

Effect of Lasalocid on Weight Gains,
Rumen Fermentation and Forage
Intake of Stocker Cattle
Grazing Winter Wheat
Pasture

By

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CHAPTER I

INTRODUCTION

Wheat pasture is a high quality forage available for grazing cattle during the winter months over much of the southern United States. Estimates of wheat forage crude protein exceed 21% of dry matter (NRC, 1984). However, wheat forage protein may exceed 30% of dry matter and is typically highly soluble in the rumen. Daily weight gains of cattle grazing wheat pasture can exceed 2 lbs., although can be quite variable as availability of forage may be short at times, and winter weather may alter grazing and forage availability.

Daily gains of cattle are a key figure in determining profitability of a wheat pasture stocker enterprise. Supplemental feeding programs offer a means of increasing daily gains, and adding stability to the stocker cattle enterprise. Feeding grain to stocker cattle is a convenient means of supplementing feed to wheat forage, however feed efficiency of grains for wheat forage supplementation has been shown to be quite poor, 9.2 lbs grain/ lb. gain or 16.2 lbs

grain/lb. gain if wheat forage is not limiting (Elder et al., 1967).

Ionophores, lasalocid and monensin, can be easily incorporated into grain feed mixes. Lasalocid has been shown to increase daily gains (Horton, 1983) and increase feed efficiency in feedlot cattle. Monensin increased daily gains 15% in stocker cattle grazing small grains and rye pastures. (Ellis et al., 1983).

The mode of action of ionophores is not completely understood. Several studies have reported that the molar proportions of acetic and butyric acids decreased while molar proportion of propionic acid increased when lasalocid was fed. (Davis, 1978; Brown and Davidovich, 1979; Thonney et al., 1981; Bartley and Nagaraja, 1982). Changes in rumen production of acids that are more efficiently utilized may explain part of the response to lasalocid, however other actions probably also aid in increased performance. Lasalocid decreased microbial protein synthesis, methane production and lactic acid production (Bartley and Nagaraja, 1982). By altering ruminal microbial metabolism and growth, ionophores improve nutrient digestibility and utilization in ruminants. Ferrell (1982) reported lasalocid, monensin and salinomycin fed to steers in a high energy ration improved digestibility of dry matter and organic matter. Ricke et al., 1981, showed lambs fed lasalocid had improved N retention.

Limited information is available as to the effect of lasalocid on performance of cattle grazing wheat or small grains pastures. Therefore, the objective of this research is to evaluate the effect of lasalocid on performance, rumen fermentation and forage intake of stocker cattle grazing winter wheat pasture.

CHAPTER II

REVIEW OF LITERATURE

Lasalocid is a polyether ionophore that has been shown to be effective in improving performance of feedlot cattle (Raun et al., 1976, Thoney et al., 1981). Pressman et al., (1967) were the first to classify polyether antibiotics as ionophores because of their ability to induce cation permeability in biological membranes by carrying ions across lipid-by-layer membranes, as lipid insoluble complexes. The mechanism(s) of action of polyether ionophores is largely related to this effect.

Lasalocid is classified as a carboxylic acid ionophore, it forms complexes with monovalent and divalent cations and aids in the transport and exchange of the cations for protons across a wide variety of biological membranes (Stuart et al., 1983). Carboxilic acid ionophores, monensin and lasalocid, have been shown to effect the relative proportions of volatile fatty acids (VFA's) produced by rumen bacteria (Baile, 1979). Ionophores may also influence protein degradation in the rumen (Fuller and Johnson, 1981), digesta flow rate (Ellis and Delaney, 1981), voluntary feed consumption (Baile, 1979) and the profile if the microbial

population in the rumen (Van Nevel and Deymeyer, 1977). The mode of action of monensin and lasalocid has been shown to be quite similiar in regard to rumen fermentation (Bartley et al., 1979). Therefore, for the purpose of this review of literature on lasalocid, monensin research is sometimes compared or utilized in an attempt to illustrate the effect of lasalocid and its mode of action.

Feedlot Trials

Data compiled from 12 studies (T.M. Frye, 1983) relative to the effect of lasalocid on performance of light weight growing and finishing steers fed a wide variety of rations showed considerable benefits in feed efficiency and rate of gain for lasalocid. Lasalocid improved feed efficiency in newly weaned calves by 11.2% and average daily gain by 4.6% over control animals. Growing cattle fed lasalocid at 30 grams per ton of feed gained 9.3% more efficiently and 3.9% faster than cattle recieving no lasalocid. A summary of 7 feedlot trials on finishing steers (Frye, 1983) showed lasalocid improved average daily gain 7.9% and improved feed efficiency 7.2%.

Long term responses of growing and finishing cattle to lasalocid summarized by Horton (1983) showed cattle fed lasalocid gained 6.6%, 3.2% and 4.8% faster than control animals during the growing, finishing, and overall test

period, respectively. Feed efficiency was improved 10.4%, 6.4%, and 8.3%, respectively, by lasalocid.

In a lamb feedlot trial (Patterson et al., 1983), lambs were fed lasalocid in a ground ear corn based diet formulated to contain 82% of the NRC recommended protein level. Lambs were supplemented with soybean meal or an dehydrated alfalfa and distillers dried grains designed to be a escape protein. Lambs fed the escape protein gained 35% faster ($P < .05$) than lambs fed soybean meal. Rate of gain was improved 16% with lasalocid in escape protein diets but reduced 19% in soybean meal supplemented diets. This may be partially explained by a reduction in feed intake in lambs fed soybean meal diets with lasalocid.

Forage Trials

Lasalocid was cleared on December 20, 1984 by the Food and Drug Administration for use in pasture cattle. Information regarding the effect of lasalocid on grazing cattle is not as abundant as information on the effect of lasalocid on feedlot cattle. However, similar responses with regard to feed efficiency and rate of gain as shown in feedlot cattle might be expected.

Data pooled from sixteen lasalocid trials with grazing cattle (Miller et al., 1984), in which cattle received 0, 50, 100, 200, or 300 mg lasalocid/head/day showed that daily

gains were increased linearly ($P < .01$) through the 200 mg/day dosage of lasalocid. Spears and Harvey (1984) studied stocker steers grazing pastures containing a mixture of orchard grass, tall fescue and ladino clover receiving 0, 200, or 300 mg lasalocid per day. Lasalocid improved weight gains by 18.9% and 13.5%, respectively, over controls. Thonney et al., (1981) reported that 83, 175, or 122 mg lasalocid per day, or 149 mg lasalocid in mycelium cake resulted in a quadratic increase in rate of gain of steers fed alfalfa cubes ad libitum.

Stocker steers grazing dormant fescue pasture were fed supplemental soybean meal or an escape protein supplement made of distillers dried grains and dehydrated alfalfa (Supplements contained 50g N and 1.5 kg TDN) with and without lasalocid (Patterson et al., 1983). Steers receiving supplemental protein gained about .5 kg/day more than unsupplemented steers, however differences among treatments were not significant ($P > .10$). Lasalocid supplementation had no effect on daily weight gains ($P > .10$).

In order to determine the optimal dose of lasalocid for cattle grazing fescue pastures (Backus et al., 1981) fed stocker cattle 0, 50, 100, 200 or 300 mg lasalocid/head/day. Cattle receiving 200 or 300 mg lasalocid per day gained faster ($P < .01$) than cattle in other treatment groups. This data indicates optimal dosage level of lasalocid to be 200 mg/day. Potter et al. (1976) found similar results for stocker cattle grazing pastures consisting of alfalfa, brome

grass and ladino clover, or fed green chopped forage of the same composition. The apparent optimal dosage of monensin was 200 mg/day. Monensin increased average daily gain 17% in pasture trials, and increased daily gains 18%, and feed efficiency 14% in green chopped forage trials.

Finally, in a summary of feedlot data prepared by Frye, (1983), which compared the effect of lasalocid on 400 lb. calves fed high forage or high grain diets. Lasalocid improved daily gains by 4.6%, decreased feed intake 6.1%, and increased feed efficiency 11.2%. Responses to lasalocid tended to be greater with the high forage diets.

Mode of Action of Lasalocid

Energy Metabolism

Much of the response to ionophores can be accounted for by modified rumen metabolism. The basic mode of action of ionophores is to modify the movement of ions across biological membranes. Ionophores generally have antibiotic effects against gram positive bacteria (Westly, 1977). Lasalocid and monensin inhibit most lactate producing bacteria (Dennis et al., 1981). However, among the lactate producers, Dennis et al., (1981) found those that produced succinate as a major end product were not inhibited by lasalocid or monensin. Therefore, the reported increase in

molar proportions of propionic acid in ruminal fluid of cattle fed lasalocid, (Speers and Harvey, 1984; Brown, 1979) possibly results from selection in the rumen for succinate producing and lactate fermenting bacteria. Chen and Wollin (1979) reported that lasalocid decreased numbers of acetate and butyrate producing bacteria in in vitro studies. Lasalocid decreased rumen fluid acetic:propionic acid ratios in growing cattle (Brown, 1979; Bartley, 1984; Davis, 1978) and decreased the molar proportions of butyrate and valerate ($P < .10$) when fed at 200 mg/day (Bartley, 1984).

Lasalocid increased molar proportions of propionate ($P < .05$) in steers grazing pastures consisting of tall fescue, orchard grass, and ladino clover mixtures (Speers and Harvey, 1984), and in feedlot steers receiving high energy diets (Davis, 1978). The theory that propionate is more efficiently utilized than acetate is based on two principles. First, that propionate production in the rumen is more efficient than acetate as discussed by Hungate (1966) and Second, is evidence that propionate is utilized by host animal tissues more efficiently (Smith, 1971). Propionate appears to be more flexible as a energy source in that it has the potential to be used for gluconeogenesis or utilized directly in the citric acid cycle.

Methane is one by-product of rumen fermentation. Methane represents a loss of about 8% of gross energy intake. (Benz et al., 1980) reported that monensin fed at 27 ppm in the ration of steers reduced methane energy loss by 4%.

Monensin decreased methane production in steers (Thorton, 1981) and lambs (Joyner, 1979). Joyner also reported that monensin decreased fecal and urinary energy losses. The decrease in urinary energy loss indicates an extra ruminal effect of lasalocid. The decrease fecal energy loss indicates that lasalocid may increase metabolizable energy values of feedstuffs. Additionally, Joyner (1979) reported a decrease in heat production of lambs feed monensin, probably due to a decrease in ruminal microbial activity. Overall, monensin increased dietary energy retained by the animal. However, in steers fed low, medium, and high roughage rations, monensin did not significantly affect heat loss or dry matter digestibility (Thorton et al.,1980).

Monensin appears to be altering rumen microbial fermentation resulting in decreased methane production and inconclusively decreasing urinary and fecal losses. This response may be partially diet and/or animal dependent. The ability of ionophores to alter rumen fermentation by selection for specific rumen microbes that are more efficient in converting dietary energy to microbial energy explains part of their ability to increase gains and/or efficiency of gains of growing and finishing cattle.

Protein Metabolism

The changes in energy metabolism discussed in the previous section may only partially account for the improved performance of ruminants fed ionophores. Therefore, the effects of monensin and lasalocid on protein metabolism has been studied in order to more fully explain the mode of action of ionophores in improving cattle performance.

In a lamb digestion trial, Patterson et al., (1983), lambs fed chopped fescue hay and soybean meal or disitillers dried grains and alfalfa meal (isonitrogenous) with or without lasalocid. Nitrogen intake was not affected however, total tract nitrogen digestibility was increased with addition of lasalocid ($P < .05$). However, lasalocid may be decreasing digestion of nitrogen in the rumen. Lasalocid and monensin inhibited microbial protein production in vitro, (Bartley and Nagaraja, 1982; Van Nevel (1977, 1979). This may be due to their ability to act as a deaminase inhibitor, thereby decreasing deamination of amino acids to ammonia (Dinius et al., 1976; Van Nevel and Deymeyer, 1977, 1979; Horton, 1979; Chalupa et al., 1980). This would presumably decrease microbial protein production, because most micro organisms prefer ammonia to peptides or amino acids as a source of nitrogen. This effect is supported by results reported in vivo. Tolbert et al. (1977) reported monensin increased free amino acid concentrations in the rumen. Poos et al. (1979), reported monensin decreased flow of bacterial

nitrogen flow to the small intestine, and increased bypass of feed nitrogen by 37% in steers fed Brewers dried grains. Monensin inhibited protein degradation and increased dietary nitrogen reaching the small intestine of steers, (Whetstone et al., 1980). Owens et al., (1980) observed a 14% increase in abomasal flow of non ammonia nitrogen reaching the small intestine of steers fed monensin. In vivo results of Isichei and Bergen (1980) utilizing high concentrate and roughage diets with monensin supplementation, also tend to support the theory that ionophores increase bypass of feed nitrogen. By inhibiting dietary protein degradation in the rumen, lasalocid and monensin appear to be shifting the site of nitrogen digestion to the post-ruminal tract. This should improve efficiency of nitrogen utilization by decreasing losses associated with transfer of feed protein to microbial protein.

Crude protein level of diets did not affect weight gain or feed conversion responses to lasalocid of feedlot steers (Brethour et al., 1982). However, light weight calves fed corn silage-based rations with different protein levels showed variable daily gain responses to lasalocid. Lasalocid increased average daily gain of cattle fed corn silage without protein supplementation (ration CP= 9%), but had no effect on calves fed corn silage with soybean meal or urea supplementation to result in a dietary crude protein content of 13%. Beede et al., (1980) reported a greater gain response to monensin in cattle fed low protein diets. The

data indicate a greater advantage to feeding ionophores to cattle receiving low protein and/or high roughage diets. However this would be expected to depend partially on the quality of protein reaching the small intestine as well as quantity of protein in the ration.

Feed Intake and Utilization

Rumen fill and passage rate play an important role in ruminant nutrition as factors that influence feed intake, digestibility, site of digestion, extent of microbial fermentation, nitrogen utilization and end products of fermentation. Research with monensin (Lemenger, 1978; Pond and Ellis, 1978; Ellis and Delaney, 1981) suggests monensin may be slowing digestion by decreasing rumen turnover rate and also increasing rumen fill. Ricke et al. (1983) showed rumen liquid and solid dilution rates tended to be reduced by lasalocid and monensin. This may partially explain the increase in digestibility of dry matter and organic matter in steers fed whole shell corn diets with addition of the ionophores lasalocid, monensin and salinomycin (Ferrell, 1983). Studies with lambs have indicated lasalocid increases nitrogen digestibility, while leaving cell wall and dry matter digestibility unaffected (Petersen et al., 1981; Ricke et al., 1981). In contrast, initial dry matter digestibilities were reduced by monensin ($P < .05$) in lambs fed

ground corn diets and grain sorghum with urea or brewers dried grains as the protein sources (Poos et al., 1979). However, by 40 to 46 days after initiation of the trial, dry matter digestibility of monensin fed animals was not different than that of animals not receiving monensin. The initial decrease in digestibility may be due to palatability problems associated with feeding monensin.

Muntifering et al., (1980) found monensin had no significant effect on apparent digestibility of dry matter, gross energy or starch in steers fed a corn based diet. Digestibility of crude protein tended to be higher for steers fed monensin, but this increase was not significant. In another trial with a corn based diet, (Thorton et al., 1978), digestibilities of dry matter, crude protein and starch were improved by monensin. However, the magnitude of this improvement seemed to be dependent on the crude protein level of the ration. The trials of Muntifering et al. (1980) may have been less sensitive to the effects of monensin because of the low level of protein (10.5% dry matter basis) in the ration. Additionally, Rust et al., (1978) allowed feeding level to be free choice, whereas Muntifering et al., (1980) limited feed intake. Monensin fed cattle (Thorton et al. 1979) consumed 9% less than the control cattle. This reduced feed intake, coupled with a longer retention time, may account for the increase in feed digestibility and may not be an effect of ionophore supplementation.

Rust et al. (1978) reported in another study on the effect of level of protein and monensin supplementation on nutrient digestion in feedlot cattle and reported no protein level by monensin interaction in steers fed high moisture corn diets. In this study monensin increased digestibilities of dry matter, organic matter, starch and nitrogen to the same extent with 9.3 and 12.3 percent crude protein diets. Again the increased digestibility may be partially or totally explained by a decreased feed intake (12.3%). A slightly lowered nitrogen retention was observed in monensin fed cattle, however this may be due to a reduced nitrogen intake.

Lambs fed brewers dried grains or urea supplemented diets without monensin retained more nitrogen ($P < .05$), than lambs fed 30 mg monensin per day (Poos et al., 1979). Lasalocid, monensin and salinomycin decreased fecal nitrogen output, however had no effect on loss of nitrogen in the urine of steers fed a corn and cottonseed hull diet (Ferrell, 1983). Apparent nitrogen digestibility was enhanced by ionophores, ($P < .05$), however the nitrogen retention was not significantly affected. Ionophores increased digestibility of dry matter and organic matter ($P < .05$) and had no affect on starch digestibility.

Coccidiosis

Coccidiosis is a disease in cattle caused by infection with protozoa of EIMERIA spp. These are intercellular host specific parasites that occur in most animals, however not all are pathogenic. The disease primarily strikes young cattle, less than two years old. It results in reduced feed consumption, poor performance, mucoid diarrhea, and possibly death due to dehydration. Fitzgerald (1975) estimated that 77 million cattle less than 1 year of age would be infected that year in the United States. Coccidiosis is seen more frequently during the cool and wet times of the year. Coccidiosis is transmitted by oocysts present in the feces. These oocysts may be picked up from consuming contaminated feed, water, or licking contaminated materials.

Lasalocid is an effective anticoccidial compound for cattle and sheep when fed at high enough levels (Horton, 1982). Although research indicates dosage levels of lasalocid needed for effective control of coccidiosis are higher than for optimal performance response, it will control coccidiosis when fed at 5 mg/kg body weight in calves (Horton, 1982). Reinfection is common in severe outbreaks. Although lasalocid may aid in prevention of coccidiosis, proper care and treatment should be used to prevent the infection.

Frothy Bloat

Frothy bloat is commonly seen in cattle fed high grain low roughage diets, and cattle grazed on legume and/or wheat pastures. Bloat is caused by the inability of the animal to eructate gas produced by the rumen as fast as it is being produced. The rumen gas is commonly trapped by excessive foam produced by rumen digesta. In severe cases, bloat will lead to animal death.

Lasalocid has been demonstrated to aid in reducing the incidence of grain bloat and alfalfa bloat (Bartley et al., 1983). In grain bloat, the major foaming agent appears to be bacterial slime that traps rumen gas producing a foam. In legume bloat the primary foaming agents appear to be derived from plants. Lasalocid fed at the level of .66 mg/kg body weight effectively prevented grain bloat from developing when given to animals before feeding high grain diets. Lasalocid fed at .66 to .99 mg/kg body weight reduced the severity of legume bloat about 26% in the studies of Bartley et al. (1983).

Frothy bloat is a major cause of death in wheat pasture stocker cattle (Horn, 1983). Bloat occurs most frequently when cattle first arrive on pasture and in the early spring growth period when chemical composition of forage is changing rapidly. Because lasalocid was effective in reducing the incidence and severity of bloat in cattle grazing alfalfa pasture (Bartley et al., 1983), it is a logical assumption

that it may be beneficial in reducing bloat in cattle grazing wheat pasture.

Lactic Acidosis

Intake of high grain diets in ruminants provides starch for rapid fermentation. Large amounts of lactic acid are frequently produced. Lactic acid is a particularly strong acid ($pK=3.9$), and is produced by rumen bacteria in natural (D) and unnatural (L) forms. In an acidosis situation, rumen pH drops as lactic acid accumulates. In severe cases it may fall to as low as 4.0 causing severe rumenitis. Absorption of excessive quantities of lactic acid into the blood produces a metabolic acidosis, death may occur due to failure of hemoglobin to bind oxygen (Van Soest, 1981).

Dennis et. al. (1981) studied the effects of monensin and lasalocid on lactate producing and lactate using rumen bacteria. Their work showed lasalocid and monensin inhibited most of the lactate-producing bacteria. This work supports the findings of Chen and Wollins (1979), that lasalocid and monensin are effective in selecting for a microbial population in the rumen that produces more propionate, and less acetate, butyrate and lactate. This suggests that monensin and lasalocid may be effective in decreasing lactic acid acidosis because of their ability to select against the

major lactic acid producers, while not affecting lactic acid fermenters.

Toxicity of Lasalocid

Because lasalocid is an antibiotic it is necessary to be aware of toxicity levels to avoid overdosing . Lasalocid in high concentrations may affect biological membranes of the host animal. Galitzer et al. (1982) studied the maximum tolerable levels of lasalocid an animal could consume without detrimental effects. Signs of toxicity occurred at approximately 100 mg lasalocid/kg of body weight in cattle. Signs of lasalocid toxicity included muscle tremors, increased heart rate and respiration rates followed by anorexia and diarrhea. Death is possible in severe cases of lasalocid toxicity.

CHAPTER III

THE EFFECT OF LASALOCID ON WEIGHT GAINS, RUMEN FERMENTATION AND FORAGE INTAKE OF STOCKER CATTLE GRAZING WINTER WHEAT PASTURE

Summary

Effects of laslocid on weight gains, forage intake and ruminal fementation of stocker cattle were studied during a 2-year study on winter wheat pasture. Twenty-seven fall-weaned Hereford and Hereford x Angus heifers with mean initial weights of 215 kg were used each year. The heifers grazed a common wheat pasture for about 100 d each year, and were individually fed 1.06 kg of supplement (6 days/wk) prorated to supply 0, 100, or 200 mg lasalocid/head/day. Forage intakes and ruminal fluid pH, ammonia and VFA concentrations of the heifers were measured once each year. Fecal outputs and forage organic matter digestibilities (OMD) were estimated, respectively, by chromium dilution and use of indigestible neutral detergent fiber as an internal marker. Mean daily gains (kg), of heifers fed 200 mg lasalocid/day were .11 kg greater ($P < .05$) than heifers of the other 2

treatments. Mean OMD and forage OM intakes were not different ($P>.05$) among treatments. Ruminal ammonia concentrations (mg/dl) increased with level of lasalocid (10.57^a , 15.22^b , and 17.81^b), respectively, ($P<.05$) in year 1; however differences among means (8.32, 11.95 and 11.66) were not significant in year 2. Consistent effects of lasalocid on total VFA concentrations, and molar proportions of acetic, propionic and butyric acids were not observed. Ruminal fluid acetic:propionic acid ratio's of heifers fed 0, 100, or 200 mg lasalocid/head/day were not different ($P>.05$).

Introduction

Lasalocid is a polyether ionophore that was cleared by the food and drug administration (FDA) as a feed additive for cattle grazing pasture in December of 1984. Ionophores form lipid-soluble complexes with minerals and facilitate their transport across bilayer membranes and lipid soluble complexes (Pressman et al., 1967).

Lasalocid has been shown to increase daily gains of cattle grazing mixed pastures (Speers and Harvey, 1984) and fescue pastures (Backus, 1981). Lasalocid decreased rumen acetic acid, and increase propionic acid concentrations in vitro and in vivo, decreased rumen methane production, and inhibited microbial protein production (Bartley et al., 1979).

The objective of this research was to determine the effect of lasalocid on rumen fermentation, forage intake and daily weight gains of stocker cattle grazing winter wheat pasture.

Experimental Procedure

Cattle Performance. Twenty-seven fall weaned Hereford heifers that averaged 209 kg in year 1 (1982-83), and twenty-seven Hereford and Hereford x Angus heifers that averaged 222 kg in year 2 (1982-83) were blocked by initial weight in year 1, and initial weight within breeds in year 2, and allotted to three treatments. Treatments consisted of 0, 100 and 200 mg lasalocid/day. Heifers grazed a common winter wheat pasture for 100 and 101 days, respectively, during the 1982-83 and 1983-84 wheat pasture growing seasons. The heifers were fed in individual feeding stalls 6 days/week 1.06 kg supplement that was prorated to supply 0, 100 or 200 mg lasalocid/head/day. Ground corn was used as the carrier feed in year 1. In year 2, supplements consisted of (% as fed): ground corn, 75%; cottonseed hulls, 10%; ground alfalfa hay, 8%; liquid molasses, 7%; plus the desired amount of lasalocid. Supplements were fed in pelleted form (3/16 inch pellet) in year 2.

Initial on-test , mid-term and off-test weights were taken during the trials. All weights were measured following a 15 to 17 h drylot shrink without feed or water.

Forage Intake Trials. Wheat forage intake and digestibility of dry matter (DMD) and organic matter (OMD) were measured once during each of the 2 trials. Heifers were bolused with gelatin capsule that contained 4 g of chromic oxide twice daily (0800 and 1600 h) during 6-day preliminary and 5-day fecal collection periods in year 1, and 6-day preliminary and 4-day fecal collection periods in year 2. Fecal samples were taken from the rectum at time of bolusing, dried, and were composited across sampling times for each heifer for chromium analysis by atomic absorption spectrophotometry. Fecal outputs were calculated by the chromium dilution technique. Forage DM and OM intakes were estimated by dividing fecal outputs by forage indigestibilities. Forage DMD and OMD were determined by using indigestible neutral detergent fiber (INDF) as an internal indigestible marker (calculations are shown in figure 1 of the appendix). The INDF concentrations of fecal and hand-clipped forage samples were determined as neutral detergent fiber remaining after a 144 hour in vitro incubation with 40 ml of buffered rumen fluid. The neutral detergent fiber analysis was conducted as described by Goering and Van Soest (1970). Sodium sulfite was deleted from the neutral detergent solution, as suggested by

Robertson and Van Soest (1981), during the refluxing of forage samples.

Ruminal Fermentation Measurements. Rumen fluid samples were collected from 7 heifers per treatment at the end of each forage intake trial by aspiration through a stomach tube. Samples were obtained 4 h after feeding the lasalocid supplements. Heifers grazed wheat pasture after consuming the supplements until rumen fluid samples were obtained. Rumen fluid samples were strained through four layers of cheesecloth, and pH was immediately measured with a pH meter and glass electrode. One hundred milliliter aliquots of the strained fluid samples were acidified with 2 ml of 20% sulfuric acid and stored in an ice slurry until ammonia analyses were conducted within 2 h by a modification of the magnesium oxide distillation method (Horwitz, 1975). Ten milliliters of acidified ruminal fluid, 1 g of magnesium oxide and .5 g of powdered pumice stone, and 1 ml of CaCl_2 (25% w/v in water) and five drops of caprylic alcohol were added to macro-Kjeldahl flasks. Five-milliliter aliquots of the strained ruminal fluid were prepared for VFA analysis by deproteinization with 1 ml of 25% w/v meta-phosphoric acid that contained 2-ethylbutyric acid as an internal standard. Samples were centrifuged at $25,000 \times g$ for 20 minutes and the supernatants were refrigerated until analyzed for VFAs by gas chromatography.

Statistical Analysis of Data. Analysis of variance was conducted using the General Linear Model of the Statistical

Analysis System (Helwig and Council, 1979) for a completely randomized block design. Initial weight gain analysis for year one was conducted using initial weight (breed), and treatment as sources of variation. The initial model for year 2 weight gains included, treatment, initial weight, breed, breed X treatment interaction, and initial weight within breed. The models were reduced when sources of variation were not significant components of the model ($P > .15$). Initial weight block in year 1, and initial weight block, breed, breed X treatment, and weight block(year) in year 2 were not significant sources of variation ($P > .15$), and therefore dropped from the model. The data were combined and analyzed across years with treatment, year, and year X treatment sources of variation.

Forage intake data was analyzed using the same model as that used in the analysis of weight gain data, as were rumen fermentation data. However, a year by treatment interaction occurred for acetic, propionic and butyric acids ($P < .10$), therefore the rumen fermentation data were analyzed by year with treatment as the source of variation, and reported as such. Analysis of variance results for final models of weight gain, rumen fermentation, and forage intake are reported in appendix tables 12-18.

Results and Discussion

Cattle Performance. In year 1, two heifers in the 0 mg lasalocid/day treatment group died from bloat, and in year 2 one heifer died of pneumonia in the 0 mg lasalocid/head/day treatment group, and one heifer in the 100 mg/day group was removed from the trial because of coccidiosis.

Effects of lasalocid on weight gains of the heifers in year 1 are shown in table 1. During the first 57 days of year 1, daily gains of heifers that received 200 mg lasalocid/day were greater than gains of heifers that received 0 or 100 mg lasalocid/day. However, differences among treatments were not significant. During the last 43 days of year 1, daily gains of heifers that received 200 mg lasalocid/day were greater ($P < .05$) than those that received 0 or 100 mg lasalocid/day. Daily gains of heifers fed 200 mg lasalocid/day for the entire 100-day grazing period of year 1 were 0.10 to 0.12 kg greater ($P < .05$) than gains of heifers fed 0 or 100 mg lasalocid/day.

Daily gains for heifers in year 2 are shown in table 2. Daily gains of heifers fed 100 and 200 mg lasalocid/day were similar during the first 45 days, and were greater than those of heifers fed 0 mg lasalocid/day. However, means of the three treatments were not different ($P > .05$). During the last 56 days of year 2, daily gains of heifers fed 200 mg lasalocid/day were higher than those of heifers fed 0 or 100 mg. Mean daily gains were not different among treatments

($P > .05$). Increasing levels of lasalocid seemed to increase daily gains of heifers for the entire grazing period, but differences among treatments were not significant ($P > .05$).

Effects of lasalocid on weight gains of heifers of both years are shown in table 3. The year by treatment interaction was not significant ($P > .90$) and therefore the data were combined across years. Daily gains of heifers fed 200 mg lasalocid/day were 0.11 kg greater ($P < .05$) than those of heifers fed 0 or 100 mg lasalocid/day.

Forage Intake Trials. Effects of increasing levels of lasalocid on fecal outputs, DMD and OMD of wheat forage, and forage intakes of heifers grazing wheat pasture are shown in table 4. The year x treatment interaction was not significant ($P > .30$) for any of the measurements. Therefore data were pooled across years. Forage DM and OM digestibilities were similar for heifers fed 0, 100 and 200 mg lasalocid/day. Forage DM intakes were unusually high. However, fecal ash concentrations were also high (7.0% to 15.0%), and suggest that the heifers consumed a considerable amount of soil with the forage. Because insoluble ash appears as a cell wall component in the NDF procedure, fecal NDF concentrations expressed as a percentage of dry matter may have been biased upwards. Thus, forage DM intakes would be biased upwards by high fecal ash concentrations. Calculated intakes of forage OM would not be affected by fecal ash. However, lasalocid did not affect intake of forage OM.

Ruminal Fermentation Measurements. Ruminal fluid pH, ammonia and VFA concentrations of the heifers are shown in table 5. Because the year x treatment interaction was significant ($P < .10$) for the molar proportions of acetic, propionic, and butyric acids, the rumen fermentation data are shown for each year in tables 5 and 6.

In year 1, 200 mg lasalocid reduced rumen pH ($P < .05$). A similar, nonsignificant ($P > .05$) trend was observed for rumen pH in year 2. Rumen ammonia concentrations were increased ($P < .05$) by both levels of lasalocid in year 1. A somewhat similar trend for rumen ammonia concentrations was observed in year 2, although treatment differences were not significant.

General trends with regard to effects of lasalocid on total VFA concentrations, molar proportions of individual acids and acetic:propionic acid ratios were not apparent. Total VFA concentrations of heifers fed 200 mg lasalocid were increased ($P < .05$) in year 1. Lasalocid supplementation did not affect ($P > .4$) total VFA concentrations in year 2. Neither level of lasalocid affected ($P > .05$) the molar proportions of acetic, propionic or butyric acids, or resulted in differences ($P > .05$) in the acetic:propionic acid ratio of ruminal fluid. Isovaleric acid concentrations of heifers of year 2 were increased ($P < .05$) with increasing level of lasalocid.

These data indicate that 200 mg lasalocid/day is effective in increasing weight gains of stocker cattle on

wheat pasture. The mechanism(s) by which weight gains were increased needs further study. Alterations by lasalocid of the site of nutrient digestion and flow of nutrients to the postruminal tract (Zorrilla-Rios et al., 1985) may be involved.

Table 1. Effect of lasalocid on daily gains (kg) of heifers grazing winter wheat pasture.

Year 1

		mg lasalocid/head/day			SE ^a
		0	100	200	
No. of heifers		7	9	9	
Mean initial Weight, kg		209	210	209	
<u>Grazing Interval</u>	<u>Days</u>				
12/28-2/24	57	.68	.70	.77	.035
2/25-4/8	43	.99 ^b	.92 ^b	1.10 ^c	.037
12/28-4/8	100	.80 ^b	.79 ^b	.90 ^c	.031

^a Largest standard error of the means.
^{b, c} Means in a row with different superscripts differ (P<.05).

Table 2. Effect of lasalocid on daily weight gains (kg) of heifers grazing winter wheat pasture.^b

Year 2

		mg lasalocid/head/day			SE ^a
		0	100	200	
No. of heifers		8	8	9	
Mean initial weight, kg		223	226	220	
<u>Grazing interval</u>	<u>Days</u>				
1/13-2/27	45	1.02	1.15	1.16	.056
2/28-4/24	56	1.25	1.27	1.40	.053
1/13-4/24	101	1.14	1.22	1.30	.051

^a Largest standard error of the means.

^b Means are not different ($P < .05$).

Table 3. Effect of lasalocid on daily weight gains of heifers grazing winter wheat pasture.

Years 1 and 2 Combined

	mg lasalocid/head/day			SE ^c
	0	100	200	
No. of heifers	15	17	18	
Mean initial weight, kg	217	217	215	
Average daily gain, kg	1.03 ^a	1.03 ^a	1.14 ^b	.030

^{a,b} Means in rows with different superscripts differ (P<.05).

^c Largest standard error of the means.

Table 4. Effect of lasalocid on fecal output, digestibility of forage dry matter (DM) and organic matter (OM), and forage intake of heifers grazing winter wheat pasture.^a

Item	Mg lasalocid/head/day			SE ^b
	0	100	200	
No. of heifers	16	17	18	
Fecal output				
% of body wt				
DM	.66	.64	.68	.023
OM	.59	.58	.61	.021
Forage Digestibility, %				
DM	84.78	84.25	83.83	.370
OM	82.26	81.42	81.27	.449
Forage Intake,				
% of body wt				
DM	4.40	4.13	4.23	.187
OM	3.36	3.12	3.33	.141

^a Pooled data of years 1 and 2. Differences among treatment means are not significant ($P < .05$).

^b Largest standard error of the means.

Table 5. Effect of lasalocid on rumen fermentation.

Year 1

	mg lasalocid/head/day			SE ^a
	0	100	200	
No. of Heifers	6 ^c	7	7	
Rumen pH	6.9 ^d	6.9 ^d	6.6 ^e	.07
Ammonia (mg/dl)	10.57 ^d	15.22 ^e	17.81 ^e	1.71
Total VFA, mMoles/L ^b	96.95 ^d	109.35 ^e	128.58 ^e	8.90
VFA molar proportions, %				
Acetic	56.6	58.1	56.6	.89
Propionic	20.7	20.1	18.9	.62
Isobutyric	1.9	1.9	2.2	.18
Butyric	16.3	14.9	17.4	.86
Isovaleric	2.9	2.9	2.8	.23
Valeric	1.6	1.8	2.1	.24
Acetic:Propionic Ratio	2.7	2.9	3.0	.11

^a Standard error of the mean.^b Acetic, propionic, isobutyric, butyric, isovaleric and valeric acids.^c One heifer was removed from study because of poor quality sample.^{d,e} Means in rows with different superscripts are different (P<.05).

Table 6. Effect of lasalocid on rumen fermentation.

Year 2

	mg lasalocid/head/day			SE ^a
	0	100	200	
No. of heifers	7	7	7	
pH	7.2	7.1	7.1	.14
Ammonia (mg/dl)	8.32	11.95	11.66	1.44
Total VFA, mMoles/L ^b	74.54	83.74	77.82	9.45
VFA molar proportions (%)				
Acetic	59.8	59.0	60.0	.93
Propionic	21.0	21.7	21.7	.57
Isobutyric	1.3	1.3	1.3	.07
Butyric	14.8	15.6	13.4	.84
Isovaleric	1.2 ^d	1.4 ^d	1.7 ^e	.13
Valeric	2.0	1.9	1.8	.27
Acetic:Propionic Ratio	2.9	2.7	2.8	.10

^a Largest standard error of the means.^b Acetic, propionic, isobutyric, butyric, isovaleric and valeric acids.^{d,e} Means in row with different superscripts are different (P<.05).

CHAPTER IV

EFFECT OF LASALOCID AND SAMPLING METHOD ON RUMEN FERMENTATION IN STOCKER CATTLE GRAZING WINTER WHEAT PASTURE

Summary

Eight multicannulated hereford steers were grazed on the same wheat pasture as heifers in experiment 1. Steers recieved 0 or 300 mg lasalocid/head/day. Rumen fluid samples were taken 4 hours after lasalocid treatment by stomach tube and through a rumen cannula and analyzed for ruminal pH, ammonia and total VFA concentrations. Rumen fluid samples taken by stomach tube had higher pH values, and lower ammonia and total volatile fatty acid concentrations ($P < .001$) than the rumen fluid samples taken from rumen cannulae. Molar proportions of VFA's were higher in stomach tube samples for acetic acid ($P < .05$), and lower for propionic acid ($P < .10$). Molar proportions of butyric, iso valeric and valeric acids were lower ($P < .05$) for stomach tube samples. Stomach tube samples were more variable for rumen ammonia and total VFA

concentrations. However, in general molar proportions of VFA's were less variable than rumen cannula samples. No treatment by sampling method interaction occurred ($P>.30$). Lasalocid had no effect on ruminal pH, ammonia, or total VFA concentrations in rumen cannula samples, and did not affect ($P>.10$) molar proportions of acetic or propionic acids. Stomach tube sample results also indicate lasalocid did not affect ruminal pH, ammonia or total VFA concentrations. However, a decreased ($P<.10$) molar proportion of acetic acid, and increased ($P<.10$) molar proportion of propionic acid was observed.

Introduction

In order to examine the effect of lasalocid on rumen fermentation in experiment 1 it was necessary to obtain a rumen fluid sample by stomach tube from the heifers involved in the trial. Inserting a stomach tube is a common method of obtaining these samples, however saliva contamination of the samples led to concern over the effect of this contamination on volatile fatty acid concentrations and ruminal ammonia and pH levels. A purer sample of rumen fluid can easily be obtained with cannulated steers. Experiment 2 was conducted to examine the effect of method of rumen fluid collection on rumen fluid pH, ammonia and VFA concentrations, and to obtain

more data as to the effect of lasalocid on rumen fermentation in stocker cattle grazing winter wheat pasture.

MATERIALS AND METHODS

Eight multicannulated steers that averaged 409 kg were grazed on the same wheat pasture as cattle in experiment 1, and were randomly allotted to two treatments. Treatments consisted of 0 or 300 mg lasalocid per day. Lasalocid was administered directly into the rumen in a gelatin capsule containing lasalocid and a small quantity of ground corn as a diluent.

Rumen fluid samples were collected approximately 4 h following lasalocid treatment by aspiration through a stomach tube, similarly to the procedure used in experiment 1, and directly through the rumen cannula. Samples taken through the rumen cannula were composites of rumen fluid from the anterior dorsal, anterior ventral, posterior dorsal and posterior ventral sites of the rumen.

Rumen fluid samples were measured immediately for rumen pH using a glass electrode and pH meter. The samples were handled in a similar manner as described in experiment 1, and analyzed by the same modified magnesium oxide distillation procedure for ruminal ammonia, and standard gas chromatography procedures for ruminal VFA concentrations.

Analysis of data was conducted using the General Linear Model Procedure of the Statistical Analysis System (Helwig and Council, 1979) for a completely randomized design. Lasalocid treatment, animal within treatment, sampling method, and treatment by type interactions were used as sources of variation in the initial model to compare the effect of sampling type. To examine the effect of lasalocid, data were analyzed by sampling method with treatment source of variation. The coefficient of variation of the models were compared as an indication of variability of sampling method.

Results and Discussion

Ruminal fluid pH and ruminal ammonia concentrations are shown in table 7. Rumen fluid pH was higher ($P < .001$) in samples from stomach tubing (STS) than from samples taken from the rumen cannula (RCS). This was expected because of saliva contamination of stomach tube samples and the buffering capacity of saliva. Ruminal ammonia concentrations of STS were lower ($P < .001$) than RCS. Additionally total volatile fatty acid concentrations, shown in table 8, were lower in stomach tube samples ($P < .001$). Decreased pH values, and ammonia and total VFA concentrations are most likely a result of dilution of the samples with saliva during sampling. The molar proportion of acetic acid was higher ($P < .005$), propionic acid was lower ($P < .10$), and butyric acid

was lowerer ($P < .05$) in stomach tube samples indicating sampling method affected proportions of individual acids.

Interestingly, sample type did not interact with treatment ($P < .10$). This indicates that sampling type had no affect on relative trends of component concentrations with lasalocid treatment.

Coefficient of variations (CV) for ruminal pH and ammonia concentrations from analysis by method of sampling are shown in table 9. Coefficient of variations for ruminal ammonia concentratrions were higher in stomach tube samples. A similiar trend was noted for total VFA's (mMoles/L) shown in table 10. However CV's for the molar proportions of volatile fatty acid were higher for acetic, propionic, and butyric acids, as was CV for acetic to propionic ratio in rumen cannula samples. These data indicate ruminal ammonia, and total VFA concentrations of samples taken by stomach tube are more variable than samples taken through the rumen cannula. However, it would appear that molar proportions of VFA's from samples taken through a stomach tube are generally less variable (table 10) than samples taken through the rumen cannula.

Lasalocid supplementation had no affect on ruminal pH, ammonia or VFA concentrations in rumen cannula or stomach tube samples. In contrast, data of experiment 1 (year 1) indicated 200 mg lasalocid/head/day decreased ruminal pH ($P < .05$). In addition lasalocid decreased ($P < .10$) the molar proportion of acetic acid, and increased the molar proportion

of propionic acid ($P < .10$) and the acetic:propionic ratio ($P < .05$) in stomach tube samples. Lasalocid did not affect ($P > .10$) molar proportions of acetic, propionic or butyric acids in rumen cannula samples. Results of experiment 1 indicated lasalocid had no affect on molar proportions of VFA's, with the exception of increased molar proportion of isovaleric acid ($P < .05$) in cattle fed 200 mg lasalocid/head/day. Bartley et al. (1979) reported that lasalocid decreased ruminal acetic acid concentration and decreased the acetic:propionic acid ratio. Speers and Harvey (1984) reported lasalocid lowered the ruminal acetic acid concentration ($P < .05$), increased propionic acid ($P < .05$), and lowered butyric and valeric acid concentrations.

Results from this experiment indicate there are problems associated with sampling methods. The impact of sample type should be considered in interpreting data of this type. We know stomach tube samples in experiment 1 and experiment 2 were biased by saliva contamination as indicated by high ruminal pH values. Rumen cannula samples were less variable for rumen ammonia and total VFA concentrations, but were generally more variable for molar proportions of acids. Conclusions cannot be made about the accuracy of results from either sampling procedure. However, because we know stomach tube samples have saliva contamination, it is logical to put more faith in results from rumen cannula samples.

Table 7. Effect of type of Rumen Sampling on Rumen pH,
and Rumen Ammonia Concentrations

	Type		
	Rumen Cannula	Stomach tube	SE ^b
Rumen pH	6.06	7.58 ^a	.090
Rumen Ammonia, (mg/dl)	42.97	15.97 ^a	3.039

^a Means are different ($P < .001$).

^b Standard error of the mean.

Table 8. Effect of rumen fluid sampling type on volatile fatty acid concentrations.

	Sampling Method		
	Rumen Cannula	Stomach Tube	SE ^a
Total VFA, mMoles/L ^b	6.21	57.97 ^c	10.40
VFA Molar Proportions, (%)			
Acetic	60.3	63.7 ^d	.50
Propionic	20.8	19.8 ^f	.34
Iso-butyric	2.0	2.1	.09
Butyric	12.7	11.1 ^d	.24
Iso-valeric	2.4	2.0 ^e	.08
Valeric	1.8	1.5 ^c	.03
Acetic:Propionic Ratio	2.94	3.23 ^e	.024

^a Standard error of the mean.

^b Acetic, propionic, iso-butyric, butyric iso-valeric, and valeric acids.

^c Means differ (P<.001)

^d Means differ (P<.005)

^e Means differ (P<.05)

^f Means differ (P<.10)

Table 9. Coefficients of Variation for Models Predicting
LS Means for rumen pH and Ammonia Concentrations.

	Sampling Method	
	Rumen Cannula	Stomach Tube
Rumen pH	3.70	3.78
Rumen Ammonia (mg/dl)	25.12	53.14

Table 10. Coefficients of Variation of Models Predicting
LS Means for Volatile fatty Acid Concentrations.

	Sampling Method	
	Rumen Cannula	Stomach tube
Total, VFA mMoles/l ^a	16.68	56.26
<u>VFA Molar Proportions</u>		
Acetic	4.04	2.71
Propionic	5.97	4.00
Iso-butyric	7.42	20.91
Butyric	16.46	12.23
Iso-valeric	9.82	14.30
Valeric	1.79	18.72
Acetic:Propionic Ratio	8.53	5.77

^a Acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids.

Table 11. Ruminal pH, Ammonia, and VFA concentrations of rumen fluid samples taken by stomach tube or rumen cannula.

	Rumen Cannula			Stomach Tube		
	mg Lasalocid/head/day					
Measurement	0	300	SE ^a	0	300	SE ^a
Rumen pH	6.05	6.07	.118	7.61	7.54	.143
Rumen Ammonia	37.08	48.86	5.398	13.72	18.21	4.243
Total, VFA mm/l	146.3	146.1	12.19	52.9	63.0	16.308
<u>VFA Molar Proportions</u>						
Acetic	61.9	58.8	1.20	65.1	62.3 ^b	.86
Propionic	20.2	21.3	.62	19.1	20.4 ^b	.40
Iso-butyric	1.8	2.1	.07	2.0	2.2	.22
Butyric	12.1	13.4	1.05	10.5	11.6	.68
Iso-valeric	2.2	2.6 ^c	.12	1.9	2.1	.14
Valeric	1.7	1.9	.16	1.5	1.5	.14
Acetic:Propionic Ratio	3.08	2.76	.12	3.41	3.06 ^c	.09

^a Standard error of the mean.

^b Means within sampling method are different (P<.10).

^c Means within sampling method are different (P<.05).

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APPENDIX

Figure 1. Calculations for Indigestible Neutral Detergent Fiber, Digestibility of Forage DM and OM and Forage Intake.

$$\% \text{INDF, \% of DM} = \frac{(\text{Indigestible NDF residue, g DM}) * 100}{(\text{sample weight, g} * \% \text{DM})}$$

$$\text{DM Digestibility, \%} = \frac{(\text{INDF in forage, \% of DM}) * 100}{(\text{INDF in feces, \% of DM})}$$

$$\text{Forage DM Intake (kg)} = \frac{\text{Fecal output, kg DM}}{(1 - \text{DM digestibility})}$$

$$\% \text{INDF, \% of OM} = \frac{(\text{Indigestible NDF residue, g OM}) * 100}{(\text{sample weight} * \% \text{DM} * \% \text{OM})}$$

$$\text{OM Digestibility, \%} = \frac{(\text{INDF in forage, \% of OM}) * 100}{(\text{INDF in feces, \% of OM})}$$

$$\text{Forage OM Intake (kg)} = \frac{(\text{Fecal output, kg OM})}{(1 - \text{OM digestibility})}$$

Table 12. Analysis of variance for ruminal
fermentation measurements

Year 1

	Treatments	Error
Degrees of freedom	2	17
Sum of Squares; Variable		
pH	0.544	0.470
Ammonia	171.360	298.951
VFA molar proportions;		
Acetic	10.613	81.040
Prpionic	10.898	38.801
Isobutyric	0.496	3.124
Butyric	23.257	76.312
Isovaleric	0.080	5.600
Valeric	0.635	5.768
Acetic:Propionic Ratio	0.227	1.283

Table 13. Analysis of variance for ruminal
fermentation measurements

Year 2

	Treatments	Error
Degrees of freedom	2	18
Sum of Squares; Variable		
pH	0.073	2.370
Ammonia	56.847	222.649
VFA molar proportions;		
Acetic	16.290	93.002
Propionic	2.613	35.277
Isobutyric	0.012	0.464
Butyric	16.213	75.892
Isovaleric	0.983	1.965
Valeric	0.103	2.398
Acetic:Propionic Ratio	0.103	1.158

Table 14. Analysis of variance for weight gains.

	Treatments	Year	Treatment*year	Error
Sum of Squares	0.149	2.796	0.001	0.615
degrees of freedom	2	1	2	44

Table 15. Analysis of variance for dry matter intake (% of body weight).

	Treatments	Year	Treatment*year	Error
Sum of Squares	0.601	0.300	0.326	25.282
degrees of freedom	2	1	2	45

Table 16. Analysis of variance for organic matter intake (% of body weight).

	Treatments	Year	Treatment*year	Error
Sum of Squares	0.607	0.021	0.386	14.412
degrees of freedom	2	1	2	45

Table 17. Analysis of variance for heifer fecal output of dry matter (kg).

	Treatments	Year	Treatment*year	Error
Sum of Squares	0.080	0.368	0.106	3.046
degrees of freedom	2	1	2	44

Table 18. Analysis of variance for heifer fecal output of organic matter (kg).

	Treatments	Year	Treatment*year	Error
Sum of Squares	0.107	0.221	0.097	2.562
degrees of freedom	2	1	2	45

Table 19. Forage crude protein and indigestible neutral detergent fiber for years 1 and 2.

	Protein (% DM)	IVDMD (% DM)
Year 1 ^a	27.83	69.55
Year 2	24.93	69.83

^a Forage quality samples taken during forage intake study.

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