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The effects of hydrated sodium calcium aluminosilicate on fescue toxicosis

Paula Davis Anderson

To the Graduate Council:

I am submitting herewith a thesis written by Paula Davis Anderson entitled "The effects of hydrated sodium calcium aluminosilicate on fescue toxicosis." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Allan B. Chestnut, Major Professor

We have read this thesis and recommend its acceptance:

J.C. Waller, H.A. Fribourg

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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Date May 28, 1990

THE EFFECTS OF HYDRATED SODIUM CALCIUM ALUMINOSILICATE ON FESCUE TOXICOSIS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Paula Davis Anderson

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DEDICATION

I wish to dedicate my thesis to two very special people, my husband Brian and Dr. Carolyn Orr Straw, my major professor at Berea. Each has given me constant love, patience and support to help me continue during down times. If I had not had them I would not have sought to attain this goal. I love them and thank them both.

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ABSTRACT

Three trials were conducted to determine whether hydrated sodium calcium aluminosilicate (HSCAS) will alleviate fescue toxicosis or affect mineral metabolism. In the first of two in vitro experiments, buffered solutions indicated that ergotamine will bind with HSCAS at pH levels of pH 7.8 or less. In the second in vitro experiment, when timothy hay was the substrate incubated in ruminal fluid, recoveries of ergotamine were lower than in the first in vitro experiment which utilized buffer solutions. This suggests that feed or microbial particles adsorbed ergotamine. During in vitro post-ruminal digestion with acid-pepsin, more ergotamine was recovered from precipitated pellets when they contained HSCAS than when they did not, indicating adsorption of ergotamine to HSCAS.

A rat growth study was conducted to determine whether signs of fescue toxicosis could be reduced by the addition of HSCAS to diets containing Acremonium coenophialum infested (E+) fescue seed. Inclusion of HSCAS in diets did not appear to reduce signs of fescue toxicosis. Rats fed diets containing E+ seed consumed less feed, gained less weight and had lower gain per unit of feed than did those consuming diets containing noninfested (E-) seed. Serum prolactin concentrations (PRL) and testes weights tended to be lower in rats receiving E+ diets than in rats pair-fed E- diets.

A sheep metabolism trial was conducted to determine whether 2% dietary supplementation of HSCAS alleviated signs of fescue toxicosis and whether HSCAS interfered with mineral utilization. Serum sorbitol dehydrogenase activity suggested that HSCAS could help prevent hepatic cell destruction associated with consumption of E+ fescue. Ruminal pH and concentrations of ruminal volatile fatty acid concentrations

(VFA) and ammonia were similar among treatments. Sheep consuming HSCAS also had lower apparent absorptions of Mg, Mn and Zn than those not receiving HSCAS. Animals supplemented with HSCAS may need additional mineral supplementation with Mg, Mn and Zn.

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CHAPTER I

LITERATURE REVIEW

Tall Fescue

Tall fescue (Festuca arundinacea Schreb.) is a grass used as hay and pasture in livestock production. Tall fescue is grown in many parts of the world, including Africa, Asia, Australia, Europe, North America and South America. Fescue occupies about 14 million ha of pasture land in the United States, where it is grown primarily in the northwest and southeast regions (Buckner et al., 1979).

Tall fescue has been popular because it is a vigorous grass which produces good yields. In Virginia, hay yields of 14,000 kg/ha were produced when adequate soil moisture and fertility were available. Yields were shown to be reduced by 35 to 50% when the grass was cut often to simulate grazing. The rate of dry matter (DM) growth in uncut grass reached about 150 kg/ha/d for several weeks in early spring, then declined after anthesis (blooming or time of full bloom of a flower). During summer, DM production often exceeded 50 kg/ha/d when there was enough moisture to support growth (Buckner et al., 1979).

Fescue has a longer growth phase than do other cool season grasses such as bromegrass and orchardgrass. In Tennessee, spring growth begins in March and anthesis occurs near the first of June. Growth slows during July and August, but late growth continues into late November or early December. Thus, spring and fall are the seasons during which most growth occurs (Buckner et al., 1979).

Fescue is adaptable to many soil types and various climatic conditions. Hence,

fescue will grow on soils with either an acidic pH (4.7) or an alkaline pH (9.5). It will grow on poorly drained, cool soils and can survive flooding. It grows best in areas where the climatic conditions are mild in winter and warm with high humidity in summer (Buckner et al., 1979).

Fescue is a deep-rooted, long-lived perennial, which produces good sod due to its coarse, tough root system (Heath et al., 1973). Thus, fescue grows on land that is not suitable for other forages (Cowan, 1956).

Chemical analysis shows fescue to be a high quality forage with many utilizable nutrients. Sullivan et al. (1956) found that tall fescue was higher in nutritive value than bromegrass (Bromus spp. L.), Kentucky bluegrass (Poa pratensis L.), orchardgrass (Dactylis glomerata L.), reed canarygrass (Phalaris arundinacea L.), red top (Agrostis alba Roth), timothy (Phleum pratense L.) or tall oatgrass (Arrhenatherum elatius L.). They reached this conclusion based upon content of protein, nitrogen-free extract, fructosan, soluble ash, lignin, fiber and cellulose. Tall fescue has been shown to have a higher concentration of crude protein and higher digestibility than many warm-season grasses cut at the same stage of maturity (Buckner et al., 1979).

Signs of fescue toxicosis have frequently been exhibited by livestock consuming tall fescue. Appearance of these signs are related to the infestation of fescue plants by the endophytic fungus Acremonium coenophialum (Morgan-Jones and Gams, 1982). Neill (1941) discussed the probability of the fungus being the causative agent as early as 1941. This research went unnoticed until Robbins found the article while doing an extensive literature review in 1972. Bacon et al. (1977) identified Epichloe typhina in toxic fescue. Recently, E. typhina was renamed A. coenophialum, thus ending the dispute of nomenclature (Morgan-Jones and Gam, 1982).

Bacon et al. (1986) stated that the fungus can be transmitted only through seed produced by infected plants. They determined that the endophyte could survive in seed stored at low temperatures for extended periods of time. They determined also that the fungus can be eliminated if infected seed is stored at high temperature and humidity.

Endophytic infestation currently is determined by one of three ways: microscopic staining, callus culture or enzyme linked immunosorbent assay (ELISA; Reddick, 1988). The most common microscopic staining procedure is described by Bacon (1983) and Clark et al. (1983). This procedure may be used on either plants or seed. The callus culture for testing seed was developed by Conger and McDaniel (1983). The ELISA procedure may be used on either plants or seed to determine the presence of Acremomium species. However, positive identification of Acremomium in seed does not prove that the fungus is alive. There are three variations of ELISA used for detection of Acremomium species. They are the direct or double sandwich method, the indirect method and the protein-A sandwich method (Reddick, 1988).

Signs of Fescue Toxicosis

Stuedemann and Hoveland (1988) hypothesized that A. coenophialum is associated with disorders such as fescue foot, summer syndrome, reproductive problems, agalactia, fat necrosis and poor growth of livestock. Fescue foot was noted first by Cunningham (1949) in Australia. Garner (1973) saw from 10 to 30% of a herd exhibited signs of fescue foot. Fescue foot occurs generally in late winter and is rare in occurrence. Signs of fescue foot include arched back and soreness in hooves or total lameness, dry gangrene of tail and feet or sloughing of the hooves. One characteristic of animals exhibiting fescue foot is a red line forming at the coronary band (area between

dewclaws and hooves) of a foot with possible swelling and skin discoloration in extremities. Vasoconstriction is believed to be the major cause of the swelling and inflammation of the coronary band. Low environmental temperature may contribute to lesions which enhance dry gangrene (Jansen et al., 1956, Yates et al., 1979). Jacobson et al. (1963) conducted a study in which they administered extracts from tall fescue intraruminally to cows. The cows developed signs of fescue foot. The cows had lesions on the rear legs with enlarged lymph nodes draining these areas. The animals were then extinguished. Histologically, lymphatic hyperplasia and a thickened epidermis of the tail were observed indicating that poor circulation due to vasoconstriction caused the gangrene. Williams et al. (1975) dosed animals with fescue extracts and reported that 3 of 4 cattle exhibited signs of fescue foot. Microscopic examination revealed that calves had blood vessels with thick walls and small lumens in coronary bands and tail tips. There was also hemorrhaging in many hair follicles of the tail tips and capillaries, and small vessels were distended with blood. This study further supported the idea of vasoconstriction as the probable cause of fescue foot.

Videothermometry was used to measure the change in coronary band temperatures by Yates et al. (1979) who found an average coronary band temperature of 27 to 31 C in control animals administered an intraperitoneal isotonic saline for 12-24 d. Calves receiving intraperitoneal anion fractions of tall fescue had coronary band temperatures as low as 22 (±1) C.

Another problem often associated with consumption of Acremonium infested fescue is a group of signs collectively referred to as "summer syndrome." Animals placed under severe heat stress exhibited signs of summer syndrome (Hemken et al., 1984). Under prolonged, high ambient temperatures, heat may increase the sensitivity of the

animal to the toxin(s) in fescue or the toxins may increase the sensitivity of the animal to heat stress. Signs of summer syndrome include decreased forage intake, increased respiration rates and rectal temperatures and long, rough hair coats (High et al. 1965; Jacobson et al., 1970; Buckner et al., 1979; Johnson et al., 1982; Hoveland et al., 1983). Hemken et al., (1979) observed that dairy cattle fed fescue as soilage during July and August of two years exhibited lower average feed intakes, elevated rectal temperatures and decreased body weights when consuming G1-307 than when consuming Ky 31 or G1-306 fescues. This illustrated that these signs are exhibited during summer heat. Then Hemken et al. (1981) found, when calves fed either endophyte infested (E+) or noninfested (E-) fescues, (G1-306 and G1-307, respectively) were kept in an environmentally controlled room at 21-23 C, that there were no significant differences between the groups in DM intakes, rectal temperatures and respiration rates. However, at 34-35 C, there were significant differences in DM intakes, rectal temperatures and respiration rates between calves consuming E+ and those consuming E- fescue. This demonstrated that the environmental temperature interacted with toxins in E+ fescue to affect DM intakes, rectal temperatures and respiration rates.

Another problem often associated with fescue toxicosis is poor reproductive performance of livestock. Garret et al. (1980) observed that among mares grazing E+ fescue, 38% had prolonged gestations, 18% aborted and 9% had thickened placentas. Their foal losses were almost 3 times greater than in mares fed other forages.

Varney et al. (1987) found that rats fed a diet with 40% E+ seed failed to maintain a normal estrus cycle and became pregnant. These animals exhibited body weight losses and low uterine weights. Rats fed a diet containing 20% E+ seed did conceive but had a 2.2-day extension of their estrous cycles. Daniels et al. (1981)

administered extracts of toxic fescue to rats and found that these extracts caused the rats to abort.

McDonald (1989) reported that cows grazing E- fescue at the Highland Rim Experiment Station in Tennessee had higher pregnancy and calving rates than did those grazing on intermediate endophyte infested pastures or highly infested endophyte pastures. McKenzie (1987) reported that follicular development and germ-cell survival in ovaries of heifers grazing these pastures were affected adversely by the ingestion of E+ fescue. She hypothesized that these factors probably would cause a reduction in reproductive performance.

Agalactia (a lack of milk production) is another problem often associated with the consumption of E+ fescue. It has been demonstrated that sheep, cattle, horses, rats and rabbits had suppressed milk production when fed E+ fescue. Garett et al. (1980) observed that 53% of a group of mares grazing fescue had agalactia. Daniels et al. (1981) administered extracts of toxic fescue to rats and found that the animals did not produce milk for their pups since the pups died with empty stomachs. Daniels et al. (1984) did a similar experiment with rabbits and found that the rabbits died also from lack of milk. Studies with horses have shown that mares grazing E+ fescue had lower milk production than mares grazing other non Acremonium infested grasses (Barnett, 1985; Monroe et al., 1987). Agalactia is more common in horses than in cattle; however, beef cows fed E+ fescue generally exhibit a lower level of milk production than do cows not consuming E+ fescue (Heimann et al., 1981). Schmidt et al. (1984) reported that beef cattle consuming E+ forages had lower milk production than did those consuming E- forages. Decreased milk production due to ingesting E+ fescue has been observed also in dairy cattle. Siegel et al. (1985) and Wallner et al. (1983) reported that dairy

cows consuming E+ green chop fescue had lower milk production than did cows consuming E- green chop fescue.

Fat necrosis, another problem associated with fescue, is a condition characterized by hard fat masses located in the pelvic region of the animal. In extreme cases, fat has been shown to cover reproductive organs and reduce conception rates (Stuedemann et al., 1975; Buckner et al., 1979). Animals with fat necrosis were observed often to have digestive disturbances, bloating, reduced passage of digesta, heart and/or kidney dysfunction and decreased urinary and fecal excretions (Buckner et al., 1979; Hemken et al., 1984). Fat masses often constricted the colon and caused closure of the small intestine (Wilkinson, 1983). Fat necrosis can be confirmed by rectal palpation or by postmortem examination.

Fat necrosis was diagnosed by Williams et al. (1969) in 67% of the cows in one herd, and 31% of the cows in a second herd showed signs of fat necrosis after grazing on fescue. A survey from six Georgia herds revealed that 23% of the animals from these herds had palpable lesions of fat necrosis throughout the digestive tract. A survey taken in a Georgia slaughter house found that 2.5% (1,519 head) of the cows sampled had fat necrosis. Herd problems were initially associated with animals grazing pastures fertilized with broiler litter (Williams et al., 1969). However, Stuedemann et al. (1975) showed that fat necrosis occurred also when cows grazed fescue pastures fertilized with ammonium nitrate. Lyons (1986) reported that high nitrogen fertilization of infected fescue plants grown in a greenhouse would increase ergopeptine alkaloid concentrations in the plants. Belesky and Robbins (1988) found that ergopeptine alkaloid concentrations in the forage, when expressed per unit of endophyte infestation, were greater for pastures fertilized with a high level of N than for those with a low level of N.

Low blood cholesterol levels associated with consumption of E+ fescue indicated that altered lipid metabolism may cause fat necrosis (Stuedemann et al., 1986). The actual cause of fat necrosis is not clear, although ergot alkaloids are suspected (Stuedemann et al., 1988).

Average daily gain was reported to be lower in cattle consuming E+ than for those fed E- fescue (Yates, 1962; Yates and Tooky, 1965; Hamilton et al., 1970; Bacon et al., 1977; Hemken et al., 1977; Hoveland et al., 1980; Schmidt et al., 1982; Reed and Camp, 1986; Hoveland et al., 1988; Stuedemann and Hoveland, 1988). Schmidt et al. (1982) and Jackson et al. (1987) found that steers fed E+ fescue seed had lower intakes than those consuming E- seed. Schmidt et al. (1982) found that steers fed E+ fescue hay had lower intakes than those consuming E- hay. Lower intakes by animals on E+ pasture than those on E- pastures were reported by Chestnut et al. (1990) and Stuedemann and Hoveland (1988). Lower intake would explain at least partially the reduction in gain due to consuming E+ fescue.

Prolactin (PRL) concentrations were found to be lower in animals consuming E+ fescue. Prolactin is one of the primary indicators used in determining fescue toxicity in both bovine and ovine species (Hurley et al., 1981; Stidham et al., 1982; Wallner et al., 1983; Bond and Bolt, 1986; Henson et al., 1987). Prolactin plays a role in milk letdown. Decreased PRL concentrations were a primary cause of agalactia in mares. Wallner et al. (1983) reported that when they orally dosed cows with the endophytic plant pathogen Balansia epichloë from the Clavivipitacae family ir caused cows to have a decreased serum PRL. This pathogen also caused the surge in PRL induced by milking to be decreased. In fact, they believed that PRL plays a critical role in "mammary gland

differentiation necessary for biochemical mechanisms involved in milk synthesis" (Wallner et al., 1983).

Prolactin also regulates corpus luteum function and gonadotropin secretion in rats. These play a vital role in reproduction (Smith, 1980). In male reproduction, PRL acts upon pituitary gonadotropin release and growth of accessory glands. Prolactin increases testosterone production by acting on the Leydig cells (Bartke, 1980). Davis et al., (1977) demonstrated a positive correlation between PRL secretion and sexual maturity in ruminants and in rats.

Ergot alkaloids, such as the ones found in fescue, are alpha-adrenergic antagonists, dopamine agonists and serotonin antagonists (Floss et al., 1973). Schillo et al. (1988) hypothesized that dopamine may play a major role in decreasing PRL availability and depressing PRL concentrations. Dopamine is believed to inhibit secretions of PRL from the pituitary gland (Meltzer et al., 1979). Reduced PRL concentrations are caused by the stimulation of dopamine receptors (Nasr and Pearson, 1975).

A trial was conducted to study effects of a dopamine antagonist, spiperone, and E+ fescue hay diets on dopamine and tissue catecholamine concentrations in sheep (Henson et al., 1987). Hypothalmic and pituitary tissue were analyzed for dopamine, norepinephrine, epinephrine 3,4-dihydroxyphenylacetic acid and momoamine oxidase. Animals fed E+ fescue without spiperone had lower PRL levels than animals fed a rye (Secale cereale L.)-orchardgrass ration. The spiperone-treated sheep had elevated levels of PRL. This elevation was associated with a significant decrease in plasma dopamine. The decreased dopamine concentration caused an elevation of monoamine oxidase in the

pituitary. It was hypothesized that modifying dopamine secretion may cause symptoms associated with fescue toxicity.

Evidence suggests that PRL secretion may be controlled by the influence of a PRL releasing factor. Elasser and Bolt (1987) reported inhibition of hormone secretion due to toxic fescue, and suggested that this inhibition might be confined to PRL. Secretory responses to thyrotropin releasing hormone (TRH) and haloperidiol (HAL) suggested that the effects of PRL are mediated by the dopaminergic pathway. They found that infected fescue altered PRL levels; however, in their study, TRH was not found to be decreased. Moore (1987) found PRL levels in rats and humans to vary under certain physiological conditions such as with photoperiod, sleep and suckling. Suckled animals had elevated serum PRL because of a reduced inhibitory control of a dopamine agonist. Dopamine agonists blocked the receptor and caused a tonic inhibitory effect on PRL. When these receptors were not blocked, PRL secretion increased. He concluded that circulating levels of PRL increased and this feedback mechanism activated tuberoinfunduibular dopamine neurons. This allowed PRL to be regulated by a hormonal-neuronal feedback loop.

A recent study indicated that the dopamine antagonist metoclopramide (MC) alleviates fescue toxicosis. Administration of MC significantly increased PRL concentrations and average daily gain of steers grazing E+ fescue while decreasing body temperature. Animals treated with MC also had improved hair coats (Lipham et al., 1989). Evidence from other studies suggests that MC may be an alleviator of signs of fescue toxicosis in sheep and mares (Fitzgerald and Cunningham, 1982; Johnson and Becker, 1987).

Alkaloids

Three classes of alkaloids have been found in E+ fescue plants and have been suspected to cause poor animal performance. These include the diazaphenathrine alkaloids, perloline and perlolidine; the pyrrolizidine alkaloids, N-acetyl and N-formyl loline and the ergopeptine alkaloids, primarily represented by ergovaline. The roles of the alkaloids in various signs associated with fescue is unknown.

Bush et al. (1972) and Boling et al. (1975) found volatile fatty acid (VFA) production to be inhibited and altered in the presence of perloline. Boling et al. (1975) found that perloline decreased digestibility of crude protein and cellulose when added to lamb diets. One cause of inhibited cellulose digestion by perloline was thought to be due to poor growth of rumen bacteria. Bacterial strains found to be inhibited included: Ruminococcus albus Hungate, Bacteroides succinogenes Hungate, Butyrovibia fibrisolvens Bryant and Ruminococcus flavefaciens Sijpesteijn in in vitro culture (Bush et al., 1972). Buckner et al. (1973) reported that perloline concentrations in plants increased from March to August and declined after August. These trends were related negatively to seasonal changes in animal performance and in vitro DM disappearance in tall fescue, which was lowest when harvested in August and increased when harvested later in the fall (Boling et al., 1973).

A strain of fescue (G1-307) was developed containing reduced perloline concentration. Results from feeding studies conducted with cattle determined that perloline was probably not responsible for poor animal performance. In fact, when G1-307 was evaluated, signs of toxicity increased rather than decreased (Buckner et al., 1973; Buckner et al., 1979; Hemken et al., 1979; Steen et al., 1979; Hemken et al., 1981). Studies by Bond (1977) showed G1-307 to be more toxic than Ky 31, G1-306 or Kenhy.

The G1-307 variety of fescue had higher loline concentrations than the G1-306 variety. Animals grazing G1-307 had the poorest body condition and hair coat scores. These animals also had elevated rectal temperatures, increased heart rates and excessive salivation compared to animals grazing G1-306. This indicated that lolines might be the cause of fescue toxicosis.

Robbins et al. (1972) reported that the loline derivatives were high in G1-307 while perloline concentrations were low. They stated that loline alkaloids would cause a decrease in feed intake and a decrease in weight gain in rats. Buckner et al. (1979) reported that N-acetyl loline and N-formyl loline were not metabolized and did not inhibit cellulose digestion in vitro. They hypothesized that the loline derivatives could cause a decrease in forage intake but not inhibit digestion. Kennedy and Bush (1983) reported that loline concentrations were related to level of endophytic infestation. This led researchers to believe that lolines might be the major cause of fescue toxicosis. Kennedy and Bush (1983) demonstrated also that the effects of loline alkaloids in livestock could be altered by severe water deprivation and extreme temperatures.

Recently ergopeptine alkaloids have been investigated as a possible cause of fescue toxicosis. These alkaloids are derived from lysergic acid. Porter et al. (1979) reported that the ergot alkaloids ergosine, ergosinine and chanoclavine I. had been isolated from Epichloë typhina. Using iso-butane chemical ionization mass spectroscopy, Porter et al. (1979) identified ergosine and ergosinine as ergovaline and ergovalinine. They later reported that agroclavine, elmoclavine penniclavine and festuclavine could be identified in cultures of E. typhina (Porter et al., 1981). Ergopeptine alkaloids are currently thought to be the causative agents of fescue toxicity symptoms. Osborn et al. (1988) illustrated how signs of fescue toxcity may be induced with ergopeptine alkaloids.

Their study showed that the addition of 30 ppm ergotamine tartrate to E- diets resulted in steers exhibiting signs of toxicity similar to those exhibited by steers fed E+ fescue. Signs included increased rectal temperatures and respiration rates and reductions in feed intake, heart rate and pastern, tail tip, ear canal and coronary band temperatures.

Zeolites

Zeolites, discovered in 1756 by Baron Axel Fredrick Cronstedt, are hydrated aluminosilicates of alkali and alkaline earth cations having specific three-dimensional structures (Mumpton and Fishman, 1977). Zeolites have many functional properties. They are able to lose and gain water reversibly and exchange constituent cations without a major change in structure. The primary value of zeolites in animal agriculture is their ion exchange and adsorption properties. Zeolites have been utilized in agriculture for nutritional research, disease prevention, reduction of moisture and ammonia in manure and in many phases of aquaculture (Mumpton and Fishman, 1977).

Zeolites consist of three-dimensional frameworks of a SiO_4^{-4} tetrahedral where, in all four corners, oxygen ions are shared with an adjacent group, giving an overall Si to O ratio of 2:1. Therefore, the structures are electrically neutral. In alumino-silical zeolites, some of the silicon atoms are replaced with aluminum, giving them an affinity for positively charged monovalent and divalent cations such as Na^+ and CA^{++}. The empirical formula for an alumino-silical is $(M_{2/n}) \cdot (O \cdot Al_2O_3 \cdot xSiO_2) \cdot yH_2O$, where M represents the cation, n the valence of the cation, x the number of silicon oxides, usually ranging from 2-10 and y the number of water molecules, ranging from 2-7. In the first set of parentheses are exchangeable cations while in the second set is the structural tetrahedral framework (Mumpton and Fishman, 1977).

Zeolites have several properties which include an ability to act as a molecular sieve to separate molecules according to size. Furthermore, zeolites have an unusual charge distribution within the central cavity that allows species with permanent dipole moments to be adsorbed. This adsorption immobilizes certain molecules with electrostatic forces by forming covalent bonds (Phillips, 1987). Zeolites also have ion-exchange properties that make them useful in agriculture. The exchangeable cations may be displaced easily and exchanged by washing the zeolite in a solution of another ion. This exchange pattern is regulated by the amount of aluminum substitution for silicon (Mumpton and Fishman, 1977).

Since 1965, researchers have conducted experiments using zeolites, such as clinoptilolite and mordenite, as supplements in the diets of pigs, chickens and ruminants (Mumpton and Fishman, 1977). Quisenberry (1968) demonstrated that these zeolites increased caloric efficiency by slowing down the passage of nutrients through the digestive system of chickens. Minato (1968) observed an increase in feed efficiency and health when clinoptilolite and mordenite were added to diets of pigs, chickens and ruminants.

White and Ohlroggi (1974) used clinoptilolite and mordenite to reduce the rate of ammonia absorbed from the rumen. Animals supplemented with urea and diuret utilized more ammonia when a zeolite was present. This was determined from both in vivo and in vitro experiments. Data showed that up to 15% of the NH_4^+ in the rumen was reversibly absorbed by the zeolite. This may have allowed microbes to synthesize protein continuously while protecting animals from toxic levels of the ammonia.

Kondo et al. (1969) fed clinoptilolite at 5% of the diet to calves. This treatment improved growth rate of calves by stimulating their appetite and reducing the occurrence

of soft feces and diarrhea. Pond (1984a) used a similar treatment in sheep to determine the effect of clinoptilolite on ammonia toxicity. He found that the sheep dosed orally with 2 g of clinoptilolite had a NH_4^+ exchange capacity of 1.88 meq/g clinoptilolite/kg body weight. The exchange capacity provided protection against ammonia toxcity when the sheep were dosed orally with .5 g of urea/kg body weight. He stated that this ratio may be insufficient for animals consuming greater quantities of urea.

Pond (1984b) studied the response of lambs fed concentrate diets containing clinoptilolite and zeolite NaA. In this study, it was observed that clinoptilolite had a positive effect on the feed/gain ratio, while zeolite NaA had a detrimental effect. There was no explanation for this observation. Pond (1985) found that clinoptilolite fed at the rate of 2% of the diet will improve weight gain in intact male lambs fed a high concentrate diet. This may be due to the more constant availability of ammonia nitrogen for microbial synthesis of protein. The diet consisted of more than 11% crude protein, with 15% crude protein being the maximum level during the finishing period.

Recently, synthetic zeolites have been used to alleviate symptoms caused by mycotoxins. These mycotoxins include aflatoxins and compounds made by Fusarium molds. Mycotoxins are toxic compounds produced by fungi. They cause death or disease when consumed by animals (Phillips, 1987). Aflatoxins are biosynthesized by flavis and parasiticus species of Aspergillus fungi (Phillips, 1987). "Aflatoxins are the most widely recognized mycotoxin due to its carcinogenic potential and widespread natural occurrence" according to Huff et al. (1987). They stated also that there are at least 200 additional fungal metabolites that could be classified as mycotoxins. Aflatoxins cause many problems in cattle that are similar to those caused by E+ fescue. Signs of toxcity

include poor reproductive performance, reduced feed intake, unthriftiness and reduced weight gain (Whitlow, 1987).

Recently, a hydrated sodium calcium aluminosilicate (HSCAS), marketed under the trade name 'Novasil'[1], has been used to alleviate problems caused by ingestion of aflatoxin B_1. Phillips (1987) conducted a study using day-old chicks which were fed control diets or diets supplemented with 0.5% HSCAS, 7.5 ppm aflatoxin B_1 or 0.5 HSCAS and 7.5 ppm aflatoxin B_1. Addition of HSCAS reduced the incidence of decreased weight gain observed when chicks consumed diets with aflatoxin B_1. Livers from chicks fed HSCAS were not different from those of controls, while livers of chicks fed aflatoxin without HSCAS had a pale, friable appearance.

Phillips (1987) did a study with chickens also, using ^{14}C-labeled aflatoxin at levels calculated to deliver 20 and 80 ppb total aflatoxin. The treatments used in this study included 20 and 80 ppb total aflatoxin without HSCAS and 20 and 80 ppb aflatoxin plus either 0.1% or 0.5% HSCAS added to the diet. Samples of blood were taken from chicks at time intervals of .5, 1, 2, 4 and 6 h after feeding. At 0.5% of the diet, HSCAS reduced the level of radioactivity present in the blood of chickens throughout the test period. Feeding HSCAS at the rate of 0.1% of the diet was shown to be not as effective as the rate of 0.5% of the diet. Wyatt (1987) found that HSCAS could be used in poultry rations to reduce the effects of certain mycotoxins produced by Fusarium molds. In his study, a standard corn-soybean meal starter ration was fed without or with HSCAS (1% of diet). At the end of the three-week study, the chicks were killed and autopsy was

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[1]Englehart Corp., Cleveland, OH.

performed. Forty percent of the chicks, regardless of treatment, exhibited slight necrosis of the oral cavity, mild gizzard erosion and slight irritation of the duodenum. The chickens consuming 1% HSCAS had heavier body weights than those not receiving HSCAS. Broilers fed HSCAS (.5% of diet) had a slightly higher livability than those on control diets, indicating that the HSCAS was alleviating some of the problems caused from losses (Brake, 1987). The broilers consuming HSCAS had .05 lb better weight gain and 2.4 points better feed conversion. The improved weight gain and feed efficiency resulted in a 0.47 cents per dressed pound cost savings than when a diet without HSCAS was fed. No differences were seen in coloration, undergrades or total condemnations. The only difference they noticed was in amount of manure burns. Animals with HSCAS had fewer manure burns than those without HSCAS. Since HSCAS has the ability to adsorb aflatoxins in the digestive tract, it was hypothesized that HSCAS also may adsorb ergopeptine alkaloids and minimize detrimnental effects of consuming endophyte infested fescues.

CHAPTER II

MATERIALS AND METHODS

In Vitro Studies

The first of two in vitro experiments was designed to determine how pH would affect the binding of ergotamine, an ergopeptine alkaloid, to HSCAS. A completely randomized experimental design was utilized with a 2 X 6 factorial arrangement of treatments. Main effects were HSCAS (without or with 150 mg/tube) and pH (2.5, 5.0, 6.0, 7.5, 7.8 or 8.0). Duplicate tubes of each treatment combination were prepared with 9 ml of buffer and 1 ml of 2% tartaric acid containing 2 mg/ml ergotamine tartrate. Buffer solutions at pH of 5, 6, 7.8 and 8 were made with sodium acetate and acetic acid. The buffer at pH 2.5 was made with KH_2PO_4 and H_3PO_4. The buffer used for pH 7.5 was artificial rumen saliva commonly used for in vitro digestion studies (Tilley and Terry, 1963).

Tubes were mixed on a mechanical shaker for 1 h, centrifuged 5 min at 575 X g to precipitate HSCAS, and the supernatant decanted. Ergotamine was extracted from the HSCAS pellet and the supernatant by solvent partitioning. First, the supernatant or pellet was washed with three 10-ml aliquots of ethyl acetate in which ammonium hydroxide was added dropwise, as required, to raise the pH to a range between 8 and 10. After each addition of ethyl acetate aliquot and base, the solution was mixed on a mechanical shaker for 30 min. The organic layers were extracted by suction and composited for each tube. The ethyl acetate mixture was washed three times with 5-ml aliquots of chloroform. The chloroform layer was composited for each tube then washed

with three 5-ml aliquots of 2% tartaric acid. The acid layers were combined for each tube, and the total volume brought to 20 ml with 2% tartaric acid. Ergotamine concentrations in acid solutions were determined colorimetrically (Michelon and Kelleher, 1963).

Data were analyzed by analysis of variance using the GLM procedure of SAS (1985). Variables included in the model were pH and HSCAS and their interaction. Since the interaction was not significant, it was added to the error term. Differences among treatment means were assessed by least-squares mean separation.

The second in vitro study was designed to determine the effect of HSCAS on ergotamine recovery during ruminal and post-ruminal digestion. In vitro fermentation with ruminal fluid and digestion with acid-pepsin were carried out according to Tilley and Terry (1963). Treatments were applied in a 2 X 3 factorial arrangement in a completely randomized experimental design for both the microbial digestion experiment and the acid-pepsin digestion experiment. Each experiment was replicated twice. The main effects in both experiments were HSCAS (without or with 75 mg) and time of incubation (24, 48 or 72 h). All samples undergoing acid-pepsin digestion had been exposed previously to a 48-h digestion in ruminal fluid. Triplicate tubes of each treatment combination in each of two runs contained 0.5 g of ground timothy hay and 40 ml of artificial saliva:rumen fluid mixture in a 50:50 ratio. Ergotamine was added as 1 ml of 2% tartaric acid containing 2 mg ergotamine tartrate with the exception of blank tubes. Digestion was arrested by immediate centrifugation and extraction.

Tubes were centrifuged at 575 X g to precipitate HSCAS and other solids, and the pH of the supernatant was measured. The supernatant was separated from the

pellet, and solvent partitioning was used to extract ergotamine from both the supernatant and the pellet, as described previously.

Data were analyzed by analysis of variance using the GLM procedure of SAS (1985). Variables included in the model statement were HSCAS, time of incubation, run and all interactions. Since there was a significant interaction of run with other variables for acid-pepsin digestion, each run was analyzed separately. Interactions which were not significant were added to the error term. Differences among treatment means were determined by least-squares mean separation.

Rat Growth Study

Forty-eight white Sprague-Dawley rats, with an initial average weight of 259 g, were used in a 28-d feeding trial. Rats were blocked by weight and location within racks and allotted to one of eight dietary treatments in a randomized complete block experimental design. Animals were housed in individual cages in an environmentally-controlled room. Temperature was maintained at about 23 C and a daily 12-h light and 12-h dark cycle was maintained throughout the trial.

Animals were fed at the beginning of the 12-h dark period. Food was removed during the light cycle to prevent excessive wastage. Animals were allowed *ad libitum* access to water. Diets were formulated using 60% semipurified basal diet (BD) and 40% E+ or E- fescue seed (Table 1) which had been ground to pass a 2-mm screen. Seed designated as E+ was 80% infested with A.coenophialum and E- seed contained less than 5% infestation. Diets containing 2% HSCAS had HSCAS substituted for 1.2% BD and 0.8% E+ or E- seed. Diet compositions were: 1) 40% E+ seed and 60% BD, 2) 39.2% E+ seed, 58.8% BD and 2% HSCAS, 3) 40% E- seed and 60% BD, 4) 39.2% E-

Table 1. Composition of diets used for the rat growth study.

Ingredient	Percent of Diet (as fed basis)
Seed (E+ or E-)[a]	40.0
Corn starch	25.3
Casein	13.4
Sucrose	10.0
Corn oil	5.0
Mineral mixture[b]	3.5
Alfa cell	2.4
Vitamin mixture[c]	0.2
D-L-Methionine	0.2

[a]E+ was 80% infested with Acremonium coneophialum, E- was <.5% infested.

[b]Mineral mixture (per Kg of diet): Calcium phosphate dibasic, 17.5 g; Sodium Chloride, 2.59 g; potassium citrate monohydrate, 7.7 g; potassium sulfate, 1.82 g; magnesium oxide, .84 g; manganous carbonate (43-48% Mn), 0.122 g; ferric citrate (16-17% Fe), 0.21 g; zinc carbonate (70% ZnO), 0.056 g; cupric carbonate, (53-55% Cu), 0.01 g; potassium iodate, 0.35 mg; sodium selenite, .35 mg; Chromium potassium sulfate, 0.02 g; sucrose, 4.13 g.

[c]Vitamin mixture (per kg of diet): Thiamin·HCl, 100 mg; Niacin, 100 mg; Riboflavin, 16 mg; Ca-pantothenate, 20 mg; B_{12}, .02 mg; Pyrodoxine·HCl, 6 mg; Biotin, .6 mg; Folic acid, 4 mg; Menadione, 5 mg; Retinyl acetate, 10,000 IU; Cholicalciferol, 600 ICU.

seed, 58.8% BD and 2% HSCAS, 5) same as diet 3 with each animal pair-fed with a counterpart receiving diet 2, 7) same as diet 3 with the addition of ergotamine tartrate (ERT) at the rate of 6 μg/g of feed and 8) same as diet 4 with the addition of 6μg A/g of feed. Diets were analyzed for kjeldahl-N and gross energy (A.O.A.C., 1984).

Feed consumption and body weights were recorded daily. Rectal temperatures were taken weekly. Rats were extinguished at the end of the trial by cranial percussion. Blood samples were taken by heart puncture, allowed to coagulate and serum was harvested by centrifugation. Serum was assayed for prolactin concentrations (PRL) by radioimunioassay (McKenzie, 1987). Following blood collection, whole kidney, liver and testes were excised, weighed and frozen for further analysis. The liver was analyzed for cytochrome P-450 activity by a modification of Omura and Sato (1964) method.

Data were analyzed by analysis of variance using the GLM procedure of SAS (1985). Variables initially included in the model were diet, block and their interaction. Since neither block nor the block X diet interaction were significant, they were included in the error term. Differences among treatment means were determined by planned orthogonal contrasts.

Sheep Metabolism Study

A sheep metabolism trial was conducted to measure the effects of HSCAS on reducing signs of fescue toxicosis. In addition, the effects of HSCAS on mineral metabolism was investigated. Sixteen crossbred wethers (8 were Dorset X Finn and 8 Suffock X Hampshire) weighing about 60 kg were blocked by breed and weight and randomly assigned to treatments in a randomized complete block experimental design with a 2 X 2 factorial arrangement of treatments. Treatments were: E- fescue hay; E-

hay fescue plus HSCAS; E+ fescue hay and E+ fescue hay plus HSCAS. The trial consisted of a 10-d preliminary period followed by a 7-d collection period. Animals were housed in metabolism crates to ensure a separate collection of urine and feces. Artificial incandescent light was on constantly throughout the trial.

Animals were fed twice daily (0800 and 1700 h) 55g of approximately 49.95 g corn, 4.05 g trace mineral salt and either none or 10 g HSCAS followed by 440 g of hay. The hay had been chopped in a hammer mill to pass a 2.54-cm screen. Access to hay was allowed for a 1-h period twice daily. Following the access to hay, water was provided to each animal for 1 h.

During the collection period, 200 g grab samples of E+ and E- hays were obtained daily and composited within each hay treatment. A 10% sample of feces was taken daily, dried at 60 C and composited over time for each animal. Urine was collected daily in 20 ml of 6 N HCl, diluted to 1000 ml, and a 10% aliquot was composited by animal over time and stored at 4 C. Analysis of the hays and feces included kjeldahl-N and DM concentrations according to A.O.A.C. (1984), and NDF, ADF and lignin concentrations according to Goering and VanSoest (1970). One-gram samples of hay and feces were dry-ashed at 600 C then digested with 3 ml of 6 N HCl. Mineral analysis was performed on the diluted digesta by atomic absorption spectrometry for the determination of Cu, Ca, Mg, Zn, Mn, Na and K (A.O.A C., 1984). Colorimetric procedures were used for the determination of S (Häkkinen, 1963) and P (Fiske and Subbarow., 1925).

Urine samples were analyzed for concentrations of kjeldahl-N. Concentrations of Cu, Ca, Mg, Zn, Mn, Na and K were determined by dry-ashing 5 ml of urine and digesting samples in 6 N HCl, then analyzing by atomic absorption spectrometry. Urine

S was determined according to Häkkinen (1963) and P according to Hawk (1948).

Rectal temperatures and respiration rates and ambient temperature were recorded daily just prior to the evening feeding. Blood was collected via jugular venipuncture 4 h after the morning feedings on day 1, 4 and 7 of the collection period. Blood was centrifuged, serum being harvested and frozen for later analysis.

Serum PRL were determined by radioimunoassay (McKenzie, 1987). Serum Cu was determined by atomic absorption spectrometry after deproteinizing with 1 ml of tricarboxylic acid (A.O.A.C., 1984).

Activity of the following enzymes were measured in serum using the appropriate diagnostic kits[1]: alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and τ-glutamyltransferase (GGTP). Appearance of these enzymes in serum is an indicator of liver damage.

Ruminal fluid was collected at the end of the collection period via stomach intubation 4 h after morning feeding and its pH was recorded immediately. Ruminal fluid was analyzed for concentrations of ammonia nitrogen (NH_3-N) (Chaney and Marbach, 1962) and VFA (Erwin et al., 1961).

Data were analyzed using the GLM procedure of SAS (1985). Variables included in the model were infestation status, HSCAS and their interactions; if an interaction was not significant it was added to the error term. Differences among treatment means were determined by least-squares mean separation.

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[1]Sigma Chemical Co., St. Louis, MO.

CHAPTER III

RESULTS AND DISCUSSION

In Vitro Studies

Recovery of ergotamine from supernatant of buffer solutions was dependent on both HSCAS and pH. At pH levels ranging from 2.5 to 7.8, there was an apparent binding of ergotamine to HSCAS, as indicated by low recovery of ergotamine from supernatant and a high recovery from the HSCAS pellet (Table 2). However, at pH 8, ergotamine did not bind to HSCAS, as indicated by a high recovery of ergotamine from the supernatant and no recovery of ergotamine from the pellet. Ergotamine recovery from supernatant of control tubes was similar for all pH levels between 2.5 and 7.8. At pH 8, a larger recovery was observed than at pH levels 2.5, 5, 6 or 7.8. Total recovery of ergotamine from HSCAS treated tubes at pH of 2.5, 5, 6, 7.5 or 7.8 was incomplete and lower than at pH 8. Total recoveries of ergotamine were lower from tubes treated with HSCAS than from control tubes at pH 5.0, 6.0, 7.5 and 7.8 and tended to be lower ($P<.1$) at pH 2.5. Lower recovery of ergotamine from tubes with HSCAS at 7.8 or less may have been due to irreversible binding of some ergotamine to HSCAS. These results suggest that ergot alkaloids could bind to HSCAS at physiological pH levels found in the digestive tract. These results indicate also that ergotamine does not bind to HSCAS at a pH of 8. Organic extractions should be at pH 8 or above.

In the second in vitro study, the effects of fermentation with rumen fluid on ergotamine recovery were not different among runs; therefore, the data were combined for analysis. Recovery of ergotamine from the supernatant after in vitro fermentation

Table 2. Recovery of ergotamine from buffered solutions without (control) or with 150 mg hydrated sodium calcium aluminosilicate (HSCAS) at various pH levels.

	Control	HSCAS (%)			
pH	Supernatant/Total	Supernatant	Pellet	Total	SE
2.5	93.0^{a,c}	8.0^{a,d}	78.0^{a}	86.0^{a,c}	1.74
5.0	89.5^{a,c}	6.0^{a,d}	68.5^{a}	74.5^{a,d}	1.74
6.0	85.5^{a,c}	6.0^{a,d}	71.0^{a}	77.0^{a,d}	1.74
7.5	98.5^{a,b,c}	2.0^{a,d}	73.0^{a}	75.0^{a,d}	1.74
7.8	94.5^{a,c}	2.5^{a,d}	74.5^{a}	77.0^{a,d}	1.74
8.0	100.5^{b}	106.0^{b}	0.0^{b}	106.0^{b}	1.74

^{a,b}Means within the same column with different superscripts differ ($P < .05$).

^{c,d}Means within supernates or within totals within the same row with different superscripts differ ($P < .05$).

with rumen fluid was not different between those without or with 75 mg HSCAS treatments (Table 3). These results are different from those obtained when ergotamine was added to buffer solutions in experiment 1 and suggest that ergotamine was binding to microbial or feed particles. After 24 h, there appeared to be some release of bound ergotamine in tubes with or without HSCAS, because ergotamine recovery from supernatant was higher after 48 and 72 h of fermentation than after 24 h. This may be due to greater digestion of feed particles or microbial cell lysis. Ergotamine recovery from pellets containing HSCAS was greater than that from pellets without HSCAS at 24 and 72 h. Recovery of ergotamine from pellets with HSCAS was higher at 24 h than at 48 h, but there was no difference between 24-h and 48-h tubes without HSCAS (time interval X HSCAS interaction). The reason for reduced recovery of ergotamine from pellets with HSCAS at 48 h is not known. Total recovery of ergotamine was higher at 72 h than at 24 h or 48 h and was higher from tubes containing HSCAS than from those without HSCAS at 24 h and 72 h. A HSCAS interaction was observed with respect to total recovery similar to that observed with respect to recovery from pellets. Total recoveries of ergotamine averaged 51% and 57% from tubes without and with HSCAS, respectively. These were lower than total recoveries of ergotamine in experiment 1, which averaged 92 and 76% from tubes without and with HSCAS, respectively. The low total recovery after fermentation could have been due to binding of ergotamine to compounds other than HSCAS, preventing their recovery by the organic extraction procedures used in this study. This hypothesis is supported by a greater recovery of ergotamine from tubes containing HSCAS than from those without HSCAS.

Ergotamine recoveries during the acid-pepsin digestion stage were not similar among runs; thus, runs were analyzed separately. Data from the first run are shown in

Table 3. Effect of hydrated sodium calcium aluminosilicate (HSCAS) and time of indubation on recovery of ergotamine after in vitro digestion with rumen fluid.

	Without HSCAS			75 mg HSCAS			
Item	24h	48h	72h	24h	48h	72h	SE[a]
Supernatant	6.6[b]	12.5[c]	11.8[c]	5.4[b]	12.1[c]	10.8[c]	1.17
Pellet[f]	38.6[b]	36.0[b,c]	47.2[d]	53.8[e]	32.0[c]	58.2[e]	1.47
Total[f]	45.2[b]	48.5[b]	59.0[c]	59.2[c]	44.1[b]	69.0[d]	2.11

[a]SE = pooled standard error.

[b,c,d,e]Means in the same row without a common superscript are different ($P<.05$).

[f]Significant effect due to time interval X HSCAS interactions ($P<.05$).

Table 4. Ergotamine recovery from supernatant of tubes containing HSCAS was lower than from those without HSCAS. This supports the data from experiment 1 showing that ergotamine was binding to HSCAS. More ergotamine was recovered from the pellet of tubes containing HSCAS than from those without HSCAS. Again, this supports the findings in experiment 1, that ergotamine was binding to HSCAS. In run 2, as in run 1, the recovery of ergotamine from supernatant of tubes containing HSCAS was lower than that from tubes not containing HSCAS (Table 5) and ergotamine recovery from the pellet was lower for pellets without HSCAS than for those with HSCAS. Unlike in run 1, there was a time interval X HSCAS interaction in run 2 for supernatant recoveries of ergotamine. The appearance of more alkaloid in the pellets containing HSCAS than in those without HSCAS indicates that there was some binding of alkaloid to HSCAS. The reason recovery of ergotamine was lower in experiment 2 than in the first in vitro experiment using buffers may be due to other compounds competing with HSCAS for binding ergotamine.

Rat Growth Study

Diets contained endophyte infested (E+) or noninfested (E-) seed fed *ad libitum*, noninfested seed which was pair-fed to E+ counterpart animals and noninfested seed with 6 ppm ergotamine tartrate (ERT). Crude protein and gross energy concentrations were similar among diets (17.0 ± .5% and 4.5 ± .1 kcal/g, respectively).

Weight gains of rats were similar when consuming diets without or with 2% HSCAS. Weight gains of rats consuming pair-fed diets were similar to gains of those consuming E+ diets (Tables 6 and 7). Rats consuming diets containing E+ diets gained less weight than those consuming E- diets. Jackson et al. (1987) reported a similar trend

Table 4. Effect of hydrated sodium calcium aluminosilicate (HSCAS) on recovery of ergotamine from in vitro tubes after acid-pepsin digestion for run 1.

Item	HSCAS		SE[a]
	Without	75 mg	
Supernatant	18.44[b]	6.22[c]	2.62
Pellet	22.66[b]	57.44[c]	2.56
Total tube	41.10[b]	63.66[c]	4.24

[a]SE = pooled standard error.

[b,c]Means in the same row without a common superscript are different (P<.05).

Table 5. Effect of hydrated sodium calcium aluminosilicate (HSCAS) and time of incubation on recovery of ergotamine from in vitro acid-pepsin digestion for run 2.

	Without HSCAS			75 mg HSCAS			
Item	24h	48h	72h	24h	48h	72h	SE[a]
Supernatant[f]	40.3[b,c]	46.0[b]	33.3[c,d]	28.3[d]	10.7[e]	12.3[e]	2.95
Pellet[g]	17.7	24.0	31.0	45.3	35.6	59.7	7.65
Total	58.0	70.0	66.0	73.7	46.3	72.0	8.38

[a]SE = pooled standard error.

[b,c,d,e]Means within main effects in the same row without a common superscript are different (P<.05).

[f]Time interval X HSCAS interactions (P<.05).

[g]HSCAS effect (P<.05).

Table 6. Performance, organ weights and serum prolactin concentrations (PRL) of rats receiving 28 d diets with or without hydrated sodium calcium aluminosilicate (HSCAS) and Acremonium coenophialum infested seed (E+) or non-infested seed (E-) fed ad libitum, pair-fed or with ergotamine tartrate (ERT).

Diet:	E+		E-		E-		E- with ERT		
Intake:	ad libitum		ad libitum		pair-fed		ad libitum		
Item % HSCAS:	0	2	0	2	0	2	0	2	SE[a]
Weight gain, g	26.61	19.06	100.27	91.08	25.69	15.04	87.83	76.60	11.23
Feed intake, g	434.2	435.0	611.1	510.7	445.7	414.3	577.2	577.2	15.75
Gain/feed	0.061	0.04	0.16	0.18	0.06	0.04	0.15	0.13	0.022
Testes wt., g	3.42	3.11	3.60	3.43	3.46	3.56	3.35	3.45	0.124
Kidney wt., g	2.01	2.04	2.47	2.32	1.94	2.04	2.46	2.24	0.077
Liver wt., g	10.03	10.72	13.08	13.16	9.77	10.23	13.56	12.43	0.574
PRL, ng/ml	28.4	14.28	14.36	54.63	83.4	37.76	62.06	48.37	21.8

[a]SE = pooled standard error.

Table 7. Planned contrasts comparing effects on performance, organ weights and serum prolactin concentrations (PRL) of rats receiving 28 d diets with or without hydrated sodium calcium aluminosoilicate (HSCAS) and Acremonium coenophialum infested seed (E+) or non-infested seed (E-) fed ad libitum, pair-fed or with ergotamine tartrate (ERT).

	Orthogonal Contrasts of Diets[a]						
Item	Diet 1 vs Diet 2	Diet 3 vs Diet 4	Diet 5 vs Diet 6	Diet 7 vs Diet 8	Diets 1 & 2 vs Diets 5 & 6	Diets 3 & 4 vs Diets 7 & 8	Diets 1 & 2 vs Diets 3 & 4
Wt gain, g/total	NS	NS	NS	NS	NS	NS	*
Feed intake, g/total	NS	*	NS	NS	NS	NS	*
Gain/feed	NS	NS	NS	NS	NS	NS	*
Testes wt., g	NS	NS	NS	NS	**	NS	*
Kidney, g	NS	NS	NS	NS	NS	NS	*
Liver, g	NS	NS	NS	NS	NS	NS	NS
PRL, ng/ml	NS	NS	NS	NS	**	NS	NS

[a]Diet 1 = E+ seed, ad libitum
Diet 2 = E+ seed, 2% HSCAS, ad libitum
Diet 3 = E- seed, ad libitum
Diet 4 = E- seed, 2% HSCAS, ad libitum
Diet 5 = E- seed, pair-fed
Diet 6 = E- seed, 2% HSCAS, pair-fed
Diet 7 = E- seed, ad libitum, 6 μg/g ERT
Diet 8 = E- seed, 2% HSCAS, ad libitum, 6 μg/g ERT

* ($P<.05$).

** ($P<.1$).

NS = no significant difference

in their study. Rats consuming ERT diets had gains similar to those of rats consuming E- diets. Therefore, the addition of 6 ppm ergotamine tartrate did not produce the detrimental effect on weight gain when rats were fed E+ fescue diets.

Osborn et al. (1988) suggested that signs of fescue toxcity could be induced with ergopeptine alkaloids. Their study suggested that the addition of 30 ppm ergotamine tartrate to E- diets resulted in steers exhibiting signs of fescue toxicosis similar to signs exhibited by steers fed E+ fescue. In the present study, 6 ppm were used because this concentration was found to be the ergovaline content found in fescue by Lyons (1986). However this level of ergotamine tartrate was not sufficient to produce signs of toxicosis.

Consumption followed the trend shown in rat weight gain except that rats receiving E- consumed more than those receiving NE-. Animals receiving E+ and NE+ treatments consumed less than those receiving E- and NE- treatments. Animals on PE- diet treatments had intakes that were similar to intakes of those on E+ diets. Therefore, it appears that the reduction in weight gain of rats consuming E+ compared to that of those consuming E- was due only to differences in feed intake. Reductions of feed intake and gain also were observed by Jackson et al. (1987) when they fed rats E+ fescue seed and compared them to those receiving E- fescue seed.

Within each diet (E+, E-, PE-, AE-), addition of HSCAS had no effect on efficiency of gain. Rat gain per unit of feed was similar in rats on E+ and PE- treatments. Gains per unit of feed were similar also among animals on E- and AE- diets. The similar gain per unit of feed was unexpected from AE- treatments when compared to the E- diets. It had been hypothesized that the weight gain per unit of feed would be higher in the control animals since an extract containing ergot alkaloids has been reported to cause rats to have a reduced gain (Jackson et al. 1987). As expected,

gain per unit of feed was greater in rats on E- treatments than in those on E+ treatments due to the trend shown with feed intake and gain.

Animals on E+ diets had lower testes weights than those on E- diets (Table 6 and 7). This was at least partially a function of the larger weight gain of rats consuming E- diets. However, rats consuming E+ diets tended ($P<.1$) to have lower testes weights than those on PE- diets. This is indicative of an effect of E+ fescue on testes development and, possibly on reproduction in animals. Kidney and liver weights of rats receiving E+ diets were lower than organ weights of those receiving E- diets, but similar to those of rats on PE- treatments. These differences probably were a function of body weight since kidney and liver weights of pair-fed animals were similar to those receiving E+ diets.

Serum PRL tended to be lower ($P<.1$) in rats receiving E+ diets than for those receiving PE- diets. Jackson et al. (1987) found that rats receiving E+ diets had lower PRL concentrations than had those receiving pair-fed E- diets in 2 of 3 sampling times. They reported also that PRL of rats consuming E- diets was similar to those of rats consuming E+ treatments *ad libitum* in 2 of 3 sampling periods.

Cytochrome P-450 concentrations are not reported because of a negative reading which is believed to have been caused by interfering substances in the rat livers. Analysis was conducted for cytochrome B_5 which was thought to be the interfering substance. However, this was not the cause for the interference; thus, the procedure had to be abandoned due to a lack of tissue.

Sheep Metabolism Study

Rectal temperatures (Table 8) on days 1, 3 and 7 were greater in sheep receiving E+ hay than in sheep receiving E- hay and mean daily temperatures tended to be higher ($P<.1$) in sheep receiving E+ hay than in sheep receiving E- hay. Rectal temperatures were not affected by the addition of HSCAS. Respiration rates (Table 9) were similar among treatments. Concentrations of PRL (Table 10) were similar among hay treatments, but were lower in sheep receiving no HSCAS supplementation than in those receiving a 2% dietary HSCAS supplementation. The similarity of PRL among hay treatments may have been due partially to photoperiod. Studies have indicated that PRL may not decrease when the photoperiod is decreased. For example, in the fall, PRL concentrations may be as great as concentrations measured in the spring. Chestnut et al. (1989) reported that, during the spring grazing period, a quadratic decrease in PRL was observed in steers grazing fescue pastures with increasing levels of infestation. However, at the end of the summer grazing period there were no differences in PRL among steers grazing tall fescue pastures of different levels of infestation. During this study, overhead lights were left on 24 h/d in an effort to counteract the decreased photoperiod due to seasonal light patterns and dreary weather conditions for the 10 d adjustment and 7 d collection period. However, the lighting may not have been sufficient to counteract the seasonal photoperiod effect.

Blood enzymes indicative of liver disorders were used in this study as a clinical diagnosis to determine if the alkaloids in E+ fescue cause liver dysfunction. Piper (1989) suggested that liver damage may occur in the presence of alkaloids in fescue. He stated that " . . . the liver may initially remove toxins, but with prolonged grazing and subsequent liver damage toxin degradation is retarded." He found that sheep grazing

Table 8. Rectal temperatures of sheep fed Acremonium coenophialum infested tall fescue (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infestation		HSCAS (%)		
Day	E+	E-	0	2	SE[a]
	---------- C ----------				
1	39.4[b]	39.2[c]	39.2	39.4	.05
2	39.3	39.3	39.2	39.2	.06
3	39.4[b]	39.2[c]	39.2	39.3	.06
4	39.5	39.3	39.4	39.4	.08
5	39.4	39.3	39.3	39.3	.08
6	39.5	39.5	39.9	39.5	.09
7	39.6[b]	39.4[c]	39.4	39.5	.08
Mean	39.4[d]	39.3[e]	39.3	39.3	.05

[a]Pooled standard error.

[b,c]Means within main effects with different superscripts differ (P<.05).

[d,e]Means within main effect with different superscripts differ (P<.07).

Table 9. Respiration rates of sheep fed Acremonium coenophialum infested tall fescue (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infestation		HSCAS (%)		
Day	E+	E-	0	2	SE[a]
	Breaths Per Minute				
1	104	107	108	104	5.4
2	109	105	108	106	4.7
3	111	108	111	108	4.7
4	130	121	120	132	10.5
5	123	125	119	129	11.5
6	111	125	120	116	7.4
7	111	118	109	119	10.3
Mean	117	113	113	116	5.6

[a]Pooled standard error.

Table 10. Serum prolactin concentrations (PRL) of sheep fed Acremonium coenophialum infested tall fescue (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infestation		HSCAS (%)		
Item	E+	E-	0	2	SE[a]
PRL, ng/ml	47.7	45.5	36.5[b]	56.7[c]	6.6

[a]Pooled standard error.

[b,c]Means within main effects with different superscripts differ (P=.05).

E+ perennial ryegrass had elevated aspartate amino transferase levels (AST) with no significant difference in alkaline phosphotase (ALP) and τ-glutamyl transferase (GGTP). Bond et al. (1984) reported that steers grazing G1-307 fescue had higher levels of ALP than had those grazing G1-306 or Ky-31 fescue in 2 of 3 sampling times. In the present studies, serum sorbitol dehydrogenase activity (SDH) also was measured since it indicates immediate liver damage. When SDH is elevated, it is indicative of hepatocellular injury or liver cell destruction. However, it does not remain elevated over an extended period of time even if the problem persists. Thus, it is useful as a measure for early detection of liver damage. Alkaline phosphotase is useful in diagnosing long-term general liver damage which SDH might not indicate. Alkaline phosphotase is useful to indicate hepatobiliary disease and bone disorders. Gamma-glutamyl transferase determination is used as a tool to eliminate or indicate what is specifically wrong. An elevation in GGTP is indicative of cholelithiasis, hepatic neoplasms, hepatitis, liver cirrhosis or liver metastasis. Elevation of alanine aminotransferase (ALT) and AST may further clarify the liver dysfunction. These enzymes measure liver necrosis, hepatitis, pulmonary infarction, myocardial infarction and poisoning by substances such as medications. In the present study, SDH activity levels were elevated in sheep consuming E+ hay without HSCAS supplementation. Sheep receiving E+ hay with HSCAS had similar SDH activity levels as those receiving E- diets (Table 11). This indicated that there was liver cell destruction or hepatocellular injury caused by the consumption of E+ fescue hay. This indicated also that the sheep consuming HSCAS were being protected from some toxic compounds in E+ fescue. Activity levels of ALP, AST, ALT and GGTP were similar among treatments (Table 11).

Water consumption was reduced by sheep consuming E+ hay without HSCAS as

Table 11. Activity of sorbitol dehydrogenase (SDH), alkaline phosphotase (ALP), τ-glutamyl transferase (GGTP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum of sheep fed Acremonium coenophialum infested tall fescue (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infestation				
	E+		E-		
Enzyme	0 HSCAS	2% HSCAS	0 HSCAS	2% HSCAS	SE[a]
SDH, U/l[b]	17.2[d]	8.4[e]	8.2[e]	9.7[e]	1.67
ALP, U/l	136.8	80.8	97.8	75.7	26.52
GGTP, units/ml[c]	35.0	36.5	32.7	39.7	2.13
AST, U/l	17.5	19.5	19.3	21.4	2.79
ALT, U/l	16.4	14.8	14.3	16.7	2.79

[a]Pooled standard error.

[b]International units/liter.

[c]One unit of GGPT activity is defined as the amount of activity that will liberate 1 nanomole of p-nitroaniline per minute at 25 C under conditions specified as sigma diagonostics procedure no. 415.

[d,e]Means in the same row with different superscripts differ (P<.05).

compared to that of those on other treatment combinations (Table 12). Barth et al. (1989) reported that water intakes of sheep were similar when comparing E+ fescue hay treatments to E- hay treatments during the collection period. They reported that, during the preliminary period water intakes tended to be higher in sheep consuming E+ hay than in those consuming E- hay. As expected, forage consumption was similar among treatments (Table 12). Ruminal pH and concentrations of ruminal VFA and ammonia were similar among treatments (Table 13).

No differences on effect of nitrogen metabolism by treatment were observed (Table 14). Barth et al., (1989) reported that fecal N excretion was lower for sheep consuming E- hay than sheep on E+ hay treatments. They reported also that intake of N and urinary excretion of N were similar in sheep consuming E+ hay and in sheep consuming E- hay.

Serum Cu concentrations were similar among treatments (Table 15). Stoszek et al. (1986) reported a linear relationship between blood plasma Cu and ceruloplasmin oxidase activity. They reported that tall fescue produced rapid Cu depletion in unsupplemented cattle and that it affected intermediary Cu metabolism. However, Bond et al. (1984) reported no effect of E+ fescue consumption on serum Cu levels in cattle.

Infested fescue hay contained higher concentrations of Zn, Mn and K, than noninfested hay which had greater concentrations of Na, Ca and S (Table 16). Because E+ hay had a higher concentration of Ca than E- hay and HSCAS contained Ca, sheep consuming E+ hay or 2% HSCAS had larger Ca intakes than sheep consuming E+ hay or diets without HSCAS, respectively (Table 17). Fecal excretion of Ca by sheep was greater for those receiving E+ hay than by those receiving receiving E- hay. The elevated fecal excretion of Ca by sheep consuming E+ hay may have been due to E+

Table 12. Water and forage dry matter consumption by sheep fed Acremonium coenophialum infested (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infestation			
	E+		E-	
Item	0 HSCAS (SE)[a]	2% HSCAS (SE)	0 HSCAS (SE)	2% HSCAS (SE)
Water, ml/d	1278[b] (51.0)	1458[c] (45.8)	1472[c] (48.6)	1447[c] (47.3)
Forage, g/d	702 (37.1)	767 (37.1)	753 (37.1)	768 (37.1)

[a]Standard error.

[b,c]Means in the same row with different superscripts differ (P<.05).

Table 13. Volatile fatty acid concentrations, ruminal pH and ruminal ammonia concentrations in sheep fed Acremonium coenophialum infested tall fescue (E+) or noninfested (E-) tall fescue hay without or with 2% hydrated sodium clacium aluminosilicate (HSCAS).

	Fescue Infestation		HSCAS (%)		
Item	E+	E-	0	2	SE[a]
Acetic acid, μm/ml	39.5	34.5	36.2	37.7	5.19
Propionic acid, μm/ml	15.6	14.5	14.8	15.3	1.07
Butyric acid, μm/ml	4.7	4.2	4.8	4.1	0.56
Isobutyric acid, μm/ml	1.7	1.6	1.7	1.6	0.55
Valeric acid, μm/ml	1.0	0.9	1.0	1.0	0.09
Isovaleric acid, μm/ml	1.0	1.0	1.0	1.1	0.17
pH	6.5	6.7	6.6	6.6	0.06
NH_3, μg/ml	1.2	1.1	1.2	1.0	0.07

[a]Pooled standard error.

Table 14. Nitrogen utilization by sheep fed Acremonium coenophialum infested tall fescue (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infection		HSCAS		
Parameter	E+	E-	0	2%	SE[a]
N intake, g/d	11.67	10.82	11.04	11.45	0.407
Fecal excretion, g/d	5.67	5.67	5.38	5.98	0.270
Urinary N excretion, g/d	4.20	4.37	4.33	4.24	0.282
Apparent N absorption, % of intake	51.62	47.49	51.46	47.65	1.666
Retained N, % of intake	15.31	7.15	12.04	10.77	2.480

[a]Pooled standard error.

Table 15. Serum Cu concentrations of sheep fed Acremonium coenophialum infested tall fescue (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infestation		HSCAS (%)		
Item	E+	E-	0	2	SE[a]
Cu, mg/ml	0.07	0.07	0.07	0.07	±.004

[a]Pooled standard error.

Table 16. Mineral composition of feedstuffs used to formulate diets for the sheep metabolism study.

Mineral	E+ Hay[a]	E- Hay[b]	Corn	Mineral Mix	HSCAS[c]
Zn, mg/g	0.015	0.012	0.035	1.80	0.015
Mn, mg/g	0.121	0.108	0.009	0.085	0.05
Cu, mg/g	0.004	0.003	0.005	0.035	0.002
Na, mg/g	0.112	0.231	0.058	222.33	0.164
K, mg/g	18.76	15.52	4.46	12.89	0.200
Ca, mg/g	2.08	2.45	2.09	105.41	16.82
Mg, mg/g	3.27	3.40	0.14	22.40	0.59
S, mg/g	2.84	4.35	6.02	1.72	2.96
P, mg/g	2.29	2.26	3.61	41.37	0.32

[a]E+ hya = fescue infested with the endophytic fungus Acremonium coenophialum.

[b]E- = non-infested fescue hay.

[c]HSCAS = hydrated sodium calcium aluminosilicate.

Table 17. Utilization of Ca, P, Na and K by sheep fed Acremonium coenophialum infested tall fescue (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infestation		HSCAS (%)		
Mineral	E+	E-	0	2%	SE[a]
Calcium:					
Intake, g/d	5.28[c]	5.48[d]	5.27[c]	5.54[d]	0.018
Fecal excretion, g/d	4.04[c]	3.68[d]	3.68	4.00	0.098
Urinary excretion, g/d	0.15	0.22	0.20	0.17	0.025
Apparent absorption, % of intake	23.48[c]	32.84[d]	20.17	27.79	1.831
Retention, % of intake	20.64[c]	28.78[d]	26.37	24.73	1.812
Phosphorus[b]:					
Intake, g/d	2.60	2.66	2.60	2.67	0.067
Fecal excretion, g/d	2.53	2.53	2.47	2.58	0.104
Apparent absorption, % of intake	3.01	5.16	4.96	3.22	2.731
Sodium:					
Intake, g/d	2.05[c]	2.16[d]	2.10	2.11	0.004
Fecal excretion, g/d	1.00	1.01	0.99	0.92	0.098
Urinary excretion, g/d	0.59	0.52	0.61	0.50	0.082
Apparent absorption, % of intake	51.36	53.32	52.86	56.44	0.001
Retention, % of intake	22.70	29.16	23.80	33.70	0.0001
Potassium:					
Intake, g/d	17.41[c]	14.96[d]	15.88	16.48	0.590
Fecal excretion, g/d	1.82	1.36	1.44	1.74	0.180
Urinary excretion, g/d	5.19[c]	7.47[d]	6.90	5.77	0.740
Apparent absorption, % of intake	89.63	90.91	90.87	89.66	1.082
Retention, % of intake	60.41[c]	40.91[d]	47.76	53.56	3.961

[a]Pooled standard error.

[b]Phosphorus was not detectable in urine samples.

[c,d]Means within main effect with different superscripts differ (P(.05).

hay having more Ca in an unutilizable form than was the Ca in E- hay. Calcium absorption may be limited by parathyroid hormone secreted by the parathyroid, vitamin D_3 produced in the kidney, calcitonin secreted from the thyroid gland, or by the formation of insoluble Ca salts. These factors may have been the cause of decreased Ca absorption in E+ fescue; however, none of these factors was determined in this study. Percent of apparent absorption of Ca was greater for sheep receiving E- hay than for those receiving E+ hay. This corresponds to the increase Ca intake in animals consuming E- hay. Powell et al. (1978) and Pendlum et al. (1980) found mean Ca concentrations in tall fescue of 4 mg/g, higher than in the present study. However, percent apparent absorption of Ca by lambs consuming tall fescue in those studies ranged from 19 to 24%. Similar results were found in the present study.

Phosphorus intake, excretion and apparent absorption were not affected by endophyte infestation of hay or supplementation with HSCAS. Phosphorus concentrations in fescue hays were similar to those observed by Powell et al. (1978) and Pendlum et al. (1980). Urinary P was not detectable with the colorimetric procedure used in the present study. Powell et al. (1978) reported that lambs receiving tall fescue had very low urinary P, ranging from 0 to 0.06 g/d. Pendlum et al. (1980) reported urinary P excretion of lambs consuming fescue was low, ranging from 0.03 to 0.06 g/d. Apparent absorption coefficients of P were similar for the present study when compared to the findings of Powell et al. (1978) and Pendlum et al (1980). Baker and Chung (1989) also reported that HSCAS did not affect P utilization when added (1%) to poultry diets.

Sodium intake by lambs was greater for those receiving E- hay than for those receiving E+ hay (Table 17). The elevated intake of Na by lambs on E- hay was due to

the larger concentration of Na in E- hay as compared to that in E+ hay. Apparent absorption, urinary excretion or retention of Na were not affected by endophyte infestation of hay or supplementation with HSCAS. Neither Powell et al. (1978) nor Pendlum et al. (1980) reported Na values from their studies.

Intake of K was greater for lambs consuming E+ fescue than for those consuming E- fescue hay (Table 17). This was due to the higher K content of E+ hay as compared to that of E- hay. Urinary excretion of K was lower in sheep consuming E+ hay than in sheep consuming E- hay. The reason for this is not known. Fecal excretion of K and apparent absorption as a percent of intake were similar among treatments. Powell et al. (1978) and Pendlum et al. (1980) reported mean K concentrations of tall fescue of 20 to 38 mg/g and apparent absorption coefficients of 86 to 95%. Similar results were reported in the present study.

Intake, fecal excretion and percent of apparent absorption of Mg were similar among hay treatments (Table 18). Urinary excretion of Mg by sheep consuming E+ fescue was lower than that by sheep consuming E- fescue hay. Dietary intake and urinary excretion of Mg were similar among HSCAS treatments (Table 18). Fecal excretion of Mg by sheep was lower for those receiving diets without HSCAS than for those receiving the 2% HSCAS supplement. The apparent absorption and retention coefficients were lower in sheep consuming the 2% addition of HSCAS, indicating that HSCAS caused more Mg to be excreted in the feces and that the animal was not able to utilize the Mg that otherwise would be available. Powell et al. (1978) and Pendlum et al. (1980) reported mean Mg concentrations of tall fescue of 1.8 to 2.6 mg/g and apparent absorption coefficients of 23 to 48 %, similar to those in the present study.

Intake and apparent absorption of S by sheep on E- diets was greater than by

Table 18. Utilization of Mg, S, Cu, Mn and Zn by sheep fed Acremonium coenophialum infested tall fescue (E+) or non-infested (E-) tall fescue hay without or with 2% hydrated sodium calcium aluminosilicate (HSCAS).

	Fescue Infestation		HSCAS (%)		
Mineral	E+	E-	0	2%	SE[a]
Magnesium:					
Intake, g/d	3.15	3.36	3.20	3.31	0.100
Fecal excretion, g/d	1.41	1.52	1.30[c]	1.62[d]	0.180
Urinary excretion, g/d	0.17[c]	0.29[d]	0.26	0.20	0.021
Apparent absorption, % of intake	55.63	54.83	59.40[c]	51.07[d]	1.71
Retention, % of intake	50.33	46.31	51.55[c]	45.09[d]	1.61
Sulfur:					
Intake. g/d	2.84[c]	4.15[d]	3.43	3.55	0.081
Fecal excretion, g/d	1.46	1.32	1.19	1.59	0.171
Urinary excretion, g/d	0.71	1.28	1.18	0.80	0.232
Apparent absorption, % of intake	49.46[c]	68.10[d]	64.31	53.22	4.723
Retention, % of intake	24.88	37.09	30.92	31.15	8.118
Copper[b]:					
Intake, g/d	0.005[c]	0.004[d]	0.004	0.004	0.0001
Fecal excretion, g/d	0.003	0.003	0.003	0.003	0.0003
Apparent absorption, % of intake	25.18	20.52	28.35	17.35	7.15
Manganese[b]:					
Intake, g/d	0.12[c]	0.10[d]	0.10	0.10	0.004
Fecal excretion, g/d	0.09	0.10	0.08[c]	0.11[d]	0.004
Apparent absorption, % of intake	18.76[c]	5.72[d]	25.86[c]	-1.38[d]	2.745
Zinc[b]:					
Intake, g/d	0.033[c]	0.031[d]	0.032	0.032	0.0005
Fecal excretion, g/d	0.022	0.021	0.022[c]	0.030[d]	0.0010
Apparent absorption, % of intake	33.43	29.39	46.17[c]	16.65[d]	3.213

[a]Pooled standard error.

[b]Was not detectable in urine samples.

[c,d]Means within main effect with different superscripts differ (P<.05).

sheep on E+ diets (Table 18). The HSCAS had no effect upon S intake or apparent absorption by the sheep. Fecal and urinary excretion and percent S retained were similar among treatments. Powell et al. (1978) and Pendlum et al. (1980) reported that the S concentrations in tall fescue ranged from 1.8 to 3.4 mg/g. Similar values were reported in the present study. Apparent absorption coefficients of this study were similar to those of both Powell (1978) and Pendlum (1980). Powell was unable to quantify urinary S, and Pendlum et al. (1980) reported urinary S excretion was .42 to .78 g/d.

Intake of Cu was greater by sheep receiving E+ fescue than by sheep consuming E- fescue (Table 18). This increase in Cu intake was due to a higher Cu concentration in E+ hay than in E- hay. The HSCAS did not affect Cu intake by sheep. Fecal excretion and percent of apparent absorption of Cu was similar among treatments.

Intake and percent of apparent absorption of Mn was greater in sheep consuming E+ diets than for those consuming E- diets. This increase in Mn intake was due to a higher Mn concentration in E+ hay than in E- hay. Fecal excretion of Mn was similar between the forage treatments. The HSCAS supplemented sheep had a higher fecal excretion and a lower percent of apparent absorption of Mn than sheep not receiving HSCAS. This indicates that HSCAS interfered with absorption of Mn.

Intake of Zn was greater for sheep consuming E+ diets than for those consuming E- diets. This increase in Zn intake was due to a higher Zn concentration in E+ hay than in E- hay. Fecal excretion and apparent absorption were similar among sheep receiving the two hay treatments. The addition of HSCAS did not affect the intake of Zn by sheep. However, sheep supplemented with HSCAS had greater fecal excretion of Zn than sheep not receiving HSCAS. The percent of apparent absorption of Zn was

greater in animals not receiving HSCAS as compared to those receiving HSCAS. This indicates that HSCAS interfered with absorption of Zn.

CHAPTER IV

INTERPRETIVE SUMMARY

Three studies were conducted to determine whether HSCAS would alleviate signs of fescue toxicosis. These studies included: in vitro studies, a rat growth study and a sheep metabolism study. These studies demonstrated that supplemental feeding of HSCAS did not alleviate most signs of fescue toxicosis. Although in vitro studies indicated that ergotamine would bind to HSCAS, results from in vitro fermentation and acid-pepsin digestion indicated that the ergotamine was binding also to substances other than HSCAS.

The rat experiments illustrated that the classic signs of fescue toxicosis were not alleviated by dietary supplementation with HSCAS. Differences in weight gain and feed efficiency were due to differences in feed intake rather than for other toxic effects.

The sheep metabolism study also indicated that dietary addition of HSCAS did not alleviate classic signs of fescue toxicity. Sheep consuming E+ fescue had higher levels of SDH activity in their sera than sheep consuming E- fescue. Levels of SDH in serum may be a useful indicator of toxic effects of E+ fescue on livestock. Supplementation with HSCAS reduced elevated SDH activity associated with the consumption of E+ fescue hay, indicating that HSCAS prevented liver cell destruction in sheep consuming E+ hay. The use of HSCAS reduced absorption of Mg, Mn and Zn, indicating that additional supplementation of these minerals may be required when feeding 2% dietary HSCAS.

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