AGRICULTURAL EXPERIMENT STATION • UNIVERSITY OF KENTUCKY COLLEGE OF AGRICULTURE, LEXINGTON, KY, 40546

UK UNIVERSITY OF KENTUCKY College of Agriculture

2010
Research and Extension
Beef Report

UKAg RESEARCH • University of Kentucky • Lexington, Kentucky 40546

2010
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Contributing Authors

University of Kentucky

Department of Animal and Food Sciences

L.H. Anderson
A.L. Ballou
D. Bullock
W.R. Burris
N.A. Elam
A.P. Foote
D.L. Harmon
A. Heenan
G. Hibbard
R.B. Hightshoe
V.B. Holder
S.W. El-Kadi
S.E. Kitts
K.B. Knight
A.F. Koontz
F. Korthaus
E. Lane
K.M. Laurent
J.W. Lehmkuhler
J.C. Matthews
K.R. McLeod
L.T. Porter
J.H. Randolph
G. Rentfrow
K.K. Schillo
G.L. Sipe
S.M. Speight
C.C. Taylor-Edwards
L. Van Rensberg
E.S. Vanzant
M. Xu

Department of Plant and Soil Sciences

L.P. Bush

UK Veterinary Diagnostic Laboratory

M. Steinman

USDA ARS Forage Animal Production Unit

G.E. Aiken
K.R. Brown
J.M. Carter
J.M. Johnson
J.L. Klotz
J.R. Strickland

Industry, Government, and Other Universities

USDA Henry A. Wallace Beltsville Agricultural Research Center

R.L. Baldwin VI
E.E. Connor
T.H. Elsasser
S. Kahl
T.S. Rumsey

D.E. Axe
ANTG, Petersburg, Virginia

D.G. Burrin
USDA ARS Children's Nutrition Research Center, Houston, Texas

T. Dietrich
Kentucky Department of Agriculture, Frankfort, Kentucky

J.J. Holst
Department of Biomedical Sciences, The Panum Institute, University of Copenhagen, Denmark

K.K. Kreikemeier
Life Products Inc. Norfolk, Nebraska

N.B. Kristensen
Department of Animal Health and Bioscience, Faculty of Agricultural Sciences, Aarhus University, Denmark

M. Rinker
Distributors Processing Inc. Porterville, California

J.M. Tricarico
Alltech Inc., Nicholasville, Kentucky

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Introduction

The *2010 Research and Extension Beef Report* provides summaries of completed and ongoing research and extension efforts related to the beef cattle industry. This work is carried out by faculty, staff, and students in a variety of disciplines in both the UK Department of Animal and Food Sciences and the USDA Forage Animal Production Research Unit.

The report highlights advances in understanding of basic scientific principles of livestock production as well as applied research from which producers and the industry can benefit. Extension educational programs, on-farm demonstrations, and other activities help transfer this knowledge to producers so they can adopt of management changes as appropriate.

For more information related to particular projects, please contact the faculty members involved.

Acknowledgements

Undergraduate Students

Stephanie Ballinger
Jessica Brownfield
Matt Dintelmann
Carly Farrell
Claudia Gering
Keri Grace
Spencer Guinn
Jamie Laity
Kevin Mattingly
Todd Miller
Travis Miller
Tyler Miller
Aric Meyer
Michelle Ogden
Aaron Ross
Michelle Simon
Justin Spengler
Nolan Taylor
Margie Ward
Josh Winslow

Industry Support

Juan Tricarico, Alltech Inc., Nicholasville, KY
Dirk Axe, ANTG Research & Development, Petersburg, VA
Mike Rincker, Distributors Processing Inc., Porterville, CA
Rick Stock, Cargill Incorporated, Blair, NE
John Hutcheson, Intervet/Schering Plough Animal Health, Summit, NJ

Stocking Rates and Gain in Growing Cattle: Lessons from the Literature

E.S. Vanzant

Summary

In a meta-analysis of pasture stocking rate studies in the United States over the past 50 years, 69% of the variation in stocking rate effects on average daily gain were accounted for by gain at low stocking rate, presence of grass and legume, and latitude of the research site. This work demonstrates the potential for quantitative prediction of stocking rate effects on cattle performance and represents a first step in the development of decision support software for cattle graziers.

Introduction

Selection of proper stocking rates (SR) is arguably the most important management decision faced by graziers, but the relationship between SR and animal performance is potentially affected by a large number variables, including forage and cattle type, environmental conditions during the forage grazing season, length of the grazing season, etc. The many variables that can influence this relationship make it quite difficult to anticipate the effects of SR decisions at a given point in time at a given location. Generally speaking, within ranges of SR that are typical for an area, as SR increases, average daily gain (ADG) decreases. Although some studies have shown the response to be curvilinear, it can be shown that linear models (incorporating a threshold response at low SR) adequately describe the data for practical purposes. Over the past several decades, there have been numerous research studies designed to evaluate the effects of SR on growth of stocker cattle. However, to our knowledge, there have been no attempts to compile the results from these numerous studies in such a way as to draw quantifiable conclusions from them. The purpose of this study was to review pertinent research literature from studies on pasturelands in the United States (excluding rangeland studies) in order to derive quantitative relationships between SR and gains for growing cattle. A unique approach was developed for this synthesis, in which the relationship between ADG and SR was defined by the slope of the regression line of ADG on SR. In general terms, this quantity (the SLOPE) describes the responsiveness of ADG to changing SR.

Materials and Methods

Data Sources

The National Agricultural Library's AGRICOLA database was searched for research studies that were conducted on pastureland (as distinct from rangeland) in the United States, reported in refereed research journals[1] since 1960, and included ADG responses of growing cattle to changes in SR. Ultimately, 26 independent reports, comprising 58 individual comparisons of ADG and SR were retained for analysis. Table 1 shows the variables recorded for each study. The influence of SR on ADG was evaluated by determining the slope of the regression of ADG on SR. When no effect of grazing intensity was detected, the y-intercept value described the response (representing the average ADG at all SR). When the data fit a threshold model (i.e., SR effect only at high SR), SLOPE came only from the portion of the curve in which ADG was influenced by SR.

Table 1. Variables recorded from each report for use in calculations and multiple regression equations.

Variable	Units
Stocking rate	lb live weight/acre
Average daily gain	lb/day
Animal weight	lb
Grazing start and end dates	Used to calculate length of grazing period in days
Location of research site	Latitude and longitude in decimal degrees
Average monthly precipitation	inches
Average forage mass	lb/acre
Forage type	Grass vs. legume, C3 vs. C4 photosynthetic pathway

Basis for Assumptions

Because forage mass is affected by SR, we calculated the theoretical average forage mass in the absence of grazing pressure. Where data were available (26 observations) measured forage mass (averaged across the grazing season) was regressed against SR, and the y-intercept of this relationship for each study was the estimate of 'zero grazing' forage mass.

The y-intercept of the regression of ADG on SR (Y-INTERCEPT ADG) can be viewed as an integrated measure of forage quantity and nutritive value. In studies with no effect of SR on ADG (i.e., slope = 0), this response represents the average ADG across SR. With a threshold model, the Y-INTERCEPT ADG would overestimate the true "maximum" ADG on a given forage base. Thus, we also calculated a presumptive maximum ADG based on the threshold model (MAX ADG). When available from the data, this value was the threshold ADG, otherwise MAX ADG was presumed to be the ADG at the lowest SR used in the study. Though likely underestimating the true maximum ADG in some studies, it is likely that the error would be smaller than that introduced using the y-intercept approach.

Statistical Procedures

For each independent variable, two transformations were assessed, one to maximize the normality of the distribution of the independent variable, the second to maximize the correlation with the target variable, "SLOPE." Additionally, body weight was included in the model without transformation and as metabolic body weight (weight$^{.75}$). Not all data were avail-

1 Two papers from experiment station reports were included in order to provide data from geographical regions that were underrepresented in the database and because all of the essential data were available from the non-refereed reports.

able for all studies. Specifically, to evaluate potential influence of precipitation (n=21 observations) and forage mass (n = 26 observations), these variables were regressed against SLOPE in univariate models. Neither had significant effects, and both were excluded from subsequent multivariate regression approaches. Models were fit using a stepwise multiple regression method using SAS statistical analysis software.

Results and Discussion

A four-variable model using all 58 observations accounted for 69% of the variation in SLOPE (Table 2; Figure 1). The strongest predictor of the slope of the ADG response to increasing SR was the estimate of gain at a theoretical zero SR (Y-INTERCEPT ADG). The greater the gain of cattle at low SR, the more rapid the decrease in ADG with increasing SR. This relationship was not only evident across the entirety of the data set, but also within most individual reports.

This relationship can be clarified by looking at some specific situations. Increased growth of individuals (i.e., higher Y-INTERCEPT ADG) means greater grazing pressure at an equivalent live weight. In one study, implanting cattle increased ADG and increasing SR was associated with decreasing ADG with implanted but not with non-implanted steers. The growth-promoting implant would have increased forage demand for each pound of animal live weight, thus explaining this response. In another study, lighter steers gained more than heavy steers, with a greater negative impact of increasing SR on ADG in the lighter, more rapidly growing steers. In a forage cultivar comparison study, forages supporting higher levels of ADG (associated with greater forage intake per unit of live weight) had steeper SLOPE values. Accordingly, we would predict that any factor improving forage nutritive value and intake would increase the rate at which forage is removed in response to increasing SR, and thereby accelerate the rate of decrease in ADG with increasing SR.

Occasionally, increased grazing intensity improves ADG. Such improvements are likely attributed to increased diet quality. Decreased fiber and lignin concentrations, increased digestibility, and changes in protein concentration and degradability can moderate the effect of grazing intensity on animal performance and likely affect the slope of the ADG response. However, the overwhelming influence of grazing intensity on animal performance appears to occur through decreased intake consequent to decreases in forage mass.

Much smaller, but still significant portions of the variation in SLOPE were explained by variables coding for the presence of grass and/or legumes and for differences in latitude. The "all legume" studies used alfalfa, whereas a number of legume species were present in mixed grass-legume associations. The coefficients associated with these indicator variables indicate that ADG is affected to a greater extent in mixed grass-legume stands than in grass- or alfalfa-only pastures. The relationship between grazing intensity and animal performance is likely more complex in stands containing mixtures of species than in monoculture stands because of variable effects of grazing intensity on the growth, persistence, and nutrient composition of different species. This result should be viewed with caution because the number of studies was small, and it is possible that it is an artifact of these particular experiments.

Table 2. Significant terms in the multivariate regression of factors affecting the slope of the average daily gain (ADG, lb·d-1) response to increasing stocking rate (SR; lb initial live weight·ac-1).

Variable	Estimate ± SE	P > F	Partial R2	Model R2
Intercept	0.1850 ± 0.4113	0.67		
Y-int gain†	-0.4951 ± 0.0470	<0.001	56.25	56.25
Legume‡	-0.4253 ± 0.0949	<0.001	5.74	61.98
Grass§	-0.3150 ± 0.1400	0.03	4.60	66.59
Latitude¶	0.0208 ± 0.0104	0.05	2.42	69.00

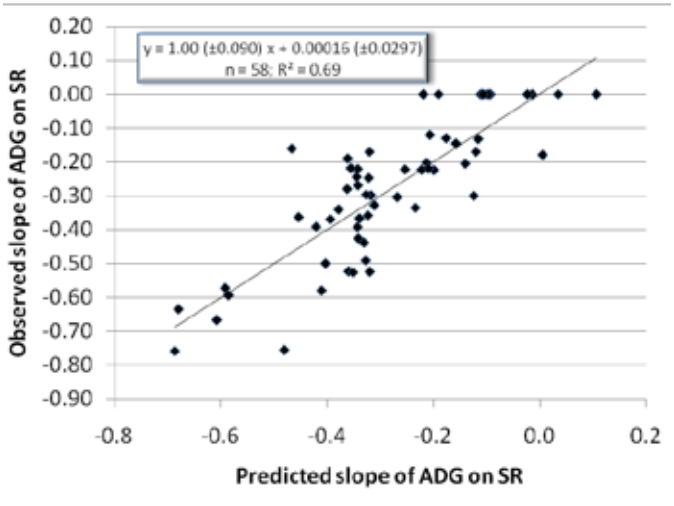
† *Y-intercept gain = estimated ADG at stocking rate of zero, determined from the y-intercept of the regression of ADG on SR.*
‡ *Legume = dummy variable coded as 0 for studies without legume present and 1 for studies with legume present in stand.*
§ *Grass = dummy variable coded as 0 for studies without grass present and 1 for studies with grass present in stand.*
¶ *Latitude = latitude of research site, expressed in decimal form of degrees.*

The small latitude effect indicates that after accounting for the major effect of Y-INTERCEPT ADG, an increase in SR at northern latitudes has a somewhat smaller effect on ADG than a similar increase at southern latitudes. These latitude effects could be mediated through differences in the forage species base among regions.

Implications

The most important decision for optimizing use of grazed forages is the establishment of proper stocking rates. This work represents a unique approach for synthesizing data from numerous stocking rate studies conducted on pasturelands in the United States over the past several decades. Results indicate that we can obtain reasonable estimates of the influence of stocking rate on ADG, mainly by accounting for the potential of the forage base to support gain at low stocking rates. Thus, this work represents a first step in the development of decision support software for cattle graziers.

Figure 1. Regression of observed vs. predicted slope of ADG on stocking rate (SR). Predicted slope was determined from the multiple regression equation detailed in Table 2.



Stocking Rates for Early-Season Grazing of Endophyte-Infected Tall Fescue

E.S. Vanzant

Summary

For stocker cattle grazing endophyte-infected fescue for 50 to 70 days in the spring, each 1000 lb/ac increase in stocking rate resulted in a decrease in ADG that ranged from 0.15 to 0.67 lb/d. Exclusion of grazing in the summer months is warranted in situations in which alternative forages may be available for use during the time when gains on endophyte-infected fescue are generally quite low. Although the large variation in response to stocking rate makes it difficult to draw generalizations regarding optimal stocking rates, the magnitude of these relationships was related to the average daily gain of cattle at low stocking rates. Thus, computer models designed to assist managers in making stocking rate decisions can focus on estimating ADG at low SR without need for sophisticated approaches to predicting SR effects on production functions. Until models are available to help predict optimal SR on a year-by-year basis, results from this study suggest that with approaches similar to those used in the present study, the average optimal stocking rate will be around 1300 lb/ac. Results from an economic model based on these data demonstrate the tremendous importance of the stocking rate decision on profitability from grazing enterprises.

Introduction

Endophyte-infected (E+) fescue is the predominant forage in grazing programs throughout Kentucky and the southeastern United States. Cattle gains on E+ fescue suffer particularly during mid to late summer, in part because alkaloid compounds produced by the endophytic fungus decrease the animals' ability to dissipate heat. One strategy to optimize cattle performance in E+ fescue-based systems is to refrain from grazing during the warmer parts of the season and to utilize alternative forages during this period.

The most important management decision affecting grazing animal performance is the selection of stocking rate. For nearly all forages studied, increasing stocking rate (above some critical value) results in linear decreases in average daily gain (ADG). However, the presence of endophyte and associated alkaloids could alter this standard relationship with E(+) fescue. Alkaloid compounds are more heavily concentrated in seed and stem tissues, and the quantity of these tissues can be decreased with heavier stocking rates. Furthermore, some research has shown that daily growth rates of tall fescue can be enhanced with more intense utilization. For these reasons, it is possible that the decrease in ADG per unit increase in stocking rate may be substantially less for E+ fescue than for other forages. Additionally, optimal stocking rates will depend on the intended period of use of a given forage base. Thus, if the intent is to utilize forage only during the spring, optimal stocking rates would be higher than under season-long grazing.

The objective of this study was to determine the relationship between stocking rate and growth performance for stocker cattle grazing E+ fescue during the spring grazing period. Because stocking rate-gain relationships are highly variable from year to year, this study was conducted across a four-year time span in order to provide an estimate of how much year to year variation to expect. This information can be used as a guideline for establishing optimal stocking rates for E+ fescue in the upper mid-south region.

Materials and Methods

Stocking rate studies were conducted on endophyte-infected fescue pastures (7.5 ac each) in each of four years at the University of Kentucky Animal Research Center in Woodford County. In year 1, four SR (ranging from 500 to 2240 lb live weight/ac) were evaluated on eight pastures (two replicates per SR treatment). It was determined that the highest stocking rate in this study was insufficient to accurately characterize the response curve. Therefore, in each of the subsequent years, five stocking rates (ranging from 520 to 2740 lb live weight/ac) were used, each of which was replicated twice, requiring 10 pastures in each of these years. The pastures that were used in years 2 through 4 were different than the pastures used in the first year. Furthermore, in years 2 through 4, SR treatment assignments to pastures were maintained so that cumulative effects of applying SR over years would be included in the assessment. Details of the experimental methods used in each year are shown in Table 1. Grazing was initiated about May 7 in years 1 and 4 and about April 20 in years 2 and 3 and continued until the last week of June with one exception. In the third year, pastures with cattle on the highest SR were destocked after 28 days of grazing because dry conditions resulted in inadequate forage to sustain the cattle (Figure 1). Growing steers were used throughout, with the exception that in year 4 one replicate of steers and one replicate of heifers was used for each SR. In each year, cattle were stratified by weight and randomly assigned to

Table 1. Details of experimental methods in each of four years.

Year	Total Animals	Avg Initial Wt, Lb	Grazing Days	Notes
1	119	538	49	Four stocking rates evaluated
2	228	541	63	Five stocking rates evaluated
3	192	628	66	Both replicates of heaviest stocking rate destocked after 28 d of grazing due to dry conditions; five stocking rates
4	169	679	49	One block of steers, one of heifers; five stocking rates

Figure 1. Precipitation during one to two months preceding grazing (March/April) and during the grazing phase of each year of the experiment compared with long-term average precipitation for the central Bluegrass region of Kentucky.

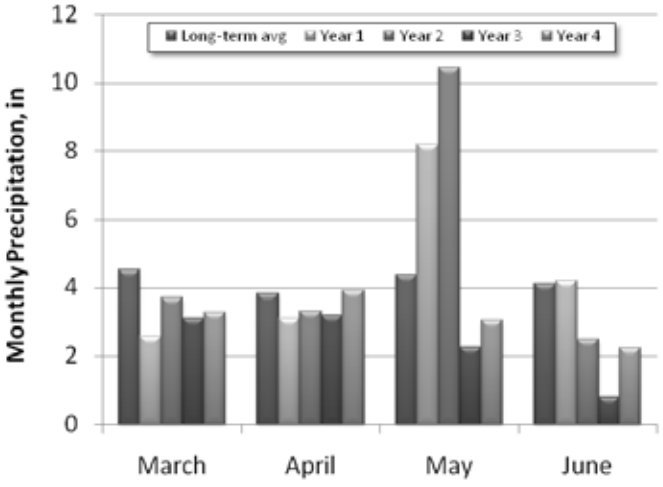
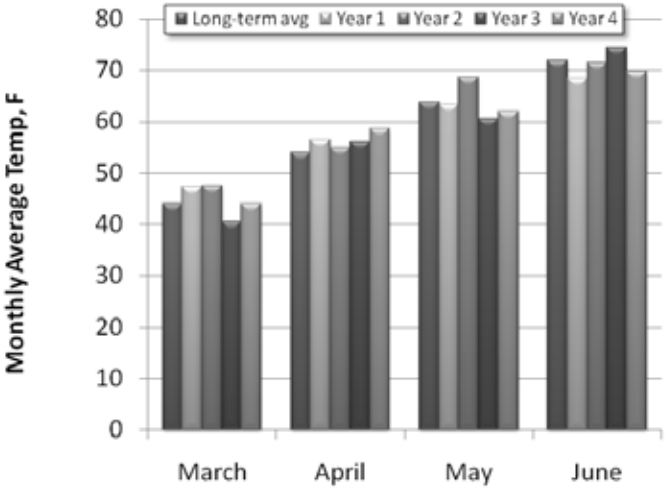


Figure 2. Average monthly temperatures during one to two months preceding grazing (March/April) and during the grazing phase of each year of the experiment compared with long term average monthly temperatures for the central Bluegrass region of Kentucky.



each pasture in such a way as to equalize as nearly as possible the average weight (and variance of weights) of cattle in each pasture, and provide the appropriate numbers to establish the various stocking rates. At the beginning of grazing in each year, cattle were implanted with a TBA/estradiol implant (Revalor® G) and vaccinated against respiratory disease and clostridia, with booster vaccinations administered 28 days later. All cattle were dewormed with Safeguard® on days 0 and 28 of each grazing study.

Pastures were fertilized with 50 lb N/ac in March and clipped in late May or early June of each year to a stubble height of approximately 10 in to remove seed heads. Cattle had free choice access to mineral and water throughout and were weighed following an overnight shrink at the beginning and ending of the grazing period in each year. After cattle were removed in late June of each year, forages were stockpiled for fall use (an additional 50 lb/ac N was applied in August or September of each year). Stockpiled forage was grazed during the fall/winter of each year by either growing animals or mature cows. All pastures were stocked at similar SR and otherwise treated similarly during the stockpile phase.

The effect of SR on ADG was analyzed using the GLM procedure of SAS by incorporating SR as a covariate in an analysis of variance of the effect of year on ADG. No differences were detected between steer and heifer response curves for the year 4 data. Thus, gender was removed from the model. A SR by year interaction (P = 0.04) revealed that slopes varied by year. Therefore, regression equations for the effect of SR on ADG were developed separately for each year of the study.

Results and Discussion

Across years, precipitation preceding the start of grazing averaged about 0.9 inches below the long-term average (Figure 1). May precipitation varied from 6 in above normal in year 2 to 3 inches below normal in year 3. June precipitation was normal in year 1 and below normal in the remaining three years. The most notable temperature deviation occurred in May of year 2, when it was 5°F above normal (Figure 2). The warmer, wetter conditions during May of year 2 would have supported higher forage production of lower quality. Accordingly, ADG of cattle in year 2 was below that seen in either of the "surrounding" years (year 1 or 3; Figure 3). In year 4, the second consecutive year of dry conditions, the lowest gains were observed. In all years, ADG decreased linearly with increasing SR, agreeing with most of the relevant literature. However, the extent to which SR influenced ADG varied from -0.15 lb ADG to -0.67 lb ADG per 1,000 lb/ac increase in SR. This variation makes selecting ideal SR difficult in any given year. Gain per acre curves were calculated from the above regressions along with grazing days determined in the studies (Figure 4). These curves are (necessarily) curvilinear and peak at some SR well above the SR that yields maximum ADG. The sharp decline in gain/acre in year 3 when transitioning from SR of 2200 to 2500 lb/ac primarily resulted

Figure 3. Influence of stocking rate on average daily gain of cattle grazing endophyte-infected fescue during a spring grazing period in each of four years.

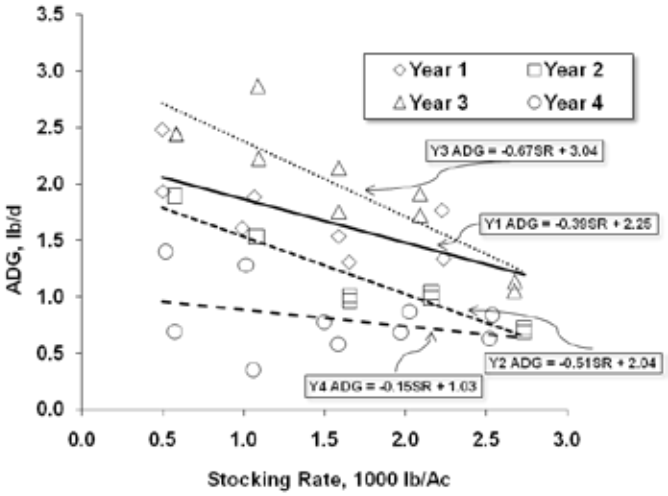
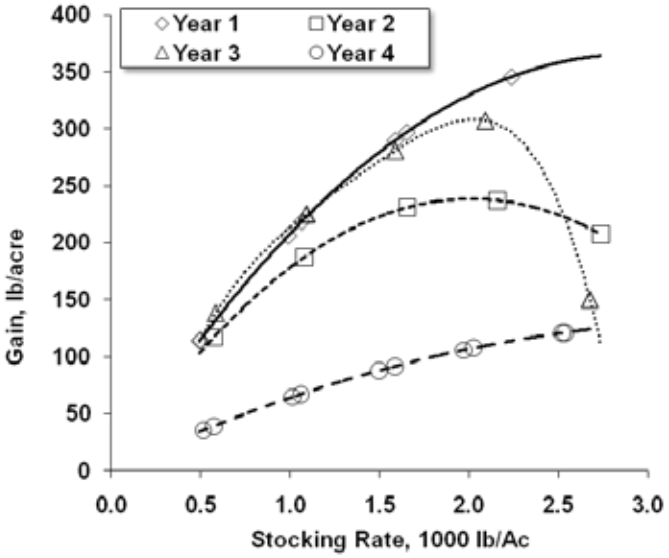


Figure 4. Influence of stocking rate on gain per acre for cattle grazing endophyte-infected fescue during a spring grazing period in each of four years. The plotted points represent gain per acre calculated from the ADG production functions and number of grazing days measured in the experiments described in the text, explaining why all of the points fall exactly on the response curves.



from the dramatically shortened grazing season for the heavy SR treatment, showing the cost of setting SR too high. Although such SR can be maintained during years of adequate rainfall, high SR is much more risky with adverse growing conditions.

The usefulness of this information to stocker producers is shown in Figures 5 and 6. A computer model was constructed from these data that also accounted for fixed and variable costs of stocker production. Figure 5 shows gain per acre and the influence of SR on net profits per 100 acres in a year with relatively high cattle growth rates (year 3). One net profit curve used a theoretical buy-sell margin of $0.05/lb, and for illustrative purposes, no buy-sell margin existed in the other. Figure 6 shows the same curves for a year with low-growth conditions (year 4). Four key concepts can be drawn from these figures. First, optimum SR, from an economic standpoint, is less than the SR that yields maximum gain per acre. Second, high SR can catastrophically influence net profit, particularly when adverse conditions require destocking. Such effects will be compounded by maintaining high SR over long periods of time. Third, market factors, particularly the buy-sell margin, substantially affect optimal SR. Finally, the optimal SR for generating net profit varies tremendously from year to year. These data, however, demonstrate that the challenge of predicting the slope of the ADG response curve (which is necessary for determining optimal SR) is eased somewhat because of its relationship with gain of cattle at low SR. In this study, differences in ADG at low SR explained 84% of the variation in the slope of the ADG curves. Thus, models can be developed to estimate optimal SR based only on estimates of market factors and gain at low SR.

Implications

Average daily gain of stocker cattle grazing endophyte-infected fescue for about 60 days during the spring decreased at rates between 0.15 and 0.67 lb/d per 1000 lb/ac increase in SR. Economic models show that this large variation has substantial influence on the selection of SR for maximum net profit. With the conditions experienced during the four years of this study, optimal SR would have varied from about 300 to about 1700 lb/acre. The wide range of environmental conditions across these four years allows for this data set to provide a reasonably robust estimate of an average optimal SR for early-season grazing of E(+) fescue in the central Bluegrass region, which (with particular estimates of market conditions) is around 1300 lb live weight/acre.

Figure 5. Influence of stocking rate on net returns to a grazing operation with growing cattle grazing endophyte-infected fescue during a spring grazing period in an example year with high growth rates. Production responses from year 3 were used in conjunction with a computer model that accounted for fixed and variable costs to generate these curves.

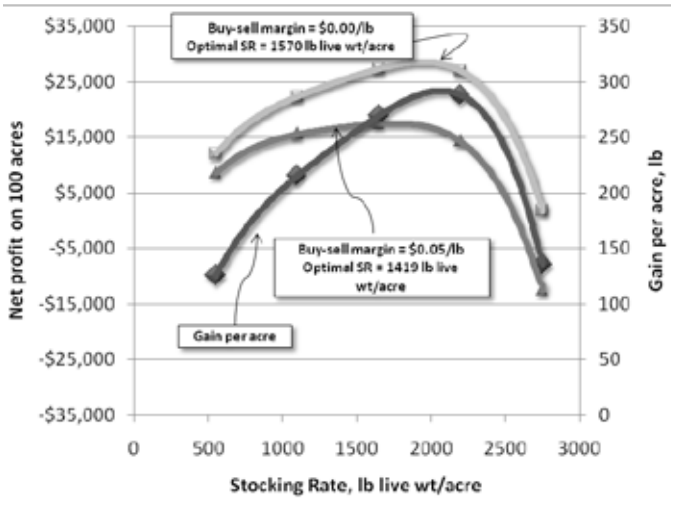
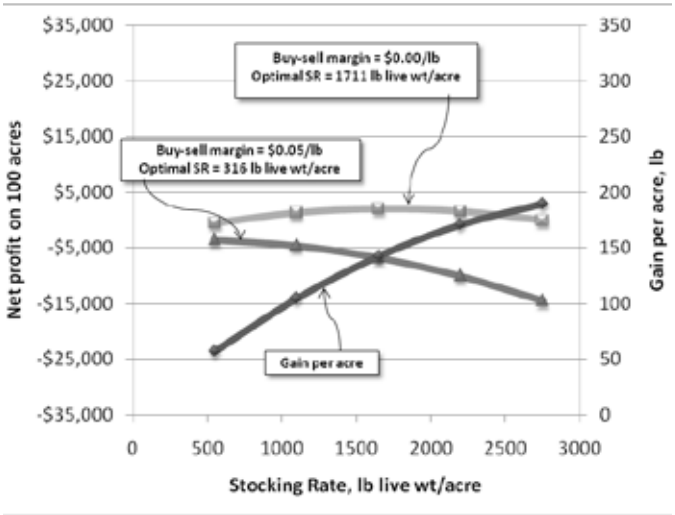


Figure 6. Influence of stocking rate on net returns to a grazing operation with growing cattle grazing endophyte-infected fescue during a spring grazing period in an example year with poor growth rates. Production responses from year 4 were used in conjunction with a computer model that accounted for fixed and variable costs to generate these curves.



Grazing Evaluation of a Novel Endophyte Tall Fescue Developed for the Upper Transition Zone

J.M. Johnson and G.E. Aiken

Summary

A grazing experiment determined that a late-maturing tall fescue developed by the University of Kentucky has potential for grazing in the upper transition zone of the United States. Steers were grazed in a 2-yr experiment using variable stocking rates to compare steer performance and physiology and forage productivity of KYFA9301 infected with the nontoxic AR584 novel endophyte (NE9301) to Kentucky 31 infected with the toxic wild-type endophyte (KY31), endophyte-free KYFA9301 (EF9301), and 'Jesup' infected with non-toxic AR542 (MaxQ). Fescue-endophyte combinations were assigned to 1.0-ha pastures in a randomized complete block design with three replications. Average daily gains among nontoxic fescues were similar and greater than KY31. Rectal and skin temperatures and prolactin concentrations were similar among the nontoxic fescues and were improved relative to those for KY31. Stocking rates in the latter half of the grazing were highest for KY31, were higher for EF9301 than for MaxQ and NE9301, and tended to be higher for NE9301 than for MAXQ. Results indicate that KYFA9301 supports higher stocking rates in the late spring and early summer than MaxQ, making it an option to KY31 in the upper transition zone.

Introduction

Poor cattle performance on tall fescue has been linked to a toxicosis caused by a fungal endophyte that infects most plants of Kentucky 31 tall fescue and produces ergot alkaloids that induce a toxicosis in cattle. Symptoms of "fescue toxicosis" include poor weight gain, reduced conception rates, retention of rough hair coats, depressed serum prolactin concentrations, and increased core body temperatures. New Zealand's AgResearch Ltd. has developed novel endophytes that can provide the desired animal performance associated with endophyte-free fescue but with the stand persistence of toxic endophyte-infected fescue. The commercialization of novel endophytes for tall fescue was initiated with insertion of AgResearch's endophyte AR542 into Jesup tall fescue and its commercial release under the trade name Jesup MaxQ.

A late-maturing tall fescue experimental population, KYFA9301, was developed by T. D. Phillips of the Department of Plant and Soil Sciences at the University of Kentucky. As an endophyte-free fescue variety adapted to the upper transition zone, this led to the infection of KYFA9301 with AgResearch NZ AR584, a novel endophyte with improved viability over extended storage (Hill and Roach, 2009).

A 2-yr grazing experiment was conducted to evaluate steer performance and forage productivity of KYFA9301 infected with the AR584 novel endophyte (NE9301) in comparison to 'Jesup' MaxQ infected with AR542 novel endophyte (MaxQ), Kentucky 31 tall fescue infected with toxic wild-type endophyte (KY31), and endophyte-free KYFA9301 (EF9301).

Materials and Methods

The grazing experiment was conducted in 2008 and 2009 at the University of Kentucky Animal Research Center in Woodford County. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at UK (2008-0289). Four tall fescue-endophyte combinations were assigned at planting to twelve 1.0-ha pastures in a randomized complete block design with three replications. Combinations were Kentucky-31 (KY31) tall fescue infected with the wild-type endophyte, KYFA9301 endophyte-free (EF9301), KYFA9301 infected with AR584 endophyte (NE9301), and 'Jesup'-MaxQ infected with AR542 endophyte (MaxQ). Forty-eight tester steers were blocked by body weight for assignment to pastures (four testers/pasture). Pastures were grazed from May 6 to July 23, 2008 (76 d), and from April 2 to June 25, 2009 (84 d).

Forage mass was determined at four-week intervals using a disk meter to determine compressed canopy height (100 to 175 measurements/pasture). For three disk-meter locations per pasture, forage under the disk was clipped to the soil surface and dried in a forced-air oven for calibration of a regression equation to estimate forage mass (kg DM/ha). Disk meter measurements were taken one day prior to making stocking rate adjustments on days 28 and 56 with put-and-take steers to maintain mean herbage masses of 2500 + 200 lb/acre. There also was a need to make stocking rate adjustments in the latter part of grazing in each year when forage growth was substantially reduced.

Shrunken bodyweights were taken at initiation and termination of grazing each year for calculation of average daily gain (ADG). Animal physiological measurements and rectal and skin temperatures were collected in the late afternoon on days 28, 56, and at study completion along with collection of jugular blood for serum prolactin assay. Serum prolactin concentrations were assayed using a radioimmunoassay. Carrying capacity (grazing days) and stocking rate (lb BW/acre) were determined for each pasture at initial, midpoint, and final days of grazing. Body weights at the midpoint and final days of grazing were estimated for each pasture by adjusting initial BW using ADG (mean initial BW + (days on pasture x ADG)) x steers/ha.

Single tillers were collected from 25 randomly chosen plants on June 9 and July 7 in 2009. Endophyte infection percentages were determined using an immunoblot procedure. Kentucky bluegrass (*Poa pratensis*) encroached in 2009, following dry weather in the late summer and fall of 2008. Botanical composition of each pasture was determined on June 24 using double sampling. Rainfall data was collected from a national weather station located at the Bluegrass Airport in Lexington (approximately 8 miles from the experimental site).

Animal and pasture responses were analyzed using mixed models. Orthogonal contrasts were used for effects of fescue-

Table 1. Mean average daily gain (ADG) and total body weight (BW) gain for steers grazing wild-type endophyte Kentucky 31 (KY31), 'Jesup' infected with the AR542 novel endophyte (MaxQ), KYFA9301 infected with AR584 novel endophyte (NE9301), and endophyte-free KYFA9301 (EF9301) tall fescues. Means are 2-yr averages across 3 replications.

					Contrasts[1]		
Item	KY31	MaxQ	NE9301	EF9301	KY31 vs. Nontoxic 2	EF9301 vs. Novels	MaxQ vs. NE9301
ADG, lb/day	1.41	1.85	1.79	1.76	0.0016	0.4364	0.7520
Total BW gain, lb/acre	265	324	313	325	0.0078	0.7520	0.6250

[1] *P-values for orthogonal contrasts*

Table 2. Physiological measures in steers grazing wild-type endophyte Kentucky 31 (KY31), 'Jesup' infected with the AR542 novel endophyte (MaxQ), KYFA9301 infected with AR584 novel endophyte (NE9301), and endophyte-free KYFA9301 (EF9301) tall fescues. Means are 2-yr averages across 3 replications.

					Contrasts[1]		
Item	KY31	MaxQ	NE9301	EF9301	KY31 vs. Nontoxic	EF9301 vs. Novels	MaxQ vs. NE9301
Rectal temperature, °F	104.5	103.8	103.6	103.6	0.0004	0.0001	0.0001
Skin temperature, °F	99.0	97.7	97.7	97.5	0.1803	0.5688	0.7533
Serum prolactin, ppb	82	230	207	194	0.8205	0.9449	0.2282

[1] *P-values for orthogonal contrasts*

endophyte combination and interactions between combination and year. The PDIFF option of SAS was used for means separations for other response variables.

Results and Discussion

Rainfall was low in 2008 and less than the 39-yr average for May, June, and July, whereas in 2009 it was consistently higher than the long-term average for the full duration of grazing. Low rainfall in 2009 resulted in mean herbage mass for that year (2206 ± 23 kg DM/acre) being slightly less than the targeted mass. Forage mass in 2009 was 2475 ± 22 kg DM/acre.

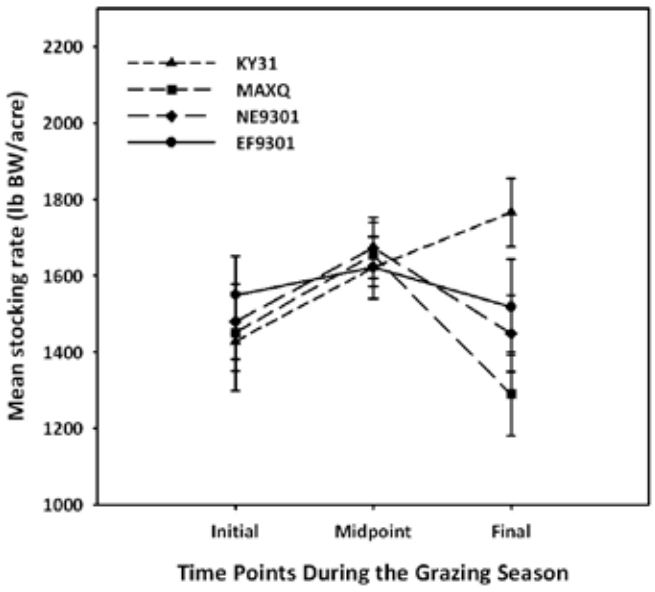
Endophyte infection percentages, averaged over the two sampling dates for KY31, MaxQ, NE9301, and EF9301, were 66.8 ± 4.1, 81.2 ± 2.1, 86.3 ± 2.7, and 2.8 ± 0.9%, respectively. Infection percentages were less than 5% in the EF9301 pastures, which were likely of little consequence to animal performance or physiology. Infection percentages in KY31 pastures were lower than anticipated but were high enough to affect animal performance.

Percentage bluegrass was lowest ($P < 0.05$) in KY31 (32.3%) pastures, and was 38.2, 46.5, and 42.1% for MaxQ, NE9301, and EF9301, respectively. The three nontoxic fescue pastures did not differ in bluegrass percentage ($P > 0.10$). The moderate percentages in the pastures could have caused interference in steer weight gain and physiology measures; however, bluegrass matured two to three weeks earlier than tall fescue, shortly after the start of grazing, and cattle were observed selectively grazing tall fescue. This was also indicated by the presence of only bluegrass seed heads and few tall fescue seed heads. Therefore, the confounding caused by bluegrass was likely minimal.

Steer weight gain (ADG and gain lb/ac) among MaxQ, AR584, and EF9301 were comparable and were greater ($P < 0.10$) than KY31 (Table 1). Both rectal and skin temperatures among AR584, MaxQ, and EF9301 were comparable and were lower ($P < 0.10$) than KY31 (Table 2). Serum prolactin concentrations also were comparable among the three nontoxic varieties and higher ($P < 0.10$) than KY31. Weight gain and physiological measures were, therefore, similar among the three nontoxic fescue treatments and were favorable for steers grazing nontoxic fescues compared to those grazing toxic KY31.

Pasture carrying capacity was higher for KY31 than for the nontoxic pastures in both years ($P < 0.05$), which was likely a

Figure 1. Means and standard errors for stocking rate on a BW basis at the initial, midpoint, and final day of grazing for steers grazing wild-type endophyte Kentucky 31 (KY31), 'Jesup' infected with the AR542 novel endophyte (MaxQ), KYFA9301 infected with AR584 novel endophyte (NE9301), and endophyte-free KYFA9301 (EF9301) tall fescues. Means are 2-yr averages across 3 replications.



reflection of reduced forage consumption by KY31 steers as ambient temperatures increased rather than increases in forage growth rates. Carrying capacity was 190 ± 3 steers days/acre for KY31 and averaged 170 ± 7 steer days/acre for the three nontoxic fescues. Stocking rates (lb BW/ac) at initial and midpoint days of grazing did not differ among fescue-endophyte treatments (Figure 1). Over the latter half of grazing, KY31 had a higher ($P < 0.01$) stocking rate than the nontoxic fescues, and the rate for EF9301 was higher ($P < 0.10$) than for the novel endophyte pastures. Further, the stocking rate for NE9301 tended ($P = 0.12$) to be greater than for MaxQ. Productivity of the later maturing KYFA9301 apparently provided greater carrying capacities than MaxQ in late June/early July.

Implications

Results of the 2-yr grazing experiment indicated that animal performance and physiology, and pasture forage growth with KYFA9301 infected with the AR584 novel endophyte makes it a viable option for alleviating fescue toxicosis in the upper transition zone and providing higher carrying capacities in the late spring and early summer. Endophyte-free KYFA9301 provided a higher carrying capacity than the two novel endophyte fescues, but a 2-yr grazing experiment is too short to conclude that an endophyte is not needed for KYFA9301.

Performance and Physiology of Steers Grazing Toxic Tall Fescue as Influenced by Feeding Soybean Hulls and Implanting with Steroid Hormones

J.M. Carter and G.E. Aiken

Summary

A grazing experiment with steers grazing toxic tall fescue indicated that feeding pelleted soybean hulls (PSBH) combined with steroid hormone implants (SHI) can increase steer weight gain and that PSBH can reduce the severity of fescue toxicosis Ergot alkaloids produced by a fungal endophyte that infects tall fescue adversely affect cattle weight gain and physiology. Sixty-four steers were grazed on endophyte-infected (E+) KY31 tall fescue for 77 days in 2007, and 60 steers were grazed for 86 days in 2008 to evaluate the combined effects of SHI and PSBH on steer performance and physiology. With or without feeding PSBH treatments (5.0 lb/steer/day) were randomly assigned to six 7.5-acre pastures. Treatments of with or without SHI (200 mg progesterone – 20 mg estradiol) were assigned to two groups of steers within each pasture. Average daily gain (ADG), rectal temperature, serum prolactin, and hair coat rating responses to PSHI and SHI were measured. Combining feeding PSBH with SHI can provide cost- effective increases in ADG and PSBH and can reduce the severity of toxicosis to make it a management option in producing stocker calves on toxic tall fescue.

Introduction

Tall fescue is a cool-season perennial grass that is predominantly utilized for forage in the transition zone, an area extending from the temperate Northeast to the subtropical Southeast. The fungal endophyte is responsible for producing ergot alkaloids, with ergovaline being the primary ergot alkaloid. The endophyte contributes to agronomic qualities that promote sustainability. These attributes include ease of establishment, heat and drought tolerances, plant vigor, long growing season, resistance to insect feeding, and minimizing of soil erosion.

[1] Mention of trade names or commercial products in the article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

Despite its productivity and persistence, endophyte-infected fescue is toxic to grazing livestock and consequently suppresses animal productivity. Symptoms of this malady include lowered body weight (BW) gains, decreased reproductive performance, depressed serum prolactin concentrations, rough hair coats during the summer months, and reductions in grazing time and feed intake. The annual cost of the endophyte is likely close to $1 billion with current cattle prices.

Materials and Methods

The grazing trial was conducted with 64 steers (mean initial BW = 661 lb) from April 19 to July 5 in 2007, and with 60 steers (mean initial BW = 644) from April 29 to July 24 in 2008. Treatments were assigned as a split-plot design with three replications. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at UK (00996A2006). The main plot treatment was with or without feeding of SBH and the sub-plot treatment was implant (Synovex®-S; Fort Dodge Animal Health, Fort Dodge, IA) vs. no implant. Feeding treatments were assigned to pastures, and implant treatments were assigned as groups within pastures. Steers were blocked by BW for assignment to treatments. Steers assigned to the feeding treatment were group-fed to provide daily consumptions of 5.0 lb/steer (as fed). Steers were placed on pastures for a seven-day adjustment period prior to taking initial body weights. Unshrunk body weights were taken on days 1, 28, and 56 in both years, and final body weights were taken on day 77 in 2007 and on day 86 in 2008. On the final weigh day, hair coats were rated as sleek (< 25% coverage by rough hair), transitional (25 to 75% coverage by rough hair), or rough (75 to 100% coverage of rough hair). Blood also was collected from the jugular vein to obtain serum for assay of prolactin using a radioimmunoassay.

Ultrasonography was used to measure growth in cross-sectional area of the ribeye and external fat thickness as an

indicator of body condition. Ultrasound scans were taken the day after taking final BW with an Aloka SSD-500V (Tokyo, Japan) instrument with a 3.5-MHz linear array transducer (UST 6049). A certified ultrasound technician scanned between the 12th and 13th ribs to acquire cross-sectional area of the ribeye (REA) and rib fat thickness (1213RFT). A second scan was taken between the pin and hook bones over the rump to measure rump fat thickness (RFT).

Disk meter height was recorded for 50 random locations within each pasture at 14-d intervals. Calibration samples were collected by clipping forage below the disk meter plate to the soil surface at five locations per pasture. In addition, tiller samples from 50 individual plants were taken per pasture on June 7, 2007 to determine endophyte infection level using an immunoblot assay.

Cost of additional weight gain was analyzed relative to the breakeven costs for PSBH with four different cattle-selling prices. Costs of PSBH per incremental pound increase in ADG (cost of additional gain) were calculated over a range of PSBH costs of $20 to $330/ton PSBH at $10 intervals. Cost of additional gain was analyzed using PROC TTEST of SAS to determine corn cost needed for the cost of additional ADG to be lower ($P < 0.05$) than breakeven costs. Breakeven costs were set to reflect cattle selling prices of $.80, $.90, $1.10, and $1.20/lb live weight.

Results and Discussion

Forage mass was greater in 2007 (3518 ± 95 lb/ac) than in 2008 (2367 ± 95 lb/ac). Although rainfall was greater in 2007, forage mass was likely not low enough in either year to limit steer ADG. Tillers averaged 80 ± 9.4% endophyte infection levels.

Highest recorded weight gains were obtained from the combined treatment of PSBH and SHI ($P < 0.05$; Table 1). The combination of treatments provided a 71% increase ($P < 0.05$) over the control treatment ($P < 0.001$), whereas feeding SBH without implantation and implantation without feeding SBH provided increases of 32 and 13%, respectively, over the control treatment. Although feeding SBH and steroid implantation both increased ADG, additive effects from combining these substantially increase ADG.

Ultrasonography indicated that SHI and PSBH treatments alone had no effect ($P > 0.10$) on REA (Table 1). Combining PSBH with SHI resulted in a 15.6% increase in LDA over that of the control treatment, a 14.0% increase over SHI-only, and a 13.1% increase over feeding PSBH-only. Feeding PSBH, with or without SHI, resulted in an 11% in 1214RFT and 20% increase in RFT. Feeding PSBH likely promoted fat deposition because of increased energy intake. Interactivity between PSBH and SHI apparently promoted greater REA, and PSBH had a greater effect than SHI on the deposition of external fat.

Serum prolactin concentrations were not affected ($P > 0.05$) by SHI, but were higher with PSBH (106 ppb) than without (37 ppb). Therefore, feeding PSBH resulted in a near threefold increase in prolactin concentrations. Serum prolactin is often used as a marker of toxicosis because low prolactin concentrations have been consistently detected in cattle exhibiting fescue toxicosis.

There were reductions ($P < 0.05$) in percentages of steers with rough hair coats with either PSBH or SHI treatments (Figure 1). There were no effects of either treatment on percentage of transitional hair coat ratings. Percentage of cattle with sleek hair coats tended ($P < 0.10$) to be greater with feeding PSBH but not with SHI. Therefore, SHI treatment did not increase percentages of transitional and sleek hair coat ratings even though the treatment reduced the percentage of rough hair

Figure 1. Frequencies of hair coat ratings for steers grazing toxic endophyte-infected tall fescue and were a) with or without daily feeding of pelleted soybean hulls (PSBH) and b) with or without steroid hormone implantation (SHI). Hair coats were rated as being sleek (less than 25% coverage by rough hair), transitional (25 to 75% coverage by rough hair), or rough (greater than 75% coverage of rough hair).

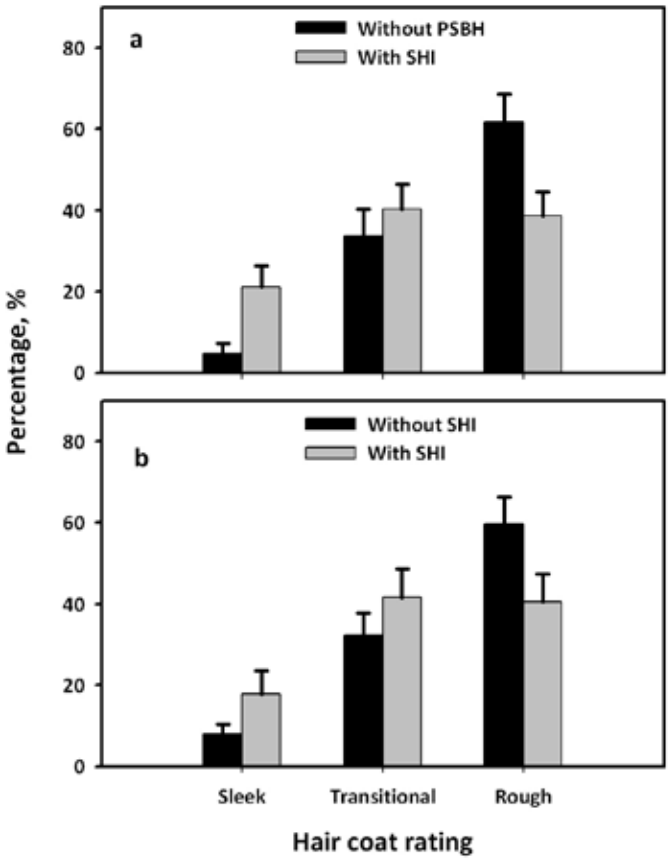


Table 1. Average daily gain (ADG) and final ultrasound measures of ribeye area (REA), 12 to 13th rib fat thickness (1213RFT), and rump fat thickness (RFT) for steers grazing toxic tall fescue with different combinations offeeding pelleted soybean hulls (PSBH) and steroid hormone implants (SHI). Means are averages over 2 yr.

	PSBH/SHI[1]				
Item	**No/No**	**No/Yes**	**Yes/No**	**Yes/Yes**	**SEM[2]**
ADG, lb/day	1.59 c	1.79 c	2.09 b	2.71 a	0.1
REA, in[2]	7.7 b	7.9 b	7.9 b	9.0 a	0.1
1213RFT, in	0.17 b	0.17 b	0.19 a	0.19 a	0.01
RFT, in	0.16 b	0.14 c	0.17 a	0.19 a	0.01

[1] *Means within rows with different letters (a, b, c) are significantly different ($P < 0.05$).*

[2] *Standard error of the mean.*

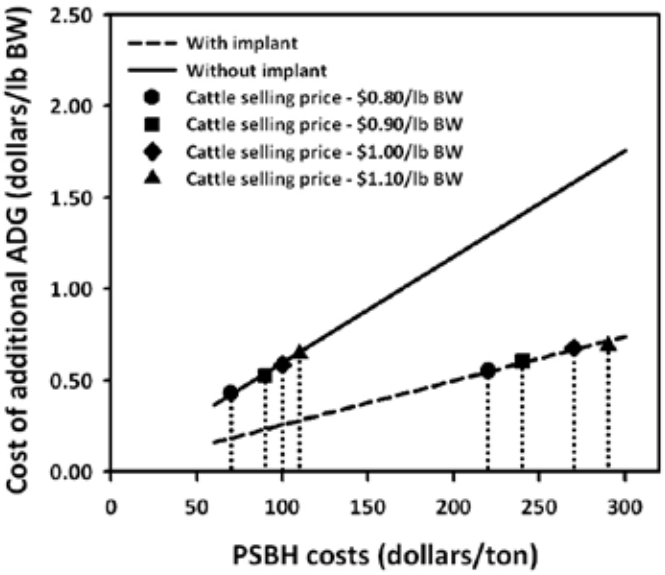
coats. Feeding PSBH apparently promoted some shedding of winter hair coat. Feeding PSBH at the consumption rate used in the experiment could have overcome a nutrient deficiency to trigger shedding, but reduction in rough hair coats with SHI could be associated with a hormonal triggering of shedding.

Feeding SBH without implantation provided positive net returns with SBH purchased at low costs (Figure 2). The breakeven cost for SBH daily fed at 5.0 lb/steer was $110/ton with a high cattle price ($1.20/lb). When steroid implants were combined with SBH, the breakeven cost of SBH was $220/ton for the $0.80/lb cattle price and $290/ton for the $1.20/lb cattle price. The additive effect of implanting on ADG can generate costs of additional weight gain that are below the breakeven with high PSBH costs and a wide range of cattle prices.

Implications

Results of the experiment indicated that feeding PSBH in combination with SHI can cost effectively provide the ADG needed for profitable stocker production. Furthermore, feeding SBH can improve the shedding of rough and hair coats and increase prolactin concentrations, suggesting that feeding SBH can reduce the severity of toxicosis. Feeding PSBH in combination with SHI can substantially increase beef calf ADG and reduce the severity of toxicosis, making it a management option for producing stockers on toxic tall fescue.

Figure 2. Cost of additional weight gain of steers grazing toxic endophyte-infected tall fescue with daily feeding of pelleted soybean hulls (PSBH, 2.3 kg/steer/day, as fed) and with or without steroid hormone implants. Costs of PSBH are designated that are below ($P < 0.05$), the break-even cost of the additional weight gain for four cattle prices. Does not necessarily reflect net profit from additional weight gain because negative profit margins are not accounted for in the analysis.



Effect of Ergot Alkaloids on Bovine Foregut Vasculature

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

Summary

Ergot alkaloids induce vasoconstriction of bovine foregut vasculature. Ergovaline induced the greatest response in ruminal artery, while ergovaline and ergotamine induced the greatest response in ruminal vein. Lysergic acid did not stimulate a contractile response in either the ruminal artery or vein. Ergonovine caused vasoconstriction of the ruminal artery but not the vein. A greater maximal arterial response was observed for ergonovine, ergocornine, and ergovaline. The arterial and venous responses were not different for ergocryptine, ergocristine, ergotamine, and lysergic acid. The EC-50 (concentration to produce 50% of maximal concentration) for the alkaloids was not different for the ruminal vein. The EC-50 for ergotamine for the ruminal artery was greater than the other alkaloids. These data indicate that ergot alkaloids have the potential to induce vasoconstriction of core body vasculature of cattle, which could alter nutrient absorption.

Introduction

Tall fescue is a cool-season grass that is prevalent in pastures of the eastern United States. Farmers have preferred tall fescue because of its many desirable agronomic attributes. However, an endophytic fungus associated with tall fescue produces toxic alkaloids. Consumption of these alkaloids by cattle causes many symptoms, referred to as fescue toxicosis. Symptoms of fescue toxicosis include inability to tolerate heat, increase respiration rate, depressed intake, and dry gangrene of the tail and back legs. Many of these symptoms are likely caused by vasoconstriction of peripheral vasculature, which prevents heat dissipation and warmth of the extremities in cold temperatures. The ergot alkaloids produced by the endophyte in association with tall fescue are thought to be responsible for the observed vasoconstriction of peripheral vasculature.

The objective of this study was to determine the vasoconstrictive potential of ergovaline, ergotamine, ergocryptine, ergocristine, ergonovine, ergocornine, and lysergic acid.

Materials and Methods

Segments of right ruminal artery and vein were collected from the ventral coronary groove of predominately Angus heifers (n = 10; BW = 1099 ± 20 lb) shortly after slaughter and placed in a modified Krebs-Henseleit buffer on ice. Vessels were cleaned of excess connective tissue and fat, sliced into 2-3 mm segments, and suspended in a multi-myograph chamber with 5 mL of continuously oxygenated Krebs-Henseleit buffer (95%O_2/5% CO_2; pH 7.4; 37˚C). Arteries and veins were equilibrated to 1.0 g and 0.5 g respectively for 90 min followed by addition of 120 mm KCl. Increasing concentrations of each compound were added to the respective chamber every 15 min following buffer replacement. Data were normalized as a percentage of the contractile response induced by KCl, and EC-

Figure 1. Ruminal artery response to increasing concentrations of ergot alkaloids.

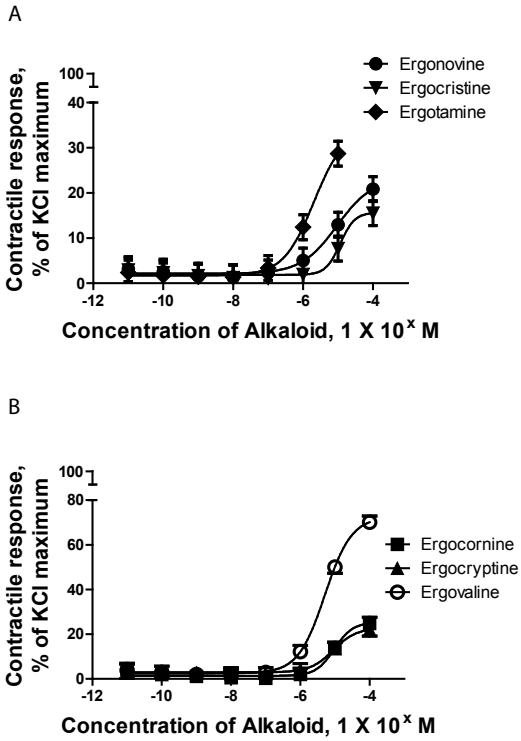
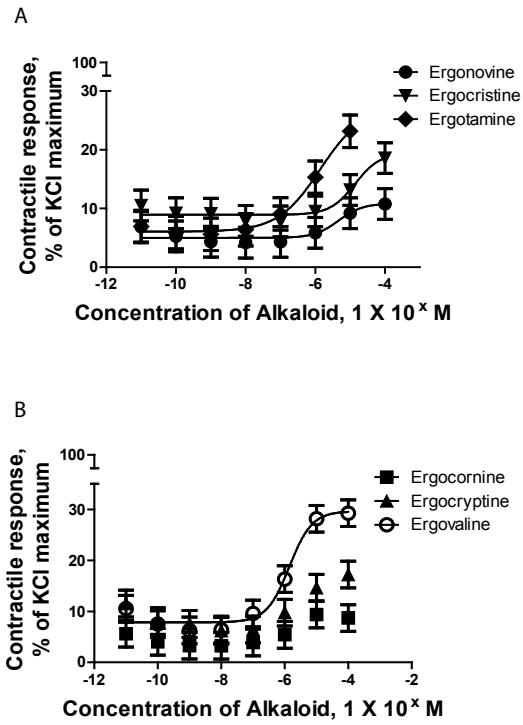


Figure 2. Ruminal vein response to increasing concentrations of ergot alkaloid.



50 estimates were calculated using GraphPad Prism by fitting data to the line:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC50-X})\times\text{Hill Slope})}).$$

Data were analyzed as a completely randomized design using Proc Mixed of SAS.

Results and Discussion

Lysergic acid did not cause a contractile response in the ruminal artery or vein (data not shown). Dose response curves for ruminal artery are shown in Figure 1. Contractile responses were first observed for ergovaline and ergotamine at 1×10^{-6} M concentration. The first contractile response was observed at 1×10^{-5} M for ergonovine, ergocornine, and ergocryptine, while ergocristine did not elicit a response until the 1×10^{-4} M addition. Ergovaline caused the greatest contractile response in the ruminal artery. The EC-50 for the ruminal artery and vein is shown in Table 1. The EC-50 for ergotamine within ruminal artery was greater than the other alkaloids, indicating a higher concentration of ergotamine is required to elicit half of the maximum contraction induced by ergotamine.

Contractile responses of ruminal vein were not detected for ergonovine and ergocornine. Dose response curves for ruminal vein are shown in Figure 2. The lowest dose where a response was statistically greater than the lowest point was 1×10^{-6} M for ergovaline, 1×10^{-5} M for ergotamine, and 1×10^{-4} M for ergocryptine and ergocristine. The greatest response observed in ruminal vein was produced by ergovaline and ergotamine. The EC-50 for the alkaloids within ruminal artery did not differ.

Implications

Addition of ergot alkaloids to *in vitro* culture of bovine ruminal artery and ruminal vein produced a contraction similar to the responses observed in peripheral bovine vasculature exposed to ergot alkaloids. The contractile response produced by these ergot alkaloids present in endophyte-infected tall fescue have the potential to alter blood flow to the foregut of cattle grazing infected tall fescue. Alterations in blood flow and blood drainage from the foregut of cattle grazing endophyte-infected tall fescue could negatively affect nutrient absorption from the foregut of these animals, which could contribute to the observed reduction in growth and performance in cattle experiencing fescue toxicosis.

Table 1. EC-50 for ruminal artery and ruminal vein dose response.

	EC-50							
	Ergonovine	**Ergocornine**	**Ergocryptine**	**Ergocristine**	**Ergotamine**	**Ergovaline**	**SEM**	**P <**
Artery[1]	1.1E-05a	1.2E-05a	8.6E-06a	1.2E-05a	3.0E-05b	5.6E-06a	4.9E-06	0.01
Vein	-	-	1.7E-05	1.2E-04	2.0E-05	2.0E-06	5.0E-05	0.26

[1] Means on the same line with different letters (a, b) differ (P<0.05).

Tall Fescue Alkaloids Bind Serotonin Receptors in Cattle

J.L. Klotz, J.R. Strickland, L.P. Bush, K.R. Brown, and G.E. Aiken

Summary

The serotonin (5HT) receptor $5HT_{2A}$ is involved in the tall fescue alkaloid-induced vascular contraction in the bovine periphery. This was determined by evaluating the contractile responses of lateral saphenous veins biopsied from cattle grazing different tall fescue/endophyte combinations. The contractile responses of the biopsied blood vessel segments to different alkaloids (ergovaline, ergotamine, and ergocornine) were evaluated in the presence or absence of an antagonist or blocker of the $5HT_{2A}$ receptor. The presence of the antagonist significantly reduced the contractile response to all three alkaloids in all three pasture types. A better understanding of how tall fescue alkaloids cause peripheral vasoconstriction will lead to better solutions for summer slump and fescue foot manifestations of the fescue toxicosis syndrome.

Introduction

Alkaloids produced by the fungal endophyte *Neotyphodium coenophialum* that is found in tall fescue (*Lolium arundinaceum*) have been shown to cause peripheral vasoconstriction, which contributes to the symptoms that are collectively referred to as the fescue toxicosis syndrome. The alkaloids are thought to elicit their effects by interacting with adrenergic, dopaminergic, and serotonergic receptors. These three types of receptors are found throughout the mammalian body but all have different structural and functional classifications. Alkaloids have been shown to bind and activate D_2 dopamine receptors that are found in the higher neural centers of the basal ganglia and anterior pituitary, promote functional changes in a_2-adrenergic receptors in bovine saphenous veins, and activate $5HT_{2A}$ and $5HT_{1B/1D}$ receptors in rat and guinea pig blood vessels. Serotonin receptors have been linked to ergovaline- (an ergot alkaloid) induced vascular contractions in bovine umbilical and uterine artery preparations, but never in bovine peripheral blood vessels. Given that serotonin receptors are present in different subtypes and different active populations of these subtypes in various tissues, it was necessary to determine if ergot alkaloids interact with serotonin receptors found in peripheral bovine vasculature. Therefore, the objective of this experiment was to determine if the contractile response of bovine lateral saphenous veins to ergot alkaloids can be diminished by the presence of antagonists to select serotonin receptors and whether this is affected by grazing different tall fescue-endophyte combinations.

Figure 1. Mean contractile responses of lateral saphenous veins isolated from year 1 steers grazing tall fescue pastures with a novel endophyte (MAXQ/AR542; n = 5; EC = 2.3 x 10 M), wild-type endophyte (KY31; n = 5; EC = 2.4 x 10 M) endophyte free (EF; KYFA9301; n = 5; EC = 4.2 x 10 M) to increasing concentrations of serotonin.

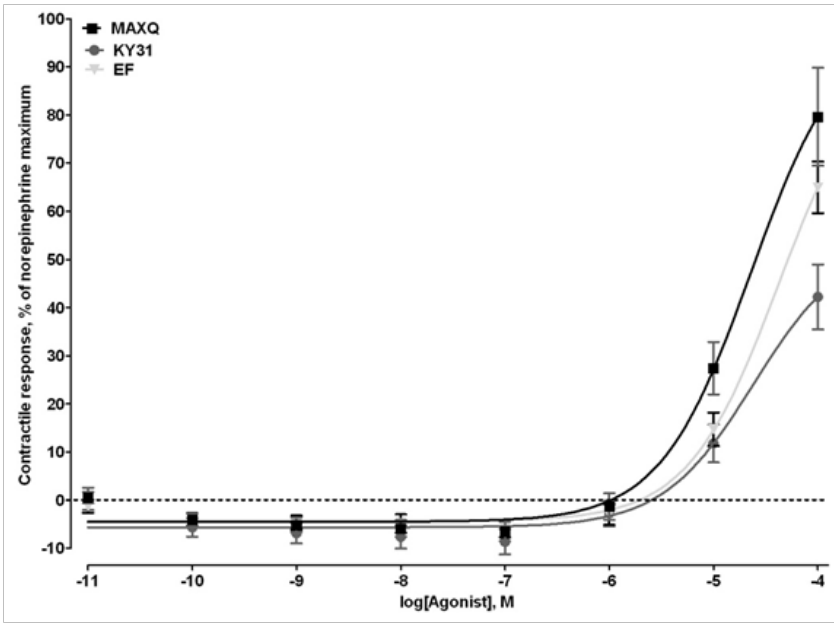
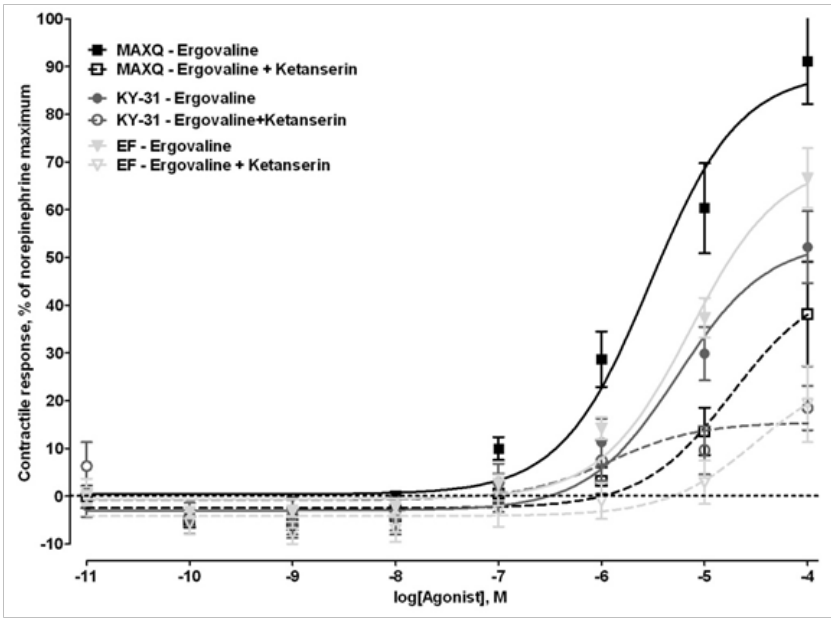


Figure 2. Mean contractile responses of lateral saphenous veins isolated from year 1 steers grazing tall fescue pastures with a novel endophyte (MAXQ/AR542; n = 6; EC = 2.9 x 10 M), wild-type endophyte (KY31; n = 6; EC = 5.5 x 10 M) endophyte free (EF; KYFA9301; n = 6; EC = 7.3 x 10 M) to increasing concentrations of ergovaline. Ergovaline responses in the presence of 10 mM ketanserin are indicated with dashed lines for MAXQ (n = 6; EC = 1.8 x 10 M), KY31 (n = 5; EC = 1.2 x 10 M), and EF (n = 6; EC = 3.1 x 10 M).



Materials and Methods

Animals and Pastures

Biopsies of lateral saphenous veins were conducted over a two-year period (2008 and 2009) in 35 mixed breed steers (362 ± 6.3 kg) following the completion of a separate grazing trial. In year 1 the steers grazed for 84–98 d on 3 ha pastures of Kentucky-31 (KY31) tall fescue infected with the wild-type endophyte (n = 6), an endophyte free tall fescue (EF; KYFA9301; n = 6), or a Georgia Jesup infected with a novel endophyte (MAXQ; AR542; n = 6). In year 2 the same number of steers grazed for 108–124 d on the same 3 ha KY31 and endophyte-free pastures, but instead of MAXQ, steers grazed a KYFA9301 pasture infected with a different novel endophyte (AR584; n = 5). The novel endophyte pasture was changed because there was a significant bluegrass infestation in the MAXQ pasture that would have likely confounded the treatment. In each year, one animal was biopsied from each pasture type per biopsy day.

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 section was then cleaned of external adipose and connective tissues and sliced into 2 – 3 mm cross-sections. These 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 a reference for all experimental additions.

Experimental additions for year 1 consisted of increasing concentrations of 5HT, ergovaline, and ergovaline + 10 mM ketanserin (a selective $5HT_{2A}$ antagonist). In year 2, the experimental additions to the vein cross-sections in the myograph were increasing concentrations of the ergot alkaloids ergotamine and ergocornine in the presence and absence of 10 mM ketanserin. The ketanserin was included as a constituent of the Krebs Henseleit buffer solution.

Figure 3. Mean contractile responses of lateral saphenous veins isolated from year 2 steers grazing tall fescue pastures with a novel endophyte (AR584; n = 5; EC = 8.2 x 10 M), wild-type endophyte (KY31; n = 6; EC = 8.1 x 10 M) endophyte free (EF; KYFA9301; n = 6; EC = 7.0 x 10 M) to increasing concentrations of ergocornine. Ergocornine responses in the presence of 10 mM ketanserin are indicated with dashed lines for AR584 (n = 5; EC = 1.3 x 10 M), KY31 (n = 6; EC = 5.0 x 10 M), and EF (n = 6; EC = 1.1 x 10 M).

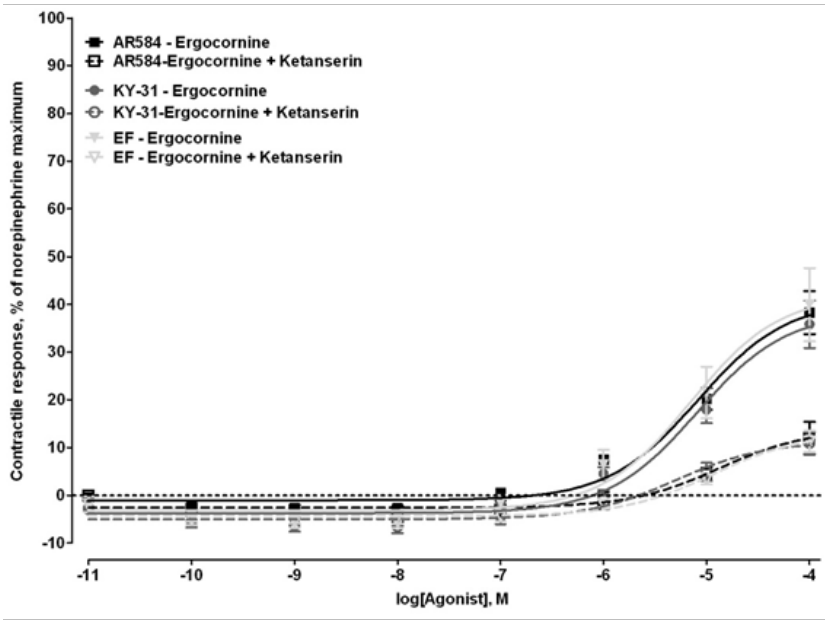
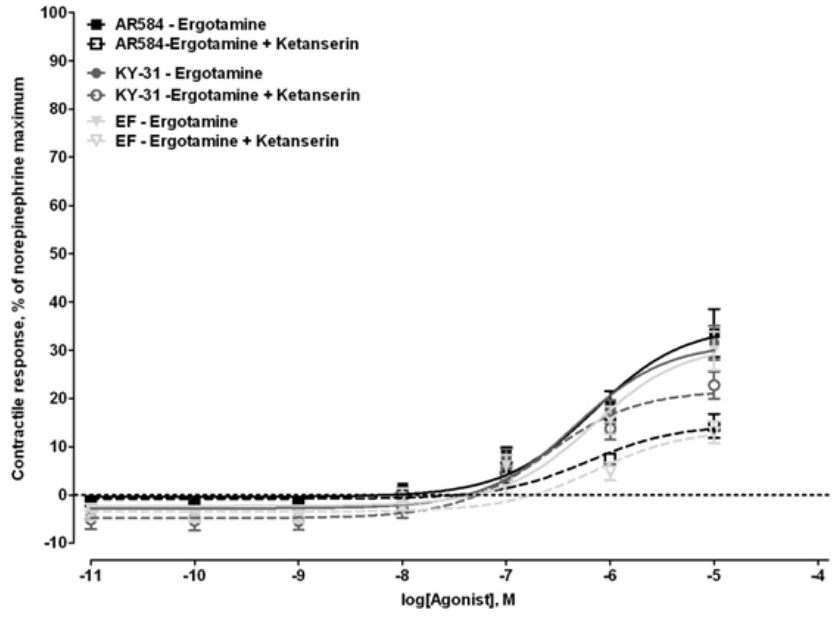


Figure 4. Mean contractile responses of lateral saphenous veins isolated from year 2 steers grazing tall fescue pastures with a novel endophyte (AR584; n = 5; EC = 6.9 x 10 M), wild-type endophyte (KY31; n = 6; EC = 4.6 x 10 M) endophyte free (EF; KYFA9301; n = 6; EC = 6.8 x 10 M) to increasing concentrations of ergotamine. Ergotamine responses in the presence of 10 mM ketanserin are indicated with dashed lines for AR584 (n = 5; EC = 6.0 x 10 M), KY31 (n = 6; EC = 2.4 x 10 M), and EF (n = 6; EC = 7.1 x 10 M).



Data and Statistical Analyses

Data from each experiment were recorded, digitized (LabChart version 7.1; ADInstruments, Colorado Springs, CO), and normalized to the reference addition of norepinephrine. Thus, the form that all contractile response data are presented is percentage of norepinephrine maximum. Data were plotted, and a nonlinear regression line was fit using GraphPad Prism (version 5.00, GraphPad Software, San Diego CA), which was used to determine EC_{50} (50% effective concentration—the concentration of agonist that gives a response halfway between bottom and top plateaus) for each treatment.

The contractile response data were analyzed as a completely randomized design with a pasture x alkaloid treatment factorial arrangement using the mixed models procedure of SAS (version 9.1, SAS Inst. Inc., Cary, NC), with steer as the experimental unit. All differences discussed as significant are $P < 0.05$.

Results and Discussion

Year 1: 5HT and Ergovaline

There was a significant pasture effect on the contractile response to serotonin (Figure 1) and ergovaline (Figure 2). The steers on MAXQ pasture had the greatest response to both serotonin and ergovaline, whereas the steers that grazed KY31 had the lowest ($P < 0.05$). This observation has been reported in other experiments with similar pasture treatments. It is possible that the blood vessels collected from animals that have been on toxic endophyte-infected tall fescue (i.e., KY31) are already constricted and less responsive to external stimuli.

The presence of the 10 mM ketanserin in the Krebs Henseleit buffer solution significantly reduced the contractile response to ergovaline across all pastures (Figure 2). The percent change in maximal contractile response induced by ketanserin was a 71% drop for EF, a 65% drop for KY31, and a 58% drop in the MAXQ veins. There was a significant a pasture x ketanserin effect between veins isolated from the EF and MAXQ pastures. This may be a result of differing receptor populations affected by the antagonist.

Year 2: Ergocornine and Ergotamine

The alkaloids ergotamine and ergocornine were chosen for evaluation because they are structurally similar to ergovaline. Unlike in year 1, there was no pasture effect for either alkaloid (Figures 3 and 4). This lack of pasture effect may have resulted from drastic differences in ambient temperature and precipitation between years. It could have resulted in differing alkaloid exposure levels and loads as a result of these environmental differences.

As in year 1, there was a significant decrease in contractile response of both ergocornine (Figure 3) and ergotamine (Figure 4) in the presence of ketanserin. This decrease closely resembled the observed decrease in contractile response to ergovaline in the presence of ketanserin (Figure 2). There was not a significant interaction between pasture and the presence of ketanserin for ergocornine (Figure 3), as the percent decrease was between 69% and 71% for all three pastures. There was no interaction between pasture and antagonist treatments for ergotamine, but there was a much smaller decrease in the KY31 veins (28%) compared to the EF and the AR584 veins (56% and 58%, respectively).

Implications

Antagonism of the $5HT_{2A}$ receptor significantly reduced contractile responses to ergovaline, ergotamine, and ergocornine in all fescue-endophyte combinations evaluated. The involvement of this receptor in alkaloid-induced contractile response has only been reported previously in rodent models, and this is the first report of this effect in bovine peripheral vasculature. This substantiates that the $5HT_{2A}$ receptor is involved in tall fescue alkaloid-induced vascular contraction. Receptor populations and corresponding intracellular signaling pathways may be affected by exposure to ergot alkaloids. Administration of antagonists for receptors like $5HT_{2A}$ may be effective in reducing the fescue toxicosis symptoms related to vasoconstriction.

Development of a Precise, Repeatable Model for Fescue Toxicosis

A.F. Koontz, L.P. Bush, K.R. McLeod, D.L. Harmon

Summary

This study was designed to examine the efficacy of a fescue seed extract for inducing fescue toxicosis in cattle. Four ruminally cannulated Holstein steers were utilized in a four-phase crossover design experiment. The basal diet was endophyte-free fescue hay fed *ad libitum*. In phases 1 and 2, steers were ruminally dosed twice daily with 1 kg, either ground endophyte-infected seed (S_{E+}) or ground endophyte-free fescue seed (S_{E-}) for 7 d. In phases 3 and 4, steers were ruminally dosed twice daily with an extract from endophyte-infected seed (E_{E+}) or endophyte-free fescue seed (E_{E-}) for 7 d. During d 4-7 of each phase, room temperature was increased to 32°C (Heat Stress [HS]). Steers on S_{E+} and E_{E+} had a reduction in total intake and heart rate. Rate of intake was also reduced for S_{E+} and E_{E+} during HS. Core body temperature and respiration rate were elevated during HS with S_{E+} and E_{E+} dosing. Blood pressure was only measured during E treatment. Systolic pressure was unaffected by treatment, while diastolic pressures were higher with E_{E+} dosing. These data indicate a fescue seed extract model is able to mimic the symptoms of fescue toxicosis induced by seed.

Introduction

Tall fescue is grown on more than 15 million acres of land in the United States alone, and more than half of these fields are infected with the endophyte *Neotyphodium coenophialum*. This endophyte provides positive aspects such as drought and heat tolerance. However, the ergot alkaloids produced by this endophyte cause health and production issues in animals grazing the infected fescue, resulting in negative economic effects for producers. The decrease in productivity caused by fescue toxicosis has been estimated to cost United States producers more than $600 million per year.

The clinical symptoms of toxicosis include reduced weight gain and feed intake, decreased milk production, reduction in reproductive efficiency, tissue necrosis, and rough hair coat. Physiological signs and blood profile changes can be used to diagnose less severe cases of fescue toxicosis. Increased respiration rate, increased core temperature, and decreased skin temperature have been shown to be highly correlated to the onset of fescue toxicosis. Monitoring these measurements would allow for immediate evaluation of the activity level of administered alkaloids.

Most research examining the effects of fescue alkaloids on animal performance uses ground seed added to a basal diet to mimic grazing infected pasture. Because one of the factors associated with fescue toxicosis is a reduction in feed intake, alteration of alkaloid intake level can occur using this methodology. A more precise method would be to dose the animals with an extract containing the alkaloids found in toxic fescue. However, little research has been done to determine the bioactivity of such extracts. The goals of this experiment were: 1) To develop a set of standard measurements to determine the onset of fescue toxicosis and 2) to determine the ability of a fescue seed extract to cause the symptoms of fescue toxicosis in Holstein steers.

Materials and Methods

Animals Model and Experimental Design

Four ruminally cannulated Hereford steers were used in a four-phase crossover experiment. Steers were housed in temperature and humidity controlled rooms. Animals were fed once daily at 0800. The basal diet consisted of endophyte-free fescue hay topdressed with 40 mg trace mineralized salt. Water was available *ad libitum* throughout the trial.

Each phase consisted of a 7d measurement period. A minimum of two weeks washout was allowed between phases. During phases 1 and 2, two randomly selected steers were ruminally dosed with 0.5kg ground endophyte-infected fescue seed (E+), and two control steers were ruminally dosed with 0.5kg ground endophyte-free fescue seed (E-) twice daily (at 0800 and 1600). Fescue seed was ground to pass through a 2-mm screen. During phases 3 and 4, steers were ruminally dosed with an ethanol extract of either E+ and E- fescue seed. Environmental temperature was maintained at 22°C on D1-3 (thermoneutral, TN), then increased to 32°C on D4-7 to simulate a heat-stress (HS) situation.

Measurements

Feed intake was measured continually via feed bunks attached to load cells. Orts were collected, weighed, and recorded from the previous day prior to each day's 0800 feeding. At 0900 and 1500 on d 1-7, skin surface temperature was measured using an infrared thermometer. Measurements were taken in the center of a 75- x 100-mm clipped area over the rib. Three measurements were taken at each time point to provide an average. At each time point, respiration rate was measured by flank movement counts.

During ruminal cannulation, each steer was implanted with a radio telemetry device to continually measure and record core temperature. Heart rate was continually measured using a telemetry device attached to a heart-girth band. Room temperature and humidity were recorded throughout the experiment via continuous data-logger. Blood samples were taken via jugular venipuncture to on d 1 and 7 relative to the beginning of each phase and analyzed for serum prolactin by RIA.

Fescue Extract Preparation

Fescue seed extract was prepared from packed ground seed in an extractor column. Extraction utilized 80% ethanol over approximately 48 h. After extraction, the ethanol was evaporated off and the remaining residue freeze-dried in the dark then ground with mortar and pestle, maintaining clod with liquid nitrogen. Single-dose amounts of ground extract were packaged in cellulose paper and stored in the dark at -5°C to ensure stability until immediately prior to ruminal dosing.

Statistical Analysis

The data were analyzed with the mixed procedure of SAS (2007) with individual measurements as the experimental unit except for core temperature and heart rate, which were averaged by hour within a day prior to analysis. Animal and phase were considered random effects, while treatment and temperature were fixed effects. Data are presented as least squared means. Effects were considered significant at $P \leq 0.05$.

Results and Discussion

Ground Seed

Classic symptoms of fescue toxicosis include elevated core body temperature, increased respiration, and increased susceptibility to heat stress. Dosing steers with ground E+ seed resulted in the physiological changes seen in Table 1.

Heat stress reduced ($P = 0.001$) intake, meals per hour, and tended to reduce rate of intake ($P = 0.06$). Respiration rate, core temperature, and skin temperature were elevated ($P < 0.0001$) during heat stress. Animals dosed with E+ seed had increased ($P < 0.01$) respiration rates, heart rates, and core temperatures as compared to E-dosed animals. In addition, total feed intake was reduced during E+ dosing ($P < 0.0001$). Skin temperature over

Table 1. Comparison of physiological measurements between steers dosed with SE- and SE+ at 22°C and 32°C.

	Treatment[1]				P =		
	SE-		SE+		Main Effects		Interaction
Item	22°C	32°C	22°C	32°C	Endophyte	Temp	E*T
Intake (kg/d)	7.43a	7.40a	7.01a	5.34b	<0.0001	0.0003	0.0004
Rate of intake (log kg/h)	1.44a	1.07b	1.02b	1.06b	0.1319	0.0595	0.0644
Meals (per h)	10.2a	8.3bc	9.2ab	8.0c	0.1134	<0.0001	0.3302
Respiration rate (bpm)	38.4a	54.1b	38.5a	77.9c	0.0089	<0.0001	0.0096
Heart rate (bpm)	81.07a	84.08b	72.28c	71.61c	<0.0001	0.1566	0.0260
Core temperature (°C)	37.87a	38.07b	37.48c	38.15b	<0.0001	<0.0001	<0.0001
Skin temperature (°C)	35.1a	36.4b	35.4a	36.8b	0.1526	<0.0001	0.7454

[1] *Values within a row with differing letters (a, b, c, d) are significantly different (P < 0.05).*

the ribs was not affected by E+ treatment ($P = 0.15$). All indicators except rate of intake, meals per hour, and skin temperature showed a significant ($P < 0.05$) interaction between treatment and temperature, with E+ animals being more sensitive to the elevated ambient temperature.

From these results, it was concluded that the seed was able to induce toxicosis and the set of measurements was able to detect the onset of toxicosis in these animals.

Fescue Extract

After validation of the model for determining the onset of toxicosis in steers, the same measurements were used to evaluate the viability of the fescue seed extract to induce toxicosis. A summary of these results can be seen in Table 2.

Heat stress significantly reduced ($P < 0.05$) all feed-intake measurements and heart rate. Respiration rate and core temperature were increased ($P < 0.0001$) during heat stress. Diastolic blood pressure tended to decrease ($P = 0.06$) with heat stress, while systolic pressure was unaffected ($P = 0.18$). Skin temperature was unaffected by temperature ($P = 0.23$) or treatment ($P = 0.74$). Treatment with E+ extract resulted in increased ($P < 0.05$) respiration rate, heart rate, and core temperature as compared to E- treatment. In addition, total feed intake and rate of intake were reduced ($P < 0.05$) during E+ extract dosing. Heart rate, core temperature, and respiration rate showed significant interactions ($P < 0.05$) between temperature and treatment. As with ground seed dosing, animals dosed with E+ extract were more sensitive to the elevated temperature.

These changes are comparable to those seen in cattle grazing endophyte-infected pastures during summer months and previous research examining fescue toxicosis. Therefore, it was concluded that the extract of the fescue seed is able to induce fescue toxicosis in steers.

Implications

Ruminal dosing of an ethanol extract of tall fescue seed is able to mimic the symptoms of fescue toxicosis in Holstein steers. Ruminal dosing removes the issues of palatability and reduced intake level from research where determination of ergot alkaloid intake level is a fundamental factor. Utilization of this extract for future fescue toxicosis research may provide a more precise, repeatable model for future research.

Table 2. Comparison of physiological measurements between steers dosed with EE- and EE+ at 22°C and 32°C.

	Treatment[1]				P =		
	EE-		EE+		Main Effects		Interaction
Item	22°C	32°C	22°C	32°C	Endophyte	Temp	E*T
Intake (kg/d)	9.14a	8.29a	8.50a	6.70b	0.0432	0.0207	0.3342
Rate of intake (per h)	1.58a	1.41a	1.36a	1.08b	0.0054	0.0011	0.4734
Meals (per h)	13.2a	9.8b	11.9c	7.3d	<0.0001	<0.0001	0.2093
Respiration rate (breaths/min)	38.3a	45.8b	38.3a	602c	0.0030	<0.0001	0.0031
Heart rate (beats/min)	80.6a	76.0b	67.9c	66.4c	<0.0001	<0.0001	0.0435
Core temperature (°C)	38.4a	38.5b	38.2c	38.6d	0.0166	<0.0001	<0.0001
Skin temperature (°C)	35.2a	35.4a	35.3a	35.4a	0.7426	0.2270	0.8196
Systolic blood pressure, mm Hg	98.5a	99.7ab	110.2b	98.2a	0.2028	0.1791	0.1098
Diastolic blood pressure, mm Hg	51.5a	49.3a	63.1b	54.8a	0.0800	0.0643	0.2536

[1] *Values within a row with differing letters (a, b, c, d) are significantly different (P < 0.05).*

The Effect of Dietary Protein on Growth and Immunological Response in Growing Steers

E.S. Vanzant, E. Lane, K.R. McLeod, M. Steinman, and J.W. Lehmkuhler

Summary

Reducing dietary protein concentrations from 100% to 80% of the National Research Council (NRC) metabolizable protein requirements in corn silage-based diets for growing steers reduced growth, gain:feed, and immunological function. A novel approach for assessing immunological function provided results that were consistent with growth responses to dietary protein manipulation. In general, responses support the NRC estimates of metabolizable protein requirements for growth, although gains with low metabolizable protein diets exceeded those predicted by the NRC model.

By establishing health and performance responses to specific levels of metabolizable protein, results from this study can be used to improve the formulation of diets for growing steers on corn silage-based diets with the intent of optimizing economic returns.

Introduction

Newly arrived feedlot steers often experience significant stress during pre- and post-feedlot arrival activities. The stressors encountered before and after feedlot arrival can lead to numerous health and production problems that may include immune system incompetence, decreased digestive efficiency, decreased dry matter intake (DMI), depressed weight gain, and negative alterations in metabolic function.

Supplementing rations with ruminally undegradable intake protein (UIP) can increase the quantity of and/or shift the profile of the amino acid flow to the lower gastrointestinal tract. The crude protein (CP) requirement of the animal has long been the established standard for balancing dietary protein. The *1996 Nutrient Requirements of Beef Cattle* evaluates protein requirements using a metabolizable protein (MP) system. The MP system accounts for ruminal degradation of protein and separates requirements into the needs of microorganisms and the needs of the animal. However, incorporation of protein degradability information into ration formulation has not become widespread, partly because of limited availability of research demonstrating tangible benefits to balancing diets based on DIP and MP requirements.

There is currently little information connecting level and source of protein to immune response in receiving cattle. Additionally, more data are needed in order to evaluate how well the NRC estimates of MP requirements predict growth responses in beef cattle. There is some evidence that current estimates of MP needs may overestimate the actual requirements of some growing cattle. The objectives of this study were to evaluate how well the NRC MP system predicts growth responses in receiving steers fed corn silage-based diets with differing protein degradabilities and to determine whether protein nutrition affects the ability of receiving steers to mount an immunological response to a novel antigen challenge.

Materials and Methods

Animals and Feeding

One-hundred ninety-two crossbred steers purchased at local auction (initial BW 571 lb) were used in a 2 x 2 factorial experiment. Steers were stratified by weight and assigned randomly within strata to 48 pens of four steers each. Pen groups were assigned randomly within weight blocks to receive one of four experimental diets. Rations were formulated to provide either 80% or 100% of NRC MP requirements for 2.5 lb/d gain using protein sources which were high (soybean meal) or low (SoyPlus) in ruminal degradability (Table 1). Diets were formulated using the Level 1 model of the NRC using a DIP requirement value of 11% of Total Digestible Nutrients (TDN) (Table 2). Treatments were as follows: 1) low Metabolizable Protein (MP) diet formulated to meet 80% of MP requirement using SoyPlus, 2) high MP diet formulated to meet 100% of MP requirement using SoyPlus, 3) low MP diet formulated to meet 80% of MP requirements using Soybean Meal (SBM), or 4) high MP diet formulated to meet

Table 1. Ingredient composition of experimental diets (% of DM).

	Dietary Treatment			
	SBM		SoyPlus	
Ingredient	Low MP	High MP	Low MP	High MP
Corn silage	65.4	66.0	65.7	66.2
Cracked corn	25.8	10.3	25.4	18.7
Soybean meal	5.6	21.4	0	0
SoyPlus	0	0	5.8	12.3
Dicalcium phosphate	0.49	0.16	0.46	0.30
Limestone	1.21	1.30	1.20	1.22
Potassium chloride	0.67	0.06	0.67	0.42
TM salt	0.80	0.80	0.80	0.80

Table 2. Formulation targets of experimental diets (% of requirements).

	Dietary Treatment			
	SBM		SoyPlus	
Nutrient	Low MP	High MP	Low MP	High MP
Crude protein	89	144	88	109
Degradable protein	96	157	86	99
Metabolizable protein	80	100	80	100
MP allowable ADG, lb	1.65	2.51	1.65	2.51
Net energy allowable ADG, lb	2.49	2.49	2.49	2.49

Table 3. Chemical composition of consumed rations (% of DM except as noted).

	Dietary Treatment			
	SBM		SoyPlus	
Item	Low MP	High MP	Low MP	High MP
Crude protein	10.9	17.9	10.9	12.6
Degradable protein, % of CP	68.0	68.8	53.2	52.7
Neutral detergent fiber	36.5	30.4	31.2	31.4
Acid detergent fiber	14.1	14.9	14.4	13.9
Acid detergent lignin	2.0	1.8	2.1	2.3

100% of MP requirements using SBM. Diets were fed once daily at 0800. Samples of feed ingredients were collected once weekly. Feed refusals from each pen, when present, were weighed weekly, and composite samples for each treatment group were collected for nutrient analysis.

Vaccination Schedule

Steers were weighed on d 0, 28, and 56 of the experiment. On d 0, steers were vaccinated (Vista Once-SQ, 20/20 Vision 7 with SPUR; Moraxella ovis) and drenched with an oral anthelmintic (Safe-Guard). In order to assess the humoral immune response to a novel antigen, steers were inoculated on d 0 and 28 with a Leptospirosis vaccine (L5-SQ). Blood samples were obtained via jugular venipuncture on d 0, 28, and 56 for measurement of antibody titers to Leptospira serovar hardjo (LSH). Steers were removed from treatments and placed on endophyte-infected tall fescue pastures from d 28 to 56. Residual effects of treatments on immunological status were evaluated in 160 of the steers (evenly representing treatments and blocks) by measuring the antibody titer at d 56.

Table 4. Performance of growing steers as affected by source and level of metabolizable protein.

	Dietary Treatment					Probability of Greater F-value		
	SBM		SoyPlus					
Item	Low MP	High MP	Low MP	High MP	SEM	Source x Level	Source	Level
ADG, lb	2.32	2.70	2.18	2.667	0.108	0.62	0.41	<0.01
Final BW, lb	639	651	642	647	7.3	0.27	0.99	0.01
DMI, lb/d	14.6a	13.9b	14.4a	14.8a	0.16	<0.01	0.03	0.33
Gain: Feed	0.16	0.19	0.15	0.18	0.01	0.61	0.10	<0.01

a,b Means with different superscripts differ (P<0.05).

Table 5. Serum *Leptospirosis* titers as affected by source and level of metabolizable protein.

	Dietary Treatment					Probability of Greater F-value		
	SBM		SoyPlus					
Item	Low MP	High MP	Low MP	High MP	SEM	Source x Level	Source	Level
28-d titer, log	2.24	2.38	2.09	2.37	0.060	0.23	0.15	<0.01
56-d titer, log	3.40	3.44	3.41	3.44	0.061	0.95	0.89	0.63

Laboratory Analyses

In addition to routine nutrient analysis of feedstuffs, ruminal protein degradability of feedstuffs was determined by an in situ technique in which feed samples were incubated, in porous Dacron bags, in the rumens of steers fed a 75% forage, 25% concentrate diet. Samples were either unincubated or incubated in the rumen for 12 or 96 hours, after which N remaining was determined. Microbial N remaining in the bags was assessed using a 15N isotope infusion and was subtracted from the total remaining N. A mathematical model was constructed from the N remaining in the bags from the various incubation times to calculate protein degradability for each of the feedstuffs.

Statistical Analysis

Data were analyzed using a model appropriate for a randomized complete block design with the SAS GLM procedure. Pen was the experimental unit, and the model included terms for weight block, protein source, MP level, and the protein source x MP level interaction. When interactions were significant, means were separated using protected ($P < 0.10$) Fisher's LSDs.

Results and Discussion

Source and level of MP did not interact to affect ADG, final body weight, or gain:feed (Table 4). Reducing MP from 100 to 80% of NRC requirements decreased ADG and final weight, whereas source of protein did not affect these responses. Gain:feed was improved by about 20% with high as compared with low MP diets. Animals consuming the SBM diets tended ($P = 0.10$) to have higher gain:feed ratios than those consuming SP diets. Observed gains on the low MP diets were substantially greater than predicted by the NRC model (observed gains of 2.32 and 2.18 lb/d vs. predicted gain of 1.65 lb/d). There was a source x level interaction for intake, which was lower with the high MP SBM treatment as compared with the other three treatments. In this diet, SBM, a feedstuff with a relatively high ruminal degradability, was used to balance the MP requirements, resulting in a very high overall protein content in the ration (~18% CP; Table 3). The high level of dietary and ruminally degradable protein with this treatment likely resulted in high concentrations of NH_3 in the ruminal fluid and blood, which could have depressed voluntary intake.

Increasing MP was also associated with greater 28-d antibody titers to *Leptospirosis* in response to vaccination on d 0 (Table 5). There were no differences detected in the 28-d antibody titers between SBM and SP nor any treatment effects in the 56-d antibody titers in response to the booster vaccinations on d 28. Because the 56-d measurement represented effects subsequent to placing all animals on a common diet, this indicates a lack of residual effects of dietary treatments on the ability to mount a humoral immunological response.

This study demonstrates that reducing MP to 80% of NRC requirements results in reduced ADG, final body weights, gain:feed ratios, and ability to mount an immunological response to an antigen challenge. These data support the concept of formulating diets using NRC MP guidelines, although they also suggest that the NRC model overestimates the influence of MP deficiency on growth. However, because of the diet formulation strategy used, diets varied in their provision of degradable intake protein as well as MP. Thus, additional research is necessary to clarify whether the results were in fact a response to MP, per se.

Implications

Reducing dietary protein concentrations from 100% to 80% of NRC MP requirements in corn silage-based diets for growing steers reduced growth, gain:feed, and immunological function. Though the general response was in accord with predictions from the NRC model, these data suggest that the NRC model overestimates the impact of an MP deficiency. Accurate prediction of gain and health responses to protein levels below requirements is essential for designing growing diets for optimal economic efficiency.

Effects of Direct-Fed Microbial and Vitamin/Mineral Supplementation on Health and Growth Performance of Stressed Calves

S.E. Kitts, E.S. Vanzant, R.B. Hightshoe, D.L. Harmon, K.K. Kreikemeier, J.W. Lehmkuhler, and K.R. McLeod

Summary

The objective of the current study was to determine the effects on growth performance and health of stressed calves of a direct-fed microbial (DFM) supplement containing *Enterococcus faecium* and *Lactobacillus acidophilus* (Formula 120 G) administered daily and a gel containing vitamins and mineral-amino acid chelates (Battle®) that was administered orally upon arrival (52.8 g per steer) and when calves were eligible for subsequent treatment according to the established protocol. In agreement with previous observations, calves receiving Formula 120 G had greater dry matter intake (DMI) over the course of the entire experiment relative to those receiving no Formula 120 G. Additionally, these calves had greater average daily gain (ADG) during the initial 28 d and decreased mortality, which may have resulted from stimulation of the immune system. Conversely, calves receiving the Battle® treatment had lower ADG during the initial 28 days of the experiment and increased morbidity, which was unexpected. Preliminary observations had shown improvements in growth performance as well as decreases in morbidity and mortality in "downer" dairy cows with the administration of Battle® gel. However, other research has shown daily supplementation of vitamins and minerals to weaned calves to have variable results; therefore, the lack of positive response to the Battle® gel in the current experiment may be related to insufficient supplementation, as these calves were administered the gel only upon arrival and during medicated treatments.

Introduction

The original concept of feeding direct-fed microbials to ruminants evolved from the idea that pathogenic bacteria (*E. coli* and other coliforms) have the ability to thrive in the hindgut under "stressful" conditions, such as weaning and shipping. Under these conditions, cattle are more susceptible to bacterial infections, such as those involved in the development of bovine respiratory disease (BRD), which is a major problem in weaned calves and causes serious economic losses for producers. Therefore, the hypothesis arose that daily administration of large amounts of "beneficial" microbes during periods of intense stress would prevent the establishment of pathogenic bacteria and allow the colonization of microbes such as *Lactobacillus acidophilus* and *Enterococcus faecium*, which preliminary research in our lab has shown to increase DMI and ADG in weaned calves. The proposed mechanism by which DFMs accomplish these improvements is unclear; however, it is suggested that they compete with pathogens in the lower gut for space and nutrients, detoxify undesirable compounds, and produce antibacterial compounds. Although little research has been conducted to assess the effects of DFMs on growth performance and health of weaned calves, research involving the use of *Propionibacterium* P15, *Lactobacillus acidophilus,* and *Enterococcus faecium* in feedlot cattle has shown a decrease in the incidence of ruminal acidosis and improvements in body weight (BW) gain.

In addition to the administration of DFMs, supplementing vitamins and minerals in the diets of newly weaned or received cattle has been shown to decrease morbidity and improve growth performance. Although results have been variable, B vitamins and vitamins C and E are the most commonly supplemented; benefits from vitamin E are most likely mediated through effects on the immune system. Likewise, the addition of certain minerals to receiving diets can have beneficial effects on the immune system, thereby improving feed intake and BW gain. Although the mineral requirements of stressed calves do not differ from those of unstressed animals, feed intake is generally less by stressed calves, so it is necessary either to increase mineral concentrations in the feed, or supplement minerals in some other manner. Among the most important minerals for boosting the immune system are zinc and copper. Not only are these minerals important for the overall health of stressed animals, but the form (oxides vs. mineral-amino acid chelates) in which they are given is important as well. Preliminary research involving a gel (Battle®) containing a mixture of vitamins and mineral-amino acid chelates has shown improvements in growth performance and a decrease in morbidity in dairy cattle.

Although research has shown positive effects of DFMs on growth performance of feedlot cattle and decreased morbidity in weaned calves with vitamin/mineral supplementation, there is a paucity of information regarding the effects of DFMs when fed in conjunction with a vitamin/mineral supplement. Therefore, the objective of the current study was to determine the effects on growth performance and health of stressed calves of a direct-fed microbial (DFM) supplement containing 5 x 10^8 CFU *Enterococcus faecium* and *Lactobacillus acidophilus* (Formula 120 G) per steer per day and a gel containing vitamin supplements and mineral-amino acid chelates (Battle®) that was administered orally upon arrival (52.8 g gel/steer) and when calves were eligible for subsequent treatment according to the established protocol.

Materials and Methods

Animals and Treatments

The protocol for the research discussed in this report was approved by the University of Kentucky Institutional Animal Care and Use Committee. Two hundred Angus crossbred steers were purchased from commercial sale yards in Central Kentucky. Cattle were shipped from the sale yard to the University of Kentucky Animal Research Center (UKARC) in two, 100-head lots. Previously, we have shown this method of purchasing cattle to result in 30-50% morbidity and approximately 2% mortality. Upon arrival at UKARC, cattle were immediately processed

and allotted to pen and treatment. They received *ad libitum* hay and water for 24 h prior to treatment implementation. Routine processing included the following: 1) recording of individual body weight, 2) ear tag placement for animal ID and fly control, 3) administration of Synovex®-S growth implant (ear implant), 4) administration of a viral vaccination (Bovi-Shield™4), 5) a clostridial vaccination (Ultrabac®7), 6) deworming using ivermectin (Merial, Duluth, GA), 7) 'tipping" of horns, and 8) recording of rectal temperatures. Cattle were allotted randomly to pen, with each receiving block generating five pens per treatment. Treatments were arranged as a 2 x 2 factorial design and included the following: 1) control (no Formula 120 G, no Battle®), 2) Formula 120 G, no Battle®, 3) Battle®, no Formula 120 G, and 4) Formula 120 G + Battle®. Because adjacent pens shared a common water source and concern existed regarding cross-contamination between those animals receiving/not receiving DFMs, treatments were assigned randomly to "pen pairs." These pairs were generated using pairs of treatments 1 and 3 and treatments 2 and 4.

Cattle were weighed on two consecutive days at the beginning (day 0 and 1) and end (day 55 and 56) of the experiment, with an interim weight collected on day 28.

Diets and Feeding

Each pen of cattle received a common diet *ad libitum* once daily, and the amount of feed offered to each pen was adjusted to minimize feed refusals. The diet was a total mixed ration (TMR) composed of corn silage, cracked corn, and a protein/mineral supplement (Table 1). The diet was formulated to meet the metabolizable energy and protein requirements of 227-kg calves gaining 1.09 kg/d. Rumensin® (Elanco, Greenfield, IN) was added to the diet to supply 400 mg Rumensin®/steer/d. Individual diet ingredients were sampled weekly, and composition of the TMR was adjusted to reflect changes in the DM content of those ingredients. Additionally, orts were determined weekly and a sub-sample collected and composited across treatments for DM analysis.

A ground corn meal carrier containing 0 or 5 x 10^8 CFU *Enterococcus faecium* and *Lactobacillus acidophilus* (Formula 120 G) per steer per day was topdressed over the TMR at the time of feeding. Direct-fed microbial supplements were mixed twice weekly to maintain the integrity of the microbes.

Identification and treatment of sick cattle

Cattle were examined daily at time of feeding throughout the experiment and removed from the pen and treated if the following criteria were met: 1) visual appearance of sickness (e.g., lethargic, emaciated, coughing, runny nose) and 2) a rectal temperature of 40˚ C or greater. Each animal was treated no more than three times during the experiment, and the order of medication was as follows: 1) a single, subcutaneous injection of Micotil® (10 mg/kg BW; Elanco, Greenfield, IN), 2) a single, subcutaneous injection of Nuflor® (40 mg/kg BW; Schering-Plough Animal Health, Summit, NJ), and 3) a single, subcutaneous injection of Liquamycin LA-200® (0.03 mL/kg BW; Pfizer Animal Health, Exton, PA) and a sulfa drug bolus (Sustain III®, one bolus/91 kg BW; Durvet Inc., Blue Springs, MO). These medications and dosage levels were sufficient to provide a 3-d treatment; therefore, it was required that cattle were non-responsive to a medication for greater than three days before they were eligible for subsequent treatment. If an animal appeared dehydrated at any time during the experiment, fluid therapy (Entrolyte® H.E., 178 g/trt; Pfizer Animal Health, Exton, PA) was administered. Cattle receiving the Battle® treatment (Table 2) were administered 52.8 g gel/steer during initial processing and during each medicated treatment. After all treatments, steers were returned to their pens.

Results and Discussion

The objective of the current study was to determine the effects on growth performance and health of stressed calves of a direct-fed microbial (DFM) supplement containing *Enterococcus faecium* and *Lactobacillus acidophilus* (Formula 120 G) and a

Table 1. Experimental diet.

Ingredient	% DM
Corn silage	48.50
Cracked corn	37.00
Corn gluten meal	11.00
Limestone	1.24
Dicalcium phosphate	0.70
Ground corn	0.50
Trace-mineralized salt[1]	0.50
Urea	0.32
Choice white grease	0.20
Vitamins A,D,E[2]	0.02
Rumensin-80	0.01

[1] 92.00% NaCl, 5,500 ppm Zn, 4,790 ppm Mn, 1,835 ppm Cu, 9,275 ppm Fe, 115 ppm I, 65 ppm Co, and 18 ppm Se.
[2] 8,800 IU/g vitamin A, 1,760 IU/g vitamin D, and 1.1 IU/g vitamin E.

Table 2. Battle® Gel ingredient composition.

Ingredient	% DM
Protein, µg/kg	134.77
Non-protein nitrogen, µg/kg	86.08
Sodium chloride, µg/kg	56.09
Calcium, µg/kg	10.08
Phosphorus, µg/kg	21.24
Sodium, µg/kg	15.99
Potassium, µg/kg	33.03
Sulfur, µg/kg	6.06
Magnesium, µg/kg	6.09
Zinc, mg/kg	2.67 x 103
Iron, mg/kg	174.70
Copper, mg/kg	2.71 x 103
Manganese, mg/kg	1.15 x 103
Cobalt, µg/kg	388.73
Selenium, mg/kg	12.57
Vitamin A, IU/kg	10.77 x 105
Vitamin D, IU/kg	3.67 x 105
Vitamin E, IU/kg	6.09 x 103
Thiamine (vitamin B1), mg/kg	11.11
Riboflavin (vitamin B2), mg/kg	16.61
Niacin (vitamin B3), mg/kg	21.99
Choline (vitamin B4), mg/kg	99.96
D-Pantothenic acid (vitamin B5), mg/kg	11.07
Pyridoxine (vitamin B6), mg/kg	48.91
Biotin (vitamin B7), mg/kg	112.63
Cyanocobalamin (vitamin B12), mg/kg	244.86

gel containing vitamin supplements and mineral-amino acid chelates (Battle®) that was administered orally (52.8 g gel/steer) upon arrival and when calves were eligible for subsequent treatment according to the established protocol. Based on previous observations using the stressed calf model, we showed there was no correlation between rectal temperature of calves upon arrival from the sale yard and subsequent incidence of BRD and/or elevated rectal temperature; i.e., calves did not consistently have elevated rectal temperatures both days (d 0 and 1) upon initiation of the experiment. This lack of correlation could be due to the residual effects of fescue toxicosis, as weaned calves in Central Kentucky are usually grazed on tall fescue pastures. Therefore, contrary to most treatment protocols for newly-weaned calves, steers in the current experiment were not administered the initial treatment of Micotil® for elevated rectal temperatures until d 6 of the experiment. The dispensation of DFMs and Battle® gel in this experiment had differential effects on growth performance, morbidity, and mortality. While steers receiving Formula 120 G demonstrated improvements in DMI and ADG, steers given Battle® had decreased ADG and greater morbidity. The reason for these discrepancies is unclear; however, most previous research involving administration of DFMs and vitamin/mineral supplements to weaned calves has shown variable responses, which are likely due to differences in previous nutrition and environment as well as feed intake.

Table 3. Effect of feeding Formula 120G and Battle® on growth performance of stressed calves.[a,b]

	-F120G		+F120G			P<		
Item	**-Battle**	**+Battle**	**-Battle**	**+Battle**	**SEM[c]**	**F120G**	**Battle**	**X[d]**
Period 1, 1-28 d								
Initial BW, kg	246	248	248	247	3.01	0.95	0.78	0.58
DMI, kg/d	4.92	4.62	5.44	5.15	0.16	0.05	0.18	0.98
ADG, kg/d	1.80	1.63	2.02	1.79	0.07	0.03	0.03	0.61
Gain: DMI, g/kg	366.70	354.86	369.00	348.68	0.01	0.89	0.30	0.76
Period 2, 29-56 d								
DMI, kg/d	8.44	8.38	8.79	8.51	0.17	0.27	0.41	0.57
ADG, kg/d	1.81	1.84	1.72	1.87	0.07	0.77	0.44	0.57
Gain: DMI, g/kg	215.74	219.64	196.34	218.83	0.01	0.43	0.32	0.46
0-56 d								
DMI, kg/d	6.68	6.50	7.12	6.83	0.14	0.04	0.12	0.65
ADG, kg/d	1.81	1.73	1.87	1.83	0.05	0.22	0.38	0.77
Gain: DMI, g/kg	270.29	267.20	262.36	268.00	0.01	0.56	0.83	0.48
Final BW, kg	347	347	352	350	3.69	0.49	0.84	0.86

[a] *F120G = 5 x 108 CFU* Lactobacilus acidophilus *and* Enterococcus faecium *per animal per day.*
[b] *Battle® = 52.8 g/steer administered upon arrival and during each medicated treatment.*
[c] *Standard error of the mean calculated from analysis of variance using n = 10.*
[d] *Interaction of F120G x Battle.®*

In the current experiment, calves receiving Formula 120 G had an 11% increase ($P \leq 0.05$; Table 3) in both DMI and ADG during the initial 28 days of the experiment relative to those receiving no Formula 120 G. Over the course of the entire experiment, DMI increased ($P = 0.04$) in these cattle by 6%, and mortality tended ($P = 0.08$; Table 4) to decrease from 8% to 3% compared to those steers fed no Formula 120 G. These improvements in growth performance agree with our previous observations in which cattle fed the same level of *Lactobacillus acidophilus* and *Enterococcus faecium* (5 x 10^8 CFU per animal per day) had improvements of 12% and 7% in DMI and ADG, respectively, over the course of a 63-d experiment with weaned steers and heifers. Other research has shown these microbes to decrease the occurrence of acidosis in feedlot cattle, presumably by assisting the rumen microflora in adapting to the presence of lactic acid. Although the mechanism by which *Lactobacillus acidophilus* and *Enterococcus faecium* improved growth performance in the current experiment is unknown, it has been suggested that these microbes elucidate their effects in the lower gut instead of in the rumen. *Lactobacillus acidophilus* produces lactic acid, which may lower small intestinal pH and thus inhibit the growth of pathogenic microbes. By inhibiting the growth of undesirable bacteria, beneficial microbes are allowed to flourish and facilitate digestion in the animal. These bacteria also produce nutrients such as vitamins, which can assist in stimulating the immune system of the stressed calf. In the current experiment, it is possible that the addition of *Lactobacillus acidophilus* and *Enterococcus faecium* to the diet of stressed calves improved DMI and ADG and decreased mortality by exerting effects in the lower gut and stimulating the immune system.

Calves receiving the Battle® treatment had an 11% decrease ($P = 0.03$; Table 3) in ADG during the initial 28 days of the experiment, and while DMI was unaffected ($P = 0.18$) by Battle® during this period, it tended ($P = 0.12$) to decrease by 0.24 kg/steer/d over the course of the entire experiment. This group also had 29% more animals treated during initial treatments on d 6 of the experiment (Table 4). These results were unexpected, as

Table 4. Effect of feeding F120G and Battle® on morbidity and mortality of stressed calves.[a,b]

		-F120G		+F120G			P <		
Item		**-Battle**	**+Battle**	**-Battle**	**+Battle**	**SEM[c]**	**F120G**	**Battle**	**X[d]**
No. of animals treated	1- time	38	36	24	38	7.11	0.30	0.30	0.19
	2- time	6	12	4	12	4.06	0.64	0.04	0.64
	3- time	4	8	4	0	3.00	0.31	1.00	0.31
d6 mass treatment		18	22	16	22	7.21	0.39	0.02	0.39
Total number of animals treated		48	56	32	50	11.47	0.20	0.15	0.52
Mortality, %		6.00	10.00	2.00	4.00	3.32	0.08	0.22	0.64

[a] *F120G = 5 x 108 CFU* Lactobacilus acidophilus *and* Enterococcus faecium *per animal per day.*
[b] *Battle® = 52.8 g/steer administered upon arrival and during each medicated treatment.*
[c] *Standard error of the mean calculated from analysis of variance using n = 10.*
[d] *Interaction of F120G x Battle.®*

previous observations using Battle® had shown improvements in growth performance as well as decreases in morbidity and mortality in "downer" dairy cows. While some studies supplementing various levels of B vitamins and vitamin E have shown improvements in ADG and decreases in the occurrence of BRD in weaned calves, other research using a supplement containing either 5 or 10 g/steer of B-vitamins and supplements containing vitamin E and/or selenium failed to alter BW gain, feed efficiency, or morbidity. Additionally, providing supplemental levels of minerals such as zinc, copper, and selenium can have beneficial effects on immunity and growth performance of stressed calves. However, the form (oxide vs. amino acid chelate) in which these minerals are presented to the calf can have profound effects on the availability and subsequent performance of the animal. For example, studies comparing the use of zinc methionine vs. zinc oxide and copper lysine vs. copper oxide have shown the amino acid-chelated forms of these minerals to result in greater improvements in DMI and ADG and decreased morbidity and development of BRD in weaned steers as assessed by an infectious bovine rhinotracheitis virus (IBRV) challenge. Steers receiving the Battle® treatment in the current experiment received most minerals in the form of amino acid chelates, which should result in high availability of these minerals to the animal. In conjunction with the supplemental A, D, E, and B vitamins in the Battle® gel, providing these minerals was expected to improve growth performance and decrease morbidity in weaned steers. Although the reason is unclear, this treatment had negative effects on ADG and morbidity in the current experiment. However, the lack of a positive effect could be related to the fact that the steers in this experiment received the Battle® gel only upon arrival and with administration of medicated treatments. This protocol differs from other research in which animals provided with additional vitamins and minerals receive the supplement daily in the total mixed ration. Therefore, it is possible that in the current experiment, steers receiving the Battle® treatment simply did not receive sufficient amounts of the supplemental gel to elicit positive physiological effects.

Implications

The results of the current experiment show that addition of *Lactobacillus acidophilus* and *Enterococcus faecium* to the diet of stressed calves improved DMI and ADG and decreased mortality. While the mechanism by which these improvements occurred is unknown, it is likely that these bacteria inhibited the growth of pathogenic bacteria and stimulated the immune system of the steers. The improvements in growth performance are in agreement with our previous observations using these DFMs in weaned calves; however, more research is needed to determine the mechanism by which these improvements occur. The reason for the negative effects of Battle® gel on ADG and morbidity is unclear, as previous observations had shown a decrease in morbidity of "downer" dairy cows. However, other research has shown variable responses of weaned calves to vitamin and mineral supplementation; additionally, it is possible that daily supplementation would elicit a positive growth response, whereas administration of the gel with medicated treatment alone had no positive effect.

Effects of Supplemental Slow-Release Urea vs. Normal Urea on Performance of Growing Beef Steers Fed Corn Silage

G. Hibbard, D.L. Harmon, K.R. McLeod, and E.S. Vanzant

Summary

One hundred and eighty Angus cross steers (330 ± 0.2 kg) were fed diets composed of corn silage and supplemented with urea or slow-release urea (SRU) to evaluate the effects on feed intake, gain, and gain efficiency. The experimental design was a randomized complete block design with a 2 x 4 + 1 factorial arrangement of treatments. Treatments included: 1) no supplemental urea, 2) 0.4% normal urea, 3) 0.4% slow-release urea, 4) 0.8% normal urea, 5) 0.8% slow- release urea, 6) 1.2% normal urea, 7) 1.2% slow-release urea, 8) 1.6% normal urea, and 9) 1.6% slow-release urea, each fed for 56 days. Over the entire 56-day experiment, steer gain increased with supplemental nitrogen and tended to be greater for urea than SRU; however, there was a urea source × level interaction. Gain of steers receiving urea increased with 0.4% dietary urea with little change through 1.6% urea. Gain for the SRU treatment increased at the 0.4 and 0.8% treatments and was maintained at 1.2% SRU, but it declined for the 1.6% treatment. Dry matter intake was not affected by urea source but increased with supplemental nitrogen and was consistent for all levels of nitrogen supplementation. Gain/feed increased with supplemental nitrogen and tended ($P < 0.09$) to be greater with urea than SRU; however, there was a urea source × level interaction. Gain/feed of steers receiving urea increased with 0.4% dietary urea, and there was little change through 1.6% urea. Gain/feed for the SRU treatment increased at the 0.4 and 0.8 treatments and was maintained at 1.2% SRU but it declined for the 1.6% treatment. This study did not show any advantage of the slow-release urea, and in fact, it was detrimental at the highest level of inclusion.

Introduction

Dietary urea is rapidly hydrolyzed upon entry into the rumen resulting in a rapid peak in rumen ammonia concentrations within the first hour after feed is consumed. Ruminal carbohydrate degradation and subsequent microbial growth is a much slower process, with peak activities occurring four to six hours post-feeding. For many years researchers have sought means to synchronize ruminal urea degradation with

carbohydrate availability. Various means of inhibiting ruminal urease activity have been tried but have provided only short-term regulation as the microorganisms adapt to the presence of the inhibitors. Alternatively, the urea could be modified to control its rate of release so that ammonia release more closely parallels carbohydrate digestion. A greater synchrony of these processes may improve the efficiency of incorporation of non-protein nitrogen into microbial protein and thereby improve the overall efficiency of nitrogen use, particularly for animals fed low-protein diets. Therefore, the objective of this experiment was to determine the effects of supplemental slow-release urea vs. normal urea on performance of the growing beef steers fed corn silage.

Materials and Methods

One hundred and eighty Angus cross steers (330 ± 0.2 kg; mean ± SEM) were fed diets composed of corn silage and supplemented with varying amounts of normal urea or slow-release urea to evaluate the effects on feed intake, gain, and gain efficiency. Animals were blocked by weight and feeding location and assigned randomly to one of the nine treatments (four steers per pen, five pens per treatment) in a randomized complete block design with a 2 x 4 + 1 factorial arrangement of treatments. Treatments included: 1) no supplemental urea, 2) 0.4% normal urea, 3) 0.4% slow-release urea, 4) 0.8% normal urea, 5) 0.8% slow-release urea, 6) 1.2% normal urea, 7) 1.2% slow-release urea, 8)1.6% normal urea, and 9) 1.6% slow-release urea. All diets (Table 1) contain 45% of corn silage produced in 2004, 45% of corn silage produced in 2005, and 10% supplement (all components on a dry-matter basis). The corn silage produced in 2005 was drought-stressed and had minimal grain content. The intermediate urea levels were accomplished by blending proportions of the control and the 1.6% urea treatment supplements. Diets were fed daily as a completely mixed ration to achieve *ad libitum* intakes.

Animals were weighed on days 0, 1, 28, 55, and 56. Initial and final weights were taken over two days and averaged. Blood samples were collected by jugular venipuncture on day 28, and serum was collected for urea nitrogen analysis.

Total feed offered was measured daily. Feed refusals were collected from feed bunks every Tuesday morning before the steers were fed. The feed refusals from each treatment were weighed and a sample collected and composited within each treatment and analyzed for DM. Feed ingredients were analyzed for DM content and diets adjusted accordingly every Tuesday to correspond with weigh days and collection of the feed refusals. Samples for diet composition were collected weekly and composited for the experiment and analyzed for dry matter, crude protein, NDF, and ADF.

The data were analyzed as a randomized complete block design with a 2×4+1 factorial arrangement of treatments. To accomplish this analysis, data were first analyzed as a randomized complete block design with nine treatments using a model that included just block and treatment effects. The data were then analyzed as a 2×4 factorial structure by eliminating the basal control treatment, and the treatment sums of squares were partitioned into urea source, level of urea, and their interaction. These factors were tested using the error terms from the analysis including all nine treatments, and the F-test and probabilities were computed by hand. The effects of urea level were tested for linear, quadratic, and cubic effects using contrast statements; however, when a level-by-urea-source interaction occurred, the linear, quadratic, and cubic effects within each urea source were determined using contrast statements. Differences were considered significant when $P < 0.05$ occurred.

Results and Discussion

Diet compositions are listed in Table 1. We collected weekly samples of all ingredients and composited those for analysis at the completion of the experiment. The crude protein concentrations obtained were consistent between the two urea sources, with diets ranging from 9.1 to 12.3% crude protein.

As designed, initial body weights were not different between treatments (Table 2). However, final body weights increased quadratically ($P < 0.001$) with increasing urea supplementation.

During the initial 28 days of the experiment gain was not affected by urea source, but gain did increase quadratically ($P < 0.001$) with increasing urea supplementation. This increased gain with urea supplementation was accompanied by a linear ($P < 0.01$) increase in dry matter intake (DMI) as urea supplementation increased. Gain/feed also increased quadratically ($P < 0.001$) with level of supplementation. These results demonstrate a response to supplemental nitrogen and show that nitrogen was limiting growth.

During days 29-56 of the experiment, gain was lower than the initial 28 days, probably because of the influences of gastrointestinal tract fill during the initial 28 days; however, the patterns of response to treatment were very similar. Gain increased quadratically ($P < 0.001$) with increasing urea supplementation;

Table 1. Composition of experimental diets fed to steers to compare urea or slow-release urea (SRU) addition.

Feedstuff	Control	0.4 Urea/ SRU	0.8 Urea/ SRU	1.2 Urea/ SRU	1.6 Urea/ SRU
Corn silage (2004)	45.00	45.00	45.00	45.00	45.00
Corn silage (2005)	45.00	45.00	45.00	45.00	45.00
Ground corn	6.93	6.53	6.13	5.73	5.33
Dicalcium phosphate	0.30	0.30	0.30	0.30	0.30
Limestone	0.44	0.44	0.44	0.44	0.44
Soybean meal	1.00	1.00	1.00	1.00	1.00
Trace mineral-salt	0.50	0.50	0.50	0.50	0.50
Feed grade fat	0.30	0.30	0.30	0.30	0.30
Deccox 10	0.50	0.50	0.50	0.50	0.50
Vitamin pre-mix	0.03	0.03	0.03	0.03	0.03
Urea or SRU	0.00	0.40	0.80	1.20	1.60
Composition[a]					
Crude protein-urea	9.13	9.91	10.70	11.48	12.26
Crude protein-SRU		9.88	10.63	11.38	12.13

[a] Composition values (% of diet dry matter) represent values from composite samples taken throughout the study.

Table 2. Body weights, growth performance, dry matter (DM) intake, and 28-d blood urea concentration from steers consuming silage-based diets supplemented with urea or slow-release urea (SRU).

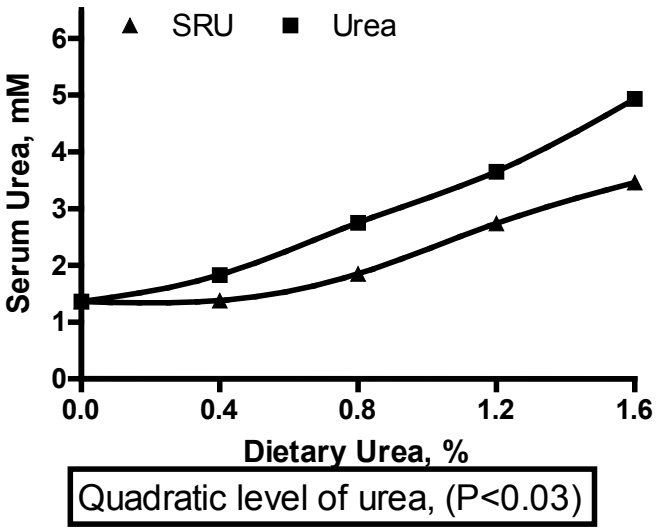
	Treatment										Main Effects, P<			Contrasts, P<[a]		
Item	Control	Urea 0.4 Urea	SRU 0.4 SRU	Urea 0.8 Urea	SRU 0.8 SRU	Urea 1.2 Urea	SRU 1.2 SRU	Urea 1.6 Urea	SRU 1.6 SRU	SEM	Urea	Level	Urea* Level	L	Q	C
Initial weight, kg	326	332	330	330	332	330	331	332	330	2.27	0.92	0.99	0.79	.10	.14	.21
Final weight, kg	373	396	386	395	398	395	398	398	388	3.79	0.23	0.39	0.15	.00	.00	.32
Days 1-28																
Gain, kg/d	1.33	1.50	1.48	1.59	1.58	1.57	1.64	1.55	1.41	0.068	0.56	0.16	0.54	.03	.00	.60
DM Intake, kg/d	7.10	7.50	7.39	7.63	7.74	7.52	7.67	7.62	7.62	0.163	0.75	0.52	0.85	.01	.06	.57
Gain/Feed,	18.58	19.88	20.00	20.86	20.37	20.92	21.27	20.19	18.48	0.700	0.38	0.09	0.47	.16	.00	.35
Days 29-56																
Gain, kg/d	0.36	0.77	0.53	0.74	0.78	0.74	0.77	0.82	0.64	0.062	0.06	0.28	0.06	.00	.00	.26
DM Intake, kg/d	6.62	7.57	7.01	7.65	7.31	7.65	7.52	7.67	7.37	0.167	0.01	0.35	0.64	.00	.01	.45
Gain/Feed,	5.53	10.14	7.55	9.55	10.69	9.68	10.23	10.87	8.75	0.760	0.17	0.39	0.05	.00	.00	.26
Days 0-56																
Gain, kg/d	0.38	0.52	0.46	0.53	0.54	0.53	0.55	0.54	0.47	0.019	0.07	0.03	0.04	.00	.00	.68
DM Intake, kg/d	6.87	7.54	7.20	7.64	7.53	7.58	7.60	7.65	7.49	0.153	0.19	0.42	0.72	.00	.01	.47
Gain/Feed,	5.61	6.82	6.34	6.93	7.13	6.95	7.18	7.04	6.24	0.17	0.09	0.01	0.01	.00	.00	.90

[a] *Main effect contrasts refer to linear (L), quadratric (Q) and cubic (C) contrasts across levels of supplemental urea.*

however, there was a tendency ($P < 0.06$) for an interaction with level of supplementation and urea source. Steers receiving urea responded cubically ($P < 0.01$) to increasing urea, while steers receiving SRU responded quadratically ($P < 0.01$). Gain of steers receiving urea increased from control to 0.4% dietary urea, whereas gain was slightly lower for the two intermediate levels of supplementation before increasing at the 1.6% level. Gain for the SRU treatment increased at the 0.4 and 0.8 treatments, was maintained at 1.2% SRU, but declined for the 1.6% treatment. A like pattern of response was seen in gain/feed (urea × level, $P < 0.05$) during days 29-56. Efficiency of gain for steers receiving urea was increased with the 0.4% urea level being lower than the next two levels and the 1.6% level showing the greatest response (cubic, $P < 0.01$). Efficiency of gain for the SRU treatment increased at the 0.4 and 0.8 treatments, was maintained at 1.2% SRU, but declined for the 1.6% treatment (quadratic, $P < 0.001$).

Over the entire 56-day experiment, steer gain increased with supplemental nitrogen (quadratic, $P < 0.001$) and tended ($P < 0.07$) to be greater for urea than SRU; however, there was a urea source × level interaction ($P < 0.03$). Gain of steers receiving urea increased with 0.4% dietary urea with little additional change through 1.6% urea (cubic $P < 0.03$). Gain for the SRU treatment increased at the 0.4 and 0.8 treatments, was maintained at 1.2% SRU, but declined for the 1.6% treatment (quadratic, $P < 0.001$). Dry matter intake was not affected by urea source but increased with supplemental nitrogen and was consistent for all levels of nitrogen supplementation (quadratic, $P < 0.001$). Since feed intake was consistent across source and amount of nitrogen supplementation, gain/feed closely paralleled the changes seen in gain. Gain/feed increased with ($P < 0.001$) supplemental nitrogen and tended ($P < 0.09$) to be greater with urea than SRU; however, there was a urea source × level interaction ($P < 0.01$). Gain/feed of steers receiving urea increased with 0.4% dietary urea, and there was little change through 1.6% urea (cubic, $P < 0.04$). Gain/feed for the SRU treatment increased at the 0.4 and 0.8 treatments, was maintained at 1.2% SRU, but declined for the 1.6% treatment (quadratic, $P < 0.001$).

Figure 1. Serum urea concentrations of steers fed either urea or slow-release urea (SRU) at 28 days of experiment.



Blood samples were collected at day 28 to assess serum urea concentrations as an indicator of nitrogen intake and availability (Figure 1). Serum urea concentrations increased with increasing nitrogen supplementation (quadratic, $P < 0.03$) and were greater ($P < 0.001$) for urea than SRU. These data demonstrate the difference in nitrogen availability between urea and SRU and show that we did obtain the predicted differences in nitrogen intake that were sought.

Our aim was to test if SRU would be used more efficiently as a source of supplemental nitrogen. If nitrogen is limiting growth(and it clearly was in this study, since animals responded to supplemental nitrogen with increased feed intake, gain, and

gain/feed), then a more efficient source of supplemental nitrogen would increase performance at a lower level of inclusion. One concern would be that our lowest inclusion gave near maximal response. However, at the 0.4% inclusion the response to SRU was generally lower than urea, thus we believe we had a valid test between the two nitrogen sources.

One intriguing aspect of the results of this experiment was the reduced performance for the SRU treatment at the 1.6% inclusion. The reduced performance was seen throughout the experiment and was unrelated to feed intake, as gain/feed was reduced, as was gain. If nitrogen was limiting, one would expect that increased supplementation would relieve that deficiency, not exacerbate it. It remains unclear whether this group of animals was just more poorly performing by chance or increased supplementation of SRU somehow changed the digestion dynamics and thus the nitrogen availability.The blood samples collected at day 28 suggest that nitrogen availability was as expected, thus the reasons for this response remain unclear.

Implications

The aim of this experiment was to test the slow-release nitrogen source in comparison to urea. We thought that if the slow-release urea were used more efficiently it would improve animal growth at lower amounts of inclusion. This study did not show any advantage of the slow- release urea, and in fact it was detrimental at the highest level of inclusion.

Distiller's Grains and a Direct-Fed Microbial for Finishing Beef Steers

E.S. Vanzant, F. Korthaus, K.R. McLeod, G. Rentfrow, J.W. Lehmkuhler, and K.K. Kreikemeier

Summary

Regardless of level of inclusion of modified distiller's grains, including a direct-fed microbial product in the diet for rapidly gaining steers resulted in a boost in gain of about 0.25 lb/d. Gain and dry matter intake were maximized when modified distiller's grains were included in the diet at 20% of the DM as a replacement for the grain portion of the diet. Carcass characteristics were generally unaffected by either direct-fed microbials or distiller's grains.

Results of this study provide guidelines for cattle feeders interested in taking advantage of increased availability of distiller's by-products and current direct-fed microbial technologies.

Introduction

Increased use of corn for ethanol production has resulted in large quantities of distiller's grains available for incorporation into livestock diets over the past several years. Distiller's grains are available in a variety of forms, particularly varying in their moisture content. Considerable research has been conducted with both wet and dry distiller's grains. Less research has been conducted with modified distiller's grains (MDG), which contain about 50% moisture and are becoming an increasingly popular marketed product of distilleries.

Additionally, the inclusion of direct-fed microbial (DFM) products is receiving much interest in finishing diets for cattle, in part because of public concern over the use of antibiotics and other growth promotants. Direct-fed microbials are preparations of live microorganisms that have been shown to improve performance in animals, particularly those under stress.

Benefits of DFM are usually thought to be related to effects on gut microbial populations (both ruminal and postruminal). However, diet composition may also be important with respect to effects of DFM. Diet composition could interact with DFM by directly affecting the substrates available for microbial growth or through effects on ruminal pH and concentrations of fermentation end-products that could influence the ability of the introduced microorganisms to become established. Additionally, the extent of biohydrogenation of fatty acids can be affected by ruminal microbial populations, pH, and other ruminal factors that could be directly or indirectly affected by introduction of DFM. Thus, it is feasible that DFM inclusion could interact with dietary lipids to influence the fatty acid composition of the carcass.

Little research has been done to evaluate potential interactions between DFM and dietary ingredients on animal performance or carcass characteristics. Thus, this study was conducted to assess the impact of increasing quantities of MDG and potential interactions between MDG and DFM inclusion on performance and carcass characteristics of beef steers consuming high moisture, corn-based finishing diets.

Materials and Methods

Animals and Feeding

One hundred ninety-two crossbred steers (851 lb) were used in a 112-d finishing study with a 3 x 2 factorial arrangement of treatments. Treatments were 0, 20 or 40% modified distiller's grains plus solubles (MDG) with or without added DFM (Vit-E-Men 10-G). Cattle were randomly assigned to one of 48 pens (4 hd per pen), within weight strata, and pens were randomly assigned to treatment. Cattle were vaccinated (Vista Once-SQ, 20/20 Vision 7 with SPUR) and drenched with an oral anthelmintic (Safe-Guard) prior to the start of the study and implanted (Revalor®-S) on day 28. Initial and final body weights were determined by weighing steers on two consecutive days, and interim weights were obtained every 28 days.

Diets (Table 1) were formulated to be isocaloric and balanced to meet requirements of steers growing at 3.5 lb/d. Steers were adapted to the final diets across 21 d. In the MDG diets, MDG replaced high moisture corn (HMC) and cracked corn. The ratio of high moisture corn to cracked corn was 70:30. Two different supplements were used, one for the control diet and one for the 20 and 40% MDG diets. The control supplement was a 51% CP supplement based on corn gluten meal and urea (NPN provided 39% of the supplement CP) designed to equalize the dietary CP of the control diet with the 20% MDG diet (Table 2). The MDG supplement contained 72% corn gain. Supplements included monensin, tylosin, and thiamine (to reduce the risk of polioencephalomalacia, which was not observed in this study). Limestone was incorporated in the supplements at a level to maintain Ca concentrations at 0.8% of diet DM.

Steers were fed once daily at approximately 0800 h and given unlimited access to water. Five-hundred grams of ground corn carrier either with or without DFM was topdressed over the experimental diets at the time of feeding.

Samples of feed ingredients were collected once weekly. Feed refusals from each pen, when present, were weighed weekly, and composite samples for each treatment group were collected for nutrient analysis.

Laboratory Analyses

In addition to routine nutrient analysis of feedstuffs, ruminal protein degradability of feedstuffs was determined by an *in situ* technique in which feed samples were incubated, in porous Dacron® bags, in the rumens of steers fed a 75% forage, 25% concentrate diet. Microbial N correction was accomplished through use of an ^{15}N isotope infusion.

Steers were transported to a commercial processing facility in Joslin, IL and harvested according to humane slaughter procedures. Carcass data were collected following a 24h chill (4° C) using a CVSBeefCam®. Yield grade was calculated using measurements recorded by the beef camera and kidney, pelvic and heart fat (KPH) was determined by a certified USDA meat grader.

Table 1. Ingredient composition of experimental diets (% of DM).

Ingredient	0% MDG	20% MDG	40% MDG
Corn silage	5	5	5
Alfalfa haylage	5	5	5
High moisture corn	56	43	28
Cracked corn	24	17	12
Supplement	10	10	10
MDG	-	20	40

Statistical Analysis

Data were analyzed with the SAS GLM procedure as a randomized complete block design. Pen was used as the experimental unit. Model terms included weight block, DFM, MDG, and the DFM x MDG interaction. For MDG effects, means were separated using linear and quadratic contrasts. In the presence of interactions, Fisher's protected ($P < 0.10$) LSD were used to separate treatment means.

Results and Discussion

There were no interactions ($P > 0.19$) for any of the reported response variables. Therefore, tables show main effect responses. Additionally, the lack of interactions indicates that, within the range of dietary differences induced in this study, diet composition did not influence the response to DFM. Actual fat content and protein degradability of the MDG differed substantially from the pre-study estimates. Thus, measured fat content and protein degradability values of the complete rations differed somewhat from target values (Table 2). Cattle gains were unaffected by level of MDG for the first eight weeks of the study, decreased linearly with increasing MDG during the next four-week period, and increased linearly with MDG during the final four weeks of the study (Table 3). Combined across the 112-d finishing period, MDG inclusion resulted in a quadratic effect on gain, with maximum gain with 20% MDG inclusion. Conversely, quadratic effects were evident for dry matter intake (DMI) during the first 12 weeks, with no detectable effect of MDG during the final week of the study. As seen with gain, intake responded quadratically across the entirety of the feeding period, with maximal intake with 20% MDG. Consequently, gain:feed ratios were unaffected by level of MDG.

Table 2. Nutrient composition of experimental diets.

Nutrient	Diet		
	0% MDG	20% MDG	40% MDG
Formulated Composition			
NEm, Mcal/lb	0.93	0.92	0.91
CP, % of DM	14.2	14.2	18.6
DIP[a], % of CP	63.6	50.4	43.4
NPN[b], % of CP	13.9	-	-
Crude fat, % of DM	4.1	5.9	7.6
Ca, % of DM	0.80	0.80	0.80
P, % of DM	0.35	0.51	0.67
Ca: P	2.3	1.6	1.2
Analyzed Composition			
CP, % of DM	14.6	14.9	17.0
DIP[a], % of CP	69.7	70.1	64.1
Crude fat, % of DM	3.00	4.23	5.48

[a] *DIP = degradable intake protein.*
[b] *non-protein nitrogen.*

Depressions in intake and consequent effects on gain with high levels of MDG are likely explained by the high fat concentrations typical of distiller's grains, although high sulfur concentrations may also contribute to such effects. The MDG used in this study contained 9.4% fat and 0.8% S (both on DM basis). In general terms, responses from this study agree with literature reports for both wet and dry distillers' grains, in which maximal gain and intake have been found to occur with around 20 to 30% inclusion rates.

Gains were numerically greater with DFM inclusion in all periods, although significance was only detected in the second

Table 3. Effect of modified distiller's grains on growth and dry matter intake by finishing steers.

Item	MDG, % DM			SEM[b]	P-Value[a]	
	0	20	40		Linear	Quadratic
Initial BW, lb	869	869	871		-	-
Final BW, lb	1340	1369	1356	11.7	0.41	0.16
BW gain, lb, d-1						
d0 – d28	2.91	3.15	2.87	0.179	0.80	0.22
d28 – d56	5.56	5.80	5.84	0.152	0.20	0.58
d56 – d84	5.00	4.98	4.63	0.134	0.06	0.31
d84 – d112	3.40	3.92	3.95	0.176	0.03	0.25
d0 – d112	4.21	4.48	4.32	0.088	0.45	0.09
DMI, lb, d-1						
d0 – d28	19.0	19.8	18.8	0.33	0.68	0.03
d28 – d56	23.2	24.2	23.6	0.29	0.32	0.02
d56 – d84	25.6	27.0	26.0	0.33	0.44	<0.01
d84 – d112	25.6	26.5	26.4	0.53	0.25	0.42
d0 – d112	23.4	24.5	23.8	0.26	0.34	0.01
Gain: Feed	0.181	0.184	0.183	0.0031	0.64	0.63

[a] *Probability of a greater F-statistic. MDG x DFM interaction (P ≥ 0.48) for all response variables.*
[b] *SEM = standard error of the mean, n = 16 pens.*

Table 4. Effect of direct-fed microbial on growth and dry matter intake by finishing steers.

Item	DFM (-)	DFM (+)	SEM[a]	P-Value[b]
Initial BW, lb	869	871	-	-
Final BW, lb	1340	1369	9.5	0.04
BW gain, lb, d-1				
d0 – d28	2.91	3.06	0.146	0.46
d28 – d56	5.58	5.89	0.126	0.08
d56 – d84	4.81	4.94	0.110	0.45
d84 – d112	3.59	3.92	0.143	0.11
d0 – d112	4.21	4.45	0.077	0.04
DMI, lb, d-1				
d0 – d28	19.1	19.3	0.26	0.68
d28 – d56	23.7	23.7	0.22	0.94
d56 – d84	26.0	26.4	0.26	0.31
d84 – d112	25.6	26.7	0.44	0.81
d0 – d112	23.6	24.0	0.22	0.18
Gain: Feed	0.179	0.186	0.0025	0.07

[a] *SEM = standard error of the mean, n = 24 pens.*
[b] *Probability of a greater F-statistic. MDG x DFM interaction (P ≥ 0.48) for all response variables.*

Table 5. Effect of modified distiller's grains on carcass characteristics of finishing steers.

	MDG, % DM Basis				P-Value[a]	
Item	0	20	40	SEM[b]	Linear	Quadratic
HCW, lb	816	831	820	7.1	0.57	0.12
REA, in^2	13.0	13.3	13.1	0.14	0.86	0.17
Fat depth, in	0.47	0.49	0.49	0.020	0.51	0.62
KPH, %	2.01	1.98	1.94	0.03	0.11	0.96
Marbling[c]	270	252	246	10.5	0.12	0.66
Yield grade	3.01	3.03	3.05	0.08	0.70	1.00
Quality grade[d]	4.16	3.93	3.98	0.12	0.31	0.37

[a] *Probability of a greater F-statistic. MDG x DFM interaction (P ≥ 0.19) for all response variables.*
[b] *SEM = standard error of the mean, n = 16 pens.*
[c] *100 = slight, 200 = small, 300 = modest.*
[d] *Standard = 1, select (-) = 2, select (+) = 3, choice (-) = 4, choice (°) = 5, choice (+) = 6.*

Table 6. Effect of direct-fed microbial on carcass characteristics of finishing steers.

Item	DFM (-)	DFM (+)	SEM[a]	P-Value[b]
HCW, lb	814	833	6.0	0.03
REA, in^2	13.0	13.3	0.12	0.08
Fat depth, in	0.48	0.49	0.016	0.59
KPH, %	2.0	2.0	0.020	0.93
Marbling[d]	258	254	8.5	0.74
Yield grade	3.03	3.04	0.077	0.92
Quality grade[e]	4.08	3.96	0.12	0.39

[a] *SEM = standard error of the mean, n = 24 pens.*
[b] *Probability of a greater F-statistic. MDG x DFM interaction (P ≥ 0.19) for all response variables.*
[d] *100 = slight, 200 = small, 300 = modest.*
[e] *Standard = 1, select (-) = 2, select (+) = 3, choice (-) = 4, choice (°) = 5, choice (+) = 6.*

four-week interval, and in the 112-d overall response (Table 4). Across the whole feeding period, DFM improved gains by 0.24 lb/d. This improvement was a consequence of increased efficiency, as DFM inclusion did not affect DMI. Typically, DFM are expected to offer benefits when animals are stressed. However, these results demonstrate the potential of DFM to stimulate growth in already rapidly gaining animals. The lack of DMI response also indicates that effects were not mediated through the control of subacute acidosis as some have suggested.

Carcass traits were unaffected by MDG (Table 5), and the only carcass-trait responses seen in response to DFM inclusion were those associated with the larger finished weight of the cattle receiving DFM (i.e., increased carcass weight and ribeye area; Table 6).

Implications

An increase in gain of about a quarter pound per day in response to DFM occurred independently of the inclusion level of MDG (from 0 to 40% of the diet DM). Furthermore, DFM increased gain and efficiency in cattle, which were gaining rapidly (4.2 lb/d) without dietary DFM addition. This indicates a greater potential role for DFM than suggested by much previous work, which has indicated that benefits were only likely in sick or stressed cattle.

Further, results from this study indicate that, when replacing the grain portion of the diet, maximal gain and intake would be expected with about a 20% inclusion level of MDG.

Effects of Chlortetracycline and Revalor®-S on Growth Performance and Carcass Quality Traits of Finishing Beef Steers

S.E. Kitts, D.L. Harmon, E.S. Vanzant, and K.R. McLeod

Summary

The objective of the current study was to ascertain the effects of chlortetracycline (CTC) and Revalor®-S, both alone and in combination, on growth performance and carcass merit of finishing beef steers. Revalor®-S increased ADG over the course of the finishing period as expected; however, the positive effect of implant on feed efficiency was partially attenuated in the presence of CTC. This attenuation appears to be a function of both DMI and ADG. Although CTC reduced DMI, the putative mechanism responsible for this interaction is unclear; it appears to be manifested through changes in both DMI and ADG, neither of which are mutually exclusive variables. Additionally, inconsistent with previous observations, carcass quality traits in the current experiment were not affected by either Revalor®-S or CTC. The fact that Revalor®-S did not negatively affect carcass quality shows that growth implants containing estrogen + synthetic androgens positively affect growth performance while not discounting carcass value. These data clearly illustrate the need for further research to identify potential interactions between anabolic implants and CTC regarding feedlot performance.

Introduction

Sub-therapeutic feeding of chlortetracycline (CTC) has been shown to have growth-promoting effects for ruminants, swine, and poultry, but the mechanisms responsible for these effects are not known. Most hypotheses for growth promotion by antibiotics in ruminants relate to effects on digestive tract microorganisms or gut wall thinning. Based on the effects of CTC on carcass composition of calves, it has been suggested that CTC may influence growth via an endocrine axis. Previously we have shown that chronic, oral administration of 350 mg CTC/steer/day elevated circulating IGF-1 concentrations and reduced plasma concentrations of growth hormone (GH), thyroid-stimulating hormone (TSH), and thyroxine (T_4) following injection of thyrotropin-releasing hormone (TRH) and growth hormone-releasing hormone (GHRH) in beef steers. Corresponding with these shifts in circulating hormone concentrations and sizes of the releasable pools were increases in both subcutaneous and intramuscular fat deposition. However, more recently we showed that oral administration of CTC over a 112-d period did not attenuate the release of GH or TSH in response to TRH + GHRH

challenges conducted at d 30, 56, and 106, and although CTC had no effect on subcutaneous fat deposition, intramuscular fat deposition tended to be greater in CTC-fed steers.

Implants containing estradiol and either progesterone or trenbolone acetate are used in finishing beef steers to improve feed efficiency and enhance lean tissue growth. Research has indicated increases in hot carcass weight (HCW), improved average daily gain (ADG), and feed efficiency as well as greater longissimus dorsi areas with the use of anabolic implants in finishing programs for beef cattle. However, it has also been demonstrated that marbling scores are lower for cattle receiving growth implants, resulting in a lower percentage of carcasses grading Choice. Current carcass pricing grids provide incentive for the development of nutritional strategies to improve the carcass grades of finished cattle from Select to Choice (Select = slight amount of intramuscular fat, Choice = moderate amount of intramuscular fat).

Although research has shown effects of CTC and anabolic implants on growth in cattle, there is a paucity of information on effects of CTC on growth performance and carcass characteristics when fed in conjunction with anabolic implants. Therefore, the objective of the current experiment was to determine if CTC and an anabolic implant containing estradiol benzoate + trenbolone acetate interact to affect growth performance and carcass characteristics of finishing beef steers. Specifically, we challenged the proclivity of CTC to promote marbling using an aggressive implant strategy that would enhance protein accretion and tend to oppose intramuscular fat deposition.

Materials and Methods

Animals and Treatments

The protocol for the research discussed in this report was approved by the University of Kentucky Institutional Animal Care and Use Committee. Ninety-six English-Continental crossbred steers were purchased from a commercial sale yard in Central Kentucky. After arriving at the University of Kentucky Animal Research Center, steers were dewormed using ivermectin (Merial, Duluth, GA), and vaccinated using Bovi-Shield™4 and Ultrabac®7 (Pfizer Animal Health, Exton, PA). Steers were housed in group pens (five steers per pen) for a 56-d backgrounding period during which they had *ad libitum* access to a 65:35 concentrate-forage diet. The group pens measured 14.6 x 2.4 m and were located on a concrete pad partially covered with a roof. The steers had continuous access to automatic waterers.

After the backgrounding period, steers were limit-fed two transition diets for an additional 30 d at 90% of the previous *ad libitum* intake. These transition diets consisted of 75:25 and 85:15 concentrate-forage, respectively, and were fed for adjustment to *ad libitum* intake of the experimental diet (Table 1). *Ad libitum* intake of the experimental diet was established incrementally over a 7-d period during the transition period, immediately prior to beginning the experiment. Steers were blocked by body weight (BW; six blocks) and assigned randomly to pen within their respective block. Pens were assigned randomly to a 2 x 2 factorial arrangement of treatments within block. Treatments included feed containing either 0 or 39.6 ppm (DM basis) CTC (Aureomycin, Alpharma Animal Health, Fort Lee, NJ) and Revalor®-S or no Revalor®-S (120 mg trenbolone acetate + 24 mg 17-β estradiol benzoate, Intervet Inc., Millsboro, DE). The level of CTC used in this study provided approximately 350 mg CTC/steer/d and was the same as the level used in previous experiments. Steers assigned to receive Revalor®-S were implanted on d 1 and re-implanted on d 63. The experimental diet was formulated using two protein supplements: protein 1 was formulated to provide 105% of the metabolizable protein (MP) requirement for large-frame steers (345 kg BW) gaining 1.60 kg/d and was fed until d 63 of the experiment; Protein supplement 2 was formulated to provide 105% of the MP requirement for large-frame steers (450 kg BW) gaining 1.20 kg/d and was fed from d 63-125 or 139 (NRC, 2000). Steers were fed daily at 0900. Once weekly, orts were measured and the amount of feed offered was adjusted to maintain approximately 10% orts. Individual diet ingredients were sampled weekly and analyzed for DM content. Weekly determinations of DM content were used in the adjustment of the amount of feed offered the following week.

Table 1. Experimental diets.

	d 0-62		d 63-139	
Ingredient, % DM	**-CTC**	**+CTC**	**-CTC**	**+CTC**
High moisture corn	53.90	53.90	53.90	53.90
Cracked corn	19.57	19.57	19.57	19.57
Alfalfa haylage	10.06	10.06	10.06	10.06
Corn silage	5.03	5.03	5.03	5.03
Feather meal	3.52	3.52	2.13	2.13
Corn gluten meal	2.80	2.80	1.68	1.68
Ground corn	3.19	3.17	5.59	5.57
Limestone	1.12	1.12	1.12	1.12
Urea	- - -	- - -	0.11	0.11
Trace mineralized salt[1]	0.50	0.50	0.50	0.50
Choice white grease	0.28	0.28	0.28	0.28
Vitamins A,D,E[2]	0.03	0.03	0.03	0.03
Aureomycin-90[3]	- - -	0.02	- - -	0.02

[1] 92.00% NaCl, 5,500 ppm Zn, 4,790 ppm Mn, 1,835 ppm Cu, 9,275 ppm Fe, 115 ppm I, 65 ppm Co, and 18 ppm Se.
[2] 8,800 IU/g vitamin A, 1,760 IU/g vitamin D, and 1.1 IU/g vitamin E.
[3] Added to supply 350 mg of CTC per day per steer.

Body weights were measured every 28 d before feeding. Initial and final BW were determined by weighing steers on two consecutive days. Ultrasound was used on a subset of steers (approximately 8-10 steers) from the heaviest blocks (blocks 5 and 6) to determine the amount of subcutaneous fat over the 12th rib on d 118. Because these steers met or exceeded 12 mm of backfat, it was determined that they had completed the finishing phase. On d 125, these steers were transported to a commercial slaughter facility and killed the following day. Subsequently, the remaining four blocks of steers completed the finishing phase on d 139 and were killed on d 140. A merit evaluation of each carcass was done according to USDA standards and performed by a qualified meat scientist the following day. Carcass quality indicators were longissimus muscle area, fat over longissimus muscle, kidney, pelvic, and heart fat (KPH), marbling, and bone maturity.

Results and Discussion

The objective of the current experiment was to determine if CTC and an anabolic implant containing estradiol benzoate + trenbolone acetate interact to affect growth performance and carcass characteristics of finishing beef steers. Because it has been demonstrated that CTC has the ability to increase subcutaneous and intramuscular fat deposition and anabolic implants containing trenbolone acetate + estradiol benzoate have been shown to reduce marbling score and the percentage of carcasses grading Choice, it is of interest to determine if CTC can transcend the antagonistic effects of an anabolic implant and increase the deposition of intramuscular fat. Over the course of the entire experiment, implanted steers had greater ADG; however, an interaction between CTC and implant for feed efficiency revealed that the presence of CTC slightly attenuated the response to implantation. Furthermore, this interaction was a result of treatment effects that occurred late in the finishing period, specifically in the last 27 d. There were no effects of CTC or Revalor®-S on carcass characteristics, most notably those involving fat deposition. These results are inconsistent with our hypothesis, considering that previous research has shown CTC and Revalor®-S to positively and negatively affect fat deposition, respectively.

Table 2. Effects of oral chlortetracycline (CTC) and Revalor®-S on dry matter intake (DMI), average daily gain (ADG), and efficiency of gain of finishing beef steers.[a]

	-CTC		+CTC			P <		
Item	-Implant	+Implant	-Implant	+Implant	SEM[b]	CTC	Implant	X[c]
Period 1, 0-28 d								
Initial BW, kg	401	402	400	401	1.55	0.11	0.53	0.75
DMI, kg/d	8.95	8.15	8.41	8.24	0.23	0.35	0.05	0.19
ADG, kg/d	1.90	2.12	1.74	2.19	0.26	0.72	0.01	0.37
Gain: DMI, g/kg	211.40	261.93	208.62	265.55	11.88	0.97	0.0004	0.79
Period 2, 29-56 d								
DMI, kg/d	9.61	9.51	9.01	9.06	0.18	0.01	0.91	0.68
ADG, kg/d	1.42	1.83	1.36	1.67	0.22	0.28	0.003	0.64
Gain: DMI, g/kg	149.29	192.84	151.28	185.83	11.48	0.83	0.004	0.70
Period 3, 57-84 d								
DMI, kg/d	9.15	8.94	8.33	9.07	0.29	0.25	0.38	0.12
ADG, kg/d	1.37	1.61	1.05	1.58	0.25	0.14	0.004	0.21
Gain: DMI, g/kg	150.60	180.03	126.12	173.61	9.64	0.13	0.001	0.36
Period 4, 85-112 d								
DMI, kg/d	8.78	9.26	9.05	9.52	0.23	0.28	0.06	0.98
ADG, kg/d[de]	1.15	1.90	1.62	1.88	0.26	0.08	0.0006	0.06
Gain: DMI, g/kg[de]	130.69	214.78	179.39	195.92	11.15	0.20	0.0004	0.008
Period 5, 113-139 d								
DMI, kg/d	9.11	10.70	9.50	10.33	0.36	0.97	0.005	0.32
ADG, kg/d[d]	0.93	1.65	1.14	1.21	0.36	0.51	0.03	0.06
Gain: DMI, g/kg	103.33	160.34	123.27	120.17	15.36	0.52	0.10	0.07
0-139 d								
DMI, kg/d	9.28	9.34	8.82	9.02	0.15	0.02	0.390	0.65
ADG, kg/d[d]	1.39	1.84	1.40	1.74	0.08	0.25	0.0001	0.18
Gain: DMI, g/kg[dfg]	152.53	200.37	159.08	190.21	3.37	0.60	0.0001	0.03
Final BW, kg[h]	587	647	586	632	11.67	0.16	0.0001	0.19

[a] *Chlortetracycline fed at 350 mg of CTC per day per steer.*
[b] *Standard error of the mean calculated from analysis of variance using n = 6.*
[c] *Interaction of CTC x implant.*
[d] *In the absence of CTC, implant means differ (P ≤ 0.02).*
[e] *In the absence of implant, CTC means differ (P ≤ 0.01).*
[f] *In the presence of CTC, implant means differ (P < 0.0001).*
[g] *In the presence of implant, CTC means differ (P = 0.05).*
[h] *Slaughtered at d 126 or 140.*

Growth Performance

It is a common practice to implant cattle in the finishing phase of growth using different ratios of estradiol benzoate and trenbolone acetate, depending on stage of finishing (e.g., d 1-70 versus d 71-140). These combinations of estrogens and androgens account for an additive growth response in cattle, commonly increasing ADG and improving feed efficiency above those of cattle receiving estrogenic implants alone. For the current experiment, ADG, dry matter intake (DMI), and efficiency of gain (BW gain per unit of DMI) are summarized in Table 2. Over the course of the entire experiment (d 0-139), CTC reduced (P = 0.02) DMI by 0.4 kg/d compared to steers receiving no CTC, while ADG increased (P = 0.0001) approximately 28% for implanted compared to non-implanted steers. Chlortetracycline and implant interacted to affect efficiency of gain for the entire experiment (d 0-139; P = 0.03). Implanted steers gained more efficiently both in the absence and presence of CTC; however, the improvement in efficiency of gain for implanted steers in the presence of CTC was only 20%, compared to a 31% increase in the absence of CTC (P < 0.0001). This finding suggests that CTC may have attenuated an improvement in efficiency of gain for implanted steers. Furthermore, this decrease in efficiency of gain is a function of the numerically lower ADG for implanted steers in the presence of CTC. Although it is not altogether surprising that in the absence of an implant, CTC had no positive effect on ADG and efficiency of gain, as we have previously shown no effect of CTC these parameters. It is a unique observation that the improved efficiency of gain for implanted steers was slightly diminished in the presence of CTC.

During the early part of the finishing phase (d 0-84), Revalor®-S increased ADG and efficiency of gain 25% and 26%, respectively, above that of non-implanted steers (P ≤ 0.01). However, in the absence of CTC, there was a greater improvement in ADG (71%) for implanted compared to non-implanted steers during the final 55 days of the finishing phase. These results demonstrate that not only did Revalor®-S increase ADG above that of non-implanted steers during the early phases of finishing, but the improvements were even more dramatic during

the latter phase of finishing, considering that non-implanted cattle normally deposit adipose tissue as a greater proportion of empty body gain during this time. Although the mechanism explaining lower ADG for steers receiving CTC in the presence of implant is unknown, an explanation may be related to thyroid hormone function. It has been demonstrated that sub-therapeutic administration of CTC decreased GH and thyroid hormone responses to a TRH + GHRH challenge in growing steers over a 91-d period. More recently, we showed a greater triiodothyronine (T_3) response for steers implanted with Synovex®-S (200 mg progesterone + 20 mg 17-β estradiol benzoate) than those receiving no implant in the absence of CTC. Triiodothyronine was not affected by implant in the presence of CTC. Although the implant used contained progesterone + estradiol benzoate, in the current experiment it is possible that, at least during the final period, the decrease in ADG for non-implanted and implanted steers in the presence of the CTC may have been associated with decreased thyroid function through an unknown mechanism which subdued the rate of BW gain.

During periods of the early finishing phase, implant and CTC decreased DMI by an average of 0.5 kg/d (d 0-28 and d 29-56, respectively; $P \leq 0.05$). Although the decrease in DMI contributed to an increase in ADG for the implanted steers (d 0-28), it did not affect efficiency of gain for the steers fed CTC (d 29-56). Over the course of the entire experiment, DMI decreased an average of 0.4 kg/d for steers fed CTC compared to steers receiving no CTC. The reason for this decrease in intake is unclear; previous studies which included oral, subtherapeutic levels of CTC in the diets of finishing steers and lambs showed no effect of CTC on DMI. Although the steers receiving CTC in the current experiment consumed less during Period 2, this decrease in intake did not translate to a significant reduction in ADG or efficiency of gain. The effects of subtherapeutic, oral administration of CTC on ADG and efficiency of gain have been shown to be variable. Although the reason for these discrepancies is unclear, it has been suggested that the effects of CTC on growth performance are more apparent under stressful conditions that are immunologically challenging to the animal. In both the current and previous studies, steers were vaccinated and backgrounded for a minimum of 30 d, and adjusted to the experimental diet prior to initiation of the experiment.

During the latter part of the finishing phase (d 85-139), interactions occurred between CTC and implant ($P \leq 0.07$) for ADG and efficiency of gain. In the absence of CTC, implanted steers gained an average of 0.74 kg/d more than non-implanted steers ($P = 0.007$), but in the presence of CTC, implant had no effect ($P \geq 0.13$). Additionally, there was a significant ($P \leq 0.07$) interaction between CTC and implant for efficiency of gain. In the absence of CTC, implanted steers gained 60% more efficiently than non-implanted steers ($P \leq 0.02$); however, implant had no effect ($P \geq 0.31$) in the presence of CTC. It is possible that the lower efficiency of gain occurring in the presence of CTC and implant during the last 27 d was due to both an increase in DMI for implanted steers ($P = 0.005$) and no positive effect of implant on ADG in the presence of CTC ($P = 0.79$).

Carcass Quality.

There were no interactions ($P \geq 0.53$) between CTC and implant for carcass quality measures (Table 3). There were no effects ($P \geq 0.22$) of treatment on longissimus dorsi area or fat cover, KPH fat, marbling, or yield grade. In previous research involving anabolic implants containing trenbolone acetate + estradiol benzoate, the effects of this implant on carcass characteristics have been variable; however, most research has shown that an implant containing estrogen + a synthetic androgen such as trenbolone acetate negatively affects marbling score and often, decreases the percentage of carcasses grading Choice. Although monetary benefit is realized through an increase in ADG and feed efficiency with these anabolic implants, a decrease in marbling and thus, lowering quality grade from Choice to Select reduces the value of a carcass. Therefore, it is of interest to develop strategies which allow producers to benefit from improved ADG and feed efficiencies associated with growth implants, while finding other compounds capable of improving marbling scores in concert with implants. The results of the current experiment showed a lack of change in longissimus area or fat cover and marbling and therefore reflects no effect of implant on compositional gain. However, these results do not preclude the possibility that compositional gain was altered during Period 4 (d 85-112), when an interaction between CTC and implant occurred for ADG. There was no effect of implant or CTC on the remaining carcass characteristics. These results disagree with most previous research showing lower marbling scores and percentage of carcasses grading Choice in steers implanted with Revalor®-S. Conversely, CTC fed to steers has increased longissimus fat cover and numerically increased marbling scores and increased the number of carcasses grading Choice. Furthermore, longissimus muscle area has been shown to be greater when Revalor®-S was used in finishing cattle. Interestingly, none of these effects were seen in the current experiment, demonstrating that, at least in this group of animals, Revalor®-S did not negatively, and CTC did not positively, affect carcass quality.

Table 3. Effects of oral chlortetracycline (CTC) and Revalor®-S on carcass quality measures in finishing beef steers.[a]

	-CTC		+CTC			P <		
Carcass quality measures	**-Implant**	**+Implant**	**-Implant**	**+Implant**	**SEM[b]**	**CTC**	**Implant**	**X[c]**
Longissimus area, cm^2	85.32	84.26	83.60	84.19	0.26	0.60	0.89	0.63
Longissimus fat cover, cm	1.21	1.12	1.14	1.07	0.03	0.81	0.60	0.64
KPH fat, %	2.06	2.04	2.10	2.04	0.03	0.53	0.22	0.53
Marbling[d]	4.41	4.07	4.33	4.25	0.20	0.80	0.30	0.53
Yield grade	2.99	3.08	3.06	3.03	0.12	0.90	0.80	0.64

[a] Chlortetracycline fed at 350 mg of CTC per day per steer.
[b] Standard error of the mean calculated from analysis of variance using n = 6.
[c] Interaction of CTC x implant.
[d] Scores: 1.00 = trace, 2.00 = slight, 3.00 = small, 4.00 = modest.

Effects of Chlortetracycline and Synovex®-S on Plasma Growth Hormone (GH) and Thyroid Hormone Concentrations Following Administration of Thyrotropin-Releasing Hormone (TRH) and GH-Releasing Hormone (GHRH) in Beef Steers

S.E. Kitts, J.C. Matthews, G.L. Sipe, K.K. Schillo, T.S. Rumsey, T.H. Elsasser, S. Kahl, R.L. Baldwin VI, and K.R. McLeod

Summary

The objective of this study was to characterize changes in circulating concentrations of GH and thyroid hormones induced by an injection of thyrotropin-releasing hormone (TRH) + GHRH in finishing beef steers receiving oral chlortetracycline (CTC) and estradiol benzoate + progesterone (Synovex®-S ear implant). Overall, this study supports the idea that Synovex®-S enhances GH and thyroid status. When considered in conjunction with previous results showing improvements in BW gain and feed efficiency for steers receiving Synovex®-S implants, these data suggest that an increase in pituitary and thyroid hormones may be associated with improvements in growth performance of cattle. There were no main effects of CTC on pituitary or thyroid hormones; however, CTC did affect hormone characteristics in the absence, but not the presence, of implant. Taken in concert, these data disagree with our previous report that CTC decreased pituitary and thyroid hormone release in response to a releasing hormone challenge. Despite the fact that CTC and estrogenic implants have been shown to have opposing effects on the composition of tissue accretion as well as pituitary and thyroid hormone status, we did not find, perhaps with the exception of T_3, that CTC mitigated the effects of implant. Furthermore, Synovex®-S may have increased BW gain through enhancement of pituitary and thyroid function.

Introduction

Sub-therapeutic feeding of chlortetracycline (CTC) has been reported to have growth-promoting effects for ruminants, swine, and poultry, but the mechanisms responsible for these effects are unknown. It is generally hypothesized that growth promotion by antibiotics in ruminants is a result of effects on digestive tract microorganisms or gut wall thinning. However, CTC-induced changes in carcass composition in calves suggest that this antibiotic may influence growth via an endocrine mechanism. Previously we have shown chronic, oral administration of CTC elevated circulating IGF-1 concentrations and reduced plasma concentrations of GH, thyroid-stimulating hormone (TSH), and thyroxine (T_4) following injection of thyrotropin-releasing hormone (TRH) and GHRH in beef steers. Corresponding with this shift in circulating hormone concentrations were increases in both subcutaneous and intramuscular fat deposition. Taken in concert, these data support the notion that CTC affects tissue deposition by suppressing pituitary responsiveness to GHRH and TRH.

Implants containing estradiol and progesterone are used in finishing beef cattle to improve feed efficiency and enhance lean tissue growth. Contrary to the reduced plasma concentrations of GH, TSH, and T_4 observed with CTC administration, estrogenic implants are considered to affect tissue deposition of cattle through increases in GH, IGF-1, TSH, and thyroid hormones. These endogenous hormones mediate the effects of estrogenic implants on protein accretion. Although it is clear that estrogenic implants repartition nutrients from fat to protein deposition, the manner in which hormone profiles are altered during repartitioning is still unclear. Moreover, no research has investigated the interaction between CTC and estrogenic implants as related to effects on growth performance, carcass characteristics, and hormone profiles.

The objectives of this study were to characterize changes in circulating concentrations of GH and thyroid hormones induced by an injection of TRH + GHRH in these steers and to determine the interactive effects of CTC and Synovex®-S.

Materials and Methods

Animals and Treatments

The protocol for the research discussed in this report was approved by the University of Kentucky Institutional Animal Care and Use Committee. Twenty-four Simmental-Angus crossbred steers were purchased from a single farm in Central Kentucky. Steers were initially housed on pasture for 30 d upon arrival at the University of Kentucky Animal Research Center, during which time they were dewormed using ivermectin (Merial, Duluth, GA) and vaccinated using Bovi-Shield™4 and Ultrabac®7 (Pfizer Animal Health, Exton, PA). After 30 d, steers were moved to group pens (four steers/pen) for a 30-d backgrounding period during which they were adapted to corn silage until a BW of approximately 340 kg was achieved. The steers had continuous access to automatic waterers.

After the 30-d backgrounding period, steers were limit-fed two transition diets for an additional 30 d at 2.25% BW for adaptation to *ad libitum* intake of the experimental diet (Table 1). Due to restrictions in the ability to sample a large number of steers on hormone challenge days, steers were separated into three groups of eight steers. The experiment start day for group 2 was one week after group 1, while group 3 started three weeks after group 1.

Ten days before each group was started on the experiment, steers were moved into individual pens containing a feed bunk and automatic waterer. During this period, steers were initially limit-fed the experimental diet at 2.0% BW, followed by a gradual step-up to *ad libitum* intake over a 9-d period. For the duration of the experiment, steers were exercised by turning them out in groups of four from 0730-0900 each day. Before feeding, individual body weights were determined once every two weeks for each block of steers; however, initial and final body weights were determined by weighing steers on two consecutive days.

Table 1. Transition and experimental diets.

	Transition		Experimental	
Ingredient, % DM	Diet 1	Diet 2	Diet (d -10-56)	Diet (d 57-112)
Corn silage	20.00	5.00	- - -	- - -
Alfalfa haylage	20.00	20.00	20.00	20.00
Cracked corn	50.00	65.00	70.00	70.00
Corn gluten meal	2.97	2.97	2.97	- - -
Ground corn	4.77	4.77	4.77	7.75
Urea	0.36	0.36	0.36	0.35
Limestone	1.07	1.07	1.07	1.07
Trace mineralized-salt[1]	0.51	0.51	0.51	0.51
Vitamins A, D, E[2]	0.02	0.02	0.02	0.02
Choice white grease	0.30	0.30	0.30	0.30

[1] 98.5% NaCl, 0.35% Zn, 0.34% Fe, 0.20% Mn, 330 ppm Cu, 70 ppm I, 50 ppm Co, and 90 ppm Se.
[2] 8,800 IU/g vitamin A, 1,760 IU/g vitamin D, and 1.1 IU/g vitamin E.

Across groups, steers were assigned randomly to a 2 x 2 factorial arrangement of treatments. Treatments included a corn meal + molasses carrier containing either 0 or 350 mg CTC (Aureomycin, Alpharma Animal Health, Fort Lee, NJ) and Synovex®-S ear implant (200 mg progesterone + 20 mg 17-β estradiol benzoate) or no Synovex®-S. The level of CTC used in this study was the same as that used in previous experiments, which had been shown to induce changes in carcass traits and pituitary and thyroid responsiveness. Steers were implanted or not implanted with Synovex®-S on d 0; after which steers initially implanted were re-implanted on d 56. The experimental diet was formulated using two protein supplements: Protein supplement 1 was formulated to provide 105% of the metabolizable protein (MP) requirement for large-frame steers (350 kg BW) gaining 1.60 kg/d and was fed until d 56 of the experiment; Protein supplement 2 was formulated to provide 105% of the MP requirement for large-frame steers (450 kg BW) gaining 1.60 kg/d and was fed from d 57-112 (NRC, 1996). The carrier or carrier plus CTC (500 g) was supplied daily at 0900. Steers were returned to the individual pens from the exercise lot and allowed one hour to consume the treatments before feeding at 1000. Orts were measured daily immediately after steers were turned out for exercise. Throughout the study, amount of feed offered was adjusted to maintain approximately 20% orts.

Releasing-hormone challenges were conducted on d 30, 56, and 106. Jugular catheters were placed in steers 12 to 18 h prior to hormone challenge, and feed was removed at 1700 on days prior to hormone challenge. On the day of hormone challenge, steers were injected via jugular catheter at 0800 with a 10-mL physiological saline bolus containing TRH (1.0 μg/kg BW) and GHRH (0.1 μg/kg BW). Relative to hormone challenge, serial, jugular blood samples (10 mL) were collected at -30, -10, 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, and 360 min. Blood samples were centrifuged (1,800 x g @ 4˚C for 30 min), plasma removed and frozen (-80˚C), and plasma concentrations of GH, TSH, T_4, and triiodothyronine (T_3) determined by RIA. Upon completion of the hormone challenge, steers were fed their respective supplements (with or without CTC) and 50% of their daily allotment of feed.

Results and Discussion

Previously, we have shown in beef steers that oral administration of CTC attenuated pituitary and thyroid hormone responses to a TRH + GHRH challenge on d 56 and increased overall fat deposition during a 91-d experiment. In contrast, anabolic implants containing progesterone + estrogen, i.e., Synovex®-S, have been shown to increase circulating concentrations of GH and thyroid hormones in response to a GH or TRH + GHRH challenge and to increase lean body tissue accretion. Thus, considering the opposing actions of these compounds, the objectives of the current study were to characterize changes in circulating concentrations of GH and thyroid hormones induced by an injection of TRH + GHRH in these steers and to determine the interactive effects of CTC and Synovex®-S. The results of the current experiment showed no effect of CTC alone on the response of pituitary or thyroid hormones to the releasing hormone challenge on d 30, 56, or 106; however, CTC x implant interactions occurred for characteristics of the T_3 response curve. Most of these interactions are explained by an effect of CTC in the absence, but not the presence, of implant. It is unclear why hormonal responses were not attentuated by CTC in this experiment as shown previously. Regardless of the discrepancies in the CTC response between our previous and current reports, there were main effects of Synovex®-S on growth performance and hormonal profiles as well as some interactive effects of CTC and Synovex®-S in the current experiment. These results provide novel data for this area of research.

Growth Hormone

Implants containing anabolic agents are typically used in finishing programs for feedlot cattle, as they promote lean tissue accretion and improve feed conversion. Because of an increase in lean tissue accretion, (i.e., protein gain), BW gain is greater for implanted compared to non-implanted cattle. It has been hypothesized that the increase in lean tissue accretion for implanted cattle is due to increases in circulating GH and thyroid hormones. In the current experiment, effects on GH response variables for implanted cattle were observed. The effects of estrogenic implant on changes in plasma GH concentrations following injection of TRH + GHRH are shown in Figure 1. The effect of time was significant ($P < 0.0001$). Although the data from the current experiment showed only a decrease ($P = 0.05$) in the time to peak for GH, there were non-significant increases ($P \leq 0.14$) in baseline concentrations and the magnitude of GH response to the TRH + GHRH challenge. Nevertheless, the overall response of GH concentrations across time to the TRH + GHRH challenge tended ($P = 0.10$) to be greater for the implanted compared to the non-implanted steers (Figure 1). Taken together, the latter observation suggests that the pituitaries of implanted steers may have been more sensitive to GHRH, leading to a quicker release of the available GH pool. Estrogen receptor-α is highly expressed in the anterior pituitary and has been shown to increase the number of GH-secreting cells. The results of our current research are consistent with the possibility that sensitivity of the pituitary to GHRH may have been greater in implanted compared to non-implanted steers.

Thyroid-Stimulating Hormone

The effects of Synovex®-S and CTC on changes in plasma TSH concentrations after TRH + GHRH injection are shown in Figure 2. The effect of time was significant ($P < 0.0001$). Over the course of the entire experiment, estrogenic implant enhanced TRH-induced TSH release as well as increased the magnitude of the T_3 response. The increase in baseline TSH concentrations for implanted steers is in agreement with previous research. In the current experiment, peak TSH concentrations after a TRH challenge tended to be higher ($P = 0.11$) for implanted compared to non-implanted steers. This most likely was a function of higher baseline concentrations and to a greater extent, larger changes in the magnitude of TSH response. Interestingly, there was no difference between implanted and non-implanted steers in the initial response (20 min, Figure 2) of TSH following TRH challenge, suggesting that the size of the readily-releasable pool was not different. In the current experiment, an implant by time interaction ($P = 0.03$) showed differences in TSH concentrations occurred at 45, 60, and 120 min ($P \leq 0.05$) following challenge for implanted vs. non-implanted steers, suggesting that these animals may have a greater ability to synthesize TSH after depletion of the releasable pool (Figure 2). It is known that in addition to stimulating the releasable pool of TSH from the pituitary, TRH also stimulates the formation of TSH mRNA, which may be responsible for subsequent stimulation of TSH synthesis.

Figure 1. Plasma growth hormone (GH) concentration in beef steers receiving no Synovex® S or Synovex® S (200 mg progesterone + 20 mg 17-β estradiol benzoate), fed the same diet top-dressed with either corn meal + molasses carrier or carrier plus 350 mg of chlortetracycline (CTC) per steer day per day and injected at time zero with 1.0 µg TRH + 0.1 µg GHRH per kg BW. Plasma GH concentrations changed with time in response to the releasing hormone challenge ($P < 0.0001$), and there was a tendency ($P = 0.10$) for a greater GH response in the implanted compared to non-implanted steers.

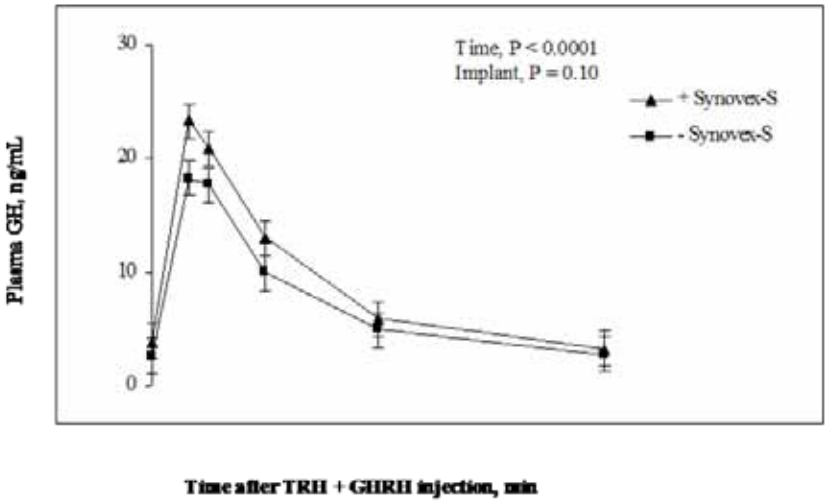
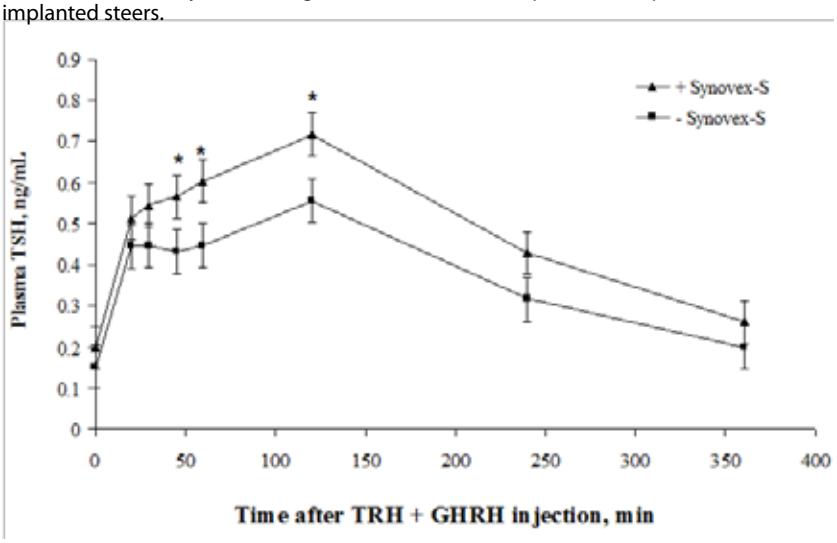


Figure 2. Plasma thyroid-stimulating hormone (TSH) concentration in beef steers receiving no Synovex®-S or Synovex®-S (200 mg progesterone + 20 mg 17-β estradiol benzoate), fed the same diet top-dressed with either corn + molasses carrier or carrier plus 350 mg of chlortetracycline (CTC) per day and injected at time zero with 1.0 µg TRH + 0.1 µg GHRH per kg BW. Plasma TSH hormone concentration changed with time in response to the releasing hormone challenge ($P < 0.0001$), and there was an interaction ($P = 0.03$) between implant and time for the TSH response. The TSH response at 45, 60, and 120 min after injection was greater (*$P = 0.05$) for implanted compared to non-implanted steers.



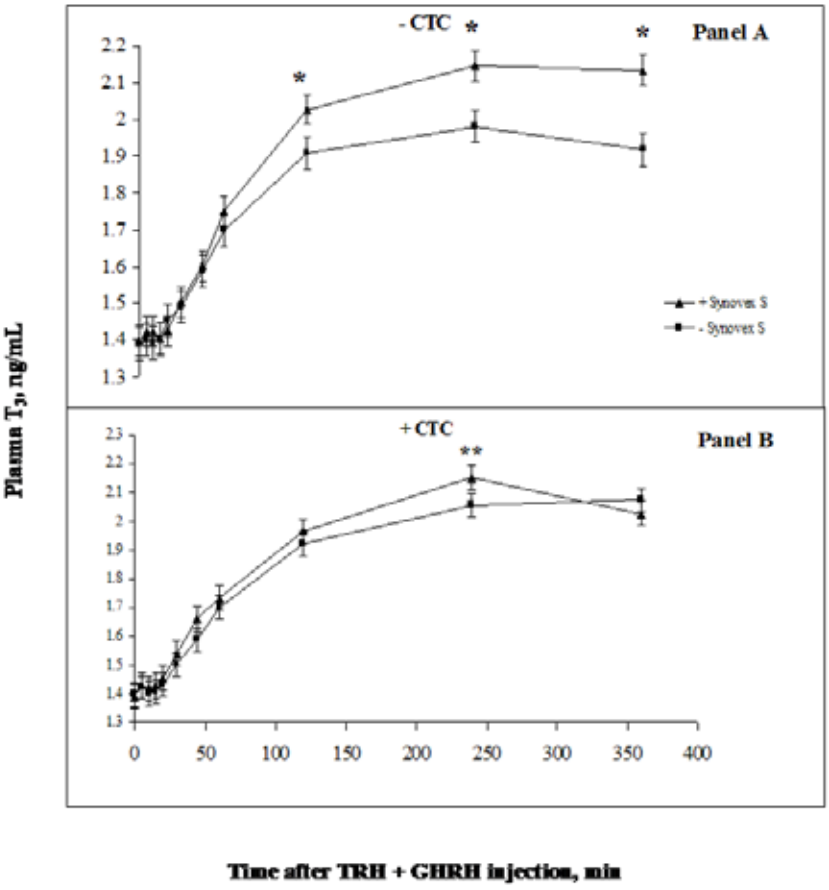
Thyroid Hormones

Thyroxine concentrations increased ($P < 0.0001$) after challenge injection; however, there were no effects ($P \geq 0.34$) of treatment on plasma T_4 concentrations. Additionally, there were no treatment effects ($P \geq 0.41$) on any other response variable for T_4. The effects of Synovex®-S and CTC on changes in plasma T_3 concentrations over time relative to injection of TRH + GHRH are shown in Figure 3. Because there was a significant CTC x Implant x Time interaction ($P = 0.004$), the means for implanted and non-implanted steers are presented in the absence and presence of CTC. In the absence of CTC, implanted steers had greater ($P \leq 0.02$) T_3 responses 120, 240, and 360 min following the releasing hormone challenge relative to the non-implanted steers. However, in the presence of CTC, implanted steers had only a tendency ($P = 0.06$) for a greater response at 240 min compared to non-implanted steers. Following 120 min post-challenge, plasma TSH concentrations began to decrease at a time when T_3 had almost reached peak concentrations (Figures 2 and 3, respectively). As expected, the peak in plasma TSH concentration occurred prior to the peak in concentration of T_3 and before the estimated peak of T_4. Because T_3, the metabolically active form of thyroid hormone, must first be converted to T_3 from T_4 in peripheral tissues, an increase in circulating T_3 occurs several hours after TRH challenge. Our data is consistent with this series of events; plasma TSH began to decrease 120 min post-challenge (Figure 2), and plasma T_3 was approaching peak concentrations (Figure 3). It is notable that in the absence of implant, T_3 time to peak increased (treatment by time interaction, $P = 0.02$) for steers receiving CTC compared to those not receiving CTC, implying

that CTC delayed the initial release of T_3 from the thyroid and/or the conversion of T_4 to T_3.

Although CTC may have decreased activity of 5'-deiodinase, which is the enzyme responsible for converting T_4 to T_3, Synovex®-S may have increased the conversion rate of T_4 to T_3 in the peripheral tissues, pituitary, liver, or kidney. The T_3 response curve demonstrated a CTC x Implant x Time interaction after the releasing hormone challenge (Figure 3). In concert with greater TSH concentrations for implanted compared to non-implanted steers (Figure 2), this interaction occurred due to greater T_3 concentrations 120, 240, and 360 min following challenge injection for implanted compared to non-implanted steers in the absence of CTC; however, these differences did not occur in the presence of CTC (Figure 3). Although TSH causes an acute stimulation of T_4 release into the plasma, it stimulates 5'-monodeiodination of only small amounts of T_4 to T_3, thus it is unlikely that the increase in circulating T_3 is due to an increase in T_3 release from the thyroid. The absence of treatment effects on circulating T_4 does not negate the possibility that, in the absence of CTC, implant increased T_4 release, which subsequently increased conversion of T_4 to T_3.

Figure 3. Plasma triiodothyronine concentration in beef steers receiving no Synovex® S or Synovex® S (200 mg progesterone + 20 mg 17-β estradiol benmam), fed the same diet top-dressed with either corn meal + molasses carrier (Panel A) or carrier plus 350 mg of chlortetracycline (CTC; Panel B) per day and injected at time zero with 1.0 µg TRH + 0.1 µg GHRH per kg BW. An interaction (P = 0.004) between CTC*Imp*Time occurred, and in the absence of CTC, implanted steers had a greater response (*P = 0.02) 120, 240, and 360 min following the releasing hormone challenge compared to non-implanted steers. In the presence of CTC, implanted steers tended (**P = 0.06) to have a greater response at 240 mm compared to non-implanted steers.



Implications

Both estrogenic growth implants and chlortetracycline have been used in cattle diets to improve body weight gain and feed efficiency as well as carcass quality, respectively. Although separate research experiments have sought to identify the hormonal mechanisms by which Synovex®-S increases lean body weight gain and chlortetracycline alters fat deposition in finishing cattle, no research has investigated the interaction of these compounds on hormonal profiles. Furthermore, little research has utilized a hormonal challenge to explore a time course of pituitary and thyroid hormone release in cattle with estrogenic implants. In contrast to previous work, this study showed no main effect of chlortetracycline on pituitary or thyroid hormone release or on growth performance. Additionally, our data showed that improvements in body weight gain and feed efficiency were associated with increased pituitary and thyroid hormone release in implanted steers. These improvements occurred despite the presence of chlortetracycline, indicating no negative associative effects between an anabolic implant promoting lean tissue accretion and the lipogenic effects of chlortetracycline.

Splanchnic Energy Metabolism in Beef Steers Consuming Graded Amounts of High-Quality Forage

K.R. McLeod, N.A. Elam, S.E. Kitts, C.C. Taylor-Edwards, E.S. Vanzant, and D.L. Harmon

Summary

Eight Angus steers fitted with vascular catheters were used to determine the relationship between forage metabolizable energy (ME) supply and visceral energy metabolism. Experimental design consisted of a replicated 4 x 4 Latin square with 28-d treatment periods and four equally spaced forage intakes ranging from 0.117 to 0.234 Mcal ME • (kg BW0.75 • d)-1, which approximates a range in ME intake from maintenance energy requirements to near *ad libitum* consumption. On d 27 or 28 of each period, simultaneous sets of arterial, portal, and hepatic blood samples were collected for determination of blood flow and net nutrient flux across the portal-drained

viscera (PDV), hepatic, and total splanchnic tissue beds. Regression analysis was used to develop models describing the relationship between ME intake and measured parameters. For all polynomial models in which a relationship between the variables was detected, the linear function of ME intake provided the best-fit model. A positive relationship was observed between ME intake and both blood flow and oxygen consumption across all tissue beds. Net PDV glucose utilization and hepatic output increased with ME intake. In contrast, greater ME intake was associated with an increase in net PDV lactate output but did not alter net hepatic lactate metabolism. Net splanchnic β-hydroxybutyrate output responded positively with ME intake and was a function of an increase in both PDV and hepatic output. Energy use (i.e., heat production) by the splanchnic tissues increased with ME intake and accounted for 56% of the total variation. These data describe important aspects of relationship between ME intake and visceral energy metabolism and provide unique estimates for defining the actual energy requirements of beef cattle consuming forage in feed prediction models.

Table 1. Regression models describing the relationship between metabolizable energy intake (MEI) and net nutrient flux across portal-drained viscera (PDV) and hepatic and splanchnic tissues in beef steers consuming forage.

Item	Regression Equation	P value	
		Intercept (β0)	Slope (β0)
Blood Flow			
PDV	162.1 (± 96.9) + 37.6 (± 7.4) × MEI	0.10	0.0001
Hepatic	437.7 (± 118.4) + 23.2 (± 9.5) × MEI	0.04	0.11
Oxygen			
PDV	-142.6 (± 56.4) - 68.7 (± 12.0) × MEI	0.37	0.0001
Hepatic	-361.8 (± 205.1) - 47.4 (± 16.4) × MEI	0.10	0.01
Splanchnic	-643.7 (± 316.7) - 222.3 (± 55.1) × MEI	0.06	0.001
Glucose			
PDV	12.08 (± 20.16) - 3.05 (± 1.54) × MEI	0.55	0.06
Hepatic	81.56 (± 34.88) + 4.77 (± 2.79) × MEI	0.04	0.11
Splanchnic	69.86 (± 33.69) + 3.61 (± 2.70) × MEI	0.06	0.20
Lactate			
PDV	14.91 (± 13.54) + 3.14 (± 1.04) × MEI	0.28	0.005
Hepatic	-94.45 (± 38.25) - 2.46 (± 3.06) × MEI	0.03	0.44
Splanchnic	-90.26 (± 28.75) + 1.29 (± 2.30) × MEI	0.008	0.59
B-hydroxybutyrate			
PDV	26.15 (± 17.86) + 2.22 (± 1.37) × MEI	0.15	0.12
Hepatic	-7.36 (± 10.30) + 2.74 (± 0.83) × MEI	0.48	0.006
Splanchnic	1.05 (± 2.48) + 6.53 (± 1.99) × MEI	0.98	0.006
Glutamine			
PDV	-1.02 (± 1.38) - 0.25 (± 0.11) × MEI	0.47	0.27
Hepatic	-1.85 (± 3.87) + 0.43 (± 0.31) × MEI	0.64	0.19
Splanchnic	-0.99 (± 3.21) + 0.01 (± 0.25) × MEI	0.76	0.96
Glutamate			
PDV	1.54 (± 1.37) - 0.13 (± 0.11) × MEI	0.27	0.22
Hepatic	8.39 (± 10.99) + 0.50 (± 0.88) × MEI	0.46	0.58
Splanchnic	8.45 (± 11.63) + 0.54 (± 0.93) × MEI	0.48	0.57
Heat production			
PDV	0.38 (± 0.41) + 0.18 (± 0.03) × MEI	0.37	0.0001
Hepatic	0.95 (± 0.54) + 0.13 (± 0.04) × MEI	0.10	0.01
Splanchnic	1.69 (± 0.83) + 0.27 (± 0.07) × MEI	0.27	0.001

Introduction

For feeding systems to accurately predict whole-body animal performance, knowledge of digestion kinetics and nutrient metabolism by the gut tissues is essential. A primary factor affecting these variables, particularly with forage diets, is variation in voluntary intake. It is well established that forage intake not only affects the amount of nutrients supplied but also has pronounced effects on passage rate and digestibility. Perhaps of equal or greater importance is the fact that there is a positive relationship between forage MEI and visceral tissue mass. Because these tissues are metabolically active and are opportunistically positioned, they have dramatic effects on net nutrient supply and whole-body energetics. Depending on diet and level of intake, current estimates indicate that visceral tissues account for 30 to 50% of whole-body energy use. However, these estimates, as well as those for net nutrient supply, are based largely on data sets utilizing one or two levels of intake, thereby limiting predictive model parameterization to linear functions. Thus, quantitative measures of the relationship between forage ME intake and visceral nutrient metabolism and heat production over a wide range of intake levels are crucial for improving current feed prediction models.

Materials and Methods

Eight Angus steers (328 ± 40 kg BW) were surgically prepared with indwelling catheters in appropriate blood vessels for measuring nutrient flux rates across the portal-drained viscera (PDV; primarily composed of tissues from the gastrointestinal tract) and liver. After allowing a minimum of 14 d for surgical recovery, steers were used in a replicated 4x4 Latin square design with each square balanced for residual effects, to study the relationship between forage intake and visceral metabolism. Treatments included post-harvested, cubed alfalfa hay fed at four equally spaced amounts of metabolizable energy ranging from 0.117 to 0.234 Mcal ME • (kg $BW^{0.75}$ • d)$^{-1}$. This range in ME intake was chosen because it reflects a span of intake from maintenance energy requirements to that approaching *ad libitum* consumption. Steers were housed individually in an environmentally controlled (23.8°C) barn with a 16-h light cycle and continuous access to water.

Each experimental period was composed of 28 d, with steers being adjusted to their respective level of alimentation incrementally over the initial 5 d. During the majority of the adaptation period, steers were fed twice daily at 07300 and 1500 h. However, beginning on d 20, steers were fed their daily feed allotment in 12 equal portions every 2-h to create steady-state conditions for nutrient flux measurements. On the day of flux measures (d 27 or 28), simultaneous samples of arterial, portal, and hepatic blood were collected into heparinized syringes at hourly intervals for 6 h beginning at 0800. Each set of blood samples were immediately placed on ice and transferred to the laboratory for processing and analysis. Portal and hepatic blood

flows were determined by a downstream dilution of a continuous infusion of p-aminohippuric acid (250 mM; pH 7.4). Whole-blood concentrations of oxygen and p-aminohippuric acid and plasma concentrations of glucose, lactate, β-hydroxybutyrate, glutamate, glutamine, and p-aminohippuric acid were determined.

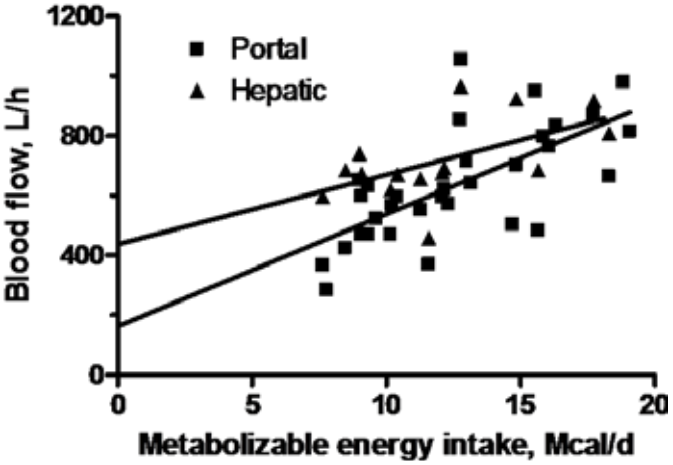
Net nutrient flux (exchange) rates were calculated as veno-arterial differences multiplied by blood flow across the PDV or splanchnic bed (includes the PDV and liver). Net hepatic flux was calculated as the difference between PDV and splanchnic flux. Heat production (measure of energy use) by each tissue bed was calculated as 4.89 Kcal/L O_2 consumed. Data were subjected to regression analysis to test the effects of ME intake on visceral metabolism. Linear, quadratic and cubic models were fitted using the maximum r^2 selection process. For each measured parameter, quadratic and cubic functions were not significant and were subsequently removed from the model. Thus, each measured parameter is described as a simple linear model and represented by the equation: y = intercept + slope (ME intake, Mcal/d). Positive y-values and slopes reflect net release or output of a nutrient by a tissue bed, while negative y-values and slopes reflect utilization or consumption.

Results and Discussion

Regression models representing the fitted data are presented in Table 1. As indicated by the positive slope values, both PDV (P = 0.0001) and hepatic (P = 0.11) blood flow increased with ME intake. However, graphic depiction of the data (Figure 1) shows that PDV and hepatic blood flows converge as ME intake approaches *ad libitum* intake. Because hepatic blood flow is comprised of inputs from both the portal vein and hepatic arteries, this convergence suggests that hepatic blood flow is underestimated and most likely is reflective of the limited data points for hepatic (n=15) compared with PDV (n=47) blood flow. Comparing the overall fitness of the models, the R^2 values show that 46% of the variation in PDV blood flow is accounted for by the presented regression equation, while the regression equation for hepatic blood flow accounts for only 32% of the variation.

A negative (P = 0.06) relationship between net PDV glucose flux and ME intake was observed. However, glucose flux across the entire splanchnic bed was unaffected by intake because of the slight positive (P = 0.11) relationship between hepatic glucose output and ME intake. Conversely, net PDV lactate release was related positively with ME intake, whereas net flux across hepatic and splanchnic tissues was not responsive to intake. This shows that although the use of glucose carbon by the PDV tissues increases with intake, the rate of glucose production by the liver is sufficient to prevent changes in glucose supply to the peripheral tissues. Because lactate is an intermediate in propionate (i.e., a major volatile fatty acid produced in the rumen) and glucose metabolism by the gut tissues, it is possible that a substantial amount of the glucose taken up by the PDV was partially metabolized and released as lactate. The observed increase in PDV lactate release with ME intake and the apparent failure to demonstrate a relationship between hepatic lactate uptake, or a net splanchinc release, most likely reflects variation in the data set, because majority lactate is taken up by the

Figure 1. Relationship between metabolizable energy intake and portal and hepatic blood flow in beef steers consuming forage.

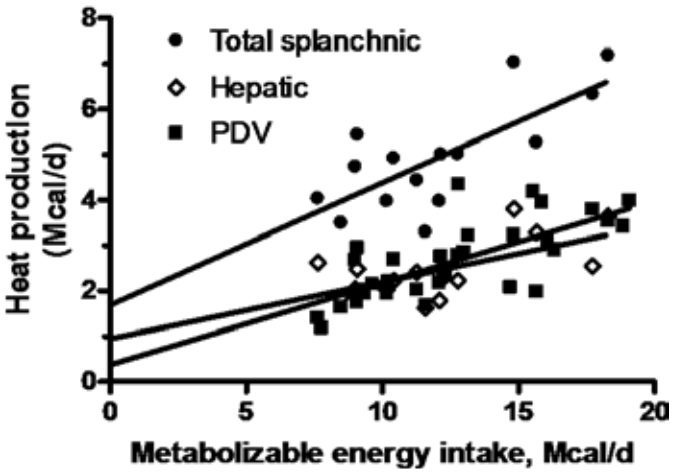


liver and used either for energy or converted to glucose, thus would account in part for the greater hepatic glucose output with increasing ME intake.

The positive relationship between ME intake and net release of β-hydroxybutyrate (i.e., intermediate of butyrate metabolism) by the tissue beds is consistent with greater ruminal butyrate production with increased forage intake. In contrast, glutamine and glutamate flux across the spanchnic beds was not influenced by ME intake, despite have been shown to be extensively metabolized by the gut tissues and involved in nitrogen shuttling in the liver.

Oxygen consumption, or use, by the splanchnic tissue bed increased (P = 0.001) with ME intake and was a function of an increase in oxygen use by both the PDV (P = 0.0001) and hepatic (P = 0.01) tissues. This increase in oxygen use is indicative of increased oxidative metabolism. Applying a heat of combustion value of 4.89 kcal/L of oxygen consumed shows that heat production, or energy use, by the PDV (P = 0.0001), hepatic (P

Figure 2. Relationship between metabolizable energy intake and heat production by the portal-drained viscera (PDV) and hepatic and splanchnic tissues in beef steers consuming forage.



= 0.01) and splachnic bed (P = 0.001) increases with ME intake and that 56% of the total variation in energy use by the splanchnic bed is explained by ME intake. A comparison of the slope values and response data depicted in Figure 2, suggests that PDV (slope = 0.18) energy use is more responsive to ME intake than that by the liver (slope = 0.13). However, as discussed for blood flow, it may be reflective of the fewer number of observations for hepatic compared with PDV energy use. Nevertheless, our regression model indicates that energy use by splanchnic tissues accounts for 27 to 30% of the total ME intake and, as indicated by the y-intercept, a substantial portion of the total fasting heat production. These quantitative estimates of energy use are consistent with previous efforts that have shown the splanchnic tissues to be metabolically active and their mass to be responsive to level of alimentation.

Implications

These results show that in steers consuming forage ranging from maintenance energy requirements to *ad libitum* intake, energy use by the splanchnic tissues is linearly related to metabolizable energy intake in a positive manner and accounts for 27 to 30% of metabolizable energy intake. Accordingly, estimates of maintenance energy requirements for cattle consuming forage, either post-harvested or fresh grazed, should account for linear changes in energy requirements necessary for maintenance of gastrointestinal and liver tissues.

Effect of Ractopamine on Whole-Body and Splanchnic Energy Metabolism in Holstein Steers

A.F. Koontz, S.W. El-Kadi, D.L. Harmon, E.S. Vanzant, and K.R. McLeod

Summary

This study was designed to examine the influence of ractopamine (RAC) on whole-body and splanchnic energy balance. Six growing Holstein steers (BW= 402kg ±39.5) surgically fitted with an arterial, portal hepatic, and mesenteric venous indwelling catheters were used in a repeated-measures study. Treatments were a basal diet of alfalfa cubes fed at 1.5x ME requirements (d 1-21) and basal plus RAC (430mg/hd•d^{-1}; d22-42). On d 14 of each period, splanchnic and portal-drained viscera (PDV) energy balance was determined as the product of arterio-venous O_2 difference and blood flow. Blood flow was determined using down-stream dilution of *p*-aminohippuric acid. Whole-body energy balance was determined on d 15-21 of each period, which included 7 d total excreta collection and 3 d of respiratory gas exchange measurements. Body weight and DM intake were greater for steers receiving RAC compared with those receiving the control diet, however, no difference was observed in either BW or DMI when expressed on a BW$^{.75}$ basis. Similarly, as a function of BW$^{.75}$, whole-body heat production as well as retained N and energy were unaffected by RAC. In contrast, RAC tended to decrease energy use by splanchnic tissues, largely due to a reduction in energy use by the PDV. These data indicate that although whole-body energy use is not affected by RAC, energy use by splanchnic tissues is decreased, thereby increasing energy use by peripheral tissues.

Introduction

Early research with beta-adrenergic receptor (βAR) agonists in livestock showed that oral administration could increase muscle mass and reduce fat deposition in sheep, swine, poultry, and cattle. This action leads βAR agonist compounds to be classified as repartitioning agents. However, the mechanisms by which these effects occur have not been fully defined.

Most research has focused on the increase in lean tissue mass that results from βAR agonist administration. Studies have shown hypertrophy of muscle fibers occurs when feeding ractopamine without a corresponding increase in number of muscle fibers. Additionally, research has indicated that RNA concentrations are increased in the muscle tissue of animals treated with βAR agonists. Some studies also show a reduction in muscle degradation as one of the mechanisms of βAR agonist action. Other research has focused on the mechanisms by which adipose mass is reduced. These studies have shown that the reduced fat deposition may be explained by increased lipolysis in combination with decreased lipogenesis.

Further research has focused on the indirect effects of βAR agonist administration. Ractopamine can reduce organ mass in growing steers; such reductions may account for N and energy savings that allow for increased nutrient availability to muscle tissues. Additionally, there is evidence that βAR agonists increase blood flow to skeletal muscles. This allows increased substrate availability to skeletal muscle as well as promoting removal of non-esterified fatty acids from adipose tissues and thereby enhancing lipid degradation.

The majority of research related to ractopamine has focused on tissue and cellular level effects or carcass composition changes. Little attention has been given to changes in energy metabolism on the organ and whole-body levels. In order to optimize the effects of βAR-agonist administration, it is necessary to fully understand its alterations to metabolism. The goal of this experiment was to determine if there is an alteration in whole-body or splanchnic energy utilization in growing steers as a result of oral RAC administration.

Materials and Methods

Animals

Six Holstein steers (402±39.6 kg) were utilized in a repeated-measures experiment. The diet consisted of alfalfa cubes fed to meet approximately 1.5x the maintenance energy requirement of the animals and topdressed with a mineral pre-mix and

Bloat-Guard®. The experimental was 48 days in length and consisted of a control period (d 1-28; CON), followed by a ractopamine test period (d 29-48; RAC); during which ractopamine was added to the diet at the maximum dose recommended by the manufacturer, 430mg hd^{-1} d^{-1}. Steers were fed equal portions every two hours during the experiment except during the nutrient balance trial, when the steers were fed once daily at 0800. Feed samples were collected daily by random grab sampling and composited over each period. Steers had *ad libitum* access to water throughout the experiment.

Blood Flow and Splanchnic O_2 Consumption

Steers were surgically fitted with chronic indwelling catheters in the mesenteric artery, mesenteric vein, hepatic portal vein, and hepatic vein. Arterial, portal, and hepatic blood samples were collected every 45 min from 1200 to 1730 on d 20 and 41. Samples were immediately analyzed for O_2 saturation and hemoglobin. Blood flow was determined by a continuous infusion of *p*-aminohippurate (PAH) into the mesenteric venous catheter. O_2 saturation and hemoglobin concentration of blood samples were used to calculate oxygen use and heat production (HP) by the splanchnic bed and portal-drained viscera as described below.

Nitrogen and Energy Balance

The excreta collection phase consisted of a 7-d nutrient balance phase where total fecal and urine collection was performed. On the middle three days (d 3, 4, and 5) the steers were placed in head-boxes for indirect calorimetry data collection. Feces and urine were collected at 0900 daily.

Urine output weight was recorded daily. A constant percentage was sub-sampled daily to contribute approximately 200 g to an acidified composite for each period and steer. This composite was stored at 0°C until analysis. The wet weight of fecal output was recorded daily for each steer. A sub-sample was taken at a constant percentage to give approximately 2 kg wet matter per day. Daily fecal samples were composited by animal and period, then stored at 0°C until analysis. Fecal samples were dried daily in a 100°C forced air oven to determine daily fecal dry-matter output.

During the indirect calorimetry phase, respiratory gas exchange was measured for three consecutive 24-hr periods in head-box style respiration chambers. Measures of inspired and expired O_2, CO_2, and CH_4 were collected at 9-minute intervals and utilized to evaluate energy balance.

Table 1. Animal weight, feed intake, and nutrient digestibility during control and ractopamine periods.[z]

	Control	Ractopamine	SE	P = [y]
Body weight (kg)	392	420	15.4	0.0002
DM intake (kg d^{-1})	9.0	9.6	0.	0.001
DM intake (kg $kgBW^{-0.75}$ d^{-1})	0.103	0.103	0.001	0.48
DMD (%)	60.6	60.6	0.83	0.95
N digestibility (%)	66.7	66.5	0.77	0.83
Energy digestibility (%)	60.0	59.7	0.92	0.84

[z] *n = 6*
[y] *Probability of a greater F statistic.*

Table 2. Nitrogen balance of steers on control and ractopamine diets.[z]

	Control	Ractopamine	SE	P = [y]
Intake N (g $kgBW^{-0.75}$ d^{-1})	3.1	3.1	0.029	0.48
Digestible N (g $kgBW^{-0.75}$ d^{-1})	2.1	2.1	0.042	0.81
Fecal N (g $kgBW^{-0.75}$ d^{-1})	1.0	1.0	0.018	0.60
Urinary N (g $kgBW^{-0.75}$ d^{-1})	1.3	1.2	0.047	0.35
Retained N (g $kgBW^{-0.75}$ d^{-1})	0.82	0.88	0.062	0.34
Retained (% of intake)	26.5	28.0	1.78	0.34

[z] *n = 6*
[Y] *Probability of a greater F statistic.*

Table 3. Energy balance of steers on control and ractopamine diets (kJ $kgBW^{-0.75}$ d^{-1})

	Control	Ractopamine	SE	P = [z]
Intake E[y]	1876.6	1865.5	18.36	0.67
Digestible E[y]	1125.0	1113.3	23.79	0.74
Fecal E[y]	751.58	752.16	19.44	0.98
Urinary E[y]	63.33	58.02	4.42	0.30
Methane E[x]	47.12	60.04	8.86	0.20
Metabolizable E[x]	1006.7	975.4	27.30	0.57
Heat production[x]	690.8	692.8	49.00	0.96
Retained E[x]	311.9	282.7	53.68	0.71
RE (% of IE)	16.50	15.29	2.82	0.77
RE (% of ME)	30.88	28.84	5.14	0.75

[z] *Probability of a greater F statistic.*
[y] *n = 6*
[x] *n = 4*

Table 4. Composition of energy gain for steers on control and ractopamine diets.

	Control	Ractopamine	SE	P = [a]
Protein (kJ $kgBW^{-0.75}$ d^{-1})[y]	122.5	131.2	9.31	0.34
Protein (% of RE)[X]	41.32	47.27	9.96	0.64
Fat (kJ $kgBW^{-0.75}$ d^{-1})[x]	190.5	157.8	48.79	0.67
Fat (% of RE)[X]	58.68	52.73	9.96	0.64

[a] *Probability of a greater F statistic.*
[y] *n = 6*
[x] *n = 4*

Composite feed and fecal samples were dried and ground prior to analysis for dry matter, ash, NDF, and ADF. Carbon, nitrogen, and heat of combustion were determined for feed, feces, and urine.

Statistical Analysis

The data were analyzed as repeated measures with the Mixed procedure of SAS, with period as a fixed effect. The statistical model used individual steer as the experimental unit. The experimental treatments were confounded by time,

Table 5. Splanchnic energy use for steers on control and ractopamine diets.

	Control	Ractopamine	SE	P = [z]
Total splanchnic (kJ d^{-1})[y]	16286	14043	1937.26	0.09
Total splanchnic (kJBW$^{-0.75}$ d^{-1})[y]	190.85	155.80	25.93	0.09
Portal-drained viscera (kJ d^{-1})[x]	8627.3	7846.9	898.94	0.31
Portal-drained viscera (kJBW$^{-0.75}$ d^{-1})[x]	100.33	85.92	9.83	0.12
PDV (%WB HP)[y]	16.09	13.62	1.76	0.27
Hepatic (kJ d^{-1})[y]	7658.4	7466.6	1502.30	0.90
Hepatic (kJBW$^{-0.75}$ d^{-1})[y]	90.53	81.90	19.12	0.64

[z] *Probability of a greater F statistic.*
[y] *n = 4*
[x] *n = 5*

as all steers were used first in the control period and second in the ractopamine period. The experiment was designed in this fashion in order to remove the possibility of long-term carryover effects of ractopamine treatment. As a result of this confounding with time, there was an increase in BW for all steers during the ractopamine period. Due to this difference in BW between the control and ractopamine periods (392kg and 420kg respectively; $P = 0.0002$), results were analyzed on a metabolic body weight (MBW; $BW^{0.75}$) basis. During the experiment, two animals had failed portal vein and hepatic vein catheters; one of these animals and a third were excluded from calorimetric analyses due to a malfunction of the oxygen analyzer. These animals are excluded from the data set where appropriate.

Results and Discussion

In the current study, there was no effect of ractopamine on digestibility of nitrogen or energy. Previous research with β-agonists has shown no change (Table 1)(Rikhardsson et al., 1991) and increased (Walker et al. 2007) dry-matter digestibility). It is possible that the age, breed, as well as level and composition of diet affect the degree to which ractopamine affects digestibility of a feedstuff. These factors are known to modulate the efficacy of ractopamine in relation to muscle gain and adipose decretion (Bell et al., 1998).

The current experiment also showed no change in the nitrogen retention of the steers when treated with ractopamine. This is in contradiction to previous research where the addition of ractopamine increased whole-body nitrogen retention (Table 2)(Anderson et al., 1989; Walker et al., 2007). The experiment of Walker et al. was confounded by time, and therefore animal weight, as in the present experiment. However, their results were not reported as a function of MBW to account for this confounding. The current study showed a strong trend for increased total nitrogen retention (9.3g d^{-1}) with no significant change in nitrogen retention on a MBW basis (0.06g d^{-1} $kg^{-0.75}$). Williams et al. (1987) also found no difference in retained N when using veal calves fed clenbuterol. In that study, nitrogen retained in the carcass increased, while non-carcass retained nitrogen decreased. This resulted in no net change in N-retention. It is possible that a similar action occurred in the present experiment; however, as no slaughter data were taken, this possibility cannot be verified.

It is possible that during the control period, the animals were already accreting lean tissue at a maximal efficiency for the given diet and the addition of ractopamine to the diet could not increase nitrogen retention or muscle gain as it customarily does without requiring an increased energy intake. Additionally, ractopamine is believed to be most effective when given in conjunction with diets that have an excess of dietary protein (Reeds and Mersmann, 1991). Due to the muscle-specific increases seen with βAR agonist treatment, it is possible that a dietary increase in methionine and phenylalanine, the limiting amino acids in muscle deposition (Bohe et al., 2003), may be necessary in order to see the full effects of βAR-agonist administration.

The data from this study do not allow for differentiation of ractopamine effects as changes in synthesis, degradation, or repartitioning. Ractopamine may have functioned as a partitioning agent, altering the ratio of nutrient use by specific tissue beds (Tables 3 and 4) without increasing whole-body retention. The 14% increase in energy retained as protein as a percentage of total RE, while there was no change ($P = 0.71$) in whole-body energy retention, may provide evidence for partitioning action. Previous studies have shown similar results without increased total energy gain. (Ricks et al., 1984a; Strydom et al., 2009)

Evidence of energy partitioning in the current experiment can be found in the alteration of energy use by the splanchnic tissues. The 13% reduction in splanchnic energy use observed in steers consuming RAC was primarily a function of a decrease (Table 5) in PDV energy use rather than hepatic energy use, or an even reduction in both tissue beds. The energy savings by the splanchnic tissues is equivalent to a 5% increase in energy use by peripheral tissues. The reduction seen in splanchnic energy use in this experiment is most likely related to a decrease in the mass of these tissues (McLeod and Baldwin, 2000). Such a reduction in organ size would cause the viscera to require fewer nutrients for maintenance, thereby reducing the amount of energy and nutrients needed for tissue maintenance and turnover (Reynolds, 2002). This would allow additional nutrients to be available for protein accretion in other areas of the body without altering whole-body nitrogen, carbon, or energy balance.

Ractopamine treatment had no effect on total HP in this experiment. In previous experiments where sheep were given cimaterol, they showed an immediate increase in daily HP; however, by d10 HP decreased to pretreatment levels (Rikhardsson et al., 1991). In this experiment, the steers were treated with ractopamine for 16 days before calorimetry data collection was begun. It is possible that if the calorimetric data collection occurred earlier in the current study that a similar effect

could have been seen. As noted above, ractopamine may result in a reduction in visceral organ mass, which could offset the increased heat production that would normally be associated with βAR-agonist treatment as a result of increased muscle mass and weight gain, thus accounting for the lack of change in heat production values seen in the current experiment. In addition, a reduction in visceral organ size and the resulting reduction in nitrogen and energy needs for these tissues may also account for part of the unchanged nitrogen and energy retentions seen with the addition of ractopamine.

Implications

The addition of ractopamine to the diet of growing Holstein steers resulted in no net change in whole-body energy or nitrogen balance after 21 days of feeding. However, there was a reduction in energy use by the splanchnic tissues. This reduction was primarily due to a decrease in energy use by the portal-drained viscera, not the hepatic tissues. The decline in splanchnic energy use without altering whole-body energy balance may indicate an increase in energy available for use by peripheral tissues. We postulate that this corresponds to an increase in muscle tissue accretion. However, muscle mass was not measured in the present study, though we did note a slight increase in total energy retained as protein, while energy retained as fat was unchanged. Based on this research, whole-body energy utilization is unaffected by ractopamine, while splanchnic energy use is decreased, thereby allowing an increase in energy available for use by peripheral tissues.

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Glucagon-like Peptide-2 (GLP-2) Alters Amino Acid Fluxes Across the Portal-Drained Viscera of Ruminant Calves

C.C. Taylor-Edwards, D.G. Burrin, N.B. Kristensen, K.R. McLeod, and D.L. Harmon

Summary

Flux of amino acids across the portal-drained viscera (PDV) is affected by GLP-2, potentially by increased small intestinal epithelial growth and thus energy and amino acid requirements of this tissue. Increased PDV extraction of Gln and alterations in PDV metabolism of arginine, ornithine, and citrulline support that intestine-specific amino acid metabolism is affected by GLP-2. However, unchanged glucose metabolism suggests GLP-2 effects on ruminant PDV glucose metabolism are more transient or less significant than for non-ruminants.

Introduction

We have shown, using calves, that GLP-2 increased small intestinal mass and epithelial mass, villus height, and crypt depth in the small intestine without affecting total body mass. The gastrointestinal tract uses amino acids (AA) as an energy source and for protein synthesis. Thus, intestinal growth caused by GLP-2 would likely also alter portal-drained viscera (PDV) utilization of AA. Furthermore, previous research has demonstrated that GLP-2 increases PDV glucose utilization in total parenteral nutrition-fed piglets, but no information is known in ruminants, which often differ in their regulation and utilization of glucose compared to non-ruminants. Thus, the aim of this experiment was to examine the effects of exogenously-administered GLP-2 on blood AA concentrations and fluxes across the PDV and utilization of glucose by the PDV.

Materials and Methods

Eight weaned Holstein calves with catheters in the carotid artery, portal vein, and mesenteric vein were paired by age and

randomly assigned to treatment: Control (0.5% BSA in saline; n = 4) or GLP-2 (50 µg/kg BW bovine GLP-2 in BSA; n = 4). Intake was 2.75% of BW (DM-basis). Treatments were injected subcutaneously every 12 h for 10 d. On day 10, para-aminohippuric acid and [U-^{13}C] glucose were continuously infused to measure portal blood flow and PDV utilization of glucose, respectively. Calves were fasted overnight, and blood samples were taken every 15 min for 150 min. Plasma was analyzed for para-aminohippuric acid, for total free AA by HPLC (phenylisothiocyanate derivitization), and for [U-^{13}C] glucose enrichment. Net PDV flux was calculated as portal plasma flow × (portal – arterial nutrient concentration), with positive values indicating net release and negative values indicating net uptake. Data were analyzed using the MIXED procedure of SAS with treatment as a fixed effect and block as a random effect.

Table 1. Arterial amino acid concentrations and PDV fluxes in calves treated with GLP-2.

	Arterial Concentration, µM				PDV FLUX, mmol/h			
	Control	GLP-2	SEM	P <	Control	GLP-2	SEM	P <
	Essential Amino Acids							
Arg	117.3	166.1	5.11	< 0.0001	3.72	1.90	0.540	0.03
Ile	117.7	102.1	8.67	0.10	4.16	1.56	0.846	0.05
Leu	134.5	102.5	9.25	0.006	4.86	2.13	0.662	0.004
Lys	64.6	52.3	4.20	0.01	3.71	1.64	0.649	0.01
Phe	47.2	38.2	2.84	0.005	3.08	1.48	0.332	0.003
Val	247.4	199.6	17.73	0.02	3.12	2.13	1.500	0.65
	Amino Acids Important for Gut Metabolism							
Gln	208.7	160.6	10.92	0.0003	-5.23	-6.87	1.043	0.12
Cit	46.9	77.4	3.30	< 0.0001	2.02	1.79	0.529	0.76
Orn	21.1	23.1	1.48	0.15	1.04	0.062	0.296	0.002

Results and Discussion

Treatment with GLP-2 for 10 d reduced (P < 0.05) arterial concentrations of the essential AA Leu, Lys, Phe, and Val (Table 1) and the non-essential AA Gln (Table 1) and Ala, Asn, and Pro (not shown). Likewise, 10-d treatment with GLP-2 reduced net PDV release of the essential AA Arg, Ile, Leu, Lys, and Phe (Table 1) and the non-essential AA Ala, Asn, Asp, Gly, Pro, and Ser (not shown). Moreover, negative correlations were found between small intestinal growth measures and arterial AA concentrations and PDV flux. For example, jejunal crypt cell BrdU labeling was negatively correlated with PDV release of Leu, Lys, Phe, Asp, Gly, Pro, Ser, and ornithine (P < 0.01). These results suggest GLP-2 increases growth and resulted in greater sequestration of AA in the PDV, perhaps for protein synthesis.

Glutamate and Gln are used for gut energy metabolism and growth, including synthesis of other AA such as Arg, Pro, ornithine, and citrulline. Arterial Gln concentrations were 23% lower with GLP-2 treatment, and net PDV uptake of Gln was unchanged; thus, the net PDV extraction of Gln was greater after GLP-2 (0.20 vs. 0.10, P < 0.0001). Greater arterial Gln extraction and reduced PDV ornithine flux may have contributed to the increased citrulline export from the gut and likely resulted in greater renal conversion of citrulline to Arg and thus greater Arg concentrations in GLP-2-treated calves. Despite greater Arg concentrations, PDV Arg release was reduced, suggesting PDV retention, perhaps to support small intestinal mucosal growth.

Despite changes in AA metabolism, whole-body glucose irreversible loss did not differ between treatments (117.4 mmol/h). Proportional glucose utilization by the PDV and non-PDV tissues was 0.41 and 0.63, respectively and was not affected by treatment. This contrasts with TPN-fed piglets, where GLP-2 increased PDV glucose uptake and extraction (Guan et al., 2003). The effects of GLP-2 on PDV glucose utilization may be a result of species adaptations in non-ruminants vs. ruminants. Although PDV use of glucose is significant, ruminants also rely on energy substrates besides glucose to spare glucose for vital functions. Availability of substrates such as Glu, Gln, propionate, and butyrate reduced glucose oxidation in ovine duodenal mucosal cells without reducing glucose uptake (Oba et al., 2008). Thus, GLP-2 may have increased Gln extraction rather than glucose to provide the energy to support growth.

Implications

These data show that intestinal specific amino acid metabolism can be dramatically altered via the application of gut regulatory peptides. Compounds like this may be tools to hasten gut repair and improve health of ruminants following gastrointestinal upset or bouts of diarrhea that can be common in calves.

Glucagon-Like Peptide-2 (GLP-2) Increases Small Intestinal Blood Flow and Mucosal Growth in Ruminating Calves

C.C. Taylor-Edwards, D.G. Burrin, J.J. Holst, K.R. McLeod, and D.L. Harmon

Summary

Glucagon-like peptide-2 (GLP-2) increases small intestinal mass and blood flow in non-ruminants, but its effect in ruminants is unknown. Eight Holstein calves with an ultrasonic flow probe around the superior mesenteric artery (SMA) and catheters in the carotid artery and mesenteric vein were paired by age and randomly assigned to treatment of control (0.5% BSA in saline; n = 4) or GLP-2 (50 µg/kg BW bovine GLP-2 in BSA; n = 4) given subcutaneously every 12 h for 10 d. Blood flow was measured on d 0 (acute) and d 10 (chronic) and included three periods: baseline (saline infusion), treatment (infusion of BSA or 1000 pmol/kg/h GLP-2), and recovery (saline infusion). On d 11, calves were euthanized and samples were obtained to determine gut mass. Infusion of GLP-2 increased SMA blood flow to 175% of baseline on d 0 but to only 137% of baseline after

chronic treatment. Compared with control, GLP-2 increased small intestinal mass by 24% by increasing epithelial mass in jejunum and ileum. These results demonstrate that GLP-2 induces similar increases in small intestinal blood flow and growth in ruminants as observed in non-ruminants. Furthermore, GLP-2 increases small intestinal blood flow in ruminants, but this response is attenuated after 10 d of GLP-2 administration.

Introduction

Glucagon-like peptide-2 (GLP-2) is a 33-amino acid hormone secreted within the gastrointestinal tract in response to luminal nutrients. The hallmark of GLP-2 action is an increase in small intestinal mucosal. A second established action of GLP-2 is a rapid increase in blood flow, specifically of vessels supplying and draining the small intestine, such as the superior mesenteric artery and portal vein. The vast majority of the existing published research investigating the effects of GLP-2 on intestinal growth and blood flow has been conducted in non-ruminant animals (rodents, pigs, humans), leaving the biological function of GLP-2 in ruminants as unknown. The digestive system of the ruminant has a more intricate stomach complex that alters foodstuffs prior to their entry into the intestines, which could potentially influence regulatory signals. Therefore, the objectives of this experiment were to examine the effects of exogenously-administered GLP-2 on superior mesenteric artery (SMA) blood flow and gastrointestinal growth in the ruminant.

Materials and Methods

Animals and surgical procedures

Eight Holstein calves (41 ± 3 d old) were fed milk replacer twice daily with water and calf starter (35.1% crimped oats, 30.1% cracked corn, 23.9% soybean meal, 7.6% molasses, and minerals and vitamins to meet requirements) available *ad libitum* before weaning (50 ± 3 d of age). Following weaning the diet was gradually adjusted to a 50:50 (w/w) mixture of alfalfa cubes and calf starter. Dietary supply of energy (1.64 Mcal net energy for maintentence/kg) and protein (19.9% crude protein) was adequate to meet normal growth requirements (24). Calves were fed this diet at 2.75% of BW before and throughout the experiment; their daily allotment was fed in two equally sized meals at 0730 and 1730 h. When calves were 97 ± 7 d of age they were surgically prepared with a 4-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) around the superior mesenteric artery along the distal duodenum. Chronic indwelling catheters were inserted in the carotid artery and portal and mesenteric veins. Catheters and flow probe cable were tunneled subcutaneously and exteriorized along the spine. Before surgery, feed and water were withheld for 24 and 12 h, respectively. On the day of surgery, calves were induced with xylazine (0.09 mg/kg) and ketamine (1.8 mg/kg), intubated, and maintained with isoflurane-oxygen (2-5% isoflurane) for the duration of the surgery. Prior to surgery calves were injected with the long-acting antibiotic ceftiofur (Excede, 6.6 mg/kg BW, Pfizer Animal Health, New York) and were administered flunixin meglumine (1.1 mg/kg BW), with additional flunixin meglumine administered following surgery as needed for analgesia. Catheter exteriorization sites and suture sites were treated daily with antimicrobial ointment. Catheter patency was maintained by biweekly flushing and filling the catheters with a solution of gentamicin sulfate (20 mg/ml) and chymotrypsin (225 U/ml). Experimental procedures began approximately 16 d after surgery (113 ± 8 d of age), once animals had maintained feed intake for a minimum of 5 d.

Experimental design

Calves were paired by age before being assigned randomly to treatment, control (n = 4) or GLP-2 (n = 4). Only two calves (one per treatment) began experimental periods at any one time. Experimental periods were 11 d in length. On d 1 (acute), a blood flow experiment was performed as described below. After the blood flow measurements, calves were given their first dose of treatment via subcutaneous injection; injections were either vehicle (0.5% BSA in saline, control) or GLP-2 (50 µg/kg BW GLP-2 in vehicle, GLP-2). The GLP-2 used for both daily injections and the blood flow experiment was synthesized (California Peptide Research, Inc., Napa, CA) based on the native bovine GLP-2 sequence. Treatments were administered by subcutaneous injection twice daily (every 12 h) for 10 d. On d 10 (chronic) a second blood flow experiment was performed as described below. Calves were slaughtered on d 11.

Blood flow measurements

On d 1 and 10 of the experiment, blood flow was monitored for 2.5 h using the chronically implanted probe to measure superior mesenteric artery blood flow. Blood flow measurements were conducted after withholding the morning feeding to minimize prandial blood flow changes, thus animals were fasted for approximately 12 hrs. The blood flow experiment consisted of three periods; 1) baseline infusion (B1 or B10 for baseline on d 1 or 10, respectively) to establish baseline blood flow during a 30-min infusion of physiological saline; 2) treatment challenge infusion (C1 or C10) in which calves were infused intravenously with their assigned treatment, either control or GLP-2 (1000 pmol•kg-1•h-1) for 60 min; and 3) saline infusion (S1 or S10) in which calves were infused with physiological saline for 60 min to observe the recovery of blood flow after treatment challenge infusion. On d 10, the blood flow experiment was started 3 to 7 h after the subcutaneous injection of treatment for that morning.

Tissue harvest

Calves were fasted for approximately 12 h before tissue harvest on d 11. Calves were euthanized using an overdose of barbituate and immediately eviscerated to obtain forestomachs (reticulorumen, omasum, and abomasum) and intestines (duodenum, jejunum, ileum, and colon). Reticulorumen, omasum, and abomasum were separated and emptied of digestive contents. After sample removal, remaining forestomachs (reticulorumen, omasum, and abomasum), intestines (small and large), cecum, and liver were rinsed with warm tap water to remove any digesta or debris, allowed to drip dry, and weighed.

Statistical analysis

The statistical model for analyzing gastrointestinal organ mass included treatment as a fixed effect with block and block by treatment as random effects. The statistical model for analyzing SMA baseline blood flow and area under the curve

(AUC) included treatment (T; control or GLP-2), day (D; acute or chronic), and their interaction as fixed effects and block as a random effect. For blood flow variables, infusion was included as a repeated measure with the subject as calf (treatment). Multiple t-tests were used to compare the effect of treatment within each infusion and to compare the differences between baseline (B1 vs. B10), treatment challenge (C1 vs. C10) or saline (S1 vs. S10) infusions within the GLP-2 treatment. A Bonferroni correction was used to correct P-values for multiple comparisons. Pearson correlation coefficients were determined between calf observations for some parameters. The blood flow probe for one calf in the GLP-2 treatment group was not measured because of flow probe failure; therefore, for SMA blood flow data n = 4 for the control treatment group but n = 3 for the GLP-2 treatment group. Results are expressed as means ± SE, and significance for treatment effects and correlations was declared at $P < 0.05$.

Results and Discussion

Intestinal mass

After 10 d of treatment, neither live BW nor empty BW was affected by treatment (Table 1). Treatment with GLP-2 for 10 d did not affect total gastrointestinal tract mass or mass of individual gastrointestinal organs except small intestine; GLP-2 increased (P = 0.03) small intestinal mass by 17% (data not shown). When expressed on an empty BW basis (Table 1), GLP-2 increased (P = 0.04) small intestinal mass by 24% and tended (P = 0.09) to decrease abomasal mass but did not affect mass of other individual gastrointestinal organs. Lengths of the small and large intestines and length:EBW were not affected by treatment. Treatment with GLP-2 increased (P = 0.02) mass:length (g/m) of the small intestine but not the large intestine.

Blood flow

Response of SMA blood flow to short-term GLP-2 infusion was dependent on previous exposure to exogenous GLP-2 (Figure 1). Analysis of AUC for SMA blood flow demonstrated that GLP-2 markedly increased SMA flow in acute calves vs. control (2482.0 ± 315.5 vs. 238.1 ± 122.1) but was less effective at increasing SMA flow in chronic calves (968.5 ± 480.5 vs. 201.2 ± 41.7; T × D P = 0.003). Likewise, infusion of GLP-2 increased SMA blood flow to 175% of baseline in the acute period but only to 137% of baseline in the chronic period, whereas control infusion did not affect SMA blood flow. Baseline SMA flow did not differ between acute and chronic exposures (47.5 ± 13.1 and 48.2 ± 13.8 L/h for GLP-2, respectively, vs. 31.2 ± 4.49 and 29.8 ± 3.88 L/h for control, respectively) although calves in the GLP-2 treatment group tended (T P = 0.09) to have a slightly higher baseline SMA flow. Blood flow of the SMA returned to baseline values during the saline infusion following GLP-2 infusion.

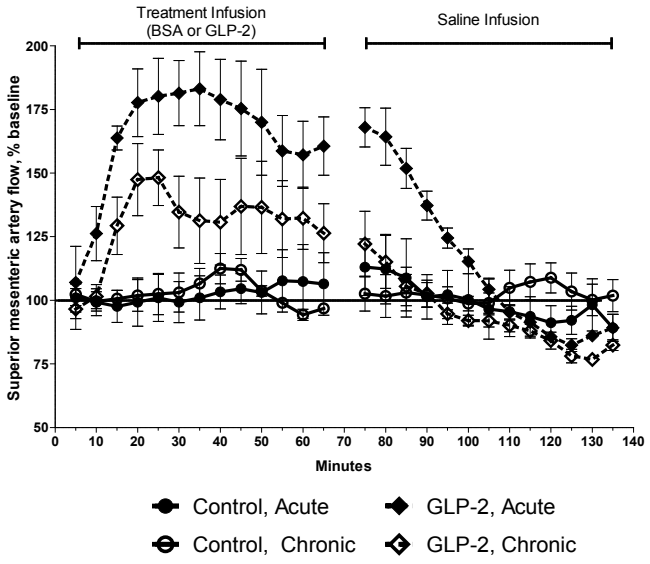
This experiment demonstrates that ruminants respond to GLP-2 administration in a similar manner to non-ruminants. Treatment with GLP-2 increases small intestinal mass. Furthermore, we show that GLP-2 infusion increases blood flow of the superior mesenteric artery when administered to calves not previously exposed to exogenous GLP-2. However, we show

Table 1. The effect of subcutaneous injection of GLP-2 (100 µg•kg BW-1•d-1) for 10 d on body weight and visceral organ mass and intestinal length as a percentage of empty body weight of Holstein calves.

	Control	GLP-2	SEM[1]	P =
Live BW, kg	137	128	8.6	0.48
Empty BW, kg	122	115	7.5	0.55
Organ, % EBW				
Total GIT	7.96	8.54	0.272	0.18
Rumen/reticulum	2.90	2.75	0.136	0.48
Omasum	0.69	0.75	0.040	0.30
Abomasum	0.61	0.54	0.040	0.09
Small intestine	2.72	3.36	0.168	0.04
Large intestine	0.89	0.94	0.065	0.44
Liver	2.29	2.13	0.085	0.23
Length, m				
Small intestine	30.7	29.8	1.54	0.72
Large intestine	5.11	5.10	0.401	0.98
Length: EBW, cm/kg				
Small intestine	25.6	26.0	1.50	0.86
Large intestine	4.30	4.41	0.394	0.85
Mass: Length, g/m				
Small intestine	106.9	129.4	4.22	0.02
Large intestine	211.3	213.0	15.28	0.94

[1] *n = 4*

Figure 1. Superior mesenteric artery blood flow as a percent of baseline blood flow in calves treated with control or GLP-2. Response to treatment challenge or saline infusion in calves not previously exposed to treatment (acute; closed symbols) or after 10 d of treatment exposure (chronic; open symbols) was evaluated. Calves in the control group were given vehicle (BSA) during both the treatment challenge infusion period and as subcutaneous injection for 10 d. Calves in the GLP-2 treatment group were given 1000 pmol•kg BW-1•h-1 GLP-2 during the treatment challenge infusion period and 100 µg•kg BW-1•d-1 GLP-2 as subcutaneous injection for 10 d. Values are expressed as means ± SE.



that chronic administration of GLP-2 significantly attenuates this blood flow response. These results extend our understanding of the actions of GLP-2 and may have implications for the use of GLP-2 in treatment of intestinal injury or adaption in developing ruminants.

Implications

These data show that ruminants respond similarly to non-ruminants when administered exogenous GLP-2. Administration of GLP-2 may be a useful means of selecting stimulating intestinal growth in animals experiencing gastrointestinal complications.

Expression of mRNA for Proglucagon and Glucagon-like Peptide-2 (GLP-2) Receptor in the Ruminant Gastrointestinal Tract and the Influence of Energy Intake

C.C. Taylor-Edwards, D.G. Burrin, J.C. Matthews, K.R. Mcleod, J.J. Holst, and D.L. Harmon

Summary

Glucagon-like peptide-2 (GLP-2) is a potent trophic gut hormone, yet its function in ruminants is relatively unknown. Exp. 1 was conducted to establish the presence of GLP-2 in ruminants and to ascertain if it was responsive to increased nutrition. Concentrations of intact GLP-2 in the blood and gut epithelial mRNA expression of proglucagon (GCG) and the GLP-2 receptor (GLP2R) were measured in four ruminally, duodenally, and ileally cannulated steers. Blood samples and ruminal, duodenal, and ileal epithelium biopsies were collected at low intake (D -6 and -3), acute high intake (D 1 and 3), and chronic high intake (D 7 and 29) periods. Exp. 2 investigated the mRNA expression pattern of GCG and GLP2R in epithelial tissue obtained from the forestomachs (rumen, omasum, and abomasum) and intestines (duodenum, jejunum, ileum, and colon) of 18 forage-fed Angus steers (260 kg BW). In Exp. 1 and 2, real-time PCR showed that expression of GCG and GLP2R mRNA was detectable in forestomach tissues, but expression was greater in small intestinal and colon tissue. High energy intake tended to increase plasma GLP-2 during the acute period and was paralleled by a 78% increase in ileal GCG mRNA expression. After this initial adaptation, duodenal GCG mRNA expression increased during the chronic high-intake period. These data demonstrate that cattle express GCG and GLP2R mRNA primarily in small intestinal and colon tissues. Increased nutrient intake increases ileal GCG mRNA and plasma GLP-2, suggesting that GLP-2 may play a role in the trophic response of the ruminant gastrointestinal tract to increased feed intake.

Introduction

The trophic effect of increased feed intake on gastrointestinal mass in ruminants is well-documented. However, the mechanisms by which nutrient intake increases epithelial hyperplasia have not been elucidated. In non-ruminants, studies suggest that GLP-2 links gastrointestinal growth to increased energy intake. Glucagon-like peptide-2 is a 33-amino acid hormone secreted by the enteroendocrine L-cell, primarily from the distal intestine. The L-cell produces GLP-2 as part of a larger precursor mRNA and protein sequence, proglucagon, which contains the sequences for glucagon, GLP-1, and GLP-2. Proglucagon mRNA (GCG) and protein is also produced in the pancreas, and it is the post-translational cleavage by tissue-specific prohormone convertase enzymes that determines the final secreted products of glucagon in the pancreas and GLP-1 and GLP-2 in the intestinal L-cell.

Effects of GLP-2 occur via the GLP-2 receptor (GLP2R). Expression of GLP2R mRNA in the non-ruminant gastrointestinal tract is highest in the proximal small intestine. This distribution also agrees with the site of the greatest response to GLP-2 administration. However, there is a paucity of information describing the biological function of GLP-2 secretion and the GLP-2 receptor in ruminants. GLP-2 could be a key nutrient responsive hormone in cattle. The objectives of Exp. 1 were to determine if 1) cattle express GCG and GLP2R mRNA in ruminal and intestinal tissues and secrete GLP-2 protein in plasma, 2) plasma concentrations of GLP-2 and gastrointestinal tissue expression of GCG and GLP2R mRNA change in response to a change in dietary energy intake, which is a known stimulus for GLP-2 secretion in non-ruminants, and 3) changes in plasma GLP-2 concentrations coincide with changes in GCG mRNA in ruminal and intestinal tissues in cattle.

Materials and Methods

Design Experiment 1

Four ruminally, duodenally, and ileally cannulated steers (417 ± 48 kg) were utilized in this experiment. For 21 d (d 21 to d 1) prior to the start of the collection period, steers were fed 0.75 × NEm (low intake). On day 0, diet provided was increased to 1.75 × NEm requirements (acute and chronic high intakes). Diet ingredient composition was 87.7% corn silage and 12.3% of a ground corn-based supplement (DM basis). Steers were weighed weekly throughout the experiment (d-21, -14, -7, 0, 7, 14, 21, 29), and the amount of feed offered was recalculated on d 0, 7, 14, and 21. Ruminal, duodenal, and ileal biopsies and blood samples were taken as described below at -6 and -3 d (low), 1 and 3 d (acute), and 7 and 29 d (chronic) relative to the change in energy intake. Steers were housed in individual stalls in a temperature (20°C) and light-controlled (12 h light: 12 h dark) room with water available *ad libitum*.

Sample collection

Animals were biopsied prior to feeding in a squeeze chute. Mild sedation with xylazine (0.088 mg/kg) was utilized for animal comfort and to reduce stress. For ruminal biopsies, ruminal contents were partially or fully evacuated, and 5-6 papillae sections were removed from the ventral ruminal sac using biopsy clippers. Intestinal (duodenal and ileal) biopsies were obtained by introducing an endoscope through the appropriate cannula. Duodenal biopsies were obtained approximately 40 to 50 cm distal to the pyloric sphincter and ileal biopsies approximately 50 to 60 cm proximal to the ileal-cecal junction. Jugular blood samples were obtained immediately after biopsies for analysis of dipeptidyl peptidase IV activity (DPPIV) and plasma GLP-2 concentrations, respectively. Relative quantitation of GCG and GLP2R expression were conducted using the relative standard curve method with expression normalized to 18S rRNA expression using real time-PCR.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The model included the fixed effect of day of sampling and the random effect of steer, with day included as a repeated measure with steer as the subject. If no difference between the two days in an intake period was found, contrasts were used to determine differences among intake periods (low, acute high, or chronic high) on mRNA expression and plasma variables. Additionally, a model including the fixed effect of tissue across sampling day and steer was used to determine the overall difference in mRNA expression between the rumen, duodenum, and ileum. Significance was declared at P = 0.05, and tendencies were declared at P = 0.10.

Design Experiment 2

Eighteen Angus steers (260 ± 17 kg) were used in this experiment. Steers were part of an experiment using three infusion treatments; 1) Control (water), or infusion of an additional 20% of ME intake as starch hydrolysate into the 2) rumen or 3) abomasum. Steers were fed alfalfa cubes (17.8% CP and 1.31 Mcal of NEM/kg, DM basis) at 1.33 × NEM in 12 meals daily (2 h intervals). Steers were individually housed in metabolism tie stalls (1.2 × 2.4 m2) in a temperature-controlled room (20°C) with water available *ad libitum*.

Sample collection

Steers were slaughtered on either d 19 or 21. Forestomachs (reticulorumen, omasum, and abomasum), intestines, and liver were obtained. Extraction of total RNA for reverse transcription, and semi-quantitative real time-PCR was performed immediately.

Statistical analysis

One animal was excluded from all analyses due to removal from the experiment. Forestomach and intestinal tissue mRNA expression data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC.) with a model including infusion treatment, tissue, and their interaction as fixed effects and period and block(period) as random effects. For mRNA expression variables, tissue was included as a repeated measure with the subject as treatment*block(period). When a significant tissue effect was detected for mRNA expression, means were compared using the Tukey-Kramer multiple comparison test.

Results and Discussion

Experiment 1

Increasing energy intake from low to high tended to increase (P = 0.07; Table 1) plasma GLP-2 concentrations by 37% in the acute period, but concentrations subsequently decreased such that GLP-2 concentrations during the chronic period were intermediate to the acute high- intake and low-intake periods but did not differ from either. Plasma DPPIV activity was not affected by a change from low intake to acute high intake, but plasma DPPIV activity tended to increase by 22% (P = 0.07) during the chronic high intake period compared to the acute high intake period because of greater (P = 0.002) DPPIV activ-

Table 1. Means of plasma variables and relative expression of proglucagon and GLP-2 receptor mRNA (to 18S rRNA) in steers changed from 0.75 × NE_M energy intake (d -6 and -3) to 1.75 × NE_M energy intake (d 1, 3, 7, and 29).

	Day							P =		
	Low		Acute		Chronic					
	-6	-3	1	3	7	29	SEM[a]	Low vs. Acute	Low vs. Chronic	Acute vs. Chronic
Plasma										
Active GLP-2, pM	14.0	10.3	15.5	17.8	12.5	16.5	2.250	0.07	0.31	0.36
DPPIV, nmol/ (ml•min)	17.9	19.1	19.1	17.1	16.3	27.7	5.98	0.85	0.11*	0.07*
Proglucagon mRNA[b]										
Rumen[c]	ND	ND	ND	ND	ND	ND				
Duodenum	1.80	0.84	0.65	0.82	2.09	1.48	0.710	0.31	0.42	0.08
Ileum	0.89	0.92	1.67	1.55	0.96	1.35	0.359	0.07	0.50	0.22
GLP-2 receptor mRNA[b]										
Rumen	0.051	0.031	0.029	0.037	0.009	0.015	0.0252	0.62	0.14	0.28
Duodenum	0.47	1.17	1.01	1.48	1.34	1.62	0.650	0.49	0.28	0.70
Ileum	0.61	0.84	1.27	1.04	1.28	2.24	0.421	0.17	0.004*	0.06*

[a] *n = 4*
[b] *Relative to 18S rRNA expression.*
[c] *ND = not detected.*
** Significance results from statistical difference of low or acute period versus D29 of the chronic period.*

ity at D29. Likewise, DPPIV activity during the chronic high intake period was numerically 19% greater (P = 0.11) than the low intake period because of greater (P = 0.002) DPPIV activity at D29.

Proglucagon mRNA (relative to 18S rRNA) was detected in duodenal and ileal epithelia but not in the rumen epithelium samples (Table 1). Changing from low intake to acute high intake numerically decreased duodenal GCG mRNA expression, whereas chronic high intake tended to increase (P = 0.08) duodenal GCG mRNA expression by 141%, relative to the acute period of high intake. In contrast to the effect of energy intake on duodenal GCG expression, there was a tendency for an increase (P = 0.07) in ileal GCG mRNA expression by 78% following the change from low to acute high intake, but then ileal expression of GCG mRNA decreased slightly such that expression during the chronic high-intake period was intermediate to that observed for the low and acute high intake periods and did not differ from either.

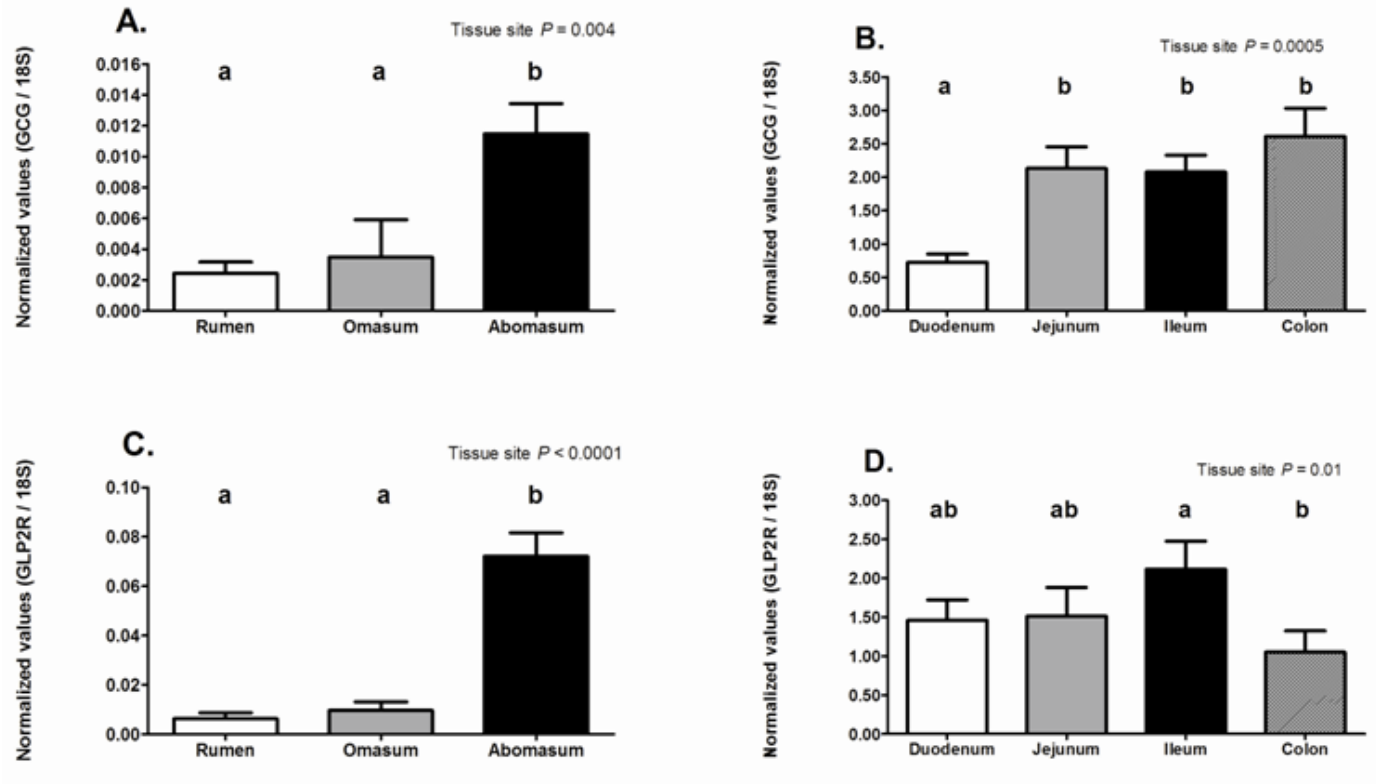
Expression of the GLP2R mRNA was detected in ruminal, duodenal, and ileal epithelia biopsies (Table 1). Across all sampling days and steers, expression of GLP2R mRNA in the duodenum and ileum was approximately 39-fold greater than that in the rumen (P < 0.0001), but expression did not differ between the two intestinal sites (P = 0.91). Duodenal GLP2R expression was not affected by changes in energy intake level. Ileal GLP2R expression did not differ between the low-intake and acute high-intake periods, but chronic high intake tended to increase (P = 0.06) ileal GLP2R expression compared to the acute high intake period because of greater (P = 0.01) expression at D29. Likewise, ileal GLP2R expression was greater (P = 0.004) during the chronic high intake period vs. the low intake period because of greater (P = 0.001) ileal GLP2R mRNA expression at D29.

Experiment 2

Proglucagon mRNA was detected in all forestomach and intestinal epithelia analyzed (Figure 1A and B). However, intestinal tissue (duodenum, jejunum, ileum, and colon) expression of GCG mRNA (relative to 18S rRNA) was approximately 5000-fold greater (P < 0.0001) than expression by forestomach epithelia (rumen, omasum, and abomasum). Within the forestomachs, abomasal expression of GCG mRNA was greater (P = 0.004; Figure 1A) than ruminal and omasal expression. Within the intestines, duodenal GCG mRNA expression was less (P = 0.0005; Figure 1B) than that in the jejunum, ileum, and colon, but expression in distal intestinal segments did not differ from each other.

Expression of GLP2R mRNA was detected in all seven tissues analyzed (Figure 1C and D). However, intestinal tissue (duodenum, jejunum, ileum, and colon) expression of GLP2R mRNA (relative to 18S rRNA) was approximately 49-fold greater (P < 0.0001) than forestomach expression (rumen, omasum, and abomasum). Within the forestomachs, abomasal expression of GLP2R mRNA was greater (P < 0.0001; Figure 1C) than ruminal and omasal expression. Within intestines, expression of GLP2R mRNA was greatest (P = 0.01; Figure 1D) in the

Figure 1. Expression (normalized to 18S rRNA, 18S) of proglucagon (GCG) mRNA by forestomach (A) and intestinal tissues (B) and GLP-2 receptor (GLP2R) mRNA by forestomach (C) and intestinal tissues (D) of growing beef steers (n = 17). Data are expressed as means ± SEM. Means without a common superscript letter differ (Tukey-Kramer test, P < 0.05).



ileum and lowest in the colon, and jejunal and ileal expression of GLP2R mRNA did not differ from either ileum or colon.

GLP-2 mRNA and its receptor and plasma GLP-2 exist in cattle and overall appear to respond similarly to other non-ruminant species in response to nutrient intake. Forestomach expression of both GCG and GLP2R mRNA is detectable but is substantially lower than intestinal expression, suggesting that other factors are likely more important in regulating forestomach epithelial growth. Distribution of GCG mRNA increased from the duodenum to the mid-intestine but did not differ among jejunum, ileum, and colon, whereas distribution of GLP2R mRNA was greatest in ileum and least in colon with duodenum and jejunum intermediate to the two. Ileal tissue appears to respond to an increase in energy intake by increasing GCG mRNA expression and likely secretion of GLP-2, thus contributing toward greater plasma GLP-2 levels, whereas duodenal GCG mRNA is less responsive to changes in dietary energy intake.

Implications

These data describe the distribution of GCG and GLP2R mRNA and provide evidence that GLP-2 changes occur prior to intestinal mass changes observed in ruminants with increasing energy intake. Thus, GLP-2 may be involved in the intestinal growth response to increasing energy intake in cattle.

Influence of Slow-Release Urea on N Balance and Gastrointestinal Nutrient Absorption In Steers

C.C. Taylor Edwards, N.A. Elam, S.E. Kitts, K.R. McLeod, D.E. Axe, D.L. Harmon

Summary

Effects of feed-grade urea (CON) or slow-release urea (SRU) on N balance and nutrient absorption were investigated. Ten steers with hepatic portal, mesenteric venous, and mesenteric arterial catheters were fed either CON or SRU at 1.6% of diet dry matter in a predominantly corn silage diet. Total fecal and urine output were measured on d 15 to 19, and nutrient absorption across the portal-drained viscera (PDV) was determined on d 21 of each period. Intake of all nutrients did not differ among treatments, and digestibility of all nutrients was similar among treatments with the exception of crude protein, which was lower for SRU than CON urea. SRU increased fecal N as a percent of intake N by tending to increase amount of N excreted daily in the feces. Both treatments increased arterial urea concentrations, but SRU consistently reduced mean arterial urea N concentration compared to CON urea. Alterations with portal ammonia flux and glutamate/glutamine metabolism suggest SRU reduces the need for ammonia disposal. However, 19% greater net transfer of urea to the gastrointestinal tract for SRU indicates increased net recycling of urea, and combined with greater fecal N excretion, these results may suggest limited availability or timing of urea availability for ruminal utilization.

Introduction

Microbial growth can be limited by asynchrony of dietary carbohydrate and nitrogen supply. Dietary urea is rapidly hydrolyzed in the rumen, thereby increasing ruminal ammonia-N concentrations before carbohydrate is available for microbial utilization. In contrast, slow-release urea (SRU) is a coated form of urea that releases N more slowly than feed-grade urea. This product has the potential to increase microbial utilization of ammonia-N, which may increase N efficiency and reduce N excretion. Previous research has demonstrated that SRU does release ammonia more slowly in the rumen and reduces the fluctuations in urea and ammonia concentrations observed when feeding feed-grade urea. However, no research has investigated the effects of urea source on N balance in steers. Therefore, the objective of this experiment was to investigate the effects of feeding feed-grade urea (CON) or SRU on N balance and nutrient flux across the portal-drained viscera in steers.

Materials and Methods

Four Holstein steers (236 ± 43 kg BW) and six Angus steers (367 ± 46 kg BW) were surgically prepared with permanent vascular catheters in the hepatic portal vein, mesenteric vein, and mesenteric artery. Animals were randomly assigned to treatment sequence within the crossover design. Treatment periods were 21 d in length with a 14-d diet adaptation followed by 5 d of N balance (d 15 to 19) and nutrient absorption across the portal-drained viscera measured on d 21. Steers were fed a corn silage based diet containing 1.6% CON or SRU on a dry matter-basis (Table 1). Diets were offered once daily at 2.0% of body weight (DM basis). Diet ingredients were sampled daily and composited by week. Orts were weighed, sub-sampled, and frozen separately. Diet ingredients and orts were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF).

Urine and feces were collected for five 24-h periods during N-balance measurements. N content was determined for all fecal and urine samples; in addition, fecal samples were analyzed for DM, OM, NDF, and ADF for digestibility calculations.

Table 1. Composition of diet for steers fed corn silage with control or slow-release urea (SRU).

Ingredient	% of DM
Corn silage	88.4
Supplement	10.0
Ground corn	73.99
Choice white grease	3.00
Dicalcium phosphate	10.21
Limestone	9.50
Trace mineral mix[1]	3.00
Vitamin ADE mix[2]	0.30
Urea or SRU	1.6

[1] Trace mineral mix contained 96.0-98.5% NaCl, 3500 mg/kg Zn, 2,000 mg/kg Fe, 1,800 mg/kg Mn, 370 mg/kg Mg, 350 mg/kg Cu, 100 mg/kg I, 90 mg/kg Se, and 60 mg/kg Co.

[2] Vitamin ADE mix contained 1.8 million IU/kg vitamin A, 3.6 million IU/kg vitamin D, and 227 IU/kg vitamin E.

Table 2. Nutrient intake and digestibilities in steers consuming urea or slow-release urea (SRU).

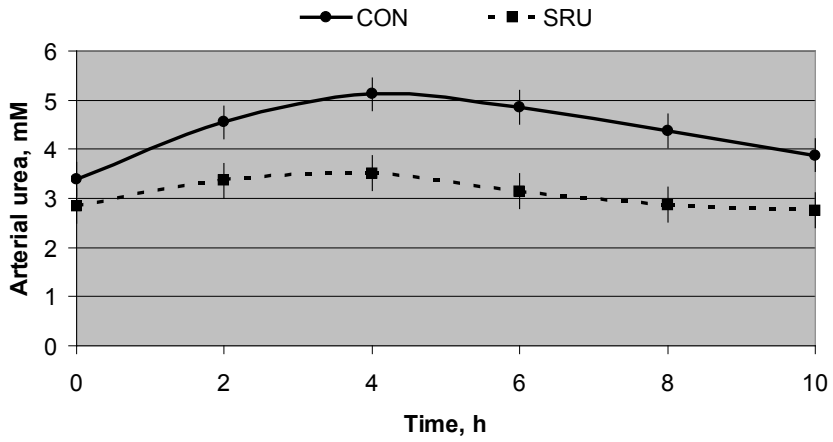
	Treatments			
	CON	SRU	SEM	P <
Intake, kg/d				
DM	6.23	6.27	0.09	0.77
OM	5.81	5.85	0.09	0.75
CP	0.79	0.76	0.02	0.30
NDF	1.94	1.95	0.03	0.73
ADF	1.11	1.11	0.01	0.74
Digestibility, %				
DM	71.55	71.76	0.54	0.80
OM	73.43	73.51	0.52	0.91
CP	99.05	98.94	0.02	0.03
NDF	46.50	45.82	1.85	0.81
ADF	45.98	44.87	1.82	0.69
Gain over 21-d period, kg	10.02	15.09	2.6	0.26

Table 3. Nitrogen balance of steers consuming urea or slow-release urea (SRU).

	Treatments			P<	
	CON	SRU	SEM	Trt	Trt x Time
N, g/d					
Intake	133.7	130.1	14.3	0.28	0.26
Urine	38.2	36.4	4.2	0.44	0.64
Feces	45.9	49.5	5.2	0.06	0.16
Retention	50.8	44.6	9.5	0.12	0.13
N, % of N Intake					
Urine	30.6	29.4	4.1	0.62	0.47
Feces	34.2	38.3	1.0	0.002	0.14
Retention	35.8	32.9	4.1	0.28	0.12

On d 21, steers were infused with 250 mM p-aminohippuric acid (PAH) starting 1 h prior to the first sample and continuing throughout the sampling period. After 1 h of infusion, time zero blood samples were taken, and steers were fed immediately. Blood samples were obtained 2, 4, 6, 8, and 10 h after feeding by simultaneously drawing hepatic portal and mesenteric arterial samples (3 mL) into heparinized syringes for analysis of PAH, oxygen saturation, and hematocrit. Blood samples (25 ml) were also taken for plasma analysis of PAH and blood metabolites.

Figure 1. Arterial plasma urea concentrations in steers consuming urea or slow-release urea (SRU).



Statistical Analysis

The model for analyzing N balance included treatment as a fixed effect and square, steer (square), and period (square) as random effects, with time used as a repeated measure with steer as the subject. Means were calculated within steer and period for PAH and metabolite concentrations in arterial and portal blood samples. Blood flow was calculated as PAH infusion rate divided by mean venoarterial PAH difference across the respective vascular beds. Net flux of metabolites across the PDV was calculated as the product of portal blood flow and portal-arterial concentration difference. All flux data were analyzed as described above with time and treatment time interaction terms included in the model.

Results and Discussion

Nutrient intakes and digestibilities are presented in Table 2. Intake of DM, OM, CP, NDF, and ADF did not differ among treatments. Additionally, digestibility of DM, OM, NDF, and ADF did not differ among treatments. However, digestibility of CP was lower for SRU compared to CON urea (98.9 vs. 99.1%, P < 0.03). This is also reflected in the N balance data presented in Table 3. Steers consuming SRU excreted more N in the feces than steers consuming CON urea, both as a total amount (49.5 vs. 45.9 g/d, P < 0.06) and as a % of N intake (38.3 vs. 34.2%, P < 0.002). There were no differences among treatments for urinary N excretion (Table 3) or urinary urea excretion (mean 53.4 g/d, P < 0.29, data not shown). Because N intake did not differ among treatments, N retention (g/d) was numerically greater (P < 0.12) for CON vs. SRU; however, there were no treatment differences when N retention was expressed as a % of N intake (P < 0.28) and body weight changes over the 21-d period did not differ (P < 0.26).

It has been demonstrated in previous experiments that SRU coating material reduces rapidity of ammonia release in the rumen. However, greater fecal N excretion of steers consuming SRU suggests that ammonia from SRU is released so slowly that supplemented N from SRU is excreted in feces. Alternatively, SRU may supply additional N to microorganisms in the lower gastrointestinal tract, resulting in increased microbial growth and loss of microbial N in the feces. Despite the reduction of ruminal ammonia release rate, there is no indication that feeding SRU limited total tract nutrient digestion in this experiment. However, more research is needed to determine effects of SRU supplementation on ruminal nutrient digestibility to assure that ruminal bacteria are not limited by ruminal N availability. Research investigating more long-term SRU supplementation is also warranted because N retention data suggests that for long-term supplementation, SRU may impact N retention.

Results from blood sampling data are shown in Table 4. Portal blood flow and plasma flow were not affected by treatment, but a treatment x time interaction was detected because of increased blood flow following feeding immediately after the zero time sample. Mean arterial urea concentrations were 41.6% greater for steers consuming CON urea compared to SRU ($P < 0.03$). Feeding CON urea increased the rapidity of urea appearance in the arterial blood, whereas steers consuming SRU urea exhibited a more consistent arterial urea concentration as shown in Figure 1 (treatment x time $P < 0.007$). Urea source did not affect portal urea flux ($P < 0.33$) and portal urea flux was negative for both treatments, indicating transfer of urea from the portal blood circulation into the gastrointestinal tract. However, the net transfer of urea to the gastrointestinal tract was 19% greater for SRU than CON urea.

Arterial ammonia concentrations did not differ among treatments, and mean portal flux of ammonia was similar among treatments, but there was a tendency for a treatment x time interaction ($P < 0.11$) because CON urea increased portal ammonia flux 2 h after feeding but was similar to SRU at other time points (Figure 2). These results again demonstrate the more steady and consistent release of ammonia from coated SRU than from a standard feed-grade urea. The reductions in blood urea concentrations and portal ammonia flux seen in this experiment are an advantage for SRU over feed-grade urea because of the negative impact that high blood urea and ammonia concentrations can have on feed intake and fertility.

SRU tended to increase arterial concentrations of glutamate ($P < 0.10$) and gastrointestinal extraction of glutamate ($P < 0.10$) compared to CON urea. In contrast, SRU decreased arterial concentrations of glutamine ($P < 0.01$) and gastrointestinal extraction of glutamine ($P < 0.03$) compared to CON urea. These shifts in glutamate and glutamine metabolism in addition to lower arterial urea concentrations are consistent with a reduced need for ammonia disposal for animals consuming SRU. Differences among other metabolites were minimal; treatment did not affect arterial concentrations or portal fluxes of O_2, glucose, lactate, or β-hydroxybutyrate, suggesting that there were no differences in gastrointestinal energy metabolism in response to urea source.

Figure 2. Portal ammonia flux of steers consuming urea or slow-release urea (SRU).

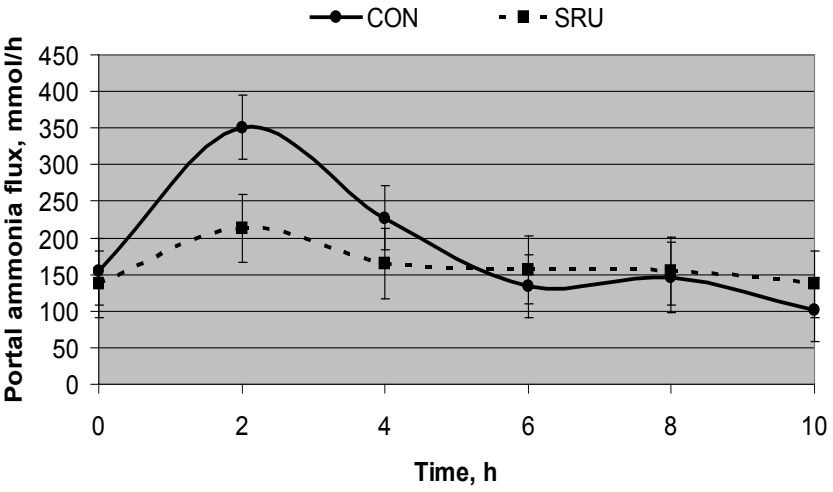


Table 4. Arterial blood (O2) and plasma metabolite concentrations and portal blood (O_2) and plasma metabolite fluxes in steers consuming urea or slow-release urea (SRU).

	Treatments			P <	
	CON	SRU	SEM	Trt	Trt x Time
Portal blood flow, L/h	859.9	844.3	96.5	0.90	0.06
Portal plasma flow, L/h	518.2	531.8	72.0	0.85	0.18
O_2					
Arterial concentration, mM	5.67	5.45	0.16	0.27	0.45
Portal flux, mmol/h	-1360.7	-1310.0	147.8	0.81	0.26
Urea					
Arterial concentration, mM	4.36	3.08	0.32	0.03	0.007
Portal flux, mmol/h	-57.7	-68.7	17.9	0.33	0.43
Ammonia					
Arterial concentration, mM	0.26	0.23	0.06	0.41	0.51
Portal flux, mmol/h	186.4	158.6	21.2	0.31	0.11
Glutamate					
Arterial concentration, mM	0.09	0.11	0.008	0.10	0.92
Portal flux, mmol/h	-2.65	-4.22	0.62	0.10	0.13
Glutamine					
Arterial concentration, mM	0.21	0.17	0.01	0.01	0.13
Portal flux, mmol/h	-9.75	-6.86	1.19	0.03	0.02
Glucose					
Arterial concentration, mM	4.60	4.68	0.24	0.70	0.43
Portal flux, mmol/h	-25.5	-20.5	6.3	0.55	0.40
Lactate					
Arterial concentration, mM	0.71	0.78	0.07	0.27	0.83
Portal flux, mmol/h	55.6	57.9	8.6	0.81	0.83
b-hydroxybutyrate					
Arterial concentration, mM	0.62	0.55	0.06	0.17	0.43
Portal flux, mmol/h	116.0	110.3	21.6	0.78	0.56

Implications

Slow-release urea reduces the rapidity of ammonia-N release and reduces shifts in glutamate/glutamine metabolism associated with disposal of ammonia. However, increased fecal N excretion and reduced plasma urea concentrations suggest although SRU may not limit total tract digestion, it may limit ruminal N utilization. The timing of SRU N release may not be fully synchronized with ruminal carbohydrate digestion despite more constant ammonia-N release. Further research is needed to determine if site of nutrient digestion and ruminal N availability are affected by slow-release urea products.

Effects of Diet and Phlorizin on Glucose Absorption from the Small Intestine of Steers

A.L. Ballou, K.R. McLeod, N.B. Kristensen, and D.L. Harmon

Summary

Adult ruminants have a limited capacity for small-intestinal starch utilization. Glucose absorption could be a limiting step in this process. This experiment evaluated the effects of forage or grain-based diets and phlorizin on small-intestinal glucose absorption. Phlorizin, an inhibitor of the glucose active transporter (SGLT1), was used to determine the proportion of glucose absorbed by SGLT1. Steers (n=6) were adapted to two isocaloric diets, high-forage and high-concentrate, at 1.5xNEm, and samples of portal and hepatic venous and mesenteric arterial blood were collected during two periods representing two abomasal infusions. Glucose (20g/h) was infused abomasally for four hours with and without phlorizin. Recovery of glucose from the PDV was 80% higher in steers adapted to the concentrate diet, and 40% lower in phlorizin-infused steers. These effects suggest absorptive capacity can be increased by dietary starch, and the system is reliant on SGLT1.

Introduction

The ruminant small intestine is not ideally suited to processing large quantities of starch as in non-ruminants, and this has led to questions about the ruminant's ability to efficiently utilize the high-starch diets fed in certain production scenarios. Of particular interest is the ruminant's capacity to absorb luminal glucose across the intestinal membrane. Identified as the major intestinal glucose transporter for several species, sodium-dependent glucose cotransporter 1 (SGLT1) has often been the focus of research quantifying the glucose-uptake capacity of ruminants. To that end, infusion of phlorizin, a glucoside and competitive inhibitor of SGLT1, allows for the analysis of sodium-dependent and –independent glucose absorption.

Our hypothesis was that if SGLT1 is the primary pathway of transcellular glucose absorption, then phlorizin should decrease glucose absorption, and this response might differ depending on dietary starch concentrations. The objective was to evaluate the capacity of the small intestine to absorb glucose at high and low dietary starch intakes in the presence and absence of phlorizin.

Materials and Methods

Six Holstein steers (222 ± 3.4 kg) were used in a crossover design with a 2x2 factorial treatment structure with two diets, forage (F) and concentrate (C), and two abomasal infusions, glucose (con) and glucose + phlorizin (phl). The steers were surgically fitted with chronic indwelling catheters in the portal, hepatic, and mesenteric veins, and in the mesenteric artery and an abomasal catheter was placed in the pyloric region of the abomasum. The animals were fed a basal diet of fescue hay at 1.5 x NEm. The energy value of the forage diet was meant to minimize the dietary starch reaching the small intestine while meeting nutrient requirements (Table 1). Feed was provided twice daily.

Sampling Procedure

On sampling days, the steers were held in sampling stalls for two 4-hr series of blood collections. The steers were first sampled while on the basal hay diet involving two abomasal infusions, glucose (control), and glucose + phlorizin. The continuous infusions consisted of 250 mM para-aminohippuric acid infused into the mesenteric vein at 1 mL/min with a 15-mL priming dose and infusion of 1.11 M anhydrous glucose into the abomasum at a rate of 100 mL/h. A temporary jugular catheter was placed for the infusion of 9.73 mM [U-^{13}C] D-glucose (99 atom %, Cambridge Isotope Labs, Andover, MA) at 1 mL/min. A 50-mL priming dose was administered immediately prior to the start of the infusion. The animals were fed prior to the sampling period. The infusions were allowed to run for 1 hr after priming to allow the concentrations of pAH and ^{13}C-glucose in the blood to reach a steady level as well as to allow the glucose infused into the abomasum to reach the small intestine. At 1 hr into the infusion, sampling started with collection of 20 mL blood from each of the portal, hepatic, and mesenteric artery catheters. Six samples were taken from each catheter in 30-minute intervals. At the end of the sixth sample collection (3.5 hr after start of infusion), a solution of 6.67 mM phlorizin in 1mL/min water (400 µmol/h) was primed (30 mL) and infused into the abomasum concurrently with the glucose already being infused. Another 1-hr period elapsed before sampling to allow time for the phlorizin to begin entering the small intestine. Another round of six sets of blood samples were collected as per the description above. Following this first sampling day, the steers were adjusted over a one-week period from their basal hay diet to a high-concentrate diet. The sampling procedure given above was repeated for each steer on the high concentrate diet.

Laboratory Analyses

Plasma was collected and analyzed on site for glucose concentration and aliquots were frozen for later analyses. Aliquots were thawed, and 0.5 mL samples were analyzed for pAH with a colorimetric assay and for ^{13}C-glucose by gas chromatography/isotope ratio mass spectrometry (GC/IRMS) after pentaacetate derivitization.

Calculations and Statistical Analysis

The data were analyzed as a crossover design with a 2x2 treatment structure using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included diet, infusion, and animal. The data were analyzed for interactions, but none were observed; all results reported are main effects. Blood sample data collected for each steer over the sampling period were averaged across time, and these means were used for the statistical analysis. Significance was set at $P \leq 0.05$. Because phlorizin infusion occurred after control in each period, phlorizin effects were confounded with time. The same was true of diet

effects; the steers were sampled first while on the forage diet, then on the concentrate diet.

The following calculations were used to determine blood flow and nutrient use/release across the PDV:

Fick principle of blood flow

$$BloodFlow = \frac{Infusion_{pAH}(mg/h)}{[pAH]_{venous} - [pAH]_{arterial}(mg/L)} = L/h$$

Net Fluxes Across PDV (Bergman et al., 1970)

$$Flux_{PDV} = BloodFlow_{Portal} \times (Cp - Ca)$$

Where Cp and Ca are the nutrient concentrations in portal and arterial blood, respectively, and a positive value indicates absorption or release of nutrients into the blood.

The following calculations were used to determine PDV utilization of arterial glucose:

Enrichment of ^{13}C glucose (Tetens et al., 1995):

$$\delta^{13}C = \left[\frac{R_{SA} - R_{ST}}{R_{ST}}\right] \times 10^3$$

Where R is the ratio between [^{13}C] and [^{12}C] abundance in glucose, SA is sample, and ST is standard.

Atom Percent [^{13}C] (Tetens et al., 1995):

$$AP = \left[\frac{[^{13}C]}{([^{13}C] + [^{12}C])}\right] \times 100$$

Portal recovery of arterial glucose (Tetens et al., 1995):

$$Portal\ rec = \frac{(AP_P - AP_B) \times [glc]_P}{(AP_A - AP_B) \times [glc]_A}$$

Where AP is atom percent, P is portal, A is arterial, B is background, and [glc] is the concentration of glucose in the blood.

Portal-drained viscera uptake of arterial glucose (Tetens et al., 1995):

$$PDV\ uptake = (1 - portal\ rec) \times [glc]_A \times PBF$$

Where portal rec is portal recovery of arterial glucose, is the concentration of glucose in the artery, and PBF is portal blood flow.

Total absorption of glucose by the PDV is defined as:

$$Net\ PDV\ glucose\ flux + PDV\ uptake\ of\ arterial\ glucose$$

Results and Discussion

Diet

Increasing dietary starch increased ($P < 0.01$) portal glucose concentration (Table 2) and tended to increase arterial glucose concentration. Portal ($P < 0.01$) blood flow increased with the concentrate diet. Feeding the concentrate diet nearly doubled net portal glucose flux ($P < 0.01$). This change in portal flux can be attributed to an increase in glucose absorption across the enterocytes of the small intestine, an effect noted by several researchers infusing or feeding high levels of starch or glucose. Though this increase in net glucose flux could be an indication of adaptation to the diet, some, maybe most, of the increase, is the result of increasing flow of dietary starch to the small intestine. As no samples of luminal contents were collected, there is no way to determine the exact change in basal luminal glucose between the forage and concentrate treatments. Looking at net portal glucose recovery (the amount of infused glucose absorbed in the small intestine), the concentrate diet resulted in a nearly twofold increase in percent of infused glucose recovered ($P < 0.01$). After accounting for PDV utilization of arterial glucose, total glucose recovery from concentrate-fed steers increased ($P < 0.01$) 40% over that of forage-fed animals. Total recovery of infused glucose was 90% on the concentrate diet.

The increase in total recovery of infused glucose in the concentrate group cannot be proven to be a result of small intestinal adaptation to diet, though that is a possibility; increased dietary starch in concentrate-fed animals will result in increased flow of starch to the small intestine and in this study could account for a net portal glucose flux of as much as 51 mmol/h. If this estimate is accurate, changes in the diet could be completely responsible for the increase in glucose absorption in concentrate-fed animals. However, an argument in favor of physiological adaptation to diet is the difference in total glucose recovery between the forage and concentrate diets. If the entirety of this increase can be accounted for by increased dietary starch in the small intestine and there is no adaptive response to diet composition, then animals fed the forage diet should maintain the same absorptive capacity as those fed starch. However, that is not the case, as forage-fed steers only assimilated 55 mmol/h of the infused glucose compared with 99 mmol/h in the concentrate group.

Inhibitor

Arterial and portal blood glucose concentrations were both increased ($P < 0.01$) by infusion of phlorizin (Table 3). This result was unexpected but could simply be a result of several hours of constant infusion of glucose into the abomasum. Portal blood flow (L/h) tended to increase with phlorizin ($P < 0.10$), but there was no effect on net portal glucose flux. There was no effect of the inhibitor on net portal glucose recovery or on PDV utilization of arterial glucose. However, there was a large numerical decrease in PDV utilization between control and phlorizin

Table 1. Composition of forage and concentrate diets fed to steers used to measure small intestinal glucose absorption.

	% (DM basis)	
Ingredient	**Forage**	**Concentrate**
Fescue hay	99.5	20.000
Cracked corn		70.000
Ground corn		5.747
Soybean meal		2.000
Limestone		1.250
Urea		0.500
TM-salt[a]	0.5	0.300
Fat		0.200
ADE premix[b]		0.003
Total	100.0	100.000
Nutrient Analysis		
Crude protein (%)	9.5	12.8
NE_m (Mcal/kg)	1.03	1.87

[a] *92.00% NaCl, 5,500 ppm Zn, 4,790 ppm Mn, 1,835 ppm Cu, 9,275 ppm Fe, 115 ppm I, 65 ppm Co, 18 ppm Se.*
[b] *8,800 IU/g vitamin A, 1,760 IU/g vitamin D, 1.1 IU/g vitamin E.*

Table 2. Glucose concentrations, blood flow, absorption, and metabolism in steers fed forage or concentrate diets.

	Diet			
Item	**Forage**	**Concentrate**	**SEM**	**P-value**
Arterial glucose, mM	4.49	4.59	0.051	0.0889
Portal glucose, mM	4.62	4.77	0.046	0.0061
Portal blood flow, L/h	350	434	15	<0.0001
Net portal glucose flux, mmol/h	41.74	74.72	6.558	0.0001
Recovery of infused glucose, %	39	68	5.9	<0.0001
PDV glucose utilization[a], mmol/h	38	23	23.4	0.526
Total glucose absorption[b], mmol/h	55	99	13.9	0.0064
Corrected recovery of infused glucose, %[c]	51	90	12.7	0.006

n = 12
[a] *PDV utilization of arterial glucose calculated by use of arterial [U-13C] D-glucose.*
[b] *Calculated as a net portal glucose flux + PDV utilization.*
[c] *Recovery of 20g/h abomasally infused glucose corrected for PDV use.*

Table 3. Effect of abomasal infusion of phlorizin on glucpse concentrations, blood flow, absorption, and metabolism in steers.

	Treatment			
Item	**Control**	**Phlorizin**	**SEM**	**P-value**
Arterial glucose, mM	4.46	4.62	0.05136	0.0095
Portal glucose, mM	4.62	4.77	0.04591	0.0060
Portal blood flow, L/h	378	406	15	0.0860
Net portal glucose flux, mmol/h	56.72	59.75	6.5575	0.6496
Recovery of infused glucose, %	52	55	5.9	0.6330
PDV glucose utilization[a], mmol/h	47	14	23.4	0.5260
Total glucose absorption[b], mmol/h	96	58	13.9	0.0157
Corrected recovery of infused glucose, %[c]	88	53	12.7	0.0134

n = 12
[a] *PDV utilization of arterial glucose calculated by use of arterial [U-13C] D-glucose.*
[b] *Calculated as a net portal glucose flux + PDV utilization.*
[c] *Recovery of 20g/h abomasally infused glucose corrected for PDV use.*

treatments resulting in lower ($P < 0.05$) total glucose absorption and corrected recovery of infused glucose. The reasons for this apparent decrease in PDV metabolism of blood glucose are unclear. However, phlorizin did decrease total glucose absorption 40%. This decrease is in agreement with reports from several studies in which infusion of phlorizin decreased glucose absorption in the small intestine.

Using the methods in the present experiment it is not possible to describe actual mechanisms of absorption, nor are we able to evaluate changes in the concentration or function of the SGLT1 protein. However, the increases in total glucose absorption in cattle fed a concentrate diet are considerable, and the effect on phlorizin infusion on total glucose absorption suggests that SGLT1 is responsible for a large proportion of total glucose transport. It is certainly possible that part or all of the adaptive response seen in this experiment is the result of changes in SGLT1 activity or expression.

Implications

The results indicate that glucose absorption in the small intestine of cattle can be affected by diet composition and provide insight into the contribution of the sodium/glucose costransporter SGLT1 to total small intestinal glucose absorption. However, the failure of phlorizin to completely inhibit glucose absorption suggests that either more inhibitor is required to bind all SGLT1 present in the small intestine or there is another system of glucose transport present in cattle.

Application of Carbohydrase Inhibitors to Moderate Ruminal Fermentation

S.M. Speight and D.L. Harmon

Summary

Carbohydrase inhibitors have been widely investigated for regulating human carbohydrate assimilation; however, their application to animal nutrition has been ignored. Four experiments were conducted to determine how commercially available α-amylase and α-glucosidase inhibitors affect rumen fermentation. *In vitro* incubations were conducted in 50-mL test tubes containing an inhibitor, 0.5 g ground corn, and 40-mL buffered rumen fluid inoculum. Rumen fluid donors were fed a 100% forage diet in Exp 1 and a 50:50 concentrate:forage diet in Exp 2-4. Incubations were conducted in duplicate at 37°C and replicated on consecutive days with pH and VFA concentra-

tions measured at 24 h. Treatments for Exp 1 and 2 were the following: no additive (CON); 12.5-37.5 mg acarbose (ACB), miglitol (MIG), or glipizide (GLI); 12.5-75 mg trestatin (TRE); and 25-100 mg Alpha Trim-W® (ATW), CarboTame® (CT), starch blocker (SB), or wheat amylase inhibitor (RWI). ACB and TRE increased pH and decreased acetate, butyrate, propionate, and total VFA in Exp 1 and 2 in a non-dose-dependent manner. The remaining six treatments (MIG, GLI, CT, ATW, RWI, and SB) failed to affect pH and VFA concentrations. In Exp 3, ACB and TRE doses were decreased to 1.2-9.5 mg. ACB increased pH and decreased acetate, propionate, butyrate, and total VFA concentrations dose-dependently. Decreasing TRE doses to 0.1-1.1 mg in Exp 4 increased pH and decreased VFA concentrations in a dose-dependent manner. Although these data suggest that trestatin is 10 times more potent than acarbose, both inhibitors have the potential to slow fermentation and could help prevent rumen acidosis in addition to resulting in greater amounts of starch reaching the small intestine where its assimilation is more efficient.

Introduction

Sub-therapeutic antibiotics are commonly fed to livestock over long-term periods to improve feed efficiency and weight gain; however, some fear that this practice will disrupt the normal flora within the gastrointestinal tract and select for antibiotic-resistant bacteria that could ultimately be transferred to humans. There have been debates over the safety of antibiotic use in livestock over the past several decades as the global concern of bacterial resistance to antibiotics in humans continues to increase. Research into alternative feed and management techniques is needed now to lessen the blow to the livestock industry that a future antibiotic ban may cause. Carbohydrase inhibitors could be one of the solutions.

Beef and dairy cattle in the United States and many parts of the world are fed high-grain diets to provide high-energy for rapid growth. Rapid fermentation of cereal grains can result in rumen acidosis, a syndrome that involves the overproduction of organic acids, particularly lactic acid, which can be detrimental to both animal health and carcass quality. Nutritional management is critical in preventing digestive disturbances associated with rumen acidosis.

Both α-amylase and α-glucosidase inhibitors have been widely investigated for regulating carbohydrate digestion and absorption in humans but to our knowledge have received little attention in livestock feeding. Compounds such as these may be beneficial to ruminants fed high-grain diets. These inhibitors could slow fermentation, thereby preventing digestive upset, and would also result in greater amounts of starch reaching the small intestine, where digestion and absorption is more efficient.

Our objective was to determine the dose-response relationship between several carbohydrase inhibitors and *in vitro* rumen fermentation as assessed by pH changes and short-chain fatty acid (SCFA) production.

Materials and Methods

Our preliminary *in vitro* experiments have looked at several plant and microbial derived α-amylase and α-glucosidase inhibitors. Acarbose (Precose®) and miglitol (Glyset®) are microbial-derived α-glucosidase inhibitors that are currently marketed by the Bayer Corporation (West Haven, CT) as treatments for human and animal type II (non-insulin-dependent) diabetes mellitus. Alpha Trim™-W (Nutricepts Inc., Carlstadt, NJ) and raw wheat amylase inhibitor extract (AHD International, Atlanta, GA) were two wheat-derived α-amylase inhibitors tested. Carbotame™ (Jarrow Formulas, Los Angeles, CA) and Starch Blocker (Whole Health Products, Boulder, CO) both contain Phase 2® starch neutralizer (Pharmachem Laboratories, Inc., Kearney, NJ), an α-amylase inhibitor extract derived from the white kidney bean (Phaseolus vulgaris). Trestatin (Roche Pharmaceuticals, Nutley, NJ) is a microbial-derived α-amylase inhibitor. Although not a carbohydrase inhibitor per se, we also tested glipizide (Glucotrol®), a sulfonylurea that is marketed by the Pfizer Corporation as another treatment for diabetes.

Experiment 1

Ground corn (0.5 g/tube) and inhibitor treatments were weighed into 50-mL polypropylene screw cap culture tubes. The treatments were as follows: 0 mg (control) and 12.5, 25, 37.5, and 50 mg/tube of each acarbose (ACB), miglitol (MIG), and glipizide (GLI); or 12.5, 25, 50, and 75 mg/tube of trestatin (TRE); or 25, 50, 75, and 100 mg/tube each of Alpha Trim-W (ATW), CarboTame (CT), Starch Blocker (SB), and raw wheat amylase inhibitor (RWI). A 40mL mixture, containing 37°C reduced McDougall's buffer (McDougall, 1949) and strained rumen fluid in a 1:2 (v/v) ratio, was dispensed into each culture tube. Strained (eight layers of cheesecloth) rumen inoculum was obtained from two steers fed a 100% forage diet mixed in a 1:1 (v/v) ratio. Immediately after inoculation, tubes were gassed with CO_2, sealed, and placed in a 37°C shaking water bath. All incubations were conducted in duplicate and were replicated on two consecutive days (one replicate per day). A pH reading (IQ150 pH meter; IQ Scientific Corp., Carlsbad, CA) and a 1-mL sample of the rumen fluid were taken from each tube for short-chain volatile fatty acid (SCFA) analysis at 24 h into the incubation. The SCFA samples were placed in 1.5-mL microcentrifuge tubes to which 200 µL of 25% (w/v) m-phosphoric acid was added, and the samples were immediately frozen (4°C) for subsequent analysis. The samples were thawed and centrifuged at 39,000 x g for 20 min at room temperature. Short chain fatty acid concentrations were determined using a HP 6890 Series GC (Agilent Technologies, Inc., Palo Alto, CA). The 4 mm glass column (1.8 m x 0.6 cm) was packed with SP-1200 (Supelco, Belfontaine, PA). Nitrogen (80 mL/min) served as the carrier gas and the FID used a combination of compressed air (200 mL/min) and hydrogen gas (20 mL/min). Operating temperatures for the injector and FID was 250°C; the column was maintained at 140°C.

Experiment 2

The experiment was set up identically to Exp. 1 except that rumen fluid donors were fed a 50% forage, 50% concentrate (corn based) diet. Samples were taken and analyzed in the same manner as those listed in Exp 1.

Experiment 3

The objective of Exp 3 was to establish a dose-dependent response in pH and VFA concentrations with ACB, GLI, MIG, and TRE. The experiment was set up identically to Exp 2. The treatments were as follows: 0 (control), 1.2, 2.4, 4.8, 7.1 and 9.5 mg/tube of ACB and TRE; or 0 (CON), 50, 100, 150, and 200 mg/tube of MIG and GLI. In order to accurately measure ACB and TRE doses used, 0.1 g of each inhibitor was mixed with 2.0 g of ground corn. A portion of each mixture (25, 50, 100, 150, and 200 mg) were added to each centrifuge tube so that each tube contained 1.19, 2.38, 4.76, 7.14, and 9.52 mg inhibitor/tube. Samples were taken and analyzed in the same manner as those listed in Exp 1 and 2.

Experiment 4

The objective of Exp 4 was to establish a dose-dependent response in pH and VFA concentrations with TRE. The experiment was set up identically to Exp 2 and 3. A portion of the diluted TRE mixture from Exp 3 was diluted again with another 2.0 g of ground corn. Once mixed, 25, 50, 75, 100, 150, 200, and 250 mg of this mixture was weighed out so that the TRE concentrations were 0 (control), 0.11, 0.22, 0.33, 0.43, 0.65, 0.87, and 1.08 mg TRE/tube. Samples were taken and analyzed in the same manner as those listed above.

Statistical Analysis

Data were analyzed in a randomized complete block design using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). Replication on consecutive days was considered the block effect in the model. Single degree of freedom orthogonal contrasts were used to discern linear and quadratic effects of treatment dose. Orthogonal contrast coefficients were obtained using the IML procedure of SAS. Differences were considered to be significant when $P < 0.05$ unless otherwise stated. When the overall F-value for dose was significant, means were separated using the LSD test.

Results and Discussion

Experiment 1

The majority of compounds tested did not prove effective in controlling fermentation. Alpha Trim, CarboTame, glipizide, miglitol, raw wheat amylase inhibitor, and starch blocker all did not affect pH or total VFA concentrations at 24H of incubation (data not shown). Acarbose and trestatin both increased pH and decreased total VFA concentration ($P < 0.001$); however, there was no dose reponse from increased concentrations as all doses produced similar changes (data not shown).

Experiment 2

The compounds tested and amounts of inhibitors were the same for Exp 2, the only difference being the diet of the donor animals contained more concentrate (50:50). The results of Exp 2 were similar to Exp 1. Only acarbose and trestatin proved effective, and all doses produced a similar response (data not shown). There were no apparent differences from the donor animal diet.

Experiment 3

In this experiment the doses of trestatin and acarbose were lowered. This produced dose-dependent responses for acarbose (Table 1) as pH increased, and acetate, propionate, and total VFA concentrations all decreased ($P < 0.001$) linearly with dose. As propionate concentration decreased there was also a linear ($P < 0.01$) decrease in propionate proportion. In contrast, trestatin addition produced a quadratic (P 0.001) increase in pH and quadratic ($P < 0.001$) decreases in acetate, propionate, butyr-

Table 1. Effect of acarbose (ACB) treatment on 24 h batch culture fermentations (Exp 3).

	Inhibitor Dose (MG)							Effects (P-Value)	
	0	1.2	2.4	4.8	7.1	9.5	SE	Linear	Quadratic
pH	**5.90**	**5.89**	**6.05**	**6.21**	**6.44**	**6.51**	**0.15**	**< 0.001**	**0.72**
VFA (mM)									
Acetate	63.42	54.04	50.91	47.36	39.75	36.44	5.30	< 0.001	0.14
Propionate	24.00	20.76	17.98	15.08	11.34	10.16	2.87	< 0.001	0.11
Butyrate	11.42	11.69	11.97	12.41	10.52	10.02	2.81	0.64	0.69
Total VFA	100.99	88.52	82.89	77.14	63.79	58.95	9.56	< 0.001	0.35
	mole/100 moles								
Acetate	62.67	61.05	61.43	61.63	62.81	62.46	2.04	0.75	0.75
Propionate	23.81	23.38	21.49	19.21	17.72	17.31	1.95	0.01	0.48
Butyrate	11.39	13.28	14.62	16.12	15.68	16.18	2.69	0.22	0.49

Table 2. Effect of trestatin (TRE) treatment on 24 h batch culture fermentations (Exp 3).

	Inhibitor Dose (mg)							Effects (P-Value)	
	0	1.2	2.4	4.8	7.1	9.5	SE	Linear	Quadratic
pH	**5.90**	**6.60**	**6.67**	**6.68**	**6.70**	**6.69**	**0.04**	**< 0.001**	**< 0.001**
VFA (mM)									
Acetate	63.42	36.79	32.42	31.53	32.05	31.48	3.03	< 0.001	< 0.001
Propionate	24.00	10.02	9.39	9.26	9.40	9.32	0.61	< 0.001	< 0.001
Butyrate	11.42	7.07	6.04	5.96	6.00	5.90	0.49	< 0.001	0.001
Total VFA	100.99	56.42	50.26	49.13	49.88	49.12	3.61	< 0.001	< 0.001
	mole/100 moles								
Acetate	62.67	65.17	64.50	64.19	64.25	64.09	0.98	0.76	0.40
Propionate	23.81	17.79	18.68	18.85	18.83	18.96	0.43	0.003	0.001
Butyrate	11.39	12.50	12.04	12.07	12.05	12.03	0.63	0.78	0.58

Table 3. Effect of trestatin (TRE) treatment on 24 h batch culture fermentations (Exp 4).

	Inhibitor Dose (MG)									Effects (P Value)	
	0	0.11	0.22	0.33	0.43	0.65	0.87	1.08	SE	Linear	Quadratic
pH	**5.56**	**5.69**	**5.75**	**5.81**	**5.85**	**6.11**	**6.17**	**6.32**	**0.39**	**< 0.001**	**0.99**
VFA (mM)											
Acetate	53.09	48.86	47.97	46.68	44.20	39.86	38.81	35.79	2.91	< 0.001	0.41
Propionate	19.01	13.99	13.28	12.57	12.00	10.74	10.65	10.07	0.57	< 0.001	< 0.001
Butyrate	16.91	21.12	19.80	18.76	16.96	11.12	10.16	7.49	1.14	< 0.001	0.003
Total VFA	91.23	86.48	83.51	80.38	75.51	63.83	61.71	55.39	4.66	< 0.001	0.45
	mole/100 moles										
Acetate	58.17	56.51	57.47	58.08	58.54	62.46	62.91	64.57	0.47	< 0.001	0.06
Propionate	20.86	16.18	15.92	15.65	15.91	16.85	17.33	18.22	0.46	0.80	< 0.001
Butyrate	18.56	24.42	23.69	23.33	22.46	17.37	16.35	13.48	0.52	< 0.001	< 0.001

ate, total VFA, and propionate proportion. Although the dose of trestatin was decreased as low as 1.2 mg per tube, there was no further response to increasing amounts (Table 2).

Experiment 4

In this experiment the dose of trestatin was further lowered as low as 0.11 mg per tube. These amounts produced a linear ($P < 0.001$) increase in pH and linear ($P < 0.001$) decrease in acetate, propionate, butyrate, and total VFA concentrations. There was a linear ($P < 0.001$) increase in acetate proportion and quadratic ($P < 0.001$) changes in propionate and butyrate proportions (Table 3) as propionate proportion decreased then increased as trestatin concentration increased, whereas butyrate proportion increased then decreased.

This could be due to an accumulation of lactate, a variable not measured in these preliminary experiments. Although this effect was interesting, further lowering pH would not help cattle suffering from rumen acidosis. There was still not a dose-dependent response to trestatin treatment for Exp 3. Trestatin levels were lowered once more to 0.11 to 1.08 mg in Exp 4 and successfully achieved the dose-dependent response in the response variables.

The data from these four experiments suggest that trestatin is more potent (effective at lower doses) or is more active than acarbose on a weight-by-weight basis. The doses of acarbose and trestatin that led to dose-dependent responses were 1.2- 9.5 mg and 0.11- 1.08 mg, respectively. Converting these doses to percentages of *in vitro* tube substrate would yield an approximate dose that could be fed to cattle for *in vivo* experiments. Acarbose could be fed at 0.26 - 2.1% of diet DM, and trestatin could be fed at 0.024 - 0.24% of diet DM. The doses of acarbose and trestatin could also be extrapolated to 1.2-28.5 g and 0.11-1.08 g *in vivo* doses, respectively, for steers with average rumen volumes of 40 L.

Implications

Based on these limited *in vitro* data, both trestatin and acarbose could be effective in moderating ruminal fermentation. Additional *in vitro* and *in vivo* research is needed to determine whether these carbohydrase inhibitors could be as effective as the antibiotics currently used in the livestock industry.

Yucca Schidigera Extract Decreases *In Vitro* Methane Production in a Variety of Forages and Diets

M. Xu, M. Rinker, K.R. McLeod, D.L. Harmon

Summary

The research was conducted to evaluate effects of YSE on 24 h *in vitro* rumen fermentation and methane production of five forages. Experimental treatments were alfalfa, fescue orchard grass, bermuda and switch grass plus 0 or 110 g/kg of YSE in a forage diet, a medium forage diet, and a low forage diet. Two ruminally-fistulated steers consuming 70% alfalfa hay and 30% concentrate were used as the donor animals. Gas emission from each fermentation vessel was measured continuously by an automated pressure transducer system. YSE did not affect pH, 24h-pressure, gas production, maximum pressure, degradation rate, lag time, and total and individual VFA concentration in forage, medium-forage, and low-forage treatments. YSE addition decreased methane production at 24 h. There were no interactions between YSE and forage source and diet type. Results suggest that YSE could have the potential to reduce methane production across a wide range of diets.

Introduction

Mojave yucca (*Yucca schidigera*), also known as the "Spanish Dagger," is a flowering plant native to the southwestern United Staters. Steroidal saponins extracts are produced commercially from *Yucca schidigera* (YSE) and have received attention from the livestock industry for many years. Additions of YSE have also been reported to reduce methane (CH_4) production. Methane represents an energy loss to the animal and contrib-

utes to greenhouse gas production. These decreases in CH_4 were accompanied by increases in total VFA concentration and propionate, suggesting a very positive effect on the energetics of the fermentation. Consequently, a means of reducing CH_4 production would be highly favorable. Therefore, the objective of this study was to examine (1) the effects of YSE on *in vitro* degradation kinetics and methane production; and (2) the interactions of YSE with differing feedstuffs.

Materials and Methods

The additive

Yucca schidigera extract used in this study was supplied by Distributors Processing Inc. (Porterville, CA).

Fermentation preparation

Ruminal contents were collected from two ruminally-fistulated steers consuming 70% alfalfa hay and 30% concentrate (Table 1). The diet was fed to supply 1.75 x NEm requirements of the steers. The steers had been previously adapted to this diet for a minimum of 14 d. Samples were prepared in sealed 260 mL fermentation vessels and fitted with automatic pressure transducers and a gas sampling port (Ankom Technology, Macedon, NY). To accurately administer YSE to the fermentation vessels,YSE was mixed with the diets at the recommended feeding level (110 mg/kg diet) prior to weighing them (0.6 g as-fed) into the fermentation vessels to provide the proper concentrations of extract in each vessel. Preliminary fermentations indicated that this was an effective dose.

Experimental design

There were three *in vitro* experiments. Each experiment used either a forage diet, a medium forage diet (0.50 forage:0.50 concentrate), or a low forage diet (0.10 forage:0.90 concentrate). The concentrate portion of the diet was supplied by the grain mix that was fed to the steers (Table 1) and the forage and concentrate mix were combined on an as-fed basis after being ground in a Wiley mill through a 2-mm screen. Within each of these diets there was a legume (alfalfa), two cool-season grasses (fescue and orchard grass), and two warm-season grasses (bermuda and switch grass; Table 2). Each fermentation treatment was replicated on four days.

Sample collection and analyses

For each treatment combination, gas pressure was monitored at 5-min intervals throughout a 24-h fermentation using an automated pressure transducer system. At termination (24 h), gas samples for methane analysis were drawn into sampling syringes and then transferred into a vacuum test tube. Gas samples were analyzed for methane concentrations by gas chromatography. Fermentation media were then analyzed for pH and VFA concentrations.

Calculations

The potential extent and rate of gas production in response to feedstuff degradation were determined using a one-pool exponential model: $P = b(1 - e^{-k(t-l)})$ where P is the cumulative pressure (psi), b is maximum pressure (psi), k is the rate of pressure (%h), t is the time (h), and l is a discrete time lag (h) using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA). In this model, it is assumed that no pressure is produced until a discrete time lag has elapsed. Gas production was calculated from the vessel pressure corrected from current atmospheric pressure (96.538 kPa) into standard atmospheric pressure (101.325 kPa).

Statistical analysis

The response variables were analyzed using the General Linear Models program of SPSS 11.5 (SPSS, Inc., Chicago, IL). The model included terms for diet (forage source), *Yucca schidigera*, and the interaction between substrate and *Yucca schidigera*. Treatment effects were considered significant if $P < 0.05$.

Table 1. Diet composition for rumen-fistulated donor steers.

Alfalfa cubes	70.00%
Ground corn	28.35%
Soybean meal	0.75%
Urea	0.23%
Fat	0.08%
Limestone	0.47%
Trace mineral-salt[1]	0.11%
Vitamins A, D, E premix[2]	0.01%
Composition, DM basis[3]	
ME	2.53 Mcal/kg
Crude protein	15.8%
NDF	35.9%
Calcium	1.27%
Phosphorus	0.24%

[1] Contains at least 920 g/kg NaCl,. 5.5 g/kg Zn, 9.3 g/kg Fe, 4.8 g/kg Mn, 1.8 g/kg Cu, 0.115 g/kg I, 0.065 g/kg Co, 0.018 g/kg Se.
[2] Contains 1,818,182 IU/kg Vitamin A, 363,000 IU/kg Vitamin D, and 227 IU/kg Vitamin E.
[3] Based on post-experiment chemical analysis of samples by Dairy One, Ithaca, NY. Fed to supply 1.75 x NEm requirements.

Table 2. Composition of feedstuffs used for *in vitro* fermentations.[1]

Item	Alfalfa	Bermuda	Fescue	Orchard Grass	Switch-grass	Grain[2]
Crude Protein, %	18.6	12.9	12.6	14.3	7.6	12.1
ADF, %	34.2	37.2	44.3	40.0	41.0	5.3
NDF, %	44.5	70.7	74.9	65.1	68.2	9.0
NFC, %	28.7	12.9	9.3	16.6	18.5	
NEm, Mcal/kg	1.19	1.06	1.01	1.12	1.08	2.11
NEg, Mcal/kg	0.64	0.51	0.46	0.55	0.53	1.43

[1] Based on post experiment chemical analysis of samples by Dairy One, Ithaca, NY.
[2] Composition was same grain mix fed to steers in Table 1.

Table 3. Effect of *Yucca schidigera* extract (YSE) and forage type on *in vitro* gas production and fermentation in forage-based diets—Experiment 1.

Forage Type	Alfalfa		Fescue		Orchard		Bermuda		Switch			P		
YSE, mg/kg	0	110	0	110	0	110	0	110	0	110	SEM	Forage	Yucca	F×Y
pH[1]	6.52	a6.49	6.50	a6.52	6.60	b6.62	6.63	b6.60	6.63	b6.66	0.011	<0.001	0.829	0.601
Pressure, kPa	27.0	a27.1	24.5	b23.99	18.6	c18.0	15.2	d13.9	13.6	d15.0	0.90	<0.001	0.745	0.692
Gas production, ml	58.3	a58.4	52.9	b51.7	40.1	c38.8	32.8	d30.0	29.2	d32.3	1.88	<0.001	0.745	0.692
Degradation rate, /h	0.080	a0.086	0.046	bc0.044	0.042	c0.033	0.065	b0.048	0.067	a0.086	0.0036	<0.001	0.893	0.119
Lag time, h	1.0	ab1.0	3.1	c3.1	2.5	b1.2	0.6	a0.2	0.7	a0	0.24	<0.001	0.189	0.805
Methane														
mol/100mol	15.76	a14.29	16.48	ab15.60	20.56	c18.88	19.74	bc17.02	18.30	bc16.92	0.430	0.002	0.027	0.939
Production, ml	9.16	a8.32	8.69	a8.08	8.22	b7.25	6.28	c5.08	5.41	c5.28	0.247	<0.001	0.001	0.208
VFA														
Total, mM	56.41	a56.09	49.99	b50.83	47.29	c46.31	39.31	d43.33	38.66	d39.60	1.054	<0.001	0.235	0.279
mol/100 mol														
Acetate	60.24	60.31	60.47	60.88	59.41	58.95	60.31	58.97	60.26	60.81	0.233	0.249	0.750	0.719
Propionate	23.91	a23.73	24.15	a24.13	21.91	b22.12	20.16	c19.67	20.65	c20.01	0.314	<0.001	0.496	0.924
Butyrate	7.78	a7.51	7.65	a7.24	8.32	b8.17	8.64	b8.06	8.39	b8.39	0.093	<0.001	0.059	0.756
Valerate	3.46	a3.66	3.43	a3.37	4.30	bc4.47	4.70	b3.99	4.62	c4.64	0.091	<0.001	0.369	0.092
Isobutyrate	1.49	a1.55	1.35	a1.48	1.99	b2.12	1.91	b1.97	1.72	ab1.77	0.061	0.002	0.426	0.997
Isovalerate	3.12	a3.25	2.95	a2.90	4.07	b4.16	4.28	b3.90	4.35	b4.38	0.100	<0.001	0.690	0.350
Acetate: Propionate	2.52	a2.54	2.51	a2.53	2.72	b2.67	2.99	c3.01	2.92	c3.04	0.038	<0.001	0.516	0.782

[1] Letters (a, b, c, d) to the left of values in the '110' columns denote main effects of forage (P < 0.05).

Table 4. Effect of *Yucca schidigera* extract (YSE) and forage type on *in vitro* gas production and fermentation in 50: 50 forage: concentrate diets—Experiment 2.

Forage Type	Alfalfa		Fescue		Orchard		Bermuda		Switch			P		
YSE, mg/kg	0	110	0	110	0	110	0	110	0	110	SEM[1]	Forage	Yucca	F×Y
pH[1]	6.28	ab6.27	6.25	a6.22	6.34	c6.37	6.27	b6.32	6.29	b6.32	0.009	<0.001	0.287	0.323
Pressure, kPa	44.1	a44.0	43.1	ab41.0	39.9	bc38.0	41.3	abc39.0	36.1	c38.1	0.76	0.030	0.532	0.824
Gas production, ml	95.0	a94.9	92.9	ab88.3	86.0	bc82.0	89.0	abc84.0	77.8	c82.2	1.58	0.030	0.532	0.824
Degradation rate, /h	0.072	0.059	0.055	0.065	0.064	0.060	0.060	0.075	0.073	0.055	0.0034	0.977	0.799	0.559
Lag time, h	1.1	0.7	1.3	1.9	1.3	0.6	2.1	1.7	1.6	1.4	0.20	0.417	0.541	0.828
Methane														
mol/100 mol	10.10	ab9.38	9.29	a9.00	10.61	b9.83	9.85	ab9.72	9.43	a8.46	0.144	0.026	0.032	0.807
Production, ml	9.56	a8.84	8.59	b7.92	9.12	ab8.08	8.69	b8.14	7.24	c6.90	0.153	<0.001	0.002	0.847
VFA														
Total, mM	68.84	a71.29	66.24	ab69.44	64.11	b65.70	66.93	b63.97	67.83	b62.89	0.580	0.007	0.890	0.068
mol/100 mol														
Acetate	55.76	55.87	54.89	55.15	55.16	55.95	55.27	54.60	53.20	54.87	0.367	0.660	0.590	0.912
Propionate	27.36	27.91	28.60	27.68	27.07	26.97	26.08	27.08	29.94	27.11	0.503	0.788	0.674	0.819
Butyrate	8.73	a8.43	8.76	a9.65	9.71	a8.84	10.26	b9.98	8.97	a9.72	0.140	0.006	0.874	0.108
Valerate	4.43	4.22	4.19	3.92	4.14	4.34	4.28	4.33	4.11	4.20	0.096	0.921	0.901	0.949
Isobutyrate	1.43	1.37	1.33	1.41	1.36	1.47	1.56	1.47	1.40	1.56	0.020	0.098	0.260	0.137
Isovalerate	2.29	a2.19	2.24	a2.19	2.55	b2.41	2.53	b2.55	2.38	b2.55	0.031	<0.001	0.656	0.324
Acetate: Propionate	2.06	2.02	1.94	2.01	2.07	2.09	2.12	2.05	1.88	2.06	0.034	0.892	0.739	0.928

[1] Letters (a, b, c) to the left of values in the '110' columns denote main effects of forage (P < 0.05).

Results and Discussion

Forage Composition

Forages (Table 2) were chosen to represent a wide range and as expected, forage source resulted in differences among CP, fiber, non-fiber carbohydrate, and energy concentrations. Crude protein content ranged from 7.6 to 18. 6%, and NDF ranged from 44.5 to 74.9%.

Fermentation Results

Changes in forage show that, as expected, forage source had a pronounced influence on most fermentation variables (Tables 3, 4, and 5). Considering the all-forage diets, only the proportion of acetate (Table 3) was not affected by forage source. Because the primary focus of our study was to characterize the influence of YSE across a wide range of diet types, we will not address specific differences occurring with forage source but rather focused on effects of YSE addition and interactions with forage source.

Table 5. Effect of *Yucca schidigera* extract (YSE) and forage type on *in vitro* gas production and fermentation in 90% concentrate diets—Experiment 3.

Forage Type	Alfalfa		Fescue		Orchard		Bermuda		Switch			P		
YSE, MG/KG	0	110	0	110	0	110	0	110	0	110	SEM[1]	Forage	Yucca	F×Y
pH[1]	6.11	ab6.13	6.09	a6.11	6.15	b6.16	6.14	b6.15	6.16	b6.15	0.006	0.024	0.541	0.910
Pressure, kPa	53.0	51.5	54.5	51.7	51.8	53.2	51.2	49.2	50.7	49.8	0.41	0.055	0.122	0.490
Gas production, ml	141.5	137.4	145.5	138.0	138.4	141.9	136.7	131.5	135.3	132.8	1.09	0.055	0.122	0.490
Degradation rate, /h	0.046	0.041	0.057	0.043	0.042	0.040	0.053	0.043	0.036	0.040	0.002	0.317	0.174	0.633
Lag time, h	0.44	0.81	1.46	1.37	1.00	0.90	0.91	1.38	1.07	1.33	0.100	0.159	0.360	0.842
Methane														
mol/100 mol	8.48	7.96	8.77	8.13	8.40	7.55	8.34	7.93	8.53	8.12	0.118	0.770	0.025	0.973
Production, ml	12.00	10.92	12.73	11.19	11.64	10.71	11.35	10.41	11.52	10.80	0.171	0.260	0.002	0.941
VFA														
Total, mM	105.2	98.31	103.43	99.09	91.12	87.90	93.80	91.18	90.62	92.23	1.695	0.066	0.355	0.949
mol/100 mol														
Acetate	48.66	48.57	49.22	48.19	49.57	48.82	47.08	46.64	46.90	46.59	0.342	0.087	0.447	0.993
Propionate	31.50	31.84	31.01	32.15	30.48	30.97	32.39	33.42	32.63	33.05	0.375	0.374	0.390	0.996
Butyrate	12.27	12.10	12.32	12.22	12.16	12.41	12.23	12.03	12.58	12.62	0.083	0.498	0.850	0.926
Valerate	4.67	a4.44	4.36	a4.38	4.59	a4.37	5.10	b4.74	4.70	ab4.52	0.058	0.031	0.078	0.848
Isobutyrate	0.99	1.11	1.11	1.09	1.12	1.13	1.12	1.09	1.13	1.17	0.022	0.495	0.354	0.704
Isovalerate	1.91	a1.93	1.98	ab1.97	2.09	c2.19	2.07	bc2.08	2.07	abc2.04	0.024	0.030	0.687	0.915
Acetate: Propionate	1.55	1.53	1.60	1.51	1.63	1.58	1.47	1.42	1.45	1.41	0.028	0.255	0.403	0.995

[1] Letters (a, b, c) to the left of values in the '110' columns denote main effects of forage (P < 0.05).

YSE addition did not affect pH, 24 h pressure, gas production, maximum pressure, degradation rate, or lag time when added to forage diets (Table 3). Addition of YSE decreased ($P < 0.05$) the methane proportion and methane production ($P < 0.001$) at 24 h but did not affect the total concentration of VFA, or VFA proportions with the possible exception of butyrate, which tended ($P < 0.06$) to be lower with YSE addition. Despite the wide range in forage quality, there were no interactions between forage source and YSE.

Results were generally similar for the medium forage diets (Table 4), Forage source affected most fermentation variables with the exception of degradation rate and lag time. Proportions of acetate, propionate, isobutyrate, and valerate were not affected by forage source. Addition of YSE did not affect pH, 24 h pressure, gas production, maximum pressure, degradation rate, lag time, VFA proportions, or concentrations. YSE addition decreased ($P < 0.05$) methane proportion and production ($P < 0.01$). There were no interactions between YSE and forage source.

As expected, forage source had little influence in the 900 g/kg concentrate diets (Table 5). Minor differences occurred for pH, pressure, and gas production ($P < 0.06$) but for all other variables, only valerate proportion ($P < 0.03$) changed with forage source. Addition of YSE tended ($P < 0.08$) to decrease valerate proportion, but otherwise only methane production was affected. Methane proportion ($P < 0.025$) and production ($P < 0.01$) were both decreased by YSE addition. There were no interactions between YSE and forage source.

It was the aim of the present experiment to determine effects of YSE among a wide range of forages and dietary forage:concentrate ratios. It is apparent that this initial goal was met, as virtually every variable measured was affected by forage source. Total gas production in the present study, which is indicative of digestibility, was ranked as alfalfa > fescue > orchard grass > bermuda = switchgrass. These results are also consistent with total VFA concentrations but not digestion rate.

It was also the aim of the present study to evaluate possible interactions between forage source and YSE. Typically, YSE addition had a minimal effect on fermentation variables and there were no meaningful interactions with forage source within any diet.

Among all forage sources and dietary forage:concentrate ratios, methane was consistently reduced by YSE addition. While YSE is inhibitory to methane production, there was no interaction between YSE and forage among diets with different forage:concentrate ratios, indicating that it may be effective with many diet types and classes of ruminants.

Implications

Across a range of forages and diets, YSE consistently reduced methane production without influencing gas production kinetics or total VFA concentrations measured following a 24 h *in vitro* fermentation. It would appear that YSE addition is a viable means to inhibit ruminal methane production. The impact of this inhibition on ruminant whole-animal energetics remains to be determined.

Influence of Energy Supply on Expression of Genes Encoding for Lipogenic Enzymes and Regulatory Proteins in Growing Beef Steers

K.R. McLeod, R.L. Baldwin VI, and E.E. Connor

Summary

Forty crossbred beef steers were used to determine the effects of metabolizable energy (ME) intake and of site and complexity of carbohydrate (CHO) infusion on expression of genes encoding lipogenic enzymes and regulatory proteins in subcutaneous (SC), mesenteric (MES), and omental (OM) adipose. Treatments were: a pelleted forage-based diet fed at 161 (LI) or 214 Kcal ME·(kg BW.75)$^{-1}$·d^{-1}; LI plus ruminal or abomasal infusion of starch hydrolysate (SH); and LI plus abomasal infusion of glucose. After 35 d, steers were slaughtered, and total RNA was isolated from harvested adipose and used for quantitative real-time RT-PCR of selected genes. Although gene expression in SC was largely unaffected by treatment, expression in visceral adipose was highly responsive to dietary ME intake and CHO. Greater dietary ME intake increased mRNA expression of rate-limiting lipogenic enzymes and regulatory proteins in MES but not OM adipose. Starch infusion did not alter gene expression in MES; however, mRNA expression of lipogenic and regulatory proteins in OM adipose was greater when SH was supplied abomasally compared with ruminally. In both the MES and OM adipose, abomasal glucose supply increased expression of lipogenic and regulatory proteins compared with abomasal SH supply. This study shows that dietary ME intake and site of digestion and complexity of CHO affect expression of lipogenic enzyme and regulatory genes of visceral adipose depots, while SC adipose appears to be more resilient to dietary manipulations.

Introduction

A comprehensive understanding of the interactions between energy supply and metabolic signals and/or pathways that regulate rate and composition of tissue accretion is necessary for accurate prediction of animal production performance. We have demonstrated that the efficiency of converting dietary carbohydrate (CHO) energy to body tissue energy in growing steers is greater if starch is digested in the small intestine rather than fermented in the rumen. However, more striking is our finding that small intestinal-starch digestion results in a disproportionate increase in the fraction of total body tissue energy being retained as fat compared with ruminal starch supply. This provides evidence that composition of tissue gain is influenced not only by the amount of energy supplied, but also the substrate through which it is supplied.

In ruminants as well as other species, fatty acid synthase (FAS*)* and acetyl-CoA carboxylase (ACC) are rate-limiting enzymes involved in the conversion of energy substrates and intermediates to fatty acids for triglyceride synthesis. In addition to the direct involvement of these enzymes, regulatory proteins such as sterol regulatory element-binding proteins (SREBP, SREBP-1, SREBP-2), SCAP, Spot-14, and carbohydrate response element-binding protein (ChREBP) have been shown to stimulate lipogenesis through up-regulation of metabolic pathways and(or) synthesis of FAS and ACC. Thus, the objectives of this study were to determine the effects of metabolizable energy (ME) intake and of site and complexity of CHO infusion on expression of genes encoding lipogenic enzymes and regulatory proteins in primary adipose depots.

Materials and Methods

Forty crossbred beef steers (243 ± 2 kg BW) with ruminal and abomasal infusion catheters were used in a randomized complete block design consisting of eight blocks containing five steers each. Within each block, all treatments were represented, and steers were assigned randomly to treatment. Across and within blocks, treatment implementation was staggered such that animal slaughters were conducted at predefined intervals to facilitate tissue sample collection while maintaining a constant 35-d treatment period. Dietary treatments included a pelleted, orchard grass based diet fed at 161 (LI) or 214 (HI) Kcal ME·(kg BW$^{0.75}$)$^{-1}$·d^{-1}; LI plus ruminal (R-SH) or abomasal (A-SH) infusion of a partial starch hydrolysate (SH); and LI plus abomasal infusion of glucose (A-G). The basal diet was fed in 12 equal portions daily at 2-h intervals. Metabolizable energy intake of the basal diet was designed to approximate 1.5 and 2.0 times maintenance energy requirements of growing steers. Based on their respective heats of combustion, infusion rates of 12.6 and 14.4 g·((kg BW$^{0.75}$)$^{-1}$·d^{-1}) were used to achieve isoenergetic infusions of SH and glucose, respectively. Accordingly, stock infusate solutions were diluted to a final weight of 5 kg using tap water and infused over a 22-h period. Total amount of infusate was equalized across treatments and infusion site by infusion of tap water (H_2O). Steers were adapted to feed intake and carbohydrate infusion incrementally over the initial 6 d of the 35-d treatment period. Feed offered and orts were recorded daily. Steers were weighed at 0900 twice weekly, and the amount of feed offered and CHO infused were adjusted weekly based on the average BW from the preceding week.

After the 35-d feeding and infusion period, steers were slaughtered using a captive bolt gun, exsanguinated, and viscera removed and placed into a visceral cart for tissue separation. Adipose samples (30 g) were taken from the omental (OM; within the lesser curvature of the abomasum), mesenteric (MES; within the sixth loop of the small intestine distal to the pylorus), and subcutaneous depots (SC; over the aitchbone). Samples were immediately frozen in liquid N_2, and maintained frozen (-80°C) until total RNA was isolated and quantified. Isolated RNA was reverse transcribed using random hexamers, and the resultant cDNA was used for quantitative real-time PCR. Primers were designed from bovine ESTs (GenBank), and resulting products verified by visualization using a 2% agarose gel containing ethidium bromide and sequencing of amplified products. Quantitative real-time PCR conditions included cDNA (78.7µg), 400nM final concentration of forward and

reverse primer and 1X final concentration SYBR Green Supermix (Bio-Rad, Hercules, CA) with cycling conditions of 95°C for 1 min; 45 cycles of 94°C for 15 sec, predetermined optimal annealing temperature for 30 sec, and 72°C for 30 sec; followed by a melting curve analysis of 95°C for 1 min; 55°C for 1 min; 80 cycles of 55°C for 10 seconds, increasing 0.5°C each cycle. The optimal annealing temperature for *FAS, ACC, spot 14,* and *SREBP-1* was 61.3°C, while the optimal temperature for *SREBP, SREBP-2, SCAP,* and *ChREBP* was 58.2°C.

Results and Discussion

Tissue samples from one steer subjected to the A-G treatment were excluded from all analyses due to inadequate intake of the basal diet (60% of feed offered). Similarly, samples from two steers assigned to the LI-H_2O were excluded because mass measurements of both visceral and carcass adipose were not consistent with their treatment cohorts (i.e., greater than 2 SD from the treatment mean), exhibiting a phenotype of more mature steers at slaughter. Additionally, data from individual samples of SC, MES, and OM adipose were excluded from data analysis because of differences in efficiency of the PCR reaction. Therefore, all data presented in the tables represent least square treatment means.

Transcription of genes encoding for rate-limiting lipogenic enzymes and regulatory proteins in the SC fat depot was largely unaffected ($P > 0.10$) by dietary ME intake and CHO infusion (Table 1). However, there were tendencies for mRNA expression of *SREB* to be greater ($P = 0.09$) for abomasal vs. ruminal SH supply and mRNA expression of *FAS* to be greater ($P = 0.07$) for A-G vs. A-SH. The general absence of a change in gene expression with either dietary ME intake or SH supply does not necessarily correspond with *in vitro* measures of fatty acid synthesis in these same tissues (data not shown). *In vitro* rates of acetate (i.e., the primary source of carbon for fat synthesis in ruminants) incorporation into fatty acids was greater in SC tissue obtained from steers receiving either greater ME intake or SH than those receiving the LI-H_2O treatment.

In contrast to SC, gene transcription in the MES fat depot was highly responsive to ME intake (Table 2). Rate-limiting lipogenic enzyme gene transcripts *FAS* and *ACC* were increased ($P = 0.03$) four- and threefold with ME intake, respectively. Consistent with these observed increases, regulatory protein gene expression was also increased ($P \leq 0.02$), with fold changes ranging from 2 to 5.5. Infusion of SH (LI-H_2O vs. R-,A-SH) and site of infusion (R-SH vs. A-SH) did not affect ($P > 0.10$) the expression of genes evaluated, with the exception of greater ($P = 0.03$) *ChREBP* mRNA expression with abomasal delivery of SH. However, gene transcription did appear to be highly sensitive to form of CHO (A-SH vs. A-G), with the number of transcripts for all genes tested being greater in A-G steers. However, mRNA transcript numbers for *FAS, SREBP,* and *SREBP-1* were not different ($P > 0.1$), while *ChREBP* tended to be increased ($P = 0.06$) and *ACC, SREBP-1, SREBP-2,* and *SCAP* mRNA were increased ($P < 0.03$) by A-G vs. A-SH. Changes in gene expression associated with ME intake were positively related to observed rates of *in vitro* fatty acid synthesis in MES adipose. However, the relationship between gene expression and fatty acid synthesis was not obvious for CHO infusion. *In vitro* rates of fatty acid synthesis were greater for SH infusion, primarily as a function of A-SH, compared to LI-H_2O, whereas form of CHO did not influence rate of synthesis.

Gene transcription in the OM fat depot was largely unaffected by ME intake and ruminal SH infusion (Table 3). However, abomasal infusion of CHO generally increased expression, with the greatest increase being observed for glucose infusion. Specifically, mRNA transcripts for *FAS* ($P = 0.03$), *ACC* ($P = 0.09$), *SREBP-1* ($P = 0.01$), *ChREBP* ($P = 0.04$), and *SCAP* ($P = 0.09$) were greater or tended to be greater for A-SH vs. R-SH. Likewise, glucose infusion further increased ($P \leq 0.05$) all transcripts, with the exception of *SREBP-1* and *ChREBP* mRNA. In these same tissues, while *in vitro* rates of fat synthesis were numerically increased with ME intake and ruminal SH, the largest increases were observed for A-SH and glucose, respectively.

Table 1. Gene transcripts (copies/µg RNA) in subcutaneous adipose from steers fed at either low (LI) or high intake (HI) and infused ruminally (R) or abomasally (A) with hydrolyzed starch (SH) or glucose (G).[a,b]

	LI				HI		Contrast[c]			
Gene	H_2O	R-SH	A-SH	A-G	H2O	SE[d]	LI-H_2O vs. HI-H_2O	LI-H_2O vs. R-, A-SH	R-SH vs. A-SH	A-SH vs. A-G
Fatty acid synthetase	831,406	1,113,900	1,075,293	1,601,155	750,933	290,629	— [e]	---	---	0.07
Aceytl-CoA carboxylase	93,606	79,643	95,911	129,244	60,474	21,712	---	---	--	---
Spot14	841,607	981,970	1,262,593	1,676,127	506,765	324,709	---	---	---	---
SREBP	78,262	71,861	101,892	102,945	54,902	13,562	---	---	0.09	---
SREBP-1	15,267	11,081	16,560	15,190	8,819	2,977	---	---	---	---
SREBP-2	8,412	13,332	8,874	1,162	7,137	2,668	---	---	---	---
ChREBP	2,914	2,101	2,878	3,350	2,289	614	---	---	---	---
SCAP	13,785	17,482	17,483	19,951	10,614	2,920	---	---	---	---

[a] *Low and high intake represent 161 and 214 Kcal ME· (kg $BW^{0.75}$) -1·d^{-1}, respectively.*
[b] *Isoenergetic infusion rates of SH (12.6 g· (kg $BW^{0.75}$) -1·d^{-1}) and G (14.4 g· (kg $BW^{0.75}$) -1·d^{-1}).*
[c] *Probability of larger F statistic.*
[d] *n = 8 except for LI-H_2O (n = 6), A-G (n = 7), and R-SH (n = 7) ; SE calculated using n = 6.*
[e] *P > 0.10.*

Implications

These results show that expression of genes that control fat deposition in cattle are responsive to energy and carbohydrate intake. Specifically, expression of these genes in the visceral adipose depots is responsive to alterations in energy intake as well as site and physical form of carbohydrate delivered. However, a clear positive relationship between genes for regulatory control and lipogenic enzymes was not established. In contrast, gene expression in the subcutaneous depot appears resilient to dietary manipulation. Finally, because there was no clearly defined relationship between lipogenic rates determined *in vitro* and gene expression, it appears that transcript number is not the sole arbiter of enzyme activity, and perhaps fat deposition, in adipose tissue.

Table 2. Gene transcripts (copies/µg RNA) in mesenteric adipose from steers fed at either low (LI) or high intake (HI) and infused ruminally (R) or abomasally (A) with hydrolyzed starch (SH) or glucose (G).[a,b]

	LI						Contrast[c]			
Gene	H_2O	R-SH	A-SH	A-G	HI H_2O	SE[d]	LI-H_2O vs. HI-H_2O	LI-H_2O vs. R-, A-SH	R-SH vs. A-SH	A-SH vs. A-G
Fatty acid synthetase	42,086	65,948	78,505	137,041	176,552	40,743	0.03	— [e]	---	---
Aceytl-CoA carboxylase	5,224	6,993	3,459	17,278	17,755	3,638	0.03	---	---	0.001
Spot14	38,155	54,141	56,127	237,823	208,655	56,243	0.05	---	---	0.02
SREBP	7,970	19,774	20,898	29,621	43,754	8,870	0.01	---	---	---
SREBP-1	2,315	1,935	2,143	2,802	5,288	824	0.02	---	---	---
SREBP-2	1,960	1,937	1,382	3,330	4,213	568	0.01	---	---	0.02
ChREBP	355	722	335	686	1,167	137	0.0005	---	0.03	0.06
SCAP	1,173	1,956	1,528	4,037	4,926	806	0.005	---	---	0.03

[a] *Low and high intake represent 161 and 214 Kcal ME· (kg $BW^{0.75}$) -1·d^{-1}, respectively.*
[b] *Isoenergetic infusion rates of SH (12.6 g· (kg $BW^{0.75}$) -1·d^{-1}) and G (14.4 g· (kg $BW^{0.75}$) -1·d^{-1}).*
[c] *Probability of larger F statistic.*
[d] *n = 8 except for LI-H_2O (n = 6), HI-H_2O (n = 6), and A-G (n = 7) ; SE calculated using n = 6.*
[e] *P > 0.10.*

Table 3. Gene transcripts (copies/µg RNA) in omental adipose from steers fed at either low (LI) or high intake (HI) and infused ruminally (R) or abomasally (A) with hydrolyzed starch (SH) or glucose (G).[a,b]

	LI						Contrast[c]			
Gene	H_2O	R-SH	A-SH	A-G	HI H_2O	SE[d]	LI-H2O vs. HI-H_2O	LI-H_2O vs. R-, A-SH	R-SH vs. A-SH	A-SH vs. A-G
Fatty acid synthetase	562,219	620,584	1,721,884	3,228,373	1,355,311	507,322	— [e]	---	0.03	0.006
Aceytl-CoA carboxylase	59,744	72,042	145,596	305,128	142,897	43,591	---	---	0.09	0.001
Spot14	973,343	706,950	1,520,000	3,742,394	1,379,500	783,350	---	---	---	0.01
SREBP	83,887	113,604	126,980	192,456	120,743	30,819	---	---	---	0.04
SREBP-1	10,738	8,870	26,604	33,819	18,265	6,516	---	---	0.01	---
SREBP-2	6,444	10,900	13,680	25,283	14,867	4,279	0.10	---	---	0.01
ChREBP	2,376	2,935	6,837	7,584	5,756	1,860	---	---	0.04	---
SCAP	15,288	17,205	33,453	52,693	31,584	9,605	---	---	0.09	0.05

[a] *Low and high intake represent 161 and 214 Kcal ME· (kg $BW^{0.75}$) -1·d^{-1}, respectively.*
[b] *Isoenergetic infusion rates of SH (12.6 g· (kg $BW^{0.75}$) -1·d^{-1}) and G (14.4 g· (kg $BW^{0.75}$) -1·d^{-1}).*
[c] *Probability of larger F statistic.*
[d] *n = 8 except for LI-H_2O (n = 5) and A-G (n = 6) ; SE calculated using n = 5.*
[e] *P > 0.10.*

The Effects of Crude Protein Concentration and Non-Protein Nitrogen Source on Nitrogen Metabolism in Steers

V.B. Holder[1], S. El-Kadi[1], J.M. Tricarico[2], E.S. Vanzant[1], K.R. McLeod[1], and D.L. Harmon[1]*

Summary

Increasing crude protein concentration resulted in increased diet digestibility and increased N retention but led to increased urinary N excretion and increased plasma urea and ammonia concentrations, whereas slow-release urea diets resulted in lower plasma ammonia and urea concentrations. The objective of the study was to compare the effects of slow-release urea and regular urea on nitrogen metabolism when fed at two concentrations of protein intake. The experiment was conducted utilizing eight growing Holstein steers (BW = 265±18kg) in a replicated 4 × 4 Latin square with a 2 × 2 factorial treatment structure. Treatment factors included dietary crude protein (CP) concentration (10.9 vs. 12.1%) and urea source (slow release vs. regular). High CP diets resulted in increased

diet digestibility and nitrogen retention but also resulted in increased urinary N excretion. High CP diets resulted in higher rumen ammonia and plasma ammonia and urea concentrations due to increased supply of degradable N, while slow-release urea diets resulted in lower rumen ammonia, lower plasma ammonia, and lower plasma urea concentrations. Increased CP concentration increased diet digestibility and N retention but also increased N pollution, whereas slow-release urea reduced blood-nitrogen values, possibly reducing the probability of urea toxicity.

Introduction

Urea is a highly concentrated source of crude protein (CP) that is used to provide rumen degradable protein (RDP) to ruminants. However, regular feed grade urea degrades rapidly to ammonia in the rumen and may result in accumulation of ammonia in the rumen. Excess rumen ammonia is absorbed into the blood, where it is detoxified to urea in the liver. A portion of this urea may be recycled back to the rumen, while the remainder is excreted in the urine. Therefore increasing the urea content of the diet inevitably leads to increased urinary excretion of urea and increased environmental N pollution. Limiting degradable N in the rumen will reduce urinary N excretion and environmental pollution but will result in a deficiency in RDP in the rumen. Controlling the release rate of urea could potentially maintain a favorable ammonia concentration for rumen microbes while limiting urinary N excretion.

Materials and Methods

The trial was conducted using eight growing ruminally cannulated Holstein steers with an average body weight of 265 ± 18 kg to evaluate N balance. The experimental design was a replicated 4 × 4 Latin square with a 2 × 2 factorial treatment structure. Treatment factors were the level of dietary crude protein (CP) and the non-protein nitrogen (NPN) source used. Dietary CP concentration was either 10.9 or 12.1% CP. NPN source was either slow-release urea (OPTIGEN™II) or regular feed-grade urea (UREA). All diets within each CP level were formulated to be isoenergetic and isonitrogenous (Table 1). Additionally, all diets were formulated to contain equivalent concentrations of NPN (20.5%) as a percentage of total dietary CP. The experiment consisted four periods, each consisting of a 13-day adaptation period followed by seven days of nitrogen balance and one day of blood and rumen sampling. Diets were offered twice daily throughout the trial, and orts were collected and weighed each morning prior to. During the nitrogen balance period, total fecal and urine output were quantified before each morning feeding. Feces were weighed, sub-sampled, and immediately frozen (-20°C). Total urine output was continuously collected and kept separate from feces by fitting each steer with a urine funnel. Urine collection vessels contained sufficient phosphoric acid to ensure a final pH of 3.0 or less. A daily sub-sample of the acidified urine was frozen (-20°C). Samples of feed, feces, orts, and urine were analyzed for total N using a Vario Max CN elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Nitrogen retention was calculated as the difference between N intake and N output (feces plus urine). Data were analyzed as a 4 x 4 replicated Latin square design using PROC MIXED of SAS.

Table 1. Ration ingredients given as % of total dry matter in steers fed 12.1% and 10.9% crude protein diets with urea or Optigen™II.

Feedstuff	12.1% CP Urea	12.1% CP Optigen™II	10.9% CP Urea	10.9% CP Optigen™II
Fescue Hay	45.56	45.52	45.64	45.60
Cracked Corn	45.56	45.52	47.68	47.64
Soybean meal	2.12	2.12	0.00	0.00
Urea	0.88	0.00	0.79	0.00
Optigen	0.00	0.97	0.00	0.87
Molasses	4.40	4.39	4.40	4.40
Limestone	0.88	0.88	0.88	0.88
Vitamin premix[a]	0.04	0.04	0.04	0.04
Trace mineral premix[b]	0.56	0.56	0.56	0.56
% CP[c]	12.90	12.61	11.40	11.00

[a] *Vitamin premix composition: 8811 ppm vitamin A, 1762 ppm vitamin D, 1100 ppm vitamin E.*
[b] *Trace mineral premix composition: 0.06% Ca, 56.34% Cl, 36.53% Na, 1.2% S, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 ppm I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, 5520.2 ppm Zn.*
[c] *Analyzed crude protein concentration of experimental diets.*

Results and Discussion

Table 1 gives the composition of the ration. Table 2 contains results on dry matter intake (DMI), diet digestibility, and N-balance. Dry matter (DM) and organic matter (OM) intake were similar in all treatments. DM and OM digestibility were higher for the 12.1% CP diets (DM: 71.3% vs. 69.4%, P=0.024; OM: 72.4 vs. 70.3, P=0.018). NPN source did not affect DM or

Table 2. Dry matter intake, diet digestibility, and N-balance in steers fed 12.1% and 10.9% crude protein diets with urea or Optigen™II.

	Treatment					Effects, P > F		
Item	12.1% CP Urea	12.1% CP Optigen™II	10.9% CP Urea	10.9% CP Optigen™II	SEM	CP	Source	CP x Source
DMI, kg/d	5.39	5.34	5.40	5.41	0.26	0.58	0.79	0.69
DMI, % BW	1.97	1.94	1.97	1.97	0.05	0.34	0.17	0.31
Digestibility, %								
DM	71.33	71.35	68.96	69.87	1.88	0.02	0.56	0.57
OM	72.32	72.39	69.93	70.70	1.83	0.02	0.60	0.66
N Balance, g/d								
N intake	125.6	122.0	111.3	107.7	5.74	< 0.001	0.07	0.98
Fecal N	43.7	44.0	47.8	44.3	4.22	0.14	0.29	0.22
Urine N	44.9	43.6	40.0	35.5	4.55	0.03	0.31	0.58
N retention[a]	37.0	34.5	23.5	28.8	5.04	0.02	0.72	0.31

[a] *N retention = N intake – N output (Fecal N + Urine N).*

OM digestibility. As expected, N intake was higher for the 12.1% CP than the 10.9% CP diets (123.8 g/d vs. 109.5 g/d, P<0.001). Fecal N output was similar in all treatments. Urinary N excretion and N retention were higher in the 12.1% than the 10.9% CP diets (Urinary N: 44.2 g/d vs. 37.8 g/d, P=0.032; N retention: 35.8 g/d vs. 26.1 g/d, P=0.018). NPN source had no effect on urinary N excretion or N retention. Blood and rumen variables are given in Table 3. The average rumen ammonia concentration was higher in 12.1% CP diets than 10.9% CP diets (9.5 mM vs. 6.7 mM, P<0.001) and higher in UREA than OPTIGEN™II diets (8.6 mM vs. 7.5mM, P<0.001). Similarly, plasma urea concentration was higher in both 12.1% CP (4.9 mM vs. 4.2 mM, P<0.001) and UREA (4.7 mM vs. 4.3 mM, P<0.001) diets. There was an interaction between dietary CP concentration and NPN source on plasma urea concentration. The difference between UREA and OPTIGEN™II was greater at 10.9% than at 12.1% dietary CP (Figures 2 and 3). The average plasma ammonia concentration did not differ between the 10.9% CP and 12.1% CP diets. However, average plasma ammonia concentration was higher in UREA than OPTIGEN™II diets (0.448 mM vs. 0.435 mM, P<0.05).

The higher diet digestibility in 12.1% CP diets is likely related to improved supply of N to ruminal microorganisms. The 12.1% CP diets increased urinary N excretion, but also increased N retention. The increase in N retention is likely due to higher diet digestibility, making more energy available to the animal. Ruminally, increased fermentable energy may result in increased capture of degradable N, and post-ruminally, increased energy supply may also increase protein deposition in the animal.

The 12.1% CP diets resulted in higher rumen ammonia and plasma urea concentrations due to increased supply of degradable N. OPTIGEN™II diets result in lower rumen ammonia, lower plasma ammonia, and lower plasma urea concentrations due to its controlled-release properties and are therefore

Figure 1. Rumen ammonia concentration (mM) over time in steers fed 12.1% and 10.9% crude protein diets with urea or controlled release urea (Optigen™II)
*Pooled standard error = 0.79mM

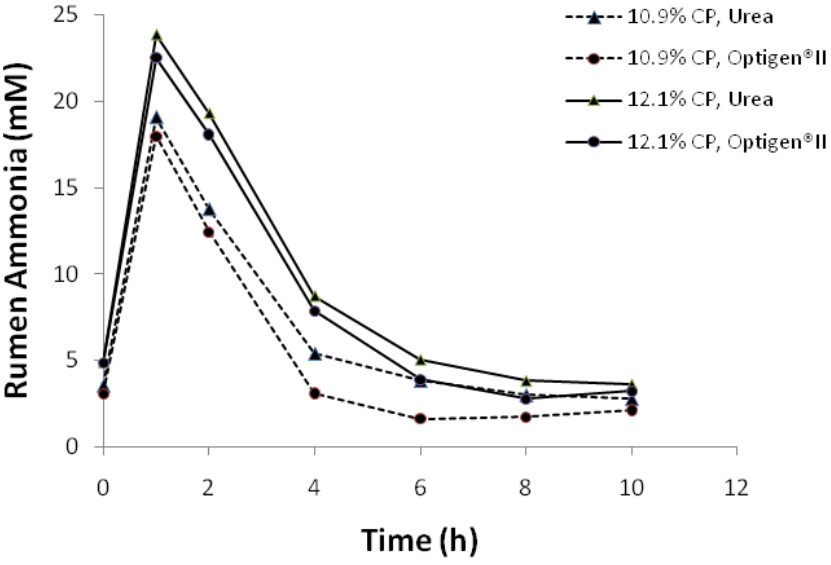


Figure 2. Plasma urea concentration (mM) over time in steers fed 12.1% and 10.9% crude protein diets with urea or controlled release urea (Optigen™II)
*Pooled standard error = 0.47mM

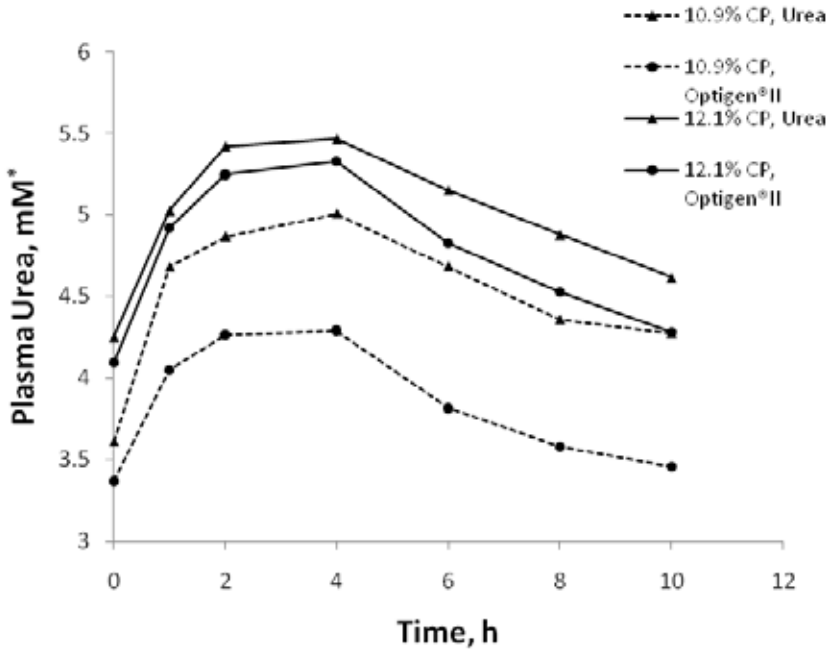


Table 3. Blood and rumen variables in steers fed 12.1% and 10.9% crude protein diets with urea or Optigen™II.

	Treatment					Effects, P > F		
Item	12.1% CP Urea	12.1% CP Optigen™II	10.9% CP Urea	10.9% CP Optigen™II	SEM	CP	Source	CP x Source
Rumen ammonia, mM	9.90	9.04	7.35	6.02	0.54	< 0.001	<0.001	0.328
Plasma ammonia, mM	0.451	0.439	0.444	0.430	0.03	0.159	<0.05	0.859
Plasma urea, mM	4.97	4.75	4.50	3.83	0.47	< 0.001	<0.001	0.010
Plasma glucose, mM	4.27	4.32	4.41	4.26	0.12	0.466	0.396	0.093

less likely than UREA to cause toxicity when fed at higher rates.

The interaction between CP level and NPN source on plasma urea may indicate that less ammonia is entering the blood at lower CP concentrations with OPTIGEN™II, possibly indicating that more N from OPTIGEN™II was utilized in the rumen. However the decrease in urinary N and increase in N retention indicative of OPTIGEN™II increasing efficiency of N use were not significant despite numerically lower urine N and higher N retention for the 10.9% CP OPTIGEN™II treatment.

Implications

It is important to look for means of reducing the impact of animal production on the environment. Efficiency of N utilization by cattle is relatively poor. Reducing N intake, particularly degradable N, is a method of reducing urinary N output. However, it is often at the expense of diet digestibility and N retention. Therefore, finding ways to reduce N pollution while maintaining production must become an important goal of ruminant researchers and producers. Reducing the degradation rate of urea has potential to reduce N excretion while maintaining RDP for rumen function. However, this was not evident from the current study.

Figure 3. Interaction between urea source and CP concentration on plasma urea concentration (mM)

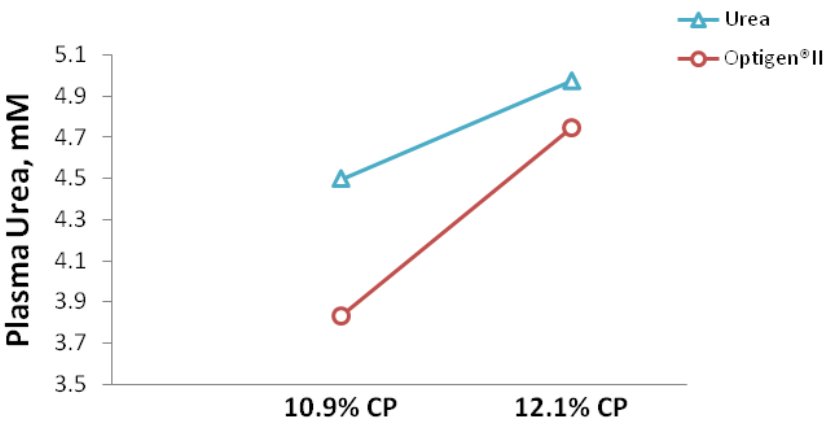
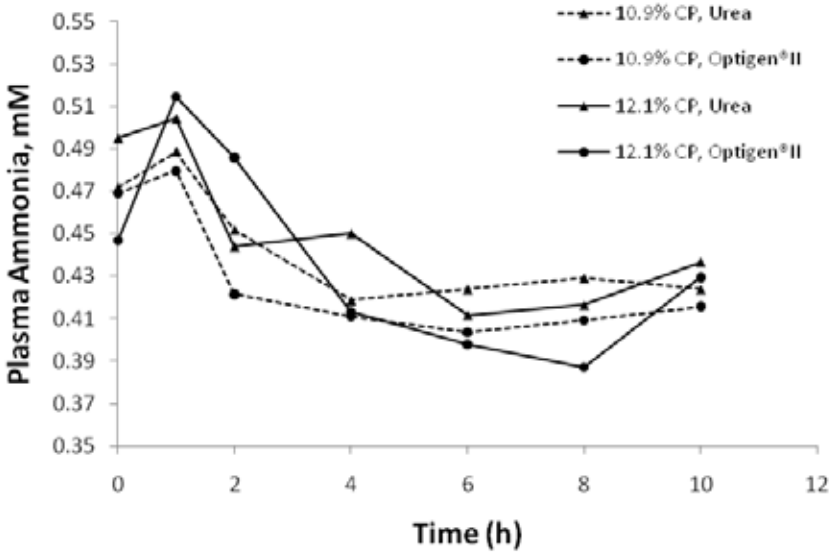


Figure 4. Plasma ammonia concentration (mM) over time in steers fed 12.1% and 10.9% crude protein diets with urea or controlled release urea (Optigen™II)



Effects of Degradable Nitrogen Level and Non-protein Nitrogen Source on Nitrogen Balance in Holstein Steers

V.B. Holder[1], D.H. Kim[1], J.M. Tricarico[2], and D.L. Harmon[1]*

Summary

Diets deficient in degradable nitrogen led to decreased nitrogen excretion but also reduced nitrogen retention and digestibility, whereas reducing the degradation rate of degradable intake protein (DIP)-reduced nitrogen retention when DIP was deficient but improved it when DIP was adequate. The objective of the study was to compare the effects of slow-release urea and regular feed-grade urea on nitrogen metabolism when fed at two levels of DIP intake. The experiment was conducted utilizing eight growing Holstein steers (BW = 460±33 lb) in a replicated 4 x 4 Latin square with a 2x2 factorial treatment structure. Treatment factors included DIP level (89% vs. 100% of requirement) and urea source (slow release vs. regular). The 100% DIP diets improved diet digestibility and nitrogen retention but also increased urinary nitrogen excretion. Slow-release urea improved nitrogen retention on 100% DIP diets but depressed nitrogen retention and increased urinary nitrogen excretion on 89% DIP diets. Slow-release urea may be beneficial to improve nitrogen utilization in diets where degradable nitrogen is not limiting.

Introduction

Urea is a highly concentrated source of crude protein (CP) that is used to provide DIP to ruminants. However, regular feed-grade urea degrades rapidly to ammonia and may result

in accumulation of ammonia in the rumen. Excess rumen ammonia is absorbed into the blood, where it is detoxified to urea in the liver. A portion of this urea may be recycled back to the rumen, while the remainder is excreted in the urine. Therefore, increasing the urea content of the diet inevitably leads to increased urinary excretion of urea and increased environmental nitrogen pollution. Limiting degradable nitrogen in the rumen will reduce urinary nitrogen excretion and environmental pollution but will result in a deficiency of rumen-degradable protein. Controlling the release rate of urea could potentially maintain a favorable ammonia concentration for rumen microbes while reducing urinary nitrogen excretion.

Table 1. Ration ingredients given as % of total dry matter in steers fed 89% and 100% of DIP requirements in diets with urea or Optigen™II.

FeedStuff	100% DIP, Urea	100% DIP, Optigen™II	89% DIP, Urea	89% DIP, Optigen™II
Cracked corn	47.27	47.22	49.14	49.10
Fescue hay	23.82	23.80	23.86	23.84
Cottonseed hulls	22.33	22.31	22.37	22.35
Soybean meal	1.86	1.86	0.00	0.00
Urea	1.04	0.00	0.95	0.00
Optigen	0.00	1.14	0.00	1.04
Molasses	1.94	1.93	1.94	1.94
Limestone	0.80	0.80	0.81	0.81
Dicalcium phosphate	0.19	0.19	0.19	0.19
Calcium sulfate	0.11	0.11	0.11	0.11
ADE premix[a]	0.04	0.04	0.04	0.04
TM premix[b]	0.60	0.60	0.60	0.60

[a] *Vitamin premix composition: 8811 ppm vitamin A, 1762 ppm vitamin D, 1100 ppm vitamin E.*

[b] *Trace mineral premix composition: 0.06% Ca, 56.34% Cl, 36.53% Na, 1.2% S, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 ppm I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, 5520.2 ppm Zn.*

Materials and Methods

The trial was conducted using eight growing Holstein steers with an average initial body weight of 209± 15 kg to evaluate N balance. The experimental design was a replicated 4 × 4 Latin square design with a 2 × 2 factorial treatment structure. Treatment factors were the level of degradable nitrogen in the diet (DIP Level) and the non-protein nitrogen (NPN) source used. DIP level was either 100% or 89% of the DIP requirements according to the National Research Council (NRC). NPN source was either slow-release urea (OPTIGEN™II) or regular feed-grade urea (UREA). All diets were formulated to be isoenergetic and isonitrogenous within DIP level (Table 1). Additionally, all diets were formulated to contain equivalent concentrations of NPN (24.9%) as a percentage of total dietary CP. The experiment consisted of four periods, each consisting of a 19-day adaptation period followed by seven days of nitrogen balance. Diets were offered twice daily throughout the trial. During the nitrogen-balance period, total fecal and urine output were quantified before each morning feeding. Feces were weighed, sub-sampled, and immediately frozen (-20ºC). Total urine output was continuously collected and kept separate from feces by fitting each steer with a urine funnel. Urine collection vessels contained sufficient phosphoric acid to ensure a final pH of 3.0 or less. A daily sub-sample of the acidified urine was frozen (-20ºC). Samples of feed, feces, and urine were analyzed for total N using a Vario Max CN elemental analyzer. Nitrogen retention was calculated as the difference between nitrogen intake and nitrogen output (feces plus urine). Data were analyzed as a 4 x 4 replicated Latin square design using PROC MIXED procedure of SAS.

Results and Discussion

Table 1 gives the composition of the diets. DMI did not differ between treatments (Table 2). DM and OM digestibility were higher for the 100% vs. 89% DIP diets (DM: 60.3% vs. 58.5%, P=0.02; OM: 60.2% vs. 58.3%, P=0.02). NPN source did not affect DM or OM digestibility. As expected, N intake was higher for the 100% vs. the 89% DIP diets (162.8 g/d vs. 146.0 g/d, P<0.0001). Nitrogen intake was higher for OPTIGEN™II than UREA diet (160.2 g/d vs. 148.6, P<0.0001). There was no effect of treatment on fecal nitrogen output. Urinary nitrogen output and absorbed nitrogen were both higher for the 100% DIP treatment (Urine N: 58.3 g/d vs. 47.7 g/d, P<0.0001; Absorbed N: 95.5 g/d vs. 80.4 g/day, P<0.0001). Similarly, urinary nitrogen output and absorbed nitrogen were both higher for OPTIGEN™II (Urine N: 57.9 g/d vs. 48.0 g/d, P<0.0001; Absorbed N: 93.4 g/d vs. 82.5 g/day, P<0.0001). Nitrogen retention was higher in 100% DIP than 89% DIP (37.2 g/d vs. 32.7 g/d, P=0.005). There was an interaction (P=0.01) between DIP level and NPN source on nitrogen retention. At 100% DIP, OPTIGEN™II had higher nitrogen retention than UREA, but at 89% DIP, UREA had high nitrogen retention (Table2).

The higher diet digestibility in 100% DIP is likely related to improved supply of DIP to rumen microorganisms. Because of variation in feed components, nitrogen intake was not equal within DIP levels (higher in OPTIGEN™II treatments), leading to possible confounding of the effects of NPN source with those of nitrogen intake. This is likely the cause for the increased urinary nitrogen excretion on OPTIGEN™II treatments. However, the fact that nitrogen retention is higher for OPTIGEN™II at 100% DIP and lower at 89% DIP cannot be explained by N intake. The interaction remains apparent even when nitrogen retention is expressed as a % of nitrogen intake (Table 2), possibly indicating improved utilization of DIP from OPTIGEN™II when fed at NRC requirements for DIP. At 89% DIP, ammonia may not accumulate in the rumen, due to the lack of degradable nitrogen. Under these circumstances, reducing degradation rate of DIP (OPTIGEN™II) appeared to reduce nitrogen retention, possibly by restricting ammonia supply to rumen microbes.

Table 2. Dry matter intake, diet digestibility, and N-balance in steers fed 89% and 100% DIP diets with urea or Optigen™II.

	Treatment					Effects, P > F		
Item	100% DIP, Urea	100% DIP, Optigen™II	89% DIP, Urea	89% DIP, Optigen™II	SEM	DIP Level	Source	DIP x Source
DMI, kg/d	7.11	7.23	7.11	7.20	0.8435	0.89	0.15	0.83
Digestibility, %								
DM	59.83	60.69	58.19	58.83	1.60	0.02	0.27	0.87
OM	59.64	60.75	57.98	58.66	1.69	0.02	0.22	0.76
N Balance, g/d								
N intake	169.46	156.17	150.98	141.03	18.10	<0.0001	<0.0001	0.31
Fecal N	67.60	67.09	66.03	65.19	7.95	0.18	0.59	0.90
Urine N	62.23	54.30	53.62	41.74	7.42	<0.0001	<0.0001	0.20
Absorbed N	101.86	89.09	84.95	75.83	10.26	<0.0001	<0.0001	0.26
N Retention[a]	39.63	34.79	31.33	34.09	4.02	0.005	0.46	0.01
Absorbed N, % of N Intake	60.18	57.02	56.04	53.75	0.91	<0.0001	0.001	0.54
Urine N, % of N Intake	36.60	34.62	35.13	29.49	1.79	0.0002	<0.0001	0.02
N Retention, % of N Intake	23.58	22.40	20.91	24.26	1.91	0.64	0.22	0.02
Urine N, % of Absorbed N	61.05	60.67	62.82	54.73	7.88	0.14	0.005	0.01
Retained N, % of Absorbed N	38.95	39.33	37.18	45.27	7.88	0.14	0.005	0.01

[a] *N retention = N intake – N output (Fecal N + Urine N).*

Implications

Reducing nitrogen content of diets will lead to decreased nitrogen excretion into the environment. However, it may also lead to decreased production related to depressed diet digestibility and nitrogen retention. When degradable nitrogen is restricted, it appears it may be counterproductive to slow the release of nitrogen in the rumen, as it may lead to decreased N retention and increased urinary nitrogen excretion. However, at higher DIP levels such as those prescribed by the NRC, reducing the rate of degradation of nitrogen in the rumen may lead to improved nitrogen retention by alleviating high rumen ammonia concentrations.

Fall vs. Spring Calving for Beef Cows on High or Low- Endophyte Fescue

J.H. Randolph, W.R. Burris, and L.H. Anderson

Summary

In a three- year study at the University of Kentucky Research and Education Center in Princeton, Kentucky adjusted weaning weights were higher for spring-born calves than for fall-born calves and were higher for calves raised on low-endophyte fescue than those raised on high-endophyte fescue. Fall -calving cows consumed more hay during the winter feeding period than did spring-calving cows, and cows on low endophyte consumed more hay than those on high endophyte. Pregnancy rates were similar for cows calving in spring or fall and for cows on low endophyte or high endophyte. Birth weights were lower, and calf survivability was higher for fall calves than for spring calves.

Introduction

Beef cattle grazing high-endophyte (HE) fescue pasture in Kentucky may experience elevated body temperature leading to increased heat stress during the summer. The heat stress is likely to decrease weight gain, lower pregnancy rate, and reduce milk production for lactating cows, resulting in lower calf weaning weights for spring calving herds. HE fescue pastures may be re-established with low endophyte (LE) fescue to avoid the endophyte-related heat stress. Also, beef herds calving in the fall may minimize the negative effects of grazing HE fescue because the critical phases of breeding and lactation are shifted to times of more favorable weather and temperature. However, the lactating cows in a fall calving herd are likely to require more feed in the winter than the dry cows in a spring-calving herd. Calf survival may also be improved for calves born in the fall over those born in the early spring because of better weather conditions. This study was done to identify advantages and/or disadvantages of fall vs. spring calving on HE or LE fescue.

Materials and Methods

Eighty cow-calf pairs per year were used in a completely randomized design with a 2 X 2 factorial arrangement of treatments. Cattle were allocated to fall or spring calving groups and HE or LE fescue pasture. Each treatment pasture consisted of 40 acres of fescue of which 10 acres were stockpiled for winter grazing. This trial was initiated in November of 2000 and was completed when three calf crops had been weaned from each treatment (late Oct 2003). The original allotment took place after calving in Sept-Oct or Feb-March of year 1.

Cows on all treatments were given one timed AI period in which they were inseminated with one of two Angus AI sires. Clean-up bulls (Hereford) were turned out about 10 days after AI and rotated after the next heat cycle. Bulls were removed about 65 days after the AI breeding date. The estrous synchronization and AI protocol was GnRH on day 0, prostaglandin F2 alpha on day 7, and GnRH with AI on day 9. Open cows were removed from the study after weaning each year, and replacement cows were randomly allotted to keep 20 pair on each treatment.

Table 1. Weaning weights (lb) average of 3 years.

	Fall LE	Fall HE	Spring LE	Spring HE
Adjusted	506.5	503.8	570.5	550.1
Actual	578.7	586.2	614.0	587.3

Table 2. Winter hay (rolls) per cow average of 3 years.

Fall LE	Fall HE	Spring LE	Spring HE
2.7	1.6	1.1	1.0

Table 3. Pregnancy rates (%) average of 3 years.

	Fall LE	Fall HE	Spring LE	Spring HE
AI	49	53	55	54
Overall	95	92	97	92

Table 4. Summary of fall vs. spring.

	Fall	Spring
AI preg. rate (%)	51	55
Preg. rate (%)	93	94
Actual weaning wt (lb)	582	601
Adj. weaning wt (lb)[a]	505	560
Avg. hay cost ($/cow)[b]	54	27
Birth weight (lb)	79	84
Calf mortality (%)	1.7	7.5

[a] *No creep feed.*
[b] *Hay valued at $25/roll.*

Calves were weighed and ID tagged at birth. Spring-born calves were weaned as needed in the fall (late Oct-early Nov), and fall-born calves were weaned in June. Creep feed was not offered. Weaning weights were adjusted for age at weaning, calf sex, and cow age. All cows had *ad libitum* access to large round bales of fescue hay after stockpiled fescue was consumed or when snow covered the ground. Cows on HE treatments received HE hay, and LE cows received LE hay. From about Feb 20 to about April 20 of each year all cows were fed 50 lb corn silage and 1 lb soybean meal per cow per day along with free-choice hay.

Results and Discussion

As shown in Table 1, adjusted weaning weights for the three-year period were higher for spring calves than for fall calves, and LE calf adjusted weaning weights were heavier than for HE calves. Spring calves were heavier than fall calves whether on HE or LE pasture. LE calves had heavier adjusted weaning weights than HE calves whether born in spring or fall. Spring-born calves have an advantage in that they have access to growing pasture constantly up until weaning, while fall calves can graze only until the stockpiled grass is consumed and then have harvested feed to supplement milk from the dam. In this trial, 50 lb corn silage

and 1 lb soybean meal per cow per day from Feb 20 to April 20 was kept constant for all treatments with no allowance being given for the fall calves that were physically big enough to eat from the feed bunk. Creep feed was not offered in this trial. It would be interesting to know if creep feed would be more of an advantage in spring or fall.

Fall-calving cows consumed more hay than spring calvers, and cows on LE consumed more hay than those on HE, as shown in Table 2. It was observed that LE cows ate up their stockpiled pasture faster (perhaps because it was highly palatable), and therefore consumed more hay. Certainly lactating fall calvers require more hay than dry, gestating spring calvers. The hay was in addition to the 50 lb corn silage and 1 lb soybean meal per cow per day fed to all cows from Feb 20 to April 20.

In this trial, treatment did not seem to have much effect on AI pregnancy rate or overall pregnancy rate. Pregnancy rate was good across all treatments, as shown in Table 3. It was expected that pregnancy rate might be improved for fall calvers because of lack of heat stress during the breeding season. If summer weather had been more severe than it was 2001-2003, perhaps there would have been some differences.

Calving losses were decreased, and calf birth weight was decreased for fall-calving cows compared to spring calvers. Over the three-year period, nine spring-born calves died at or shortly after birth, compared to a death loss of only two fall-born calves. The value of these seven extra calves should be more than enough to pay for the extra hay fed to fall calvers. Average birth weight for the three-year trial was 79 lb for fall calves and 84 lb for spring calves. Lower birth weights for fall calves may have contributed to increased survivability, as did the typically favorable weather. It was definitely a more pleasant task to weigh and ID tag newborn calves during the mild weather of Sept-Oct than in the cold, wet conditions of Feb-March. Level of endophyte did not seem to affect birth weights or calf survivability.

Implications

Spring calves were heavier at weaning, but fall calves had lower death loss. Fall-calving cows will require more winter feed, and a sound forage program is essential. Some producers may prefer to monitor cows at calving and process newborn calves during the mild weather of fall rather than the cold, muddy weather of early spring. Compared to HE, LE fescue increased weaning weights more for spring calves than fall calves.

Master Cattleman Program

L.T. Porter, W.R. Burris, and K.B. Knight

Summary

The Master Cattleman Program was developed to transform Kentucky's beef producers to be more competitive in the marketplace, more profitable, more environmentally sustainable, and more aware of industry issues.

This program has trained and certified over 3,000 beef producers. A beef producer has an average return of about $4,500 per year to his or her operation as a result of adopting recommended management practices, which producers are taught during the 10 sessions of the Master Cattleman Program. These activities should return over $13 million per year to Kentucky as a result of the economic investment made by the Kentucky Agricultural Development Board.

Table 1. Changes in production based on Master Cattleman Program FY 07 & FY 08.

Number of animals:		Multiplier	Ky. Impact
Cows in a controlled breeding/calving season	56,200	$150	8,430,000
Cows exposed to semen-tested bull	62,050	$25	1,551,250
Cows bred using estrus synchronization and AI	15,769	$70	1,103,830
Cows placed in a cross-breeding system	57,725	$18	1,039,050
Cows placed on a complete mineral	63,764	$10	637,640
Cows vaccinated	51,283	$5	256,415
Non-CPH calves implanted	28,506	$16	456,096
Non-CPH calves castrated before weaned	40,671	$5	203,355
Non-CPH calves vaccinated	56,051	$5	280,255
Calves sold in CPH Sale	28,193	$40	1,127,720
Acres hay now covered or stored inside	65,507	$50	3,275,350
Acres grazing land now rotationally grazed	90,185	$100	9,018,500
Total			$27,379,461

Introduction

The Master Cattleman Program is the flagship educational program for Kentucky cattle producers. It incorporates all phases of beef production into an intensive educational effort challenging Kentucky beef producers to be competitive and successful.

The program is an integral part of the comprehensive effort to improve Kentucky's expanding beef-forage operations. It is a collaborative effort of the University of Kentucky College of Agriculture, the Kentucky Cattlemen's Association, and the Kentucky Beef Network. The goal is to reach 10% of Kentucky beef cattle producers.

Materials and Methods

The Master Cattleman Program requires the cooperation of more than more than 20 extension specialists and associates to administer and deliver the program. Sessions begin in January and run through mid-December. Master Cattleman participants receive 40 hours of classroom instruction divided equally among the following 10 topic areas:

- Management skills for the beef business
- Forage production and utilization
- Nutrition for optimum production
- Environmental stewardship and industry issues
- Genetics for the beef herd
- Managing reproduction
- Herd Health
- Understanding the end product
- Marketing and profitability

In addition, field days are conducted at the UK Research and Education Center in Princeton and the UK Animal Research Center in Versailles. These field days allow producers to get hands-on training over two days on activities related to estrous synchronization, artificial insemination, pregnancy diagnosis, bull selection, visual appraisal, using EPDs, handling vaccines, proper injection sites and techniques, cattle handling, facility design, body condition scoring, etc.

Results and Discussion

The Master Cattleman Program was designated as a featured program by the Cooperative Extension Service in 2008. As such, it is reported on by individual, participating counties. Participants were asked to specify which activities they added or modified as a result of the Master Cattleman Program. Only 12 practices were specified in this survey, yet the results of the survey indicated a return of $27 million over a two-year period (see Table 1).

Implications

Over 3,000 Kentucky producers have benefited from the program in many ways since its inception. This program has reached nearly 8% of the estimated 38,000 beef producers in Kentucky. With continued funding, it is hoped that we would reach 10% of Kentucky's beef producers.

Participants in this program have adopted many recommended management practices as a result of this training. These practices have resulted in an approximate impact to Kentucky of $13.6 million per year (one-half of $27, 379,000 from Table 1) or about $4,500 per year per producer.

Over the next five years of this program, we plan to offer a minimum of five Master Cattleman programs per year, which should enroll at least 150 participants from 25 counties, resulting in a total of 750 participants. Based on past history of economic returns, this should yield an additional economic return of $6,122,250.

The Master Cattleman program is paying dividends to Kentucky agriculture.

Acknowledgements:

This Program is made possible with funding from the Kentucky Agricultural Development Board.

The contributions of the following individuals are gratefully acknowledged: Les Anderson, Darrh Bullock, Kenny Burdine, Garry Lacefield, Kevin Laurent, Jeff Lehmkuhler, Jack McAllister, Gregg Rentfrow, Ray Smith, and Joseph Taraba.

Summary of Kentucky Certified Preconditioned for Health (CPH-45) Feeder Calf Sales

K.M. Laurent, T. Dietrich, and W.R. Burris

Summary

Cattle producers marketed 256,672 head of feeder calves in Kentucky Certified Preconditioned for Health (CPH-45) Feeder Calf Sales during the last nine years (2001-2010). 286 sales were held in 23 locations throughout Kentucky during this time period. Calves sold in CPH-45 sales averaged $6.61 per cwt over state average prices as reported by the Kentucky Department of Agriculture (KDA) Market News Branch. The Lexington sale location conducted the most sales (57) and sold the highest volume of cattle, (73,775 head). The top five sale locations (Lexington, Owensboro, Paris, Guthrie/Hopkinsville, and Irvington) accounted for 77% of the total CPH-45 calves sold. Net added returns for producers participating in the Guthrie/Hopkinsville fall sale have been estimated each year since 1993. Participants have realized an additional estimated average return of $48.04 per head above preconditioning expenses by selling in the December CPH-45 sale as opposed to selling at weaning in October. Producers have increased returns by weaning, preconditioning, and marketing their calves in Kentucky CPH-45 sales.

Introduction

Most Kentucky cow-calf producers market their feeder calves at weaning. The stress of weaning coupled with the stress of transport and commingling with other cattle in the stockyard results in a high degree of morbidity and reduced performance. The Kentucky CPH-45 Program is a health and management program that preconditions calves for transition into the backgrounding and feedlot phase post-weaning. Although the health and management requirements to qualify for CPH-45 sales (Table 1) results in healthier calves, producers must be rewarded for their efforts through added weight gain and higher market prices to make it a viable alternative to selling at weaning. CPH-45 sales are designed to provide a market for order buyers to purchase large groups of preconditioned calves and increase the producers' likelihood of a higher return. Market data comparing prices received at all CPH-45 sales since April 1 2001 to statewide average prices were summarized. In addition, estimated net added returns per head for 600-699 lb steers selling in the Guthrie/Hopkinsville December CPH-45 sales since 1993 were calculated to determine the profitability of participating in KY CPH-45 sales.

Table 1. Kentucky CPH requirements.

1	Owned by seller a minimum of 60 days.
2	Weaned a minimum of 45 days.
3	Trained to eat feed from a bunk and drink water from a trough.
4	Dehorned and healed (no visible horns or scurs).
5	Males castrated and healed.
6	Treated for grubs and lice according to label recommendations for time of year.
7	Dewormed with an endectocide a maximum of 60 days before the sale.
8	Vaccinated for Clostridia (7-way) subcutaneously in the neck.
9	Vaccinated and boostered for IBR, PI3, BVD, and BRSV (booster injection for viral diseases must be modified live vaccine)
10	Vaccinated for *Manheimia haemolytica (pasteurella)*
11	Identified with official Kentucky CPH tag.
12	Heifers are guaranteed open at time of sale and steers are guaranteed not to be bulls.
13	Calves must have access to a free choice mineral supplement which contains a minimum 1,400 ppm copper (no copper oxide), 26 ppm selenium, 3,000 ppm zinc, 3,000 ppm manganese and 18- 25% salt based on a 4 oz. daily intake. No other salt available.

Materials and Methods

CPH-45 Prices vs. Statewide Average Prices

Market data comparisons were obtained from KDA Market News Branch for CPH-45 sales held the last nine sale years (April 1, 2001- March 31, 2010). Sale years begin April 1 and end March 31. CPH-45 prices were compared to statewide averages for the same week for the same sex, grade, and weight of cattle as reported by KDA Market News reporters. CPH-45 prices for USDA Large and Medium Frame, No. 1 and 2 (LM1-2) steers were compared to statewide prices for USDA Large and Medium Frame, No. 1 (LM1) steers. Both CPH-45 prices and statewide prices are weighted averages. For example, the average price for all LM1-2 CPH-45 steers weighing 500-599 lb sold in Lexington on 4/2/09 was $114.94/cwt. The weekly average statewide price for all LM1 steers weighing 500-599 lb reported that same week was $103.08/cwt. The difference was $11.86/cwt. A total of 1692 price comparisons were then arithmetically averaged and summarized by sale year, sale location, sex, and weight of cattle.

Estimated Net Added Returns

The Guthrie/Hopkinsville sale is the oldest continuously operating CPH-45 sale in the state. The CPH-45 program began in Hopkinsville in 1980 and was known as the Pennyrile Area CPH Sale. In 2005, the Pennyrile committee moved the sale to Guthrie and renamed the sale the Kentucky-Tennessee CPH-45 Advantage Sale to reflect the addition of Tennessee producers. Since 1993, estimated net added returns per head have been calculated each year to determine the profitability of selling a 675 lb steer in the Guthrie/Hopkinsville CPH-45 December sale vs. selling the same calf weighing 550 lb at weaning in October. A 50-day preconditioning period and an average daily gain of 2.5 lb/day were assumed for these estimates. Calf value at weaning was determined by multiplying the average statewide price reported by KDA for LM1 500-599 lb steers in October by 550 lb minus a commission charge of 3% and $3 per head. Calf value at the December CPH-45 sale was determined by multiplying the average CPH-45 price for all LM1-2 600-699 lb steers by 675 lb minus a commission charge of 3% and $3 per head. Net added returns were determined by subtracting the calf's value at wean, feed

Table 2. Difference in CPH-45 prices vs. statewide average prices, by sale year (2001-2010) ($/cwt., LM 1-2).

	Sales	Head	4 wt. Heifer	4 wt. Steer	5 wt. Heifer	5 wt. Steer	6 wt. Heifer	6 wt. Steer	7 wt. Heifer	7 wt. Steer	8 wt. Steer	Avg. Heifer	Avg. Steer	Avg. Overall
2001-02	22	21,616	2.42	3.59	3.02	4.86	3.51	4.37		4.13		2.98	4.24	3.70
2002-03	33	31,789	4.56	7.37	5.01	6.26	4.65	5.79		4.13		4.74	5.89	5.40
2003-04	29	26,172	6.71	9.90	7.79	8.54	6.19	7.24		6.11		6.90	7.95	7.50
2004-05	27	27,603	8.61	7.63	8.23	9.53	8.38	7.01		4.20		8.41	7.09	7.66
2005-06	30	29,903	5.58	9.78	7.25	11.37	6.21	7.28		6.77		6.34	8.80	7.75
2006-07	36	33,241	6.29	7.77	5.96	9.29	6.75	8.04		6.36		6.33	7.87	7.21
2007-08	36	30,070	7.82	8.28	9.52	9.15	8.09	8.14		6.53		8.48	8.02	8.22
2008-09	38	30,114	4.67	7.18	7.29	7.66	6.48	6.64	6.09	4.36	1.52	6.13	5.47	5.77
2009-10	35	26,164	6.46	10.00	7.03	8.92	6.92	6.18	4.17	4.57	2.88	6.14	6.51	6.35
Total/Average	286	256,672	5.90	7.94	6.79	8.40	6.35	6.74	5.13	5.24	2.20	6.27	6.87	6.61

cost, veterinary cost (vaccine, dewormer,), sale tag, and interest (calf's value at weaning for 50 days) from the calf's value at the CPH-45 sale. Feed cost was calculated using rations formulated for approximately 2.7 lb average daily gain and prices based on 3-ton bulk delivery. Corn, soybean meal, and hay rations were used exclusively from 1993-2004. Soybean hull, corn gluten feed, distillers dried grains, and hay rations were used 2005-09. Hay quality was assumed to be 10% protein and 50% TDN. Vaccine and dewormer costs were determined by local suppliers. Interest rates were obtained from a local lending institution.

Results and Discussion

CPH-45 Prices vs. Statewide Average Prices

The difference in CPH-45 prices as compared to statewide average prices by sale year in $/cwt are presented in Table 2. Price comparisons from 286 sales consisting of 256,672 head were analyzed. 1,610 out of 1,692 price comparisons (95%) showed an advantage to CPH-45 prices. The average differences in prices ranged from a low of $3.70 in 2001-02 to a high of $8.22 in 2007-08, with an average advantage to CPH-45 prices for all years combined of $6.61/cwt. Steers, in most cases, sold at a slightly higher advantage than heifers. Calves weighing 400-699 lb sold at a higher advantage than 700-899 lb calves. Table 3 summarizes sale data and price differences by sale location. 23 different locations held CPH-45 sales during this time period. Lexington had the most sales (57) and sold the most cattle (73,775 head) of any site. The top five sale locations (Lexington, Owensboro, Paris, Guthrie/Hopkinsville, and Irvington) sold 197,773 head of feeder calves, or 77% of the total. The Danville and Richmond sales had the highest average differences in prices at $9.75 and $9.38, respectively.

Table 3. Difference in CPH-45 prices vs. statewide average prices, by sale location (2001-2010) ($/cwt, LM 1-2).

	Location	Sales	Head	4 wt Heifer	4 wt Steer	5 wt Heifer	5 wt Steer	6 wt Heifer	6 wt Steer	7 wt Heifer	7 wt Steer	8 wt Steer	Avg. Heifer	Avg. Steer	Avg. Overall
1	Lexington	57	73,775	8.13	12.59	8.58	12.40	6.93	8.85	4.65	6.87	2.75	7.07	8.69	7.97
2	Owensboro	56	51,662	5.60	3.52	7.07	7.44	6.86	7.43	6.90	6.33	4.17	6.61	5.78	6.15
3	Paris	36	33,871	8.16	14.34	8.87	12.50	5.75	7.05	1.66	4.68	-0.32	6.11	7.65	6.96
4	Guthrie/ Hopkinsville	25	24,048	6.20	6.22	8.31	8.29	8.01	6.74	9.24	7.04	7.12	7.94	7.08	7.46
5	Irvington	17	14,417	7.74	7.29	8.50	8.41	6.71	5.58	7.82	5.39	4.80	7.69	6.29	6.91
6	Springfield	20	9,295	3.86	5.84	5.58	9.26	6.21	7.26	2.70	4.00	0.69	4.59	5.41	5.05
7	Russellville	10	8,600	4.22	5.93	3.55	3.93	5.14	3.48	10.39	3.54	6.41	5.83	4.66	5.18
8	Richmond	10	7,852	9.80	13.92	9.14	12.95	8.41	10.24	2.93	7.18		7.57	11.07	9.32
9	Mt. Sterling	9	6,361	6.34	9.46	7.02	7.71	6.44	9.81	4.88	5.12		6.17	8.03	7.10
10	Maysville	11	4,536	0.12	1.86	4.43	4.15	5.15	4.25	3.33	2.95	2.30	3.26	3.10	3.17
11	Danville	4	3,753	7.67	14.18	7.27	13.59	9.07	10.36		6.12		8.01	11.06	9.75
12	Glasgow	4	3,691	7.19	3.79	5.70	6.04	5.49	4.94		3.79		6.12	4.64	5.28
13	Lancaster	3	2,560	6.01	3.19	6.75	9.85	4.56	9.24		6.75		5.77	7.26	6.62
14	Flemingsburg	3	2,189			2.67	1.31	1.64	4.25		3.95		2.15	3.17	2.76
15	Bowling Green	2	1,931	4.55		4.66	0.99	4.97	2.83		2.09		4.73	1.97	3.35
16	Stanford	6	1,849	7.03	7.30	8.19	7.99	5.96	6.31	-1.15	5.74	-1.07	5.01	5.26	5.15
17	Owenton	2	1,616		10.20	5.16	10.06	6.14	5.33		5.29	2.07	5.65	6.59	6.32
18	Marion	2	1,370	12.01	12.25	8.17	7.39	7.40	6.65		0.81		9.19	6.77	7.81
19	Hopkinsville	4	1,090	3.71	1.83	3.13	5.04	4.82	2.90		3.54	-7.49	3.89	1.16	2.19
20	Monticello	2	949			0.53	-1.72	1.85	2.96		3.58		1.19	1.61	1.44
21	Russell Springs	1	598	-1.11	7.23	0.54	1.16	5.33	1.37		2.30		1.59	3.02	2.40
22	Campbellsville	1	487			6.15	2.29	5.79	5.04		3.75		5.97	3.69	4.60
23	Paintsville	1	172	0.61	8.94	2.59	5.85	3.21	3.95		7.90		2.14	6.66	4.72

Estimated Net Added Returns

The profitability of holding 550 lb steers from October until the Guthrie/Hopkinsville December CPH-45 sale is estimated in Table 4. Market conditions and feed cost are two critical factors affecting profits in short-term preconditioning. The average price reported by KDA for October 500-599 lb LM1 steers from 1993-2009 was $86.97/cwt. The average price received for 600-699 lb LM1-2 steers at the Guthrie/Hopkinsville CPH-45 sale during that same time period was $87.21/cwt. This means, on the average, the heavier 6-weight steer actually sold at a positive margin in the CPH-45 sale as compared to the lighter 5-weight steer in October. Cost of gain fluctuates with the price of feed. Estimated feed cost per lb of gain ranged from $0.27 to $0.47 during this time period. The average feed cost per lb of gain was $0.33. Veterinary expense/sale tags increased from $12 to $15 per head. Interest rates have fluctuated between 6 and 9 percent. Estimated net added returns for a 675-lb steer in the Guthrie/Hopkinsville December CPH-45 sale ranged from -$19.46 (2008) to $100.34 (2005), with an average return of $48.04 per head. Negative returns only occurred in two years (2006 and 2008). In 13 out of the last 17 years, it is estimated that producers benefited from selling these calves in the December CPH-45 sale.

Table 4. Estimated net added returns—Hopkinsville 1993-04; Guthrie 2005-09—50 days 2.5 ADG (Oct - Dec).

Year	550 lb Steer in Oct ($/cwt[1])	675 lb Steer CPH ($/cwt[2])	Cost of Gain ($/lb[3])	Est. Net Returns ($/Head[4])
1993	86.80	85.18	0.33	$37.02
1994	72.64	75.72	0.29	$56.39
1995	60.36	66.07	0.37	$49.35
1996	55.24	65.18	0.36	$72.41
1997	77.62	78.80	0.35	$42.21
1998	70.24	69.00	0.31	$22.73
1999	82.76	87.74	0.27	$83.14
2000	90.38	91.42	0.28	$64.90
2001	83.56	84.01	0.29	$52.15
2002	79.62	84.15	0.32	$70.58
2003	98.46	102.72	0.33	$89.56
2004	110.63	103.58	0.27	$36.62
2005	115.82	117.82	0.27	$100.34
2006	108.01	95.48	0.37	(-$17.33)
2007	103.93	102.81	0.47	$40.04
2008	91.70	83.04	0.43	(-$19.46)
2009	90.80	89.82	0.36	$35.98
Average	86.97	87.21	0.33	$48.04

[1] KDA state average for LM1 steers 500-599 lb in October.
[2] Average price for all LM1-2 steers 600-699 lb sold in Guthrie/Hopkinsville December CPH-45 sale.
[3] Average estimated feed cost of gain October – November. Rations formulated for 2.7 lb average daily gain. Rations: (1993-2004) Corn – 8 lb; SBM – 1.5 lb; Hay – 5.5 lb (2005-09) Soyhulls – 7.4 lb; Corn Gluten – 3.6 lb; Hay – 4.0 lb (2005-09) Soyhulls – 9 lb; DDGS – 2 lb; Hay – 4.0 lb
[4] Estimated Net Added Returns = ((CPH-45 value – commission (3%/$3/head)) – feed cost, veterinary/sale tags ($12-$15/head), interest (6-9%), (October value – commission).

Implications

The KY CPH-45 program has a 30-year history of providing a viable marketing option for Kentucky cattle producers. Summary data in this report shows that 95% of the time producers have received prices above the state average for their cattle. Data also suggests that when the health and management protocol of CPH-45 are coupled with a proper feeding program and an established sale site, net added returns should be realized nearly 90% of the time.

Adequacy of Refrigeration Units for Storing Animal Health Vaccines

K.B. Knight, W.R. Burris, and K.M. Laurent

Summary

Forty refrigeration units across the state of Kentucky that were being used to store cattle vaccines were monitored with data-loggers to record internal temperatures. Only 61% of the units tested were maintaining the temperature in the desired range (35 to 45°F). Almost a third (31%) of the units were frequently below the desired temperature range. Refrigeration units that are used to store vaccines should be used solely for that purpose. Internal temperature should be adjusted and closely monitored so that it stays in the desired range.

Introduction

One of the most important factors in storing animal health products is proper refrigeration. Drug manufacturers are responsible for ensuring that all vaccines are stored at proper temperatures at all times during the manufacturing and delivering of product, but after the product is purchased it becomes the producer's responsibility to keep that product at the correct temperature. The Animal and Plant Health Inspection Service (APHIS) requires that all vaccines be kept refrigerated at between 35˚ and 45˚F. When vaccines are stored at improper temperatures, the vaccines should be removed and discarded because the effectiveness of the product has been compromised. Vaccines that are stored at <35˚F are considered to be more damaging than vaccines stored at >45˚F. When vaccines are stored at below recommended temperatures it causes the antigens to begin separating from the adjuvant. Typically, producers are very good at keeping their vaccines refrigerated after purchase. However, many of the refrigeration units are often older models that have been moved to the barn or working areas, and they may not keep the inside temperatures at the recommended range. Different environments and conditions can cause a refrigerator to either over-cool the vaccine or fail to keep it cool enough, potentially rendering the product ineffective.

Table 1. Average temperature (°F) of refrigeration units tested in Kentucky.

Average Temp[a]	35˚-45˚	Below 35˚	Above 45˚	Below 32˚	Average Low Temp	Average High Temp
37.5˚	64%	31%	4.9%	13.9%	33.6˚	43˚

[a] *Desired temperature range is between 35 and 45°F.*

Materials and Methods

Forty WatchDog® data-loggers were used (Spectrum Technologies, Inc.,. Plainfield, IL) to record temperatures in refrigeration units that contained vaccines. Each data-logger was programmed to record the internal temperature of the refrigerator every 10 min for up to 55 days. The data-loggers were sent to county extension agents and extension associates, who placed them in refrigerators of area producers, retail companies, and veterinary clinics. Once the data-logger had been placed in the desired location, it remained there for the minimum of 49 hours before being removed. A survey was conducted for each refrigerator with information concerning location, type (side by side, freezer on top, etc.), age, number of products in refrigerator, number of expired vaccines, number of opened vaccines, types of human food/drink in refrigerator, other types of species vaccines, and any rabies vaccines.

Results and Discussion

The results of this demonstration are shown in Table 1. Data were obtained from 40 refrigerators throughout Kentucky, and only 64% of those tested were cooling in the correct temperature range (35˚-45˚F). The types of refrigerators that were tested included side by side, freezer on top, freezer on bottom, walk-in, and front glass. Of the refrigerators that were not cooling at recommended temperatures, 4.9% of them were too warm (over 45˚F), and 31% were too cold (below 35˚F). Colder temperatures have the potential to cause the most damage to the vaccines. In units that were too cold, 13.9% were at or near freezing.

Implications

Producers should place a thermometer in the refrigerator and periodically check temperature levels when vaccines are present. Units should be immediately adjusted to operate in the desired temperature range (35-45°F). Producers should dispose of any vaccines that have been stored at improper temperatures. Appropriate storage and administration of vaccines is necessary to ensure that cattle are properly immunized.

By-product Feeds for Postweaning Beef Calves

W.R. Burris, J.H. Randolph, K.M. Laurent, and J.W. Lehmkuhler

Summary

Two feeding trials were conducted with weaned calves with different by-product feeds and varying levels of intake. Calves fed combinations of soyhulls with either corn gluten feed, dried distiller grains, or modified distillers grains to attain a level of 15.2% crude protein gained similarly: 3.5, 3.7 or 3.4 lb/hd/day, respectively. In trial 2, calves fed the soyhull-corn gluten feed diet at 1.5, 2.0, 2.5% bodyweight or *ad libitum* intake gained 2.25, 2.58, 3.09, and 3.56 lb/hd/day, respectively.

All diets and all levels of intake used in this trial elicited acceptable levels of performance for post-weaning feeding of farm-raised calves.

Introduction

Home-raised calves can make rapid and efficient gains during short post-weaning periods. By-product feeds such as soyhulls and distillers grains are readily available and generally less expensive than some commercial feeds. These and many other by-product feeds can form the basis of diets for weaned calves. However, the relative value of each feed and the level at which to feed them is not clear.

Materials and Methods

Trial 1

Forty-five fall-born calves (24 steers and 21 heifers) averaging about 600 lb were weaned in late May and divided into three groups according to sex and weight. They were immediately assigned to one of three treatments:

1. 67% soyhulls and 33% corn gluten feed
2. 82% soyhulls and 18% dried distillers grains
3. 69% soyhulls and 31% modified distillers grains

Each diet contained 15.2% crude protein. Calves were fed their respective treatment once daily. Calves receiving treatments 1and 2 were fed 18 lb daily, and calves on treatment 3 received 20 lb/day so that dry matter intake was equal. Calves were fed for 51 days.

Trial 2

Thirty-nine spring-born steer calves were weaned in October and placed in drylot for two weeks. Calves were allotted by breed and weight into four groups and received a supplement containing 67% soyhulls and 33% corn gluten feed at (1) 1.5% body weight, (2) 2.0% body weight, (3) 2.5% body weight, or (4)

Table 1. Performance of preconditioning calves fed different by-product diets (51 days).

Item	67% SH 33% CG	82% SH 18 DDGS	69% SH 31% MDG
Calves	15	15	15
Init. wt, lb	607	604	611
Final wt, lb	787	792	784
ADG	3.5	3.7	3.4
Conc. feed intake, lb	18	18	20

ad libitum intake. During the trial, each group of calves was fed in a 4-acre grazed over paddock with free-choice fescue hay. The feeding period was 45 days.

Results and Discussion

Trial 1

Performance of calves that were fed either (1) 67% soyhulls (SH) and 33% corn gluten feed (CGF), (2) 82% SH and dried distillers grains (DDG), or (3) 69% SH and 31% modified distillers grains (MDG) is shown in Table 1. Average daily gain was similar for all treatments, 3.5, 3.7, and 3.4 lb/hd/day for SH/CGF, SH/DDS,and SH/MDG, respectively.

Trial 2

Performance of calves that were fed a mixture of SH/CGF at varying levels of intake is shown in Table 2. Hay intake decreased as concentrate intake increased. Average daily consumption was 9, 12, and 15 lb for the 1.5, 2., and 2.5% BW levels that were hand-fed. Daily consumption was 21.9 lb/hd for calves with *ad libitum* intake. Calf daily gains were 2.25, 2.58, 3.09, and 3.56 lb/day for 1.5, 2.0, 2.5, and *ad libitum*, respectively. Calves tended to be more efficient at the 2.0 and 2.5% BW levels, although all treatments were similar and acceptable. Feed costs per lb of gain was similar for the first three levels (which were hand-fed) and slightly higher for those that received their feed from a feeder.

Table 2. Performance of preconditioning calves at varying levels of feed intake.

	1.5% Body Wt (9 lb Conc.)	2.0% Body Wt (12 lb Conc.)	2.5% Body Wt (15 lb Conc.)	*Ad Libitum* (Self-Fed Conc.)
No. head	10	10	10	9
Avg. start wt[a]	618	603	613	615
Avg. end wt	719	719	752	775
Days on feed	45	45	45	45
Avg. daily gain	2.25	2.58	3.09	3.56
Avg. daily conc.[b]	9	12	15	21.9
Avg. daily hay[c]	6.0	5.1	4.7	2.0
Feed/gain, lb	6.7	6.6	6.4	6.7
Feed cost ¢/day	82.5	98.4	116.3	150.4
Feed cost/lb gain, ¢	37	38	38	42

[a] *Calves were weaned 2 weeks prior to trial. During the trial, calves were fed in a 4-acre, grazed-over paddock.*
[b] *Concentrate was a 2/3 soyhull: 1/3 corn gluten mix valued at $130/T.*
[c] *Hay valued at $80/T.*

Implications

Calves that are retained on the farm of origin and feed for short periods of time can make rapid and efficient gains. By-product feeds like soyhulls, corn gluten feed, and distillers grain can form the basis of their concentrate intake. Performance was very acceptable as long as calves received between 1.5% of their body weight and *ad libitum* intake (3.6% BW). Feed efficiency was somewhat lower with *ad lib* intake but may be a viable option for producers who want to avoid daily hand-feeding and have calves that won't get too fat in a short-term feeding program.

Summary of Kentucky Beef Cattle Performance Data Acquired through the Integrated Resource Management Performance and Financial Records Program

D. Bullock, A. Heenan, and L. Van Rensberg

When keeping track of basic on farm production data such as number of cows exposed to the bull, calves born, birth dates, weaning weights and dates, it is important to be able to integrate this information into a production record-keeping program that can provide producers with a summary of results. These reports can often be used as valuable management and decision-making tools that can aid the producer in culling, selection, and breeding decisions.

Records in any form are encouraged, but to fully benefit from this effort it is necessary to have an easy-to-understand report that can help in management decisions. For over 20 years, Kentucky producers have been utilizing CHAPS (Cow Herd Appraisal and Performance Software) for recording beef production records, and from 2000-2009, these records have been accumulated by the Kentucky Beef IRM Committee. These values are the averages of participating CHAPS herds. The data includes information from Kentucky herds during the period of 2000-2008.

When looking at these values, it is important to keep in mind that pregnancy percentage (Table 1), calving percentage and weaning percentage are based on total number of cows exposed to the bull and will therefore reflect true reproductive efficiency. Replacement rate is also based upon the number of exposed females and is calculated by looking at newly exposed females (purchased or raised) divided by total females exposed. The most important of these values is the weaning percentage. This value reflects how good a job was done in getting the cows bred, having live calves, and keeping those calves alive through weaning. Kentucky's weaning percentage was 88%, which is a very high value. Unfortunately, this value is probably inflated. There are many new producers in the data set, and often only the cows that

had calves the first year get included in the data set. This means that many first-year record keepers have 100% weaning, which is not very likely. Taking this into consideration, the actual value would probably be less than 85%, which is still very respectable.

The numbers of calves born within the first 21, 42, and 63 days of calving season are a summary of aherd's production by 21-day intervals or calving distribution within the calving season. Calving distribution tables can give us a very good indication of how well our genetics matches our management. If you have high percentages born early in the calving season, you are meeting your cattle's nutritional needs. If you have a high percentage born in the middle or even late in the calving season, you are getting enough nutrition to your cattle at the time they need it for rebreeding. This could mean that the genetic potential of your herd is too high for the nutrition you are providing. This will require either reducing the growth and/or milking ability of your herd to better fit the nutrition you are providing or proving better nutrition to fit the needs of your cattle. In this data set, 54% of the cattle are calving in the first 21 days. This is decent but not great. More concerning is that 16% are calving after the first 63 days. Those calves will likely have weaning weights that are much lighter than the average, and their dams will have a difficult time rebreeding.

The actual weaning weight refer to average weights obtained at weaning and is not adjusted. These are the pounds that you are actually selling. However, there are many factors that affect those weights, including age of the calf, age of its dam, and its growth rate. Adjusted 205-day weaning weight has been adjusted for sex of the calf and the dam's age; which gives us a better indicator of the growth potential of the calf. Weaning weights are very important and needed to evaluate differences in mothering ability of the cows and to measure differences in growth potential of calves. To fairly evaluate and compare calves of different sexes and those raised by dams of different ages, it is necessary to adjust individual calf records to a standard basis, thus the 205-day weight adjustment. In the Kentucky data set, steers averaged 548 lb and heifers averaged 507 lb for an overall average of 516 lb. These weights are in the range of what would be expected when raising calves on fescue with some interseeded legumes. We would also expect about a 40-50 lb difference between steers and heifers. When the calves were adjusted to 205 day weights and adjusted for age of dam and sex, their average weight was 521 lb.

One of the most important production values to look at is probably pounds weaned per exposed female. The more open females at calving time, death losses, and low-weight calves, the lower the pounds weaned per exposed female. The more profitable producers tend to have more pounds weaned per exposed female than the lower-profitability group. Our weaning weight per cow exposed was 448 lb.

Table 1. Kentucky average performance information from CHAPS herds (2000-2008).

Standardized Performance Analysis Factors	
Pregnancy %	93
Pregnancy loss %	0.18
Calving %	93
Calf death loss %	4.0
Weaning %	88
Replacement rate %	10.5
Calf death loss based on # born	3.9
Average age at weaning (days)	218
% calves born within 21 days	54
% calves born within 42 days	75
% calves born within 63 days	84
% calves born after 63 days	16
Steers actual weaning weight	548
Heifers actual weaning weight	507
Average actual weaning weight	516
Lb weaned/exposed female	448
Critical success factors:	
Average daily gain (lb)	2.1
Weight per day of age (lb)	2.4
Birth weight	74
Adjusted 205-day wt	521
Cow age	6.4

Based on today's prices, let's assume that calves are worth $1.25 per pound. If you multiply that by the weaned weight per cow exposed, it will indicate how much income was generated per cow exposed for breeding, which gives a value of $560. For most producers, that should cover the cost of maintaining those cows. However, if the price for the calves was only $.85, the income per cow would be only $380.80. It would be difficult for many Kentucky producers to realize a profit under that price structure.

These values give an indication of the average production level of Kentucky producers who participate in the performance records program. These values can be used to compare your operation with those operations but should not be used as a scale to measure your operation. Because of differences in cost of production and other very important economic measures, being a high-producing producer does not ensure high profitability. It is a simple matter of determining how much cost is going into the cow herd compared to how much income is coming back.

Forage Analyses: Survey of Forage Laboratories and Professionals

J.W. Lehmkuhler and E.S. Vanzant

Summary

Surveys of forage testing laboratories and forage/livestock professionals were conducted to determine potential sources of variation in forage quality estimates as well as how users of these services applied information received. Twenty-six laboratories completed the online survey. These laboratories collectively processed more than 320,000 forage samples in 2008. Energy values were calculated using 12 different equations by laboratories, with the Penn State NEL-ADF (52.6%) and OARDC (36.8%) summative equations having the highest frequency of use. Frequency of check sample use varied among laboratories, with most laboratories running a check sample in run while some laboratories did not run any check samples. County-based extension agents were more apt to follow published forage sampling procedures than other classes of professionals. Forage

protein value was deemed the most important constituent, followed by energy for agents, researcher/teaching professionals, state specialists, and consultants, while regional specialists put energy above protein. Custom spreadsheets were the most commonly utilized tool for balancing ruminant diets, followed by the 2000 Nutrient Requirements of Beef Cattle, National Research Council (NRC). Based on the information collected, it is recommended that the industry and laboratories consider a standard equation ,which is always reported allowing comparisons across regions/laboratories. Further, use of published forage sampling procedures should be followed as often as possible to ensure a representative sample is collected for analysis.

Introduction

Forages provide the foundation for the beef industry in the southeast region of the United States. The ability to match animal nutrient needs with nutrient supply is critical in minimizing purchased feed inputs and improving overall profitability of the operations. Few beef operations sample forage for nutrient analyses. Furthermore, those that obtain forage analyses are not well informed on how to make use of the information. This results in a quick departure from this tool and a need for educational programs.

Today's rapid technological advances have afforded the beef producer with a vast amount of data and information often exceeding the producer's ability to process all the information and obtain value from the knowledge. Forage testing is not exempt from this phenomenon. Advances in technology such as near infrared spectroscopy (NIRS) and inductively coupled plasma (ICP) spectrometry have increased laboratories capacities for analyzing forages and feedstuffs. Yet the question remains as to what information is the most critical and how to best determine these values.

Knowledge regarding the digestibility of forage is essential in evaluating forage quality and predicting animal performance. Figure 1 illustrates the predicted daily gain data for increasing total digestible nutrient (TDN) concentration in the diet. On average, a single point change in estimated TDN would translate into an expected difference in ADG of over 0.09 lb/d. However, direct measurement of forage digestibility requires intensive procedures and is expensive. Thus, indirect methods are required to speed up laboratory throughput, reduce costs, and improve overall efficiency. The use of NIRS has become widely accepted for forage quality analyses. Development of predictive equations for the composition as well as digestibility estimates allow for the required throughput and efficiency.

We were interested in procedures laboratories are utilizing for forage digestibility estimates as well as how professionals may use this information. Web-based survey instruments were developed and utilized to gather information related to this topic.

Materials and Methods

Certified Forage Testing Labs

A web-based survey instrument was developed using Zoomerang®. Contact information for certified forage testing laboratories was gathered. A link to the survey tool was emailed to 52 laboratories. Follow-up reminders were sent twice to the laboratories. A total of 26 laboratories completed the online survey. The majority (19) were commercial laboratories, while seven of the respondents were university-based laboratories. Data were exported to an Excel spreadsheet for summarization.

Professionals/Specialists

An email was circulated by the department head using the listserv containing the email contacts for the department heads/chairs of animal/range science departments. In this message a link was embedded for accessing the web-based questionnaire. Additionally, a targeted email with the web link was sent to 27 beef specialists across 20 states. Another direct mailing was conducted targeting beef and dairy consultants. A total of 37 consultants were emailed the link for the online survey. Again, the results were exported to a spreadsheet for summarization. Two email requests were sent to the presiding president of the National Association of County Agricultural Agents to disseminate the web link to members. An additional 80 agents across 11 states were also directly emailed the link. A direct mailing was also performed to agricultural extension agents within the Cooperative Extension Service in Kentucky.

Results and Discussion

Laboratories

A total of 26 laboratories completed the online web questionnaire. These laboratories represented in excess of 320,000 samples processed during 2008, with the number samples being processed ranging from 100 to 119,000. Of these laboratories, three provided strictly NIRS analyses, while the remaining provided wet chemistry or a combination of wet chemistry and NIRS.

Laboratories used a variety of equations to express the energy value of forages (Figure 2). The predominant equations being utilized were the Pennsylvania State Net Energy for Lactation (NEl) equation based on ADF and the recent Dairy NRC equation. Why is this of interest? Based on information from the National Forage Testing Association, which provides certification for forage testing laboratories, there is a range in predicted TDN% of 6.6%-8.4% depending on which equation is utilized. Reviewing Figure 1, this range in TDN can have a substantial impact on predicting performance from a particular forage and more importantly on determining the economic value of a forage.

Laboratories used a variety of techniques for determining forage dry matter and/or Neutral Detergent Fiber (NDF) digestibility. Use of glassware (beakers/flasks) in a water bath was the most common technique (six responses), with just over 191,000 samples being analyzed in using this approach. The Ankom system was the second most common technique (four responses), reported with slightly less than 33,000 samples analyzed using the system. Additionally, the most frequently requested time point for reporting digestibility estimates was 30 hr, followed by 48 hr. Over 200,000 samples had digestibility estimates reported at 30 hr, whereas approximately 57,000 samples were reported at 48 hr.

Quality control is important for all laboratories. Frequency of check sample analysis analyzed varied, but most laboratories

analyzed check samples with every run. Ideally, 100% of laboratories would analyze check samples with some frequency. However, it was noted that three of the 26 laboratories completing the survey did not run any additional check samples other than those required for passing certification.

Specialist and Agent Responses

A total of 70 respondents categorized as "specialists" completed the survey, while 26 county-based agricultural agents responded to the request.

Specialists were relatively evenly distributed across the regional specialist (n=19), state specialist (n=26), and research/teaching faculty (n=21), with only four being industry consultants. The specialists spent the majority of their time consulting with beef cow-calf, stocker, and feedlot operations. The majority had 20+ years of experience, while eight specialist respondents had one to five years of experience. The distribution of forage samples submitted to laboratories for analysis varied. Twenty-three respondents submitted less than 20 samples annually, 19 indicated they submitted 21-50, 10 submitted 51-100, and 15 reported submitting more than 100 forage samples for analyses.

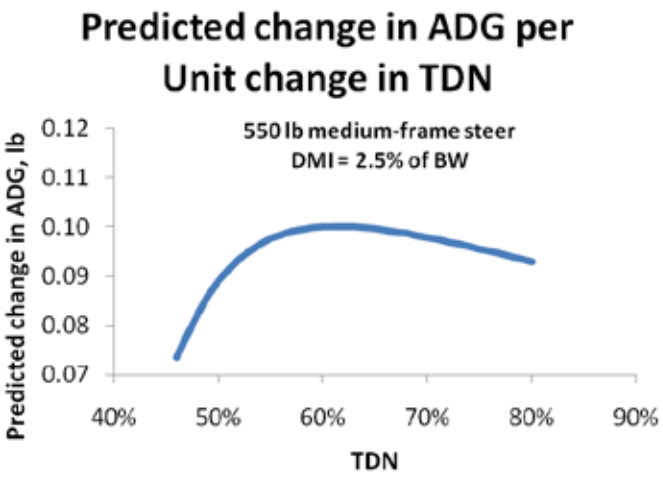
County-based agents were similar in distribution as specialists, with the majority have more than 20 years of experience. The majority indicated spending the majority of their time consulting with beef cow-calf operations. Agents submitted fewer samples than specialists, with the majority of agents submitting fewer than 20 samples annually.

Agents were more likely to sample forages following published sampling techniques than were others. Agents also indicated using book values or other published values for forage analysis more frequently than others, reflecting the lower sample-submission information.

When selecting a forage testing laboratory, the respondents generally reported their most important criteria as laboratories that provided specific analytic procedures and had established quality control measures and/or per sample cost. Consultation services were also important criteria, as was the geographical location.

When asked what software packages were utilized, users of forage testing laboratories indicated the use of a customized spreadsheet most frequently (n=29), followed closely by the most recent Beef NRC (n=19). Several indicated the information was not entered into a software package (n=14), while other software packages such as Oklahoma's Cowculator, Iowa's BRANDS, Spartan Beef, Dairy NRC, Kansas Balancer/Grower, CowBytes, and the Cornell CNCPS model were used.

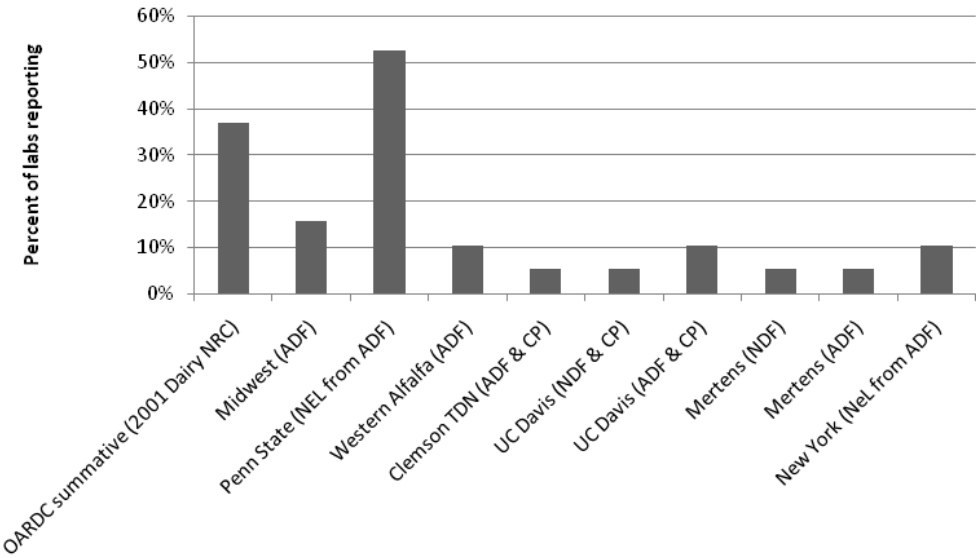
Figure 1. Predicted change in daily gain for a 550 lb feeder calf as the Total Digestible Nutrient (TDN) content of the diet increases.



Implications

There appears to be a need to continue to standardize estimates of forage energy concentrations provided by forage testing laboratories. There needs to be continued improvement by clients and laboratories to reduce the errors associated with sampling and analysis. Check samples, for instance, should be included by all laboratories with every run. Further, the development of a universal energy predication equation for beef cattle would allow for improved animal performance predications and value assessment of forages.

Figure 2. Energy equation reported by 26 laboratories conducting forage analyses in 2008.



Forage/Management Systems for Cow-calf Production

W.R. Burris, L. Anderson, J.H. Randolph, D. Bullock, and J.W. Lehmkuhler

Summary

Calves born in the fall were lighter at birth and had heavier actual weaning weights than calves born in the spring. Fall calves that received creep feed were heavier than those that did not receive creep feed. Spring-born calves on the high-endophyte, continuous grazing system were weaned earlier than calves from rotational grazing systems due to diminishing forage supply, but weaning weights were similar for all spring systems.

Fall-calving cows required more feed than spring calving cows. Among the spring-calving systems, the continuous grazing system required the most feed. Body condition scores were favorable for cows on all systems, indicating an adequate nutritional status for all groups under the conditions (winter feeding and stocking rate) of this three-year study.

Introduction

Fescue forms the base of most grazing systems in middle eastern half of the United States (Burns and Chamblee, 1979; Poor et al, 2000). Most of this fescue is infected with the endophyte *Neotyphodium coenophialum,* which can reduce cattle performance. Fescue can be stockpiled for winter grazing but sometimes undergoes near-dormancy (summer slump) in July and August.

Beef producers generally want to graze cattle as much as possible, which minimizes the need for stored forage or other supplemental feed. Warm-season grasses such as bermuda grass can fill in during the summer slump, and fescue, with stockpiling, can extend the winter-grazing period.

The benefits of rotational grazing, summer grasses, and different calving seasons are important considerations in developing grazing and management systems for cow-calf production.

Materials and Methods

Seventy-five cow-calf pairs of predominately Angus breeding were used in a three-year trial to evaluate five different forage/management systems for beef production. Cows were allotted by breed into five groups of 15 and randomly assigned to one of the treatments shown in Table 1.

Table 1. Experimental treatments (forage/management systems).

Treatment	SCH	SRH	SRL	FRH	FRHCr
Calving season	Spring	Spring	Spring	Fall	Fall
Grazing mgt	Continuous	Rotational	Rotational	Rotational	Rotational
Pasture arrangement					
Paddocks	1	6	6	6	6
Fescue, acres	24	20	20	20	24
Bermudagrass-rye	0	4	4	4	0
Total acres	24	24	24	24	24
Endophtye level	High	High	Low	High	High
Creep feed	No	No	No	No	Yes

Table 2. Performance of cattle on different forage/management systems (1.6 acres/cow), 3 years.

	Forage Management System[1]				
Calving season	Spring	Spring	Spring	Fall	Fall
Grazing mgt	Continuous	Rotational	Rotational	Rotational	Rotational
Endophyte level	High	High	Low	High	High
Item creep feed	No	No	No	No	Yes
Cow data, avg.					
Wt @ breeding, lb	1,320	1,283	1,287	1,326	1,276
Timed AI preg. rate, %	50.9a	60.1ab	49.7a	62.5ab	71.4b
Overall pregnancy rate, %	82.4	88.8	93.6	88.6	95.3
Calving loss, %	8.9	11.1	6.7	4.4	8.9
Cow BCS[2], breeding	5.7a	5.3b	5.6a	5.2b	5.3b
Cow BCSd, mid-July	5.6a	5.4a	5.7s	6.1b	6.2b
Supplemental feed, lb/cow					
Hay	2,415	1,840	2,185	3,335	2,875
Corn silage	1,393	1,440	1,387	1,840	1,840
Calf data, avg					
Birth date	Mar 6	Mar 6	Mar 11	Sept 30	Sept 24
Birth wt, lb	85.6a	89.7a	88.1a	80.2b	79.4b
Weaning date	Sept 6	Oct 17	Oct 17	June 1	June 1
ADG, lb	2.30a	2.18b	2.29a	2.16b	2.27a
Adj. 205 day wt, lb	573.1a	555.4ab	572.2a	536.8b	560.5a
Actual wean wt, lb	575.7a	580.3a	590.2a	608.5b	650.3c
Creep feed (soyhulls), lb/hd	- -	- -	- -	- -	821

[1] Means on the same line with different letters (a, b, c) differ (P<.05).
[2] BCS = body condition score: 1 = very poor; 9 = obese.

Cows that were determined to be non-pregnant by ultrasound or had a calf to die were replaced. Cows were artificially inseminated using one round of timed AI, and Angus "clean-up" bulls were placed with the herds for 45 days. Pregnancy (by AI or natural service) was determined using ultrasound technology 45 days after removal of the bulls. Bulls were rotated to a different group after 21 days. Spring-born calves were weaned when pasture conditions deteriorated in the fall. Fall-born calves were weaned on June 1.

Statistical analysis was by the GLM procedure of SAS (Statistical Analysis System) using the model of treatment, year, and treatment*year, with cow age as a covariant.

Results and Discussion

Performance of cows and calves is shown in Table 2. Cows on all treatments had similar body weights of about 1,300 lb. Body condition score of cows at breeding were lowest (P<.05) in the spring cows for cows on the SRH treatments and probably reflects the lower amount of supplement feed received due to longer grazing without supplementation. Fall cows had higher mid-July (P<.05) body condition scores than spring cows simply because they were not lactating at that time.

Pregnancy rates for all treatments were not significant (P<.05). However, there was a trend for an increase in rotational over continuous grazing, low endophyte over high, and fall over spring. Pregnancy rate for timed AI was highest for the FRHCr treatment.

Supplemental feed was greatest among the spring cows for the continuously grazed group (SCH) because one year was a drought year and cows in this treatment group had to be fed hay during the summer. Spring cows in the low-endophyte group (SRL) generally required more hay than the spring-rotational-high endophtye (SRH) group because the low-endophtye fescue became limiting in the fall before the high-endophtye fescue did. Supplemental winter feed for both fall calving groups were higher than spring calving groups.

Calves born in the fall had lower (P< .05) birth weights than those born in the spring by about 8 lb. Calves on the continuous grazing group (SCH) were weaned earlier than the other spring groups but had similar weaning weights. This probably indicates that the other calves did not gain much weight from Sept 6 until Oct 17. Actual weaning weights of calves on the fall treatments were higher (P<.05) than those on the spring treatment, reflecting their increased age at time of weaning. Creep-fed fall calves (FRHCr) were heavier (P<.05) at weaning than FRH calves, although the FRH calves had access to creep-graze winter annuals (Rye). Creep fed calves received 821 pounds of soy hulls and were 42 lb heavier than their creep-grazed contemporaries. Overseeded rye was probably not beneficial, especially to the spring-calving groups. We observed a large flush of growth in the spring and very little growth during the winter.

Table 3 shows a partial budget (only those costs that are different are shown) using the actual results that were observed with these treatments during this three-year period. Results are shown for each group (15 cows and calves) on an annual basis. All "improved" systems gave positive results over the "unimproved" (SCH) group. The spring group on the low-endophyte fescue had the best results of the spring-calving groups. The fall group with creep feed had the greater advantage. These differences were primarily due to lower reproductive rates and the subsequent cost of replacing the non-pregnant cows.

Implications

Improved management systems were advantageous over continuous grazing for cow-calf production. Most of the difference in feed required occurred in one year during a summer drought. In good forage years, there was little difference in calf performance, although reproductive performance tended to be higher for improved systems.

Table 3. Differing costs and income per year for different forage/management systems (15 cows on 24 acres).

	Forage Management System				
Calving season	Spring	Spring	Spring	Fall	Fall
Grazing mgt	Continuous	Rotational	Rotational	Rotational	Rotational
Endophyte level	High	High	Low	High	High
Item creep feed	No	No	No	No	Yes
Income					
Weaned calves[a]	$8,635.50	$8.704.50	$8.853.00	$9,127.50	$9.754.50
Differing costs	$2,000.00	$1,666.67	$1,166.67	$1,166.67	$1,000.00
Replacement cows[b]	$1,671.90	$1,359.00	$1,541.25	$2,289.90	$2,031.15
Feed[b]	- -	- -	- -	- -	615.75
Creep feed[d]	- -	$80.00	$80.00	$80.00	- -
Bermudagrass pasture[e]	- -	$80.00	$80.00	$80.00	- -
Rye[f]	- -	$36.00	$36.00	$36.00	$36.00
Electric fence[g]	$3,671.90	$3,221.67	$2,903.92	$3,652.57	$3,682.90
TOTAL	$4,963.60	$5,482.83	$5,949.08	$5,474.93	$6,071.60
Income minus differing costs	- -	$519.83	985.48	511.33	$1,108.00
Advantage over unimproved system, $/cow	- -	$34.62	65.70	34.09	$73.87

[a] Calves valued @ $1.00 per pound.
[b] Replacement cow cost equals the difference in purchase cost and salvage of cows (1100 - 600 = 500). Cows were replaced whenever they were determined not to be pregnant or lost a calf.
[c] Hay 75 $/T, corn silage 30 $/T.
[d] Creep feed = soyhulls @ 100 $/T.
[e] Bermudagrass = 200 $/acre cost of establishment prorated over 10 years.
[f] Rye = 20 $/acre to overseed.
[g] Electric cross fence 1.50 $/acre/year.
NOTE: This is not a total budget and does not consider all costs of production—only those costs which differ by treatment.

Fall calving systems offer a viable alternative to spring calving. Performance was best for cows on the fall calving system with calves receiving creep feed. Creep grazing calves on cereal rye was a questionable value during this trial.

Stocking rate (1.6 acres per cow) in this trial was adequate for grazing the cattle but did not provide additional hay for winter feeding.

Literature Cited

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3-2011