritional program providing energy dense feeds with adequate protein, mineral, and vitamins will help build the immune system. A healthy calf is a productive calf.

The objective of the present work was to investigate the effects of weaning and transportation stress, supplementation frequency, and fescue type on the health and performance of calves during the backgrounding period, as well as the effect of supplement frequency on the rumen environment.

REVIEW OF LITERATURE

Cattle Health and Stress

The cattle industry is plagued by the economic losses resulting from morbidity and mortality of newly received cattle. Nearly 84 % of all deaths at commercial feedlots can be attributed to BRD (USDA, 2004; Orr, 1990). During weaning, marketing, and transportation the calf is exposured to a variety of infectious agents. Proper nutritional management before and after stress induction can reduce the severity of BRD infections by boosting the immune system.

Stress can be defined at the sum of biological reactions to any adverse stimulus, which can be physical, mental, emotional, internal, or external that disrupts homeostasis and may lead to disorders. For cattle stress can include but is not limited to weaning, transportation, deprivation of feed and/or water, crowding, infectious agents, environmental changes, castration, dehorning, deworming, vaccinating, and human interaction (Church and Hudson, 1999; Fluharty and Loerch, 1994; Price et al., 2003). Stressed calves tend to eat less than unstressed calves, resulting in a need for careful nutritional management. Galyean et al. (1999) stated that high concentrate receiving diets increase morbidity rate and severity of morbidity in stressed calves. Vitamin E, Se, Cu, and Zn are all important components of a nutritional supplement for stressed calves to help enhance immune function (Galyean et al., 1999). Stress in cattle results in an increase of acute phase proteins such as haptoglobin (Arthington et al., 2003), white blood cell counts, and fibrinogen (Fb), decreased keyhole limpet hemocyanin (KLH) response and interferon-γ (Hickey et al., 2003), decreased serum Zn and increased Cu (Orr, 1990), and increased creatine kinase (CK) (Cole et al., 1988).

White blood cell analysis is a common procedure to determine immune function. Leukocytes include neutrophils, monocytes, eosinophils, basophils, and lymphocytes, each with

a unique function in the body's defense system. Neutrophils are phagocytotic and bacteriocidal, are mobilized during periods of stress or pathogen invasion, and have a rapid turnover rate of a few hours (Latimer, et al., 2003). Lymphocytes are important for cell and humoral mediated immunity and are long-lived compared to other leukocytes (Latimer, et al., 2003). The neutrophil:lymphocyte (N:L) ratio may be increased due to stress or illness. The normal N:L ratio for healthy cattle is 0.25 (Latimer, et al., 2003). Blood urea nitrogen (BUN) can be elevated due to high-protein diets or increased protein catabolism during fever, shock, stress, or tissue necrosis (Latimer, et al., 2003). The urea in BUN is produced by the liver from ammonia, filtered by the glomerulus, and then reabsorbed by the renal tubules. The reference interval for BUN concentrations in beef cattle is 7.8-24.6 mg/dL (Latimer, et al., 2003). Creatine kinase is an enzyme found in cardiac and striated muscle and brain tissues. Creatine kinase converts ADP to ATP by catalyzing high energy creatine phosphate bonds to provide energy in the muscles. The reference interval for CK in beef cattle is 14.4-107.0 IU/L (Latimer, et al., 2003). Elevated serum CK levels indicate damage of either muscles or the brain (Latimer, et al., 2003). Fibrinogen is a plasma protein essential for blood coagulation. The reference interval for Fb concentrations in beef cattle is 100-600 mg/dL (Latimer, et al., 2003). In large animals, such as cattle, Fb is a more sensitive indicator of inflammation than total white blood cell counts. An increase in Fb concentration could indicate inflammation due to injury or pneumonia.

Arthington et al. (2003) studied the effects of transportation and commingling on acute-phase protein response, growth, and feed intake of newly weaned beef calves. In two separate experiments, groups of 32 and 36 Brahman crossbred calves were randomly allotted to four treatments: transported and commingled, not transported and commingled, transported and not commingled, and not transported and not commingled. For both experiments commingling was achieved by penning study calves with two outsourced calves. In Exp. 1, calves that were

transported traveled for 3 h, whereas in Exp. 2, transported calves traveled for 3 h, then were housed overnight, and transported for an additional 3 h. In Exp. 1, BW and jugular blood samples were obtained at weaning, 1, 3 and 7 d post transport and for Exp. 2 at weaning, 1, 5, 9, 13, 17, and 21 d post transport. Commingled calves tended to have higher DMI than non-commingled calves. Transported calves lost more ($P < 0.05$) BW than non-transported calves following weaning. Serum amlyoid-A, cortisol, haptoglobin, and ceruloplasmin concentrations increased on each sampling day. Fibrinogen and ceruloplasmin concentrations were higher in transported calves. It was concluded that transportation induced stress affects the acute-phase protein response in newly weaned calves.

Kannan et al. (2000) examined how transportation induces a physiologic stress response and weight loss in goats. One hundred and fifty Spanish does were transported for 2.5 h and blood samples collected preload, post-load, and 0, 1, 2, 3, 4, and 18 h post-transport. Cortisol was elevated ($P < 0.05$) post-load, 0 and 18 h post-transport as compared to preload values. Creatine kinase was increased ($P < 0.05$) 1 and 2 h post-transport. The elevated CK levels were attributed to muscle damage due to bruising during transport. Blood urea nitrogen concentrations were highest at 18 h post-transport due to feed and water deprivation. Ratio of N:L was elevated at 0, 1, 2, 3, and 4 h post-transport and recovered to preload levels by 18 h post-transport. Kegley et al. (1997) reported similar increases in N:L ratio in cattle that were transported for 6 h.

Hickey et al. (2003) investigated the effect of abrupt weaning on the physiological mediators of stress and measures of immune function. Seventy-two Limousin/Charolais crossbred calves were adapted to handling, then assigned to either the non-weaned control (NW) or abruptly weaned (AW) treatments. Blood samples and behavioral scores were obtained at -168, 6, 24, 48, and 168 h after weaning. Plasma cortisol concentrations increased in AW calves ($P < 0.001$). Leukocyte concentrations were elevated at 24 and 48 h in the AW calves and N:L

ratios increased post-weaning ($P < 0.01$). In vitro interferon- γ response to mitogen concanavalin A (Con-A) and KLH were reduced ($P < 0.001$) in AW calves at weaning. It was concluded that AW calves have an increased sensitivity to social stresses due to physiological alterations in cortisol resulting in attenuated immune function. Church and Hudson (1999) reported an increase in N:L ratio up to 14 d post weaning for wapiti calves (*Cervus elaphus*).

Orr et al. (1990) investigated the concentrations of serum minerals in calves inoculated with BRD and IBRV. In Exp. 1, 80 crossbred calves were transported 24 h and commingled prior to the study and then observed daily for morbidity. Feed intake was measured from d 1 to 10, and blood samples were obtained from d 7 to 10. In Exp. 2, 100 crossbred calves were transported 24 h and commingled prior to the study then observed daily for morbidity. Blood samples were collected at the farm of origin, order buyer facility, and on d 28 and 52 after transport. Feed intake was determined for the first 28 d. In Exp. 3, 37 healthy crossbred calves were inoculated intranasally with live (Infectious Bovine Rhinotracheitis Virus) IBRV on d 8, and serum samples collected for 20 d. In Exp. 4, eight healthy calves were housed in metabolism stalls, inoculated with intranasal IBRV on d 8, and serum and urine samples were collected for 20 d. In Exp. 1, peak morbidity occurred on d 7, and morbid calves had decreased ($P < 0.05$) serum Zn concentrations on d 7, 8, and 9 while Cu concentrations were elevated ($P < 0.05$) on d 9. In Exp 2, 67 % of calves became morbid in the first 28 d (peak morbidity) and serum Zn concentrations were lower ($P < 0.05$) and Cu concentrations higher ($P < 0.05$) on d 28 than baseline values. Neither Zn nor Cu concentrations returned to baseline values after d 52. In Exp. 3 and 4, peak morbidity was noticed at 4 d post IBRV inoculation, which also corresponded with a depression in Zn and a rise in Cu concentrations. Serum Ca increased ($P < 0.05$) and P decreased ($P < 0.05$) on d 4 post IBRV inoculation in Exp 4. It was concluded that due to the

depressed feed intake, nutritional adequacy of minerals is important for calves to cope with market-transit or infection induced stress.

Backgrounding

Simpson (2000) defined backgrounding cattle as purchased weaned calves that are transported to a new facility (small feedlot or pasture) to overcome weaning stress and illness. Cattle backgrounded on pasture are often supplemented for bunk training and enhanced performance prior to entry into a feedlot. After approximately 6 wk of backgrounding, cattle are shipped to a feedlot or stockering facility. Preconditioned cattle are weaned and processed in familiar surroundings on the farm in which they were raised (Simpson, 2000). These cattle are often creep fed, vaccinated, castrated, and managed in order to minimize weight losses from weaning before shipment to a feedlot. The goal of this management program is to produce calves that are healthy and have a strong immune system. For the purpose of this thesis, backgrounding and preconditioning will be referred to collectively as backgrounding.

Wahlberg et al. (1998) backgrounded newly weaned beef calves for 42 d on three different treatments to determine health and economic benefits. Thirty calves were randomly allotted to treatments: pasture only, pasture + 1 % BW of 14.9 % CP corn + soybean meal (SBM), and hay + 1 % BW of 14.9 % CP corn + SBM in confinement. Cattle gained 0.70, 0.90, and 1.18 kg/d for pasture, pasture + grain, and hay + grain, respectively. No health problems were encountered during this trial. Cost of gain (feed cost/ kg of gain) was $ 0.22, $ 0.52, and $ 0.48 for pasture, pasture + grain, and hay + grain respectively. All three treatments, regardless of ADG or final weight, would result in net gains for the producer.

Price et al. (2003) investigated a preconditioning strategy of fenceline contact of beef calves with their dams at weaning to reduce the negative effects caused by separation anxiety on

behavior and growth rate. One hundred Angus/Hereford cross calves (BW = 206 kg) were randomly allotted to five weaning treatments: fenceline separation from dam on pasture (FP), total separation from dam on pasture (SP), total separation from dam on drylot preconditioned to hay (SDP), total separation from dam on drylot no preconditioning to hay (SDNP), and non-weaned controls on pasture (CP). Non-weaned control and FP calves spent significantly more ($P < 0.05$) time grazing and had significantly less ($P < 0.05$) vocalizations than the SP, SDP, and SDNP calves during the 7 d weaning treatment period. Ten wk post weaning CP and FP calves had higher ($P < 0.05$) weight gains as compared to SP, SDP, and SDNP. It was concluded that providing calves fenceline contact with their dams for 7 d post-weaning reduces the behavioral indications of distress that are seen following total separation. In addition, fenceline contact helps to minimize the depression in weight gain during the stressful transition of weaning resulting in heavier and, healthier calves to market.

Fluharty et al. (1994) investigated the ruminal characteristics, microbial populations, and digestive capabilities of newly weaned, stressed calves. Eight ruminally cannulated calves (BW = 245 kg) were weaned, transported, held in a sale barn without feed and water, and finally transported to a feedlot. Researchers looked at in situ DM, NDF, and N disappearance as well as concentrations of pH, ammonia, bacteria, and protozoa. Dry matter intake was suppressed on d 0 to only 62 % of DMI of d 7. No differences ($P > 0.10$) were found at 48 h for in situ DMD, NDF disappearance or concentration of total bacteria due to stress and feed withholding. Protozoal concentrations were lower ($P < 0.05$) on d 0 than 7. Fluharty et al. (1994) concluded that 24 h of feed and water withholding, weaning, and transportation did not stress the calves enough to affect ruminal bacteria and the ability to digest available substrates in the rumen.

Austin (2001) conducted three 42 d backgrounding experiments with weaned beef calves on pasture using protein and energy supplementation. In Exp. 1, 36 steers grazed stockpiled tall

fescue and were allotted to three treatments: no supplement, 15 % CP supplement at 0.5 or 1.0 % BW daily. Steers supplemented at 0.5 % BW had higher ($P < 0.05$) ADG than those supplemented at 1.0 % BW (1.30 and 0.96 kg/d, respectively). Experiment 2 consisted of 48 steers grazing stockpiled fescue with three treatments: no supplement, corn at 1 % BW, or 15 % CP supplement at 1 % BW. Steers consuming the corn and 15 % CP supplements had higher ($P < 0.05$) ADG than steers that were not supplemented (0.99, 0.89, and 0.77 kg/d, respectively). In Exp. 3, 48 steers grazed stockpiled tall fescue or fescue-alfalfa pastures with no supplement or daily supplement of 15 % CP at 1 % BW. Calves receiving supplement had higher morbidity during wk 1. At d 42, ADG was greater ($P < 0.05$) for supplemented steers. In general, forage nutritive value decreased over the 42 d study as CP decreased while NDF and ADF increased. Mineral status was highly variable on any sampling day and was not affected by treatment. Supplementation at 0.5 % BW was more efficient than at 1 % BW.

Shank (2002) investigated immune and health responses of weaning calves in three 21 d backgrounding experiments. In Exp. 1, 64 calves were backgrounding on pasture to investigate the effects of weaning stress on serum Se, leukocyte counts, and erthrocyte glutathione peroxidase activity at d –7, 0, 7, 14, and 21. Neutrophil counts increased from d –7 to 0 and then decreased from d 0 to 7, while lymphocytes followed the opposite pattern. In Exp. 2, 36 heifers received either no supplement or 15 % CP supplement at 0.5 % BW and either no injection, or on d 0 an injection of either Multi-min or Mu-Se. Whole blood Se and Cu increased while Zn decreased post-weaning. Heifers receiving Mu-Se injections gained faster than Multi-min or control heifers. In addition, supplemented heifers had higher ADG ($P < 0.05$) than unsupplemented heifers. Experiment 3 was conducted with 48 purchased steers with the same injection treatments as Exp. 2. Whole blood minerals followed the same stress response pattern

as was seen in Exp. 2. Selenium supplemented calves gained better than calves with a general mineral supplement.

Hutchinson (2003) investigated the effects of Se and vitamin E injection and supplementation on backgrounded calves. Experiments conducted in drylot and on stockpiled forage compared performance of calves receiving Se or vitamin E injections. Selenium injections did not significantly improve ADG or feed efficiency of supplements. Blood Se was higher ($P < 0.05$) on d 7, 14, 21, and 42 for both drylot and forage pasture based experiment calves that received Se injections. In another trial, forty eight steers were allotted to four pasture treatments: no fertilizer, poultry litter fed to previous cattle, poultry litter applied to field, or inorganic fertilizer applied to field and three supplements treatments: none, soyhulls (SH) + SBM, or corn + SBM at 0.5 % BW. On day 7, ADG was higher ($P < 0.05$) for steers supplemented with corn + SBM than other treatments. In an additional trial, thirty six heifers were used to compare co-product supplements when grazing stockpiled fescue. Supplementation treatments included: corn gluten feed (CGF) + SH at 0.5 % BW, CGF + SH at 1.0 % BW, and SH + SBM at 0.5 % BW. On d 14, heifer supplemented SH + SBM had higher ($P < 0.05$) cumulative gains than any other treatment. Forage and serum mineral concentrations were inconsistent throughout the studies. Backgrounded calves performed as well with co-products supplements as with the more costly traditional corn-based supplements.

Supplementation Strategy

Supplementation with protein and/or energy is often required to maximize calf performance during the fall grazing season. Cattle producers must be conscious of the costs of the supplements selected to feed and the labor required to ensure profitability (McCollum and Horn, 1990). Supplements provided to calves during backgrounding often contain corn grain and

soybean meal as well as trace minerals. Co-product feeds such as CGF, SH, wheat middlings (WM), brewers' grains (BG), and distillers' grains (DG) are an economical source of protein and energy. There are also a variety of management methods for providing supplements to forage fed cattle. Supplements are most commonly fed daily, however, there is an increasing interest in twice weekly, once weekly, or self-limited feeding strategies. A cattle producer can decrease labor inputs (time and money) by decreasing the frequency in which supplements are fed. Backgrounding cattle on pasture with other than daily supplementation schedules will aid producers in terms of time required for livestock care as well as economics.

Beaty et al. (1994) conducted three experiments to evaluate supplementation frequency and protein concentration of the diet on cattle performance, and digestion characteristics when consuming low-quality forages. Experiments 1 and 2 included 10, 20, 30 or 40 % CP concentrate fed at 14 kg DM/wk, daily or three times weekly supplementation as treatment variables, while in Exp. 3 the same supplementation frequencies and grain type (corn and sorghum) were used as treatment variables. Reducing supplementation frequency decreased forage intake ($P < 0.01$) but increased DM and NDF digestibility ($P \leq 0.03$) of the total diet. It was concluded that daily supplementation results in maximized forage intake and performance.

Scaglia et al. (2004) compared performance of unsupplemented heifers to heifers supplemented with 15 % CP CGF + SH at 0.5 % BW daily, 15 % CP CGF + SH at 1.0 % BW every other day, and 15 % CP SH + SBM at 0.5 % BW daily. Heifers supplemented every other day had higher ADG ($P < 0.05$) than unsupplemented heifers. Daily supplementation of heifers with SH + SBM daily had intermediate ADG as compared to the other treatments.

Supplementation of Protein and Energy

The NRC (2000) requirement of CP for stressed calves, such as those entering a backgrounding program is 12.5 to 14.5 % of the diet DM. Growing forages are high in rapidly

degradable protein that young cattle metabolize quickly and inefficiently; therefore supplementation with undegradable intake proteins is required for optimal performance. If pasture CP is limiting for fast growth rates, ADG can be increased with supplements that are high in bypass proteins. Protein absorption efficiency and utilization can be increased with an additional supply of bypass protein.

Cole and Hutcheson (1990) conducted a study to determine the influence of dietary CP concentration on health and performance of stressed feeder calves. Diets containing either 12 or 16 % CP were fed to 84 steers. There were no differences in morbidity or mortality between the treatments. However, cattle consuming the diet with 16 % CP tended to have fewer reoccurrences of morbidity resulting in fewer treatments per calf. During the first 14 d of the experiment calves consuming the 16 % CP diet had higher ADG ($P < 0.05$) and tended to have greater ($P < 0.10$) feed intake and gain:feed ratio than those consuming the 12 % CP diet. Eck (1988) reported greater ($P < 0.001$) DMI for calves receiving 12.5 % CP than those receiving 10.5 % CP at 6.38 and 5.98 kg/d, respectively. Average daily gains were also higher ($P < 0.001$) calves consuming the 12.5 % CP diet (0.66 and 0.50 kg/d, respectively). It was concluded that a minimum 60 % of the CP should be escape protein.

The NRC (2000) requirement of energy for stressed cattle (NE_m) is 1.3 to 1.6 Mcal/kg of DMI. The exact energy requirement for grazing cattle is not well defined because most research has been conducted in pen-fed situations. Fescue pastures in the spring and fall have an imbalanced TDN:CP ratio of 4:1 that is limiting in energy (Beck et al., 1999). Cattle growth can be depressed by the decreased protein flow into the small intestine. This imbalance could be corrected with the supplementation of energy or by-pass protein to increase the microbial protein available to the animal (Beck et al., 1999).

Fluharty and Loerch (1996) conducted a series of experiments to determine the effects of energy levels and sources on newly arrived feedlot calves. In Exp. 1, 68 steers were blocked by previous creep feeding treatment into three treatments: 75 % corn silage + 7 % corn + 14 % SBM, 30 % corn silage + 35 % alfalfa pellets + 28 % dry corn + 5 % corncobs, or 43 % dry corn + 50 % alfalfa pellets + 4 % SBM. In Exp. 2, 60 steers were fed for 28 d a 16 % CP receiving diet comprised of 70, 75, 80, or 85 % concentrate. In Exp. 3, 77 steers were allotted to a 2 x 3 factorial design with 70 or 85 % concentrate and 12.5, 16 %, or phase fed (23, 17, 14, and 12.5 % CP decreasing from wk 1-4). Previously creep fed calves consumed more ($P < 0.02$) than calves that were not previously creep fed. In Exp. 2, there were no differences ($P > 0.10$) in ADG or feed efficiency due to dietary concentrate level. There was a linear ($P < 0.02$) increase in DMI with increasing dietary concentrate level during wk 3 and wk 4. During wk 1, calves consuming the 85 % concentrate diet had greater ($P < 0.01$) DMI, ADG, and feed efficiency as compared to the 70 % concentrate diet. Calves receiving the 16 % CP concentrate and phase fed CP levels had greater ($P < 0.01$) DMI and ADG than those only receiving 12.5 % CP. Results from Pritchard and Mendez (1990) agree with the previous study. Pritchard and Mendez (1990) concluded that calves preconditioned to supplement have higher ($P < 0.001$) ADG after 28 d in the feedlot at1.38 and 1.22 kg/d, respectively. Feed intake was higher ($P < 0.001$) for calves consuming high energy (Alfalfa hay + corn) at 6.32 $kg \cdot hd^{-1} \cdot d^{-1}$ than low energy (Alfalfa hay + corn silage) at 5.43 $kg \cdot hd^{-1} \cdot d^{-1}$.

Numerous studies have concluded that supplying supplemental protein and/or energy to cattle consuming low to moderate quality forage can increase BW gains and forage intake while increasing forage digestibility. Energy and protein supplementation alter the grazing behavior of the animal, changing the energy requirements (Caton and Dhuyvetter, 1997). Grazing low quality forage can require a large expenditure of energy to consume sufficient levels of nutrients,

providing supplements decreases this energy expenditure. In addition, the energy from supplement was more efficiently used for maintenance and gain than the energy from forages (Caton and Dhuyvetter, 1997). Krysl and Hess (1993) studied the grazing behavior of cattle supplemented with varying protein and starch contents. An inverse relationship ($P < 0.05$) with starch content and daily grazing time was observed. Protein supplementation decreased grazing time ($P < 0.05$) by 1.5 h/d as compared to unsupplemented cattle. However, protein supplementation increased ($P < 0.05$) harvesting efficiency (g forage intake·kg BW $^{-1}$· min grazing $^{-1}$).

Garcés-Yépez et al. (1997) investigated the effects of supplemental energy sources and amount of supplement on forage intake, animal performance, and diet digestibility. Three supplement types (corn + SBM, WM, and SH) were fed to cattle and sheep at 0, 25, or 50 % projected TDN intake in addition to ad libitum chopped Bermuda grass hay. Hay intake was reduced ($P < 0.001$) to1.99, 1.93, and 1.63 % BW for the 0, 25, and 50 % supplementation levels respectively for all three supplement types. Total organic matter digestibility was similar for corn + SBM and SH, and was lower ($P < 0.001$) for WM. These results agree with previous results by Chase and Hibberd (1987) and Goetsch et al. (1991). Bodine and Purvis (2003) conducted a similar experiment on supplemental energy and degradable intake protein on performance, grazing behavior, intake, and digestibility in steers grazing dormant native tallgrass prairie. Supplements consisting of: 78.5 % corn + 21.5 % SBM, 78.1 % corn + 21.9 % SH, and 100 % SBM, were fed 5 d/wk at 178 g of DM/steer. Intake and digestibility of forage OM was decreased ($P < 0.01$) for steers on corn-based supplements as compared to SH and CSH-based supplements. In addition, feeding SBM improved ($P < 0.01$) efficiency of supplement by increasing added gain per unit of added supplement fed.

Co-Product Feeds

Alternative concentrate sources are a viable option for improving cattle performance. Co-product feeds, previously known as by-product feeds, may be economically beneficial products that were once considered waste from other processing procedures. Co-product feeds include CGF, SH, WM, BG, and DG. Co-product feed diets are an economical source of protein and energy for pasture fed cattle. It is often necessary to add Ca to a co-product based supplement due to a Ca:P ratio imbalance. Co-product feeds are a low cost, high quality, alternative to corn grain, SBM, and high quality hay, which are expensive supplements. The use of co-products feeds is economically important for the processing industry due to the removal of waste products, the producer attains affordable feed products, and the animal has enhanced performance. Co-product feeds can be blended together to formulate a supplement that will complement the forage-based diet with additional protein and energy that is high in bypass value and digestible fiber (Batajoo and Shaver, 1998).

Corn gluten feed is the part of commercial shelled corn that remains after the extraction of starch, gluten, and germ by the processes involved in wet milling to manufacture corn syrup or starch. Dry CGF will contain 21-22 % CP and 2.89 Mcal/kg ME. Corn gluten feed is a highly fermentable fiber source with an energy level relatively similar to that of corn (3.18 Mcal/kg ME) when fed to cattle on high roughage diets (Blasi et al., 2001). Soyhulls consist of the outer covering of the soybean, which are removed during the crushing phase of oil and meal extraction. Soyhulls contain approximately 9.4 % CP and 2.89 Mcal/kg ME. The fiber content of SH is low in lignin and therefore is highly digestible, resulting in energy levels similar to that of corn (Blasi et al., 2000). Wheat middlings consist of wheat bran, shorts, germ, flour, and offal from the commercial milling of wheat into flour. Wheat middlings contain approximately 18.8 % CP and 3.00 Mcal/kg ME. The fiber in WM is highly digestible in the rumen, therefore it does

not negatively impact fiber digestibility and subsequent forage intake like high starch feedstuffs do (Blasi et al., 1998). The nutritive value of co-products feedstuffs such as those just described is highly variable due to processing and diet composition.

Sunvold et al. (1991) conducted two studies to evaluate the use of wheat middlings as a supplement for cattle grazing bluestem range forage. In Exp. 1, 16 ruminally cannulated steers were allotted to four treatments: no supplement, 22:78 SBM:sorghum grain mix (21 % CP, 3.5 Mcal of ME/d), 100 % WM at low level (0.39 % BW, 3.5 Mcal/kg ME), and 100 % WM at high level (0.78 % BW, 7 Mcal/kg ME). In Exp. 2, 16 ruminally cannulated steers were assigned to four treatments: no supplement, 15, 20, and 25 % CP supplement. Supplements were 60% WM with various ratios of SBM and sorghum grain to achieve desired CP level. In Exp. 1, supplementation increased ($P < 0.10$) forage DMI, DM digestibility, and ruminal indigestible ADF, with high WM having the greatest response. Similar results were noted in Exp. 2. It was concluded that WM were comparable to SBM+sorghum grain as a supplement for cattle in grazing systems. Additionally, the supplementation of at least 20 % CP WM can increase the utilization of low quality forages.

Martin and Hibberd (1990) investigated the use of soy hulls as a supplement for cattle consuming low-quality hay. Twelve Hereford cows and four ruminally cannulated Hereford/Angus cross heifers were randomly allotted to one of four treatments: 0, 1, 2, or 3 kg DM from SH. Minimal decreases in hay DMI were noticed with the addition of SH to the diet. Total OM digestibility increased linearly ($P = 0.009$) with increasing SH. Soy hulls supplementation linearly increased ($P = 0.006$) ruminal VFA concentrations and molar proportions of propionate produced. It was concluded that the depression of forage intake due to SH supplementation was more than compensated for by the increased energy efficiency.

Batajoo and Shaver (1998) conducted an in situ DM, CP, and starch digestibility trial on SBM, BG, CGF, SH, and WM. Dacron bags containing 6 g (as fed) of each feed were immersed in the ventral rumen in duplicate for 2, 4, 6, 12, 24, 48, and 72 h. Ruminal degradation of DM was 63.9, 56.9, 54.6, 48.8, and 38.3 % for SBM, CGF, WM, SH, and BG respectively. Ruminal degradability of CP was 71.9, 70.3, 62.9, 58.2, and 48.9 % for WM, CGF, SBM, SH, and BG. Ruminal availability of starch was 88.0, 81.8, 76.0, 70.6, and 66.4 % for WM, SBM, BG, CGF, and SH, respectively. The values from this study provide estimates of the kinetics of ruminal degradation of feeds for the use in dynamic models of protein and carbohydrate digestion. The variability of digestion kinetics of these diverse co-product feeds allows for specialized ration formulations to meet the needs of all cattle.

Rumen Environment

Many factors influence the conditions of the rumen environment including, but not limited to diet composition, supplementation frequency, and animal health and stress (Bohnert et al., 2002). Daily supplementation of cattle grazing low quality forage with high CP concentrates maximize cattle performance better than less frequent supplementation. However, some buffering mechanisms have resulted in minimal differences in beef cattle performance due to supplementation frequency (Farmer et al., 2004). Infrequent supplementation causes greater fluctuations in the rumen environment as compared to daily or multiple times a day supplementation, which hold the rumen environment fairly uniform all the time. A concern with infrequent supplementation would be the onset of sub-acute acidosis each time the calves consume the larger amount of supplement offered (Goad et al., 1998). When concentrates are consumed the rumen pH drops due to fermentation and the production of VFA. At low ruminal pH some microbes including lactic acid users are suppressed resulting in high levels of lactic

acid (Schwartzkopf-Genswein et al., 2003). Limited information is available on how the ruminal changes due to supplementation frequency affect ruminal microbial populations, nitrogen utilization, and feedstuff digestibility in beef cattle on high forage diets (Beaty et al., 1994; Farmer et al., 2004).

Bodine and Purvis (2001) investigated the effects of supplementation on forage intake, digestion, and ruminal environment characteristics using four ruminally cannulated steers in a 4 x 4 Latin square design. Steers were fed prairie grass hay and four supplementation treatments: MINCR, mineral/vitamin mix fed with cracked corn; PROT, pelleted cottonseed-meal based protein feed; HF, high fiber WM/SH mix; and HG, high grain sorghum grain based pelleted feed. Steers were dosed with chromium sesquioxide (Cr_2O_3) for forage intake determination and with CoEDTA and ytterbium chloride labeled hay for fluid and particulate passage rates respectively. Results showed no change in hay OM digestibility ($P = 0.19$) but an increase ($P < 0.01$) in total diet OM digestibility with HF and HG as compared to MINCR and PROT fed steers. Additionally, in situ DMD was higher ($P < 0.01$) for HF, HG, and PROT as compared to MINCR treatment. Ruminal pH was higher ($P < 0.01$) and ammonia concentration was lower ($P < 0.02$) for MINCR fed steers. Fluid passage rate was lowest ($P < 0.01$) for MINCR steers whereas particulate passage rates did not vary between treatments ($P = 0.32$). Previous research has shown variable results on fluid and particulate passage rated due to supplementation (Chase and Hibberd, 1987; Martin and Hibberd, 1990; Sunvold et al., 1991). Volatile fatty acid concentrations increased with greater amounts of fermentable OM, but responses varied for protein and energy based supplements.

Bohnert et al. (2002) studied the influence of rumen protein degradability and supplementation frequency on the ruminal fermentation characteristics of steers consuming low-quality forage. Seven ruminally and duodenally cannulated steers were used in a 7 x 4

incomplete Latin square design with treatments consisting of: unsupplemented control, degradable intake protein (DIP) or undegradable protein (UIP) supplement at daily, every three or every six days. The DIP supplement consisted of SBM, and the UIP supplement consisted of a blend of expeller-processed SBM + blood meal (diets were 18 % and 60 % UIP on N basis respectively). Significant treatment by time interactions ($P < 0.05$) were observed for ruminal pH, ammonia, total VFA, and molar proportions of acetate, butyrate, and propionate on days in which all supplements were fed. On the days that all supplements were provided, ammonia was increased ($P < 0.05$) and pH was decreased ($P < 0.01$) due to CP supplementation. Bohnert et al. (2002) concluded that cattle are able to maintain a productive rumen environment (adequate fiber digestion, fluid dynamics, and particulate passage) with infrequent CP supplementation.

Carey et al. (1993) examined the influence of energy source on forage intake, digestibility, in situ forage degradation, and ruminal fermentation in beef steers fed medium-quality forage. Eight ruminally cannulated steers were used in a replicated 4 x 4 Latin square design with SBM as control, corn, barley, and beet pulp as energy sources. Total DMI was similar for all treatments, however forage DMI tended ($P < 0.10$) to be lower for the three energy supplemented groups as compared to the control. Digestibility of OM and DM was higher ($P < 0.10$) for corn and beet pulp supplemented than control or barley supplemented steers, however NDF digestibility was the opposite. Ruminal ammonia concentrations were lower ($P < 0.005$) for energy supplemented than for the control steers, but remained above the minimal 5 mg/dL (Satter and Slyter, 1974) required for proper microbial function. Volatile fatty acid response curves over time were the same for all treatments. Total VFA production was highest with beet pulp. Passage rate was similar for all treatments and ranged from 3.56 to 4.04 %/h, comparable to those reported by Chase and Hibberd (1987) for corn at 4.04 %/h.

Forage Nutritive Value and Tall Fescue

Forage nutritive value is an important determination of grazing animal performance. Forage is most nutritious during the vegetative state (Hitchcock, 1990). The nutritive value of tall fescue depends on the stage of growth. Tall fescue can be stockpiled in the fall to try to extend the grazing season. Stockpiled tall fescue loses CP while increasing NDF and ADF over time once it is stockpiled (Fritz and Collins, 1991) late in the grazing season as it becomes senescent. Austin (2001) and Hutchinson (2003) reported decreased forage mass and nutritive value with continuously stocking management during autumn backgrounding trials. As the forage matures from a vegetative to reproductive state the ADF and NDF contents increase thus decreasing digestibility (Jung and Allen, 1995). Voluntary DMI is often reduced due to rumen fill when consuming high fiber forage. The ruminant is unable to eat sufficient quantities to meet its energy demands (Jung and Allen, 1995).

Tall fescue (*Festuca arundinacea, Schreb.*) is one of the most commonly used forages in the United States. Tall fescue is a hardy cool-season perennial grass that yields high quality forage for grazing animals. Most native Kentucky 31 tall fescue pastures contain the fungal endophyte *Neotyphodium coenophialum* (Hoveland, 2003). The endophyte protects the plant from environmental stressors such as drought, insects, nematodes, disease pathogens, and grazing herbivores. Livestock grazing pasture containing endophyte-infected fescue may develop fescue toxicosis resulting in intolerance to heat, poor weight gains, and increased respiration rates. Cattle consuming endophyte-infected may also have compromised immune function due to the development of Cu deficiency (Saker et al., 1998).

Endophyte-free fescue cultivars appear to eliminate the livestock toxicity problems but are harder to develop and maintain (Peters et al., 1992). Improved cattle performance when

grazing endophyte-free as compared to endophyte-infected tall fescue has been attributed to grazing behavior (Howard et al., 1992). Howard et al. (1992) determined that cattle on endophyte-free fescue spend more time grazing with more bites per minute ($P < 0.05$) resulting in greater DMI as compared to cattle on endophyte-infected fescue. In addition to endophyte-free fescue, scientists have also developed cultivars that contain nonergot alkaloid producing endophyte-infected fescues that do not have the detrimental effects on grazing livestock (Hoveland, 2003).

Parish et al. (2003) conducted grazing studies to determine performance, toxicosis status, and grazing behavior of calves (BW = 227 kg) grazing nonergot alkaloid-producing endophytes (AR542 and AR502) or toxic endophyte infected (Kentucky 31, Jesup, and Georgia-5) tall fescue during spring and autumn grazing seasons. Cattle grazing endophyte-infected fescue exhibited signs of heat stress (panting and seeking shade) during periods of elevated environmental temperatures while cattle on the other treatments continued grazing. Rectal temperatures were elevated ($P < 0.05$) in cattle grazing endophyte-infected fescue (39.6 ˚C) as compared to cattle grazing endophyte-free (39.3 ˚C) and nonergot infected fescue (39.2 ˚C). Average daily gain was suppressed ($P < 0.05$) for endophyte-infected grazing cattle as compared to the other treatments for both spring and fall seasons with greatest suppression in the spring ($P < 0.01$). Results on ADG from this study agree with those reported by Nihsen et al. (2004). The concentration of ergot alkaloids is greatest in forage leaves and seeds in the spring resulting in the most negative effects on the cattle during that period of time (Rottinghaus et al., 1991).

Peters et al. (1992) investigated cow-calf performance, forage utilization, and ergovaline intake when grazing endophyte-infected fescue, endophyte-free fescue, or orchardgrass. Forage OM digestibility was not different between forage types but did decline ($P < 0.05$) over the grazing season. Forage intake was 18 % lower ($P < 0.05$) for cows grazing endophyte-infected

than endophyte-free or orchardgrass. However, decreased forage intake was not sufficient to justify BW loss in cows grazing endophyte-infected fescue during the trial. No apparent signs of fescue toxicosis were present. Researchers proposed that nutrient utilization and forage efficiency may be altered in cattle consuming endophyte-infected fescue. Calf ADG and weaning weights were lower ($P < 0.05$) on endophyte-infected pastures as compared to endophyte-free fescue or orchardgrass pastures.

Beconi et al. (1995) conducted a three phase investigation to determine the long term effects of endophyte-infected fescue on cattle performance in a feedlot. In phase 1 (October 24-December 19), 80 Angus calves were allotted to four treatments: endophyte-infected, endophyte-free, or low endophyte Kenhy, and low endophyte Johnstone tall fescues. In phase 2 (December 20- April 10), cattle from phase 1 were re-allotted to receive Johnstone or endophyte-infected haylage in drylot. During phase 3 (April 11- August 1), all steers received a common high concentrate finishing diet (94 % ground ear corn, 4.5 % SBM, 1.15 % limestone, and 0.35 % trace mineral salt, as fed basis). In Phase 1, ADG was highest for steers grazing Kenhy ($P < 0.05$), intermediate for those grazing Johnstone and endophyte-free, and lowest for steers grazing endophyte-infected fescue. During Phase 2, cattle consuming Johnstone haylage had greater ($P < 0.01$) DMI, ADG, and gain:feed ratio than those consuming endophyte-infected haylage. In Phase 3, cattle that had previously consumed endophyte–infected haylage had higher ($P < 0.01$) ADG and gain:feed ratios. Overall, there were no other significant differences due to previous treatment. It was concluded that the growth inhibiting effects of endophyte-infected fescue were short-term and did not affect feedlot performance (Beconi et al., 1995). Results from this study indicated compensatory gain recovery in feedlot following consumption of endophyte-infected fescue, were consistent with those of Cole et al. (1987).

BACKGROUNDING EXPERIMENTS

Objectives

The main objective of these experiments was to determine the effects of supplementation strategies on performance and health of weaned calves during the backgrounding period. Specific objectives are:

1) To determine the effects of supplementation strategy on performance and health status in newly weaned heifers grazing endophyte-infected fescue.
2) To determine the effects of stress level and supplementation strategy on the health and performance of newly weaned steers grazing endophyte-free fescue.
3) To compare health and performance of purchased calves when grazing endophyte-free, endophyte-infected, or novel endophyte fescue.
4) To investigate the changes in ruminal parameters, forage and supplement kinetics in steers fed at different supplementation frequencies.

Materials and Methods

Experiment 1: Heifer Development Study (SVAREC, Steeles Tavern, VA)

This 42-d study, which started on October 11, 2004 was conducted at the Shenandoah Valley Agricultural Research and Extension Center (SVAREC) at Steeles Tavern, VA. Thirty-six Angus crossbred heifers (237 ± 17 kg) born at SVAREC were blocked according to previous cow system and -30 d weight into six groups of six heifers. The six groups were allotted at random to six endophyte-infected (Kentucky 31) fescue paddocks (1.8 ha). Paddocks were allotted at random to one of three supplementation frequency treatments (two

replicates/treatment): 1) none, 2) every 48 h at 1.0 % BW, and 3) daily at 0.5 % BW. All groups of heifers had free access to trace mineral mixture and water.

Experiment 2: Nutrient Management Location (SVAREC, Steeles Tavern, VA)

This 42-d study, which started on October 11, 2004 was conducted at the SVAREC. Forty-eight steers (256 ± 31 kg) born at SVAREC were allotted to 12 groups of 4 steers by previous cow system and -30 d weight. The 12 groups were allotted at random to endophyte-free fescue paddocks (0.5 ha). This experiment was arranged as a 3 x 2 factorial with supplementation strategy and stress level as main factors with two replicates per treatment. Supplement treatments consisted of: 1) none, 2) every 48 h at 1.0 % BW, and 3) daily at 0.5 % BW. The two stress levels compared were: 1) low-stressed (LS) calves were weaned and held in drylot with hay and water overnight; and 2) high-stressed (HS) calves were weaned, hauled in a trailer for 2 consecutive periods of 3 h, and held overnight in drylot with water but without hay. During the grazing period all groups of steers had free access to trace mineralized salt and water.

Experiment 3: Kentland Fescue Study (Kentland Farm, Blacksburg, VA)

This 42-d study, which started on October 21, 2004 (Blocks 1 and 2) and October 26, 2004 (Block 3) was conducted at Kentland Farm near Blacksburg, VA. Thirty-six Angus crossbred steers (198 ± 11 kg) purchased from local calf sales (27 calves from Dublin VA Feeder Cattle Sales [24 experimental and 3 spare calves] and 12 calves from Narrows, VA Feeder Cattle Sales) were randomly allotted to three forage types: endophyte-free (Kentucky 31), novel endophyte (Q4508-AR542), or endophyte-infected (Kentucky 31) tall fescue (three replicates/forage, four steers/paddock). Paddocks were divided into three 0.38- 0.40 ha sub-

paddocks, which were rotationally stocked (2 weeks/sub-paddock). All steers had free access to a trace mineralized salt and water throughout the study.

Field Procedures for Experiments 1, 2, and 3. The supplement for Exp. 1 and 2 consisted of a pelleted blend of 17 % flaked corn grain, 50 % SH, and 33 % WM from Augusta Cooperative, Staunton, VA. According to NRC (2000) the supplement was calculated to provide CP (13.8 %) and ME (2.04 Mcal/kg) at levels required by stressed calves while achieving an ADG of 1.25 kg. The quantity of supplement fed was recalculated after each weigh date to reflect the average body weight of the calves in that particular paddock. Supplements were fed at 0800 at the given frequency, and sampled weekly for analysis; refusals were weighed back and discarded. On sampling days, supplements were fed after the cattle were worked. Mineral mixture for Exp. 1 consisted of 19 % NaCl, 10.7 % Ca, 6.5 % P, 1.1 % K, 11.2 % Mg, 1.9 % S, 97 ppm Co, 2500 ppm Cu, 136 ppm I, 3970 ppm Mn, 120 ppm Se, 5100 ppm Zn, 157118 IU/kg Vitamin A, 38909 IU/kg Vitamin D3, and 227 IU/kg Vitamin E (10-SMG, King Ag Products Inc., Pulaski, VA). Trace mineral salt for Exp. 2, 3 and 4 consisted of 94 % NaCl, 37 % Ca, 0.35 % Zn, 0.2 % Fe, 0.03% Cu, 0.007 % I, and 0.005 % Co (Champions Choice Trace Mineral Block, Cargill Inc., Minneapolis, MN).

Five weeks prior to weaning all calves from SVAREC (Exp. 1 and 2) were vaccinated with Pyramid 4® (Fort Dodge Animal Health, Fort Dodge, IA): infectious bovine rhinotracheitis (IBR), parainfluenza (PI_3), bovine viral diarrhea (BVD), and bovine respiratory syncytial virus, and Vision 7® (Intervet, Boxeer, Netherlands) for clostridial disease. At weaning, all calves were treated with Cydectin® pour-on anthelmintic (Fort Dodge Animal Health, Fort Dodge, IA). Purchased calves (Exp. 3) were treated with Pyramid 4® (Fort Dodge Animal Health, Fort

Dodge, IA), Vision 7® (Intervet, Boxeer, Netherlands), and Cydectin® pour-on anthelmintic (Fort Dodge Animal Health, Fort Dodge, IA) upon arrival.

In Exp.1, calves were weighed, blood samples collected, and rectal temperatures were obtained on d 0, 7, 14, 21, and 42. In Exp. 2, steers were weighed, blood samples collected, and rectal temperatures obtained on d 0 [LS, HS: at weaning (1200), HS: mid transport (1600), and LS, HS: evening when HS returned to SVAREC (2100)], 1, 4, 7, 14, 21, and 42. Blood samples were collected into Red Top and Blue Top Vacutainer™ tubes for serum and serum Zn (Exp. 3 only) respectively, K_3EDTA Vacutainer™ tubes for whole blood, and Buffered Sodium Citrate Vacutainer™ tubes for plasma via jugular venipuncture. Samples were kept on ice until arrival at the laboratory, centrifuged at 600 X g for 20 min for serum and plasma, decanted into clean plastic tubes, and frozen at –20 ˚C until further analysis. All serum samples were analyzed for plasma fibrinogen, serum prolactin, creatine kinase, and blood urea nitrogen. Blood samples from d 0 to 7 were analyzed for leukocyte ratios. Serum samples were analyzed for Cu and Zn and whole blood Se was analyzed on d 0 and d 42 for Exp. 3.

During wk 1 of Exp. 2 and 3, tympanic temperature loggers (Stow Away® XLT, Bourne, MA) were placed in the ear of two and four steers/treatment respectively. Tympanic membrane temperatures were recorded every 15 min for 7 d. Temperature data was retrieved from the loggers using BoxCar Pro® 4.0 from Onset Computer Corp (Bourne, MA).

Calf morbidity was evaluated daily at 0800. Calves were individually rated on a scale of 1 to 5 based on a subjective scoring system described in Table 2 (Perino and Apley, 1998). Sick animals were identified by the presence of nasal discharge, failure to eat or drink, coughing, and separation from the group. Cattle receiving a score of 2 or greater had the rectal temperature taken, and if above 40 °C the calf was treated with Micotil® (tilmicosin; Elanco Animal Health, Indianapolis, IN) according to label instructions. Calves requiring additional treatments received

Table 1. Morbidity scoring system[a]

Score	Description
1	Normal, no signs of disease.
2	Noticeable depression, signs of weakness are usually not apparent.
3	Marked depression, moderate signs of weakness may be apparent, but without significantly altered gait.
4	Severe depression accompanied by signs of weakness such as altered gait or lowered head.
5	Moribund, unable to rise.

[a]Adapted from Perino and Apley (1998)

NuFlor® (florfenicol; Schering Plough Animal Health Corp., Madison, NJ) for the second treatment.

Forage mass and nutritive value were determined from samples harvested on d 0, 21, and 42 for Exp. 1 and 2 and on d 0, 14, and 28 as calves were rotated to a new sub-paddock for Exp. 3. Forage samples for nutritive value were collected by walking an "X" in the paddock/sub-paddock and hand clipping representative forage at a height of 2.5 cm (grazing height) every 10-20 paces. Samples were dried in a forced draft oven at 60 °C for 48 h and stored in cloth bags until further analysis. Forage mass samples were collected by mowing two (Exp. 2 and 3) or four (Exp. 1) 3.0 x 0.5 m strips with a self-propelled push mower (American Honda Motor Company, Model HR215, Duluth, GA) at a height of 2.5 cm. Forage mass samples were dried in a forced draft oven at 60 ºC for 72 h before weighing.

Forage samples from all paddocks in Exp. 3 were collected for alkaloid analysis on October 27, 2004. Samples were collected following the same procedures as forage quality samples. Forage samples were freeze-dried (FreeZone 12, Labconco Co., Kansas City, Mo.) and ground through a 1 mm screen using a Wiley Mill (Thomas Wiley Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA). Samples were sent to Agronostics Ltd. Co. (Watkinsville, GA) for determination of alkaloid concentration via Phytoscreen Immunoblot assay kit.

Laboratory Analysis for Experiments 1, 2, and 3. Forage and supplement samples were ground through a 1 mm screen using a Wiley Mill (Thomas Wiley Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA). Forage and supplement samples were analyzed for DM by drying at 100 °C for 12 h in a Fisher Scientific Isotemp Oven. Ash was determined by placing sample at 500 °C for 2 h in a Thermolyne 30400 muffle furnace (AOAC, 2000). Crude protein was determined via the combustion method using a nitrogen analyzer (PerkinElmer Series II:

2410, Norwalk, CT) (AOAC, 2000). Neutral detergent fiber and ADF were analyzed using an adapted Goering and Van Soest (1970) method with an Ankom 200 (Fairport, NY). Forage and supplement samples were digested for mineral analysis according to Muchovej et al. (1986). Calcium, Mg, K, Cu, and Zn were then analyzed using flame atomic absorption spectrometry (PerkinElmer AAnalyst 800, Norwalk, CT). Selenium was determined in forage and feed samples using a graphite furnace on a PerkinElmer AAnalyst 800 (Norwalk, CT). Phosphorus was determined from feed samples using the plate reader colorimetric method with a molybdate reaction using a Titertek Multiskan MCC/340 (Titertek, Huntsville, AL) (Method # 965.17, AOAC, 2000).

Blood urea nitrogen (BUN) and creatine kinase (CK) were determined using a Beckman SYNCHRON CX SYSTEMS ® (Beckman Instruments, Inc., Brea, CA) blood chemistry analyzer (Beckman Coulter kit # 442750 and 442635 respectively). Analysis for BUN and CK followed the AOAC (2000) procedures using an enzymatic reaction being read at 340nm. The change in absorbance between sample and reagents is directly proportional to concentration of BUN or CK of the serum sample. The activator in the CK assay kit is monothioglycerol. Plasma fibrinogen (Fb) was analyzed according to Latimer et al. (2003) using a Reichert Vet 360. Leukocyte ratios were analyzed at the Clinical Laboratory Services, Virginia Maryland Regional College of Veterinary Medicine via a smear, stain (Wescor 7120), and differential analysis according to Sink et al. (2004). Blood smears were prepared by placing one drop of fresh blood on a warm, dry glass slide then spread with another slide held at a 30 º angle. White blood cell type counts were taken and reported as a percent of WBC. Neutrophil:lymphocyte (N:L) ratios were compared. Serum Cu and Zn were analyzed using flame atomic absorption spectrometry (PerkinElmer AAnalyst 800, Norwalk, CT). Whole blood Se was determined using a graphite furnace on a PerkinElmer AAnalyst 800 (Norwalk, CT).

Experiment 4: Rumen Environment Study (Kentland Farm, Blacksburg, VA)

This 42 d study started on October 31, 2004. It was conducted at the Kentland Farm near Blacksburg, VA. Three ruminally cannulated (690 ± 60 kg) steers grazing endophyte-free fescue were utilized in a 3 x 3 Latin Square design with three supplement treatments: 1) none, 2) every 48 h at 1.0 % BW divided into 2 meals, and 3) daily at 0.5 % BW. The supplement consisted of the pelleted blend used in Exp. 1 and 2. Steers were supplemented in individual pens at 0600 and 1800 according to protocol. A 12 d adaptation period to the supplementation strategy was allowed prior to the 3 d sample collection period. Steers were weighed at the beginning of each period.

Field Procedures for Experiment 4. Steers were dosed with 8 g Cr_2O_3 on d 5-12 at 0600 and 1800, and fecal samples were collected on d 11-14 at 0600 and 1800 for forage intake estimation (Bodine et al., 2001). Forage intake was estimated as: DMI (g/d) = DMFO (g/d) / (1-DMD). Steers were dosed with 100 g of encapsulated Yb-labeled forage (8123 ppm Yb) for determination of particulate passage rate at 0600 on d 0 of each sampling period. Ytterbium forage binding was prepared according to Teeter et al. (1984). Forage was ground through a 6 mm screen in Wiley Mill (Thomas Wiley Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA), boiled for 1 h in a modified NDF solution prior to soaking overnight in a 7.8232 g Yb/L solution. Chromium sesquioxide and Yb labeled forage were placed into Torpac #SuO7 gelatin capsules (Torpac Inc., Fairfield, NJ) for dosing (1 and 24 capsules, respectively per dosing). During the 3-d sampling period ruminal fluid and particulate were collected via the cannula at 0, 2, 4, 6, 8, 12, 24, 36, 48 h post dosing. Particulate and ruminal fluid was collected from four quadrants of the rumen and then composited. Ruminal fluid was removed from

particulate by filtering through four layers of cheesecloth. Ruminal fluid samples were analyzed for pH, VFA, and ammonia-N. The ruminal fluid pH was determined immediately after being pulled from the rumen. Five ml ruminal fluid samples for VFA analysis were stored with 5 ml internal standard and 1 ml 25 % metaphosphoric acid. Eight ml of ruminal fluid was stored in 2 ml 25 % metaphosphoric acid for rumen ammonia determination. All samples were frozen upon collection until further laboratory analysis could be conducted. Forage and supplement samples for in situ analyses were incubated in the rumen for the same time intervals as above. Forage and supplement samples were ground through a 2 mm screen using a Wiley Mill (Thomas Wiley Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA) sealed in 10 x 20 cm Dacron bags pore size 50 μm, and incubated in the rumen in triplicate.

Laboratory Analysis for Experiment 4. Volatile fatty acid (acetate, propionate, and butyrate) concentrations from ruminal fluid were analyzed using gas chromatography (GC) on an Agilent Technologies 6890N Network GC System. The GC column used was a DB-WAXetr 30 m x 0.53 mm I.D., 1.0 μm with He carrier at 37 cm/sec. Ruminal fluid samples were centrifuged at 800 x g for 10 min and filtered through a 0.45 μm filter (D26-45, MP Biomedicals, Aurora, OH). Ruminal ammonia-N was determined using plate reader method according to Chaney and Marbach (1962) on a Titertek Multiskan MCC/340 (Titertek, Huntsville, AL). In vitro dry matter digestibility was determined according to Tilley and Terry (1963) using a Daisy II Incubator (Ankom, Fairport, NY). Fecal and particulate samples were dried at 60 ºC in a forced draft oven for 72 h, ground through a 1mm screen in Wiley Mill (Thomas Wiley Model 4, Philadelphia, PA), and wet ashed according to Muchovej et al. (1986) prior to analysis for Cr and Yb, respectively. Chromium concentration was analyzed according to Bodine et al. (2001) and Yb concentration according to Teeter et al. (1984) by way of atomic absorption spectrometry using a

PerkinElmer AAnalyst 800 (Norwalk, CT). Determination of in situ digestibility was calculated according to Nocek (1985) and Vanzant et al. (1998). Crude protein concentration was determined using the combustion method with a PerkinElmer Series II: 2410 nitrogen analyzer (Norwalk, CT) without a correction for purine derivatives.

Statistical Analysis

Data from Exp. 1 and 3 were analyzed using Proc MIXED (SAS, 2001) procedure for analysis of variance in a complete randomized block design with repeated measures. Experiment 2 data was analyzed using Proc MIXED (SAS, 2001) for a 3 x 2 factorial design with repeated measures. Pasture was the experimental unit for all variables. Experiment 4 data was analyzed using the Proc Mixed procedure in SAS (2001) for repeated measures within a 3 x 3 Latin Square design. Dry matter disappearance was calculated with Proc NLIN according to Orskov and McDonald (1979) with model as $P = A + B (1\text{-}e^{-ct})$ where A = readily degradable fraction, B = slowly degradable fraction, and C = rate of digestion for B. Differences in DM disappearance and forage intake were analyzed with Proc Mixed. Models included the fixed effect of paddock as block; supplement, stress, and forage, for the corresponding experiments, as treatments, and sampling dates as repeated measures. All means were reported as least squares means. The autoregressive, ar(1), covariance structure provided the best fit data for all analyses as compared to unstructured, un, and compound symmetry, cs. Standard errors were calculated in SAS with the estimation of similar variances between treatments on any day. Differences were determined for the repeated measures using Tukey's adjusted P-values. Contrasts were conducted to determine linearity and quadratic trends in all data.

Results

Chemical Composition of the Supplement

The chemical composition of the supplement used in Exp. 1, 2, and 3 is presented in Table 2. The supplement was designed to provide 13.8% CP and the analyzed value was 14.05% CP. Analyzed values for supplement mineral content were similar to those estimated using NRC (2000).

Experiment 1: Heifer Development Study (SVAREC, Steeles Tavern, VA)

Forage Mass and Nutritive Value. Forage mass and chemical composition data are presented in Table 3. There were no differences in forage availability or chemical composition between treatments at any sampling date. Paddocks (n=2) for the daily supplemented heifers on average had lower ($P < 0.05$) forage mass than paddocks for the heifers that were not supplemented. Forage nutritive value and mass declined over the 42 d of the study. Forage mass and availability declined linearly ($P < 0.001$) for all paddocks. Ash content of the forage decreased linearly and quadratically ($P < 0.001$) over the 42 d study. Neutral detergent fiber and ADF increased linearly ($P < 0.001$). Crude protein declined linearly ($P < 0.0001$) with an average loss of three percentage units over the 42 d period.

Forage mineral composition data is presented in Table 4. The average Cu concentration was lower ($P < 0.05$) in paddocks with heifers supplemented daily (3.66 ppm) as compared to the paddocks in which heifers were not supplemented (4.36 ppm) or were supplemented every 48 h (4.27 ppm). All other mineral concentrations in forages were not different ($P > 0.05$) between treatments at any sampling date. Forage K declined linearly ($P < 0.0001$) and quadratically ($P < 0.005$) over the 42 d study. Additionally, forage P declined linearly ($P < 0.01$).

Table 2. Chemical composition of supplement[a]

Item	Concentration
Dry matter, %	90.53
Ash, %	4.76
Crude Protein, %	14.05
Nitrogen, %	2.25
Neutral detergent fiber, %	43.87
Acid detergent fiber, %	25.19
Calcium, %	0.35
Magnesium, %	0.34
Phosphorus, %	0.55
Potassium, %	0.99
Copper, ppm	6.00
Zinc, ppm	87.63
Selenium, ppb	230.03

[a]DM basis

Table 3. Forage mass and chemical composition[a] in Exp. 1 (n=2)

		Supplementation frequency		
Day	Item	None	Every 48 h	Daily
0	Forage Mass, kg/ha[b]	4439^{jx}	4137^{jkx}	3216^{kx}
	Forage Availability, kg DM/kg BW[c]	5.61^{x}	5.21^{x}	4.10^{x}
	Ash, %[d]	9.19^{x}	9.35^{x}	8.92^{x}
	NDF, %[e]	55.08^{x}	55.02^{x}	55.27^{x}
	ADF, %[f]	28.15^{x}	28.1^{x}	26.94^{x}
	CP, %[g]	19.74^{x}	18.63^{x}	19.9^{x}
21	Forage Mass, kg/ha[b]	2986^{y}	2337^{y}	2510^{y}
	Forage Availability, kg DM/kg BW[c]	3.74^{xy}	3.44^{y}	3.08^{xy}
	Ash, %[d]	9.14^{x}	9.55^{x}	9.28^{x}
	NDF, %[e]	58.75^{y}	58.95^{y}	58.16^{x}
	ADF, %[f]	29.87^{y}	30.43^{y}	29.71^{y}
	CP, %[g]	15.92^{x}	17.00^{xy}	17.45^{x}
42	Forage Mass, kg/ha[b]	2763^{y}	2532^{y}	2049^{y}
	Forage Availability, kg DM/kg BW[c]	3.28^{y}	2.93^{y}	2.37^{y}
	Ash, %[d]	8.13^{y}	8.05^{y}	8.13^{y}
	NDF, %[e]	65.53^{z}	63.29^{z}	63.71^{z}
	ADF, %[f]	32.84^{z}	31.25^{z}	31.98^{z}
	CP, %[g]	15.87^{y}	16.07^{y}	16.22^{y}

[a]DM basis

[b]SE=378

[c]SE=0.50

[d]SE=0.21

[e]SE=1.12

[f]SE=0.63

[g]SE=0.60

[j,k] Means within a row with different superscripts are different ($P < 0.05$)

[x,y,z] Means within column with different superscripts are different ($P < 0.05$)

Table 4. Forage mineral composition[a] in Exp. 1 (n=2)

		Supplementation frequency		
Day	Mineral	None	Every 48 h	Daily
0	Ca, %[b]	0.50	0.45	0.55
	Mg, %[c]	0.33	0.34	0.32
	K, %[d]	3.17[x]	3.12[x]	3.03[x]
	P, %[e]	0.32	0.32	0.32
	Cu, ppm[f]	5.2[ix]	5.0[ix]	3.6[j]
	Zn, ppm[g]	19.9	20.6	20.2
	Se, ppb[h]	89.7[xy]	74.0	33.2
21	Ca, %[b]	0.45	0.44	0.46
	Mg, %[c]	0.33	0.36	0.35
	K, %[d]	2.84[xy]	3.05[x]	2.91[x]
	P, %[e]	0.29	0.31	0.31
	Cu, ppm[f]	3.4[y]	4.0[y]	3.8
	Zn, ppm[g]	15.8	16.7	17.0
	Se, ppb[h]	2.1[x]	2.1	25.3
42	Ca, %[b]	0.46	0.48	0.51
	Mg, %[c]	0.30	0.33	0.32
	K, %[d]	2.04[y]	2.06[y]	2.10[y]
	P, %[e]	0.29	0.27	0.27
	Cu, ppm[f]	4.5[xy]	3.8[y]	3.6
	Zn, ppm[g]	19.1	17.5	16.3
	Se, ppb[h]	153.9[y]	14.4	12.7

[a]DM basis

[b]SE=0.03

[c]SE=0.02

[d]SE=0.13

eSE=0.02

[f]SE=0.3

[g]SE=1.8

[h]SE=30.2

[i,j]Means within a row with different superscripts are different ($P < 0.05$)

[x,y]Means within column with different superscripts are different ($P < 0.05$)

Forage Ca, Mg, Cu, Zn, and Se did not change over the 42 d study. Selenium content of the forage in these paddocks was highly variable.

Animal Performance. Performance data for heifers are presented in Table 5. No differences were observed between supplementation treatments at any sampling date. There were no significant differences in cumulative ADG due to supplementation treatments. Weights increased linearly ($P < 0.0001$) and responded quadratically ($P < 0.005$) due to an initial weight loss from d 0 to 7. Average daily gains responded linearly and quadratically ($P < 0.01$ and $P < 0.0001$, respectively), following a similar pattern as BW. Heifers supplemented daily appeared to have the greatest gain per hectare (161 kg/ha), with those supplemented every 48 h were intermediate (148 kg/ha), and the heifers that were not supplemented appeared to have the lowest gain per hectare (113 kg/ha). Additionally, daily supplemented heifers had numerically higher supplement efficiency at 6.77 kg supplement/kg of additional gain as compared to 8.27 kg supplement/kg of additional gain for heifers supplemented every 48 h.

Animal Health. Heifer health parameters are presented in Table 6. No differences were observed between treatments on any day for morbidity, rectal temperature, or leukocyte ratios. The N:L ratio was higher ($P < 0.01$) on d 0 than d 7 for all treatments. Rectal temperatures were not significantly different between treatments or sampling dates. All heifers received a morbidity score of 1 throughout the study. Three heifers were medicated due to elevated rectal temperatures on d 14.

Serum and Plasma Analyses. Serum concentrations of BUN are presented in Table 7. No treatment differences were detected on d 0-21. There was a sampling day by supplement interaction ($P < 0.05$) for BUN concentration. On d 42, heifers receiving supplement every 48 h

Table 5. Heifer performance record in Exp. 1 (n=2)

		Supplementation frequency		
Measurement	Day	None	Every 48h	Daily
Weight, kg[a]	0	237.9^{xy}	238.7^{xy}	235.5^{xy}
	7	229.8^{x}	236.8^{x}	230.3^{x}
	14	236.8^{xy}	241.8^{xy}	238.5^{xy}
	21	241.4^{y}	249.4^{y}	244.8^{y}
	42	254.9^{z}	261.0^{z}	259.6^{z}
ADG, kg/d[b]	0-7	-1.16^{x}	-0.27^{x}	-0.74^{x}
	7-14	1.00^{y}	0.74^{y}	1.17^{y}
	14-21	0.65^{y}	1.07^{y}	0.90^{y}
	21-42	0.64^{y}	0.55^{xy}	0.71^{y}
	0-42	0.40	0.53	0.57

[a]SE=4.5

[b]SE=0.22

[x,y,z]Means within column with different superscripts are different ($P < 0.05$)

Table 6. Heifer health record in Exp. 1 (n=2)

		Supplementation frequency		
Analysis	Day	None	Every 48 h	Daily
N:L ratio[ac]	0	0.50[x]	0.49[x]	0.59[x]
	7	0.28[y]	0.27[y]	0.31[y]
Temperature, ˚C[d]	0	39.9	40.2	39.9
	7	39.4	39.6	39.8
	14	39.4	39.6	39.7
	21	39.8	40.2	40.1
	42	39.4	39.8	39.8
Morbidity score[b]	0-7	1.00	1.00	1.00
	7-14	1.00	1.00	1.00
	14-21	1.00	1.00	1.00
	21-42	1.00	1.00	1.00
Number treated	0-7	--	--	--
	7-14	--	--	--
	14-21	--	--	3
	21-42	--	--	--

[a]Neutrophil:Lymphocyte ratio

[b]Based on a subjective scoring system (1=healthy, 5=moribund)

[c]SE=0.04

[d]SE=0.2

[x,y]Means within column with different superscripts are different ($P < 0.05$)

Table 7. Heifer serum and plasma analyses in Exp 1 (n=2)

		Supplementation frequency		
Analysis	Day	None	Every 48h	Daily
Blood urea nitrogen, mg/dL[a]	0	19.10	18.82^{x}	18.37
	7	18.96	19.01^{x}	16.66
	14	17.96	14.36^{y}	16.97
	21	20.02	20.04^{x}	20.58
	42	18.38^{de}	15.55^{dxy}	19.71^{e}
Creatine kinase, IU/L[b]	0	663.0^{x}	501.3^{x}	571.6^{x}
	7	219.5^{y}	208.0^{yz}	203.2^{y}
	14	188.2^{y}	171.7^{z}	175.9^{y}
	21	173.9^{y}	136.9^{z}	156.4^{y}
	42	207.1^{y}	340.6^{xy}	231.5^{y}
Fibrinogen, mg/dL[c]	0	156.3	201.3	147.9
	7	185.4	193.8	275.0
	14	170.8	257.5	193.8
	21	143.3	161.3	191.7
	42	146.9	103.8	166.7

[a]SE=0.77

[b]SE=64.6

[c]SE=32.9

[d,e]Means within a row with different superscripts are different ($P < 0.05$)

[x,y,z]Means within column with different superscripts are different ($P < 0.05$)

had lower ($P < 0.05$) BUN concentrations than the heifers that were daily supplemented while heifers that received no supplementation were intermediate. The BUN levels fluctuated ($P < 0.05$) by sampling day in the heifers supplemented at every 48 h intervals, while the heifers that were not or were daily supplemented had relatively steady BUN levels.

Serum CK concentrations are presented in Table 7. No treatment differences were observed at any sampling date. There was a linear and quadratic ($P < 0.001$ and $P < 0.0001$ respectively) decline in CK concentration during the 42 d study.

Plasma Fb concentrations are also presented in Table 7. There were no treatment differences of Fb concentrations at any sampling date. Fibrinogen decreased linearly ($P < 0.05$) and quadratically ($P < 0.0001$) during the 42 d of this study.

Experiment 2: Nutrient Management Location (SVAREC, Steeles Tavern, VA)

Forage Mass and Nutritive Value. Forage mass and nutritive value data are presented in Table 8. There were no differences in forage mass, availability, or nutritive value between treatments at any sampling date. However, forage nutritive value and mass decreased over the 42 d of the study. Forage mass and availability declined linearly ($P < 0.0001$). Neutral detergent fiber and ADF increased linearly ($P < 0.001$ and $P < 0.01$, respectively) throughout the study. Crude protein declined linearly ($P < 0.05$) with an average loss of two percentage units CP over the 42d. The ash content of the forage did not change over the experimental period.

Forage mineral composition data is presented in Table 9. There were no differences in forage mineral composition between treatments at any sampling date. A linear decrease was observed for Mg, K, and P ($P < 0.001$) while an increase was observed for Cu and Se ($P < 0.0001$ and $P < 0.01$, respectively). Forage Ca and Zn concentrations did not change during the 42 d study.

Table 8. Forage mass and chemical composition[a] in Exp. 2 (n=2)

		Low stress			High stress		
		Supplementation frequency			Supplementation frequency		
Day	Item	None	Every 48h	Daily	None	Every 48h	Daily
0	Forage Mass, kg/ha[b]	4902^{x}	4753^{x}	5362^{x}	5942^{x}	6075^{x}	5793^{x}
	Forage Availability, kg DM/kg BW[c]	2.35^{jlx}	2.30^{jx}	2.70^{jkx}	2.91^{klx}	3.04^{kx}	2.76^{jkx}
	Ash, %[d]	6.89	6.75	6.91	7.96	8.05	7.63
	NDF, %[e]	60.67^{x}	60.53^{x}	60.52^{x}	59.64^{x}	59.11^{x}	57.07^{x}
	ADF, %[f]	30.24^{x}	30.42^{x}	30.52^{x}	30.03^{x}	29.05^{x}	28.47^{x}
	CP, %[g]	16.17	15.44	15.60	15.65	15.85	16.63
21	Forage Mass, kg/ha[b]	4605^{x}	3877^{xy}	4263^{x}	4040^{x}	3757^{y}	3491^{y}
	Forage Availability, kg DM/kg BW[c]	2.16^{jx}	1.77^{jkx}	2.10^{jkx}	1.94^{jky}	1.84^{jky}	1.56^{ky}
	Ash, %[d]	6.86	6.86	7.44	7.31	7.78	7.72
	NDF, %[e]	62.31^{xy}	62.88^{xy}	60.75^{x}	61.84^{x}	61.98^{xy}	60.55^{x}
	ADF, %[f]	31.72^{xy}	32.69^{xy}	31.19^{x}	31.77^{x}	36.21^{xy}	31.01^{x}
	CP, %[g]	14.37	13.75	15.29	15.07	15.43	14.55
42	Forage Mass, kg/ha[b]	1723^{y}	1827^{y}	1411^{y}	1604^{y}	1664^{y}	1381^{z}
	Forage Availability, kg DM/kg BW[c]	0.80^{y}	0.84^{y}	0.67^{y}	0.76^{y}	0.79^{z}	0.60^{z}
	Ash, %[d]	6.24	6.73	6.53	6.27	7.38	7.14
	NDF, %[e]	67.19^{y}	67.71^{y}	68.02^{y}	68.19^{y}	67.38^{y}	67.11^{y}
	ADF, %[f]	35.85^{y}	34.07^{y}	37.31^{y}	36.39^{y}	36.79^{y}	36.00^{y}
	CP, %[g]	14.47	14.46	13.24	14.36	14.39	14.12

[a]DM basis

[b]SE=379

[c]SE=0.17

[d]SE=0.64

[e]SE=1.03

[f]SE=3.81

[g]SE=1.13

[j,k,l]Means within row with different superscripts are different ($P < 0.05$)

[x,y,z]Means within column with different superscripts are different ($P < 0.05$)

Table 9. Forage mineral composition[a] in Exp. 2 (n=2)

		Low stress			High stress		
		Supplementation frequency			Supplementation frequency		
Day	Mineral	None	Every 48h	Daily	None	Every 48h	Daily
0	Ca, %[b]	0.52	0.48	0.50	0.43	0.42	0.45
	Mg, %[c]	0.36	0.35	0.33	0.33	0.31	0.34
	K, %[d]	1.62	1.72	1.59	2.05	2.26	2.02
	P, %[e]	0.38	0.33	0.30	0.41	0.35	0.40
	Cu, ppm[f]	1.9[x]	1.5[x]	2.9	2.8[x]	3.0	1.9[x]
	Zn, ppm[g]	22.6	22.7	19.5	21.4	23.9	23.4
	Se, ppb[h]	122.4	118.4	115.5	104.2	177.7	102.4
21	Ca, %[b]	0.45	0.45	0.47	0.48	0.42	0.41
	Mg, %[c]	0.35	0.34	0.33	0.33	0.32	0.37
	K, %[d]	1.45	1.34	1.78	1.63	1.90	1.89
	P, %[e]	0.37	0.29	0.33	0.38	0.39	0.34
	Cu, ppm[f]	4.4[xy]	5.0[xy]	4.6	4.4[xy]	3.8	5.2[xy]
	Zn, ppm[g]	19.1	21.1	19.9	19.9	21.6	21.9
	Se, ppb[h]	135.9	171.9	136.4	188.7	112.1	133.1
42	Ca, %[b]	0.46	0.49	0.48	0.46	0.43	0.46
	Mg, %[c]	0.33	0.31	0.29	0.29	0.29	0.30
	K, %[d]	1.04	1.20	0.86	1.03	1.28	1.30
	P, %[e]	0.28	0.29	0.24	0.32	0.30	0.33
	Cu, ppm[f]	5.8[y]	5.2[y]	6.2	5.9[y]	5.8	6.6[y]
	Zn, ppm[g]	19.7	23.7	23.9	22.2	21.7	22.7
	Se, ppb[h]	148.9	163.8	139.8	139.8	129.8	163.7

[a]DM basis

[b]SE=0.02

[c]SE=0.02

[d]SE=0.32

eSE=0.03

[f]SE=0.6

[g]SE=2.3

[h]SE=14.8

[x,y,z]Means within column with different superscripts are different ($P < 0.05$)

Animal Performance. Performance data for steers are presented in Table 10. No supplement frequency by stress level interactions were observed, therefore data are presented as main effects. No differences were observed between supplementation or stress treatments at any sampling date. There were no significant differences in cumulative ADG due to supplementation treatments. Weights increased linearly ($P < 0.0001$) and responded quadratically ($P < 0.0005$) due to an initial weight loss from d 0 to 4. There was a stress level by sampling time interaction ($P < 0.05$) with the high stress calves having lower BW at the late sampling on d 0 (240.5 and 246.1 kg , respectively). There was a supplementation frequency by sampling day interaction ($P < 0.05$) with daily supplemented calves having the highest weights and calves supplemented every 48 h being intermediate on d 42. Average daily gains responded quadratically ($P < 0.0001$) as steers lost weight during the first 2 wk and then gained weight the last 4 wk. Supplemented calves tended ($P < 0.10$) to have higher ADG as compared to the calves that were not supplemented. Gain per hectare appeared lowest for steers that were not supplemented (58 kg/ha) and appeared highest for daily supplemented steers (169 kg/ha), while steers supplemented every 48 h had intermediate gains (127 kg/ha). The gain per hectare was also numerically higher for the high stress steers (124 kg/ha) as compared to the low stress steers (112 kg/ha). Supplement efficiency was numerically greater for daily supplemented steers at 4.74 kg supplement/kg of additional gain than steers supplemented every 48 h at 6.92 kg supplement/kg of additional gain.

Animal Health. Steer health parameters are presented in Table 11. No supplement frequency by stress level interactions were observed, therefore data are presented as main effects. No differences were observed between supplementation or stress treatments at any sampling date

Table 10. Steer performance record in Exp. 2

		Stress level (n=6)		Supplementation frequency (n=4)		
Measurement	Day	Low	High	None	Every 48h	Daily
Weight, kg[a]	0, 1200 h	256.0^{xy}	257.4^{x}	259.6^{yz}	254.8^{xy}	255.8^{x}
	*0, 1600 h	--	245.4	--	--	--
	0, 2100 h	246.1^{x}	240.5^{w}	245.5^{x}	240.9^{wx}	243.4^{w}
	1	241.4^{x}	240.6^{w}	243.1^{x}	239.9^{x}	240.1^{w}
	4	252.0^{xy}	251.6^{wx}	249.0^{xy}	252.2^{x}	254.2^{x}
	7	257.5^{y}	256.0^{x}	255.2^{y}	258.2^{xy}	258.0^{x}
	14	256.4^{xy}	256.0^{x}	255.1^{y}	254.2^{x}	259.3^{xy}
	21	264.0^{y}	265.4^{y}	263.4^{z}	263.6^{y}	267.1^{y}
	42	270.0^{z}	272.9^{z}	266.9^{z}	270.6^{z}	276.9^{z}
ADG, kg/d[b]	0-7	0.22^{x}	-0.10^{y}	-0.62^{y}	0.48^{xy}	0.31
	7-14	-0.17^{y}	-0.10^{y}	-0.02^{xy}	-0.56^{y}	0.19
	14-21	1.08^{x}	1.34^{x}	1.19^{x}	1.33^{x}	1.11
	21-42	0.29^{x}	0.36^{x}	0.17^{x}	0.33^{xy}	0.47
	0-42	0.33	0.37	0.17	0.38	0.50

*Means not included in repeated measures

[a]Stress SE=5.47, Supplement SE=6.70

[b]Stress SE=0.35, Supplement SE=0.43

[w,x,y,z]Means within column with different superscripts are different ($P < 0.05$)

Table 11. Steer health record in Exp. 2

		Stress level (n=6)		Supplementation frequency (n=4)		
Analysis	Day	Low	High	None	Every 48h	Daily
N:L ratio[ac]	0	0.41^{yz}	0.40^{yz}	0.34^{z}	0.37^{yz}	0.51^{y}
	1	0.52^{hy}	0.87^{gx}	0.22^{hy}	1.10^{gx}	0.26^{hx}
	4	0.71^{x}	0.60^{y}	0.77^{x}	0.59^{y}	0.60^{y}
	7	0.20^{z}	0.28^{z}	0.24^{yz}	0.16^{z}	0.32^{y}
Temperature, °C[d]	0, 1200 h	40.2	40.1	40.1	40.1	40.3
	*0, 1600 h	--	40.4	--	--	--
	0, 2100 h	39.6	40.0	39.6	39.9	39.8
	1	39.7	39.2	39.5	39.4	39.5
	4	39.3	39.3	39.6	39.2	39.1
	7	39.6	39.3	39.5	39.3	39.6
	14	39.5	39.5	39.3	39.5	39.6
	21	39.8	39.6	39.5	39.7	39.9
	42	39.1	39.0	39.9	39.0	39.3
Morbidity score[be]	0-7	1.00^{t}	1.00	1.00	1.00	1.00^{t}
	7-14	1.00^{t}	1.00	1.00	1.00	1.00^{t}
	14-21	1.02^{ju}	1.00^{i}	1.00^{i}	1.00^{i}	1.03^{ju}
	21-42	1.00^{t}	1.00	1.00	1.00	1.00^{t}
Number treated	0-7	--	--	--	--	--
	7-14	1	1	1	--	1
	14-21	2	1	2	--	1
	21-42	--	--	--	--	--

*Means not included in repeated measures

[a]Neutrophil:Lymphocyte ratio

[b]Based on a subjective scoring system (1=healthy, 5=moribund)

[c]Stress SE=0.07, Supplement SE=0.09

[d]Stress SE=0.10, Supplement SE=0.12

[e]Stress SE=0.00, Supplement SE=0.00

[g,h]Means within a row with different superscripts are different ($P < 0.0005$)

[i,j]Means within a row with different superscripts are different ($P < 0.05$)

[t,u]Means within column with different superscripts are different ($P < 0.0005$)

[x,y,z]Means within column with different superscripts are different ($P < 0.05$)

for morbidity, rectal temperature, or leukocyte ratios. The N:L ratio responded linearly and quadratically ($P < 0.0001$) with the highest ratios occurring on d 1 and 4. The average N:L ratio was higher ($P < 0.05$) for the high stress calves as compared to the low stress calves. On d 1, high stress calves had higher ($P < 0.05$) N:L ratio than the low stress calves. Rectal temperatures showed a linear ($P < 0.0001$) decline over sampling dates. Most steers received a morbidity score of 1 throughout the study. Morbidity scores increased during wk 3 then returned to normal. Only one steer was given a morbidity score of 2 for not coming to the bunk and showing signs of laminitis and was treated accordingly. Additional steers were treated on sampling dates due to elevated rectal temperatures. For all steers, the highest number of treatments occurred between d 14 and 21.

Tympanic temperature data was not analyzed statistically due to unequal replications within treatments due to the fact that some loggers failed to record accurately. Hourly tympanc temperature averages are presented in Figure 1. Visual appraisal of data shows the HS treatment cattle had elevated temperatures at approximately 1900. Lowest temperatures (less than 38.6º C) were observed around 0800. Day 0, hourly tympanic temperatures are presented in Figure 2. High stress calves appear to have higher temperatures than LS calves from (1300 to 1600), which corresponds to the transport period. Daily tympanic temperature averages are presented in Figure 3. High stress treatment calves appear to have elevated temperatures as compared to low stress treatment calves on d 0 and 1. In the remaining 5 d, both treatments appear to have similar temperatures.

Figure 1. Hourly tympanic temperature logger averages (d 0 to 7) in Exp. 2

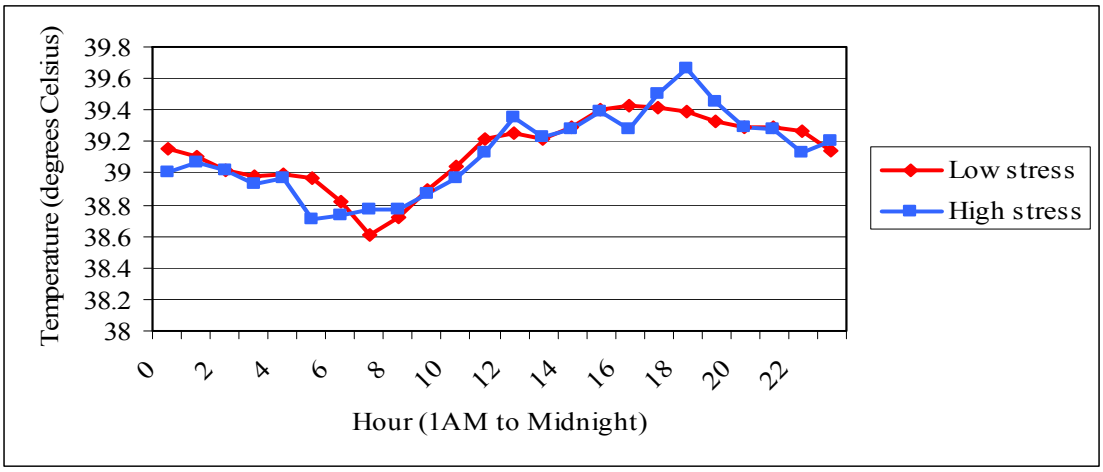


Figure 2. Day 0 hourly tympanic temperature logger averages in Exp. 2

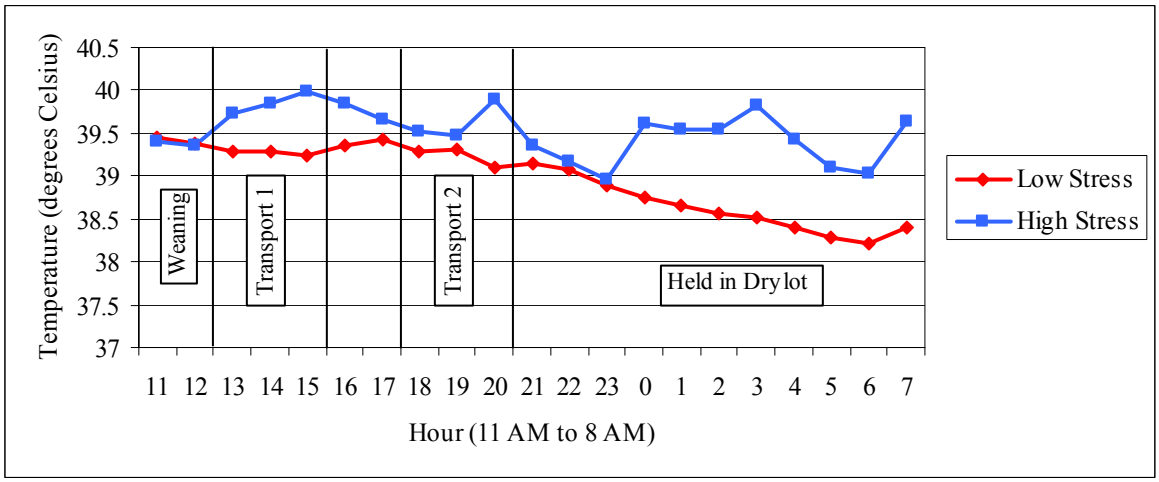
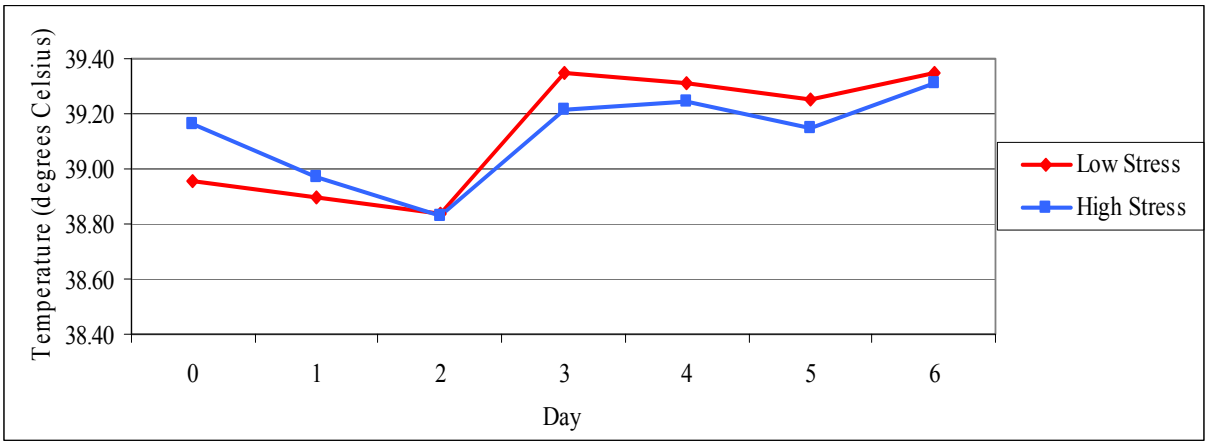


Figure 3. Daily tympanic temperature logger averages in Exp. 2



Serum and Plasma Analyses. Serum concentrations of BUN are presented in Table 12. No supplement frequency by stress level interaction was observed therefore data is presented as main effects. No treatment differences were detected at any sampling date. Blood urea nitrogen concentration had a linear decrease ($P < 0.05$). There was a sampling day by supplement interaction ($P < 0.0001$) for BUN concentration. The BUN levels fluctuated ($P < 0.05$) by sampling day in the steers supplemented at 48 h intervals, while the steers that were not or that were daily supplemented had relatively steady BUN levels.

Serum CK concentrations are presented in Table 12. No supplement frequency by stress level interaction was observed therefore data is presented as main effects. No stress or supplementation treatment differences were observed on any day. There was a linear and quadratic ($P < 0.0001$) decline in CK concentration during the 42 d study.

Plasma Fb concentrations are also presented in Table 12. No supplement frequency by stress level interaction was observed therefore data is presented as main effects. There were no stress or supplementation treatment differences on Fb concentrations on any day. Fibrinogen decreased linearly ($P < 0.01$) and quadratically ($P < 0.0001$) during the 42 d of this study.

Experiment 3: Kentland Fescue Study (Kentland Farm, Blacksburg, VA)

Forage Mass and Nutritive Value. Forage mass and chemical composition data are presented in Table 13. There were no differences in nutritive value between fescue types at any sampling date. On average, forage mass and availability were lower ($P < 0.005$) for endophyte-free paddocks as compared to novel endophyte and endophyte-infected paddocks. On d 14, forage mass and availability were lower ($P < 0.05$) for endophyte-free paddocks than endophyte-infected while novel endophyte paddocks were intermediate. Neutral detergent fiber

Table 12. Steer serum and plasma analyses in Exp. 2

		Stress level (n=6)		Supplementation frequency (n=4)		
Analysis	Day	Low	High	None	Every 48h	Daily
Blood urea nitrogen, mg/dL[a]	0, 1200 h	16.54^{x}	17.54^{x}	17.45^{x}	16.38^{xz}	17.28^{x}
	*0, 1600 h	--	16.65	--	--	--
	0, 2100 h	16.08^{x}	16.34^{xz}	16.28^{xz}	16.07^{xz}	16.27^{xz}
	1	13.74^{yx}	15.07^{yz}	14.49^{yz}	14.06^{yz}	14.67^{xz}
	4	15.33^{xz}	14.84^{yz}	14.88^{yz}	15.43^{yz}	14.94^{xz}
	7	14.39^{xz}	15.03^{yz}	15.21^{yz}	14.94^{yz}	13.98^{yz}
	14	15.15^{xz}	15.63^{xz}	16.58^{xz}	13.51^{yz}	16.08^{xz}
	21	17.05^{x}	17.52^{x}	17.50^{x}	18.12^{x}	16.25^{xz}
	42	15.68^{xz}	16.95^{xz}	18.77^{x}	14.08^{yz}	16.08^{xz}
Creatine kinase, IU/L[b]	0, 1200 h	714.1^{x}	912.4^{x}	779.9^{x}	751.2^{x}	908.7^{x}
	*0, 1600 h	--	2079.5	--	--	--
	0, 2100 h	1784.4^{y}	2021.8^{y}	2488.3^{dy}	1465.5^{ey}	1755.5^{dey}
	1	1633.4^{y}	1619.5^{y}	1715.2^{y}	1388.6^{y}	1739.4^{y}
	4	301.2^{x}	293.1^{x}	278.0^{x}	324.3^{x}	289.1^{x}
	7	206.7^{x}	236.2^{x}	238.1^{x}	229.3^{x}	196.9^{x}
	14	162.1^{x}	226.8^{x}	201.6^{x}	217.9^{x}	163.8^{x}
	21	201.8^{x}	187.3^{x}	189.0^{x}	230.0^{x}	164.6^{x}
	42	246.7^{x}	264.1^{x}	282.1^{x}	226.0^{x}	258.2^{x}
Fibrinogen, mg/dL[c]	0, 1200 h	91.0	75.7	80.7^{t}	82.8	86.46^{t}
	*0, 1600 h	--	162.1	--	--	--
	0, 2100 h	188.9	156.6	152.6^{tu}	163.0	202.6^{tu}
	1	151.0	213.5	195.31^{u}	168.8	182.8^{tu}
	4	214.6	219.8	262.5^{u}	198.4	190.6^{tu}
	7	213.5	224.0	246.9^{u}	196.9	212.5^{tu}
	14	219.4	206.3	191.7^{tu}	218.8	228.1^{u}
	21	158.7	162.5	150.0^{tu}	199.0	132.8^{tu}
	42	103.8	113.2	126.6^{tu}	103.1	95.8^{tu}

*Means not included in repeated measures

[a]Stress SE=0.83, Supplement SE=1.03

[b]Stress SE=129.4, Supplement SE=158.4

[c]Stress SE=22.2, Supplement SE=27.2

[d,e]Means within a row with different superscripts are different ($P < 0.05$)

[t,u]Means within column with different superscripts are different ($P < 0.10$)

[x,y,z]Means within column with different superscripts are different ($P < 0.05$)

Table 13. Forage mass and chemical composition[a] in Exp. 3 (n=3)

		Fescue type		
Day	Item	Endophyte-free	Novel	Endophyte-infected
0	Forage Mass, kg/ha[b]	1528	2049	2058
	Forage Availability, kg DM/kg BW[c]	0.77	1.02	1.03
	Ash, %[d]	8.94	8.69	9.07
	NDF, %[e]	55.62	55.83	53.75
	ADF, %[f]	28.21	28.50	27.81
	CP, %[g]	18.08	17.26	18.42
14	Forage Mass, kg/ha[b]	1636[j]	1915[jk]	2325[k]
	Forage Availability, kg DM/kg BW[c]	0.75[j]	0.86[jk]	1.11[k]
	Ash, %[d]	8.88	8.50	9.15
	NDF, %[e]	54.33	56.95	54.24
	ADF, %[f]	28.23	30.28	28.18
	CP, %[g]	18.01	17.12	15.93
28	Forage Mass, kg/ha[b]	1697	2113	2073
	Forage Availability, kg DM/kg BW[c]	0.72	0.88	0.92
	Ash, %[d]	8.92	8.87	9.20
	NDF, %[e]	57.15	57.20	59.06
	ADF, %[f]	25.38	26.45	26.27
	CP, %[g]	16.58	15.21	16.07

[a]DM basis

[b]SE=140

[c]SE=0.18

[d]SE=0.43

[e]SE=1.47

[f]SE=0.88

[g]SE=0.76

[j,k]Means within a row with different superscripts are different ($P < 0.05$)

increased linearly ($P < 0.05$) while ADF increased linearly and quadratically ($P < 0.01$ and $P < 0.01$, respectively) with time. Crude protein declined linearly ($P < 0.01$) with time for an average of two percentage units. Forage mass, availability, and % ash did not change significantly between samplings as calves were rotated to a new sub-paddock.

Forage mineral composition data is presented in Table 14. There were no differences in forage K, P, and Se composition between fescue types at any sampling date. The average forage Cu content was higher ($P < 0.01$) for the novel endophyte (4.69 ppm) than for endophyte-infected fescue (4.30 ppm) while endophyte-free fescue was intermediate (4.66 ppm). The average forage Mg concentration was lower ($P < 0.05$) for novel endophyte (0.34 %) than endophyte-free (0.36 %) or endophyte-infected fescue (0.36 %). The average Zn concentration was lower ($P < 0.005$) for endophyte-infected (18.03 ppm) than novel (19.98 ppm) or endophyte-free fescue (19.94 ppm). Forage Ca concentrations declined linearly and quadratically ($P < 0.0001$ and $P < 0.01$ respectively). The Mg content of the forage declined linearly and quadratically ($P < 0.01$ and $P < 0.001$ respectively). Forage Cu declined in concentration linearly ($P < 0.001$) and quadratically ($P < 0.01$). Additionally, forage Zn concentration declined linearly ($P < 0.005$).

Forage alkaloid concentration data is presented in Table 15. Alkaloid concentrations were not analyzed statistically. Alkaloid concentration in endophyte-free paddocks was higher than expected due possible contamination with endophyte-infected fescue. There are multiple alkaloids found in tall fescue. The exact alkaloid that causes fescue toxicosis is not known.

Animal Performance. Performance data for steers is presented in Table 16. Weights increased linearly ($P < 0.0001$) and quadratically ($P < 0.0005$) during the 42 d of this study. Calves grazing novel endophyte fescue on average had higher ($P < 0.05$) weights than those grazing endophyte-infected fescue. Average daily gains responded quadratically ($P < 0.05$), with

Table 14. Forage mineral composition[a] in Exp. 3 (n=3)

		Fescue type		
Day	Mineral	Endophyte-free	Novel	Endophyte-infected
0	Ca, %[b]	0.33[xy]	0.35[x]	0.36[x]
	Mg, %[c]	0.36[xy]	0.34	0.37
	K, %[d]	2.87	2.93	2.92
	P, %[e]	0.35	0.37	0.35
	Cu, ppm[f]	5.6	5.8	5.5
	Zn, ppm[g]	20.4	21.1	19.3
	Se, ppb[h]	137.1	140.7	154.3
14	Ca, %[b]	0.35[x]	0.31[xy]	0.36[x]
	Mg, %[c]	0.40[x]	0.36	0.39
	K, %[d]	2.81	2.89	2.94
	P, %[e]	0.38	0.37	0.35
	Cu, ppm[f]	4.0	3.9	3.8
	Zn, ppm[g]	20.2	19.5	18.0
	Se, ppb[h]	152.7	165.8	150.8
28	Ca, %[b]	0.29[y]	0.27[y]	0.29[y]
	Mg, %[c]	0.33[y]	0.32	0.33
	K, %[d]	3.07	3.03	3.09
	P, %[e]	0.36	0.32	0.33
	Cu, ppm[f]	4.4	4.4	3.6
	Zn, ppm[g]	19.2	19.3	16.8
	Se, ppb[h]	173.8	180.8	168.1

[a]DM basis

[b]SE=0.01

[c]SE=0.01

[d]SE=0.20

eSE=0.01

[f]SE=0.5

[g]SE=0.7

[h]SE=18.8

[x,y]Means within column with different superscripts are different ($P < 0.05$)

Table 15. Alkaloid concentrations for fescues in Exp. 3

Fescue type	Alkaloid (ng/g)
Endophyte-free	1261
Novel	1476
Endophyte-infected	2726

Table 16. Steer performance record in Exp. 3 (n=3)

		Fescue type		
Measurement	Day	Endophyte-free	Novel	Endophyte-infected
Weight, kg[a]	0	197.7^{x}	200.7^{x}	199.1^{x}
	7	204.4^{xy}	206.4^{xy}	202.3^{x}
	14	219.9^{y}	222.2^{y}	210.6^{xy}
	21	229.8^{yz}	234.1^{yz}	217.8^{yz}
	42	241.3^{z}	246.4^{z}	233.1^{z}
ADG, kg/d[b]	0-7	0.95	0.80	0.46
	7-14	2.21	2.27	1.19
	14-21	1.41	1.70	1.03
	21-42	0.55	0.58	0.73
	0-42	1.04	1.09	0.81

[a]SE=3.6

[b]SE=0.58

[x,y,z]Means within column with different superscripts are different ($P < 0.05$)

lower ADG during wk 1 as compared to the remaining 5 wk. On average, calves grazing novel endophyte fescue tended to have higher (P = 0.07) ADG than those grazing endophyte-infected fescue. Gain per hectare was numerically greatest for novel endophyte fescue (150 kg/ha) and lowest for endophyte-infected fescue (114 kg/ha). Endophyte-free fescue appeared to be intermediate (143 kg/ha).

Animal Health. Steer health parameters are presented in Table 17. No differences were observed between supplementation treatments at any sampling date for morbidity, rectal temperature, or leukocyte ratios. The N:L ratio was higher (P < 0.01) on d 0 than d 7 for all treatments. On average, N:L ratio was higher in calves grazing endophyte-free fescue as compared to the calves grazing novel or endophyte-infected fescues. Rectal temperatures had a quadratic (P < 0.01) response with time for all fescue types. Morbidity scores were not different between forage types or periods, with most calves receiving a score of 1. During wk 3 one calf on novel endophyte fescue was giving a morbidity score of 2 then died later that day due to pneumonia. Administration of medications occurred on sampling days due to elevated rectal temperatures. The highest frequency of medications occurred between d 0 and 7 (5 administrations). There were also five calves that were treated for laminitis for a total of nine treatments (several calves required multiple treatments for multiple feet). The incidences of laminitis occurred during from d 7 to 21.

Tympanic temperature data was not analyzed statistically due to unequal replications within treatments due to the fact that some loggers failed to record accurately. Hourly tympanic temperature averages are presented in Figure 4. Lowest temperatures were observed on average around 0900 and highest temperatures were observed around 1700. Daily tympanic temperature averages are presented in Figure 5.

Table 17. Steer health record in Exp. 3 (n=3)

		Fescue type		
Analysis	Day	Endophyte-free	Novel	Endophyte-infected
N:L ratio[ac]	0	0.72[gx]	0.57[hx]	0.61[ghx]
	7	0.29[y]	0.26[y]	0.23[y]
Temperature, °C[d]	0	39.8	39.8	40.0
	7	40.3	40.2	40.5
	14	40.2	40.1	40.4
	21	40.1	40.1	40.0
	42	39.8	40.1	39.8
Morbidity score[be]	0-7	1.00	1.00	1.02
	7-14	1.00	1.00	1.00
	14-21	1.00	1.02	1.02
	21-42	1.00	1.00	1.00
Number treated	0-7	2	1	2
	7-14	0	0	0
	14-21	0	0	3
	21-42	0	0	0
Laminitis Cases	0-7	0	0	0
	7-14	0	1	2
	14-21	1	2	3
	21-42	0	0	0

[a]Neutrophil:Lymphocyte ratio

[b]Based on a subjective scoring system (1=healthy, 5=moribund)

[c]SE=0.02

[d]SE=0.2

[e]SE=0.01

[g,h]Means within a row with different superscripts are different ($P < 0.05$)

[x,y]Means within column with different superscripts are different ($P < 0.05$)

Figure 4. Hourly tympanic temperature logger averages (d 0 to 7) in Exp. 3

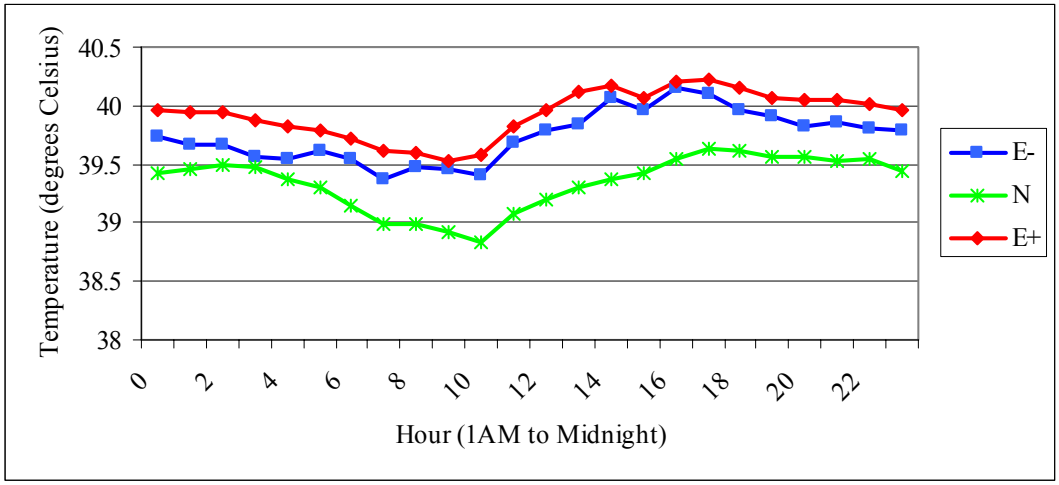
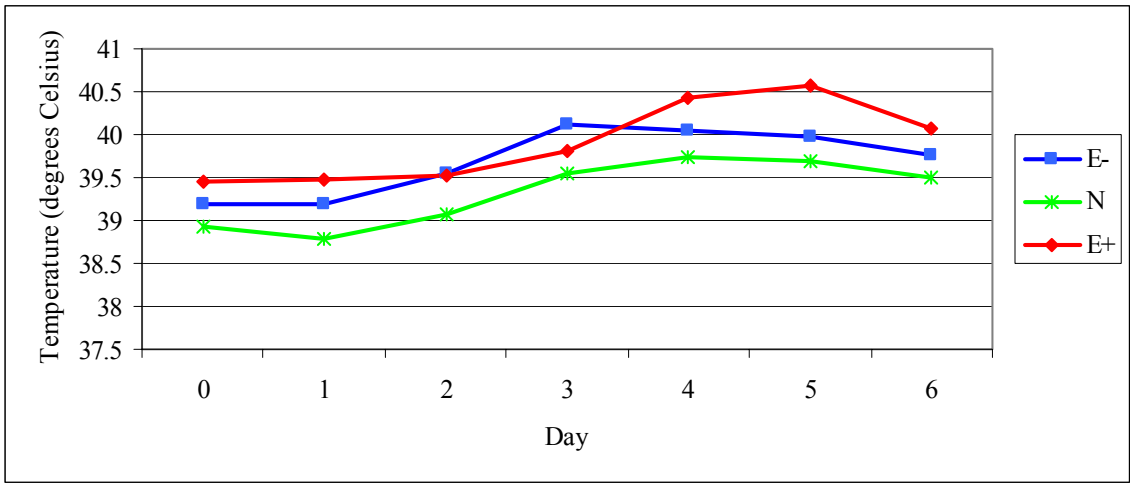


Figure 5. Daily tympanic temperature logger averages in Exp. 3



Temperature patterns are similar for all fescue types, however calves grazing endophyte-infected fescue appear to have higher temperatures than calves grazing endophyte-free or novel endophyte fescue by d 4.

Serum and Whole Blood Mineral Analyses. Serum Cu, Zn, and Se concentrations in steers on d 0 and 42 are reported in Table 18. Serum Cu concentrations were greater ($P < 0.0001$) on d 42 than on d 0 for all calves. No differences were observed in serum Zn concentrations between treatments or sampling days. Whole blood Se concentrations were lower ($P < 0.05$) on d 42 than on d 0 for all treatments. On average calves grazing endophyte-free had higher ($P < 0.05$) Se concentrations (60.97 ppb) than those on novel endophyte fescue (31.58 ppb), while calves on endophyte-infected were intermediate (52.77 ppb).

Serum and Plasma Analyses. Serum concentrations of BUN are presented in Table 19. No treatment differences were detected at any sampling date. Serum BUN concentrations fluctuated for steers on all fescue types with the highest levels occurring on d 7.

Serum CK concentrations are presented in Table 19. No treatment differences were observed at any sampling date. There was a linear and quadratic ($P < 0.0001$) decline in CK concentration during the 42 d study for all fescue types.

Plasma Fb concentrations are also presented in Table 19. There were no treatment differences of Fb concentrations at any sampling date. Fibrinogen decreased linearly ($P < 0.05$) during the 42 d of this study.

Table 18. Steer serum and whole blood mineral analyses in Exp. 3 (n=3)

		Fescue type		
Analysis	Day	Endophyte-free	Novel	Endophyte-infected
Serum Cu, ppm[a]	0	0.42[x]	0.41[x]	0.39[x]
	42	0.76[y]	0.75[y]	0.77[y]
Serum Zn, ppm[b]	0	0.80	0.67	0.82
	42	0.88	0.82	0.77
Blood Se, ppb[c]	0	69.3	33.6	54.8
	42	52.7	29.5	50.7

[a]SE=0.04

[b]SE=0.07

[c]SE=7.4

[x,y]Means within column with different superscripts are different ($P < 0.05$)

Table 19. Serum and plasma analyses in Exp. 3 (n=3)

Analysis	Day	Fescue type		
		Endophyte-free	Novel	Endophyte-infected
Blood urea nitrogen, mg/dL[a]	0	13.20^{y}	13.30^{xy}	13.53^{y}
	7	17.11^{x}	18.37^{x}	19.58^{x}
	14	14.76^{xy}	13.01^{xy}	15.13^{xy}
	21	13.57^{xy}	11.11^{y}	13.83^{y}
	42	14.30^{xy}	14.11^{xy}	14.75^{xy}
Creatine kinase, IU/L[b]	0	696.4^{x}	1178.3^{x}	962.8^{x}
	7	197.3^{y}	273.2^{y}	276.5^{y}
	14	370.4^{xy}	196.7^{y}	154.9^{y}
	21	252.7^{xy}	221.6^{y}	163.0^{y}
	42	215.0^{xy}	251.7^{y}	230.6^{y}
Fibrinogen, mg/dL[c]	0	195.1	221.5	297.0
	7	204.2	150.0	245.8
	14	166.7	219.4	235.3
	21	250.0	231.3	230.1
	42	118.8	150.7	178.6

[a]SE=1.34

[b]SE=143.8

[c]SE=45.4

[x,y]Means within column with different superscripts are different ($P < 0.05$)

Experiment 4: Rumen Environment Study (Kentland Farm, Blacksburg, VA)

In Situ Analysis. Residual supplement and forage DM after in situ analysis are presented in Table 20. No significant differences in supplement and forage DM disappearance were observed at any sampling time due to supplementation treatment. Forage and supplement DM disappeared quadratically ($P < 0.0001$) over the 48 h. Dry matter kinetics of forage and supplement are presented in Table 21. Readily and slowly digested fractions, rate of slowly digested fraction, and effective degradability of supplement were not affected by supplementation frequency. No differences in readily digested fraction or rate of slowly digested fractions of the forage were observed. The slowly digested fraction of the forage was lower ($P < 0.05$) for daily supplementation than every 48 h supplementation while no supplementation was intermediate. The effective degradability of the forage was higher for no supplementation than with either daily or every 48 h supplementation.

In situ CP content of forage and supplement are presented in Table 22. Crude protein content was not different between supplementation treatments at any sampling time. Forage and supplement CP decreased linearly and quadratically ($P < 0.0001$) over the 48 h period.

Ruminal pH, Ammonia, and Volatile Fatty Acids. Ruminal fluid pH levels are presented in Table 23. No differences in pH level were observed between supplementation treatments at any sampling time. The ruminal fluid pH levels did not change significantly between sampling times.

Ruminal ammonia concentrations are presented in Table 23. No differences in ruminal ammonia concentration were observed at any sampling time due to supplementation treatment.

Table 20. In situ forage and supplement disappearance in Exp. 4 (n=3)

		Supplementation frequency		
In situ	Hour	None	Every 48h	Daily
Supplement DM	0	35.1	42.1	36.2
disappearance, %[a]	2	42.0	42.3	45.7
	4	45.7	51.3	48.2
	6	49.8	50.1	56.4
	8	54.5	60.2	57.5
	12	61.3	59.9	63.9
	24	76.2	72.2	73.6
	36	84.3	78.6	83.1
	48	91.7	87.1	88.4
Forage DM	0	32.6	36.5	34.3
disappearance, %[b]	2	35.6	37.3	37.7
	4	41.7	40.5	38.0
	6	41.9	40.6	42.1
	8	49.1	53.4	47.8
	12	60.1	57.0	59.1
	24	73.1	70.5	71.1
	36	79.4	75.7	78.5
	48	84.1	81.3	81.9

[a]SE=1.2

[b]SE=1.0

Table 21. Dry matter disappearance kinetics of forage and supplement in Exp. 4 (n=3)

	Supplementation frequency			
Fraction	None	Every 48h	Daily	SE
Forage				
Readily degradable, %	32.13	29.47	33.36	1.16
Slowly degradable, %	59.37[ab]	60.38[a]	57.07[b]	0.45
Rate of slowly degradable digestion, %/h	4.47	4.85	4.17	1.13
Effective degradability,%	67.72[a]	66.18[b]	65.82[b]	0.17
Supplement				
Readily degradable, %	38.63	38.63	38.37	2.92
Slowly degradable, %	52.97	52.67	50.56	0.48
Rate of slowly degradable digestion, %/h	3.26	5.01	5.87	1.13
Effective degradability,%	72.08	71.93	71.67	0.69

[a,b]Means within a row with different superscripts are different ($P < 0.05$)

Table 22. In situ supplement and forage crude protein in Exp. 4 (n=3)

		Supplementation frequency		
In situ residual	Hour	None	Every 48h	Daily
Supplement CP, %[a]	0	12.7	12.8	12.5
	2	11.5	11.8	11.6
	4	11.1	11.1	10.8
	6	10.9	11.2	10.7
	8	10.3	10.4	10.6
	12	9.3	9.8	9.2
	24	8.9	8.8	8.1
	36	9.9	9.0	8.5
	48	10.6	10.1	9.2
Forage CP, %[b]	0	18.3	18.3	18.4
	2	17.9	18.0	17.6
	4	17.6	17.9	17.1
	6	16.7	17.1	16.4
	8	15.6	15.8	15.2
	12	11.3	13.5	12.7
	24	11.3	11.7	11.0
	36	12.3	12.2	12.3
	48	13.3	12.8	12.6

[a]SE=0.38

[b]SE=0.48

Table 23. Ammonia concentration and pH levels in ruminal fluid of steers in Exp. 4 (n=3)

		Supplementation frequency		
Analysis	Hour	None	Every 48h	Daily
pH[a]	0	6.6	6.5	6.6
	2	6.5	6.2	6.1
	4	6.5	6.0	6.0
	6	6.7	6.2	6.3
	8	6.8	6.4	6.3
	12	6.4	6.2	6.0
	24	6.5	6.2	6.4
	36	6.3	6.3	6.2
	48	6.7	6.6	6.7
Ammonia, mg/dL[b]	0	7.2^{x}	8.2^{xz}	10.5^{x}
	2	13.6^{xyz}	17.3^{y}	18.0^{y}
	4	18.0^{z}	19.4^{y}	19.1^{y}
	6	14.4^{xz}	14.3^{xz}	12.3^{xy}
	8	15.2^{xz}	12.7^{xyz}	10.9^{xy}
	12	15.7^{xz}	14.1^{xyz}	14.6^{xy}
	24	6.7^{x}	7.0^{z}	12.3^{xy}
	36	14.12^{xz}	15.6^{xy}	15.3^{xy}
	48	7.6^{x}	8.1^{xz}	11.1^{xy}

[a]SE=0.3

[b]SE=1.7

[x,y,z]Means within a column with different superscripts are different ($P < 0.05$)

Ruminal ammonia concentration linearly increased ($P < 0.005$) for all supplementation treatments. Diurnal variation was most pronounced in steers when not supplemented or supplemented every 48 h, with lowest ($P < 0.05$) ammonia concentrations at 0600 h (0, 24, and 48 h).

Concentrations of acetate, propionate, and butyrate are presented in Table 24. No differences in acetate concentration due to supplementation treatment were observed at any sampling time. Acetate concentration did not change significantly with time for any supplementation treatment. On average, the acetate concentration tended to be higher ($P < 0.10$) for daily supplemented steers than those supplemented every 48 h. No differences in propionate concentration were observed at any sampling time due to supplementation treatment. Propionate concentrations followed a quadratic ($P < 0.01$) pattern with highest concentrations at 6 h. On average, the propionate concentration tended to be higher ($P < 0.10$) for daily supplemented steers as compared to those supplemented every 48 h. No differences in butyrate concentration were observed at any sampling time due to supplementation treatment. Butyrate concentration responded quadratically ($P < 0.01$) over the 48 h.

Passage Rates and Intake Estimation. Particulate passage rate and dry matter intake estimation data are presented in Table 25. Particulate passage rate data was not analyzed statistically. Particulate passage rate appears faster (approximately 0.5 %/h) with daily supplementation than with no supplementation or supplementation every 48 h.

Dry matter intake was greater ($P < 0.05$) for supplemented steers as compared to the non supplemented controls. The DMI as a percent BW was also higher ($P < 0.05$) with daily and every 48 h supplementation than without supplementation.

Table 24. Volative fatty acid concentrations due to supplementation frequency in Exp. 4 (n=3)

		Supplementation frequency		
VFA	Hour	None	Every 48h	Daily
Acetate, μmol/ml[a]	0	31.25	32.40	31.21
	2	41.87	49.11	54.96
	4	39.26	33.47	46.09
	6	40.19	40.01	47.40
	8	34.15	31.76	40.72
	12	46.22	40.68	52.10
	24	31.98	37.79	36.30
	36	46.97	35.23	45.88
	48	42.05	34.08	37.03
Propionate, μmol/ml[b]	0	7.64[x]	8.04	7.30[x]
	2	11.02[xy]	13.53	15.77[xy]
	4	10.34[xy]	9.63	13.07[xy]
	6	11.07[xy]	11.28	13.03[xy]
	8	9.61[xy]	9.06	11.21[xy]
	12	15.03[y]	12.00	16.22[y]
	24	8.90[xy]	9.70	8.94[xy]
	36	15.65[y]	10.49	13.77[xy]
	48	11.05[xy]	8.43	9.26[xy]
Butyrate, μmol/ml[c]	0	3.65	4.00	3.78[x]
	2	5.58	7.82	8.55[xy]
	4	5.46	5.02	6.82[xy]
	6	6.28	6.65	7.15[xy]
	8	5.67	5.36	6.19[xy]
	12	8.60	7.32	9.37[y]
	24	4.16	5.33	4.48[xy]
	36	8.22	6.08	7.73[xy]
	48	5.31	4.05	5.08[xy]

[a]SE=4.45

[b]SE=1.32

[c]SE=0.97

[x,y]Means within a column with different superscripts are different ($P < 0.05$)

Table 25. Particulate passage rate and dry matter intake in Exp. 4 (n=3)

	Supplementation frequency			
Rate	None	Every 48h	Daily	SE
Particulate Passage, %/h	2.52	2.39	3.19	--
Dry matter intake, kg/d	13.12[a]	15.59[b]	15.43[b]	0.44
Dry matter intake, % BW/d	1.86[a]	2.21[b]	2.19[b]	0.06

[a,b]Means within a row with different superscripts are different ($P < 0.05$)

Discussion

Forage Mass and Composition

In general, forage CP levels decreased and NDF and ADF increased as the forage matured. Forage mass and forage availability decreased significantly with time in Exp. 1 and 2. These paddocks consisted of stockpiled tall fescue and were continuously grazed during the 42 d study. Forage mass declined greatly as the cattle consumed the standing forage and there was minimal regrowth since forages began a dormant state for winter. The decreasing CP content of stockpiled fescue has been reported by Fritz and Collins (1991) and Beconi et al. (1995). Neutral detergent fiber and ADF concentrations (55 and 30 %, respectively) at the beginning of Exp. 1 and 2 and for the entire Exp. 3 were similar to those reported by Hitchcock et al (1990) for high quality vegetative regrowth of Kentucky 31 tall fescue. At the end of Exp. 1 and 2, NDF and ADF concentrations had increased nearly 10 and 5 percentage units respectively, significantly affecting forage quality. Jung and Allen (1995) reported that as NDF and ADF concentrations in forage increases DMI of the forage decreases. In Exp. 1 and 2, forage NDF and ADF concentrations increased resulting in possible decreased DMI which could explain the decrease in animal performance during the last three weeks. Forage mass and nutritive value remained relatively constant in Exp. 3 due to rotational stocking of the cattle and because forages were not stockpiled. Forage availability and nutritive value were similar for each of the three rotations as steers moved to a new sub-paddock. Each sub-paddock was at the same stage of maturation when the calves entered, therefore fresh forage high in nutritive value was available to the calves every 2 wk as compared to continuous stocking. At each rotation, forage mass was less for endophyte-free fescue as compared to endophyte-infected fescue. Peters et al. (1992) also reported lower yields for endophyte-free as compared to endophyte-infected fescue at 3,500 and 5,000 kg/ha DM, respectively.

Animal Performance

Cattle on Exp. 1 and 2 did not achieve the targeted 1.25 kg/d ADG when supplemented with a blend of corn grain, SH, and WM. The decreasing forage availability and quality in Exp. 1 and 2 may have been a limiting factor for cattle gains. As forage quality decreases, palatability, intake and digestibility decrease. Calves in Exp. 1 and 2 lost weight during wk 1 of the study. Previous research has attributed weight loss in the first week of receiving cattle to decreased DMI as a result of stress (Fluharty and Loerch, 1996; Lofgreen, 1981). High stressed calves in Exp. 2 lost 6.6 % BW due to transportation and feed deprivation, while in the same period of time LS calves that had had access to hay lost 3.9 % BW on d 0. Arthington et al. (2003) reported similar weight losses due to 3 h transport (at 6.5 and 3.9 % BW loss for transported and non-transported respectively).

In Exp. 1 and 2, supplemented cattle performed better than non-supplemented cattle ($P > 0.05$). Austin (2001) and Hutcheson (2003) backgrounded calves on forage based diets with supplementation at 1 % BW and 0.5 % BW. Both studies concluded that supplementation at 0.5 % BW is more efficient than supplementation at 1 % BW. Austin (2001) reported steers supplemented at 0.5 % BW had higher ($P < 0.05$) ADG than steers supplemented at 1.0 % through d 28. Although the effect of supplementation frequency (daily at 0.5 % BW or every 48 h at 1 % BW) on ADG in Exp. 1 and 2 cannot be directly compared, contradictory trends were observed. In Exp. 1, heifers supplemented every 48 h had numerically higher final body weights than daily supplemented heifers. These results agree with the findings of Scaglia et al. (2004) where heifers supplemented with 15 % CP corn gluten feed + soy hulls at 0.5 % BW daily had higher ($P < 0.05$) ADG than heifers supplemented 15 % CP corn gluten feed + soy hulls at 1.0 % BW every other day. However, conflicting results were observed in Exp. 2 where steers

supplemented daily had numerically higher final body weights and ADG as compared to the every 48 h supplemented steers. Supplement efficiency may have increased in Exp. 1 and 2 as forage nutritive value and mass became limiting. Supplement efficiency can be defined as the increased gain due with the addition of supplement. The efficiency of the supplement increases as the unit gain per unit supplement fed increases. As the forage quality decreases calves are forced to utilize supplements more efficiently resulting in higher gains. Greater supplement efficiency was also observed in the daily as compared to every 48 h supplemented calves in Exp. 1 and 2.

Fescue type has an effect on calf performance in the fall during a backgrounding program. Calves grazing endophyte-free or novel endophyte fescue (Exp. 3) had numerically higher final weights and ADG than those grazing endophyte-infected fescue. Additionally gain/ha was greater for endophyte-free and novel endophyte as compared to endophyte-infected fescue in spite of lower forage mass and forage availability. Parish et al. (2003) reported similar results with higher ($P < 0.05$) ADG and gain/ha for calves (240 kg BW) grazing endophyte-free or novel endophyte fescue as compared to endophyte-infected during spring and fall grazing seasons. Gain per hectare reported by Parish et al. (2003) was higher than the present study at 226 kg/ha for endophyte-free and 165 kg/ha for endophyte-infected.

Body weight losses were observed during wk 1 in Exp. 1 and 2, while not in Exp. 3. Initial BW on d 0 in Exp. 1 and 2 were not shrunk weights (calves were not withheld feed and water). The loss in BW can be attributed to loss of ruminal fill due to the stress that occurred during weaning. Calves recovered their d 0 BW by d 14 and 7 for Exp. 1 and 2, respectively. In Exp. 3 , d 0 BW could be considered shrunk weights because calves were purchased from auctions then transported to the research farm to initiate the study. As a result calves appear to gain weight during wk 1, however much of this weight could be attributed to ruminal fill. The

HS calves in Exp. 2 were held in drylot overnight without access to hay therefore d 1 BW could be considered shrunk weights. The cumulative ADG in Exp. 2, from d 1 (shrunk weight) to d 42 would be 0.77 kg. The d 1 to 42 cumulative ADG from Exp. 2 is still lower to the ADG observed in Exp. 3 at 0.98 kg. Forage mass and nutritive value was better in Exp. 3 than Exp. 2 indicating that the forage may have been the limiting factor for animal performance in Exp. 1 and 2.

Animal Health

Animal health was not significantly impacted by supplementation strategy or tall fescue type. The lack of energy and protein supplementation effects on health parameters agree with previous research on receiving cattle (Berry et al., 2004). Berry et al. (2004) did not find differences in acute phase proteins, haptoglobin, fibrinogen, or morbidity due to dietary energy and starch concentrations. Researchers found that acute phase proteins, haptoglobin, and fibrinogen concentrations varied in response to morbidity, and that haptoglobin could possibly be used to predict morbidity.

Morbidity incidence was low in all experiments. Morbidity was monitored closely in these experiments because of previous reports of high incidences with receiving stressed calves during the backgrounding period (Lofgreen, 1981; Cole and Hutcheson, 1990; Galyean, 1999). There were only a few days in which a calf received a score of 2 for morbidity (total of 5 calves received a score of 2). There was a slightly higher incidence of morbidity in purchased calves (Exp. 3) than in calves born at the SVAREC (Exp. 1 and 2). One calf in Exp. 3 received a score of 2 for appearing slightly dehydrated, later that day the calf died. Upon necropsy by the Virginia-Maryland Regional College of Veterinary Medicine, cause of death was determined as pneumonia. The data from this calf was excluded in the analysis. A replacement calf was placed in the pasture to maintain the stocking density. The majority of calves treated, were so on

sampling days due to elevated rectal temperatures but not based on morbidity scores. Similarly to morbidity, number of treatments was greater for purchased calves (Exp. 3) than for SVAREC produced calves (Exp. 1 and 2). There was an incidence of laminitis in Exp. 3. Between d 7 and 21, five calves were treated for a total of nine laminitis cases. History of these calves could not be obtained; therefore the cause of laminitis could not accurately be determined. There are multiple possible causes of laminitis including: grain acidosis, grass founder, injury in the working facility, or pneumonia.

Rectal temperatures were not significantly different between treatments in any of the experiments on sampling days. Rectal temperatures were 0.5 °C higher in HS calves at the late sampling on d 0 than LS calves in Exp 2. Tympanic temperature logger data from Exp. 2 show HS calves as having higher tympanic temperatures on d 0 than LS calves. The elevated temperatures for the HS calves at 1900 can be attributed to elevated temperatures on d 0 during transportation. Diurnal variation can be observed from tympanic temperature logger data in Exp. 2 and 3 with lowest temperatures from 0700 to 1000 and highest temperatures at 1400 to 1600. Air temperatures were 10 ºC warmer on d 3 to 5 in Exp. 3, corresponding with the higher tympanic temperatures. No differences in rectal temperatures were observed in Exp. 3 due to fescue type over the 42 d study. Tympanic temperature logger data show a slight elevation in temperature for endophyte-infected as compared to endophyte-free or novel endophyte fescue on d 4 and 5. These results do not agree with those of Parish et al. (2003), which showed significantly higher ($P < 0.05$) rectal temperatures for calves grazing endophyte-infected fescue (40.2 °C) as compared to endophyte-free fescue (39.8 °C) during the fall grazing season in Georgia.

The N:L ratios were higher on d 0 than 7 for Exp. 1 and 3. In Exp. 2, highest N:L were observed on d 4. On d 1, N:L was greater for HS calves than LS calves. Similar N:L responses to

transportation were reported in steers (Kegley et al., 1997) and goats (Kannan et al., 2000). Kegley et al. (1997) transported steers (263 kg BW) for 6 h resulting in a N:L ratio of 0.62 post transport and a return to normal levels at 0.18 N:L 1 wk later. The observed difference in N:L on d 1 of Exp. 2 between supplementation frequencies should not be considered because calves had not yet received supplement at that point in time. Weaning stress in cattle has been previously reported as causing a rise in N:L within the first 48 h (0.61) with recovery to normal levels by 168 h (0.47) (Hickey et al., 2003).

Serum and Plasma Analyses

Blood urea nitrogen was used in these studies as a biological indicator of dietary crude protein intake. The BUN concentrations observed in all studies were in the normal range. Cole et al. (1988) observed increased ($P < 0.05$) BUN concentrations due to marketing and transport stress on beef calves due to feed and water withholding. Immediately following transport BUN concentrations were 2.2 mg/dL higher than pre-transport and returned to pre-transport values by 24 h post-transport (Cole et al., 1988). Blood urea nitrogen in Exp. 2 was 1.33 mg/dL higher for the HS calves as compared to LS calves on d 1, indicating a slight transportation and feed restriction response. In Exp. 1 and 2, the lowest BUN concentrations, d 14 and 42, for calves supplemented every 48 h correspond with days in which the supplementation was 24 h before the sampling, while higher BUN concentrations, d 7 and 21, correspond with days in which the supplementation was 48 h before the sampling. Previous research has shown fluctuations in BUN concentrations with less frequent supplementation. Cole and Hutcheson (1990) showed a direct relationship between dietary CP concentration and BUN levels in beef calves. As CP content of the diet increased, BUN concentrations increased ($P < 0.05$) due to the greater N intake (Cole and Hutcheson, 1990).

In these studies, CK was used as an indicator of calf stress and health status following weaning. The CK concentrations followed a similar pattern for all studies, with significantly higher values on d 0 (d 0 to 4 in Exp. 2), then a return to approximately normal levels for the remainder of the 42 d. On d 0 of Exp. 2, all steers within a stress treatment were handled and penned together regardless of supplementation treatment, therefore the difference in CK at the evening sampling between supplementation frequency treatments was not due to supplementation treatment. In Exp. 2, CK concentrations were numerically higher for HS calves as compared to LS calves on d 0 at 2100. These results agree with those of Kannan et al. (2000) who observed in goats an increase ($P < 0.05$) in CK due to 2.5 h transportation. Concentrations of CK peaked at 260 IU/L 2 h post-transport (normal CK of a goat is 16.3-46.6 IU/L). Conversely, Cole et al. (1988) reported no differences in CK due to transportation in feeder calves. The elevation of CK on d 0 for all three experiments can be attributed to weaning stress.

Fibrinogen was used in these studies as an indicator of stress and health after weaning. Fibrinogen concentrations had a quadratic response in all experiments, however all measurements were in the reference interval for cattle. The low Fb concentrations correspond with the low rate of illness observed in this study. Hickey et al. (2003) reported a similar trend in Fb concentrations in heifers and bulls after weaning with highest levels observed 48 h postweaning (825 mg/dL and 600 mg/dL for weaned bulls and heifers, respectively). Fibrinogen concentrations were not affected by stress level in Exp. 2. Cole et al. (1988) did not observe differences in Fb concentrations due to transportation while Arthington et al. (2003) observed higher Fb concentrations due to transportation. Arthington et al. (2003) reported higher Fb concentrations 1 d after transport (408 mg/dL) as compared to weaning (221 mg/dL).

Serum mineral concentrations are important for health and immunity during stress. The calves in Exp. 1 and 2 were raised at the research station and previous mineral supplementation

was known, therefore serum minerals were not analyzed. In previous studies conducted at SVAREC (Austin, 2001; Hutchinson, 2003) it was concluded calves from SVAREC have mineral concentration in the normal range. Experiment 3 used purchased calves of unknown mineral supplementation history, therefore initial and final serum minerals were obtained. Copper, Zn, Cr, and Se are considered as some of the most critical minerals for stressed calves (Galyean et al., 1999). Calves in Exp. 3 were grazing Se marginally deficient forages and were not supplemented with Se in the mineral mix, therefore levels declined significantly over the 42 d. The forage in Exp. 1 was Se deficient, but in this case heifers received Se in the mineral supplement. In Exp. 3 Cu and Zn concentrations increased over the 42 d study due to mineral supplementation. On d 0, serum Cu levels were considered deficient, however they were at a normal level on d 42. Orr et al. (1990) reported decreased ($P < 0.05$) Zn for morbid as compared to healthy steers (0.69 ppm and 0.97 ppm, respectively) and increased Cu for morbid as compared to healthy steers (1.30 ppm and 1.03 ppm, respectively).

In Situ Analysis

Dry matter disappearance kinetics of the supplement was not affected by supplementation frequency. The readily degradable fraction, slowly degradable fraction, rate of slowly degradable fraction digestion, and overall effective degradability of the supplement was not different for any treatment. The readily degradable and rate of slowly degradable digestion of the forage was not effected by supplementation frequency. The slowly degradable fraction was highest for supplementation every 48 h and lowest for daily supplementation, while no supplement was intermediate. The overall effective degradability was highest for no supplement and decreased with increasing supplementation frequency. These results agree with Carey et al. (1993), whereas Chase and Hibberd (1987) reported increased rate of DM digestibility while Bodine et al. (2001)

and Garcés-Yépez et al. (1997) reported increased rates of in situ DM digestibility. The discrepancies in previous research, for DM digestibility can be attributed to the varying levels of UIP and DIP in the supplements as well as the types of forages fed. Forage in the present Exp. was of high nutritive value and the supplement consisted of readily digestible fiber sources that are considered to be high in UIP (corn at 43 %, SH at 42 % and WM at 23 %).

Forage and supplement CP concentrations decreased linearly from 0 to24 h of the present trial and then increased from 36-48 h for all supplementation frequencies. There were no treatment differences in CP content, which agree with the results of Carey et al. (1993). These results differ from those of Bodine and Purvis (2001) where they were feeding low quality forage as compared to medium/high quality forage in the previous studies mentioned. The increase in CP at 36 and 48 h could be attributed to microbial protein from the bacteria that were tightly bound to the forage and were not rinsed away in the washing process.

Ruminal pH, Ammonia, and Volatile Fatty Acids

No supplementation frequency by time interactions were observed for ruminal pH. Although not significantly different, pH was numerically lower for supplemented steers from 2 to 24 h post supplementation. The pH level for the daily supplementation appeared lower than the every 48 h supplementation except at 6 and 24 h post supplementation. The lowest pH value observed was at 4 h for daily supplementation when pH dropped below 6.0. Most researchers agree that fiber-digesting bacteria are hindered when pH drops below 6.0 (Mertens and Loften, 1980; Bodine and Purvis, 2001). Although pH dropped below 6.2 and approximated 6.0 for supplemented steers, no apparent affect on in situ DM digestibility was observed in the present study. Results of pH levels due to supplementation were comparable to the findings of Carey et al. (1993).

Numerically, ruminal ammonia concentration was higher for daily and every 48 h supplementation at 2 and 4 h post supplementation as compared to the non-supplemented control. Although not significant, ruminal ammonia is lower for daily supplementation at 8 h than the non-supplemented or every 48 h supplemented steers However at 24 h, daily supplemented steers had the highest ($P = 0.90$) ruminal ammonia. Ruminal ammonia concentrations due to supplementation were comparable to those reported by Carey et al. (1993) when feeding SBM as a protein supplement. They reported highest ruminal ammonia at 4 h (16 mg/dL) and lowest at 24 h (8 mg/dL) post supplementation. All ruminal ammonia concentrations in the present study were well above the 5 mg/dL level required for optimal microbial protein synthesis reported by Satter and Slyter (1974). The ruminal ammonia peaks in this study occurred rapidly (2-4 h) post supplementation whereas Bohnert et al. (2002) and Farmer et al (2004) reported a 6-24 h delay in ruminal ammonia peaks after infrequent protein supplementation. The delayed peak concentration, reduced peak amplitude, and prolonged peak elevation with infrequent protein supplementation indicates the rumen can buffer ammonia concentrations to maintain fiber digestion. The ruminal ammonia levels of the present study and previous studies indicate that ruminants can sufficiently maintain ammonia levels between supplementation periods to support adequate microbial function.

Volatile fatty acid concentrations were not significantly affected by supplementation or supplementation frequency at any sampling time. Total VFA concentrations appear higher for daily supplemented steers than non-supplemented or every 48 h supplemented steers. Martin and Hibberd (1990) and Bodine and Purvis (2001) also reported higher ($P < 0.05$) total VFA (63.5 and 74.9 mM; 71.1 and 84.3 mM, respectively for the two studies) concentrations for energy-supplemented steers as compared to controls. Acetate concentrations did not significantly change over time, nor were there any differences between supplementation treatments in the present

study. Propionate and butyrate concentrations followed a quadratic trend over time for all supplementation treatments. No differences between supplementation treatments in propionate or butyrate concentrations were observed in the present study. In addition, molar proportions of acetate:propionate were not different due to supplementation frequency. Carey et al. (1993) reported no differences in acetate:propionate ratio when supplementing energy from SBM, corn, barley or beet pulp to steers. Farmer et al. (2004) observed a decrease in acetate concentration and increase in propionate concentration in the acetate:propionate ratio (3.5 and 5.0 acetate:propionate, respectively) as well and an increase in butyrate concentration (10.0 and 7.8 mol/100mol, respectively) from 4 to 24 h post supplementation for 2 d/wk supplemented steers as compared to daily supplementation. Proportions of acetate:propionate were similar for daily supplemented as compared to control steers although total concentrations of acetate and propionate were higher ($P < 0.05$) with daily supplementation. Supplementation can increase VFA concentrations in the rumen.

Passage Rate and Intake

Particulate passage rate was not affected by supplementation frequency. Beaty et al. (1994) also reported no effect of supplementation frequency (7 vs 3 d/wk) on indigestible ADF passage rate of steers consuming low-quality hay. Furthermore, Judkins et al. (1987) reported that supplementation of protein from cottonseed meal cake has no effect on particulate fill (12.8 or 12.7 g/kg BW, respectively) or passage rate (3.35 or 3.36 %/h, respectively) as compared to controls in ruminants grazing medium to low quality forages. Bohnert et al. (2002) reported increased ($P = 0.07$) DM fill for supplemented steers as compared to control steers (41.9 and 42.3 g/kg BW, respectively) on day of supplementation, however infrequently supplemented steers had lower ($P < 0.05$) DM fill than control (42.1, 39.3, and 37.2 g/kg BW, respectively) at 2

or 5 d post supplementation. The IADF passage rate was higher ($P < 0.10$) on any day for supplemented steers as compared to controls (1.58 and 1.81 %/h). The particulate passage rate from the present study for non-supplemented steers (2.52 %/h) was similar to that observed by McCracken et al. (1993) for steers grazing endophyte-free fescue in November at 2.6 %/h.

Dry matter intake as kg/d or as % BW was greater for both daily and every 48 h supplementation as compared to no supplementation. Supplement accounts for approximately 3.5 kg/d while DMI was only increased approximately 2.4 kg/d, therefore estimated forage intake was decreased approximately 1 kg/d due to supplementation. Average DMI was similar for daily and every 48 h supplementation frequencies. Judkins et al. (1987) reported increased ($P < 0.05$) total DMI with similar forage DMI supplemented steers as compared to control steers. Beaty et al. (1994) observed higher ($P < 0.05$) DMI of straw with 7 d/wk supplementation as compared to 3 d/wk. In the present study, total DMI was increased, however there was an associative effect due to supplementation. Similar associative effects were reported by Carey et al. (1993) with energy supplementation with corn, barley, or beet pulp as compared to SBM supplemented controls. Garcés-Yépez et al. (1997) reported that supplements containing highly digestible fiber (soybean hulls) had less negative associative effects on forage intake than high starch (corn-soybean meal) supplements. McCracken et al. (1993) reported DMI of 1.53 % BW/d for steers grazing endophyte-free tall fescue in the fall, which was slightly lower than the 1.86 % BW/d observed in the present study for the non-supplemented steers.

CONCLUSIONS

Results from this study indicate that supplementation of beef calves during the backgrounding period can improve animal performance. Supplementation of high fiber co-products every 48 h at 1 % BW may provide equal or greater animal performance than supplementation daily at 0.5% BW. Previous research indicates that supplementation every 48 h can improve calf ADG during the backgrounding period. More research in necessary to determine the impact of supplementation frequency on backgrounded calf performance and ruminal environment flucuations. Calves consuming supplement did not achieve the ADG that NRC predicted for the ration. Supplement refusals were minimal, indicating palatability and consumption was not a limiting factor for cattle performance. Performance limitations could be attributed to significantly decreasing stockpiled forage mass and nutritive value during the backgrounding period. Supplementation of high fiber co-product feedstuffs at 0.5 % BW were not sufficient to greatly enhance calf performance when forage became limiting.

Cattle health, immune, and stress responses to weaning and transportation did not appear to be significantly impacted by supplementation of a high fiber co-product blend during the receiving period when forage quality is adequate. Data from Exp. 1 and 2 indicate the importance of high quality forage or adequate supplementation for enhanced calf performance during the backgrounding period. The transportation stress imposed in Exp. 2 did not significantly impact any of the stress parameters measured, health, or performance of the steers. The transportation stress and feed withholding in Exp. 2 was designed to simulate handling of cattle at marketing without the aspect of commingling. Previous research implicating similar stresses have reported significant differences in health and gain, however that was not seen in the

present study. More replications may have resulted in significant differences for this study due to the fact that numerical differences were observed for CK and N:L ratios during wk 1.

Supplement fed was a blend of co-product feeds designed to have adequate balance of DIP and UIP according to NRC. Ruminal environment and kinetic measurements in Exp. 4 indicated that the rumen function was adequate with infrequent supplementation of a high fiber co-product based feedstuff. Previous research indicates that supplements fed at low frequencies must be balanced in energy and protein to maintain forage intake, bacterial fiber digestion, and microbial protein synthesis in the rumen. Co-product feedstuff quality can be highly variable, therefore chemical composition analysis is important. Supplements, such as the highly digestible fiber co-product blend used in these experiments should compliment forage to promote and healthy ruminal environment to maintain forage utilization and intake while enhancing cattle performance. Experiment 4 results showed that decreasing supplementation frequency did not decrease forage or supplement digestibility, negatively impact ruminal pH, ammonia, or VFA concentrations. Dry matter intake was greater for supplemented steers indicating additive effects maintaining similar for DMI. Every other day supplementation could provide an alternative to daily supplementation to decrease labor costs for cattle producers.

Results from Exp. 3 indicate that calves backgrounded on endophyte-free or novel endophyte fescue will perform better than those grazing endophyte-infected fescue in spite of higher forage availability and similar quality. Calves grazing endophyte-infected fescue did not appear to develop fescue toxicosis. Average daily gains were lower for calves on endophyte-infected fescue as compared to novel or endophyte-free fescue, however rectal temperatures, mineral status, BUN, CK, and Fb concentrations were all similar.

IMPLICATIONS

Backgrounding programs on high quality forage with supplementation either daily or every 48 h can improve calf performance while providing an opportunity to overcome detrimental effects associated with weaning and transportation stress at marketing. Ruminants can maintain a healthy ruminal environment for microbial function and nutrient utilization with supplementation every 48 h or daily, resulting in comparable performance. Backgrounded calves gain better on novel endophyte or endophyte-free tall fescue as compared to endophyte-infected tall fescue.

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