AN ABSTRACT OF THE THESIS OF

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Title: Evaluation of Perennial Ryegrass Straw as a Forage Source for Ruminants.

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David W. Bohnert

We conducted two experiments evaluating perennial ryegrass straw as a forage source for ruminants. Experiment 1 evaluated digestion and physiological variables in steers offered perennial ryegrass straw containing increasing levels of lolitrem B. Sixteen ruminally cannulated Angus X Hereford steers (231 ± 2 kg BW) were blocked by weight and assigned randomly to one of four treatments (**TRT**). Steers were provided perennial ryegrass straw at 120% of the previous 5-d average intake. Prior to straw feeding, soybean meal (**SBM**) was provided (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein. Low (**L**) and high (**H**) lolitrem B straws (<100 and 1550 ppb, respectively) were used to formulate TRT diets: **LOW** (100% L); **LOW MIX** (67% L:33% H); **HIGH MIX** (33% L:67% H); **HIGH** (100% H). Intake and digestibility of DM and OM, and ruminal pH, total VFA, and NH_3-N were not affected by increasing lolitrem B concentration ($P > 0.13$). Ruminal indigestible ADF (**IADF**) fill increased linearly ($P = 0.01$) and IADF passage rate (%/h) decreased linearly ($P = 0.04$) as lolitrem B level increased. Experiment 2 evaluated performance and production of 72 Angus X Hereford cows (539 ± 5 kg

BW) consuming perennial ryegrass straw containing increasing levels of lolitrem B during the last third of gestation. Cows were blocked by body condition score (**BCS**) and randomly assigned to one of three TRT. Cows were provided perennial ryegrass straw ad libitum and supplemented with SBM (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein. Mixtures of a L and H lolitrem B straw (467 and 2017 ppb, respectively) were used to formulate TRT diets: LOW (100% L); MIX (50% L:50% H); HIGH (100% H). Thirteen of 24 cows on the HIGH TRT exhibited signs of ryegrass staggers and were removed from the study. Dry matter intake was not affected ($P > 0.12$) by increasing lolitrem B concentration; however, estimated DM digestibility decreased linearly ($P < 0.01$) as lolitrem B concentration increased. Lolitrem B concentration did not influence pre- or post calving weight or BCS change ($P > 0.10$). These data suggest that feeding perennial ryegrass straw containing up to 1550 ppb lolitrem B does not adversely affect nutrient digestion or physiological response variables in steers. However, providing straw with a lolitrem B concentration of approximately 2000 ppb resulted in 54% of cows exhibiting signs of ryegrass staggers. Blending of H and L straws appears to be a successful management practice.

Keywords: Alkaloid, Beef Cattle, Endophyte, Lolitrem B, Perennial Ryegrass, Straw

Evaluation of Perennial Ryegrass Straw as a Forage Source for Ruminants.

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Michael J. Fisher, Author

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CONTRIBUTION OF AUTHORS

In Chapter 2, D. W. Bohnert provided cannulated steers, gestating cows, and facilities for this study. He also assisted with experimental design, data collections, laboratory analysis, and data interpretation. C. J. Ackerman assisted with experimental design and data collections. C. S. Schauer assisted with data collections, laboratory analysis, and data interpretation. T. DelCurto provided cannulated steers, assisted with experimental design, and assisted with data collection. A. M. Craig provided laboratory analysis of lolitrem B and ergovaline within perennial ryegrass straw samples. E. S. Vanzant and D. L. Harmon provided laboratory analysis of volatile fatty acid samples. Additionally, F. N. Schrick provided laboratory analysis of serum prolactin.

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Introduction

The Pacific Northwest is known for its production of grass seed. Consequently, a great deal of agricultural emphasis and effort is put toward the production of grass seed in Oregon where approximately 573,000 acres were harvested in 2001 (2001-2002 Oregon Agriculture and Fisheries Statistics). The most abundant grass seed species is perennial ryegrass (*Lolium perenne*). In 2001, approximately 171,530 acres of perennial ryegrass were harvested in Oregon. Perennial ryegrass has been a mainstay of forage grazing systems because its palatability and digestibility have made it very popular among livestock producers (Evers et al., 1996; Hannaway et al., 1999).

The traditional manner of disposing of straw residue following seed harvest is burning. This management technique removes the residue and is instrumental in controlling disease problems (Hardison, 1960; Hardison, 1964; Conklin and Fisher, 1973; Hardison, 1976; Chilcote et al, 1981). However, the large amount of smoke produced causes adverse impacts upon the environment and may create situations that can be dangerous and/or fatal to humans (Hovermale and Craig, 2001). Much legislative activity has been connected to this problem in recent years. Consequently, the Oregon Department of Agriculture is commissioned to operate the Smoke Management Program which regulates and enforces field burning in the state of Oregon. One of the ways that the Smoke Management Program has tried to reduce field burning is by introducing various uses for the grass seed residue remaining post-harvest.

An alternative means of disposing of grass seed straw is use by ruminant livestock. Straw can be used as a low-cost winter forage resource by livestock operations in the Pacific Northwest; however, a majority of the straw is exported (several thousand tons from the state of Oregon) to countries within the Pacific Rim, primarily Japan, Korea, and Taiwan. These countries imported 286,414 tons of Oregon's perennial ryegrass straw during the 2000-2001 market year (Young, 2001).

In recent years, much of the grass seed industry's focus has been on producing "turf-type" grasses (Evers et al., 1996; Hannaway et al., 1999). Many of the "turf type" varieties are infected with the endophytic fungi, *Neotyphodium lolii* and/or *N. coenophialum*. (The *Neotyphodium* fungi were formerly known by the name *Acremonium*; Clement et al., 1997.) These varieties are appreciated by the landscape industry due to the benefits that the plant host derives from the symbiotic relationship it shares with the endophyte. The endophytic fungi live between cells within the plant, deriving nourishment and a means of reproduction from the plant (Joost, 1995). In return, the endophytes help the grass to be hardy, vigorous, resistant to the effects of trampling and insect herbivory, and resilient to grazing and mowing (Glenn and Bacon, 1997; Hannaway et al., 1997). However, this can be a problem because *N. lolii* and *N. coenophialum* produce the ergot alkaloids lolitrem B and ergovaline, respectively. Consumption of these alkaloids can have toxic effects on livestock. Lolitrem B can cause reduced performance, muscle tremors, incoordination, and death in ruminant livestock (Clay, 1988). In the case of ergovaline, signs and symptoms of toxicosis include reduced performance, increased body temperature, lameness, gangrene, and spontaneous abortion (Joost, 1995).

Recently, Japan has expressed concerns relating to impaired health and performance of cattle consuming imported perennial ryegrass straw (Schnitzius et al., 2001). Consequently, the objective of this research was to evaluate the affect of increasing lolitrem B concentration in perennial ryegrass straw on physiological response variables, ruminal fermentation characteristics, straw intake and digestibility, animal performance, milk production, and serum prolactin level in ruminants.

Review of the Literature

The Ruminant Animal

Ruminant is a term used to describe a group of animals that are cud-chewing. In other words, they regurgitate previously consumed feed, in the form of a bolus, and further masticate it. The word ruminant is derived from the Latin word *ruminare*, which means to chew over again (Church, 1988a). Ruminants have had an impact on hunting and agricultural societies over the past several thousand years. Beyond being a human food source, they have provided skin and fiber for clothing, traction and power for conducting work, pest control, cultural status, and entertainment. There are 155 to 165 ruminant species currently in existence (Church, 1988a; Van Soest, 1994). Van Soest (1994) suggests that there are six species of domesticated ruminants. These include cattle, sheep, goats, buffaloes, reindeer, and yaks. Church (1988a) reported that archeological evidence puts the domestication of sheep, goats, and cattle at least 11,000, 9,000, and 8,500 years ago, respectively.

The Ruminant Stomach

The digestive tract of ruminant animals differs from that of monogastric animals. While there are similarities between the two groups, ruminants have evolved to have a series of compartments prior to the gastric stomach. These compartments, collectively known as the forestomach, are referred to as the reticulum, rumen, and omasum (Frandson, 1986). In the forestomach, ingested feed materials are soaked with digestive secretions and subjected to digestion by microorganisms. Researchers have isolated approximately 200 different species of bacteria and approximately 20 species of protozoa that are included in the rumen microbial population (Russell and Hespell, 1981; Baldwin and Allison, 1983; Martin, 1994). Additionally, approximately 8% of the ruminal microbial population of ruminants consuming high-roughage diets is made up of fungi (Yokoyama and Johnson, 1988). While their significance is not completely clear, there are indications that fungi assist in the digestion of fiber (Yokoyama and Johnson, 1988).

The reticulum is the first compartment of the forestomach and is followed by the rumen. The reticuloruminal fold separates these two compartments; however, they are often considered a single organ, referred to as the reticulorumen (Van Soest, 1994). Ingesta has the potential for free exchange between the reticulum and rumen. The major portion of fermentation, including production and absorption of volatile fatty acids (**VFA**) and ammonia, takes place within the reticulorumen (Russell and Hespell, 1981; Fahey and Berger, 1988; Merchen, 1988; Owens and Goetsch, 1988; Van Soest, 1994; Merchen et al., 1997).

Ruminants have the capacity to consume large volumes of course, poor quality forage from which they extract nutrients for growth and maintenance (Ammerman and Henry, 1985). One of the mechanisms which enables utilization of low-quality forage is rumination. Rumination is a process where contact of course digesta particles against the rumen and/or reticulum wall stimulates regurgitation of previously masticated food material (Hungate, 1966). The material is then remasticated and reswallowed. This process results in the digesta being "mechanically" broken down into smaller particles that are more readily saturated with digestive secretions and "chemically" broken down (Schalk and Amadon, 1928). The process of mechanically breaking down the digesta disrupts plant tissues creating physical barriers to digestion and provides more surface area for microbial attachment (McAllister et al., 1994). Larger particles are retained in the reticulorumen for further digestion while smaller particles are circulated to the omasum for passage to the intestines.

The third compartment of the forestomach is the omasum. This segment of the digestive tract contains many leaves that prevent the passage of large digesta particles while absorbing water and nutrients. Also, some digesta grinding may occur within the omasum (Hungate, 1966; Acker, 1983). Pumping digesta from the reticulum to the abomasum is one of the major functions of the omasum (Ruckebusch, 1988; Van Soest, 1994). In cattle, the omasum is larger than in many other ruminant species (Hofmann, 1988). Also, it is believed that a greater amount of absorption takes place within the omasum of cattle compared with other ruminant species (Frandson, 1986; Hofmann, 1988; Van Soest, 1994). Van Soest (1994) suggests that potentially 30-60% of the water and 40-69% of the VFA that enters the omasum is absorbed. Additionally, he notes that a considerable amount of sodium, potassium, and other ions are absorbed from the omasum.

The compartments of the forestomach and their associated microflora allow the ruminant to utilize roughage as a main source of nutrients. As previously mentioned, mechanical breakdown and digestion of feed material by ruminal microorganisms takes place in the reticulorumen. Additionally, this is where bacterial protein is formed (Hungate, 1966; Acker, 1983; Ammerman and Henry, 1985; Lyford and Huber, 1988; Van Soest, 1994). Clark et al. (1992) state that rumen bacteria contain approximately 50% crude protein (**CP**) and provide the majority of CP (amino acids, peptides, true proteins, etc.) flowing to the small intestine of ruminants consuming low-quality forage (Merchen and Bourquin, 1994).

Rumen bacteria are able to synthesize many of the amino acids that are essential for the normal growth and production of their ruminant host. These include tryptophan, histidine, leucine, lysine, methionine, phenylalanine, tyrosine, and valine (Allison, 1969). Therefore, most ruminants consuming low-quality forage obtain the majority of these amino acids through intestinal digestion of ruminal microorganisms.

Another benefit that ruminants derive from the microbial population of the forestomach is synthesis of B vitamins (Hungate, 1966; Maynard et al., 1979; Acker, 1983; Ammerman and Henry, 1985; Miller et al., 1988, Taylor, 1994; Van Soest, 1994). Ruminants are unable to synthesize these essential vitamins on their own. However, there are specific ruminal bacteria that can produce them (Van Soest, 1994). The synthesized vitamins are absorbed as the bacteria are digested farther down the digestive tract.

After exiting the omasum, digesta is passed to the fourth compartment of the ruminant stomach, the abomasum. While the abomasum is a compartment of the stomach, it is not part of what is collectively called the forestomach. This is where the similarities between the ruminant and monogastric digestive systems begin. Sometimes referred to as the "true stomach" (Maynard et al., 1979) or "gastric stomach", this is the only ruminant stomach that has the ability to secrete gastric juices

(Merchen, 1988). Van Soest (1994) suggests that digesta flow into the abomasum, as well as abomasal secretion of gastric juices, is a nearly continuous process; as opposed to an intermittent procedure in non-ruminant species.

Rumen Fermentation

The ruminal degradation of consumed feedstuffs takes place in an environment void of oxygen (anaerobic) and is referred to as ruminal fermentation. The idea that fermentation occurs in the rumen was first suggested in the late 1600's by Peyer (Hungate, 1966; Ammerman and Henry, 1985).

Ruminal Volatile Fatty Acids

The process of ruminal fermentation creates organic compounds (i.e. VFA, lactic acid, and ethanol) which retain much of the original energy that existed in the previous substrate (Van Soest, 1994). Volatile fatty acids produced during fermentation provide the mature ruminant with its major source of utilizable energy (Ammerman and Henry, 1985). Owens and Goetsch (1988) state that 50-85% of the metabolizable energy utilized by ruminants consuming forage-based diets comes from VFA. However, this is not necessarily an efficient system. Methane, heat of fermentation, and heat of assimilation are all significant sources of energy lost as a result of microbial fermentation (Merchen and Bourquin, 1994). As a result Rémond et al. (1995) state that ruminants have access to only 50-75% of the metabolizable energy contained in the original feedstuff.

Ruminal pH

Rumen pH is very important for ruminal digestion. If pH becomes too low or too high it can inhibit ruminal fermentation and nutrient absorption. The optimal pH level for cellulolytic microorganisms is 6.7, with a normal activity range from 6.2 to 7.2 (Van Soest, 1994). Saliva buffering and VFA absorption are used to keep ruminal pH at a relatively constant rate (Owens and Goetsch, 1988). Church (1988b) suggests that the bicarbonate and phosphate buffers secreted in saliva make buffering capacity one of saliva's most important functions. Owens and Goetsch (1988) state that the average pK for VFAs is 4.1 and they become non-dissociated as the rumen pH descends towards that level. Because VFA are more readily absorbed when in a non-dissociated state, this allows for greater absorption.

Ruminal Ammonia Nitrogen

Many species of rumen bacteria use ammonia nitrogen (NH_3-N) as their primary source of nitrogen (Russell and Hespell, 1981; Owens and Zinn, 1988; Van Soest, 1994), especially those that digest complex carbohydrates (Owens and Zinn, 1988; Van Soest, 1994). It has been estimated that approximately 80% of rumen bacteria can survive and grow with NH_3-N providing their only source of nitrogen (Baldwin and Allison, 1983). Some species of the rumen microbial population produce ammonia through the degradation of both proteins and non-protein nitrogen (NPN).

Another source of NH_3-N is nitrogen recycling. Recycling can be equivalent to 10-15% of a ruminant's dietary nitrogen intake (Owens and Zinn, 1988). In this process, plasma urea enters the rumen via diffusion or within salivary secretions (Owens and Zinn, 1988; Van Soest, 1994). Owens and Zinn (1988) suggest that 15 to 50% of the total urea recycled (in ruminants consuming forage diets) enters the rumen via salivary secretions. Urea is hydrolyzed to NH_3-N and carbon dioxide by microbial urease (Russell and Hespell, 1981; Owens and Zinn, 1988; Van Soest, 1994).

Oregon's Grass Seed Industry

Seed production of cool-season forages and turf-type grasses is a very important part of Oregon's agricultural economy. Grass seed production contributed approximately 573,000 acres of harvested crop in 2001 (2001-2002 Oregon Agriculture and Fisheries Statistics). The mild, moist winters and dry summers of the Oregon valleys have contributed to making Oregon the world's major producer of seed for these types of grasses (Conklin and Fisher, 1973). A significant percentage of the US seed production of bluegrass, orchard grass, and tall fescue comes from Oregon. Furthermore, essentially all of the annual ryegrass, perennial ryegrass, bent grass, and fine fescue seed produced in the US is a product of Oregon (Conklin et al., 1989). Also, approximately two-thirds of all cool-season grass seed that is produced in the United States is from Oregon (Young and Barker, 1997).

History of Grass Seed Production in Oregon

The Willamette Valley of Western Oregon has long played a major role in grass seed production. The north half of the valley is comprised of rich, loamy, well drained soils. This has made the area favorable for the production of diversified crops such as grains, vegetables, and fruits. However, the southern half of the valley consists of clay soils that lack effective drainage. This created a situation in which the farmers in the southern half of the valley were unable to compete with those in the northern half. During the 1920's, farmers in the southern Willamette Valley were introduced to the idea of producing grass seed as an alternative crop (Young and Barker, 1997). It was believed that this might prove to be a viable agricultural product that could withstand the area's poorly drained clay soils. As a result, the grass seed industry has steadily increased in the region. In 1970, the Plant Variety Protection Act was established. This granted proprietary protection and exclusive production and marketing rights to private plant breeders. The result was a dramatic improvement in the genetics of many cool-season grasses.

Open Field Burning of Grass Straw Residue

Oregon's grass seed industry was plagued with disease problems in the 1930's (Conklin et al., 1989). In 1943, the first case of blind seed disease of perennial ryegrass in the US was discovered (Hardison, 1948). This greatly reduced seed production in the Pacific Northwest. An effort to control the spreading disease problem led to the introduction of open field burning. Approximately half of the perennial ryegrass acreage was burned following harvest in 1948. The resulting successful disease control initiated the adoption of post-harvest burning of straw residue and field stubble as a universal management practice in 1949 (Hardison, 1960; Conklin and Fisher, 1973; Hardison, 1976; Chilcote et al., 1981). Field burning was soon credited for having multiple benefits to the grass seed industry. In his proceedings article, 'Justification for burning grass fields', Hardison (1964) noted the following list of benefits of post-harvest burning.

> [Primary benefits:
> (A) Control of numerous diseases and particularly several serious diseases (blind-seed disease, ergot, and grass seed nematode).
> (B) Elimination of the sexual stage of many pathogenic fungi, thereby reducing the opportunity for production of new, more virulent races.
> (C) Direct weed control by incineration of seeds and by heat-killing certain weed plants.
> (D) Indirect weed control by providing a clean soil surface that is necessary for uniform distribution and root absorption of soil-active herbicides in control of winter-annual weeds.
> (E) Return to the soil of potash and other minerals from crop residues.

(F) Stimulation of better yields by thinning or overcoming part of the "sod-bound" effect.
(G) Prevention of fall heading in perennial ryegrass, thereby eliminating ergot-infested heads and avoiding ergot poisoning in livestock, game animals, and birds.
(H) Destruction of certain mites, insects, and rodents.
(I) Avoidance or reduction of pesticide residues by the control of numerous pests without pesticides.
(J) Low-cost removal of straw, which has become an economic necessity in production of many grass seed crops.

Secondary benefits:
(A) Elimination of smothering.
(B) Greatly improved efficiency of fertilizers.
(C) Renewal of annual ryegrass fields by replanting directly with rangeland seeders (no-till planting).
(D) Preventative fire control by removing the extreme danger of uncontrolled fires in late summer.]

By the 1950's and early 1960's, post-harvest field burning was considered to be the most valuable cultural practice of grass seed production; however, the smoke being produced was beginning to be considered a nuisance by the general public (Hardison, 1964; Conklin and Fisher, 1973, Chilcote et al., 1981). During the 1960's, it was estimated 17% of the annual air pollutants in Oregon were derived from field burning (Conklin and Fisher, 1973). In 1969, there were 5,142 complaints directly related to field burning. In August of that year, Oregon governor McCall initiated temporary emergency bans on field burning after the cities of Eugene and Springfield were inundated with smoke (DEQ, 1988). Additionally, there were two motor vehicle deaths in 1969 that were attributed to the smoke hazard from field burning (Conklin and Fisher, 1973). This was followed by a series of legislative actions throughout the 1970's. As a result, acreage "phase-down" of permitted burning was established. Additionally, the Oregon State Legislature assigned regulatory power over field burning to the Department of Environmental Quality (**DEQ**) which was appointed the task of implementing a smoke management program (Conklin et al, 1989). A large volume of smoke related complaints (3,783) were again reported in 1988. During August of that year, another motor vehicle accident was attributed to field burning smoke. This was a chain reaction incident on Interstate-5 in which seven people were killed and 38 others injured (DEQ, 1988). Following this incident, legislation was introduced that further "phased-down" the total acres of grass seed residue that could be burned. Additionally, a stronger, renewed effort was launched to find alternative ways to dispose of straw residue.

Alternative Means for Disposal of Straw Residue

The amount of post-harvest grass seed residue that needs to be contended with is continually increasing. This is due to increased seed production and/or decreases in the allowable acreage burned. Many producers have been left struggling with the challenge of what to do with their crop's by-product, straw.

Several ideas have been investigated in the search for alternative means of straw disposal. One technique is to flail chop the residues and return it to the soil. However, concerns have been expressed regarding disease proliferation (Department of Agriculture, personal communication). Some producers compost the straw residue and market it as a soil conditioner (Edgar, 1996). Other producers have marketed it as a mulch product to berry farms, Christmas tree farms, mushroom producers, and vineyards (Department of Agriculture, personal communication). Additionally, the

Oregon Department of Transportation annually utilizes straw residue as a soil erosion preventative at construction sites. Several other alternative uses of grass straw, ranging from production of paper to insulation board, have been investigated (Conklin et al., 1989). However, concern over a stability of supply has hampered progress in this direction. Other research has been conducted evaluating grass seed straw as an alternative fuel source; however, this has proved to be cost prohibitive (Conklin et al., 1989). An alternative disposal technique that appears to exceed the potential of the other alternatives is utilization of grass seed straw as a forage source for ruminants.

Straw as a Forage Source

Straw is a major feed source for ruminants in Third World countries (Van Soest, 1994). Yet, in the United States, it is estimated that less than 1% of the total straw supply is used as a forage resource (Han, 1978). While grass straw is generally a low-quality forage source, the ruminant animal and its microbial population can utilize grass seed straw with proper nutritional management. Consequently, grass straw can be used as a low-cost forage resource by livestock operations.

Recently, straw exports have increased (Young, 2001). A large quantity of Oregon's straw is exported to countries within the Pacific Rim, primarily Japan, Korea, and Taiwan. These countries imported 286,414 tons of Oregon's perennial ryegrass straw during the 2000-2001 market year (Young, 2001). However, producers in these countries have recently expressed animal health and performance concerns associated with endophyte-infected grasses (Miyazaki et al., 2001; Schnitzius et al., 2001).

Perennial Ryegrass

Perennial ryegrass is considered a high-value grass crop and is the leading species for grass seed production in the state of Oregon (2001-2002 Oregon Agriculture and Fisheries Statistics). The 2001 Oregon harvest for this crop covered approximately 171,530 acres yielding approximately 249 million pounds of seed valued at $98.2 million.

Perennial ryegrass, also known as English ryegrass, is native to Europe, portions of Asia, and Northern Africa; however, it is currently grown throughout the world. It is considered a temperate, perennial grass that is quick establishing, of exceptional nutritional quality, and performs with cool-season productivity (Hannaway et al., 1997). Among cultivated cool-season grasses, ryegrass seedlings have the greatest rate of growth, giving it a competitive edge in establishment (Griffith and Chastain, 1997). Also, because it is tolerant of poorly drained soils having a pH between 5.0 and 8.3, perennial ryegrass is suited to mild, wet climates. Optimal growth occurs during periods of mild temperature (ranging from 20 to 25°C), with dormancy possible during periods of extreme heat (Evers et al., 1996; Hannaway et al., 1997). Although not known for being a winter-hardy grass, Casler and Walgenbach (1990) and Novy et al. (1995) proved that it could survive and be productive in the harsh climate of Wisconsin. This combination of characteristics has made perennial ryegrass successful in the Pacific Northwest. Additionally, in the US it is used as a forage source within irrigated intermountain valleys, the Midwest, and the Northeast (Evers et al., 1996).

Perennial ryegrass is a flexible grass having multiple uses. Traditionally, it has been grown as pasture forage and for harvest as silage and/or hay. The palatability and digestibility of perennial ryegrass is greater compared with other cool-season perennial grasses (Novy et al., 1995; Evers et al., 1996; Hannaway et al., 1997; Hannaway et al., 1999). Jung et al. (1976) contribute the increased digestibility to greater total nonstructural carbohydrates.

Endophyte and Alkaloids in Perennial Ryegrass

While perennial ryegrass is considered to be a premium feedstuff for ruminants, there are potential health concerns. Some varieties of perennial ryegrass are infested with endophytic fungi (Aldrich-Markham and Pirelli, 1995; Hannaway et al., 1997; Hannaway et al., 1999). These endophytes have a symbiotic relationship with their plant host. The fungi live in the intercellular space within the plant's tissue, deriving nourishment and a mode of reproduction from the plant (Joost, 1995). In exchange, the fungi provide the plant with protection from herbivory (Cheeke, 1998). Also, endophytes and their respective alkaloids provide benefits to the host plant that go beyond herbivory protection. Cunningham et al. (1993) reported that perennial ryegrass had enhanced drought tolerance when infected with endophytes. Plant persistence and stress tolerance have also been attributed to endophyte infection (Funk and White, 1997). These types of benefits have made endophyte-infected perennial ryegrass varieties ("turf-type") favored by the landscaping industry.

The Impact of Alkaloids When Consumed

Perennial ryegrass staggers is a neurological disorder that affects several animal species. Fletcher and Harvey (1981) are credited with correlating perennial ryegrass "staggers" with consumption of endophyte infected perennial ryegrass. It is generally believed that the causative fungus for the condition is *Neotyphodium lolli.* Gallagher et al. (1981) extracted the toxin believed to be responsible for the condition, the ergot alkaloid lolitrem B. It has since been documented that perennial ryegrass staggers can be caused by intake of lolitrem B (Nicholson, 1989; Galey et al., 1991; Powell and Petroski, 1992; Cunningham et al., 1993; Odriozola et al., 1993; Miles et al., 1994; Hovermale and Craig, 2001; Tor-Agbidye et al., 2001). Although there are several alkaloids in perennial ryegrass, lolitrem B is generally accepted as being the primary concern. The other major "problem" alkaloid that is often found in perennial ryegrass is ergovaline (Lane et al., 1997). It is derived from the endophytic fungus *Neotyphodium coenophialum* and is more noted for its infestation of tall fescue (*Festuca arundinacea*).

Lolitrem B

Hovermale and Craig (2001) suggested that a lolitrem B concentration greater than 1800 ppb could be expected to cause clinical signs of ryegrass staggers in cattle and sheep consuming perennial ryegrass. The condition manifests itself after seven to fourteen days of exposure to high (> 1800 ppb) levels of lolitrem B. Clinical symptoms include incoordination, staggering, tremors, head shaking, and collapse (Aldrich-Markham and Pirelli, 1995; Cheeke, 1995). While death can arise from the disorder, it is usually associated with misadventure (i.e. stumbling off of a cliff, entering a pond to cool off and drowning, etc.; Cheeke, 1998). Animals suffering from perennial ryegrass staggers should be removed from the causative feed source. Clinical signs normally subside in two to fourteen days.

Prestidge (1993) suggests that the economical impact resulting from subclinical effects can be of more consequence than the actual clinical symptoms. These can include inhibited average daily gains, reproduction, and lactation. Additionally, environmental stressors (ie. heat or cold stress) may also have a role in an animals response to lolitrem B intake. Experimentation using endophyte infected perennial ryegrass straw as a forage source is not available. Much of the reported lolitrem B data refers to case studies or grazing experiments.

In 1989 and 1990, cattle herds and sheep flocks grazing perennial ryegrass pastures in northern California began exhibiting symptoms of ryegrass staggers (Galey et al., 1991). These animals demonstrated neck and head tremors while at rest and exhibited a stiff gait and arched back when they walked. On occasion, the afflicted animals would collapse and enter a tetanic seizure when disturbed. These animals recovered after one to two weeks of isolation from the causative feedstuff.

Fletcher and Sutherland (1993) conducted a trial to evaluate liveweight change of lambs grazing perennial ryegrass infected with varying types and/or levels of endophyte. Lambs were assigned to one of three treatments: endophyte-free perennial ryegrass, endophyte wild-type (containing lolitrem B endophyte), and 187BB perennial ryegrass (containing an endophyte but no lolitrem B). Lambs on the endophyte wild-type treatment lost weight while lambs on the other two treatments gained weight. Also, lambs that consumed endophyte-free perennial ryegrass had greater liveweight gains compared with wild-type and 187BB perennial ryegrass varieties.

Eerens et al. (1997a) used pastures with endophyte-free or endophyte-infected ryegrass to evaluate lamb performance. Ewes with lambs were allotted to pastures at lambing and remained there until lambs were weaned. The authors reported greater weight gains by lambs from lactating ewes grazing endophyte-free perennial ryegrass pastures as opposed to those in infected pastures. In another study, Eerens et al. (1997b) investigated gestation length of ewes grazing endophyte-free or endophyte-infected ryegrass. They observed a delay of 4.5 days in parturition for ewes grazing infected perennial ryegrass/white clover pasture.

Using pastures containing endophyte-free or endophyte infected perennial ryegrass, Easton and Couchman (1999) rotationally grazed cattle during an 84-day study. Heifers grazing the endophyte-free pastures had higher gains than those grazing endophyte-infected perennial ryegrass. Also, serum prolactin levels were reduced in heifers consuming the endophyte-infected grass. Additionally, the authors reported that heifers grazing endophyte-infected perennial ryegrass appeared to be heat stressed, as noted by excessive salivation. However, no difference was seen in rectal temperature of heifers grazing endophyte-free and endophyte-infected perennial ryegrass.

Ergovaline

Animal health concerns related to *N. coenophialum* were first reported by Bacon et al. (1977). Hemken et al. (1984) and Johnson et al. (1985) suggest that these concerns include reduced weight gain, decreased milk production, lowered feed intake, rough haircoat, increased respiration rate, and high rectal temperatures. Hovermale and Craig (2001) documented the critical levels for ergovaline toxicity. In cattle, clinical signs normally appear when levels are 400-750 ppb. Critical levels for sheep are slightly higher, at 500-800 ppb. A brief sample of research and case studies follow that support the affect of ergovaline on animal health and/or performance.

During the winter of 2001-2002, an outbreak of fescue toxicosis occurred in Eastern Oregon (D. Bohnert, personal communication). Producers on three ranches were utilizing tall fescue straw as a forage source when colder temperatures (two weeks of temperatures below 0°C) occurred. It is believed that decreased peripheral blood flow to the extremities caused many of the cows to suffer severe frostbite to their feet. Approximately 600 cows had to be destroyed due to this incident. Total long-term losses from the three ranches approached $1.25 million.

Research conducted by Dawe et al. (1997) randomly assigned steers to eight pastures containing highly *N. coenophialum*-infected tall fescue or low-infected tall fescue. Results indicated that steers consuming highly *N. coenophialum*-infected tall fescue had decreased average daily gain, serum prolactin, and antibody response to immunization with tetanus toxoid compared to steers grazing low-infected tall fescue.

Piper et al. (1997) were able to induce ergovaline toxicosis symptoms in rats fed a fescue seed diet infected with 5 ppm ergovaline. Decreases were observed in feed intake, weight gain, and serum prolactin in rats consuming endophyte-infected seed diets compared with those consuming endophyte-free seed diets.

Aldrich et al. (1993) fed an endophyte-free or an endophyte-infected (381 ppb of ergovaline) tall fescue seed diet to Angus heifers. Heifers consuming the endophyte-infected diet exhibited decreased serum prolactin. A follow up experiment used Holstein steers consuming similar treatments (285 ppb of ergovaline in the endophyte-infected diet); however, this study was conducted in one of two environments (22°C:60% humidity or 32°C:60% humidity). The resulting data indicated that the endophyte-infected diet reduced feed intake by 10% and digestibility of dry matter and organic matter by 9%. They suggested that while intakes were affected by both the diet treatment and the environmental treatment, digestibility was solely affected by the diet treatment. Additionally, serum prolactin and skin vaporization were less in the steers consuming the endophyte-infected diet while rectal temperature was increased.

In a Missouri experiment, two 120-day trials were conducted to determine performance of cows grazing endophyte-infected tall fescue compared with endophyte-free. Cows grazing endophyte-infected tall fescue lost three times as much body weight and had a 25% reduction in milk production compared with cows grazing endophyte-free tall fescue (Peters et al., 1992).

Conclusion

While there is an abundant supply of grass seed straw, there is a need for further research evaluating it as a forage source. It is well documented that some of the alkaloids contained within the grass straw have the potential to create health problems if not properly managed. Producers who utilize grass straw would benefit from research that defines how to manage it as a feedstuff. Additionally, research with ruminants consuming perennial ryegrass-straw could help the grass seed industry in promoting this valuable by-product, while expanding current markets. Presently, perennial ryegrass straw can be exported to other countries if the lolitrem B concentration is below 1800 ppb. However, many foreign producers have expressed concerns about the performance and production of livestock consuming perennial ryegrass straw and would like to have the acceptable level lowered to 900 ppb (R. Anderson and S. Van Mouwerik, National Hay Association, Export Committee, personal communication; Miyazaki et al., 2001). Such action could drastically limit the amount of perennial ryegrass straw exported from Oregon.

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Evaluation of Perennial Ryegrass Straw as a Forage Source for Ruminants

Abstract

We conducted two experiments evaluating perennial ryegrass straw as a forage source for ruminants. Experiment 1 evaluated digestion and physiological variables in steers offered perennial ryegrass straw containing increasing levels of lolitrem B. Sixteen ruminally cannulated Angus X Hereford steers (231 ± 2 kg BW) were blocked by weight and assigned randomly to one of four treatments (**TRT**). Steers were provided perennial ryegrass straw at 120% of the previous 5-d average intake. Prior to straw feeding, soybean meal (**SBM**) was provided (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein. Low (**L**) and high (**H**) lolitrem B straws (<100 and 1550 ppb, respectively) were used to formulate TRT diets: **LOW** (100% L); **LOW MIX** (67% L:33% H); **HIGH MIX** (33% L:67% H); **HIGH** (100% H). Intake and digestibility of DM and OM, and ruminal pH, total VFA, and NH_3-N were not affected by increasing lolitrem B concentration ($P > 0.13$). Ruminal indigestible ADF (**IADF**) fill increased linearly ($P = 0.01$) and IADF passage rate (%/h) decreased linearly ($P = 0.04$) as lolitrem B level increased. Experiment 2 evaluated performance and production of 72 Angus X Hereford cows (539 ± 5 kg BW) consuming perennial ryegrass straw containing increasing levels of lolitrem B during the last third of gestation. Cows were blocked by body condition score (**BCS**) and randomly assigned to one of three TRT. Cows were provided perennial ryegrass straw ad libitum and supplemented with SBM (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein. Mixtures of a L and H lolitrem B straw (467 and 2017 ppb, respectively) were used to formulate TRT diets: LOW (100% L); MIX (50% L:50% H); HIGH (100% H). Thirteen of 24 cows on the HIGH TRT exhibited signs of ryegrass staggers and were removed from the study. Dry matter intake was not affected ($P > 0.12$) by increasing lolitrem B concentration; however, DM digestibility decreased linearly ($P < 0.01$) as lolitrem B concentration increased. Lolitrem B concentration did not influence pre- or post calving weight or BCS change ($P > 0.10$). These data suggest that feeding perennial ryegrass straw containing up to 1550 ppb lolitrem B does not adversely affect nutrient digestion or physiological response variables in steers. However, providing straw with a lolitrem B concentration of approximately 2000 ppb resulted in 54% of cows exhibiting signs of ryegrass staggers. Blending of H and L straws appears to be a successful management practice.

Keywords: Alkaloid, Beef Cattle, Endophyte, Lolitrem B, Perennial Ryegrass, Straw

Introduction

In the Pacific Northwest, grass seed is a major agricultural product (2001 – 2002 Oregon Agriculture and Fisheries Statistics, Oregon Department of Agriculture, Salem, OR). One of the most common is perennial ryegrass (*Lolium perenne*).

Burning has been the traditional way of straw disposal following seed harvest; however, the large amount of smoke produced causes adverse impacts upon the environment and can create situations that prove dangerous and/or fatal to humans (Hovermale and Craig, 2001). An alternative way of disposing of grass-seed straw is use by ruminant livestock. Straw can be a low-cost, winter forage resource for livestock operations in the Pacific Northwest; however, a majority of the straw is exported (several thousand tons from the state of Oregon) to countries within the Pacific Rim, primarily Japan, Korea, and Taiwan. These countries imported 286,414 tons of Oregon's perennial ryegrass straw during the 2000-2001 market year (Young, 2001).

In recent years, much of the grass seed industry's focus has been on producing "turf-type" grasses (Evers et al., 1996; Hannaway et al., 1999). Many of the "turf-type" perennial ryegrass varieties contain the endophytic fungus, *Neotyphodium lolii*. This can be a problem because *N. lolii* produces the ergot alkaloid lolitrem B, which can have toxic effects when consumed by livestock (Tor-Agbidye et al., 2001). Recently, producers from Japan have expressed concerns relating to impaired health and performance of cattle consuming imported perennial ryegrass straw (Schnitzius et al., 2001). Therefore, the objective of our study was to evaluate the effect of increasing lolitrem B concentration in perennial ryegrass straw on physiological response variables, ruminal fermentation characteristics, straw intake and digestibility, performance, and milk production in ruminants.

Materials and Methods

Experiment 1: Digestion/Physiology Study

Sixteen Angus X Hereford, ruminally cannulated steers (231 ± 2 kg BW) were used in a Randomized Complete Block design (Cochran and Cox, 1957). Steers were blocked by weight and assigned randomly to one of four treatments (**TRT**). Animals were housed in individual pens (2 X 4 m) within an enclosed barn with continuous lighting. Each steer had unrestricted access to fresh water and a trace mineralized salt block (≥ 96.000% NaCl, ≥ 0.200% Mn, ≥ 0.100% Fe, ≥ 0.100% Mg, ≥ 0.050% S, ≥ 0.025% Cu, ≥ 0.010% Co, ≥ 0.008% Zn, and ≥ 0.007% I). In addition, all steers received an intramuscular injection of vitamins A, D, and E (500,000, 50,000, and 1,500 IU, respectively; Vitamin E-AD 300, Agrilabs, St. Joseph, MO) at trial initiation to safeguard against deficiency. Perennial ryegrass straw was provided at 120% of the previous 5-d average intake at 0730, with feed refusals from the previous day determined before feeding. Prior to straw feeding (0700), soybean meal (**SBM**) was provided (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein assuming an 11% microbial efficiency (NRC, 1996). Mixtures of a low (**L**) and high (**H**) lolitrem B straw (<100 and 1550 ppb, respectively; Table 1) were used to formulate TRT diets. The TRT were **LOW** (100% L), **LOW-MIX** (67% L:33% H), **HIGH-MIX** (33% L:67% H), and **HIGH** (100% H). Nutrient content of straws and SBM is provided in Table 1. Experimental procedures for this study were approved by the Institutional Animal Care and Use Committee at Oregon State University.

Table 1. Feedstuff nutrient content (DM Basis)

	Experiment 1			Experiment 2			
Item	Low[a] Perennial Ryegrass Straw	High[a] Perennial Ryegrass Straw	Soybean Meal	Low[a] Perennial Ryegrass Straw	High[a] Perennial Ryegrass Straw	Meadow Hay	Soybean Meal
CP, %	4.6	5.5	45.6	5.4	6.2	6	51.8
OM, %	95	95	90	95	95	89	92
NDF, %	63	64	20	67	64	60	16
ADF, %	33	34	6	36	33	31	4
IADF[b], %	16	15	N/A	18	18	N/A	N/A
Lolitrem B, ppb	<100	1550	N/A	467	2017	N/A	N/A
Ergovaline, ppb	<10	160	N/A	40	200	N/A	N/A

[a]Low and High are indicative of lolitrem B concentration.
[b]Indigestible ADF.

The experimental period was 25 d, with the first 13 d used as an adaptation period. At 0700 on d 14, each steer was intraruminally pulse-dosed with 4 g of Co-EDTA in a 150-ml aqueous solution (Uden et al., 1980). The Co marker was administered throughout the rumen using a stainless-steel probe with a perforated tip. Rumen fluid (approximately 100 ml) was collected by suction strainer (Raun and Burroughs, 1962; 19-mm diameter, 1.6-mm mesh) at 0 (prior to feeding), 3, 6, 9, 12, and 24 h after straw feeding. Samples were immediately analyzed for pH and sub-sampled by placing 5 ml of rumen fluid in 1 ml of 25% (wt/vol) meta-phosphoric acid and stored (-20°C) for later analysis of NH_3-N and volatile fatty acids (**VFA**). Also, 20 ml was stored (-20°C) for later analysis of Co concentration. Frozen NH_3-N and VFA samples were prepared for analysis by thawing, centrifuging (15,000 X g; 10

min), and collecting the supernatant. Volatile fatty acids were analyzed as described by Harmon et al. (1985) and NH_3-N by a modification (sodium salicylate was substituted for phenol) of the procedure described by Broderick and Kang (1980) using a UV/VIS spectrophotometer (Spectronic 710 Spectrophotometer, Bausch & Lomb, Inc., Rochester, NY). Frozen Co samples were prepared for analysis by thawing, centrifuging (2,000 X g; 20 min), and collecting the supernatant. Cobalt concentration in ruminal fluid was analyzed by atomic absorption using an air/acetylene flame (Model 351 AA/AE spectrophotometer, Instrumentation Laboratory, Inc., Lexington, MA.) Ruminal liquid fill and dilution rate were estimated by regressing the natural logarithm of Co concentration against sampling time as described by Warner and Stacey (1968).

Intake and orts were monitored throughout the trial; however, official measurements were taken on d 14 through 19 and d 15 through 20 for intake and orts, respectively. Samples (approximately 200 g) of L straw, H straw, and SBM were collected on d 14 through 19 and composited. Orts samples were collected and a sub-sample obtained (10% wet-weight) on d 15 through 20. Samples were dried in a forced-air oven (55°C; 48 h) and reweighed for calculation of DM. Orts samples were composited by steer. Straw, SBM, and orts samples were ground in a Wiley mill (1-mm screen).

On d 15, reticulorumen contents were manually removed (Lesperance et. al., 1960) 4 h post-feeding to determine TRT effects on ruminal indigestible ADF (**IADF**) fill and passage rate. Reticuloruminal contents were weighed, thouroughly hand-mixed, and sub-sampled in triplicate (approximately 400 g). Remaining ruminal contents were then replaced into the appropriate steer. Samples were weighed, dried in a forced-air oven (55°C; 96 h), reweighed for DM, composited by steer, and ground as described previously.

Steers were fitted with fecal bags at 0630 on d 16. Bags were changed once every 24 h for a total fecal collection period of 6 d. Each day, fecal samples were weighed, hand-mixed, and a 2.5% sub-sample (wet-weight) collected. Samples were weighed, dried in a forced-air oven (55°C; 96 h), reweighed for DM, composited by steer, and ground as described previously.

Ground samples were later analyzed for DM and OM (AOAC, 1990), N (Leco CN-2000, Leco Corporation, St. Joseph, MI), and NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Also, samples were analyzed for IADF as described by Bohnert et al. (2002). Average fecal IADF recovery was 96.4 ± 1.4%. Digesta kinetics techniques described by Van Soest (1994) were used to determine IADF passage by dividing IADF intake by the quantity of IADF in the rumen 4 h after feeding. Straw samples were analyzed for lolitrem B and ergovaline as described by Hovermale and Craig (2001).

Physiological variables were measured at 1300 on d 16 through 21 to determine heart rate (**HR**; audibly monitored with a stethoscope in the area behind the left front elbow), respiration rate (appraised by flank movement), and rectal temperature. In addition, 10 ml of blood was collected from the jugular vein by venipuncture 4 h post-feeding on d 22 through 25. Blood was immediately transferred to a vacutainer tube and allowed to clot overnight. Samples were then centrifuged

(1,500 X g; 15 min) and serum harvested and stored (-20°C) for prolactin analysis as described by Hocket et al. (2000; Intra-assay CV = 5.59).

Data were analyzed as a Randomized Complete Block using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Steer, TRT, and block were included in the model. Contrast statements were: 1) linear effect of increasing lolitrem B concentration; 2) quadratic effect of increasing lolitrem B concentration. Results with a P value ≤ 0.05 were considered statistically significant. Data for ruminal pH, NH_3-N, and VFA, collected at fixed timepoints post-feeding, were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, TRT, block, hour, and TRT X hour. Also, physiological variables and serum prolactin, collected 4 h after feeding on fixed days, were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, TRT, block, day, and TRT X day. The same contrasts described above were used to partition TRT effects for ruminal pH, NH_3-N, VFA, physiological variables, and serum prolactin. All results with a P value ≤ 0.05 were considered statistically significant.

Experiment 2: Performance/Production Study

Experimental procedures for this experiment were approved by the Institutional Animal Care and Use Committee at Oregon State University. Seventy-two spring calving Angus X Hereford cows (approximately 200 d gestation) were stratified by body condition score (**BCS**; 1 = emaciated, 9 = obese; Herd and Sprott, 1986) and assigned randomly to one of eighteen drylot pens (four cows/pen; six pen/TRT) and one of three TRT in a Randomized Complete Block design (Cochran and Cox, 1957). All cows had unrestricted access to fresh water, a loose mineral mix (≥ 21.000% NaCl, 2600 ppm Mn ≥ 12.000% P, ≥ 11.000% Ca, ≥ 2.500% Mg, ≥ 2.500% K, 3000 ppm Zn, 2000 ppm Cu, 140 ppm Se, 60 ppm Co, 60 ppm I, ≥ 136,078 I.U./kg. vitamin A, and ≥ 27 I.U./lb vitamin E), and trace mineralized salt (≥ 95.000% NaCl, ≥ 3500 ppm Mn, ≥ 3500 ppm Zn, ≥ 2300 ppm Fe, ≥ 120 ppm I, ≥ 90 ppm Se, and ≥ 60 ppm Co). Cows had ad libitum access to perennial ryegrass straw. Also, SBM was provided (0.1% BW; CP basis) at 0700 to meet the estimated requirement for degradable intake protein assuming an 11% microbial efficiency (NRC, 1996). Low and H lolitrem B straws (467 ppb and 2017 ppb, respectively; Table 1) were used to formulate TRT diets. The TRT were **LOW** (100% L), **MIX** (50% L:50% H), and **HIGH** (100% H). Following parturition, cows and calves were removed from experimental TRT, placed in a common pasture (7.3 ha) that had been harvested for hay earlier in the year, and managed as a single group. Cows were provided approximately 11.3 $kg \cdot hd^{-1} \cdot d^{-1}$ (DM basis) of meadow hay and supplemented with SBM (2.7 $kg \cdot hd^{-1} \cdot d^{-1}$) on Monday, Wednesday, and Friday for approximately 53 ± 1.4 d (completion of weigh-suckle-weigh procedure). Samples of meadow hay and SBM were collected weekly. Samples were dried in a forced-air oven (55°C; 48 h) and reweighed for calculation of DM. Straw, meadow hay, and SBM samples were ground in a Wiley mill (1-mm screen) and composited by period for analysis of NDF, ADF, N, DM and OM as described in Experiment 1. Nutrient content of straw, meadow hay and SBM is provided in Table 1. Additionally, L and H straw samples were analyzed for lolitrem B and ergovaline as described by Hovermale and Craig (2001).

A visual appraisal of all cows was conducted daily (up to parturition; while receiving experimental TRT) at 0630 with cows resting and then walking within their pen. Daily "clinical sign" scores were assigned to cows based on an evaluation scale adapted from Galey et al. (1991; Table 2). Cows receiving a score of 3 or higher were removed from the experiment by placing them, with minimal excitement, in an isolated, quiet pen, and providing free choice meadow hay and fresh water. Once clinical signs receded, cows were turned out to pasture with meadow hay provided at approximately 11.3 $kg{\bullet}hd^{-1}{\bullet}d^{-1}$ (DM basis) and supplemented with SBM (2.7 $kg{\bullet}hd^{-1}{\bullet}d^{-1}$) on Monday, Wednesday, and Friday.

Table 2. Perennial Ryegrass Staggers Clinical Sign Evaluation Scale[a]

Score	Clinical Signs
0	No clinical signs
1	No resting tremors or incoordination; Low-intensity tremor and incoordination with handling
2	No resting tremors or incoordination; Moderate-intensity tremors and incoordination with handling
3[b]	Spontaneous low-intensity tremors and incoordination at rest; Moderate to severe tremors and incoordination with handling
4	Pronounced resting tremors and incoordination; Convulsive tremors and severe incoordination with handling
5	Severe spontaneous tremors and incoordination at rest usually accompanied by convulsive episodes

[a]Adapted from Galey et al., 1991.
[b]Removal from study occurred with a scale reading of 3.

Cow BW and BCS were measured at study initiation, d 28, and every 14 d thereafter until calving. All weights were obtained following an overnight shrink (16 h). Body condition score of cows was evaluated independently by three trained technicians. The same technicians were utilized throughout the trial. Also, following parturition (within 24 h), cow weight, cow BCS, and calf weight were obtained.

Eighteen cows (one cow/pen; six cows/TRT) were randomly selected and dosed with an intra-ruminal Cr-releasing device (**IRCRD**; Captec, Nufarm, Auckland, New Zealand) on d 28. Fecal grab samples were collected on d 35-39 at 0730. Fecal samples were dried in a forced-air oven (55°C; 96 h), composited by cow, and ground as described previously. Samples were later analyzed for DM and IADF as described in Experiment 1. Fecal samples were prepared as described by Williams et al. (1962) and analyzed for Cr by atomic absorption spectroscopy (air/acetylene flame; Model 351 AA/AE Spectrophotometer, Instrumentation Laboratory, Inc., Lexington, MA). Chromium payout rate (950.9 ± 12.9 mg/d) for the IRCRD was validated using 4 steers in Experiment 1 and was 103 ± 1.4% of the IRCRD distributor's estimated payout. Fecal output was estimated by dividing IRCRD Cr payout by fecal Cr concentration. Also, DM digestibility was estimated using IADF as an internal marker (Cochran and Galyean, 1994). Consequently, DMI was estimated as fecal output divided by diet indigestibility.

Approximately 53 ± 1.4 d post-calving (May 7, 2003), milk production was estimated using a weigh-suckle-weigh (**WSW**) procedure after an 8 h separation (Williams et al., 1979). Calf excretory (fecal and urinary) losses during suckling were considered minimal and were not collected as suggested by Lampkin and Lampkin (1960).

Data were analyzed as a Randomized Complete Block using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Pen, TRT, and block were included in the model. Contrast statements were: 1) linear effect of increasing lolitrem B concentration; 2) quadratic effect of increasing lolitrem B concentration. Results with a P value ≤ 0.05 were considered statistically significant.

Results and Discussion

We are unaware of previous research that has evaluated the effect of feeding perennial ryegrass straw containing increasing lolitrem B on physiological response variables, ruminal fermentation characteristics, straw intake and digestibility, performance, and milk production in ruminants. Traditionally, nutritional management concerns with perennially ryegrass have been associated with lolitrem B while concerns with tall fescue have been associated with ergovaline. However, Stamm (1992) conducted a survey of several grass straws, harvested in the Willamette Valley of Oregon, and reported the concentration of ergovaline contained within the various species and varieties. Results indicated that 42% of the perennial ryegrass fields sampled had straw containing greater than 200 ppb ergovaline while only 14% of the tested tall fescue fields had an ergovaline concentration greater than 200ppb. This suggests that perennial ryegrass has the potential to cause symptoms of both lolitrem B and ergovaline toxicosis. However, ergovaline level within the current study was below the critical threshold (400-750 ppb) suggested to cause fescue toxicosis in cattle (Hovermale and Craig, 2001).

Experiment 1: Digestion/Physiology Study

Neither straw nor total DMI was affected by increasing lolitrem B concentration ($P > 0.61$); straw and total DMI averaged 19.4 g/kg BW and 21.8 g/kg BW, respectively (Table 3). Similarly, straw and total OM intake was not affected by increasing lolitrem B concentration ($P > 0.60$); with straw and total OM intake averaging 18.4 g/kg BW and 20.6 g/kg BW, respectively. These results are inconsistent with those of Bluett et al. (2001). They allowed lambs to graze one of two cultivars of perennial ryegrass (Aries HD, 3420 ppb lolitrem B and 160 ppb ergovaline; Yatsyn 1, 2420 ppb lolitrem B and 450 ppb ergovaline). Lambs consuming the grass with a higher concentration of lolitrem B had 12% greater herbage intake than lambs consuming the variety with a lower lolitrem B level. However, this data may have been influenced by the ergovaline concentration of the two varieties (the variety with higher lolitrem B had lower ergovaline than the variety with lower lolitrem B).

Previous work has indicated that consumption of ergovaline can depress intake when livestock are under environmental stress. Work by Hemken et al. (1981) noted that DMI of Holstein calves consuming endophyte-free or endophyte-infected tall fescue and maintained in an environment of 23°C or less did not differ. However, they reported that when temperature exceeded 34°C, a marked decrease in DMI was

Table 3. Effect of increasing lolitrem B concentration on nutrient intake and diet digestibility in steers consuming perennial ryegrass straw

Item	Treatment[a]					*P*-Value[c]	
	LOW	LOW-MIX	HIGH-MIX	HIGH	SEM[b]	Linear of LB	Quadratic of LB
Daily DM Intake, g/kg BW							
Straw	19.7	19.7	19.4	18.9	1.15	0.62	0.81
Supplement	2.4	2.4	2.4	2.4			
Total	22.1	22.1	21.8	21.3	1.15	0.62	0.81
Daily OM Intake, g/kg BW							
Straw	18.7	18.7	18.4	17.9	1.09	0.61	0.81
Supplement	2.2	2.2	2.2	2.2			
Total	20.9	20.9	20.6	20.1	1.09	0.61	0.81
Daily N Intake, g/kg BW	0.326	0.335	0.341	0.345	0.0103	0.20	0.82
Daily NDF Intake, g/kg BW	12.8	13.0	12.9	12.7	0.73	0.88	0.81
Total Tract Apparent Digestibility, %							
DM	59.9	61.9	59.2	58.9	0.87	0.19	0.23
OM	60.6	63.1	60.8	60.3	0.88	0.44	0.14
NDF	51.6	55.4	52.4	51.9	1.46	0.72	0.16
IADF[d] Intake, g/kg BW	3.1	3.1	2.9	2.8	0.17	0.19	0.78
IADF[d] Fill, g/kg BW	6.7	7.0	6.6	7.7	0.28	0.01	0.06
IADF[d] Passage, %/h	1.93	1.82	1.86	1.51	0.115	0.04	0.31

[a] LOW = 100% low straw (<100 ppb lolitrem B); LOW-MIX = 67% low straw:33% high straw (1550 ppb lolitrem B); HIGH-MIX = 33% low straw:67% high straw; HIGH = 100% high straw.

[b] n = 4.

[c] Linear of LB = linear effect of increasing lolitrem B concentration; Quadratic of LB = quadratic effect of increasing lolitrem B concentration.

[d] Indigestible Acid Detergent Fiber.

observed in calves consuming endophyte-infected compared with endophyte-free tall fescue. This agrees with results observed in the current study which had an average environmental temperature of 9.3 ± 0.26°C. Therefore, no difference in intake was expected because of increasing ergovaline. Also, data suggest that lolitrem B and ergovaline may have a similar affect on DMI.

Intake of N and NDF was not affected ($P > 0.19$) by increasing lolitrem B concentration. Also, apparent total tract DM, OM, and NDF digestibility did not differ ($P > 0.13$) between diets. Similarly, Bluett et al. (2001) reported no difference in OM digestibility by lambs grazing two perennial ryegrass varieties containing different levels of lolitrem B (3420 vs. 2420 ppb). Additionally, our results agree with those of Stamm et al. (1994). Stamm et al. (1994) noted no difference ($P > 0.10$) in straw and total DMI or apparent digestibility of DM and NDF by steers consuming straw with an increasing alkaloid (ergovaline) concentration. Ergovaline increased from < 10 to 160 ppb in the current study and from < 50 to 475 ppb in Stamm et al. (1994).

No TRT effects were observed for IADF intake ($P > 0.18$); however, ruminal IADF fill increased linearly ($P = 0.01$) and IADF passage rate (%/h) decreased linearly ($P = 0.04$) as lolitrem B level increased (Table 3). It is possible that reticulo-rumen smooth muscle activity may have been reduced as lolitrem B concentration increased, subsequently reducing ruminal IADF passage. Smith et al. (1997) inhibited gastrointestinal tract smooth muscle activity in sheep by dosing lolitrem B into the jugular vein. Furthermore, McLeay et al. (1999) noted that lolitrem B inhibited ($P < 0.001$) the frequency of reticular and ruminal contractions in sheep compared with those not receiving lolitrem B. However, the lack of a difference in DM and OM intake as lolitrem B level increased suggests that this probably did not occur in the current study.

No TRT X hour interactions ($P > 0.08$) were noted for ruminal NH_3-N, pH, total VFA, or molar proportions of acetate, propionate, isobutyrate, butyrate, isovalerate, or acetate:propionate ratio. Therefore, only overall TRT means are discussed. We did observe a TRT X hour interaction ($P = 0.01$) for the molar proportion of valerate; however, after reviewing the data, we concluded that the interaction did not appear biologically relevant. Consequently, we are only reporting overall TRT means for valerate.

Increasing lolitrem B concentration did not affect ($P > 0.15$) ruminal NH_3-N, pH, or total VFA (Table 4). Molar proportions of propionate, isobutyrate, butyrate, valerate, and acetate:propionate ratio, were not affected by increasing lolitrem B level ($P > 0.09$). However, a quadratic influence was observed for acetate and isovalerate ($P < 0.05$), with the greatest molar proportion of acetate occuring with the LOW-MIX and HIGH-MIX diets, while the greatest molar proportion of isovalerate occurred with the LOW and HIGH diets. It is not readily apparent why molar proportions of acetate and isovalerate responded in this manner. While other ruminal fermentation data evaluating increasing lolitrem B levels within perennial ryegrass is unavailable, Stamm et al. (1994) did report ruminal fermentation parameters of cattle consuming tall fescue straw with increasing concentration of another alkaloid ergovaline. Similar to the current study, the authors did not report a TRT difference in ruminal NH_3-N. However, contrary to results of Experiment 1, they did observed a linear decrease for ruminal pH as ergovaline content of the diet increased. Additionally, they reported

Table 4. Effect of increasing lolitrem B concentration on ruminal fermentation dynamics in steers consuming perennial ryegrass straw

	Treatment[a]					*P*-Value[c]	
Item	LOW	LOW-MIX	HIGH-MIX	HIGH	SEM[b]	Linear of LB	Quadratic of LB
NH_3-N, mM	2.9	3.0	3.2	3.7	0.59	0.35	0.73
pH	6.6	6.2	6.6	6.5	0.06	0.16	0.36
Total VFA, mM	81.7	81.0	80.2	86.7	3.53	0.39	0.33
Molar proportion, mol/100mol							
Acetate	69.0	70.7	69.2	68.4	0.42	0.10	0.01
Propionate	17.2	16.8	17.7	17.9	0.41	0.14	0.56
Isobutyrate	0.66	0.65	0.75	0.72	0.562	0.26	0.87
Butyrate	11.4	10.2	10.6	11.1	0.46	0.86	0.11
Isovalerate	1.06	0.83	0.93	1.22	0.107	0.24	0.04
Valerate	0.72	0.70	0.76	0.70	0.453	0.98	0.70
Acetate:Propionate ratio	4.0	4.2	3.9	3.9	0.10	0.10	0.24
Rumen Liquid Fill, ml/kg BW	152.7	161.4	152.5	148.5	12.64	0.71	0.63
Ruminal Dilution Rate, %/h	14.5	14.2	13.7	13.0	0.68	0.14	0.78

[a] LOW = 100% low straw (<100 ppb lolitrem B); LOW-MIX = 67% low straw:33% high straw (1550 ppb lolitrem B); HIGH-MIX = 33% low straw:67% high straw; HIGH = 100% high straw.

[b] n = 4.

[c] Linear of LB = linear effect of increasing lolitrem B concentration; Quadratic of LB = quadratic effect of increasing lolitrem B concentration.

that total VFA increased linearly as ergovaline concentration increased. The current study utilized a high alkaloid straw that contained 3 times less ergovaline than that used by Stamm et al. (1994; 160 ppb vs. 475 ppb). Consequently, it is possible that steers in Experiment 1 did not receive a high enough ergovaline dose to cause an effect on ruminal pH or total VFA.

There was no difference ($P > 0.13$) in rumen fluid fill or dilution rate as lolitrem B concentration increased (Table 4). Again, there is no other data of which we are aware evaluating increasing lolitrem B on rumen fluid dynamics. However, our results agree with those reported by Forcherio et al. (1992). They evaluated energy and protein supplementation effects on ruminal fermentation by cows consuming endophyte-infected tall fescue hay and did not see a difference in ruminal fluid passage rate as alkaloid concentration increased. However, Hannah et al. (1990) reported a linear decrease in ruminal fluid fill and a linear increase in ruminal fluid dilution rate of lambs consuming diets with increasing ergovaline concentration. This may have been due, in part, to a linear decrease in ruminal digestibility of OM, NDF, and cellulose, as alkaloid concentration increased.

No TRT X day interactions ($P > 0.32$) were noted for serum prolactin, HR, respiration rate, or rectal temperature. Therefore, only overall TRT means are discussed. Alkaloid (lolitrem B and ergovaline) concentration did not influence serum prolactin or HR ($P > 0.41$); however, a quadratic effect ($P = 0.03$) was noted for respiration rate, with the greatest values occurring with the LOW-MIX and HIGH-MIX diets (Table 5). Rectal temperature increased quadratically ($P = 0.03$) as lolitrem B increased, with the highest temperature observed with the HIGH-MIX TRT. These

Table 5. Effect of increasing lolitrem B concentration on serum prolactin and physiological variables in steers consuming perennial ryegrass straw

Item	Treatment[a]				SEM[b]	P-Value[c]	
	LOW	LOW-MIX	HIGH-MIX	HIGH		Linear of LB	Quadratic of LB
Serum Prolactin, ng/ml	10.0	5.7	11.2	4.4	4.63	0.60	0.79
Heart Rate, beats/min	82	77	78	77	4.7	0.55	0.70
Respirations, breaths/min	29	34	32	29	1.6	0.94	0.03
Rectal temperature, °C	38.7	38.9	39.2	38.9	0.09	0.05	0.03

[a] LOW = 100% low straw (<100 ppb lolitrem B); LOW-MIX = 67% low straw:33% high straw (1550 ppb lolitrem B); HIGH-MIX = 33% low straw:67% high straw; HIGH = 100% high straw.

[b] n = 4.

[c] Linear of LB = linear effect of increasing lolitrem B concentration; Quadratic of LB = quadratic effect of increasing lolitrem B concentration.

results may be because of increased ergovaline in the diet as lolitrem B level increased. Paterson et al. (1995), in their review of the effects of fescue toxicosis on beef cattle productivity, stated that animal temperature and respiration rate are normally increased, and serum prolactin decreased, with increasing ergovaline intake. However, the magnitude of change observed in temperature and respiration rate as lolitrem B level increased in the current study is small (0.5°C and 5 breaths/min, respectively). Also, our quadratic effects do not correspond with what is normally expected following a linear increase in ergovaline intake (linear increase in temperature and respiration rate; Paterson et al., 1995). Therefore, the response observed for temperature and respiration rate may not be related to ergovaline intake. This is supported by the lack of a TRT effect on serum prolactin. Additionally, Hemkin et al. (1981) reported that Holstein calves consuming endophyte-free or endophyte-infected tall fescue did not have different respiration rates or rectal temperatures at environmental temperatures of 23°C or less. However, calves consuming endophyte-infected tall fescue did have higher respiration rates and rectal temperatures than calves consuming endophyte-free tall-fescue when maintained at an environmental temperature of 34° C or greater. In the current study, the average environmental temperature was 9.3 ± 0.26°C. This suggests that our study was conducted in an environment not conducive to the same physiological response trends reported by Paterson et al. (1995). Also, Stamm et al. (1994) reported no difference in HR, respiration rate, or rectal temperature of steers consuming tall fescue straw with increasing ergovaline concentration. However, these authors reported a weekly decrease in serum prolactin as ergovaline concentration increased. This contradicts serum prolactin results in the current study, probably because of the lower ergovaline concentration in our study compared with Stamm et al. (1994; 160 ppb vs. 475 ppb).

Experiment 2: Performance/Production Study

During the course of the study, 13 of 24 (54%) cows consuming the HIGH endophyte-infected diet were observed to have a "clinical sign" score of three or higher indicating that they suffered from ryegrass staggers. These cows were removed from the study. The first removal occurred on d 8 and the last on d 43. It is of interest to note that none of the 13 cows were observed to have difficulty calving and their calves appeared healthy. One cow on the HIGH TRT expired during the trial. On multiple days a "clinical signs" score of 2 had been assigned to her; however, she had received a 0 for the two days previous to her death. Cause of death was inconclusive following necropsy and histopathilogical analysis.

Pre- and post-calving BW and BCS change were not affected ($P > 0.10$) by increasing lolitrem B concentration (Table 6). This suggests that across TRT, cows maintained themselves equally and entered the lactation period in similar body condition. These data concur with Eerens et al. (1997a) who noted no difference in pre-lambing BW change by ewes grazing endophyte-free or endophyte-infected perennial ryegrass/white clover pasture.

Table 6. Effect of increasing lolitrem B concentration on dry matter intake, digestibility, and performance in cows consuming perennial ryegrass straw

	Treatment[a]				*P*-Value[c]	
Item	LOW	MIX	HIGH	SEM[b]	Linear of LB	Quadratic of LB
Initial						
Weight, kg	527	547	558	9.3		
Body Condition Score	5.4	5.4	5.4	0.02		
Pre-calving change[d]						
Weight, kg	55.8	53.9	51.4	5.31	0.57	0.96
Body Condition Score	-0.14	-0.05	0.06	0.076	0.11	0.94
Post-calving change[e]						
Weight, kg	7.8	15.2	19.4	6.13	0.22	0.85
Body Condition Score	-0.03	-0.11	-0.08	0.079	0.64	0.62
DMI, g•kg BW^{-1}•d^{-1}	11.7	11.3	13.6	1.34	0.13	0.81
DM Digestibility, %	51.6	49.8	48.8	0.51	< 0.01	0.57
Days to Calving[f]	68	65	63	2.1	0.13	0.81
Calf Birth Weight, kg	39.8	38.6	39.7	1.15	0.96	0.45
Calf Gain, kg/day of age[g]	1.10	1.06	1.04	0.071	0.58	0.93
Milk Production, kg[g]	11.7	11.3	13.6	0.57	0.03	0.078

[a] Low = 100% low straw (467 ppb lolitrem B); Mix = 50% low straw:50% high straw (2017 ppb lolitrem B); High = 100% high straw.
[b] n =6.
[c] Linear of LB = linear effect of increasing lolitrem B concentration; Quadratic of LB = quadratic effect of increasing lolitrem B concentration
[d] Within 14 d of calving.
[e] Within 24 h post-parturition.
[f] Measured from study initiation (January 10, 2003) to parturition.
[g] Obtained 53 ± 1.4 d post-calving.

Similar to DMI of steers in Experiment 1, DMI of cows was not affected by increasing lolitrem B concentration. Also, work by Bluett et al. (2001) in which lambs were allowed to graze one of two varieties of perennial ryegrass (Aries HD, 3420 ppblolitrem B and 160 ppb ergovaline; Yatsyn 1, 2420 ppb lolitrem B and 450 ppb ergovaline) resulted in 12% greater herbage intake among lambs grazing grass with a higher concentration of lolitrem B than lambs grazing perennial ryegrass with a lower lolitrem B level. However, these data are confounded by a greater concentration of ergovaline in the perennial ryegrass variety with the lower lolitrem B concentration compared with the variety containing higher lolitrem B. As research with lolitrem B is limited, a comparison of DMI from previous work evaluating ergovaline level follows.

Our data agrees with Stamm et al. (1994), who reported no difference in DMI among steers consuming tall fescue straw with increasing concentration of the alkaloid ergovaline. However, in contradiction to the results of Stamm et al. (1994), Bluett et al. (2001), and Experiment 1, a linear decrease ($P < 0.01$) in digestibility of perennial ryegrass straw was observed as alkaloid level increased. Digestibility (%) of LOW, MIX, and HIGH TRT was 51.6, 49.8, and 48.8, respectively. It is not readily apparent why this difference occurred, however, it may have been because of variety differences and/or harvest conditions of the two straws used to obtain required endophyte levels.

Eerens et al. (1997b) reported delayed parturition in ewes grazing endophyte-infected perennial ryegrass pasture compared to ewes grazing endophyte-free perennial ryegrass/white clover pasture. This is contrary to the results of our study, which had no difference ($P > 0.12$) in days to calving among the three TRT groups. It may be possible that an alkaloid/species interaction exists that accounts for delayed parturition in sheep (Eerens et al., 1997b) compared with cows. Also, calf birth weight did not differ ($P > 0.44$) across TRT. This is similar to results reported by Eerens et al. (1997a), in which lamb birth weight was not affected by ewes grazing endophyte-infected or endophyte-free pasture.

Calf gain/day of age at WSW was not affected ($P > 0.57$) by TRT. This contradicts other research which has shown a performance difference in nursing young while the dam is consuming endophyte-infected grass. Eerens et al. (1997a) reported greater ($P < 0.05$) weight gains by lambs of ewes grazing endophyte-free perennial ryegrass/white clover pasture compared with lambs of ewes grazing endophyte-infected pasture. Additionally, Peters et al. (1992) reported that calves of cows grazing endophyte-infected tall fescue had lower ($P < 0.05$) daily gains than calves of cows grazing endophyte-free tall fescue. In the current study, cows received meadow grass hay during lactation instead of endophyte-infected straw. This may have contributed to similar calf gain/day of age among TRT.

On May 7, 2003, calf weight was recorded and WSW was conducted (53 ± 1.4 d post-calving). Surprisingly, a linear increase ($P = 0.03$) in milk production was noted as lolitrem B level increased. This is contradictory to research conducted by Lean (2001). In a case study, Lean (2001) reported that Holstein-Fresian dairy cows had a 4.6 liter reduction in milk production when consuming perennial ryegrass silage that contained a high concentration of ergovaline compared to cows consuming a similar perennial ryegrass silage containing a low concentration of ergovaline. Also, in a study with tall fescue, Peters et al. (1992) reported that daily milk consumption of calves nursing cows grazing endophyte-infected tall fescue was 25% lower ($P < 0.05$) than that of calves nursing cows grazing endophyte-free pasture. It is possible that there could have been a stronger ergopeptide influence in the experiments conducted by Peters et al. (1992) and Lean (2001) compared to the current study. In his review, Oliver (1997) suggests that it is the ergopeptide class of alkaloids that influences serum prolactin (an indicator of potential milk production). Additionally, in Experiment 1, there was no difference in serum prolactin as lolitrem B concentration increased. This suggests that milk production should not have been negatively affected by increasing lolitrem B level within the straw, which is what we observed. Furthermore, differences in milk production within our study may have been the result of removing "stagger" cows from the HIGH TRT which decreased the number of observations used in obtaining pen means.

Implications

Feeding perennial ryegrass straw with greater than 2000 parts per billion lolitrem B can cause neurological disorders that increase management concerns. However, blending of low and high lolitrem B straws can be a successful management alternative. Therefore, perennial ryegrass straw containing lolitrem B levels similar to those used in the current study has value as a forage source for ruminants. This information should provide the grass-seed industry, importers of ryegrass straw, and livestock producers with valuable information concerning safe feeding practices for use with ruminant livestock.

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Appendix

List of Abbreviations

ADF	Acid Detergent Fiber
AOAC	Association of Official Analytical Chemists
BCS	Body Condition Score
BW	Body Weight
°C	Celsius
Co-EDTA	Colbalt-Ethylenediaminetetraacetic Acid
CP	Crude Protein
d	Day
DEQ	Department of Environmental Quality
DM	Dry Matter
DMI	Dry Matter Intake
g	Gram
H	Perennial Ryegrass Straw Containing a High Concentration of Lolitrem B
h	Hour
ha	Hectare
hd	Head
HR	Heart Rate
IADF	Indigestible Acid Detergent Fiber
IRCRD	Intra-Ruminal Chromium Releasing Device
IU	International Unit
kg	Kilogram
L	Perennial Ryegrass Straw Containing a Low Concentration of Lolitrem B
m	Meter
min	Minute
ml	Milliliter
N	Nitrogen
NDF	Neutral Detergent Fiber
NH_3-N	Ammonia Nitrogen
NPN	Non-Protein Nitrogen
NRC	National Research Council
OM	Organic Matter
ppb	Parts Per Billion
ppm	Parts Per Million

Appendix (Continued)

SBM	Soybean Meal
TRT	Treatment
VFA	Volatile Fatty Acid
wk	Week
WSW	Weigh-Suckle-Weigh
wt/vol	Weight by Volume