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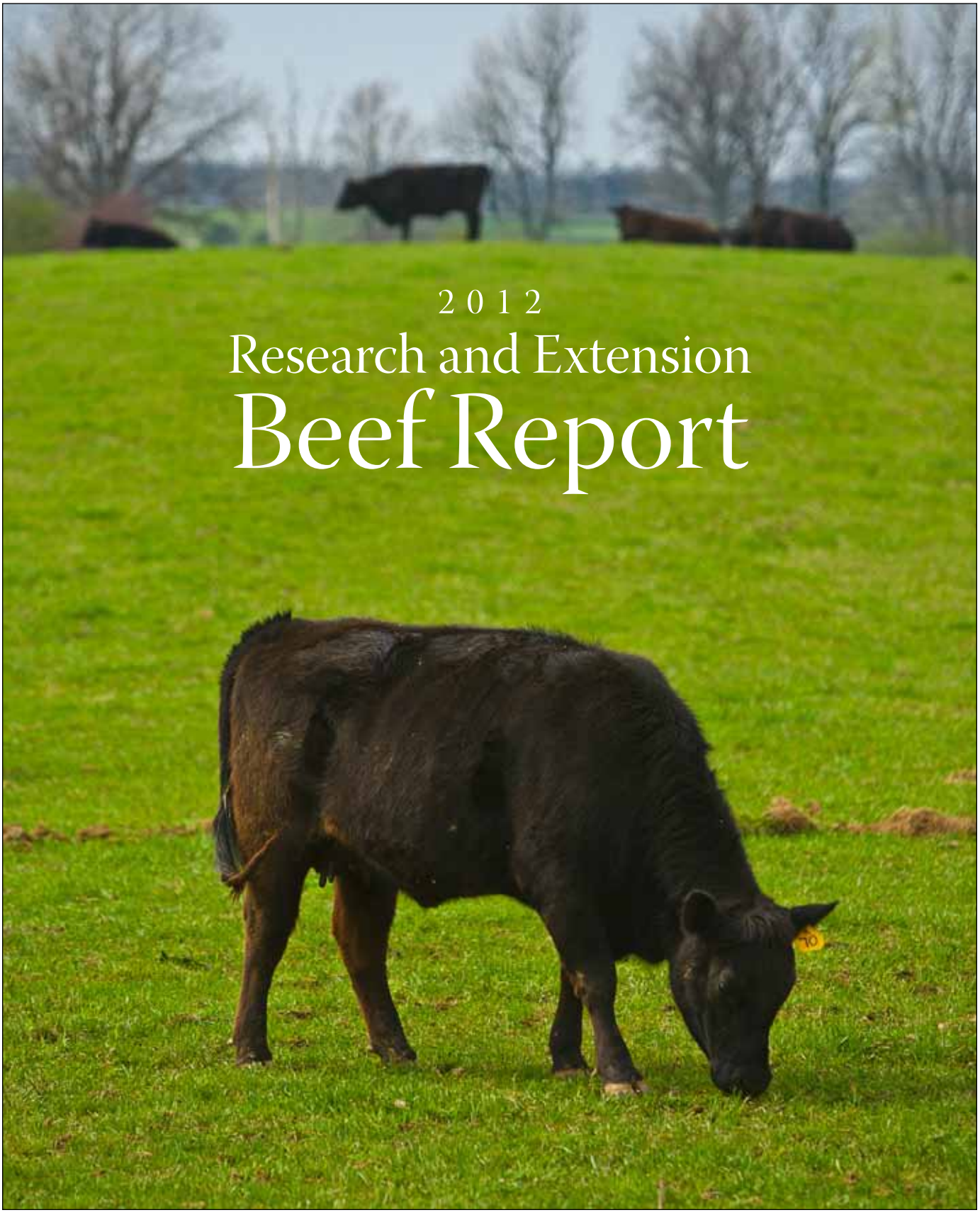

2012

Research and Extension Beef Report

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2012 Research and Extension Beef Report

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Foreword

The faculty and staff of the Department of Animal and Food Sciences within the College of Agriculture at the University of Kentucky, along with the Dean of the College of Agriculture, Dr. Smith, Associate Dean of Research, Dr. Cox, and Associate Dean of Extension, Dr. Henning, and our colleagues in the USDA Forage Animal Production Research Unit are pleased to present the 2012 Research and Extension Report. The report provides summaries of completed and on-going research and extension efforts related to the beef cattle industry involving faculty, staff and students within a variety of disciplines.

The intent of this report is to provide highlights of our research and extension activities. We have a vested interest in the beef industry in the state and nation. We hope this report provides a window into our programs. We believe that after viewing this report, a greater appreciation will be garnered with respect to our involvement in the multiple fields of study related to beef production. The faculty, staff and student activities are advancing our understanding of basic science principles of livestock production as well as applied research that producers and the industry can benefit from immediately, as well as in the future. Extension educational programs, on-farm demonstrations, and other activities aid in transferring this knowledge to producers, allowing for increased awareness and adoption of management change.

I hope you find this report informative and enjoyable to read. You are encouraged to contact the faculty members for additional information related to their research. Additionally, please contact me if you have general questions or comments regarding this year's report. Lastly, the use of product names in this report is not an endorsement of the product and is for the reader's convenience.

Dr. Jeff Lehmkuhler
Assistant Professor of Beef Cattle Extension and Editor

Alteration of Basal Metabolic Rate in Holstein Steers During Fescue Toxicosis

A.F. Koontz, A.P. Foote, D.H. Kim, L.P. Bush, J.L. Klotz, K.R. McLeod, and D.L. Harmon

Summary

The results of this study indicate that consumption of E+ tall fescue by cattle results in a reduction in basal metabolic rate. Six ruminally cannulated steers were weight-matched and pair-fed during a two period crossover experiment. Each period consisted of two temperatures (22°C and 30°C). During each segment, one steer per pair was ruminally dosed twice daily with ground endophyte-infected fescue seed (E+), the other with ground endophyte-free fescue seed (E-). On d8 of each segment, animals were moved to individual metabolism stalls fitted with indirect calorimetry head-boxes. Rumen contents were removed, weighed and subsampled. The reticulorumen was washed and filled with a buffer and an E+ or E-fescue seed extract was added at 12h intervals. After a 12h wait heart rate (HR), urine production, O_2 consumption, and CO_2 production were recorded for 16h. There was no difference in intake between endophyte treatments by design; however, intake decreased at 30°C. Increased temperature had no effect on other measurements. HR was unaffected by fescue treatment or temperature. DM of rumen contents as well as total rumen DM/kg $BW^{.75}$ increased in E+ animals. O_2 consumption decreased and CO_2 production tended to be reduced in E+ animals. Fasting heat production was reduced in E+ animals, suggesting that animals consuming E+ fescue use less energy for maintenance.

Introduction

Tall fescue is grown on more than 15 million hectares of land in the United States and more than half of these fields are infected with the fungal endophyte *Neotyphodium coenophialum*. This endophyte provides drought and heat tolerance to the grass. However, the ergot alkaloids produced by this endophyte cause health and production issues when consumed by grazing animals. This decrease in productivity has been estimated to cost United States beef producers more than $600 million per year.

Animals consuming infected fescue have a 10-150% reduction in intake and can have significant weight losses. It is unlikely that this reduction in weight is due solely to reduced intake. Changes in organ mass, gene expression, and stress can affect energy metabolism, altering nutrient availability and use. A reduction in intake can modify energy use as a result of reduced visceral organ mass. Whole body energy use may also be altered by alkaloid consumption. Several studies report that consumption of ergot alkaloids and reduced energy intake may interact to alter energy metabolism in rats consuming endophyte-infected tall fescue. However, minimal research has been conducted to examine and separate the effects of reduced energy intake and alkaloid consumption.

The majority of research on fescue toxicosis has relied on animal consumption of seed or hay to introduce alkaloids into the system. This experiment used a ruminally dosed animal model so as to avoid the possibility of a reduction in intake altering the quantity of alkaloids ingested by the animal over the course of the experiment. In addition, pair-feeding was utilized to separate the effects of reduced energy intake and alkaloid consumption on energy metabolism. The goal of this experiment was to use these methods to evaluate the interaction between consumption of endophyte-infected tall fescue and environmental temperature on basal metabolism in Holstein steers.

Materials and Methods

Animals Model and Experimental Design

Six Holsteins steers (BW=348 ±13 kg), surgically fitted with ruminal cannulas, were weight-matched into pairs for a two period cross-over experiment. Each period consisted of two temperatures, one each at 22°C and 30°C. Steers were housed in individual pens in temperature- and humidity-controlled rooms and fed once daily. The basal diet consisted of alfalfa cubes fed at 1.5x NE_m, top dressed with 40g trace mineralized salt. Water was available *ad libitum* throughout the experiment.

During the first 7d at each temperature, one steer per pair was ruminally dosed twice daily with ground endophyte-infected tall fescue seed (E+); the other animal in each pair received ground endophyte-free tall fescue seed (E-). Steers were offered alfalfa cubes at 1.5x NE_m during E+ dosing. During E- dosing, intake was restricted to be equal to the intake of the assigned E+ pair on the corresponding day.

Measurements

At 0700 on d8 at each temperature animals were moved to individual metabolism stalls fitted with indirect calorimetry head-boxes. In order to minimize the time between feeding and measurement of fasting heat production (FHP), rumen contents were evacuated. Contents were weighed and subsampled for dry-matter, covered with hay, and stored at 39°. Following evacuation, the reticulorumen was rinsed with warm (39°C) physiological saline, emptied, and filled with a buffer solution at 39°C.

During buffer incubation an E+ or E- fescue seed extract was added at 12h intervals to maintain treatment presentation to the animal. After buffer introduction animals were fasted for 12h prior to data collection. A 12h fast has been shown to be sufficient to bring the respiratory quotient of cattle to a stable baseline level following rumen evacuation. The collection period consisted of a 16h determination of heat production via indirect calorimetry. Inspired and expired air was analyzed for O_2 and CO_2 concentrations at 9 min intervals. Air flow was measured by individual mass flow meters and maintained at 600 L/min. In addition, heart rate was continually measured.

Urine was collected during the 16h FHP determination via continuous suction using a rubber funnel. Urine acidity was reduced to pH < 3 by adding H_3PO_4 to the collection. Urine output weight was recorded and subsampled for each period and steer. Samples were stored at 0°C prior to nitrogen analysis.

Whole-body HP over the 16h collection period was calculated by indirect calorimetry using a modification of the Brouwer equation as follows:

$$HP\ (kcal) = 3.869\ (L_{O2}) + 1.195\ (L_{CO2}) - 1.431\ (g_{UN})$$

Where HP is heat production, L_{O2} is oxygen consumed (L), L_{CO2} is carbon dioxide produced (L), and g_{UN} is urinary nitrogen excretion (g).

Following FHP determination, rumen contents were replaced and animals returned to individual pens and basal diets for 7d before repeating the procedure at 30°C. After the second FHP determination, animals were returned to individual pens for a 21d washout period prior to the cross-over period. The cross-over period was identical to the first with each steer within a pair on the alternate endophyte treatment.

Statistical Analysis

The data were analyzed using the Mixed procedure of SAS, with individual steer as the experimental unit. Animal and period were considered random effects, while endophyte treatment (E) and environmental temperature (T) were fixed effects. Data were analyzed for effects of treatment, temperature and the interaction of ExT. Indirect calorimetry data (O_2 consumption, CO_2 production, and respiratory quotient) and heart rate were averaged across the 16h measurement period prior to analysis. Treatment effects were considered significant at $P \leq 0.05$.

Results and Discussion

Both seed and extract dosing provided 4.1 mg•hd^{-1}•d^{-1} total ergovaline (ergovaline and ergovalinine). This level, as well as the 7d ruminal dosing method, were previously shown to be sufficient to induce fescue toxicosis.

Historically the most severe effects of fescue toxicosis are seen in the summer, as natural vasorelaxation necessary for thermoregulation at elevated ambient temperatures is contradicted by ergot alkaloid induced vasoconstriction, reducing the ability of the animal to dissipate heat. However, in this experiment, no interaction between treatment and temperature was observed ($P > 0.10$), and increased environmental temperature had no effect except to reduce dry matter intake by 17% ($P = 0.004$). There was no difference in intake between endophyte treatments ($P = 0.931$) due to the pair-feeding design. This lack of intake difference and effect of elevated environmental temperature indicates that observed differences can be attributed to the alkaloids present in endophyte-infected tall fescue.

Treatment with endophyte-infected seed increased the dry matter percentage ($P < 0.0001$) and total dry matter weight ($P < 0.0001$) of rumen contents, while total weight of rumen contents was not different between treatments ($P = 0.149$). Considering that the animals in this study were pair fed, these data suggest that there is alteration of rumen kinetics, possibly resulting in a reduction in particulate passage from the rumen of animals consuming endophyte infected tall fescue. Heart rate was unaffected by endophyte treatment ($P = 0.953$) or temperature ($P = 0.555$). Bradycardia has been previously shown to occur during fasting and following rumen evacuation. Thus, the lack of difference in heart rate between endophyte treatments is likely due to the rate already being at a physiological minimum due to fasting, with no opportunity available for further depression by ergot alkaloid ingestion.

In the present study, oxygen consumption was reduced ($P = 0.040$) in E+ dosed animals, while carbon dioxide production tended to be reduced ($P = 0.070$). These changes led to a lower calculated fasting heat production ($P = 0.006$) for animals dosed with E+ fescue seed. This is in contrast to previous work showing no difference in heat production between endophyte treatments in steers fed *ad libitum*. Other work has observed a reduction in heat production in lambs consuming a diet containing endophyte-infected fescue at 1.5% of body weight. As maintenance energy can be defined as the sum of FHP and energy used for digestion, the use of a rumen evacuation methodology for determination of fasting heat production may provide a more accurate indication of maintenance energy requirements than the traditional 48-72h fast.

Possible mechanisms underlying a reduction in FHP during fescue toxicosis in cattle are 1) reduced service organ size and 2) alteration of gene expression, resulting in changes in energy

Table 1. Comparison of physiological measures and gas production between steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed at 22°C and 30°C[1]

	Treatment					P =[2]		
	E-		E+			Main Effects		
Item	22°C	30°C	22°C	30°C	SEM	Endophyte	Temperature	
Body Weight (kg)	348	349	348	346	13.4	0.675	0.955	
Intake (kg DM/kg BW$^{.75}$)	81.5	67.5	81.6	68.1	4.76	0.931	0.004	
Rumen Contents (g/kg BW$^{.75}$)	492	509	555	540	48.7	0.149	0.614	
Rumen Contents (%DM)	5.8	6.9	12.5	11.6	1.22	<0.0001	0.972	
Rumen Contents (g DM/kg BW$^{.75}$)	32.0	35.5	65.5	67.4	0.812	<0.0001	0.955	
Heart Rate (beats/min)	51.1	48.1	47.5	51.0	5.34	0.953	0.555	
O_2 Consumption (L/kg BW$^{.75}$)	13.7	14.3	12.9	13.0	0.565	0.040	0.630	0.525
CO_2 Production (L/kg BW$^{.75}$)	9.9	10.5	9.3	9.8	0.423	0.070	0.989	0.110
Heat Production (kcal/kg BW$^{.75}$)	51.52	56.31	45.89	46.01	3.00	0.006	0.332	0.356

1 Data are presented as least squares means of animals dosed with E- and E+ treatments (n = 6).
2 Probability of a greater F statistic.

use. While neither of these were measured in the present experiment, both have been shown to occur in previous research regarding fescue toxicosis.

As much as 25% of whole-body energy use can be attributed to the hepatic tissues, thus a decrease in organ size would represent a reduction in maintenance energy requirements.

Several studies have shown that cattle entering the feedlot after grazing endophyte-infected tall fescue pasture exhibit compensatory gain and greater feed efficiency. The data presented here may provide a cause for this observation. If the consumption of E+ fescue causes a reduction in maintenance energy requirements, the animals would then be able to utilize the high levels of energy in feedlot diets more efficiently. Steers subjected to intake restriction during the growing phase, then re-fed, have been shown to have decreased maintenance requirements. After cessation of alkaloid intake and adaptation to a concentrate diet, animals no longer exhibit this increased efficiency and growth.

Implications

Ingestion of endophyte-infected tall fescue results in decreased fasting heat production in cattle. This is indicative of a reduction in maintenance energy requirements and may be related to a decrease in liver size or other metabolic activity in animals grazing endophyte-infected pastures. In addition, a reduction in metabolic rate may lead to the compensatory gain often observed in cattle entering the feedlot after grazing endophyte-infected pastures.

Ergovaline Recovery from Digested Tall Fescue Seed Heads

B.M. Goff, G.E. Aiken, and W.W. Witt

Summary

Steers were shown to selectively consume tall fescue seed heads during grazing, and there was near total release of ergovaline from these tissues during digestion, regardless of the maturity of the seeds. Seed heads were collected from pastures grazed by Angus-cross steers from early May until mid-June. Pastures were also monitored at this time for the grazing of seed heads by the cattle. Samples were digested with two-stage acid-pepsin procedure and the ergovaline concentration of all materials determined. Steers did not graze tall fescue seed heads until the first week of June and removed portions from ~80% of the seed heads by mid-June. The percentage of the ergovaline released during digestion decreased slightly between early and mid-June (100 to 96%). However, the ergovaline concentration of the seed heads increased during this period (1.66 to 4.41 ppm), and resulted in a larger total amount of alkaloids that may potentially be absorbed by the animal. Management strategies that reduce the reproductive growth of tall fescue in pastures, such as mowing or grazing at high stocking densities, are necessary to reduce the effects of fescue toxicosis, as they prevent the consumption of plant tissues that contain high concentrations of ergot alkaloids, all of which are readily released into the rumen during digestion.

Introduction

Ergot alkaloids produced by the fungal endophyte (*Neotyphodium coenophialum*) of tall fescue (*Lolium arundinaceum*) is a frequent issue faced by cattle producers in the southeastern US. These alkaloids, particularly ergovaline, have been shown to act as vasoconstrictors and lead to the symptoms of fescue toxicosis. Ergovaline concentrations within the seed heads of tall fescue are known to be up to three times higher than within leaf tissue, which makes the reproductive growth of the grass a management concern. To further complicate matters, there are documented reports of steers and geldings selectively grazing these tissues when grazing tall fescue pastures at low stocking rates. While previous studies have estimated the release of alkaloids from plant tissues in the ruminant digestive system, the focus was primarily on vegetative tissues. The objective of this study was to quantify the degree tall fescue seed heads were grazed upon by steers, and to estimate the amount of ergovaline released from these tissues during digestion.

Materials and Methods

Tall fescue seed heads were collected approximately weekly from 6 May to 17 June 2010 from three endophyte-infected KY-31 tall fescue pastures at the C. Oran Little Research Farm in Versailles, KY. Pastures were stocked with Angus cross steers at rates of 1.1 steers/acre. Seed heads were removed at their base to reduce excessive stem material within the sample, and their approximate stage of maturity visually estimated. The amount of total and grazed seed heads was estimated from 2.7 ft^2 quadrats collected at 50 random locations within each pasture.

Samples were freeze-dried and ground with a Wiley mill to pass through a 4 mm sieve. This larger particle size was done in an effort to replicate the size of materials entering the rumen following mastication. The in vitro organic matter digestibility (IVOMD) of the coarse-ground material was determined using a two-stage acid pepsin digestibility procedure adjusted for ash content. Replicates of the digested residues, as well as the initial undigested material, were reground to pass through a 1 mm sieve for determination of ergovaline using high-performance liquid chromatography (HPLC). All data reported is on a dry matter basis and was analyzed as a randomized complete block design (RCBD) with pasture and collection date as the block and whole plot, respectively. Significance was determined at a $P < 0.05$ level.

Results and Discussion

Steers did not begin to graze tall fescue seed heads until early June. On 4 June, 60.7% of the seed heads present had been grazed. Before this date, there was minimal seed head removal (< 10%). The selective grazing of these plant tissues increased to 67.9 and 78.8% on 11 and 17 June, respectively. The number of seed heads decreased slightly over this period (9.24 to 6.38 seed heads/ft^2), although the difference between dates was not significant ($P < 0.10$).

Table 1. Stage of maturity, *in vitro* organic matter digestibility (IVOMD), ergovaline concentration (undigested and digested), and percent of ergovaline released from tall fescue seed heads collected during 2010[1].

Date	Stage of Maturity	IVOMD	Ergovaline		
			Undigested	Digested	Released
		%	ppm		%
6 May	Shortly after boot stage, 1st floret visible	65.9ab	1.36a	0.00a	100.0a
13 May	Florets fully emerged	62.8c	1.32a	0.00a	100.0a
18 May	Seed head fully emerged and elongated	63.4bc	1.92a	0.00a	100.0a
4 June	Post-anthesis/fertilization	64.7abc	1.66a	0.00a	100.0a
11 June	Milky endosperm	67.4a	3.78b	0.09b	97.7b
17 June	Hard-dough endosperm	67.4a	4.41b	0.18c	95.9c

[1] Letters refer to significant difference at $P < 0.05$ level.

Initially, seed heads were beginning to emerge from the boot stage, with only a few florets visible (Table 1). Tall fescue seed heads were fully emerged and the branches of their panicles fully expanded by 18 May. When noticeable grazing by the steers occurred, the florets had shed the pollen and were beginning the initial stages of seed development. The seed continued to mature and were at the hard-dough endosperm stage of development on the last collection date (Table 1).

The IVOMD of the seed heads declined between 6 May and 13 May (Table 1) before reaching a maximum digestibility of 67.4% on the last two harvest dates (11 and 17 June, Table 1). The initial decline in IVOMD after 6 May is believed to be due to a majority of florets being immature and enclosed by the boot leaf. The steady increase in IVOMD after 13 May is most likely due to the accumulation of nonstructural carbohydrates within the developing seed. Because these carbohydrates are easily degradable by rumen microflora, their presence would dilute the impact of any fibrous tissue, such as the pericarp, that would developed during maturation.

The ergovaline concentration of the seed heads collected between 6 May and 4 June were relatively constant (1.36 to 1.92 ppm; Table 1), and were not significantly different ($P > 0.05$). Ergovaline concentrations increased within the seed heads to 3.78 and 4.41 ppm on for 11 and 17 June, respectively. Other researchers have reported similar trends for ergovaline concentration in tall fescue seed over the growing season, but reported concentrations were not as great as those seen in the present experiment.

Ergovaline was not detected in the digested residues of the seed heads collected between 6 May and 4 June and had only minute concentrations remaining in the residues of seed heads collected on 11 and 17 June (Table 1). This translated to 97.7 and 95.9% of the ergovaline being released for seed heads in the later stages of seed development (11 and 17 June), compared to approximately 100% with younger tissues (Table 1).

Although the percentage of ergovaline released from seed heads was slightly reduced in more mature seed, its effect on animal productivity is likely minimal as the total dosage of alkaloid received by the animals would be greater than if the steer would have consumed the seed heads earlier in the season. Thus, there is a need to remove seed heads from tall fescue pastures early before animals have the opportunity to selectively graze upon these tissues, possibly reducing the risk of production losses due to fescue toxicosis.

Implications

Results of this experiment indicated that a minimum of 96% of ergovaline present within tall fescue seed heads may be potentially released within the rumen during, and *in vivo*, these estimates may be higher due to the further reduction in seed particle size. Steers on these pastures actively exhibited symptoms of fescue toxicosis by the end of the grazing season. This is at least partially due to these animals consuming seed heads at a time when their concentrations of ergovaline were at the highest levels. Management strategies aimed at preventing reproductive growth in stands, such as heavy grazing, mowing, or chemical suppression, are needed to reduce losses in animal productivity.

Prevention of seed set is also used to limit the re-infection of pastures and the spread of infected seed in hay and manure.

Chemical Suppression of Seed Head Emergence in Toxic Endophyte-Infected Tall Fescue Pastures

G.E. Aiken, W.W. Witt, B.M. Goff, and I.A. Kagan

Summary

Results of a two-year grazing experiment with steers indicated the seed heads of toxic endophyte-infected (E+) tall fescue can be chemically suppressed to increase average daily gain (ADG) and reduce the severity of fescue toxicosis. Six, 7.5-acre pastures of toxic endophyte-infected tall fescue pastures were either treated or untreated with Chaparral® herbicide (Dow AgroSciences LLC, Indianapolis, IN 46268) to determine if suppression of seed head emergence can increase ADG and alleviate fescue toxicosis. Pastures were grazed with 8 steers per pasture from 9 April to 1 July 2009 and 6 April to 7 July 2010. Seed head densities on treated pastures were less than 10 reproductive tillers/yd^2, whereas untreated pastures had 94 and 57 reproductive tillers/yd^2 in 2009 and 2010, respectively. Concentrations of two ergot alkaloids combined, ergovaline and ergovalinine, were threefold greater in seed heads than in leaf blades. ADG on treated pastures was 39% greater than on untreated pastures. Steers on treated pastures had twofold greater serum prolactin concentrations (i.e., low concentrations are a marker of toxicosis). Emergence of seed heads can be chemically suppressed to enhance forage quality and alleviate seed heads as a highly toxic source of ergot alkaloids.

Introduction

Tall fescue is a cool-season perennial grass that is the predominant forage in Kentucky. A fungal endophyte infects most plants of Kentucky 31 tall fescue and produces alkaloids that impart tolerances of the grass to stresses from dry soils, heat, and grazing, but also produces ergot alkaloids that cause fescue toxicosis. Symptoms of the malady in cattle include rough hair coats during the summer, elevated body temperature, labored respiration, and decreased prolactin concentrations. Poor weight gain and diminished market value of beef calves exhibiting symptoms of toxicosis have limited the use of tall fescue for commercial stocker production.

Chaparral herbicide has no grazing restriction and its application to tall fescue pastures could potentially increase calf daily weight gain and alleviate fescue toxicosis by suppressing emergence of seed heads. A 2-yr grazing experiment was conducted to evaluate steer and pasture responses to application of Chaparral herbicide on toxic E tall fescue pastures. All pastures were encroached by Kentucky bluegrass; therefore, a second objective was to determine if competitiveness of fescue with bluegrass is reduced by suppression of fescue maturation.

Materials and Methods

The grazing experiment was conducted at the C. Orin Little Animal Research Center in Woodford County. With or without Chaparral® herbicide treatments were assigned to six, 7.5-ac pastures of toxic E+ tall fescue as a randomized complete block design with three replications. The herbicide was sprayed at 2 oz/ac on 4 April 2009 and 31 March 2010. Nitrogen fertilizer was applied at 70 lb N/ac on 17 March 2009 and 26 March 2010.

Forty-eight crossbred steers (initial BW: 2009 = 614 ± 48 (SD) lb; 2010 = 555 ± 30) were blocked by BW for random assignment to pastures (stocking rate = 1.1 steers/ac). Cattle were weighed following a 12- to 14-h fasting from feed and water on the initial (9 April in 2009 and 6 April in 2010) and final days of grazing (1 July in 2009 and 7 July in 2010). The pastures were continuously stocked. Jugular blood was collected from each steer prior to fasting on the final day to analyze serum prolactin as a marker of toxicosis.

Forage availability was estimated at 2-wk intervals with a falling disk meter. Disk meter height was recorded for 50 randomly chosen locations within each pasture. Calibration samples for regressing sample dry weights over disk meter heights to estimate forage mass were collected on two dates in each year by clipping forage below the disk meter plate to the soil surface at 3 random locations per pasture. Single tillers from 25 randomly chosen fescue plants were clipped at the crown in each pasture on 5 June 2009 to estimate endophyte infection percentages using immunoblot test kits (Agrinostics Ltd. Co., Watkinsville, GA). Seed heads/yd^2 was determined for each pasture by counting reproductive tillers of fescue within a 0.2 yd^2 ring placed in 10 random locations on 30 June 2009, and a 0.3 yd^2 quadrat placed in 50 random locations on 17 June 2010. Encroachment of Kentucky bluegrass was observed in all pastures early in the first year; therefore, percentages of tall fescue in above-ground herbage were estimated on 19 May 2009 and 6 July 2010 using point-transects (n = 500 points/pasture).

Reproductive tillers (n = 25) were collected from untreated pastures on 20 May 2009 and 26 May 2010 for hand-separation into leaf blade, sheath, stem (only in 2010), and seed head components. Vegetative tillers (n = 25) also were collected from treated and untreated pastures and separated in leaf blade and sheath components. Tiller components were analyzed for ergovaline combined with ergovalinine. At approximately 2-wk intervals, whole tillers were collected from 25 randomly chose plants in each pasture. Dried tillers were analyzed for N (CP = %N x 6.25), water-soluble carbohydrates (WSC), and in vitro dry matter digestibilities (IVDMD).

All responses were statistically analyzed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). Sampling date was evaluated as a repeated measure in analyses of CP, WSC, and IVDMD in whole tillers.

Results and Discussion

Endophyte infection percentages in the pastures averaged 89.3%. There was a tendency ($P < 0.10$) for higher percentages of tall fescue in untreated than in treated pastures (Table 1). Tall fescue percentage in the aboveground herbage did not

decrease ($P > 0.10$) in treated pastures from 2009 to 2010, indicating that the herbicide did not reduce competiveness of fescue with bluegrass. Following herbicide treatment, tall fescue yellowed and did not exhibit growth for a 2- to 3-wk period. Following this lag period, tall fescue actively grew and cattle were visually observed to selectively graze tall fescue over bluegrass. Most bluegrass tillers readily set seed, which could have resulted in bluegrass contributing more than tall fescue to the aboveground forage dry matter yield. Seed head populations were greater ($P < 0.001$) in untreated than treated pastures in both years, demonstrating that the herbicide treatment suppressed seed head emergence of tall fescue. Mean forage availability was less ($P < 0.10$) in treated than untreated pastures. The higher availability in untreated pastures was partly due to the higher quantity of stems and seed heads,

Concentrations of ergovaline and ergovalanine in vegetative tillers differed minimally between treated and untreated pastures (Table 2). In untreated pastures, ergovaline was generally greater in leaf blades and sheaths of vegetative tillers than of reproductive tillers. A distinctive difference between the treatments was in the presence of seed heads in untreated pastures, which had threefold greater ergovaline than leaf blades. Steers were visually observed to consume seed heads in an early stage of development. Compared to untreated pastures, whole tillers in treated pastures were higher in WSC and IVDMD in both years and in CP in 2009 (Table 3). The higher nutritive values for treated fescue were likely related to the suppressed maturation of tall fescue in these pastures.

Averaged over the 2 years, steer ADG in treated pastures was 39% higher ($P < 0.05$) than in untreated pastures (Table 1). Serum prolactin concentrations in steers grazing treated pastures were twofold greater than in those grazing untreated pastures. Lower serum prolactin in steers grazing treated pastures suggests a reduction in the severity of toxicosis for steers grazing treated pastures. A non-toxic control treatment was not part of the experiment; therefore, it cannot be determined if toxicosis was alleviated. Alkaloid analysis of plant tissues, however, indicated that steers on treated pastures were exposed to ergot alkaloids and likely subjected to some degree of toxicosis.

Table 1. Pasture and steer responses to toxic E+ tall fescue pastures that were with or without application of Chaparral® herbicide in 2009 and 2010.

	2009		2010		
Item	**With**	**Without**	**With**	**Without**	**SEM[1]**
Pasture:					
Forage availability, lb DM/acre	2665 ***	3334	3462 *	3726	89
Percentage tall fescue, % [2]	51.0 *	61.1	52.0 *	72.3	6.1
Seed head density, #/acre	6 ***	94	4 ***	57	7
Steer:					
Average daily gain, lb/day	2.00 **	1.22	2.10 **	1.74	0.1
Serum prolactin, ppb [3]	58 ***	9	143 ***	86	8.6

1 Standard error of the mean.
2 Percentage of tall fescue in aboveground fresh biomass relative to Kentucky bluegrass.
3 Parts per billion.
*, **, *** Effect of treatment at $P < 0.10$, $P < 0.05$, and $P < 0.01$, respectively.

Table 2. Parts per million of ergovaline plus ergovalinine concentrations in plant parts of toxic E+ tall fescue on 20 May 2009 and 26 May 2010 in pastures that were with or without Chaparral® herbicide.

		Chemical treatment		
		With	Without	
Year	**Plant Part**	**Vegetative**	**Vegetative**	**Reproductive**
2009	Blade	0.43	0.43	0.31 c
	Sheath	1.47	1.24	0.84 d
	Seed			1.32 d
2010	Blade	0.40 a	0.26 b	0.24 c
	Sheath	1.84 a	0.93 b	0.73 d
	Stem			0.93 d
	Seed			1.26 e

a Vegetative tillers differ ($P < 0.05$) between with and without Chaparral® treatments.
b Vegetative and reproductive tillers differ ($P < 0.05$) between with and without Chaparral® treatments.
cde Plant parts of reproductive tillers with different letters differ significantly ($P < 0.05$).

Table 3. Percentages of crude protein (CP), water soluble carbohydrates (WSC), and in vitro dry matter digestibility (IVDMD) in whole tillers for toxic E+ tall fescue pastures that were with or without application of Chaparral® herbicide in 2009 and 2010.

	2009		2010		
Item	**With**	**Without**	**With**	**Without**	**SEM[1]**
CP	13.9 ***	10.5	13.2	13.6	0.5
WSC	14.7 **	12.8	12.6 **	11.8	0.6
IVDMD	74.3 **	67.3	80.1 **	78.3	1.6

1 Standard error of the mean.
*, **, *** Effect of treatment at $P < 0.10$, $P < 0.05$, and $P < 0.01$, respectively.

Implications

This research identified a management approach to increase post-weaning weight gain and reduce the severity of toxicosis on toxic endophyte-infected tall fescue. Therefore, the management approach has potential use for stocker production in the fescue belt. Reduction in forage availability with the pasture treatment also indicates that grazing management will be necessary to increase pasture carrying capacities and sustain cattle performance with chemical suppression of tall fescue seed heads.

Constriction of Bovine Vasculature Caused by Endophyte-Infected Tall Fescue Seed Extract is Similar to Pure Ergovaline

A.P. Foote, D.L. Harmon, K.R. Brown, J.R. Strickland, K.R. McLeod, L.P. Bush, and J.L. Klotz

Summary

A mixture of ergot alkaloids does not increase the contractile response of peripheral bovine vasculature above that observed for pure ergovaline but may increase the contractile response of foregut vasculature. Preliminary data indicated that an extract of tall fescue seed induced a greater contractile response in ruminal artery and vein than ergovaline. To determine if the increased contractility was due to the presence of other ergot alkaloids or a non-alkaloid effect of the extraction procedure, the contractile response of bovine saphenous veins, right ruminal artery and right ruminal veins to a mixture of ergot alkaloids with similar concentrations to the endophyte-infected tall fescue seed extract, an endophyte-free tall fescue seed extract, and pure ergovaline were evaluated. Contractile response of the saphenous vein, ruminal artery, and ruminal vein were not different for the endophyte-infected tall fescue seed extract, the mixture of ergot alkaloids, and pure ergovaline. The endophyte-free tall fescue seed extract failed to induce a contractile response in any of the tissues. These results indicate that ergovaline is likely the main ergot alkaloid responsible for vasoconstriction.

Introduction

The association of endophyte-infected tall fescue consumption with vasoconstriction is well documented. Ergot alkaloids have long been implicated as the causative agents of vasoconstriction and likely contribute to most of the observed symptoms of the fescue toxicosis syndrome. Most studies related to fescue toxicosis and vasoconstriction have focused on ergovaline alone; however, in vitro studies have shown that ergot alkaloids other than ergovaline, including ergonovine, ergotamine, ergocristine, ergocryptine, and ergocornine, can induce contractile responses in bovine lateral saphenous vein. Preliminary results (Figure 1) showed that an extract of endophyte-infected tall fescue seed (E+EXT) serially diluted based on ergovaline concentration induced a greater contractile response in ruminal artery and vein preparations in vitro compared with pure ergovaline. Findings from this experiment led to the development of a hypothesis that the presence of ergot alkaloids other than ergovaline in the extract are responsible for the increased contractile response. The objective of the current experiment was to determine if the greater contractility produced by the extract is attributed to the presence of the other ergot alkaloids. This was accomplished by using the bovine lateral saphenous vein bioassay to represent peripheral vasculature and the right ruminal artery and vein bioassay to represent core vasculature, to compare E+EXT with an endophyte-free tall fescue seed extract (E-EXT), ergovaline alone, and a mixture of commercially available ergot alkaloids (ALK) mixed to mimic the E+EXT alkaloid concentrations.

Materials and Methods

Experiment 1

Angus-cross heifers (n = 10; BW = 1,099 ± 20 lb) were utilized in an initial test of E+EXT vasoactivity. Sections of right ruminal artery and vein were collected from the ventral coronary groove shortly after slaughter and placed in a modified Krebs-Henseleit buffer on ice. Vessels were cleaned of excess connective tissue and fat, cut into 2- to 3-mm segments and suspended in a multi-myograph chamber with 5 mL of Krebs-Henseleit buffer. Ruminal veins and arteries were equilibrated to 0.5 and 1.0 g of resting tension respectively for 90 min with buffer replaced every 15 min. Ruminal arteries and veins were then exposed to the reference compound 120 mM KCl prior to beginning the addition of treatments. Increasing concentrations of E+EXT and ergovaline were added to the respective chamber every 15 min following buffer replacement.

Experiment 2

Saphenous veins, right ruminal arteries, and right ruminal veins were collected from Holstein steers (n = 6; BW = 1,098 ± 62 lbs.) shortly after slaughter. Vessels were handled as described in Exp. 1. The saphenous veins were equilibrated to 1.0 g for 90 min and 0.1 mM norepinephrine was used as the reference compound. Increasing concentrations of E+EXT, ALK, ergovaline, and E-EXT were added as described in Exp. 1.

Preparation of Extracts and Treatments

Ground endophyte-free or endophyte-infected tall fescue seed was packed in columns and filled with 80% ethanol. Seed was allowed to steep for 12 h followed by elution of the column with 80% ethanol. Ethanol was evaporated and the remaining residue was freeze-dried and ground under liquid N. To further purify the extract, 300 g of extract was suspended in 150 mL of H_2O and shaken for 5 min. A hexane liquid-liquid extraction was performed a total of 6 times, discarding the hexane phase. Chloroform was then added to the aqueous phase and shaken a total of 6 times, discarding the aqueous phase each time. Chloroform was removed by rotary evaporator under vacuum. The residue was solublized in 80% methanol to generate final stock extracts. Three separate purified extracts were utilized in these experiments, an E+EXT for Exp.1, an E+EXT for Exp. 2, and an E-EXT for Exp. 2.

After analyzing the E+EXT for Exp. 2 by ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS-MS), the ALK treatment was prepared by dissolving ergovaline, ergotamine, ergocornine, ergocryptine, ergocristine, lysergic acid, and ergonovine in 80% methanol. The ALK and E-EXT treatments were analyzed by UPLC/MS-MS. The resulting concentration of ergot alkaloids in the myograph chamber at the highest concentration are presented in Table 1.

Data Collection and Analysis

The maximum observed tension following each treatment addition was recorded. Contractile responses for the treatments were normalized to the contractile response induced by a reference compound. To measure potency of treatments, an EC_{50}, the concentration required to produce half the maximum response, was calculated using a sigmoidal concentration response curve with a variable slope (slope set to 1 for the ruminal vessel data). Data were analyzed as a completely randomized design using Proc Mixed of SAS.

Table 1. Concentration of ergot alkaloids in the endophyte-infected tall fescue seed extract (E+EXT) and ergot alkaloid mixture (ALK) used in Exp. 2 as measured by UPLC MS/MS.[1]

	Concentration, M		
Alkaloid	**E+EXT**	**ALK**	**E-EXT**
Ergovaline	3.2×10^{-6}	5.9×10^{-6}	ND[2]
Ergotamine	4.3×10^{-8}	1.1×10^{-7}	7.5×10^{-11}
Ergocornine	4.0×10^{-8}	1.0×10^{-9}	1.3×10^{-13}
α-Ergocryptine	2.0×10^{-9}	1.0×10^{-8}	2.6×10^{-12}
Lysergic Acid	9.0×10^{-9}	1.1×10^{-8}	ND
Ergocristine	2.0×10^{-9}	2.8×10^{-8}	6.5×10^{-12}
Ergonovine	1.5×10^{-10}	1.4×10^{-10}	ND

[1] Values represent the working concentrations of the ergot alkaloids present in the myograph chamber at the greatest treatment concentration.
[2] ND = Not detected.

Results and Discussion

Experiment 1

Results from Exp. 1 using the right ruminal artery and vein are shown in Figure 1. The E+EXT induced a greater response than ergovaline (47.9% versus 30.6% of KCl maximum response) in the ruminal artery at 10^{-6} M ergovaline ($P = 0.018$). The extract appeared to produce a greater response than ergovaline treatment for the ruminal vein at the 10^{-7} M ergovaline level (17.5% versus 9.6% of KCl maximum response), although there was not an interaction of treatment and concentration. The results from Exp. 1 with the bovine right ruminal artery indicated that either the chemical nature of the extract in general contributes to a greater observed contractile response compared with identical concentrations of pure ergovaline or that other alkaloids are present in the extract and contribute to the overall contractile response.

Experiment 2

Results from Exp. 2 are shown in Table 2 and Figure 2. The E+EXT had a lower calculated E_{max} (maximum contractile response) than ergovaline ($P < 0.0001$) with ALK intermediate to the other treatments (Table 3) for the saphenous vein. This lower E_{max} for the E+EXT resulted in a lower EC_{50} for E+EXT than ALK or ergovaline ($P = 0.008$). Although the Hill slopes for

Table 2. The EC_{50}, Hill slope, and E_{max} least square means (± SEM) for an ergot alkaloid mixture (ALK), endophyte-infected tall fescue seed extract (E+EXT), and pure ergovaline in bovine lateral saphenous vein and EC_{50} for the treatments in the right ruminal vein and artery in Exp. 2.[1]

	Saphenous vein			Ruminal vein	Ruminal artery
Treatment	**log EC_{50}**	**Hill slope**	**E_{max}**	**log EC_{50}**	**log EC_{50}**
ALK	-5.91 ± 0.17[a]	0.51 ± 0.04[b]	86.14 ± 4.02[b]	-5.87 ± 0.15[a]	-5.17 ± 0.30[a]
E+EXT	-6.48 ± 0.17[b]	0.69 ± 0.04[a]	61.51 ± 4.02[c]	-6.99 ± 0.15[b]	-5.69 ± 0.19[a]
Ergovaline	-5.62 ± 0.17[a]	0.51 ± 0.04[b]	105.70 ± 4.02[a]	-6.22 ± 0.15[a]	-5.37 ± 0.18[a]

[a,b] Means within column with differing superscripts differ ($P < 0.01$).
[1] Log EC_{50} = measure of potency of a treatment, expressed as the log of the molar concentration of ergovaline required to induce 50% of the maximal contractile response for each treatment;
E_{max} = maximal contractile response extrapolated by the model used to fit the data, expressed as percentage of 0.1 mM norepinephrine contractile response.

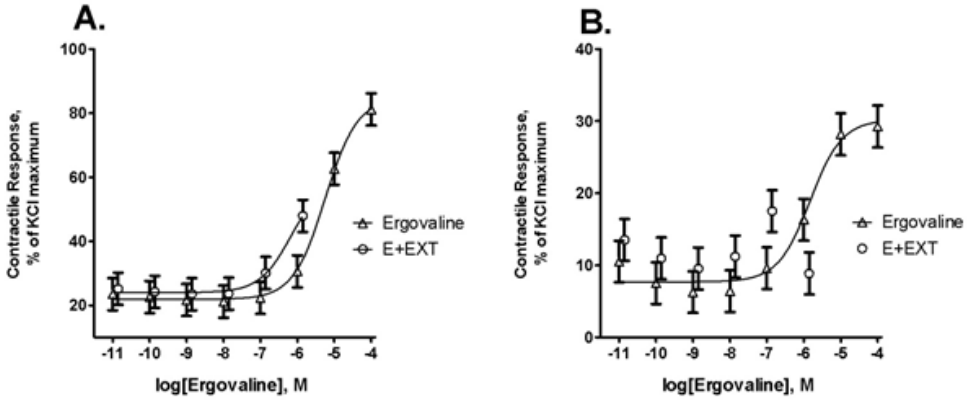


Figure 1. Exp. 1 contractile response (±SEM) of bovine right ruminal artery (A) and vein (B) to increasing concentrations of ergovaline and an endophyte-infected tall fescue seed extract (E+EXT; n = 10 for each treatment) standardized to ergovaline concentration. The contractile response was dependent upon both treatment and concentration ($P = 0.0045$) for the right ruminal artery; however for the ruminal vein, the effect of concentration was significant ($P < 0.0001$) but effect of treatment ($P = 0.139$) and the interaction ($P = 0.16$) were not significant. Nonlinear regression lines represent the fitting of data to a sigmoidal concentration response curve. Missing regression lines indicate data could not be fit to the concentration response model.

the E+EXT were greater ($P = 0.006$) than the ALK and ergovaline slopes, this difference likely has no physiological relevance. In the graphical representation of the data (Figure 2A) the observed contractile responses all follow the same basic line, regardless of treatment. Even though the extrapolated E_{max} is different for these treatments, the data suggest that ergovaline is mostly responsible for the local vasoconstriction of peripheral vasculature.

The E+EXT treatment also displayed a similar trend for a lower EC_{50} than ALK and ergovaline in the ruminal vein bioassays (Table 3); however it should be noted that only one or two concentrations of the treatments tested resulted in a response in the ruminal artery and vein bioassays and therefore interpretation of the EC_{50} data for the ruminal vessels is tenuous. Graphical representation of the ruminal artery (Figure 2B) and ruminal vein (Figure 2C) data shows that there is likely no difference in the response to increasing concentrations for these three treatments. The results from this experiment differ from the results of Exp. 1 (Figure 1).

To test the possibility that the extraction process included a compound that is vasoactive in this bioassay and not one of the measured ergot alkaloids, an extract of endophyte-free tall fescue seed was titrated in the saphenous vein and ruminal artery and vein bioassays (Figure 3). The E-EXT failed to induce a contractile response in all vessel types.

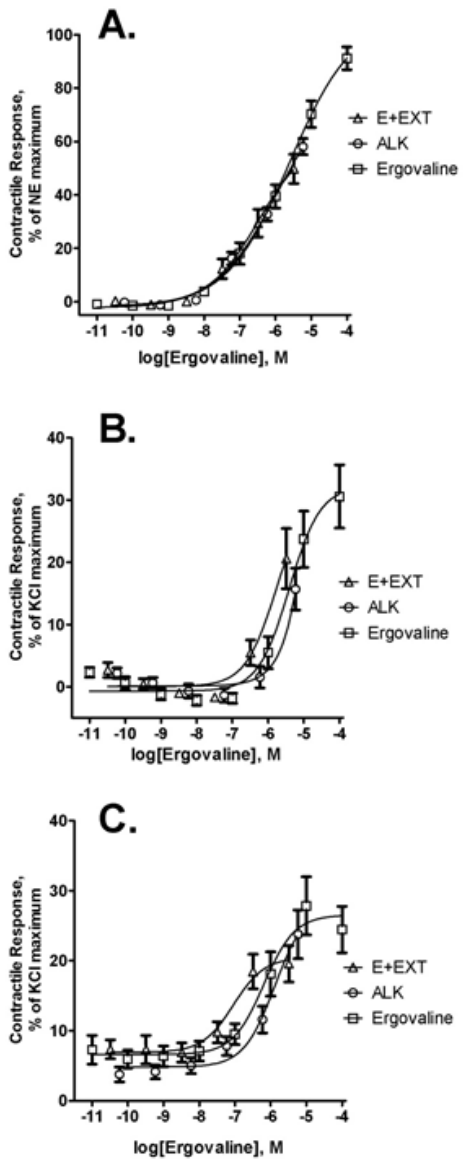


Figure 2. Exp. 2 contractile response (±SEM) of bovine lateral saphenous vein (A), right ruminal artery (B), and right ruminal vein (C) to ergovaline, an endophyte-infected tall fescue seed extract (E+EXT) standardized to ergovaline concentration and a mixture of ergot alkaloids (ALK; n = 6 each) that reflects the alkaloid profile of E+EXT. Regression lines represent the fitting of data to a sigmoidal concentration response curve.

Implications

Data from these experiments indicate that an extract of endophyte-infected tall fescue seed is capable of inducing a contractile response similar to a mixture of ergot alkaloids and ergovaline alone. Results support ergovaline as being primarily responsible for vasoconstriction, especially in the peripheral vasculature.

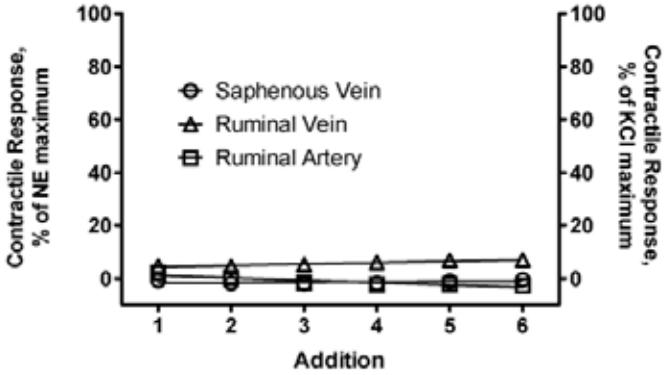


Figure 3. Exp. 2 contractile response (±SEM) of bovine lateral saphenous vein, ruminal artery, and ruminal vein to an endophyte-free tall fescue seed extract (E-EXT). The E-EXT was serially diluted from the stock concentration and additions of the E-EXT were added in order of increasing concentration (undiluted extract is addition 6). Saphenous vein data is normalized to the contractile response to 0.1 mM norepinephrine and the ruminal artery and vein are normalized to the contractile response to 120 mM KCl. The E-EXT failed to induce a contractile response in any of vessels used in these bioassays ($P > 0.11$).

Ergot Alkaloids Reduce Rumen Epithelial Blood Flow and Volatile Fatty Acid Absorption

A.P. Foote, N.B. Kristensen, J.L. Klotz, D.H. Kim, A.F. Koontz, K.R. McLeod, L.P. Bush, and D.L. Harmon

Summary

Ergot alkaloids have been shown to induce vasoconstriction of both peripheral and ruminal vessels. Constriction of ruminal vessels could lead to a reduction in epithelial blood flow thereby reducing nutrient absorption. The objectives of this experiment were to determine if steers receiving endophyte-infected or endophyte-free tall fescue seed have decreased rumen epithelial blood flow and volatile fatty acid absorption rates in the presence of differing levels of ergot alkaloids. Ruminally cannulated steers received endophyte-infected or endophyte-free seed for 7 days. On day 8 a washed rumen experiment was conducted. Three buffer treatments including a control, a low concentration of ergot alkaloids, and a high concentration of ergot alkaloids were incubated and epithelial blood flow and volatile fatty acid absorption were evaluated. Results show that ergot alkaloids induce a large reduction in epithelial blood flow as well as a reduction in volatile fatty acid absorption. The observed alterations in nutrient absorption could contribute to the decreased growth rates of cattle consuming endophyte-infected tall fescue.

Introduction

Ergot alkaloids produced by endophyte (*Neotyphodium coenophialum*)-infected tall fescue (*Lolium arundinaceum*) causes constriction of peripheral vasculature, reducing blood flow to the extremities. Recent in vitro research also indicates that ergot alkaloids cause vasoconstriction of right ruminal arteries and veins. Absorption of nutrients from the rumen is dependent on blood flow to the absorptive surface of the rumen. Ergot alkaloids could potentially decrease blood flow to the rumen epithelium and consequently decrease nutrient absorption from the rumen. A decrease in nutrient absorption from the rumen could contribute to the observed unthriftiness of cattle consuming endophyte-infected tall fescue. An experiment was conducted to determine if ergot alkaloids alter rumen epithelial blood flow and volatile fatty acid (VFA) absorption from a washed reticulorumen of steers receiving endophyte-infected (E+) of endophyte-free (E-) tall fescue seed.

Materials and Methods

Animals and Treatments

Eight ruminally cannulated Holstein steers (562 ± 2.9 lb) were used in this experiment. Steers were paired by weight, housed in individual pens in a climate controlled barn and pair-fed alfalfa cubes at 1.5 × NE_m. The experiment was conducted at thermoneutral (TN; 72°F) and at heat stress (HS; 86°F) ambient temperatures.

Treatments were arranged in a randomized complete block split-plot design with seed treatment and period as the whole plot and buffer treatment during the washed rumen experiment as the sub-plot. One steer from each pair was assigned to receive ground E+ seed (0.015 mg ergovaline · kg BW^{-1} · day^{-1}) twice daily via the rumen cannula for 7 days. The remaining steer of each pair was assigned to receive ground E- seed (0 mg ergovaline) twice daily for 7 days. On day 8, a washed rumen experiment was conducted. After the 7 day seed treatment and washed rumen experiment was conducted at both TN and HS temperatures, the seed treatment for each steer was switched and the entire experiment was repeated.

Washed Rumen Experiment

On day 8 of the experiment, a washed rumen experiment was conducted. Steers were weighed and a jugular catheter was placed. An initial serum sample was collected for prolactin analysis and a plasma sample was collected for background deuterium oxide (D_2O) analysis. Rumen contents were removed, weighed, sampled for dry matter analysis, and placed in a warm water bath or force draft oven. The rumen was washed once with warm tap water and three times with warm saline. Three buffer treatments were incubated in the rumen as shown in Figure 1. The three buffer treatments were control (CON; excipient), 1×EXT (0.015 mg ergovaline· kg BW^{-1}), and 3×EXT (0.045 mg ergovaline · kg BW^{-1}). Each buffer treatment consisted of an equilibration buffer with the composition shown in Table 1 with the addition of excipient or extract and a sampling buffer which was identical to the equilibration buffer with the addition of Cr-EDTA and D_2O.

The washed rumen experiment was conducted as shown in Figure 1. Following the incubation of the equilibration buffer, the sampling buffer for each treatment was incubated and sampled at 0, 5, 10, 15, 20, and 30 minutes. Blood was sampled at 5, 15, and 30 minutes. The buffers were weighed before and after incubation in the rumen.

A mathematical model was developed using the buffer weights and Cr-EDTA concentrations to predict flow of liquid out of the rumen, flow physiological water into the rumen, and volume of water prior to buffer entry into the rumen. The model allowed a calculation of rumen liquid at each sampling time point. Using the calculated volume and the measured concentrations, a ruminal pool of D_2O and VFA was calculated at each sampling time point. The change in ruminal pool corrected for outflow in liquid and influx in physiological water was as-

Table 1. Chemical composition of the ruminally incubated buffer for the washed rumen experiment.

Item	Content (mmol/kg)
$NaHCO_3$	24.0
NaOH	95.0
$KHCO_3$	30.0
K_2HPO_4	2.0
$CaCl_2$	1.5
$MgCl_2$	1.5
Acetic Acid	72.0
Propionic Acid	30.0
Butyric Acid	12.0
Isovaleric Acid	2.0
Valeric Acid	1.3

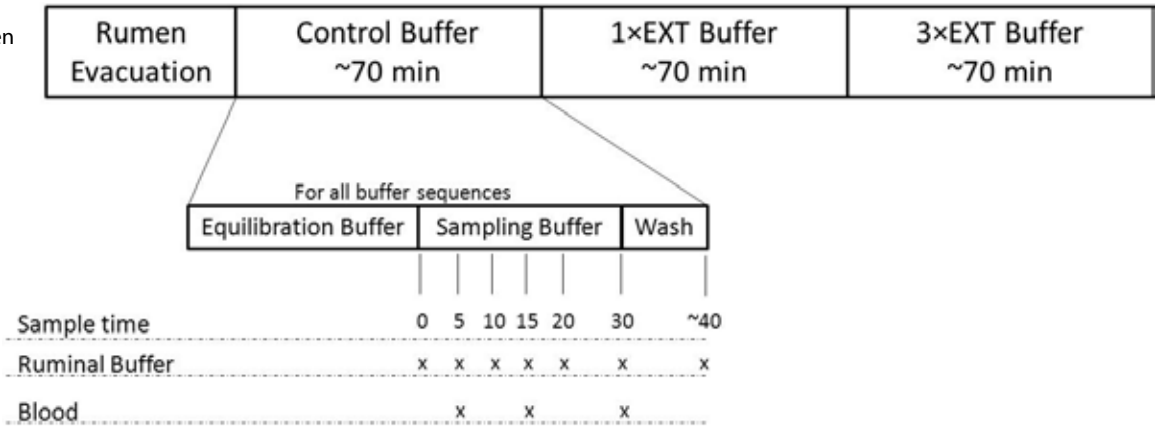


Figure 1. Timeline and outline of washed rumen experiment conducted after 7 days of steers receiving E+ or E- seed.

sumed to be absorption. Clearance of D_2O was calculated as the absorption relative to the buffer and blood concentration and is assumed to be equivalent to rumen epithelial blood flow.

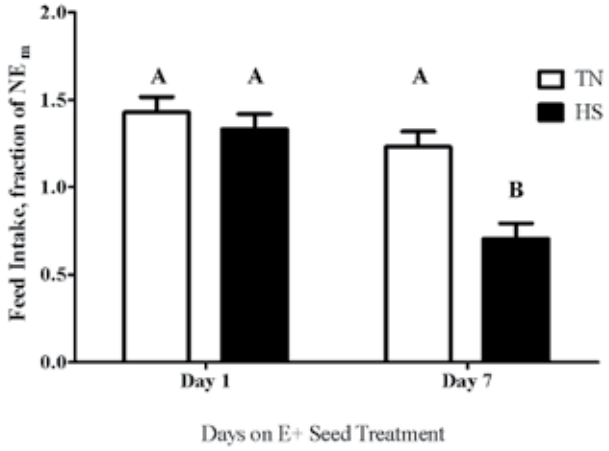
Results and Discussion

Induction of Fescue Toxicosis

The change in feed intake during the 7 days steers received E+ seed is shown in Figure 2. Intake was reduced only during the HS portion of the experiment. Previous reports have shown similar feed intake results during heat stress. It is also common for intake to be depressed during TN conditions; however this is more common when cattle are fed *ad libitum.*

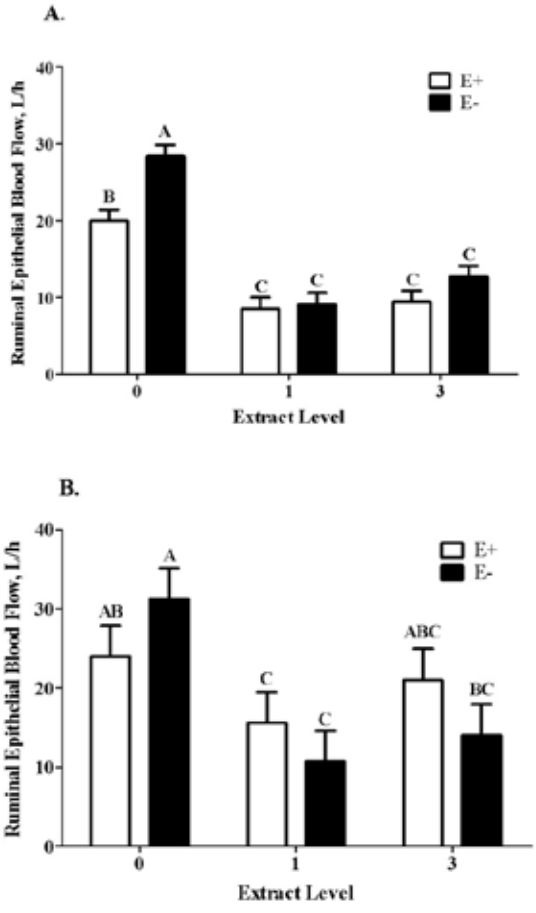
Serum prolactin concentrations are shown in Figure 3. At TN prolactin was lower in steers receiving E+ seed at both day 0 and day 8, however prolactin concentrations were not different from 0.0 ng/mL for E+ steers on day 8, indicating a slight reduction in prolactin in E+ steers. At HS, prolactin was lower on day 8 than day 0 for both groups of steers. Reduced intake and prolactin concentrations are common measures of

Figure 2. Feed intake of steers on day 1 and day 7 of receiving an endophyte-infected tall fescue seed.



[A, B] Bars with unlike letter differ; temperature × day $P = 0.018$

Figure 3. Serum prolactin concentration of steers prior to and following receiving endophyte-infected (E+) or endophyte-free (E-) tall fescue seed at TN (A)[1] and HS (B)[2] ambient temperature.



[1]Animals housed at thermoneutral conditions (72°F). Effect of seed $P = 0.005$; effect of day $P = 0.14$
[2]Animals housed at heat stress conditions (86°F). Effect of seed $P = 0.33$; effect of day $P < 0.0001$

the induction of fescue toxicosis and these data indicate that E+ steers received adequate levels of tall fescue seed to induce fescue toxicosis within 7 days.

Blood Flow and VFA Absorption

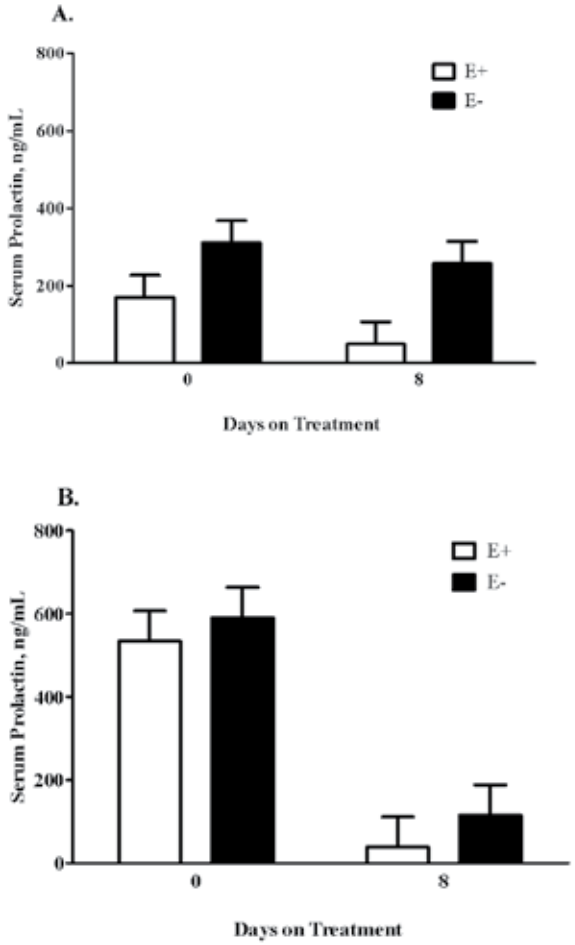
Rumen epithelial blood flow data is shown in Figure 4. The approximately 30% reduction in epithelial blood flow observed in steers receiving E+ seed compared to E- steers during the CON buffer incubation indicates that endophyte-infected tall fescue seed reduces blood flow to the absorptive surface of the rumen. Incubating ergot alkaloids in the washed rumen at either the 1× or 3× level caused an approximate 50 – 60 % reduction in blood flow to the rumen epithelium in steers at TN temperatures.

At HS conditions, epithelial blood flow was reduced with the inclusion of ergot alkaloids at the 1× and 3× level, although blood flow increased slightly during the 3×EXT incubation. The effect of the seed treatment on blood flow during the CON buffer incubation was not statistically significant but did have the same numerical trend observed at TN.

VFA absorption from the washed rumen is shown in Table 2. At TN absorption of acetate, propionate, and butyrate is greatly reduced when ergot alkaloids are included in the washed rumen incubation; however there is no difference in the low and high concentration. Steers receiving E+ seed tended to have lower rates of acetate, butyrate, and isovalerate absorption than E- steers at TN. At HS conditions, absorption rates of acetate, propionate, butyrate, and valerate were lower during the incubation of the 1×EXT and 3×EXT treatments.

VFA absorption is strongly correlated to blood flow to the absorptive surface and these data follow this concept. However, comparing the blood flow and VFA absorption data for E+ and E- steers during the control buffer incubation, E+ steers had 30% lower blood flow than E- steers, but there were only small differences in VFA absorption rates. This could indicate that steers receiving E+ seed absorb more VFA for every unit of blood flowing to the rumen epithelium. This could indicate a

Figure 4. Ruminal epithelial blood flow at thermoneutral (A)[1] and heat stress (B)[2] conditions.



[1]Experiment conducted at thermoneutral conditions (72°F). Bars with unlike letters differ – seed × extract level $P = 0.038$.
[2]Experiment conducted at heat stress conditions (86°F). Bars with unlike letters differ – seed × extract level $P = 0.005$.

Table 2. Volatile fatty acid absorption from the washed rumen of steers receiving E+ or E- seed at TN and HS conditions with increasing levels of ergot alkaloids incubated ruminally.

VFA Absorption, mmol/h	E-			E+			SEM (n=6)	P-value		
	Control	1×EXT	3×EXT	Control	1×EXT	3×EXT		Seed	Buffer	Seed × Buffer
Thermoneutral										
Acetate	320.59	201.26	221.42	314.78	128.20	134.87	42.19	0.13	<0.01	0.61
Propionate	177.32	88.57	75.36	152.17	86.56	67.40	19.42	0.47	<0.01	0.83
Butyrate	57.46	39.81	37.39	48.17	31.97	28.39	5.60	0.07	<0.01	0.99
Valerate	5.90	5.53	5.61	6.12	4.02	4.82	0.58	0.18	0.17	0.40
Isovalerate	6.46	4.59	4.99	4.78	3.73	3.86	0.74	0.05	0.13	0.85
Heat Stress										
Acetate	263.71	162.49	179.56	264.15	178.90	122.52	40.90	0.77	<0.01	0.50
Propionate	141.18	95.92	82.02	122.62	78.25	64.59	21.47	0.50	<0.01	0.99
Butyrate	54.40	35.81	34.91	42.12	30.95	30.12	7.04	0.40	<0.01	0.67
Valerate	8.59	5.46	6.00	6.62	5.51	5.83	0.98	0.59	<0.01	0.13
Isovalerate	6.06	4.77	5.15	5.69	3.78	4.78	0.97	0.62	0.08	0.87

difference in epithelial metabolism of the VFA or a disruption in the barrier function of the epithelium, allowing VFA to more freely pass through the epithelium through a paracellular route.

Implications

Results from this experiment clearly demonstrate that ergot alkaloids present in endophyte-infected tall fescue reduce blood flow to the absorptive surface of the rumen. The reduction in blood flow is accompanied by a decrease in VFA absorption from the washed rumen. This reduction in VFA absorption could be partially responsible for the reduced growth rate and unthriftiness of cattle consuming common endophyte-infected tall fescue.

Vascular Activity Increases with Time Off of Tall Fescue

J.L. Klotz, G.E. Aiken, A.P. Foote, J.R. Bussard, K.R. Brown, B.M. Goff, D.L. Harmon, and J.R. Strickland

Summary

Cattle continue to recover from depressed vasoactivity (vasoconstriction) beyond 60 days after removal from endophyte-infected tall fescue pasture and after prolactin (an indicator of fescue toxicosis) returned to physiologic levels. This was determined by evaluating the contractile responses of lateral saphenous veins biopsied from cattle at different time points relative to their removal from a tall fescue pasture across 2 years. It was evident that no peripheral vascular recovery occurred within the first 28 days, but thereafter (days 42 and 63) increases in contractile response to different agonists were observed. These findings indicate that for a complete recovery from fescue toxicosis, animals should be removed from tall fescue pastures and fed a non-toxic diet for at least 6 weeks.

Introduction

Consumption of ergot alkaloids found in endophyte-infected tall fescue alters cardiovascular function. In addition to reduced body weight in cattle coming off of tall fescue pastures, the combined stresses of fescue toxicosis and transportation of stocker cattle can result in increased mortality. Grazing exposure to alkaloids has been shown to have an effect on the contractile responses induced by agonists of α_2-adrenergic and serotonergic receptors (both shown to bind ergot alkaloids in peripheral vessels). Previous research has demonstrated a suppressed contractile response in cattle grazing endophyte-infected tall fescue. It was hypothesized that as time off of tall fescue pasture increased, so too would the magnitude of contractile response. Therefore, the objective of this experiment was to determine if the contractile response of lateral saphenous veins to ergot alkaloids and agonists for serotonin$_{2A}$, α_{2A}-, and α_{2C}-adrenergic receptors changes as the time off of endophyte-infected tall fescue increases.

Materials and Methods

Animals and Pastures

In both years, 24 predominantly Angus steers (year 1 = 787 ± 6 lbs; year 2 = 795 ± 8 lb) grazed Kentucky-31 pastures (7.5 acres) for 126 days (year 1) or 88 days (year 2) prior to removal. In both years, all steers were removed from pastures and placed in a dry lot, and fed a corn-silage and soybean hull mixed diet.

Lateral saphenous veins were biopsied in year 1 at 0 (n = 6), 7 (n = 6), 14 (n = 5), and 28 d (n = 4) off of tall fescue pastures. In year 2, the biopsy dates were extended and occurred on 0 (n = 6), 21 (n = 6), 42 (n = 6), and 63 d (n = 6) off of tall fescue pasture. Jugular vein blood samples were collected the day of biopsy for measurement of serum prolactin.

Myograph Experiments

Once the biopsied section of lateral saphenous vein was removed from the steer, it was placed in a cold Krebs-Henseleit buffer and transported to the laboratory. The vein was then cleaned of external adipose and connective tissues and sliced into 2 to 3 mm cross-sections. Cross-sections were suspended on luminal supports on a multi-myograph, which permitted the observation and recording of the vessel's contractile responses. The suspended vessel was submersed in continuously gassed Krebs-Henseleit buffer that was replaced in 15-min intervals. The vein cross-sections were equilibrated to a 1 g tension for 1.5 hour and then exposed to a $1x10^{-4}$ M addition of norepinephrine that was used as a reference for all experimental additions.

Experimental additions for year 1 consisted of increasing concentrations (1×10^{-11} to 1×10^{-4} M) of ergovaline, TCB2 (a high affinity serotonin$_{2A}$ receptor agonist), guanfacine HCl (GF; α_{2A}-adrenergic receptor agonist) and (R)-(+)-*m*-nitrobiophenyline oxalate (NBP; α_{2C}-adrenergic agonist). In year 2, the experimental additions to the vein cross-sections in the myograph were increasing concentrations of ergotamine (1×10^{-11} to 1×10^{-4} M), and the range of additions of TCB2, GF, and NBP was decreased to focus in more on the response area of the additions ($5x10^{-8}$ to $1x10^{-4}$ M).

Data and Statistical Analyses

Data from each experiment were recorded, digitized, and normalized to a reference addition of norepinephrine. Thus, the form that all contractile response data are presented is % of norepinephrine maximum. Data were plotted and a nonlinear regression line was fit.

The contractile response data were analyzed as a completely randomized design for main effects of days off of pasture, agonist concentration, and the interaction using the mixed models procedure of SAS, with steer as the experimental unit. All differences discussed as significant are $P < 0.05$.

Results and Discussion

In year 1, there was no interaction of days off of pasture with agonist concentration for any of the treatment compounds in

the 28-day interval evaluated. This demonstrated that even though steers appeared to recover from the alkaloid exposure that occurred during grazing (as indicated by serum prolactin increasing from day 0 = 24.3 ±13.8 to day 28 = 111.3 ±30.5 ng/mL), there was little change in contractile response to ergovaline (Figure 1A), TCB2 (Figure 2A; the serotonin$_{2A}$ receptor agonist) and the adrenergic agonists GF (Figure 3A), and NBP (Figure 4A).

In year 2, the same receptor agonists were used, but ergovaline was replaced with a similar acting ergopeptine alkaloid, ergotamine. The biopsy timeline was also changed to 3-week intervals to extend the evaluation period out an additional 5 weeks. Contractile response was greatest for all 4 compounds tested at day 63. The response to ergotamine (Figure 1B) and TCB2 (Figure 2B) both had significant interactions between days off of tall fescue and concentration. For ergotamine, the response was lowest at day 0, highest at day 63, and responses on days 21 and 42 were not different. For TCB2, days 0 and 21 were not different (similar to year 1), but days 42 and 63 were each greater than days 0 and 21.

It is apparent that serotonin receptors seem to be affected more in the lateral saphenous vein than adrenergic receptors. Although GF (Figure 3B) and NBP (Figure 4B) did not have a significant interaction for days off of pasture x agonist concentration, there was an obvious suppression of contractile response attributable to ergot alkaloid exposure when comparing the response curves at day 0 to day 63.

Implications

These data demonstrate that significant changes in peripheral vasoactivity occur beyond one month after cessation of ergot alkaloid exposure. These changes occur well beyond the time interval necessary for prolactin levels to increase (low concentrations are frequently used as an indication of fescue toxicosis) to normal levels. This would suggest that cattle might still be recovering from fescue toxicosis when they would be diagnosed otherwise if prolactin were used as the lone indicator of recovery.

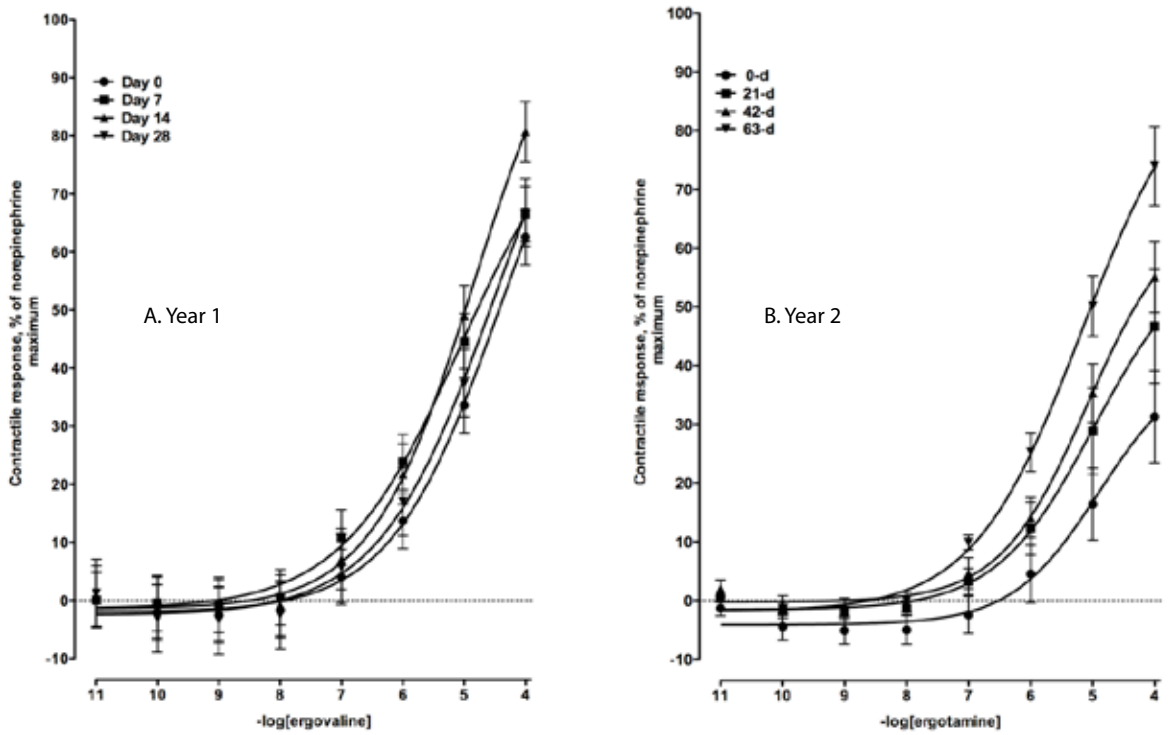


Figure 1. Concentration response to additions of ergovaline for year 1 (A) and ergotamine for year 2 (B; days off of pasture x agonist concentration interaction $P < 0.05$)

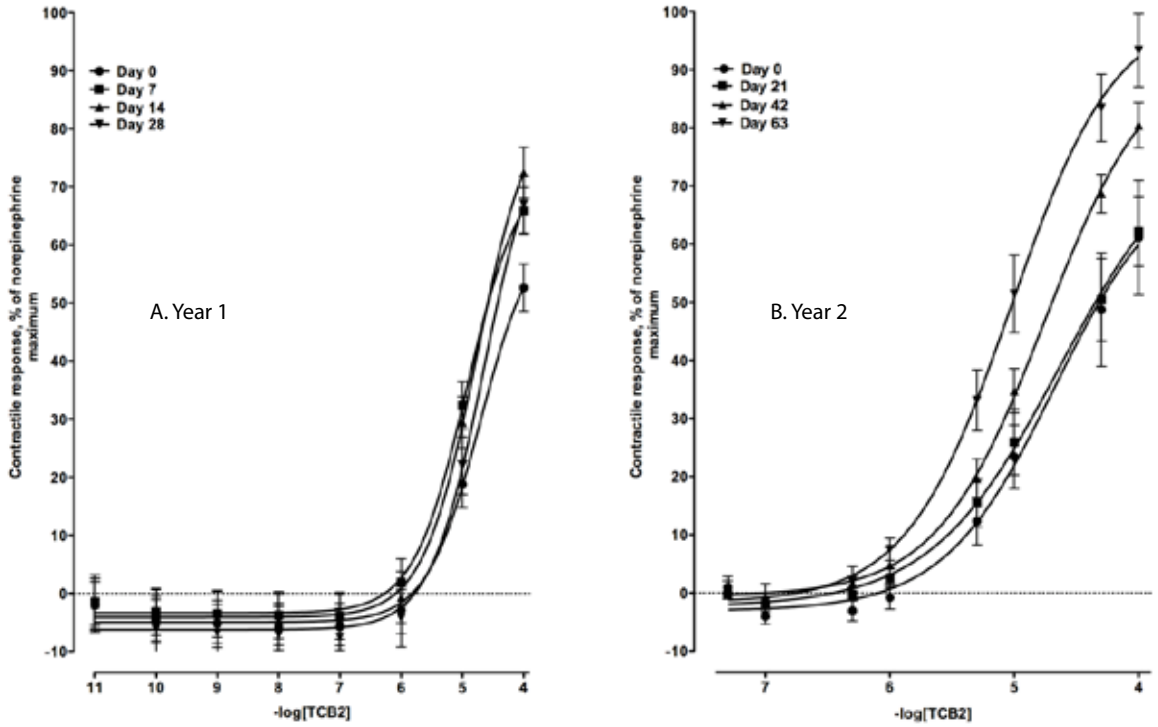


Figure 2. Concentration response to additions of TCB2 (serotonin$_{2A}$ agonist) for year 1 (A) and year 2 (B; days off of pasture x agonist concentration interaction $P < 0.05$)

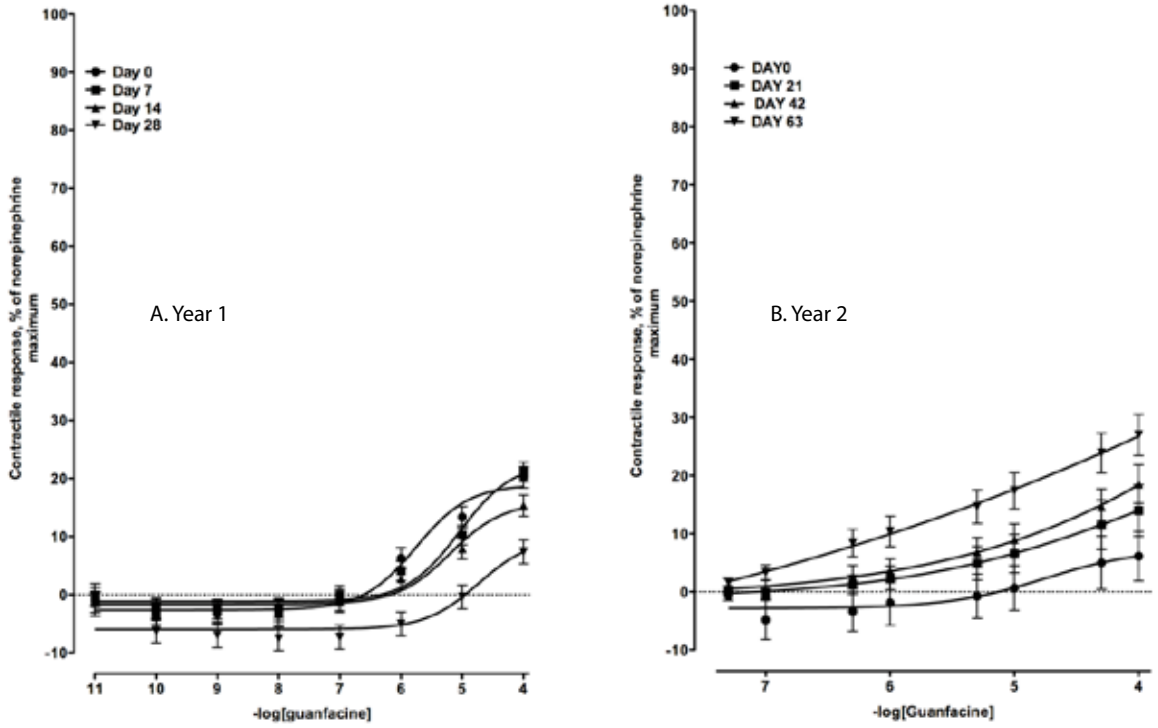


Figure 3. Concentration response to additions of guanfacine (α_{2A}-adrenergic receptor agonist) for year 1 (A) and year 2 (B)

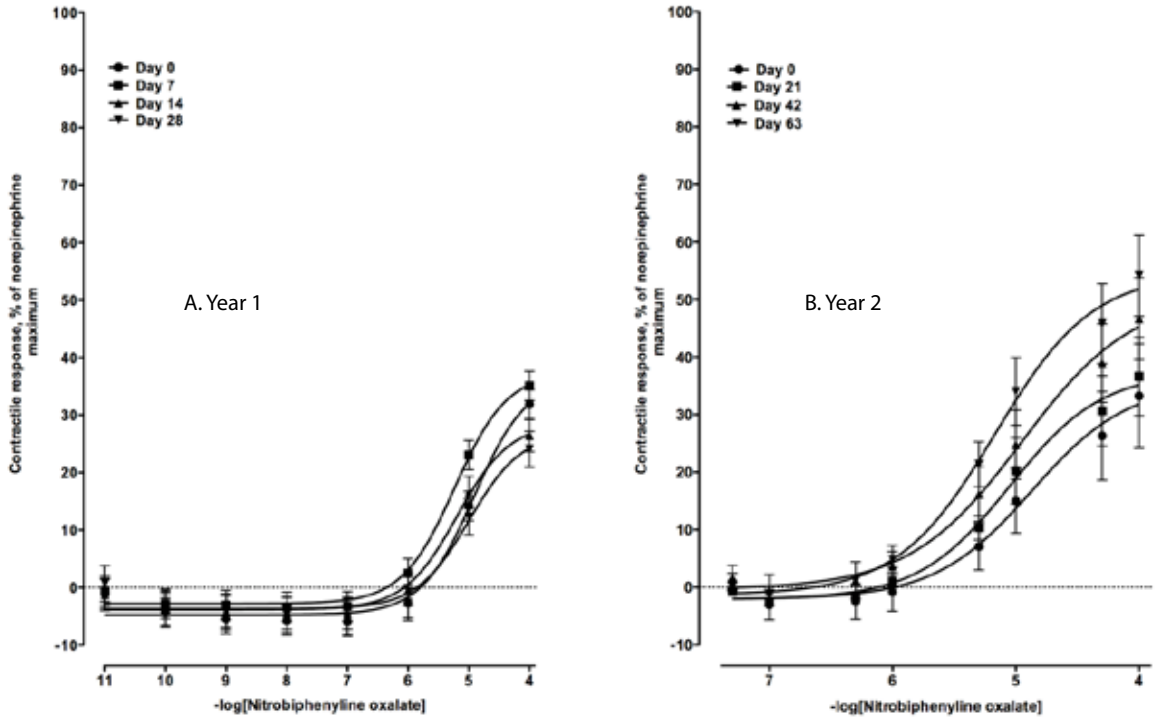


Figure 4. Concentration response to additions of (R)-(+)-*m*-nitrobiophenyline oxalate (NBP; α_{2C}-adrenergic agonist) for year 1 (A) and year 2 (B)

Effects of Basal Diet on Ruminal Disappearance of Optigen®II and Urea *In Situ*

V.B. Holder, J.S. Jennings, J.M. Tricarico, and D.L. Harmon

Summary

Urea disappearance from Optigen®II and feed grade urea is more rapid on high forage diets than high concentrate diets. The effect of basal diet on disappearance of urea from Optigen®II and feed grade urea in situ was evaluated. A method was developed to measure urea disappearance directly instead of measuring DM or N disappearance. The method involved dissolving residues in solution and measuring the urea concentration of the resulting solutions. The rate of urea disappearance as well as the rate and extent of urea disappearance was greater for high forage than high concentrate diets. Optigen®II could be customized to specific diet, and urea may be less toxic to high concentrate fed animals.

Introduction

In order to study the effects of slow release urea on N metabolism and production in cattle, it is important to characterize the ruminal behavior of Optigen®II under different circumstances. Therefore *in situ* methodologies were developed in order to study the behavior of Optigen®II and urea in the rumen. These methodologies focus on determining the actual urea disappearance instead of utilizing traditional techniques based on DM or N disappearance. Traditional *in situ* assays cannot be used for urea and other soluble compounds as the required rinsing procedures would result in solubilization of the *in situ* residues, leading to over estimation of ruminal degradability. In addition, measuring urea directly negates the need to correct for microbial contamination in the residue. The objective of this experiment was to determine the effects of basal diet on the ruminal degradation of urea and Optigen®II. The hypotheses was that Optigen®II and urea would degrade more rapidly in animals fed a high concentrate diet than those fed a high forage diet.

Materials and Methods

Experimental design

The degradation of urea and Optigen®II were determined on a 70% concentrate diet and a 100% forage diet respectively. The 100% forage diet consisted of *ad libitum* fescue hay plus a vitamin-mineral supplement. The 70% concentrate diet consisted of 30% of fescue hay and cottonseed hulls (CSH) blend and 70% of a cracked corn and soybean meal blend (70% concentrate diet, Table 1). Additionally, diets were top dressed with 0.1% urea in order to adapt animals to urea in the diet. In order to limit the total amount of urea fed to each animal, separate experiments were conducted for determination of urea and Optigen®II disappearance respectively. For determination of urea disappearance, 4 Angus steers (average BW = 310 kg) were randomly assigned to either the 100% forage or 70% concentrate diets (n = 2). For determination of Optigen®II disappearance, 4 Angus steers (average BW = 282 kg) were randomly assigned to either the 100% forage or 70% concentrate diets. For the Optigen®II experiment, treatments were crossed over and the experiment was repeated (n = 4). Urea samples were incubated in the rumen for 0, 5, 10, 15, 20, 25, 30, 40, 50 and 60 min. Optigen®II samples were incubated in the rumen for 0, 1, 2, 4, 6, 8, 10 and 24 h.

Sample preparation and ruminal incubation

For each time point, triplicate 10.0 g samples of Optigen®II or urea were weighed out into polyester bags (R510, 5x10 cm, 50 µm pore, Ankom Technology, Macedon, NY), and were placed into a single weighted mesh bag at each incubation time. Bags were placed in the ventral rumen sequentially and then removed simultaneously at the end of the incubation to achieve the appropriate incubation times. Upon removal, bags were immediately flash frozen in liquid N and stored at -80°C until they were processed.

Processing of in situ *residues*

For each polyester bag, a clean plastic funnel was placed in a 500 mL medicine bottle and the frozen polyester bag was cut into 4 to 5 pieces above the funnel. Then 350 mL of 1M HCl was used to rinse all residues including the bag into the medicine bottle. The bottles were capped and placed in a 100°C water bath for 25 min in order to dissolve Optigen®II granules and urea into the solution. After incubation, bottles were agitated and a sample of the liquid portion was collected for urea analysis.

Calculations

Urea disappearance was calculated by expressing urea lost from the bags, as a percentage of initial urea. The final amount of urea in the *in situ* residues was determined by multiplying the concentration of the resulting solution by the volume of the solution (mmol/L x L = mmol urea). Percent urea disappearance is then calculated by expressing the weight (g) of urea remaining in the residue as a percentage of initial urea and subtracting from 100 to get disappearance.

Statistical analysis

Data were analyzed as a split plot in time with steer as the main plot and time (h) as the subplot. Differences among treatments were considered to be significant when $P < 0.05$, whereas when $P > 0.05$ but < 0.10 differences were considered to indicate a trend.

Results and Discussion

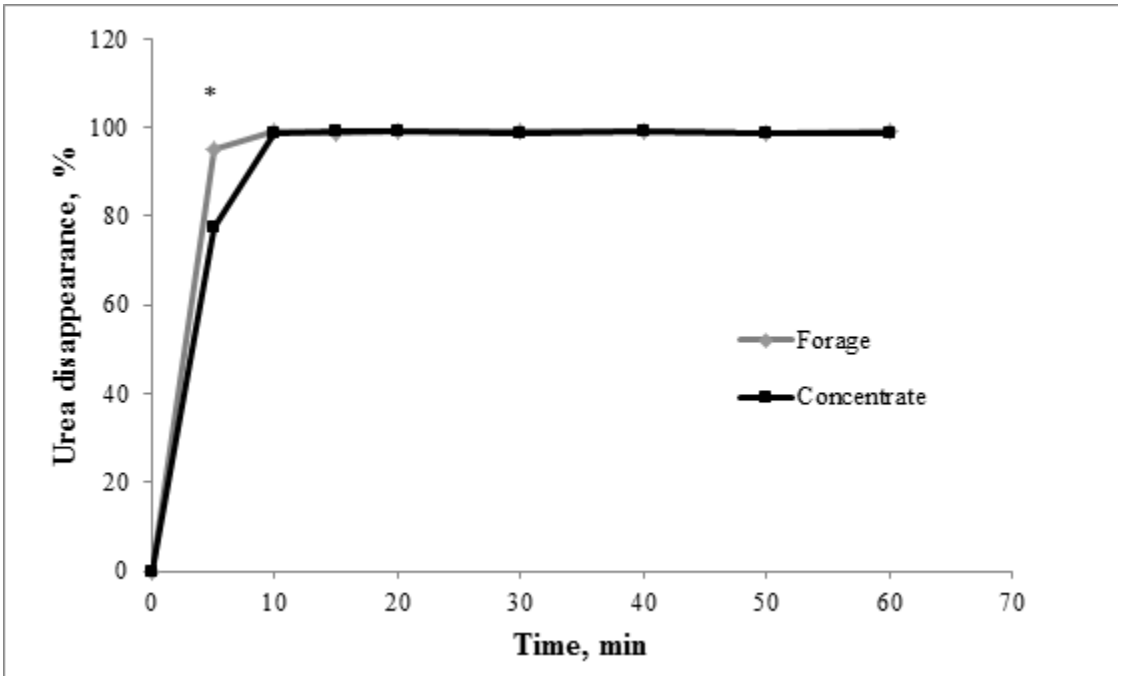
Urea disappearance from both feed grade urea and Optigen®II was affected by basal diet. For feed grade urea, the extent of disappearance had already reached a maximum (~99%) by 10 min of incubation in the rumen. However, there

was a significant difference between forage and concentrate diets on the percentage urea disappearance at 5 min of ruminal incubation. Forage diets had a higher percentage of urea degraded (95.3 vs. 77.5%, $P < 0.05$, Figure 1) at 5 min. For Optigen®II, basal diet had an effect on ruminal urea disappearance with average disappearance being higher for forage than for concentrate diets (65.8 vs. 58.6%, $P < 0.0001$, Table 2). All time points for Optigen®II from 2 to 24 h had significantly higher urea disappearance for forage vs. concentrate diets ($P = 0.0005$, Figure 2). Differences in ruminal urea disappearance at 5 min for feed grade urea and for all time points from 2 to 24 h for Optigen®II samples indicates a marked effect of basal diet on urea and Optigen®II degradation in the rumen. It is possible that the microbial populations prevalent in a high forage diet may have a higher urease production than populations prevalent in the high concentrate diets. Additionally, it is known that pH affects urease activity, with urease activity at its highest between 6.8 and 8.5, and that urease activity is completely eliminated at a pH below 3. High concentrate diets may have lead to a depression in rumen pH and depressed ruminal pH may have resulted in a depression of urease activity in the rumen and the subsequent depression of degradation of urea from both Optigen®II and urea. Additionally, pH is known to affect ruminal lypolytic activity, with rumen lypolysis being significantly depressed at pH below 6. Enzymatic degradation of the lipid coating of Optigen®II granules is the proposed mechanism by which urea is released. Therefore, reduction in lypolysis in the rumen of animals fed a high concentrate diet may have lead to slower release of urea from Optigen®II granules.

Implications

The effect of basal diet on Optigen®II urea disappearance may have some practical application for utilizing Optigen®II products of varying degradability. It is possible that Optigen with higher tested degradation rates can be recommended for higher concentrate diets and that less degradable batches may be indicated for forage dominated diets. The practical application of the effect of diet on urea degradation rate may be of limited practical consequence, except for a possibility that concentrate fed animals may be slightly more tolerant of urea than their forage fed counterparts. This statement would require further investigation.

Figure 1. Ruminal urea disappearance of feed grade urea in animals fed 100% forage and 70% concentrate diets



* Treatments differ at indicated time point ($P < 0.05$)

Table 1. Ingredient composition of the 70% concentrate diet, Experiment 3.

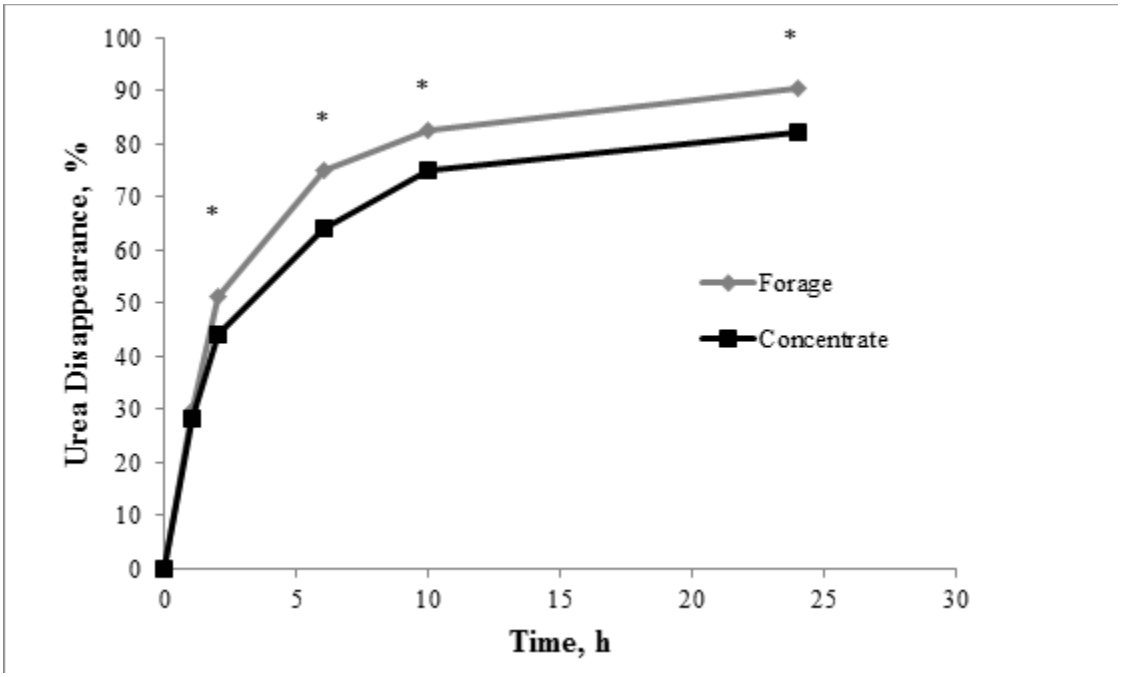
Ingredient	% of DM
Hay	19.0
Corn	55.9
CSH	11.0
Soybean meal blend[1]	14.0
Urea	0.1
Totals	100.0

[1] Soybean meal plus vitamin and mineral premix.

Table 2. Effects of basal diet on the disappearance of urea from feed grade urea and Optigen®II in the rumen.

	LSM for Diet			P-value		
Item	Forage	Concentrate	SEM	Diet	Time	Diet*Time
Urea Disappearance, %	98.5	96.2	0.9	0.224	<0.0001	<0.0001
Optigen Disappearance, %	65.8	58.6	2.5	<0.0001	<0.0001	0.0005

Figure 2. Ruminal urea disappearance from Optigen® in animals fed 100% forage and 70% concentrate diets



* Treatments differ at indicated time point ($P < 0.05$)

Evaluation of a Rapid Determination of Heat Production and Respiratory Quotient in Holstein Steers Using the Washing Rumen Technique

D.H. Kim, K.R. McLeod, J.L. Klotz, A.F. Koontz, A.P. Foote, and D.L. Harmon

Summary

The objective of this study was to validate use of the washed rumen technique for rapid measurement of fasting HP and RQ. The plateau of RQ values was 0.87 ± 0.01 and 0.72 ± 0.01 for unwashed and washed rumen, respectively. The RQ decreased to approximately 0.7, 8 h after washing the rumen. Mean RQ after washing rumen were 0.78, 0.74, and 0.73 (SEM = 0.01) for time segments 0 to 8 h, 9 to 16 h, and 17 to 24 h, respectively. Mean fasting HP after washing rumen was 18.75, 16.84, and 16.72 (SEM = 0.35) kJ/(h·kg$^{0.75}$) for time segments 0 to 8 h, 9 to 16 h, and 17 to 24 h, respectively. There were no significant differences in RQ and fasting HP (P = 0.225 and P = 0.810, respectively) between the time segment of 9 to 16 h and 17 to 24 h. Thus, an accurate measurement of fasting HP can be obtained using a shorter-term measurement with the washed rumen technique. This approach provides an alternative to the traditional 48 h fasting time, or measurements made during the third and fourth day after starvation.

Introduction

Traditional measurement of maintenance energy requirements in ruminants used estimates of fasting HP during the 3rd and 4th day of fasting. The fasting HP measured by calorimetry is a measure of fasting metabolism, which by definition, equates to NE_m. However, this approach has limitations, specifically the actual severity of stress and decline of physical activity induced by the extended fasting period required.

It was hypothesized that using the washed rumen technique, in conjunction with accurate fasting HP estimates from short-term caloric assessment, would emulate a fasting state of metabolism rapidly and as a result provide a more robust measure of fasting HP compared with the traditional fasting methodologies while still excluding most of the energy required for digestion, related tissue deposition, and activity. Therefore, this study was conducted to evaluate the use of the washed rumen technique for rapid measurement of fasting HP and RQ and to compare this with heart rate (HR) and core temperature (CT) as indicators of a basal metabolic rate.

Materials and Methods

Animal, Feeding, and Management

Eight Holstein steers (322 ± 30 kg), each surgically fitted with a ruminal cannula, were used. Steers were offered free access to water and were fed once daily (0700 h) alfalfa cubes (composition on % DM basis: CP = 16.5; ADF = 37.2; NDF = 51.9; NE_m = 1.24 Mcal/kg) top-dressed with a mineral pre-mix at 1.5 × NE_m based on body weights.

Experimental Procedure and Measurement

The experiment was conducted as follows: 10 d of feed adaptation at 1.5 × NE_m, 1 d for measurement of respiratory gases at 1.5 × NE_m (unwashed rumen), followed by 1 d for measurement of respiratory gases at fasting (washed rumen), and finally 7 d to ensure re-establishment of normal intake. Respiratory gases (O_2, CO_2, and CH_4) were measured for 24 h following the 0700 h feeding. The CT was measured using the radio telemetry device, and HR was measured using a radio telemetry transmitter attached to a heart-girth band. The following day at 0700, the contents of the reticulorumen were removed using a vacuum, followed by rinsing with 10 L of tap water (39°C) and futher rinsed again 3 times with 10 L of saline (39°C). Ruminal buffer (NaCl = 96; $NaHCO_3$ = 24; $KHCO_3$ = 30; K_2HPO_4 = 2; $CaCl_2$ = 1.5; $MgCl_2$ = 1.5 mmol/kg of buffer) with Cr-EDTA (53.27 mmol Cr/kg of buffer) was aerated with a mixture of 75% N_2 and 25% CO_2 before incubation in the rumen (Kristensen and Harmon, 2004). The ruminal buffer (15 kg) was placed in the rumen after completion of the washing. After adding buffer, respiratory gases were collected for analysis.

The contents from the reticulorumen were stored in a plastic barrel covered with straw and warmed (39°C) until reintroduction into the rumen at the end of the gas exchange measurement. After the calorimetry measurements were completed, the ruminal buffer was pumped out. The ruminal contents were reintroduced into the rumen, and the steers were returned to individual pens and fed.

Calculations and Statistical Methods

Heat production was calculated using the equation of Brouwer (1965).

The plateau of RQ was estimated using non-linear regression analysis to a one-phase decay equation using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA) and the following equation:

$$Y(t) = A \cdot e^{-kt} + \text{Plateau}$$

where t is time in hours, Y(t) is the RQ value, A is the difference between Y value at time zero and at plateau, Plateau is the Y value at infinite time, and k is the rate constant.

The statistical model used individual steer as the experimental unit. The CT, HR, HP, and RQ for each steer were averaged over day and within each hour, and then analyzed using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC) with animal as random effects, and treatment (washed and unwashed rumen), hour, and treatment × hour as fixed effects. Means are presented as least squared means.

Results and Discussion

Comparison of mean hourly CT, HR, HP, and RQ between unwashed rumen and washed rumen models are presented in Table 1. Mean hourly CT was lower for washed rumen steers ($P < 0.001$), however, there was an interaction with sampling hour ($P < 0.001$) as CT for steers with a washed rumen were relatively stable and CT for unwashed rumen steers declined over the sampling period with a nadir at 20 hours (Figure 1). Data for mean hourly HR was lower for washed rumen steers than that of unwashed ($P < 0.001$); again, there was an interaction with sampling hour ($P < 0.001$). The HR for steers during the washed rumen segment was relatively stable whereas HR declined in unwashed rumen steers over the sampling period (Figure 2). Hourly HP was lower ($P < 0.001$) for the washed rumen steers and interacted with sampling hour ($P < 0.001$). For washed rumen steers, HP was relatively stable whereas the steers maintained on alfalfa cubes at 1.5 × NE_m during the experiment declined over the sampling period (Figure 3). A similar pattern was seen for RQ in that RQ was lower for the washed rumen steers ($P < 0.001$) but interacted with sampling hour ($P < 0.001$; Figure 4). The washed rumen steers had RQ that were relatively stable while the unwashed rumen steers RQ declined over the sampling period.

In the present study, by removing rumen contents we estimated the time for RQ to decline and stabilize by using a one-phase decay exponential equation.

Figure 1. Hourly CT patterns of Holstein steers on unwashed (n = 4; ◆) and washed (n = 4; ■) rumen. The steers of unwashed rumen were fed with alfalfa cubes at 1.5 × NE_m based on BW. The steers of washed rumen were incubated ruminal buffer of 15 kg in the reticulorumen up to 24 h. Error bars are SEM.

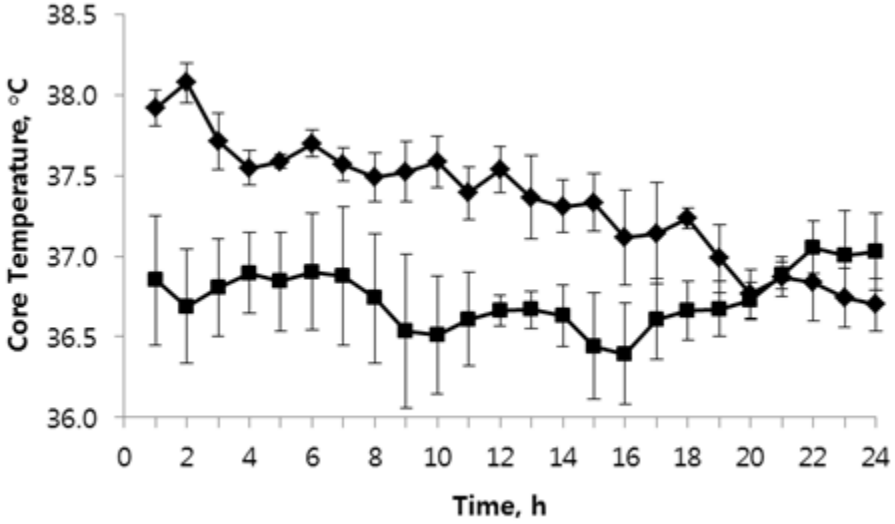


Figure 2. Hourly HR patterns of Holstein steers on unwashed (n = 8; ◆) and washed (n = 7; ■) rumen. The steers of unwashed rumen were fed with alfalfa cubes at 1.5 × NE_m based on BW. The steers of washed rumen were incubated ruminal buffer of 15 kg in the reticulorumen up to 24 h. Error bars are SEM (partly covered by the symbols).

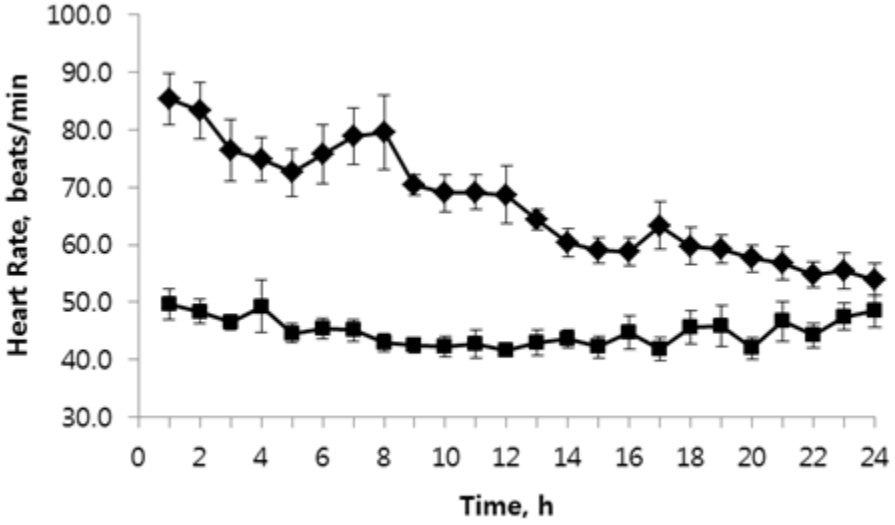


Table 1. Comparison of physiological measurements between steer with unwashed and washed rumens.

Item[1]	Unwashed Rumen	Washed Rumen	SEM	P -value		
				Treatment (T)	Hour (H)	T × H
Core Temperature, °C	37.33	36.76	0.05	<0.001	0.375	0.006
Heart Rate, beats/min	66.93	44.89	0.66	<0.001	<0.001	<0.001
Heat Production, kJ/(h·$kg^{0.75}$)	26.70	17.44	0.20	<0.001	<0.001	<0.001
Respiratory Quotient	0.91	0.75	0.01	<0.001	<0.001	<0.001

[1] Data are presented as least squared means of animals were fed with alfalfa cubes at level of 1.5 × NE_m based on the BW (unwashed rumen) and incubation of ruminal buffer of 15 kg in the reticulorumen up to 24 h (washed rumen). Core temperature: both unwashed and washed rumen are n=4; heart rate: unwashed rumen is n=8, washed rumen is n=7; heat production and respiratory quotient: both unwashed and washed rumen are n=8.

The RQ had fallen to the 0.7 range by 8 h (range = 6.55 to 10.25) after removing the rumen contents. The estimated equation is as follows: $Y = 0.0957e^{-0.1251t} + 0.7189$ ($r^2 = 0.5163$). This implies that RQ in the washed rumen model can be measured starting at 8 h and continuing through 24 h. This was confirmed by dissociating the data into three segments; 0 to 8 h, 9 to 16 h, and 17 to 24 h. The mean RQ from 8 to 16h and from 17 to 24h were not different ($P = 0.225$) in the washed rumen steers. Mean fasting HP was also not different ($P = 0.810$) between the same time periods. The data in the washed rumen steers were as follows: mean RQ was 0.78, 0.74, and 0.73 (SEM=0.01) for the time segments of 0 to 8 h, 9 to 16 h, and 17 to 24 h, respectively; mean fasting HP was 18.75, 16.84, and 16.72 (SEM=0.35) kJ/(h·kg $^{0.75}$) for the time segments of 0 to 8 h, 9 to 16 h, and 17 to 24 h, respectively.

A fasting state was achieved using the washed rumen technique in this study; CT, HR, HP, and RQ were 36.74 ± 0.06 °C, 44.08 ± 0.68 beats/min, 418.55 ± 13.30 kJ/(d·kg $^{0.75}$), and 0.73 ± 0.003, respectively. The values are stable from 8 to 24 h after removal of rumen contents. The rapid nature of inducing the fasting state may minimize stress on the animal and minimize changes in organs and tissues that can occur with 3 to 4 d of fasting, thereby providing a more accurate estimate of fasting HP.

Implications

Accurate measurement of the factors contributing to maintenance energy expenditures and to efficiencies of energy use for maintenance is a necessary part of developing an understanding of the animal's energy economy. Emptying the rumen presents the unique characteristic of shutting down the main source of energy into the animal in minutes. Therefore, the washed rumen technique may permit determination of accurate estimates of the energy required for maintenance within shorter time periods than traditional fasting approaches, and this approach may provide an alternative to the traditional 48 h fasting time, or measurements made during the third and fourth day of starvation.

Figure 3. Hourly HP patterns of Holstein steers on unwashed (n = 8; ◆) and washed (n = 8; ■) rumen. The steers of unwashed rumen were fed with alfalfa cubes at 1.5 × NE_m based on BW. The steers of washed rumen were incubated ruminal buffer of 15 kg in the reticulorumen up to 24 h. Error bars are SEM (partly covered by the symbols).

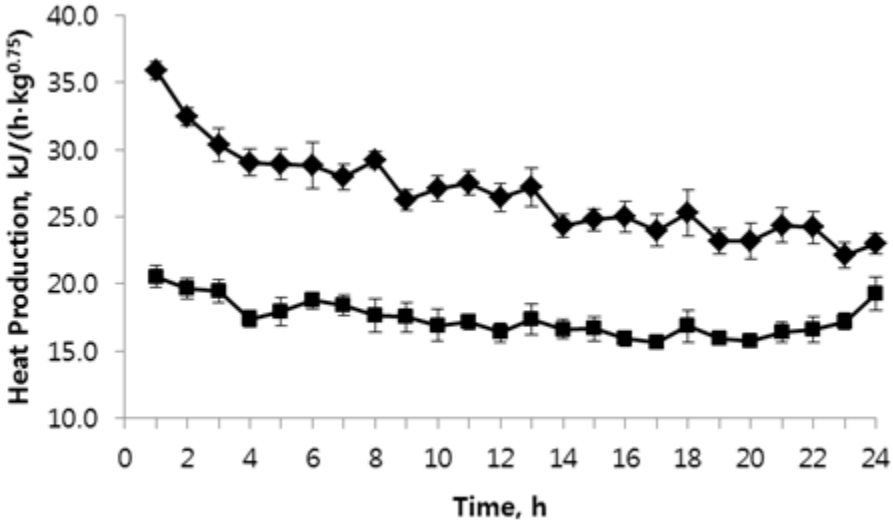
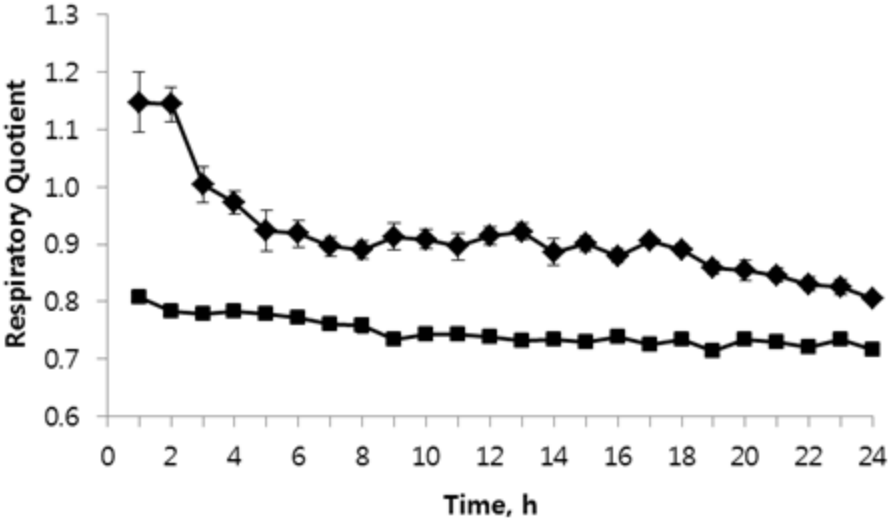


Figure 4. Hourly RQ patterns of Holstein steers on unwashed (n = 8; ◆) and washed (n = 8; ■) rumen. The steers of unwashed rumen were fed with alfalfa cubes at 1.5 × NE_m based on BW. The steers of washed rumen were incubated ruminal buffer of 15 kg in the reticulorumen up to 24 h. Error bars are SEM (partly covered by the symbols).



Effect of Degradable Intake Protein Supply on Utilization of Direct-Fed Microbials in Receiving Steers

N.M. Kenney, E.M. Vanzant, J.W. Lehmkuhler, D.L. Harmon, and K.R. McLeod

Summary

One hundred and ninety-two crossbred beef steers were fed five levels of degradable intake protein (DIP) with or without direct-fed microbials (DFM), a mixed culture primarily containing *Lactobacillus acidophilus* and *Enterococcus faecium* (Vit-E-Men probiotic 10G), to determine if DIP supply affects response to DFM in receiving cattle. Summary results are representative of responses over the entire 56 day study. No significant differences were observed in intake; however, DFM-fed steers exhibited a cubic increase in average daily gain with increases in DIP supply. Additionally, DFM steers exhibited an earlier stabilization of fecal pH. Innate immune response was measured by exposing steers to an antigen to which they were previously naive, and a leptospirosis vaccination, and measuring serum titer levels over time post-booster. No differences in innate immune response were observed. Ultimately, the greatest DFM-mediated increases in animal performance were observed above DIP requirement, indicating that animal response to DFM is dependent on DIP supply. Degradable intake protein supply should be taken into consideration when developing a feeding strategy for receiving cattle which includes the use of DFM in order maximize animal performance response.

Introduction

Direct fed microbials have been found to have positive effects on animal performance, such as increased average daily gain, dry matter intake, and growth efficiency, when included in receiving and finishing cattle diets. However, the magnitude and consistency of animal responses to DFM have been variable. To date, the exact mechanism of action of DFM is unknown, though previous work has shown that DFM have some capacity to alter rumen fermentation (i.e. VFA concentration, lactate utilization, microbial population) and possibly immune function. Supply of degradable intake protein (DIP) has major impacts on ruminal digestion, volatile fatty acid production, and microbial protein production and subsequently animal performance measures. Given the previously described modes of action, it is possible that response to DFM may be influenced by supply of DIP. However, to date there is a paucity of data addressing this potential interaction. An interaction between DFM and DIP supply may explain at least part of the variability observed in previous DFM experiments. As such, this study was designed to determine if DIP supply affected animal performance response to DFM.

Table 1. Receiving Diets Composition (DM basis)

		% Inclusion DM Basis			
Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fescue Hay	20.0	20.0	20.0	20.0	20.0
Cotton Seed Hulls	20.0	20.0	20.0	20.0	20.0
50/50 HMC/Cracked Corn	44.32	44.32	44.32	43.87	43.11
Amino Plus	12.7	9.26	5.821	4.875	4.533
Soybean Meal	0	3.44	6.879	8.125	9.067
Urea	0	0	0	0.15	0.31
Limestone	1.3	1.3	1.3	1.3	1.3
Potassium Chloride	0.5	0.5	0.5	0.5	0.5
TM premix - Salt	0.75	0.75	0.75	0.75	0.75
Vitamin premix, A,D,E	0.05	0.05	0.05	0.05	0.05
Choice White Grease	0.38	0.38	0.38	0.38	0.38
Total	100	100	100	100	100

Materials and Methods

One hundred and ninety-six crossbred beef steers (617 ± 55 lb) were used in a randomized complete block design. Steers were blocked by initial weight. Treatments were arranged in a 5 × 2 factorial, with 5 levels of DIP (80, 90, 100, 110, and 120% of DIP requirement) fed with and without DFM. Degradable intake protein requirement was calculated as 11% of TDN. Differences in protein degradability were achieved by altering the ratio of soybean meal to treated soybean meal (Amino Plus®) and amount of urea (Table 1). Steers were immediately transitioned from a forage based diet to the final experimental diet on d 1 of the study. Direct-fed microbial consisted of a mixed bacterial culture, primarily *Lactobacillus acidophilus* and *Enterococcus faecium* mixed in a corn carrier (1 billion cfu/hd/d). Steers were fed once daily, the DFM or corn-carrier control treatment was fed as a top-dress on the experimental diet. Body weight and feed refusals were recorded weekly for 56 days. Fecal pH was determined by rectal grab sample on day 7 and 14. Animals were vaccinated against *Leptospirosis* on day 0 and re-immunized at day 14. Blood samples were collected 2, 3, 4 and 6 weeks post re-immunization for measurement of antibody titers to *Leptospira* serovar hardjo. Titers are expressed as the natural log of the reciprocal of the highest titer dilution exhibiting agglutination. Performance data was analyzed using the GLM procedure in SAS. Fecal pH was analyzed as repeated measures using the MIXED procedure in SAS, with block as a random effect. Means were separated using orthogonal polynomial contrasts.

Results and Discussion

Animal Performance

Dry matter intake was not affected by DFM or DIP treatment over the 56 day trial (Table 2). During the first 28 days of the trial, a significant cubic DIP by DFM interaction was ob-

Table 2. Effect of DFM and DIP on animal performance and immune response.

		DIP, % of Requirement													
		Control					DFM						P Value		
		80	90	100	110	120	80	90	100	110	120	SEM	DFM	DIP	DFM × DIP
Initial BW, lb		620	613	618	613	611	614	615	617	611	614	2.25	0.48	0.06	0.30
DMI, lb/d	0 – 28 d	18.5	16.3	18.1	16.9	17.8	17.4	18.4	19.1	16.8	18.7	0.7	0.25	0.11	0.24
	29 – 56 d	20.8	20.2	21.4	20.8	20.6	20.3	21.7	21.6	20.9	22.5	0.6	0.11	0.41	0.23
	0 – 56 d	19.7	18.3	19.8	18.9	19.2	18.9	20.1	20.4	18.8	20.6	0.6	0.13	0.21	0.20
ADG, lb	0 – 28 d	3.19	2.88	3.45	3.15	3.05	2.81	3.38	3.49	3.13	3.95	0.22	0.13	0.11	0.05[1]
	29 – 56 d	3.24	3.35	3.33	3.46	3.19	3.35	3.44	3.46	3.35	3.6	0.17	0.25	0.96	0.65
	0 – 56 d	3.22	3.11	3.39	3.3	3.12	3.08	3.41	3.47	3.24	3.78	0.15	0.09	0.26	0.08[2]
Growth Efficiency, lb/lb	0 – 28 d	0.174	0.174	0.193	0.187	0.171	0.162	0.184	0.183	0.189	0.211	0.009	0.32	0.11	0.05[3]
	29 – 56 d	0.156	0.167	0.157	0.167	0.155	0.166	0.158	0.16	0.163	0.161	0.007	0.81	0.88	0.72
	0 – 56 d	0.164	0.171	0.173	0.176	0.162	0.164	0.17	0.171	0.174	0.184	0.006	0.41	0.42	0.21
Leptospirosis Titer		6.78	6.09	6.37	6.33	6.4	6.24	6.22	6.09	6.24	6.19	40.4	0.50	0.11	0.54

1 Contrasts - DFM × DIP $P = 0.02$; No Significance in Control; Linear in DFM $P = 0.005$; Cubic in DFM $P = 0.03$.
2 Contrasts – DFM × DIP $P = 0.03$; No Significance in Control; Linear in DFM $P = 0.02$; Cubic in DFM $P = 0.05$.
3 Contrasts – DFM × DIP $P = 0.03$; No Significance in Control; Linear in DFM $P = 0.002$.

served for ADG which resulted in a tendency for an interaction over the entire 56 day trial (Figure 1). In the absence of DFM, ADG was similar across all DIP levels, whereas in the presence of DFM, ADG increased with increasing level of DIP in a cubic fashion; greatest response occurred at 120% of DIP requirement. The depression in ADG observed for DFM fed animals receiving 110% of DIP requirement, compared with those fed 100 or 120%, is difficult to explain. However, this is likely attributable to differences in dry matter intake; although not significant, the same cubic trend was observed in intake. Direct-fed microbials increased growth efficiency linearly with increasing level of DIP supply during the first 28 days of the trial, whereas efficiency was similar across DIP levels in the absence of DFM (DIP by DFM interaction). Growth efficiency was similar across treatments in the second 28-day period, such that efficiency over the entire 56-day study was unaffected by treatment. Taken together, the growth performance data suggests that in order to maximize the positive effects of DFM, animals must be fed above DIP requirements.

Fecal pH

Fecal pH was measured on day 7 and 14 of the trial as an indicator of animal adaptation to the receiving diet. Degradable intake protein had no effect on fecal pH. In the presence of DFM there was no change in pH over time (6.64) (Figure 2). However, in the absence of DFM fecal pH decreased from 6.71 to 6.62 on day 7 and 14, respectively. The earlier stabilization of fecal pH in the DFM steers may be indicative of an earlier shift in the microbial population of the lower gut as a result of the change in diet. These results combined with the increases in growth efficiency during the first 28 days suggest that DFM may play a role in dietary adaptation.

Immune Response

Previous work has demonstrated differences in morbidity and mortality as a result of DFM inclusion in receiving and finishing diets. Steers were subjected to a leptospirosis challenge, consisting of an initial vaccination on day 0 followed by a booster on day 14, in order to determine if DFM mediated changes in the innate immune response. As expected, peak titers were measured two weeks post administration of the booster and declined over time thereafter (Figure 3). No treatment effects or interactions were detected, indicating that the innate immune response is not responsible for differences in morbidity and mortality previously observed in DFM fed animals.

Implications

Positive animal performance response to DFM is dependent on DIP supply and in order to maximize the positive effects of DFM, DIP requirements must be met or exceeded. Previous DFM work has not accounted for the effect of DIP supply on DFM response, which likely explains part of the variation previously observed.

Figure 1. Effect of DFM and DIP on 0 to 56 d Average Daily Gain.

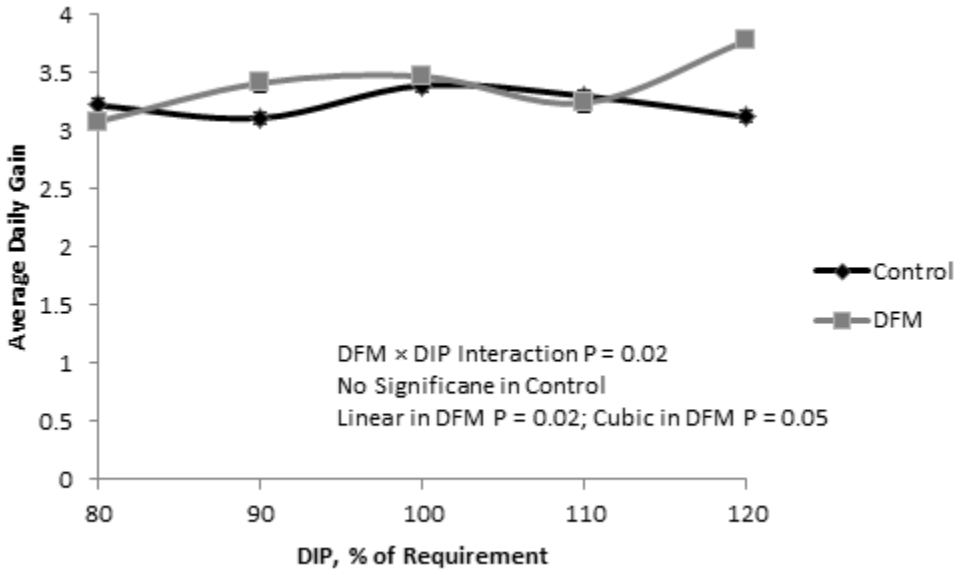


Figure 2. Effect of DFM on fecal pH.

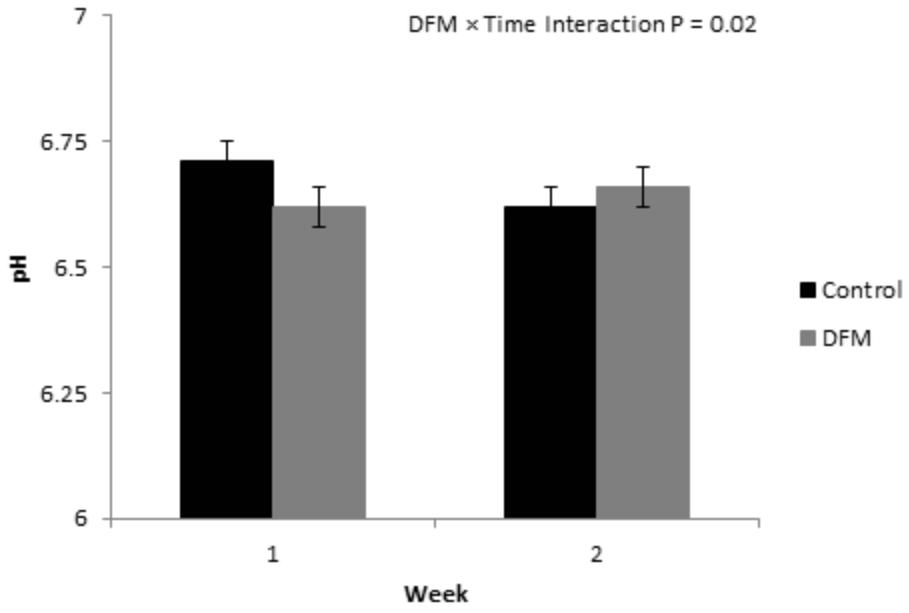
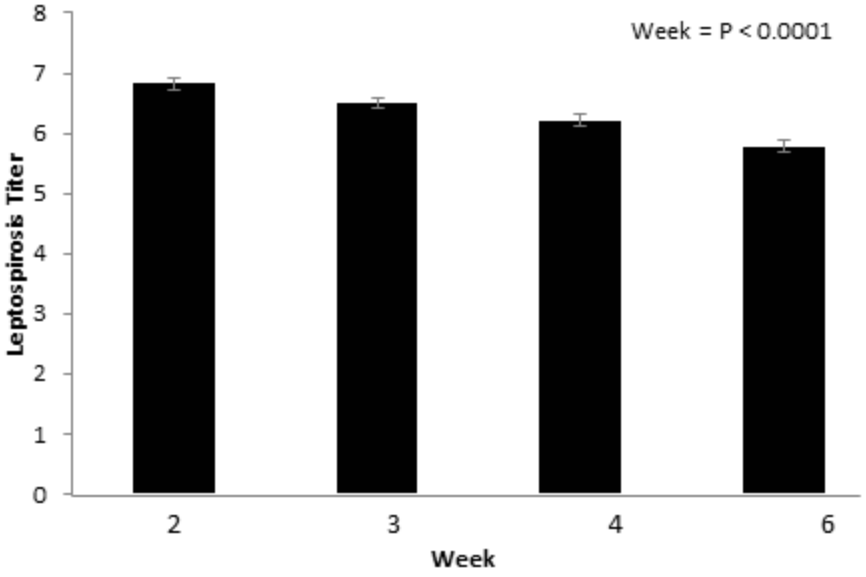


Figure 3. Effect of time on Leptospirosis titer.



Survey Results from Master Cattleman Program Genetics Session

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Summary

Producers participating in the genetics session of the Master Cattleman program are equipped with rapid response devices to answer a series of questions during the presentation. These questions were developed to get a sense of the common perceptions of producers on certain topics and to provide teachable moments. The results of these surveys indicate a high level of misconceptions pertaining to coat color, branded products, heterosis, proper use of expected progeny differences and genetic control of horned versus polled.

Introduction

Beef producers are under increasing pressure to manage their operations at a high level of efficiency in order to achieve profitability. Input costs such as feed, fertilizer and fuel are increasing at far greater rates than general inflation. While cattle prices have enjoyed high levels as well, it is still imperative for beef producers to get as much output from their resources as possible.

The Master Cattleman program is the major educational effort of the University of Kentucky's Beef Integrated Resource Management (IRM) team. This program has educated more than 3,500 beef producers since 2000 on all phases of the beef industry (business management, environmental stewardship, facilities and handling, reproduction, genetics, nutrition, forages and end product). The genetics session is typically 3 hours in length and focuses on developing a targeted breeding program that fits the producers' resources and utilizes crossbreeding and sound selection practices. Rapid response devices are used to get a measure of demographics of the participants, get a measure of practice utilization, highlight some misconceptions in the beef industry, and provide some teachable moments. The purpose of this article is to show the results of some of these questions and discuss possible implications.

Materials and Methods

Survey Questions

Survey questions were asked throughout the 3-hour genetics session of the Master Cattleman program and the results were instantly posted and discussed. The devices were also used in a bull purchasing exercise that emphasizes the importance of matching genetics to the environment and management.

Three hundred ninety-five people participated in sixteen presentations. These sessions were conducted from 2010 through 2012.

The following is a list of the questions asked during the presentation:

Do you use your IRM calendar as a management tool?
a. Yes
b. No

How important is coat color in your bull selection?
a. Very important
b. Somewhat important
c. Not very important

What traits do you think color impacts?
a. Birth weight
b. Weaning weight
c. Carcass traits
d. Color
e. All of the above

How much Angus breeding is required for Certified Angus Beef (CAB)?
a. 100%
b. 75%
c. 50%
d. 25%
e. 0%

Which of the following has the greatest level of milk production?
a. Holstein
b. Jersey
c. Holstein-Jersey cross

Which of the following has the highest milk fat percent?
a. Holstein
b. Jersey
c. Holstein-Jersey cross

Which of the following has the highest reproduction rate?
a. Holstein
b. Jersey
c. Holstein-Jersey cross

What is the best way to select for a trait such as weaning weight?
a. Actual weight
b. Weaning weight ratio
c. Weaning weight EPD
d. Combination of any of the above

Which bull is most likely to give horned calves?
a. Smooth polled bull
b. Scurred bull
c. Can't tell

Did you like the answering devices?
a. Yes
b. No

The answers to these questions are stored on the computer and were retrieved and analyzed using simple percentages.

Results and Discussion

The results of the survey were varied across the sessions; however, they clearly indicate that there are considerable misconceptions about branded beef products, heterosis, the proper use of expected progeny differences and the implications of scurs on the horn/poll genotype.

Each participant in the Master Cattleman program is given an IRM calendar at the start of the course and are encouraged to use the management information provided in it to assist in overall management; 57.1% indicated they were using the calendar in this manner.

Two-thirds of the participants indicated that color was important in their bull selection decision and almost sixty percent (58.7%) felt that selecting on color impacted traits other than color alone. Only 13% of the participants correctly answered that 0% Angus is required to qualify for CAB; 36.2% thought that it required at least 50% Angus. These results may lead to a reduction in crossbreeding since many producers feel their cattle need a high percentage of Angus to qualify for these branded products.

To provide a teachable opportunity on heterosis a series of questions were asked about the impact of crossbreeding on milk production, milk fat percent and fertility in dairy breeds. Almost 42 percent correctly answered that Holstein would give the greatest level of milk; however, 50% thought that the Holstein-Jersey cross would give the most. On milk fat 82.1% correctly answered that Jersey would give the highest percent and 65.9% correctly answered that the crossbred female should have the highest level of reproduction. A discussion on the cumulative advantages of crossbreeding in beef cattle was discussed and the potential increases in salable product depending on which crossbreeding system is used.

To start the section on selection tools the participants were asked which piece or pieces of information would help them make the best selection decision for a trait such as weaning weight. The correct response of weaning weight EPD alone was answered by 28.7% of the participants, but the most popular answer was a combination of actual measurement, ratio and EPD at 61.7%. An explanation that EPDs alone is the best because it already includes all of the other information, weighted in the correct manner, was discussed.

Although the majority of participants correctly answered that scurs are not an indicator that a bull is a horned gene carrier; 48.1% thought that you could tell by looking at the bull. The genetic mechanism between horns and scurs is explained.

The final question was to get a feel for how well the participants liked using the rapid response devices; 97.7% indicated that they liked using them.

Implications

The implications of the responses to these questions are that beef producers are still unclear on factors affecting premium marketing programs, the influence of color on other traits and the best methods for selecting bulls. Their answers to the questions on heterosis indicated they were a little more aware of those benefits. These devices kept producers engaged in the presentation, provided for thoughtful discussion, provided teachable moments and were well received by the participants.

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