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Arkansas Animal Science Department Report 2010

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David L. Kreider, Editor

UNIVERSITY OF ARKANSAS
DIVISION OF AGRICULTURE

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ARKANSAS ANIMAL SCIENCE DEPARTMENT REPORT 2010

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INTRODUCTION

Welcome to the 13th edition of *Arkansas Animal Science*. Thanks to the faculty in the Department of Animal Science and especially to Dr. David Kreider who served as editor of this edition. Making this important publication a reality and ensuring the quality that stakeholders have come to expect requires time and effort, which is herein recognized. The evolution of *Arkansas Animal Science* to primarily electronic delivery reflects the speed at which information must be disseminated more so than just the economic realities of printing. Virtually all our extension and research publications are now online as well. While peer-reviewed journals are the ultimate goal for quality research, the time lines for publication and the frequent necessity to combine several trials, limit the utility of journals for early dissemination of results. Stakeholders, researchers, extension faculty and industry professionals need results as quickly as the data are statistically analyzed and determined ready for use. A professional publication such as *Arkansas Animal Science* fills this role. The research described in this report was conducted at the four main experiment stations used by the Department of Animal Science. These are the Arkansas Research and Extension Center at Fayetteville, the Southwest Research and Extension Center at Hope, the Southeast Research and Extension Center at Monticello and the Livestock and Forestry Station at Batesville. Other valuable research and extension work was conducted at numerous private farms across the state. In the modern world of Animal Science, the traditional lines between research and extension programs are increasingly disappearing. This should be apparent as one looks at the authorship of the articles in this publication. Readers are invited to view all programs of the Department of Animal Science at the departmental website at animalscience.uark.edu and the Livestock and Forestry Branch Station website at Batesvillestation.uark.edu. We want to thank the many supporters of our teaching, research and extension programs. Whether providing grants for research and extension, funds for scholarships, supporting educational and extension programs, donating facilities or horses and livestock, these friends are essential to maintaining a quality Animal Science program. We thank each and every one of you on behalf of our faculty, staff, students and stakeholders. We hope you find the research, extension and educational program reported herein to be timely, useful and making a contribution to the field of Animal Science.

Finally, this will be my last introduction to *Arkansas Animal Science*. I am retiring in February after 15 years as Department Head. I appreciate all the work of the faculty and staff and the support our stakeholders have provided in making this a good, productive department. I wish you all the very best.

Sincerely,

Keith Lusby
Department Head

INTERPRETING STATISTICS

Scientists use statistics as a tool to determine which differences among treatments are real (and therefore biologically meaningful) and which differences are probably due to random occurrence (chance) or some other factors not related to the treatment.

Most data will be presented as means or averages of a specific group (usually the treatment). Statements of probability that treatment means differ will be found in most papers in this publication, in tables as well as in the text. These will look like ($P < 0.05$); ($P < 0.01$); or ($P < 0.001$) and mean that the probability (P) that any two treatment means differ entirely due to chance is less than 5, 1, or 0.1%, respectively. Using the example of $P < 0.05$, there is less than a 5% chance that the differences between the two treatment averages are really the same. Statistical differences among means are often indicated in tables by use of superscript letters. Treatments with any letter in common are not different, while treatments with no common letters are. Another way to report means is as mean + standard error (e.g., 9.1 + 1.2). The standard error of the mean (designated SE or SEM) is a measure of how much variation is present in the data—the larger the SE, the more variation. If the difference between two means is less than two times the SE, then the treatments are usually not statistically different from one another. Other authors may report an LSD (least significant difference) value. When the difference between any two means is greater than or equal to the LSD value, then they are statistically different from one another. Another estimate of the amount of variation in a data set that may be used is the coefficient of variation (CV), which is the standard error expressed as a percentage of the mean. Orthogonal contrasts may be used when the interest is in reporting differences between specific combinations of treatments or to determine the type of response to the treatment (i.e., linear, quadratic, cubic, etc.).

Some experiments may report a correlation coefficient (r), which is a measure of the degree of association between two variables. Values can range from –1 to +1. A strong positive correlation (close to +1) between two variables indicates that if one variable has a high value then the other variable is likely to have a high value also. Similarly, low values of one variable tend to be associated with low values of the other variable. In contrast, a strong negative correlation coefficient (close to –1) indicates that high values of one variable tend to be associated with low values of the other variable. A correlation coefficient close to zero indicates that there is not much association between values of the two variables (i.e., the variables are independent). Correlation is merely a measure of association between two variables and does not imply cause and effect.

Other experiments may use similar procedures known as regression analysis to determine treatment differences. The regression coefficient (usually denoted as b) indicates the amount of change in a variable Y for each one unit increase in a variable X. In its simplest form (i.e. linear regression), the regression coefficient is simply the slope of a straight line. A regression equation can be used to predict the value of the dependent variable Y (e.g., performance) given a value of the independent variable X (e.g., treatment). A more complicated procedure, known as multiple regression, can be used to derive an equation that uses several independent variables to predict a single dependent variable. Associated statistics are r^2, the simple coefficient of determination, and R^2, the multiple coefficient of determination. These statistics indicate the proportion of the variation in the dependent variable that can be accounted for by the independent variables. Some authors may report the square root of the Mean Square for Error (RMSE) as an estimate of the standard deviation of the dependent variable.

Genetic studies may report estimates of heritability (h^2) or genetic correlation (r_g). Heritability estimates refer to that portion of the phenotypic variance in a population that is due to heredity. A genetic correlation is a measure of whether or not the same genes are affecting two traits and may vary from –1 to +1.

COMMON ABBREVIATIONS

Abbreviation	Term
ADFI	Average daily feed intake
ADG	Average daily gain
avg	Average
BW	Body weight
cc	Cubic centimeter
cm	Centimeter
CP	Crude protein
CV	Coefficient of variation
cwt	100 pounds
d	Day(s)
DM	Dry matter
DNA	Deoxyribonucleic acid
°C	Degrees Celsius
°F	Degrees Fahrenheit
EPD	Expected progeny difference
F/G	Feed:gain ratio
FSH	Follicle stimulating hormone
ft	Foot or feet
g	Grams(s)
gal	Gallon(s)
h	Hour(s)
in	Inch(es)
IU	International units
kcal	Kilocalories(s)
kg	Kilograms(s)
lb	Pound(s)
L	Liter(s)
LH	Lutenizing hormone
m	Meter(s)
mg	Milligram(s)
Meq	Milliequivalent(s)
Mcg	Microgram(s)
min	Minute(s)
mm	Millimeter(s)
mo	Month(s)
N	Nitrogen
NS	not significant
ng	nanogram(s)
ppb	parts per billion
ppm	parts per million
r	correlation coefficient
r^2	simple coefficient of determination
R^2	multiple coefficient of determination
s	Second(s)
SD	standard deviation
SE	standard error
SEM	standard error of the mean
TDN	total digestible nutrients
wk	week(s)
wt	Weight
yr	year(s)

TABLE OF CONTENTS

Performance by Spring and Fall-Calving Cows Grazing with Full Access, Limited Access, or No Access to Wild-Type Endophtye-Infected Fescue—3-Year Summary

J. Caldwell[1], K. Coffey[1], C. West[2], D. Philipp[1], J. Jennings[1], D. Hubbell, III[1], J. Tucker[1], A. Young[1],
T. Hess, D. Kreider[1], M. Looper[3], M. Popp[4], M. Savin[2], E. Kegley[1], and C. Rosenkrans, Jr[1]

Story in Brief

Replacing *Neotyphodium coenophialum*-infected tall fescue (E+) with a non-toxic endophyte-infected fescue (NE+) has improved cow performance, but producer acceptance has been slow. Our objective was to compare performance by spring (S) and fall-calving (F) cows grazing either E+ or NE+ at different percentages of the total pasture area. Gelbvieh × Angus crossbred cows (n = 178) were stratified by weight and age within calving season and allocated randomly to 1 of 14 groups representing 5 treatments: 1) F on 100% E+ (F100; 3 replications); 2) S on 100% E+ (S100; 3 replications); 3) F on 75% E+ and 25% NE+ (F75; 3 replications); 4) S on 75% E+ and 25% NE+ (S75; 3 replications); and 5) S on 100% NE+ (SNE100; 2 replications). Cows having limited access to NE+ for 4 weeks prior to breeding increased (*P* < 0.05) calving rates from S but not from F. A calving season by NE+ % interaction (*P* < 0.05) was detected for calving rates. Preweaning calf gain, actual weaning weight, ADG, adjusted weaning weight, sale price ($/lb), and calf value at weaning were greater (*P* < 0.05) from F vs. S and from SNE100 vs. S75 except for sale price which was greater (*P* < 0.05) from S75 vs. SNE100. Therefore, a fall-calving season may be more desirable for cows grazing E+, resulting in greater calving rates, cow performance, and calf BW at weaning. Limited access to NE+ may increase calving rates of spring-calving cows.

Introduction

Endophyte-infected tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh; E+] is commonly used in grazing systems in the southeastern USA because of its long-term persistence and summer survival. These superior traits are attributed to the plant being infected with the fungus *Neotyphodium coenophialum* (Bouton et al., 1993). However, this fungus produces toxins that negatively impact animal growth (Nihsen et al., 2004) and reproductive performance (Porter and Thompson, 1992).

One alterative to E+ is to plant tall fescue infected with a new or "Novel" non-toxic endophyte (NE+). Grazing NE+ has improved performance by spring-calving cows compared with E+ (Coffey et al., 2007), but producer acceptance of NE+ is slow. This slow acceptance may be attributed to the expense of planting and the uncertainty of persistence of NE+ in a year-round grazing system. The objective of this study was to compare performance of spring (S) and fall-calving (F) cows grazing E+ or NE+ at different percentages of the total pasture areas.

Experimental Procedures

This study was conducted at the University of Arkansas Livestock and Forestry Research Station near Batesville, Ark. Gelbvieh × Angus crossbred, spring (S) and fall-calving cows (F; n = 178) were stratified by weight and age within calving season and allocated randomly to 1 of 14 groups representing 5 treatments: 1) F on 100% E+ (F100; 3 replications); 2) S on 100% E+ (S100; 3 replications); 3) F on 75% E+ and 25% NE+ (F75; 3 replications); 4) S on 75% E+ and 25% NE+ (S75; 3 replications); 5) S on 100% NE+ (SNE100; 2 replications) starting January 2, 2007. The E+ areas for each replicate of the first 4 treatments and the NE+ area for the SNE100 treatment replicates was approximately 24 acres. Two separate 24-acre NE+ pastures

were divided into 3, 8-acre pastures each. Each of these 8-acre NE+ pastures was assigned randomly to one of either the S75 or F75 replicates. This combination resulted in 24 acres of E+ and 8 acres of NE+ for the S75 and F75 groups. Cows assigned to S75 and F75 treatments grazed E+ until approximately 28 d prior to the start of the breeding season (May 9, 2007, May 13, 2008, and May 12, 2009; November 27, 2007, November 20, 2008, and November 24, 2009) and 28 d prior to weaning (October 18, 2007, October 23, 2008, and October 22, 2009; May 9, 2007, May 14, 2008, and May 12, 2009). Cow/calf pairs were given access to NE+ pastures at this time. The S75 and F75 groups remained on NE+ pasture until available forage was limiting (<1000 lb/acre), and then were returned to their original E+ pasture (late May or early June). Cows assigned to F100, S100, or SNE100 treatments stayed on their assigned pasture throughout the year. Both S and F groups were placed with a bull during their respective 63-d breeding season.

All pastures were grazed using a rotational grazing system. Each of the E+ and SNE100 pastures were subdivided into 6, 4-acre paddocks and stocked at one cow/2.5 acres. Each 8-acre portion of NE+ for F75 and S75 was divided in half and cows rotated within those cells. Hay was harvested from approximately 8 acres from each pasture and was only offered back to the cows on that pasture during adverse weather conditions or when available forage was limiting. No supplemental concentrate was offered to any treatment, and trace mineralized salt was available free choice.

Cow BW and BCS (1 = emaciated, 9 = obese; Wagner et al., 1988) were evaluated at the start of the trial (1099 ± 11.6; 5.5 ± 0.07 average F weight and BCS, respectively; 1152 ± 12.2; 5.8 ± 0.08 average S weight and BCS, respectively) and at the start of each breeding season and at weaning. Calf BW was obtained at birth and at weaning. Four weeks prior to weaning, calves were gathered, vaccinated against 7 *Clostridial* strains, infectious bovine rhinotraceitis (IBR), bovine virus diarrhea (BVD), parainfluenza, bovine respiratory syncytial

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virus (BRSV), *Haemophilus somnus*, and 5 strains of *Leptospira.* Calves were gathered at weaning and re-vaccinated. Calf value was assigned by first estimating the price per pound of each calf. The price per pound was derived using a sliding price scale within calf sex based on Arkansas state average price ranges for the day the calves were weaned. Price per pound was not adjusted for calf appearance. The weaning weight of each calf was then multiplied by the derived market price per pound at weaning to obtain calf value.

Cow and calf performance measurements were analyzed using PROC MIXED of SAS (SAS Inst., Inc., Cary, N.C.) with each group of animals in a specific pasture considered the experimental unit. Planned orthogonal contrasts were used to compare 1) mean of F with the mean of the S, 2) mean of S75 and F75 with the mean of S100 and F100, 3) SNE100 with S75, and 4) interaction between S and F in their response to having 25% of their pasture area as NE+. Calf weaning weights were analyzed both as actual and adjusted 205-d weaning weights. Weaning weights were adjusted for calf age but not for age of cow. Calving rates are reported as a percent of the total number of cows that calved per treatment and were analyzed by Chi-square analysis using the same contrast statements as mentioned above in SAS. Treatment means are reported as least squares means.

Results and Discussion

Cow BW at the start and end of the breeding season and at weaning were greater ($P < 0.05$) from F compared with S and cow weight at weaning was greater ($P \le 0.05$) from S75 and F75 compared with S100 and F100 and from SNE100 compared with S75 (Table 1). Spring-calving cows lost ($P < 0.05$) more weight during the breeding season compared with F, and S75 and F75 lost ($P < 0.05$) more weight during breeding compared with S100 and F100. Cow BW at the end of the breeding season tended to be greater ($P = 0.06$) from SNE100 compared with S75 and cow BW loss during breeding tended to be greater ($P = 0.07$) from S75 compared with SNE100. Cow BCS at the start and end of the breeding season and at weaning were greater ($P < 0.05$) from F compared with S. Also, S lost ($P < 0.05$) BCS during the breeding season while F maintained BCS (-0.5 vs. 0.1 average, respectively). Cow BCS at the start of the breeding season and at weaning were greater ($P < 0.05$) from SNE100 compared with S75. Calving rates were greater ($P < 0.05$) and calving intervals were shorter ($P < 0.05$) from F compared with S. Calving rates were greater ($P < 0.05$) from S75 and F75 compared with S100 and F100 and a calving season by NE+ % interaction ($P < 0.05$) was detected for calving rates. By adding only 25% of the total pasture area as NE+, calving rates increased by 36 percentage units (80 vs. 44) from S75 compared with S100, but by only 1 percentage unit (97 vs. 96) from F75 compared with F100. No difference in calving rate ($P = 0.95$) was detected from S75 compared with SNE100, but calving interval was longer ($P = 0.05$) from S75 compared with SNE100.

Calf birth weight was greater ($P < 0.05$) from S compared with F, but calf weaning age, actual and adjusted weaning weight, calf gain, ADG, sale price, and calf value at weaning were greater ($P < 0.05$) from F compared with S. Calf actual and adjusted weaning weight, calf gain, and ADG tended to be greater ($P \le 0.09$) from S75 and F75 compared with S100 and F100, whereas sale price ($/lb) was greater $P < 0.05$) from S100 and F100 compared with S75 and F75. These factors offset each other so that calf value at weaning was not different from S75 and F75 compared with S100 and F100. Calf actual and adjusted weaning weight, calf gain, ADG, and calf value at weaning were greater ($P < 0.05$) from SNE100 compared with S75 although sale price was greater ($P < 0.05$) from S75 compared with SNE100. Daily gains by calves in this study were comparable across treatments with that previously reported by spring-born calves grazing E+ or NE+ (Coffey et al., 2007).

Therefore, fall-calving cows may have greater performance when grazing E+ compared with spring-calving cows. The performance differences between fall and spring-calving cows may be attributed to lower environmental temperatures and (or) toxin concentrations during critical times of the year when cow production requirements are highest. Furthermore, limited use of NE+ during the grazing season may improve cow weight at weaning, calf weight through weaning, and may increase calving rates of spring-calving cows by offsetting some of the negative impacts associated with grazing E+ during times of the year when tall fescue toxicosis is more severely manifested.

Implications

Based on these results, producers with predominantly E+ pastures may benefit from using fall-calving cows rather than spring-calving cows, resulting in better cow performance and heavier calves with higher value at weaning which would benefit producers selling on a cash market. Limited use of novel endophyte-infected tall fescue may benefit calving rates by spring-calving cows resulting in more pounds of calf to sell from those cows.

Acknowledgements

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Table 1. Performance by spring (S) and fall-calving cows (F) grazing with full access (S100 or F100), limited access (S75 or F75), or no access (SNE100) to toxic wild-type endophyte-infected tall fescue–3-year summary.

Item	F100	F75	SNE100	S100	S75	SEM[a]	Contrast[b]
			Treatments				
Cow weights, lb							
start of breeding	1215	1220	1155	1052	1122	46.5	W
end of breeding	1263	1221	1154	1042	1076	37.7	W,y
at weaning	1111	1172	1179	1067	1108	45.1	W,X,Y
Cow weight change, lb							
during breeding	35.0	-5.0	-8.0	-11.0	-46.0	8.76	W,X,y
Body condition score							
start of breeding	6.1	5.9	6.3	5.7	5.7	0.26	W,Y
end of breeding	6.2	6.0	5.3	5.2	5.1	0.22	W
at weaning	5.5	5.8	5.5	5.2	5.2	0.18	W,Y
Body condition score change							
during breeding	0.1	0.1	-1.0	-0.4	-0.6	0.38	W
Calving rates, %[c]	96	97	80	44	80	—	W,X,Z
Calving interval, d	366	362	364	376	374	5.2	W,Y
Age at weaning, d	233	233	231	228	227	4.0	W
Calf weights, lb							
birth	78	78	82	81	84	2.3	W
at weaning	528	557	581	490	500	22.5	W,x,Y
Adj. weaning weight[d]	473	499	522	449	459	15.8	W,x,Y
Calf gain, lb	450	479	499	409	416	23.8	W,x,Y
Daily gain, lb	1.93	2.05	2.15	1.80	1.83	0.083	W,x,Y
Sale price, $/lb[e]	1.08	1.05	0.95	1.01	0.99	0.054	W,X,Y
Value at weaning, $[f]	572	581	549	490	493	18.2	W,Y

[a]SEM = Pooled standard error of the mean.
[b]Contrasts:
  W = mean of F compared with the mean of S ($P < 0.05$).
  X = mean of S75 and F75 compared with the mean of S100 and F100 ($P < 0.05$).
  Y = mean of SNE100 compared with the mean of S75 ($P < 0.05$).
  Z = the interaction between F and S in their response to having 25% of their pasture area as NE+ ($P < 0.05$).
  Lower case letters represent statistical tendencies ($P \leq 0.10$).
[c]Calving rates represent two years from both the fall-calving and spring-calving cows.
[d]Weaning weights were adjusted for age of calf, but additive factors for age of dam were not used.
[e]Sale price/lb was determined using a sliding scale within calf sex based on the Arkansas average sale price on the actual date calves were weaned.
[f]Weaning value = actual calf weight multiplied by the sale price/lb determined for each individual calf based on a sliding scale.

Post-Weaning Performance by Spring and Fall-Born Steers Weaned from Full Access, Limited Access, or No Access to 'Wild-Type' Endophyte-Infected Tall Fescue Pastures – 2 Year Summary

J. Caldwell[1], K. Coffey[1], D. Kreider[1], D. Philipp[1], D. Hubbell, III[1], J. Tucker[2], A. Young[1],
M. Looper[2], M. Popp[3], M. Savin[4], J. Jennings[1], and C. Rosenkrans, Jr.[1]

Story in Brief

Replacing 'wild-type' endophyte-infected tall fescue (E+) with non-toxic endophyte-infected fescue (NE+) may improve calf BW at weaning, but data addressing those impacts on post-weaning performance are limited. Our objective was to determine to what extent having limited access to NE+ prior to weaning will affect post-weaning performance by spring (S) and fall-born calves (F). Gelbvieh × Angus crossbred cows were used in a study that resulted in 204 steers representing 5 treatments: 1) F on 100% E+ (F100; 3 replications); 2) S on 100% E+ (S100; 3 replications); 3) F on 75% E+ and 25% NE+ (F75; 3 replications); 4) S on 75% E+ and 25% NE+ (S75; 3 replications); and 5) S on 100% NE+ (SNE100; 2 replications). Steer actual and adjusted weaning BW, feedlot gain, age at harvest, dressing percent, and marbling scores were greater ($P < 0.01$) from F vs. S, but BW at shipping to the feedlot, feedlot ADG, ribeye area, and YG were greater ($P < 0.01$) from S vs. F. Steer actual and adjusted weaning BW were greater ($P < 0.05$) from S75 and F75 vs. S100 and F100 steers. Steer actual and adjusted weaning BW, BW at shipping to the feedlot, harvest BW, feedlot ADG, and hot carcass wt. were greater ($P < 0.05$) from SNE100 vs. S75. Therefore, after two years of post-weaning measurements, fall calving may benefit steer BW through weaning, and limited use of NE+ may benefit steer BW through weaning but may not improve performance through the feedlot phase.

Introduction

It is well documented that the'wild-type' endophyte-infected tall fescue (E+) produces toxins that reduce grazing animal performance (Nihsen et al., 2004), but the impact of these toxins on cattle after removal from E+ have been highly variable both in length and severity, making it a concern to mitigate the negative effects of E+ through later stages of production. One alternative to grazing E+ is to graze a non-toxic novel endophyte-infected fescue (NE+) that has improved spring-calving cow performance (Coffey et al., 2007). However, data addressing those impacts on post-weaning performance of calves are limited. Our objective was to compare post-weaning performance by spring (S) and fall-born calves (F) weaned from cows grazing either E+ or NE+ at different percentages of the total pasture area to determine to what extent having limited access to NE+ prior to weaning will affect post-weaning performance.

Experimental Procedures

At the start of the study, 178 Gelbvieh × Angus crossbred spring and fall-calving cows (1128 ± 11.3 lb initial BW) from the cowherd at the University of Arkansas Livestock and Forestry Branch Experimental Station (LFBES) near Batesville, Ark. were stratified by weight and age within calving season and allocated randomly to 1 of 14 groups representing 5 treatments: 1) F on 100% E+ (F100; 3 replications); 2) S on 100% E+ (S100; 3 replications); 3) F on 75% E+ and 25% NE+ (F75; 3 replications); 4) S on 75% E+ and 25% NE+ (S75; 3 replications); 5) S on 100% NE+ (SNE100; 2 replications) starting January 26, 2007. Over 2 years, these cows produced 204

steer calves. Cows assigned to S75 and F75 treatments grazed E+ until approximately 28 d prior to the start of the breeding season [May 9, 2007; May 13, 2008 (spring); November 27, 2007; November 20, 2008 (fall)] and 28 d prior to weaning [October 18, 2007; October, 23 2008 (spring); May 9, 2007; May 14, 2008 (fall)]. At this time, the cows were given access to NE+ pasture to mitigate the negative effects from grazing E+ prior to breeding and weaning. Cows assigned to F100, S100, or SNE100 treatments remained on their assigned pasture throughout the year.

Four weeks prior to weaning, the calves were gathered, weighed, and vaccinated against 7 *Clostridial* strains (Alpha 7; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Mo.), infectious bovine rhinotraceitis (IBR), bovine viral diarrhea virus (BVDV), parainfluenza, bovine respiratory syncytial virus (BRSV), *Haemophilus somnus*, and 5 strains of *Leptospira* (Elite 9-HS; Boehringer Ingelheim Vetmedica, Inc.). Calves were gathered at weaning, weighed, re-vaccinated (Elite 9-HS; Boehringer Ingelheim Vetmedica, Inc.), treated for internal parasites (Cydectin, Fort Dodge Animal Health, Overland Park, Kan.), separated from their dams, commingled, and placed in a drylot for approximately 14 d. During the 14-d weaning period the calves had ad libitum access to medium quality bermudagrass hay and water. Following the 14-d weaning period the F groups were moved to bermudagrass pastures and the S groups were moved to winter annual pastures. The F and S steers remained on either bermudagrass or winter annual pastures, respectively, until they were shipped to Oklahoma State University feedlot and fed a high concentrate diet. No supplemental concentrate was offered to any treatment, and trace mineralized salt was available to the calves free choice prior to entering the feedlot. At the end of the feedlot period, steers were harvested based on visual estimation of backfat thickness (0.50 in)

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at a commercial slaughter facility and carcass data were collected following a 24 to 48-h chill.

Calf performance measurements were analyzed using PROC MIXED of SAS (SAS Inst., Inc., Cary, N.C.) with each group of animals in a specific pasture considered the experimental unit. Planned orthogonal contrasts were used to compare 1) mean of F with the mean of the S (not including SNE100), 2) mean of S75 and F75 with the mean of S100 and F100, 3) S75 with SNE100, and 4) interaction between S and F in their response to having 25% of their pasture area as NE+. Steer weaning weights were analyzed separately as actual and adjusted 205-d weaning weights. Weaning weights were adjusted for calf age but not for age of cow. Percent choice were analyzed with the Chi-square procedure of SAS. Treatment means are reported as least squares means.

Results and Discussion

Steer actual and adjusted weaning BW, feedlot gain, age at harvest, dressing percent, and marbling score were greater ($P < 0.05$) from F compared with S, but BW at shipping to the feedlot, feedlot ADG, ribeye area, and YG were greater ($P < 0.01$) from S compared with F (Table 1). This likely reflects differences in post-weaning forage quality and environmental conditions between grazing bermudagrass during the summer compared with winter annual forages during the winter. Steer actual and adjusted BW at weaning were greater ($P < 0.05$) from F75 and S75 compared with F100 and S100, but these differences were not maintained through subsequent production phases. Actual and adjusted BW at weaning, BW at shipping to the feedlot, harvest BW, feedlot ADG, and hot carcass wt were greater ($P < 0.05$) from SNE100 compared with S75. Backfat thickness did not differ ($P \geq 0.27$) across treatments.

An overall difference ($P < 0.001$) in quality grade distribution was detected (Table 2) across treatments. The percentage of USDA Choice carcasses was greater ($P < 0.05$) from F compared with S and tended to be greater ($P = 0.10$) from F100 and S100 compared with F75 and S75.

Therefore, after two years of a three year study, it appears that fall calving may benefit steer BW at weaning and may improve the number of calves grading choice at harvest. Furthermore, weaning steer calves that have limited access to NE+ during the grazing season may improve steer BW at weaning but those benefits may not persist through the feedlot period.

Implications

Based on these results, producers having predominantly E+ pastures for their cows should consider a fall-calving season if the emphasis is on weaning weights, but availability of other forages should be considered if producers are interested in retained ownership of weaned calves. Optimal benefits beyond weaning may be achieved by avoiding exposure of calves to E+ prior to weaning, but costs of establishment of NE+ must be considered in that decision.

Acknowledgements

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Table 1. Post-weaning performance and carcass measurements by spring (S) and fall-born steer calves (F) weaned from full access (S100 or F100), limited access (S75 or F75), or no access (SNE100) to 'wild-type' endophyte-infected tall fescue pastures.

	Treatments						
Item	F100	F75	SNE100	S100	S75	SEM[a]	Contrasts[b]
Calf BW, lb[c]							
At weaning	555	585	623	483	522	25.2	W,X,Y
Adj. weaning wt.[d]	497	525	557	436	481	16.3	W,X,Y
At shipping[e]	669	682	954	855	864	32.0	W,Y
Harvest weight	1306	1330	1458	1344	1336	34.3	Y
Feedlot gain	637	645	501	488	462	52.4	W
Feedlot ADG	3.4	3.4	4.1	4.0	3.8	0.24	W,Y
Age at harvest, d[f]	585	585	560	556	553	12.1	W
Carcass measurements							
HCW, lb[g]	793	804	867	795	791	23.2	Y
Dressing, %	60.7	60.4	59.4	58.8	59.4	0.66	W
Ribeye area, in^2	13.0	13.2	15.0	14.2	14.3	0.55	W
Backfat, in.	0.47	0.47	0.45	0.47	0.49	0.029	ns
Yield grade	2.7	2.6	2.9	2.8	3.0	0.19	W
Marbling score[h]	458	432	407	388	386	14.4	W

[a]SEM = Pooled standard error of the mean.
[b]Contrasts:
  W = mean of F compared with the mean of S (not including SNE100; $P < 0.05$).
  X = mean of S75 and F75 compared with the mean of S100 and F100 ($P < 0.05$).
  Y = mean of SNE100 compared with the mean of S75 ($P < 0.05$).
  ns = no significant difference.
[c]204 steer calves were used over 2 years.
[d]Weaning weights were adjusted for age of calf, but additive factors for age of dam were not used.
[e]Shipping wt was the wt measured prior to calves being shipped to the OSU feedlot.
[f]Age from birth to harvest.
[g]HCW = Hot carcass weight.
[h]300 = Slight^0, 400 = Small^0.

Table 2. USDA Quality Grade of spring (S) and fall-born steer calves (F) weaned from full access (S100 or F100), limited access (S75 or F75), or no access (SNE100) to 'wild-type' endophyte-infected tall fescue pastures.

	Treatments					
Item	F100	F75	SNE100	S100	S75	Contrast[a]
Quality grade distribution, %						
Choice	81	67	53	39	38	W,x
Select	18	31	47	61	62	W,x

[a] Contrasts:
  W = mean of F compared with the mean of S (not including SNE100; $P < 0.05$).
  x = mean of S75 and F75 compared with the mean of S100 and F100 ($P < 0.10$).

Immune Function Responses of Spring and Fall-Born Calves Weaned from Wild-Type or Novel-Endophyte Infected Tall Fescue

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Story in Brief

Cattle grazing 'wild-type' endophyte-infected tall fescue (E+) may have a number of adverse consequences including reduced immune function. Conversely, non-toxic, novel endophyte-infected fescue (NE+) is shown to mitigate the effects of fescue toxicosis; thereby, enhancing cattle performance. A 3-year study (2007-2009) was conducted using Gelbvieh × Angus calves (484.7 ± 79.6 lb, n = 500) to determine how limited access to NE+ affects immune function in calves weaned from E+ pastures. Prior to weaning, groups of spring (S) and fall-born calves (F) grazed E+ continuously (S100 and F100, respectively), or E+ for the entire year, but grazed NE+ for 28 d just prior to weaning in either the spring or fall (S75 and F75, respectively). Additional spring-born calves also grazed NE+ continuously (SNE100). Starting in 2008, calves (1 fall and 2 spring groups, n = 262 ; subset of the 500 calves) were injected at weaning with phytohemagglutinin (PHA) in the caudal fold underneath the tail head to assess immune response. Number of neutrophils, hemoglobin concentration, and hematocrit were greater ($P < 0.05$), while number of eosinophils and basophils were lower ($P < 0.05$) for F vs. S. Number of lymphocytes and red blood cells were greater ($P < 0.05$) for S75 vs. SNE100. Number of total white blood cells and neutrophils were greater ($P < 0.05$) for S75 and F75 vs. S100 and F100. Serum prolactin concentrations at weaning were increased by removing spring and fall-born calves from E+ 28d before weaning. Within spring-born calves, skinfold thickness was greater ($P < 0.05$) for SNE100 than S100 and S75 at 0, 12, 24, and 48 h after PHA injection, indicating a greater immune response. Therefore, allowing calves temporary access to NE+ prior to weaning may enhance certain aspects of immune function.

Introduction

Cattle grazing wild-type endophyte infected tall fescue (E+) have reduced performance and immune function (Oliver et al., 2000; Rice et al., 1997). Serum copper levels were also reduced in calves grazing E+ (Coffey et al., 1992; Saker et al., 1998), which could contribute to the decreased immune function (Stabel et al., 1993). Stress of weaning compounded with problems associated with consumption of E+ may result in additive stress that affects the animal for a longer period of time (Zavy et al., 1992). Furthermore, feeding novel-endophyte infected tall fescue (NE+) reduced the symptoms of fescue toxicosis and enhanced animal performance (Realini et al., 2005). The purpose of this study was to determine the impact of grazing E+ with limited (~25%) grazing of NE+ in different seasons versus grazing E+ alone on the immune response of calves shortly after weaning.

Experimental Procedures

A total of 500 Gelbvieh × Angus crossbreed spring and fall-born calves at the University of Arkansas Livestock and Forestry Branch Experimental Station near Batesville, AR, were weaned from 1 of 5 cow-calf grazing treatments: 1) F100; fall-born calves grazing 100% E+ 2) S100; spring-born calves grazing 100% E+ 3) F75; fall-born calves grazing 75% E+ and 25% NE+ 4) S75; spring-born calves grazing 75% E+ and 25% NE+ 5) SNE100; spring-born calves grazing 100% NE+. Cow-calf pairs assigned to the F100 and S100 groups grazed E+ throughout the year and those assigned to the SNE100 groups grazed NE+ throughout the year. Cow-calf pairs that were assigned to S75 and F75 groups grazed E+ throughout most of the year, but were moved to NE+ 28 days prior to weaning to potentially reduce the negative effects of E+. All pastures were grazed using a rotational grazing system.

Calves were gathered 4 weeks before weaning, weighed, and vaccinated against 7 clostridial strains, infectious bovine rhinotraceitis, bovine viral diarrhea virus, parainfluenza, bovine respiratory syncytial virus, *Haemophilus somnus*, and 5 strains of *Leptospira*. At weaning (May 10, 2007; October 18, 2007; May 14, 2008; October 23, 2008; May 12, 2009; October 22, 2009), all calves were separated from their dams, received booster vaccinations, and blood samples were collected from the jugular vein into evacuated tubes containing EDTA (Vacutainer, BD Inc., Franklin Lakes, N.J.) to determine total and differential white blood cell (WBC) counts. Starting in 2008, 200 μg of phytohemagglutinin (PHA) in 0.1 ml of phosphate-buffered saline (pH 7.4) was injected intradermally in the caudal fold underneath the tail head of calves at weaning. The site of injection was measured using calipers prior to injection (0 h) and at 6, 12, 24, and 48h after injection. Additional blood samples were collected at 24 and 48h, and 28d post-weaning to determine serum concentration of prolactin (PRL) and cortisol. Calves had ad libitum access to medium quality bermudagrass hay and water for the following 14d.

Statistical analysis were performed by using PROC MIXED of SAS (SAS Institute, Inc. Cary, N.C.) with each group of animals in a pasture considered the experimental unit. Calf response to PHA injection was analyzed using PROC MIXED with time considered a repeated measurement. Pre-planned orthogonal contrasts were used for the blood data to compare: 1) mean of F with mean of S 2) mean of S100 and F100 with the mean of S75 and F75 3) SNE100 with S75 and 4) interaction between spring and fall treatments. Treatment means are reported as least square means.

Results and Discussion

Peripheral of neutrophils ($1 \times 10^3/\mu L$), hemoglobin, and hematocrit were greater ($P < 0.05$), while peripheral eosinophils and basophils were lower ($P < 0.05$) for F vs. S (Table 1). Oliver et al. (2000) reported reduced mean hemoglobin and concentration of eosinophils from calves grazing E+ compared with calves grazing non-toxic

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pastures. Increased eosinophils are associated with response to allergic reactions, parasites, or both, but also could be elevated in response to chronic infections (Abbas, 2009). Lymphocytes and red blood cells were lower ($P < 0.05$) for SNE100 vs. S75. Oliver et al. (2000) reported lower red blood cells from calves that consumed E+ and attributed this to the copper deficient status of those animals. However, this was not the case in this present study since there was no effect of grazing E+ on serum Cu concentrations (Tables 2 and 3) in either spring ($P = 0.80$) or fall-born ($P = 0.60$) calves. Total peripheral WBC and neutrophils were greater ($P < 0.05$) for S75 and F75 vs. S100 and F100. Increased neutrophils in peripheral blood may indicate that animals have a greater occurrence of inflammation, because these inflammatory cells are among the first to migrate toward the site of inflammation (Smith, 1994).

Serum Cu and Zn concentrations were not different ($P > 0.05$) among treatments assigned to spring-born calves (Table 2). Oliver et al. (2000) reported decreased concentrations of serum Cu from cattle grazing E+ pastures, but there was no effect on serum Zn concentrations.

Serum cortisol concentrations (µg/dL) were not affected ($P > 0.05$) by treatments (Tables 2 and 3). Zavy et al. (1992) reported that beef calves experiencing stressors, such as weaning, have increased concentrations of cortisol which have been implicated as a predisposing factor to infectious diseases and reduced immune responses in cattle. Lefcourt and Elsasser (1995) reported an increase in cortisol concentrations measured after 24h for calves that had been separated and weaned from there dams.

Serum PRL concentrations were greater ($P < 0.05$) for SNE100 and S75 than S100 at weaning, 24, and 48 h post-weaning, and greater for SNE100 compared with S75 and S100 at 28 d post-weaning. Within fall-born calves (Table 3), serum PRL concentrations were greater ($P < 0.05$) for F75 than F100 at weaning. Nihsen et al. (2004) observed reduced concentrations of PRL with steers grazing infected endophyte tall fescue (E+) compared with steers grazing NE+. Plocincki et al. (2007) found that PRL can act as a regulator of immunity to parasites. In the present study, PRL concentrations were greater ($P < 0.05$) for calves grazing SNE100, S75 in spring- born calves, and F75 of fall-born calves versus S100 and F100 which suggests the negative effect of E+ on PRL concentrations in these animals, and the benefits of short-term removal from E+ on PRL recovery.

Skinfold thickness was greater ($P < 0.05$) for SNE100 than S100 and S75 at 0h, 24h, and 48h after injection with PHA (Table 4). Within the fall-born calves, skinfold thickness did not differ ($P > 0.05$) among E+ and NE+ treatments (Table 5). Overall, the skinfold thickness measured at the reaction site increased transiently (Day effect $P < 0.01$), likely due to localized recruitment of mononuclear cells, neutrophils, and eosinophils which are considered inflammatory cells of the immune system (Kelley et al., 1982). Swelling of the injected area peaked 6 h post-PHA injection and decreased thereafter (Day effect $P < 0.01$), presumably because the number of inflammatory cells located at the injection site decreased at this time time.

Implications

Grazing toxic endophyte-infected fescue pastures prior to weaning affects white blood cell count, skinfold thickness, and prolactin hormone concentrations. Copper, zinc, and cortisol concentrations were not affected by grazing toxic endophyte-infected fescue prior to weaning. Grazing non-toxic, novel endophyte-infected fescue showed a positive effect on some of the measures of animal health used in this study. Therefore, health disorders observed in calves grazing toxic endophyte-infected fescue pastures could be minimized by grazing non-toxic, novel endophyte-infected fescue.

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Table 1. Hemogram values at weaning from spring and fall-born calves having full, limited, or no access to wild-type toxic endophyte-infected tall fescue - 3-year summary.

Item [2]	F100	F75	SNE100	S100	S75	SEM [3]	Contrasts [4]
WBC, $1 \times 10^3/\mu L$	10.19	10.87	10.22	9.45	10.39	0.57	X
Neutrophils, $1 \times 10^3/\mu L$	4.01	4.35	3.80	3.20	3.90	0.22	W,X
Lymphocytes, $1 \times 10^3/\mu L$	5.18	5.38	4.65	4.62	5.17	0.22	Y
Eosinophils, $1 \times 10^3/\mu L$	0.38	0.47	0.75	0.70	0.55	0.27	W
Basophils, $1 \times 10^3/\mu L$	0.05	0.06	0.40	0.40	0.20	0.16	W
RBC, $1 \times 10^6/\mu L$	10.15	9.90	9.50	9.70	10.20	0.17	Y
Hemoglobin, g/dl	12.74	12.60	10.64	10.13	10.54	0.70	W
HCT, %	35.03	34.26	32.70	30.70	33.20	0.59	W

Treatments [1] span header above F100–S75.

[1] F100 = fall-born calves that grazed wild type endophyte infected tall fescue (E+), F75 = fall-born calves removed from E+ to NE+ 28d prior to weaning, SNE100 = non-toxic novel endophyte-infected tall fescue (NE+), S100 = wild type endophyte infected tall fescue (E+), S75 = spring-born calves that were removed from E+ to NE+ 28d prior to weaning.
[2] WBC = white blood cells, RBC= red blood cells, HTC = hematocrit.
[3] SEM = pooled standard error of the mean.
[4] Contrasts indicated are significantly different ($P < 0.05$):
  W = mean of F compared with mean of S.
  X = mean of S75 and F75 compared with mean of S100 and F100.
  Y = mean of SNE100 compared with mean of S75.
  Z = interaction between F and S response to have 25% of pasture area as NE+.

Table 2. Serum trace mineral and selected hormone concentrations of spring-born calves having full, limited, or no access to wild-type toxic endophyte-infected tall fescue–3-year summary.

Item	SNE100	S100	S75	P	SEM [2]
Cu, ppm					
At weaning	0.67	0.65	0.66	0.80	0.13
Zn, ppm					
At weaning	1.90	1.80	1.90	0.50	0.08
Cortisol, µg/mL					
24 h post-weaning	3.55	3.01	3.24	0.28	8.30
48 h post-weaning	2.38	2.35	2.49	0.70	2.40
Prolactin, µg/mL					
At weaning	3.85 [a]	0.08 [b]	5.20 [a]	0.01	9.80
24 h post-weaning	0.67 [a]	0.05 [b]	1.09 [a]	0.01	2.10
48 h post-weaning	2.01 [a]	0.13 [b]	1.82 [a]	0.02	4.20
28 d post-weaning	6.07 [a]	3.97 [b]	3.71 [b]	0.03	5.60

[1] SNE100 = non-toxic novel endophyte-infected tall fescue (NE+), S100 = wild type endophyte infected tall fescue (E+), S75 = spring-born calves removed from E+ to NE+ for 28d prior to weaning.
[2] SEM= pooled standard error of the mean.
[a,b] = means within a row without a common subscript differ, ($P < 0.05$).

Table 3. Serum trace mineral and selected hormone concentrations of fall-born calves having full or limited access to wild-type toxic endophyte-infected tall fescue.

	Treatments[1]			
Item	F100	F75	P	SEM [2]
Cu, ppm				
At weaning	0.47	0.46	0.60	0.02
Zn, ppm				
At weaning	1.90	1.60	0.24	0.07
Cortisol, μg/mL				
24 h post-weaning	1.55	0.56	0.90	2.60
48 h post-weaning	0.91	1.03	0.50	1.10
Prolactin, μg/mL				
At weaning	1.28 [b]	25.56 [a]	0.02	22.10
24 h post-weaning	1.13	6.30	0.08	11.60
48 h post-weaning	1.37	6.79	0.08	12.30
28 d post-weaning	8.84	12.44	0.10	15.80

[1] F100 = fall calves grazed wild type endophyte infected tall fescue (E+), F75 = fall-born calves that were removed from E+ to NE+ for 28d prior to weaning.
[2] SEM = pooled standard error of the mean.
[a,b] = means within a row without a common subscript differ, ($P < 0.05$).

Table 4. Skin fold thickness measured at different times following injection with phytohemagglutinin (PHA) from spring-born calves having full, limited, or no access to wild-type toxic endophyte-infected tall fescue.

	Treatments[1]				
Item[2]	SNE100	S100	S75	P	SEM[3]
Skinfold thickness, mm					
0 h	5.40 [a]	4.90 [b]	4.70 [b]	0.01	0.11
6 h	7.70	6.70	6.80	0.06	0.34
12 h	7.40 [a]	6.50 [b]	6.40 [b]	0.02	0.61
24 h	6.70 [a]	5.90 [b]	5.80 [b]	0.01	0.20
48 h	5.90 [a]	5.30 [b]	5.30 [b]	0.01	0.21

[1] SNE100 = spring-born calves that grazed non-toxic novel endophyte-infected tall fescue (NE+), S100 = spring-born calves that grazed wild type endophyte infected tall fescue (E+), S75 = spring-born calves removed from E+ to NE+ for 28d prior to weaning.
[2] Times represent the time following interdermal injection with phytohemagglutinin (PHA) at which skinfold thickness was measured.
[3] SEM = pooled standard error of the mean.
[a,b] = means within a row without a common subscript differ, ($P < 0.05$).

Table 5. Skin fold thickness measured at different times following injection with phytohemagglutinin (PHA) from fall-born calves having full or limited access to wild-type toxic endophyte-infected tall fescue.

	Treatments[1]			
Item[2]	F100	F75	P	SEM[3]
Skinfold Thickness, mm				
0 h	5.04	4.77	0.38	0.19
6 h	6.21	6.50	0.49	0.28
12 h	6.16	6.26	0.88	0.35
24 h	5.76	5.76	0.99	0.21
48 h	5.55	5.42	0.73	0.24

[1] F100 = fall-born calves that grazed wild type endophyte infected tall fescue (E+), F75 = fall-born calves removed from E+ to NE+ for 28d prior to weaning.
[2] Times represent the time following interdermal injection with phytohemagglutinin (PHA) at which skinfold thickness was measured.
[3] SEM = pooled standard error of the mean.

In Situ Ruminal Kinetics of Dry Matter and Neutral Detergent Fiber Disappearance for the Biomass Forages Amur Silvergrass and Big Bluestem[1]

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Story in Brief

Alternative strategies, such as grazing, could minimize economic risk associated with biomass production. Minimal research is available that describes the nutritive value of biomass forages, specifically Amur silvergrass (AS; *Miscanthus sacchariflorus* (Maxim.) Benth., proprietary clone Msanag). Four ruminally-cannulated steers were used to determine ruminal *in situ* disappearance kinetics of DM and NDF for AS and 'Hampton' big bluestem (BB; *Andropogon gerardii* Vitman) harvested at vegetative growth stage on three dates (4 June, 8 July, and 31 July) in west-central Arkansas. Crude protein was greater ($P < 0.01$) on 4 June (13.0%) than either 8 July (9.5%) or 31 July (9.5%) for both forages, and similar ($P = 0.84$) for forage type across sampling dates. Neutral detergent fiber was greater ($P = 0.03$) for AS (72.8%) compared to BB (69.3%). A sampling date × forage type interaction influenced ($P < 0.02$) rate (K_d) of DM; rate of disappearance (K_d/h) of DM was slowest for AS harvested on 8 July as well as both forages on 31 July. Effective DM degradability tended to be greater for BB on 8 July (56.5%) and 4 June (55.6%) sampling dates and least for AS on all three sampling dates (mean = 39.1%). As observed for disappearance of DM, K_d for NDF disappearance was slower ($P < 0.02$) for AS than BB. Effective NDF degradability was greater ($P < 0.01$) for BB (42.5%) than AS (28.9%). It appears that AS has adequate nutritive value during the early summer, and livestock grazing of AS could be an alternative to biomass production.

Introduction

In his State of the Union Address in 2006, President Bush specifically targeted alternative sources for ethanol production, specifically switchgrass. Since then, the U. S. Department of Energy had disbursed millions of dollars in grants and funding for biofuel development. Currently, the U. S. produces approximately 750 billion dry pounds per year of biomass from perennial crops. The main uses for these biofuel forages are ethanol production and combustion.

Alternative strategies, such as grazing, could minimize the economic risk associated with biomass production; however, minimal research is available that describes the nutritive value of many of the biomass forages. *Miscanthus* is a C_4 grass native to Asia commonly used as an ornamental in the U.S. (Sanderson and Adler, 2008). Heaton et al. (2004) reported *Miscanthus* could yield twice as much biomass compared with switchgrass. Objectives of this study were to determine ruminal *in situ* disappearance kinetics of dry matter (DM) and neutral detergent fiber (NDF) of Amur Silvergrass (AS; *Miscanthus sacchariflorus* (Maxim.) Benth., proprietary clone Msanag) and 'Hampton' Big Bluestem (BB; *Andropogon gerardii* Vitman).

Experimental Procedures

Experimental Forages. The AS and BB were harvested in the vegetative growth stage on three dates (June 4, July 8, and July 31)

in west-central Arkansas. Samples were randomly taken across three replicates of each forage and oven-dried at 133°F for 48 h, then ground to pass through a 2-mm screen in a Wiley Mill.

In situ procedures. Four ruminally-cannulated steers (752 ± 40 lb) were utilized to evaluate the in situ disappearance kinetics. Steers were adapted (2.25% of BW) to a basal diet of cracked corn and common bermudagrass hay (15:85 ratio of corn:hay) for 10 d prior to the trial and were allowed ad libitum access to fresh water. Approximately 2-ounce (5 g) samples of each forage were weighed and placed into 4 × 8 inch Dacron bags. Dacron bags for each time period were placed in 14 × 18 inch mesh laundry bags and incubated in tepid water (102 °F) for 20 min before being placed in the ventral rumen. Mesh bags were incubated in the rumen for 3, 6, 9, 12, 24, 36, 48, 72, and 96 h. Following removal from the rumen, bags were rinsed in a top-loading washing machine. The 0 hour bags were rinsed immediately following incubation in tepid water for 20 min. After rinsing, samples were dried at 133 °F for 48 h until at a constant weight.

Statistics. Disappearance kinetics was calculated by nonlinear regression of the percentage of DM and NDF remaining on incubation time using the PROC NLIN procedure of SAS. A randomized complete block design with a 2 × 3 factorial arrangement of treatments using the MIXED procedure of SAS with steers representing the blocking term was used to analyze *in situ* disappearance kinetics. Least squares means were separated using PDIFF statement of SAS when protected by a significant ($P < 0.05$) treatment effect. Fraction A was defined as the immediately soluble fraction. Fraction B represented that portion

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of DM that disappeared at a measurable rate, and fraction C was defined as the portion that was undegraded in the rumen. Fraction B and C, and rate of disappearance (K_d) were determined directly from the nonlinear model. Fraction A was calculated as 100 − (B + C); similarly, the potential extent of disappearance was calculated as 100 − C.

Results and Discussion

Forage type did not influence ($P = 0.84$) CP in the current trial (mean CP = 10.6%; Table 1). Crude protein was greater ($P < 0.01$) on 4 June than 8 July and 31 July for both forages. Neutral detergent fiber was greater ($P = 0.03$) for AS compared to BB (Table 1). Acid detergent fiber tended ($P < 0.07$) to be greater for AS than BB across all sampling dates (Table 1). Dietary CP requirements of a 1,200 lb, mid-gestation cow are 6.9% CP (NRC, 1984). With CP of both forages above 9% during the duration of the trial, if dry matter intake is adequate, either forage could meet the CP requirements of cows.

Fraction A of DM was greater ($P < 0.0001$) in BB ($22.1 \pm 0.6\%$) than AS ($18.9 \pm 0.6\%$) and greater on 8 July ($23.4 \pm 0.7\%$) than 4 June ($18.7 \pm 0.7\%$) and 31 July ($19.4 \pm 0.7\%$). A sampling date x forage type interaction tended ($P \leq 0.09$) to influence fractions B and C of DM. A sampling date × forage type interaction influenced ($P < 0.02$) rate (K_d) of DM; rate of disappearance (K_d/h) of DM was slowest for AS harvested on 8 July as well as both forages on 31 July. Rate of DM disappearance was faster for both forages harvested on 4 June (Fig. 1). Effective DM degradability tended ($P = 0.09$) to be lower in AS on all collection dates than BB; BB collected 31 July had lower effective DM degradability than BB collected on 4 June and 8 July (Fig. 2). Effective DM degradability for BB was similar to that reported for bahiagrass

and bermudagrass harvested in June in west central Arkansas (Flores et al., 2006). Effective DM degradability for AS was similar to values reported for more mature bahiagrass and bermudagrass harvested in October (Flores et al., 2006).

Rate of NDF disappearance was slower ($P < 0.02$) for AS than BB (Fig. 3). Effective NDF degradability was lower ($P < 0.01$) in AS than BB in the current trial (Fig. 4). Potential extent of NDF disappearance was influenced ($P < 0.04$) by sampling date × forage type interaction. Potential extent of NDF disappearance was greatest for BB on all three sampling dates and lowest for AS on 8 July and 31 July.

We conclude the rate of disappearance for DM and NDF were slower for AS than BB. Further, the effective DM and NDF degradability were less for AS than BB. One caveat producers should consider with AS is the possibility of rapid spread via rhizomes, and AS could set seed at more southern latitudes.

Implications

It appears that Amur silvergrass has adequate nutritive value during the early summer, and livestock grazing of AS could be an alternative to biomass production. Future research should include grazing studies to determine both plant and animal performance.

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Table 1. Nutritional analysis (DM basis) of Amur silvergrass (AS) and big bluestem (BB) on three harvest dates in west-central Arkansas.

| | Harvest date | | | | | |
| | 4-June | | 8-July | | 31-July | |
Item	AS	BB	AS	BB	AS	BB
Crude protein, %[1]	12.6	13.4	9.1	9.8	9.7	9.3
Neutral detergent fiber, %[2]	72.8	70.9	71.9	67.6	73.8	69.4
Acid detergent fiber, %[3]	35.2	34.4	35.8	31.6	38.3	33.5

[1]Date effect, $P < 0.01$.
[2]Forage effect, $P = 0.03$.
[3]Forage effect, $P < 0.07$.
Pooled SE = CP ± 0.3%; NDF = 0.8%; and ADF = 0.9%.

■ 4-Jun ▧ 8-Jul ☐ 31-Jul
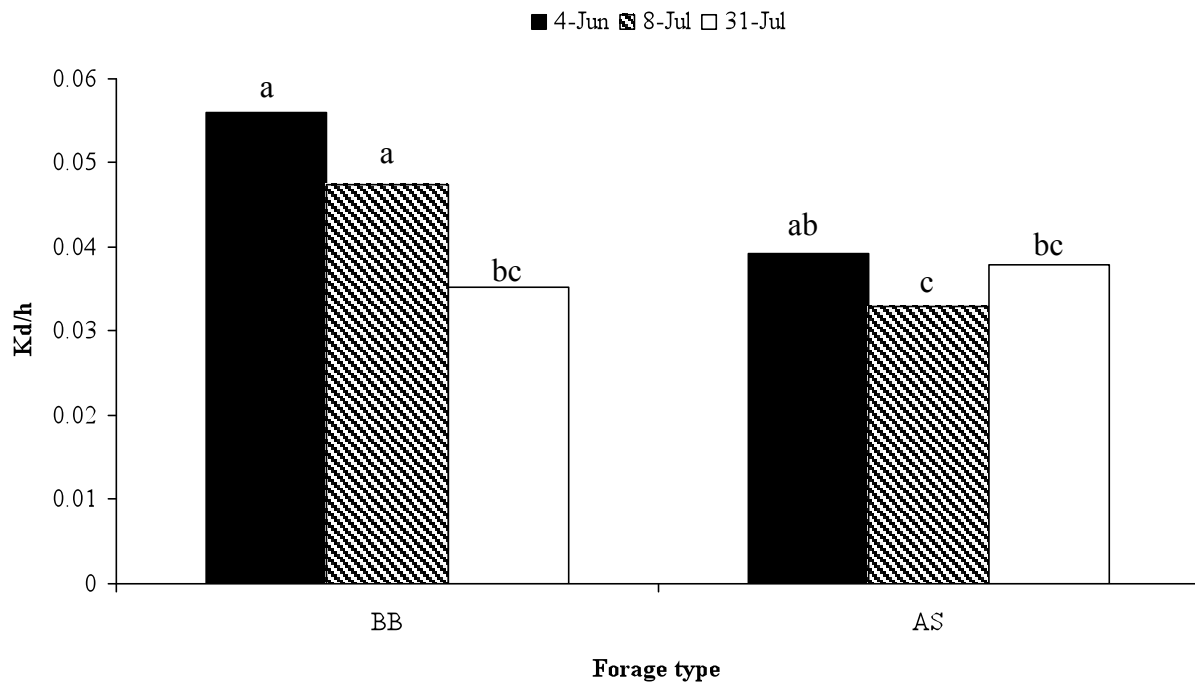
Fig. 1. Rate of DM disappearance of big bluestem (BB) and Amur silvergrass (AS); date × forage interaction; date × forage interaction, [a,b,c]*P* < 0.02.

■ 4-Jun ▧ 8-Jul ☐ 31-Jul

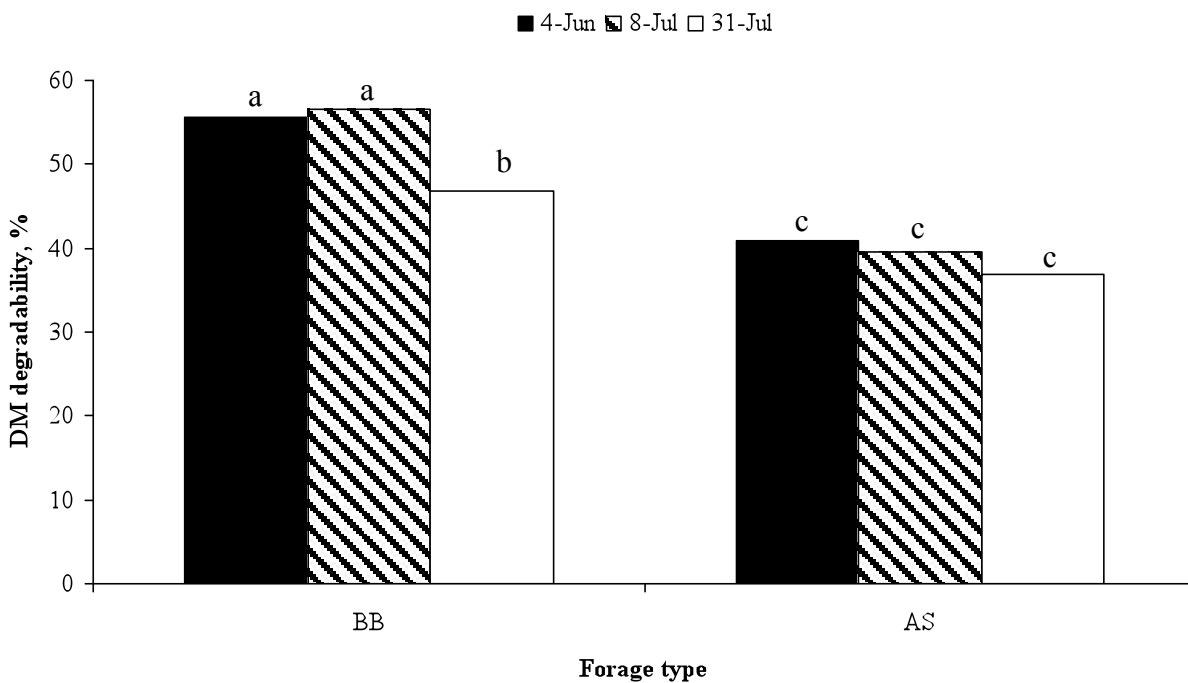**Fig. 2. Effective DM degradability of big bluestem (BB) and Amur silvergrass (AS); date × forage interaction, [a,b,c]*P* = 0.09.**
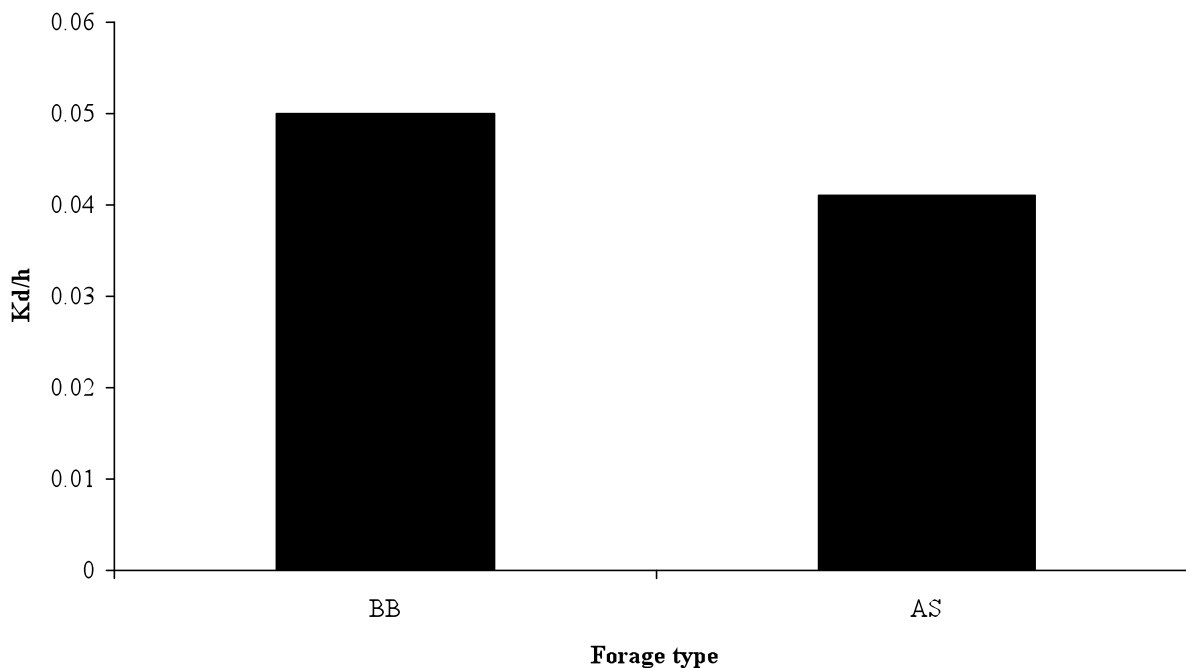
**Fig. 3. Rate of NDF disappearance of big bluestem (BB) and
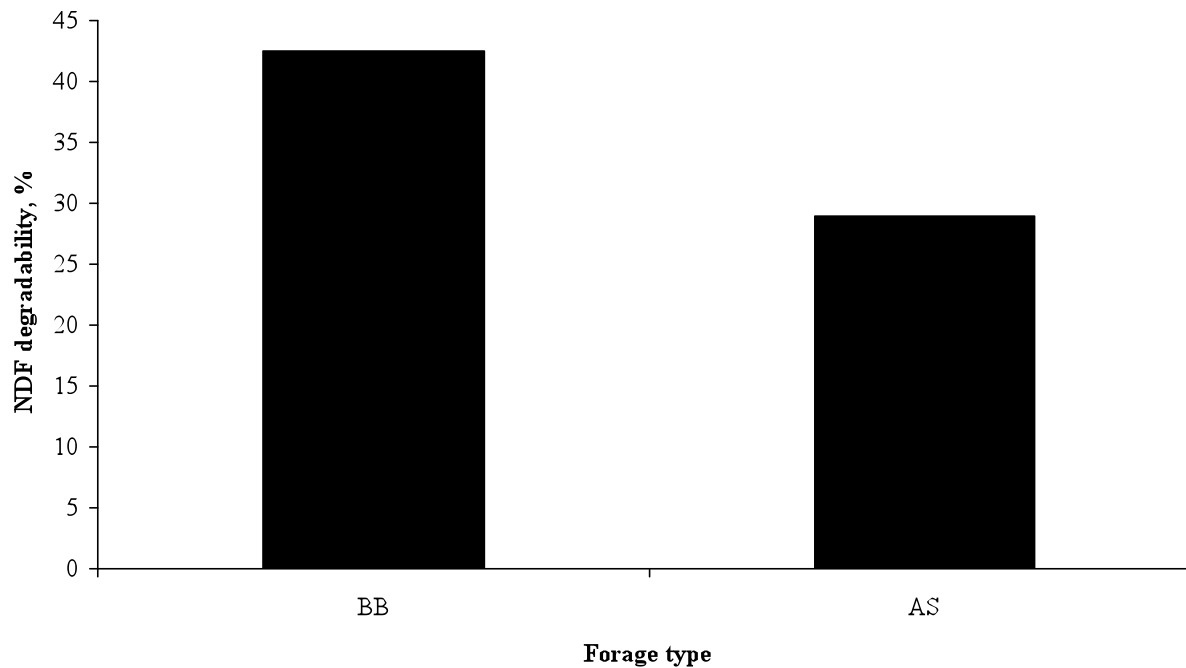Amur silvergrass (AS), *P* < 0.02.**

**Fig. 4. Effective NDF degradability of big bluestem (BB) and
Amur silvergrass (AS), *P* < 0.01.**

Growth Performance by Heifers Grazing Sod-Seeded Annual Ryegrass Pastures Fertilized with Nitrogen or Overseeded with Crimson, Ladino, or both Crimson and Ladino Clovers

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Story in Brief

Interest in substituting legumes in place of nitrogen (N) fertilizer in beef cattle grazing systems has increased with rising fertilizer prices. The objective of this study was to compare forage production and beef cattle gains from annual ryegrass pasture fertilized with N or overseeded with legumes. Gelbvieh × Angus crossbred heifers (n = 40; 535.45 ± 21.41 lb initial BW) were assigned to 1 of 8, 5-acre pastures March 15, 2010. All pastures were overseeded with 'Marshall' annual ryegrass, and were not seeded with any clover (N) or overseeded with 'Dixie' crimson (C), 'Osceola' ladino (L), or a combination of crimson and ladino clover (CL). Both non-clover and clover pastures were fertilized in an attempt to keep grazing days equal. Heifers were not turned out until March 15, 2010 and grazed until May 11. Total BW gain was not significant ($P = 0.99$) among treatments. Also, ADG was not significant ($P = 0.99$). It appears that with an equal number of grazing days, clovers may not be able to totally eliminate the need for fertilizer, but they might be able to reduce the fertilizer requirement.

Introduction

Natural gas is the primary resource used to produce ammonia which, in turn, is either directly applied as a fertilizer or used as a precursor for many common N fertilizers. Therefore, a shift in the price of natural gas will affect ammonia and N fertilizer cost. A report from the Economic Research Service (ERS) stated that ammonia prices paid by farmers increased from $227 per ton to $521 per ton between 2000 and 2006. This is forcing producers to look at alternatives to conventional fertilizer. Legumes have a somewhat unique ability to fix atmospheric N therefore reducing their own need for external N input. The primary goal of this study was to monitor forage and animal production from sod-seeded annual ryegrass either fertilized with N, or overseeded with crimson clover, ladino clover, or both crimson and ladino clover.

Experimental Procedures

Gelbvieh × Angus crossbred spring-born heifers (n = 40; 535.45 ± 21.41 lb initial BW) from the University of Arkansas Livestock and Forestry Research Station near Batesville, Ark. were transported approximately 225 miles to the Southeast Research and Extension Center (SEREC) in Monticello, Ark. Heifers remained as a group upon arrival at SEREC and were put on a dormant bermudagrass pasture and given bermudagrass hay ad libitum. The groups of heifers were then assigned randomly to 1 of 8, 5-acre pastures.

The experimental pastures consisted of common bermudagrass (*Cynodon dactylon*) that was sod-seeded by broadcasting with 30 lb/acre (actual seeding rate) of annual ryegrass (*Lolium multiflorum* cv. Marshall) after a light disking on September 28, 2009. The pastures were dragged to smooth the surface and improve the soil to ryegrass seed contact. After dragging, 2 pastures each were overseeded by broadcasting with either 9.8 lb/acre (pure live seed; PLS) of crimson clover (C; *Trifolium incarnatum* cv. Dixie), 4.5 lb/acre (PLS)[3] of ladino clover (L; *Trifolium repens* cv. Osceola), or both crimson and ladino clover (CL; 9.8 lb and 4.5 lb/acre, respectively). The seeding rates for the ladino and crimson mixed pasture were not halved when combined in order for each species to fully represent its respective grazing period. The remaining 2 pastures received no clover and were fertilized with 300 lb/acre of 19-19-19 on November 9, 2009 (N), while C, L, and CL pastures were fertilized with 300 lb/acre of 6-24-24 on November 9, 2009. On March 5, 2010, 100 lb/acre ammonium nitrate (34 lb/acre actual N) was applied to C, L, and CL, and 180 lb/acre ammonium nitrate (61 lb/acre actual N) was applied to N pastures. Application of 34 lb actual N/acre on C, L, and CL pastures was deemed necessary after the previous year's delay in the beginning of grazing because of reduced forage growth.

Heifers were stocked on their pastures on March 15, 2010 when forage biomass was great enough to begin rotationally grazing. The objective of fertilizing legume plots as well as control plots was to increase the number of grazing days for legume pastures in order to be consistent with control pastures. In 2010, grazing days were possibly affected across all pastures due to heavy use by native wildlife. The heifers stayed on their respective pastures until May 12, 2010. Each pasture was divided in half using temporary electric fencing. Calves were switched between the 2 cells every 14 d after initiation of grazing, and they were weighed every 28 days.

Calf BW, forage production, and species composition data were analyzed using PROC MIXED of SAS (SAS Inst., Inc., Cary, N.C.). The original pasture group was used as the experimental unit for all analyses.

Results and Discussion

Initial BW was not different ($P = 0.99$) among treatments (Table 1). Ending BW was not different ($P = 0.99$) among treatments (Table 1). Total BW gain and ADG ($P = 0.99$ and $P = 0.99$ respectively) did not differ among treatments (Table 1). It would seem that the less intensively fertilized legume pastures were able to produce basically equal results in terms of animal performance to traditionally fertilized

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[3] The white clover seed contained a seed coating. The seeding rate of the actual product was 6.5 lb/acre.

pastures that did not contain any legumes. After the second year of study, the outlook of using clover to reduce nitrogen fertilizer input seems better. Nitrogen fertilizer must still be used but not in as much quantity as in pastures without any legumes. If the combined cost of legume seed, establishment, and fertilizer would be less expensive than buying an increased quantity of N fertilizer without having to establish legumes, it may be beneficial to use legumes in a pasture grazing system.

Implications

Producers may use legumes and a smaller amount of commercial N in a pasture system to obtain similar animal production in comparison to pastures without legumes that were fertilized at a higher rate. However, issues with establishment and persistence should still be taken into consideration when producers consider planting a stand of legumes to lessen N input.

Table 1. Growth performance by heifers grazing sod-seeded annual ryegrass pastures with either no legumes or overseeded with crimson, ladino, or both crimson and ladino clovers spring 2010.

	Nitrogen	Crimson	Ladino	Crimson + Ladino	SEM
Initial wt., lb	531	540	536	535	21.4
End wt., lb	718	725	719	718	24.4
Total study gain, lb	187	184	183	183	14.6
Grazing days/acre	58	58	58	58	
ADG, lbs/day	3.2	3.2	3.1	3.2	0.25

Effect of GnRH Timing Following a Long-Term CIDR-Based Estrous Synchronization Protocol on Estrous Response and Conception Rates in Postpartum Beef Cows

J. G. Powell[1], R.W. Rorie[1], and T. D. Lester[1]

Story in Brief

The objective of this study was to compare the effectiveness of different timing methods of GnRH administration following long-term P4 estrous synchronization. Postpartum Angus-cross beef cows (n = 72) were equally assigned across two treatment groups. Both treatment groups received a P4 vaginal insert (CIDR) for a 14 day period. Treatment group one (TRT 1) was administered GnRH 12 days following the removal of the CIDR insert, and prostaglandin ($PGF_2\alpha$) dosing occurred 7 days post-GnRH treatment. Treatment group two (TRT 2) received GnRH dosing 2 days following the removal of the CIDR insert with $PGF_2\alpha$ dosing occurring 7 days post-GnRH treatment. Cows were observed for estrus for 96 h following $PGF_2\alpha$ dosing, and artificially insemination occurred approximately 12 h after the detection of estrus. A week after the 96 h estrus detection period, all cows were exposed to fertile bulls for 56 days. Three weeks after bull removal, all cows were evaluated for pregnancy status via transrectal ultrasonography. The percentages of cows exhibiting estrus during the 96 h period following $PGF_2\alpha$ administration were similar at 83.3 and 77.8% in TRT 1 and 2, respectively. Average time from $PGF_2\alpha$ administration until onset of estrus was less (P < 0.01) for TRT 2 at 61.8 h compared to 77.9 h for TRT 1. The artificial insemination pregnancy rates were similar at 60% and 71.4% for TRT 1 and 2, respectively. Overall (seasonal) pregnancy rates were also similar at 97.2% and 89% for TRT 1 and 2, respectively. These results indicate that time of GnRH treatment within a long-term P4 estrous synchronization protocol can be altered, reducing the overall length of the protocol by 10 days without loss of treatment effectiveness.

Introduction

Estrous synchronization and artificial insemination allow beef producers to maximize genetic improvement in their operations. However, many beef producers have been reluctant to incorporate this technology into their program. According to data in the National Animal Health Monitoring System report (2008) only 7.2% of beef cattle operations utilize artificial insemination. This reality may be due to the amount of intense management needed for these procedures. Therefore, any improvements to decrease time and labor inputs may increase the use of such technology. Synchronization programs utilizing CIDR inserts, GnRH, and $PGF_2\alpha$ have been shown to be successful for estrous synchronization in postpartum suckled beef cows (Lamb, 2010). However, the total time to complete the synchronization program can be as long as 33 days when utilizing a 14 day CIDR insert. Reducing the time between CIDR removal and GnRH dosing from 12 days to 2 days could shorten the program duration by 10 days and potentially make it more attractive for use by producers. The objective of this experiment was to characterize the response after treatment with a 14-day CIDR insert followed by the GnRH dosing either 2 days or 12 days after CIDR removal followed by a $PGF_2\alpha$ injection 1 week after GnRH dosing in postpartum suckled beef cows.

Experimental Procedures

A study was conducted at the Savoy Beef Cattle Research Unit utilizing fall calving, Angus-cross, post-partum beef cows. Throughout the study, all animals were maintained and cared for in compliance with the University of Arkansas Animal Care and Use Committee Protocol #10014. All cows (n = 72) utilized for the study were randomly and equally distributed into two treatments groups (Fig. 1) based on BCS (5.9 ± 0.3) and days postpartum (43 d ± 10). Treatment 1 (TRT 1, n = 36) cows received a controlled internal drug release insert (EAZI-Breed CIDR®, InterAg, Hamilton, NZ; 1.38g progesterone) on day 0 (November 10, 2009). On d 14 the CIDR was removed, followed by a 100 µg dose of GnRH (Factrel®, Fort Dodge, Fort Dodge, Iowa) on d 26 and a 25 mg $PGF_2\alpha$ (Lutalyse®, Pfizer, Kalamazoo, Mich.) dose on day 33. Treatment 2 (TRT 2, n = 36) cows received a CIDR insert on d 10, with removal of the CIDR on d 24. A GnRH dose was given on d 26, followed by a 25 mg $PGF_2\alpha$ dose on d 33. Cows were equipped with a heat detection patch (Estrotect®, Rockway Inc., Spring Valley, Wis.) at time of $PGF_2\alpha$ dosing.

All cows were observed at least twice daily for onset of estrus during the 96 h period following $PGF_2\alpha$ dosing, and cows were artificially inseminated approximately 12 h after the detection of estrus. Bull semen utilized for artificial insemination was from two Angus sires. Each bull was equally represented within each treatment group. Seven days following the end of the 96 h estrous detection period, all cows were then exposed to fertile Angus bulls for 56 d. Both treatment groups were evaluated for pregnancy status 21 d after the removal of fertile bulls by transrectal ultrasonography (5 MHz transducer, Aloka 500V, Aloka Inc., Tokyo, Japan). Fetal size (crown to rump length) was used to determine which pregnancies resulted from artificial insemination versus the clean-up bulls. Data were analyzed using statistical software (JMP, Version 8, SAS Inc., Cary, N.C.). Pregnancy rates were evaluated using the Chi-Square analysis, and all other data were evaluated by analysis of the variance.

Results and Discussion

Progesterone treatment of 14 d, followed by treatment with $PGF_2\alpha$, 17 to 19 d after progesterone withdrawal, has been shown to be successful for synchronizing estrus in postpartum beef cows. More recent studies have shown that the estrus response is further improved by adding a GnRH treatment about 12 d after progesterone withdrawal to synchronize follicular development in cows prior to $PGF_2\alpha$, treatment. A disadvantage of this synchronization protocol

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is that it requires over 33 d from the time treatment is initiated until estrous detection and insemination. One might expect the synchronization protocol to be equally effective with GnRH administration occurring two days rather than twelve days after progesterone withdrawal because most cows should be exhibiting large preovulatory follicles at that time, and therefore, respond to GnRH treatment. This assumption was the basis for the present study.

Results of estrus response, interval to estrus following $PGF_2\alpha$, and pregnancy rates can be found in Table 1. The percentage of cows exhibiting estrus within the 96 h period following the prostaglandin dosing was similar for both treatments with 83.3% and 77.8% for TRT 1 and TRT 2, respectively. The mean interval from $PGF_2\alpha$ dosing until estrus was detected was less ($P < 0.01$) for TRT 2 at 61.8 h compared to 77.9 h for TRT 1. These results are in agreement with Patterson et al. (2000) who reported about 80% of cows exhibited estrus 48 to 96 h after synchronization, using a similar P4-GnRH-$PGF_2\alpha$ protocol. The AI pregnancy rates were statistically similar, but TRT 2 had a numerical advantage at 71.4% compared to TRT 1 at 60%. In comparison, the study cited above reported pregnancy rates of about 65%. Seasonal pregnancy rates were 97.2% and 89% for TRT 1 and TRT 2, respectively.

In the present study desirable pregnancy rates were achieved regardless of treatment. Both treatment groups responded to synchronization management likely due to cows being in good body condition and being on average 43 days postpartum. These results indicate that time of GnRH treatment within a long-term P4 estrous synchronization protocol can be altered, reducing the overall length of the protocol by 10 days without loss of treatment effectiveness.

Implications

In this study, cows that received GnRH dosing 2 days after the CIDR removal in a 14-d CIDR-Select estrous synchronization protocol had earlier onset of estrus and equivalent estrus detection, AI pregnancy rates and seasonal pregnancy rates compared with cows that received GnRH dosing 10 days after CIDR removal. Reducing the total time of the CIDR-Select program by 10 days increased the convenience and may improve the overall utilization of this protocol.

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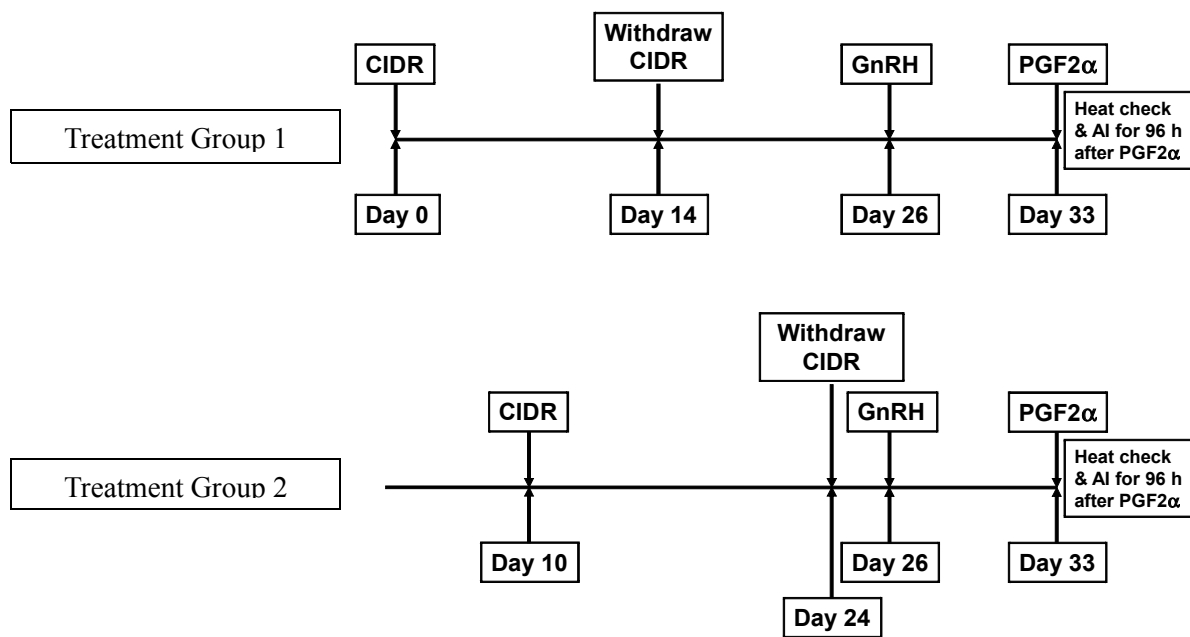
Fig. 1. Experimental protocols for estrous synchronization.

Table 1. Estrus rates, hours to estrus following $PGF_2\alpha$, and pregnancy rates.

	Estrous synchronization treatment		
Item	TRT 1	TRT 2	P value
Cows in estrus	30/36 (83.3%)	28/36 (77.8%)	0.5509
Hours, $PGF_2\alpha$ to estrus	77.9 ± 2.8	61.8 ± 2.9	0.0002
Cows pregnant to AI	18/30 (60.0%)	20/28 (71.4%)	0.3589
Seasonal pregnancy rate	35/36 (97.2%)	32/36 (89.0%)	0.6312

Variation in the Ratio of X- to Y-Chromosome Bearing Spermatozoa in Ejaculates of Semen Collected Weekly from Beef Bulls

P. A. Delgado[1], T. D. Lester[2], and R. W. Rorie[2]

Story in Brief

Previous studies have reported that the ratio of X- to Y-chromosome bearing spermatozoa may be skewed from the expected 1:1 ratio in individual ejaculates of semen. The present study was conducted to confirm the ratio of X- to Y-chromosome bearing spermatozoa varies among individual ejaculates of semen, and to determine any relationship between skewed sex ratio and parameters measured by computer assisted sperm analysis. Semen was collected from six beef bulls weekly for six consecutive weeks. After measure of sperm parameters, sperm DNA was recovered and quantitative real-time PCR was used to determine the ratio of X- to Y-bearing sperm in each ejaculate. Differences were found in the portion of X-bearing sperm among ejaculates from four of the six bulls evaluated. In some bulls, significant negative correlations ($r \geq -0.86$; $P \leq 0.05$) were found between the percentage of X-bearing sperm in an ejaculate and overall, progressive and rapid motility, and sperm track speed. Morphological assessment suggested that the percent live sperm and sperm defects may be negatively correlated ($r = -0.98$; $P < 0.05$) with the portion of X-bearing sperm in an ejaculate. However, additional studies will be needed to confirm the predictive value of sperm motility parameters or morphology in identifying ejaculates of semen with skewed sex ratio. The results do confirm that the sex ratio of semen may be skewed in individual ejaculates of semen. Future research in this area might lead to a practical, cost effective method to control the sex of offspring in livestock species.

Introduction

Sex or gender ratio is defined as the proportion of males to females in a given population. Among various species, factors that have been attributed to altered sex ratio include nutrition, season, disease, gonadotropin and steroid hormone levels, time of insemination, social status, stress, age and parity. Physiologically, an altered sex ratio might be achieved maternally by facilitating or inhibiting the transport of either X- or Y-bearing sperm through the reproductive tract, preferential selection of sperm at fertilization, or sex-specific death of embryos after fertilization (Hardy, 1997).

Another possible factor altering the sex ratio could be altered ratio of X- or Y-bearing sperm in ejaculates of semen. Chandler et al. (1998) found that Y-chromosome bearing spermatozoa in bulls varied from 24 to 84 % between ejaculates, suggesting an epididymal storage effect. The present study was conducted to confirm the findings of Chandler et al. (1998) that the ratio of X- to Y-chromosome bearing spermatozoa may be skewed in individual ejaculates of semen. Also, semen collections were analyzed for sperm morphology and by using computer assisted sperm analysis, to determine if any skewed sex ratio was reflected in measurable sperm parameters.

Experimental Procedures

Semen was collected from six mature, three to ten year old Angus bulls by electro-ejaculation weekly for six weeks. The semen was collected from mid August through September. A Hamilton Thorne Biosciences computer-assisted sperm analysis (CASA) system was used for sperm analysis. Variables measured by CASA were motility (%), progressive motility (%), rapid velocity (%), path velocity (VAP μm/s), velocity straight line (VSL μm/s), track speed (VCL μm/s), lateral amplitude (ALH μm), beat cross frequency (BCF Hz), straightness (STR %), and linearity (LIN %). After measure of sperm parameters, DNA was recovered from sperm in each collection of semen and frozen until being subjected to quantitative real-time PCR

(qRT-PCR) to determine the ratio of X- to Y-bearing sperm in each ejaculate.

The sex ratio of individual ejaculates of semen was determined using qRT-PCR procedures based on those reported by Parati et al. (2006). Forward (5′-CCA CGT CAA GCG ACC CAT-3′) and reverse (5′-AGA GCC ACC TTT CGT CTT CG-3′) primers were used to amplify a 66 bp fragment of the Y chromosome-linked SRY gene. Likewise, X-specific forward (5'-GTT GTG TTA GTT TCT GCT GTA CAA TAA AGT G-3′) and reverse (5′-GAT GGC AGG TGA GGG TAG GA-3′) primers were used to amplify a 96 bp DNA fragment on the intron 2 region of the bovine proteolipid protein gene. All primers were ordered from Invitrogen, while the FAM-labeled internal Y (5′-AAC GCC TTC ATT GTG TGG TCT CGT GA-3′) and X (5′-TGT ATA CAC ATA GCC CCT CCC TCT TGG ACC-3′) TaqMan probes were ordered from Applied Biosystems.

The PCR reaction mix consisted of 12.5 μl 2× Universal Master Mix (Applied Biosystems), 900 nM each of the appropriate forward and reverse primers, 200 nM of the appropriate TaqMan probe and 25 ng of template DNA in a volume of 25 μl. The amplification protocol consisted of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. Within each 96 well reaction plate, X and Y standard curves (1:2 dilutions; 47.5, 23.75, 11.88, 5.94 and 2.97 ng of pooled male DNA) were run in triplicate. All samples were also analyzed in triplicate, in addition the appropriate X or Y- specific primers and probes without DNA template (negative control). For quantification, the Y-specific standard curve was used, with a triplicate sample of 25 ng of DNA pooled from 6 bulls serving as the endogenous control.

A complete random design was used to analyze sex ratio data for ejaculates within bulls, using GLM procedure of Statistical Analysis System Software (SAS Institute, 2003). The Independent factor was collection weeks (1, 2, 3, 4, 5, and 6), the dependent variable was the percentage of X-chromosome bearing in each ejaculate. Differences among X-chromosome content were tested using an orthogonal contrast analysis; means were tested with LSD at 0.05 of α. Pearson

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correlation analysis used to determine any correlations between CASA variables and the ratio of X-chromosome bearing sperm in ejaculates.

Results

The overall mean of X-bearing sperm in ejaculates was 54.7%, with a range from 24.7 to 64.6% over the 6 weeks of semen collections (Table 1). Semen collected from bulls 101 and 305 did not vary in ratio of X- to Y-bearing sperm during the collection period ($P > 0.55$ and $P > 0.44$ respectively). Semen of bull 331 showed a trend ($P = 0.09$) toward skewed sex ratio between week 4 and 6 (60.5% and 49.7%, respectively), which was more pronounced from week 5 to 6 ($P = 0.03$; 64.6.5% and 49.7% respectively). There was a trend ($P = 0.09$) for the portion of X-bearing sperm to differ from week 1 (53.4%) to 4 (62.2%) in ejaculates collected from bull 2410. A difference ($P = 0.02$) also was seen in the ratio of X-bearing sperm in this bull, from week 4 to week 6 (62.2% and 49.1% respectively).

In bull 4202, the percentage of X-bearing sperm tended ($P > 0.16$) to be higher in weeks 2, 3 and 6 than in Week 5. The highest fluctuation of X-bearing sperm was found in bull 4300, varying from 24.7% to 57.2% between weeks 5 and 6; contrast analysis indicated weeks 1, 2, 3, 4, and 6 were different from week 5 of collection in content of X-chromosome ($P < 0.01$).

No significant correlations were found when different combinations of CASA parameters were evaluated across all bulls, so correlation analysis was done within each bull. The sperm parameters measured by CASA were correlated with the percentage of X-bearing, using weeks with similar significance as repetitions for analysis within a specific bull (Table 2). No correlations were found between the ratio of X-bearing sperm in semen and CASA parameters for bulls 101, 305, 4202, and 4300 ($P > 0.31$, $P > 0.11$, $P > 0.71$, and $P > 0.51$ respectively). The parameters MOT, PROG, RAPID, and VCL were correlated ($P = 0.05$, $P = 0.02$, $P = 0.06$, and $P = 0.07$ respectively) with the ratio of X-bearing sperm in ejaculates from bull 331. Other CASA variables (VSL, ALH, BCF, STR, and LIN) were not correlated with the ratio of X-bearing sperm ($P > 0.18$). The portion of X-bearing sperm in ejaculates from bull 2410 was correlated with VCL and ALH parameters ($P = 0.03$ and $P = 0.01$, respectively). The other CASA variables measured (MOT, PROG, RAPID, VSL, BCF, STR, and LIN) did not correlate with ratio of X- bearing in this bull ($P > 0.51$).

Discussion

Chandler et al. (1998) compared the sex ratio of calves born following insemination with different lots of semen collected from the same bulls, and concluded that the ratio of X- to Y-bearing sperm must vary with ejaculates. This study was designed to determine if the ratio of X- to Y-bearing sperm varies with ejaculate, and if so, determine if any variation could be predicted based on either CASA sperm parameters or morphological analysis. The results obtained from qRT-PCR in our experiment showed a sinusoidal trend in portion of X-bearing sperm of ejaculates from most of the bulls that varied at two-week intervals. This finding is similar to the results presented by Chandler et al. (2002) who reported a sinusoidal fluctuation for Y-bearing sperm every 13.5 days when semen was collected weekly. The sperm cycle in bulls is ~13.5 days, so those authors concluded that sex ratio of semen likely varies with the sperm cycle.

There has been speculation for years that Y-bearing sperm may have a more rapid motility than X-bearing sperm. A study utilizing a double swim-up procedure with bull semen has reported that the fraction of Y-bearing sperm is increased in some ejaculates following the procedure (Madrid-Bury et al., 2003). If Y-bearing sperm have more rapid velocity, then some of the sperm velocity parameters are measured by CASA might be useful in identifying a skewed sex ratio in ejaculates of semen. In the current study, sperm track speed (VCL) was found to be correlated to sperm sex ratio in 2 of the bulls evaluated. In these bulls, VCL decreased slightly as the portion of X-bearing sperm increased. Shafer-Somi and Aurich (2007) reported that sperm linearity and VCL were increased by hyperactivation. It would be interesting to investigate whether Y-bearing sperm become hyperactivated earlier that X-bearing sperm, as might be implied from our results and those of Madrid-Bury et al. (2003).

Progressive and rapid motility were both correlated with the portion of X-bearing sperm in one of the bulls evaluated. A study by Zorn et al. (2002) reported that 10 days of abstinence in humans reduces subsequent sperm progressive and rapid motility, and increases the proportion of females born. Across bulls, there was a general trend for progressive and rapid motility to decrease with increasing ratio of X-bearing sperm. Because this trend was significant in only 2 bulls, further study is needed to confirm the usefulness of these parameters in identifying ejaculates with skewed sex ratio.

In summary, these results confirm that the ratio of X- to Y-bearing sperm may be skewed in some ejaculates of bull semen. Further study is needed to determine whether sperm parameters measured by CASA or routine sperm morphology assessment are useful in identifying ejaculates with skewed sex ratio. Hopefully, this research could lead to a practical, cost effective method to control the sex of offspring in livestock species.

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Table 1. Ratio of X-chromosome bearing during six consecutive weekly collections.

Bull	Mean (± SE) percentage of X-bearing sperm by collection week					
	1	2	3	4	5	6
101	52.9 ± 3.9^a	58.1 ± 4.8^a	54.0 ± 3.8^a	53.8 ± 10.1^a	52.2 ± 4.1^a	55.4 ± 6.1^a
305	57.1 ± 3.4^a	$53.5 \pm 2.2\ ^a$	53.0 ± 3.2^a	56.0 ± 2.3^a	55.5 ± 1.5^a	53.8 ± 6.5^a
331	55.4 ± 4.9^{bc}	54.9 ± 6.9^{bc}	54.4 ± 1.2^b	60.5 ± 3.3^{ab}	64.6 ± 2.7^a	49.7 ± 1.2^c
2410	53.4 ± 0.3^b	56.6 ± 4.3^{ab}	57.0 ± 1.3^a	62.2 ± 4.1^a	56.0 ± 3.5^{ab}	49.1 ± 3.3^c
4202	56.9 ± 5.5^{ab}	58.9 ± 5.0^a	57.2 ± 2.9^a	58.0 ± 4.9^{ab}	52.7 ± 0.6^b	62.0 ± 4.2^a
4300	54.8 ± 1.5^a	51.6 ± 5.6^{ab}	51.7 ± 1.6^b	55.2 ± 1.5^a	24.7 ± 1.6^c	57.2 ± 3.1^a

[a,b,c]Means with different superscripts within rows differ ($P < 0.05$).

Table 2. Pearson correlation coefficients of computer-assisted sperm analysis parameters with the portion of X-bearing sperm in ejaculates.

Bull/ sperm analysis parameters	Coefficient of correlation	
	R	Pr > r
101		
No parameters significant	-	>0.31
305		
No parameters significant	-	>0.11
331		
MOT	-0.86**	0.05
PROG	-0.93	0.02
RAPID	-0.86	0.06
VCL	-0.84	0.07
VSL, ALH, BCF, STR, LIN		>0.18
2410		
VCL	-0.89	0.03
ALH	-0.95	0.01
MOT, PROG, RAPID, VSL, BCF, STR LIN	-	>0.34
4202		
No parameters significant	-	>0.71
4300		
No parameters significant	-	>0.51

Note: Data were individually calculated by Pearson correlation analysis; and P-value is the less value calculated in the group of variables.

Effects of Genotype on Body Weight, Cortisol, and Prolactin in Steers Grazing Toxic or Non-Toxic Endophyte-Infected Fescue

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Story in Brief

The aim of this study was to determine the effects of fescue variety (Toxic vs. Non-toxic), and single nucleotide polymorphisms (SNPs) in the prolactin (PRL) promoter region and the coding sequence of cytochrome p450 (C1286T and C994G, respectively). Specifically determined was genotype effect on steer ADG, serum concentrations of cortisol and PRL at 4 time points (d 0, 58, 114, and 176), and gene expression levels of the PRL receptor (sPRLR), chemokine factor interleukin-8 (IL-8), and tumor necrosis factor α (TNFα) at 114 d. A total of 58 steers grazed either toxic (KY-31) or non-toxic (HM4 or MaxQ) cultivars of fescue one month after weaning. Blood was collected at the four time points to determine PRL and cortisol concentrations in serum; RNA transcript levels of the three genes of interest were determined from blood collected on d 114 only. Stocker body weight on d 176, overall ADG, and PRL were less in steers grazing toxic fescue. The SNP genotypes also affected overall ADG; steers homozygous for the minor alleles (TT and GG) had the lowest weight gain. Prolactin and cortisol concentrations were least in steers homozygous for the major allele (CC). On d 58, forage × C1286T tended to influence (P = 0.07) serum PRL, with the lowest concentration in CC steers grazing toxic fescue. Similarly, a main effect of C994G (P = 0.03) was observed; CC had the lowest PRL concentration on d 114. A fescue × C994G interaction (P = 0.02) occurred for cortisol concentration on d 176, with the lowest concentration in CC steer grazing toxic fescue. There were no treatment effects on the expression of sPRLR, IL-8, and TNFα. Our findings agree with reports that steers grazing non-toxic fescue had increased ADG, although animals homozygous for the minor allele in both C1286T and C994G did not perform as well as the other genotypes. Serum cortisol and PRL concentrations were generally lower in steers consuming toxic fescue, and in animals homozygous for the major allele (CC).

Introduction

Symptoms of fescue toxicosis are typically observed in cattle grazing toxic endophyte-infected tall fescue (*Lolium arundinaceum* [Schreb.] S. J. Darbyshire). Ergot alkaloids produced by the endophytic fungus *Neotyphodium coenophialum* appear to cause these symptoms. Non-toxic endophyte strains have been developed to retain plant persistence without the deleterious effect of the toxins. Prolactin (PRL) and cytochrome p450 (CYP450) have been associated with increased tolerance to fescue toxicosis: PRL upregulates expression of heat shock proteins, and is thus related to enhanced tolerance of fescue-induced heat stress; whereas, CYP450 plays a major role in metabolizing toxins such as ergot alkaloids. To adapt to heat stress, animals dissipate heat by panting (increased respiration rates) and sweating (increased skin vaporization). Reduced blood flow may hamper heat dissipation; since cortisol is a hormone that increases blood pressure, its concentration in serum is an indicator of the animal's ability to tolerate heat stress. Similarly, greater serum PRL concentration and higher gene expression of the PRL receptor are proposed to enhance tolerance to fescue toxicosis, and consequently improve animal performance. Decreased serum PRL has been measured after consumption of toxic fescue (Paterson et al., 1995). The short form of bovine PRL receptor (sPRLR) which is encoded by a gene on Chromosome 20q17 (Schuler et al., 1997) interacts with the PRL molecule as a transmembrane receptor. Other genes of interest that may play a role in animal response to fescue toxicosis are key mediators coordinating brain-endocrine-immune responses to infection such as chemokine interleukin-8 (IL-8) and tumor necrosis factor α (TNFα). Therefore, this study was conducted to determine the effects of fescue variety and SNP genotypes on steer weight gain, cortisol and PRL concentrations in serum, and RNA transcripts of sPRLR, IL-8, and TNF-α.

Experimental Procedures

Animals. Angus-sired crossbred steers (n = 58) were weaned (213 ± 20 d of age; initial BW = 566 ± 80 lb) and backgrounded during a 30-d receiving period in preparation for the stocker phase (176 d) of the experiment. During the receiving phase, steers were given free access to clean water, mineral, and bermudagrass pasture.

Genotyping. All steers were genotyped at two single nucleotide polymorphism (SNP) sites: C1286T in the PRL promoter (Looper et al., 2010) and C994G in the cytochrome P450 3A28 (CYP3A28) coding sequence (Larson et al., 2009). Genomic DNA was extracted from the buffy coat of each anticoagulated blood sample and used as a template in polymerase chain reaction (PCR) to amplify DNA segments spanning the SNPs. The PCR primers for PRL promoter (Forward: 5′-AAGTCCCCATAAGCACACTTGG-3′; Reverse: 5′-CTAACTTTAGGGAGTTCATACTG-3′) and CYP3A28 (Forward: 5′-CAACAACATGAATCAGCCAGA-3′; Reverse: 5′-CCTACATTCCTGTGTGTGCAA-3′) amplified 501- and 565-bp DNA fragments, respectively. Following amplification, genotypes were determined by restriction fragment length polymorphism (RFLP) analysis using restriction enzymes *Xba*I and *Alu* I (New England BioLabs, Beverly, Mass.) for C1286T and C994G, respectively.

Stocker Phase. Three tall fescue varieties were stockpiled for winter grazing. In mid-September 2008, all four-acre pastures were clipped to a forage height of about 5 in followed by application of nitrogen

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(50 lb/A). Forage was allowed to grow (not grazed) until steers were assigned to pastures on November 19, 2008. The forages were endophyte-infected toxic tall fescue (KY-31; 4 pastures; 19 steers), novel endophyte-infected non-toxic MaxQ (MaxQ; 4 pastures; 19 steers), and novel endophyte-infected non-toxic HiMag 4 (HM4; 4 pastures; 20 steers). Steers were stratified by weaning weight and SNP genotype, then assigned randomly to treatment pasture.

Blood and Gene Expression Analyses. Steers were weighed and blood sampled at d 0, 58, 114, and 176 to determine serum cortisol and PRL concentrations. Expression of genes that may play a role in the immune response of cattle challenged with fescue toxin was analyzed using blood samples collected at 114 d. Serum concentrations (ng/mL) of cortisol and PRL were determined using validated radioimmunoassay. Total RNA was isolated from buffy coats using RiboPure Blood kit (Life Technologies Corp., Carlsbad, Calif.) to analyze gene expression by quantitative PCR using the StepOne Plus Real-Time PCR System (Life Technologies Corp., Carlsbad, Calif.). The 3 genes of interest (GOI) were sPRLR, IL-8, and TNFα; cyclophilin was used as endogenous control or reference (Ref) gene (Table 1). To calculate RNA transcript levels, threshold (Ct) values for the GOIs were normalized to those of the Ref gene according to the equation: $\Delta Ct = avgCtGOI - avgCtRef$. Once this was done for all repetitions, ΔCt values were grouped according to fescue type (Non-toxic or Toxic). Therefore, two ΔCt values for each gene were determined.

Statistical Analyses. Data were analyzed using pasture as the experimental unit. The statistical model analyzed for main effects of genotype and forage as well as their interaction using MIXED procedure of SAS. Genotype (C1286T and G994C) effects were analyzed separately; interactions between genotypes were not determined due to missing cells. Response variables were steer ADG, serum concentrations of cortisol and PRL, and transcript levels (ΔCt) of sPRLR, IL-8, and TNFα.

Results and Discussion

Performance. Fescue type influenced stocker BW (P = 0.03) and ADG (P = 0.03) on d 176; steers grazing toxic fescue (KY-31) weighed less (676 ± 25.3 lb) than those fed with non-toxic varieties (751 ± 17.8 lb). Overall ADG was reduced when animals grazed toxic (0.81 ± 0.11 lb/d) compared with non-toxic (1.16 ± 0.08 lb/d) fescue. Moreover, genotypes tended to affect (C1286T P = 0.06; C994G P = 0.09) overall ADG, with steers homozygous for the minor alleles having the least ADG compared with the other genotypes. For C1286T genotypes (TT steers), ADG was 0.77 ± 0.11 (Fig. 1a); for C994G, GG steers' ADG was 0.86 ± 0.09 (Fig. 1b).

Serum Cortisol and PRL. Forage type impacted (P = 0.01) PRL concentrations, which were least in steers grazing toxic fescue (Fig. 2). Our findings agree with Thompson and Stuedemann (1993) that serum PRL levels decrease in animals fed with toxic endophyte-infected fescue. Prolactin tended to be affected by a forage × C1286T interaction (P = 0.07) on d 58 as homozygous CC steers grazing toxic fescue had the lowest PRL concentration (5.4 ± 6.4 ng/mL; Fig. 3). A main effect of C994G genotype (P = 0.03) on PRL was observed

on d 114, when PRL concentration was also least in homozygous CC steers (38.7 ± 12.3 ng/mL; Fig. 4). Similarly, cortisol concentrations were influenced by a forage × C994G interaction (P = 0.02), with the lowest concentration (37.3 ± 4.6 ng/mL) in CC steers grazing toxic fescue (Fig. 5).

Decreased serum PRL concentration as a result of fescue toxicosis is well-documented (Paterson et al., 1995) and endophyte-produced toxins in tall fescue have been suggested to possess a dopamine-like activity of inhibiting PRL secretion, resulting in reduced levels of PRL in animals consuming toxic fescue. Another study reported lactating Holstein cows injected with dopamine antagonists had increased cortisol, suggesting that dopamine regulates both cortisol and PRL secretion (Ahmadzadeh et al., 2001). Although the genotypes for both C1286T and C994G seem to influence PRL concentrations, the direct effect of the SNPs on both PRL and cortisol gene products is not fully understood. In the case of C1286T, the SNP occurs in PRL's promoter region, which provides a control point in regulating gene transcription. A change from the major allele (C) resulted in higher serum PRL in animals with the minor allele (CT or TT) compared to those without the SNP. This suggests that C1286T may have increased transcription of the PRL gene, and increased PRL activity may have masked the inhibiting effect of toxins on PRL secretion. This, however, needs to be verified experimentally. In C994G, the SNP resulted in amino acid change from leucine to valine (Leu133Val) (Larson et al., 2009). The effect of this change on CYP3A28 activity and its consequent impact on PRL and cortisol concentrations remain unclear.

Gene Expression of sPRLR, IL-8, and TNFα. There were no treatment effects on gene expression (Fig. 6). However, since serum PRL concentrations varied only for d 58 (Fig. 3), resulting transcript levels for sPRLR may have been different if tested on d 58 instead of d 114.

Implications

Steers grazing novel endophyte-infected, non-toxic tall fescues do not suffer from decreased weight gains associated with fescue toxicosis. Animals homozygous for the minor alleles in both C1286T and C995G did not perform as well as those with other genotypes. Animals homozygous for the major alleles (CC) in both SNPs have decreased cortisol and PRL concentrations on d 58 and d 114, respectively, indicating periods at which steers may be more vulnerable to symptoms associated with fescue toxicosis

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Table 1. Nucleotide sequences of primers and probes used in real-time quantitative polymerase chain reaction.

Gene primer or probe		Sequence (5′ to 3′)	Accession No.
Cyclophilin (peptidyl isomerase A)	Forward	GGTCCTGGCATCTTGTCCAT	AY247029
	Reverse	TGGCAGTGCAAATGAAAAACTG	
	Probe	AATGCTGGCCCCAACACAAATGGTT	
IL-8	Forward	TGCTTTTTTGTTTTCGGTTTTTG	NM_173925.2
	Reverse	AACAGGCACTCGGGAATCCT	
	Probe	TAATCTTGCAACCCTCACCTGCTGGC	
sPRLR	Forward	CCAAAGAACACACGGAGCAA	AF027403
	Reverse	TGCCCCGGCCAGAGT	
	Probe	CGTGAAGCCCATGCACCTGGATC	
TNFα	Forward	ATCAGCCGCATTGCAGTCT	AF348421
	Reverse	CTGTGGCAAGGGCTCTTGA	
	Probe	AGACCAGGGTCAACATCCTGTCTGCC	
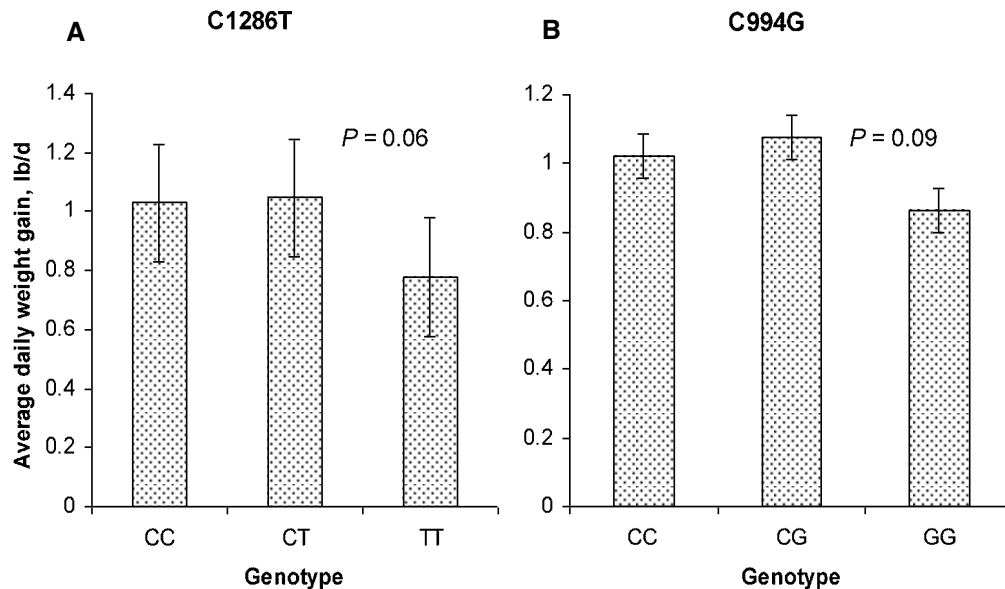
Fig. 1. Average daily gain in steers with different genotypes of C1286T (A) and C994G (B) SNPs regardless of forage grazed.
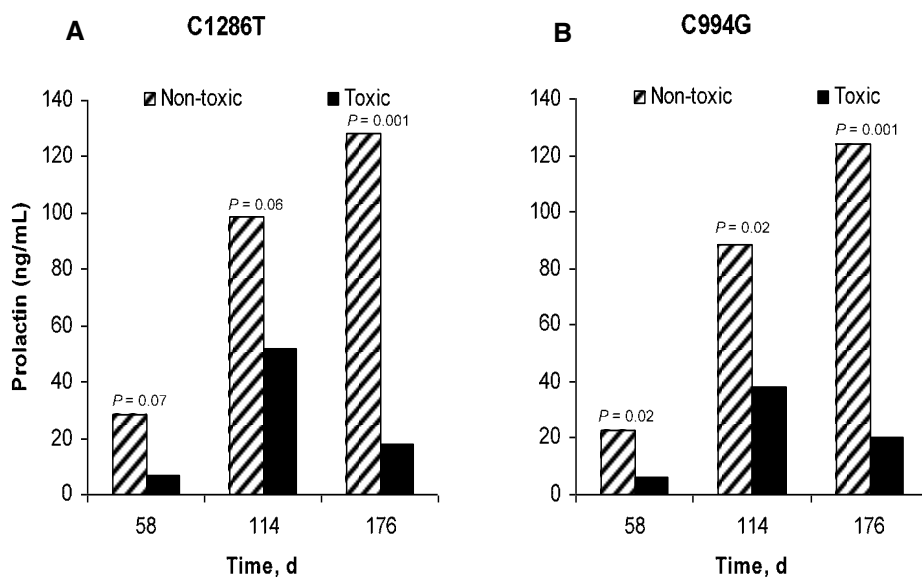
Fig. 2. Mean PRL concentration in steers grazing non-toxic or toxic tall fescue regardless of genotypes for C1286T (A) or C994G (B). Data at each time period were analyzed separately.

Day 58, Fescue x C1286T
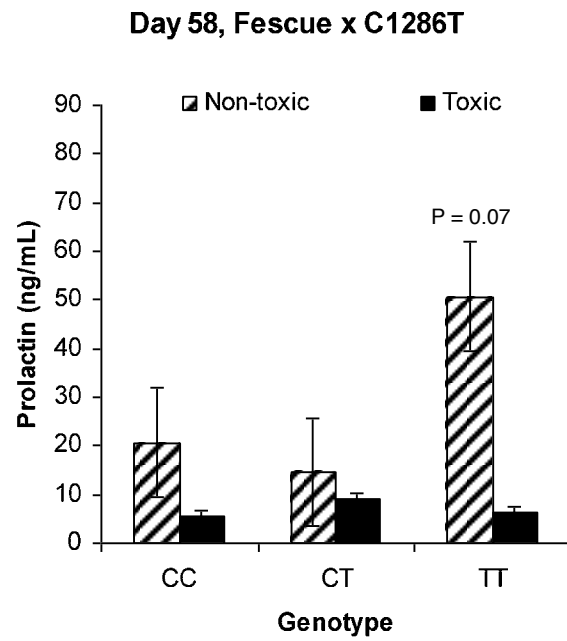
Fig. 3. Fescue x C1286T genotype interaction effect (*P* = 0.07) on PRL concentration at d 58 in steers grazing non-toxic tall fescue.

Day 114, C994G
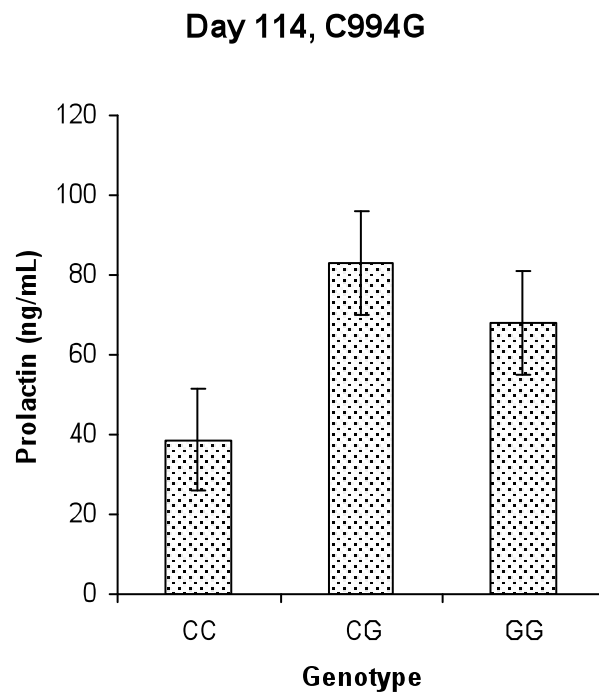
Fig. 4. Effect of C994G genotype on PRL concentration in steers (*P* = 0.03).
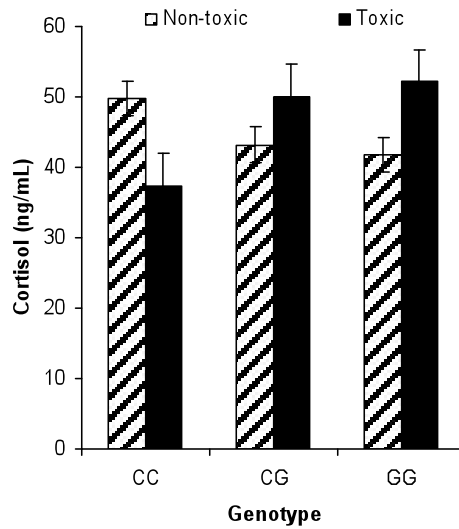
Fig. 5. Fescue x C994G genotype interaction effect (*P* = 0.02) on cortisol concentrations on d 176 in steers grazing non-toxic or toxic fescue.
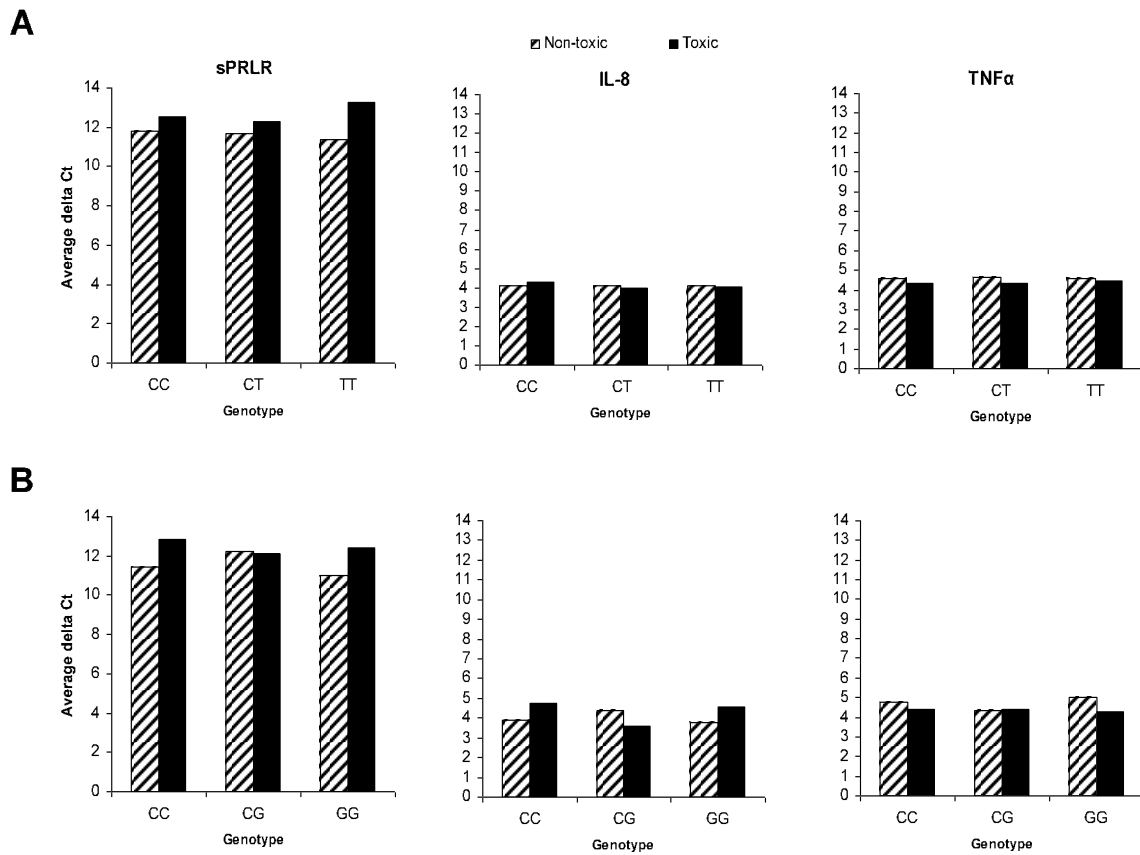
Fig. 6. Transcript levels of sPRLR, IL-8, and TNFα at d 114 in steer with different genotypes for C1286T (A) or C994G (B) and grazing non-toxic or toxic fescue (*P* > 0.05).

Identification of Single Nucleotide Polymorphisms of the Lactate Dehydrogenase-B Gene and Association with Cow and Calf Performance[1]

B. C. Williamson[2], C. F. Rosenkrans, Jr.[2], S. T. Reiter[2], J. D. Thompson[2], M. A. Sales[2], and M. L. Looper[3]

Story in Brief

Lactate dehydrogenase (LDH) is the last enzyme of the glycolytic pathway that converts lactate to pyruvate and vice versa. Objectives were to investigate the polymorphic nature of the bovine LDH-B gene, and to determine the association of those polymorphisms with performance of cows and calves. Five single nucleotide polymorphisms (SNPs) were identified: C541A, A606G, A618G, C652T, and C669T. Brahman-influenced cows (n = 90) were managed to achieve either thin (BCS = 4.3 ± 0.1) or good (BCS = 6.4 ± 0.1) body condition (BC). Cows from each BC grazed either common bermudagrass (CB; n = 3 pastures) or endophyte-infected tall fescue (EI; >85% of stand; n = 2 pastures) during a 60-d breeding period. Forty-seven percent (42/90) of cows were heterozygous for all five SNPs. Genotype of cow affected ($P < 0.01$) calf birth weight. Cows with SNPs within the LDH-B gene had smaller calves (75 ± 1 lb) at birth than cows without SNPs (79 ± 1 lb). Cows in good BC had calves with heavier adjusted 205-d weaning weights ($P < 0.03$; 530 ± 11 lb) compared with calves from thin cows (497 ± 11 lb). Pregnancy rate was influenced ($P < 0.05$) by a genotype × forage interaction with 65% of cows without a LDH-B SNP and grazing EI having a calf ($P < 0.05$). Identification of cows with specific genotypes within the LDH-B gene may be used to improve accuracies of breeding values for birth weight and assist beef producers in selecting cows that would complement their grazing system.

Introduction

Lactate dehydrogenase (LDH) is the last enzyme in the glycolytic pathway and changes pyruvate to lactate (forward; LDHf) in the absence of oxygen, and lactate back to pyruvate (reverse; LDHr) when oxygen is available. There are two subunits of the LDH gene, A and B. The B-type subunit predominates in aerobic tissues such as heart and is superior for lactate oxidation (Stock and Whitt, 1992; Markert, 1984). It is important to determine factors that allow animals to withstand common production stressors such as grazing toxic tall fescue. Looper et al. (2002) found that decreased LDHr activity has been associated with increased reproductive performance of heifers. Further, LDHr activity and physical measurements of prepartum cows could be useful in predicting the subsequent weaning weight of calves while still *in utero* (Looper et al., 2008). Objectives of the current trial were to investigate the polymorphic nature of the bovine LDH-B gene and document any associations of these SNPs with cow and calf performance.

Experimental Procedures

The animals used for this trial were located at the Dale Bumpers Small Farms Research Center in Boonville, Ark. Brahman-influenced cows (n = 90) were managed for either thin (BCS = 4.3 ± 0.1; n = 43) or good (BCS = 6.4 ± 0.1; n = 47) body condition (BC). These scores were based on the 1 to 9 scale with 1 being emaciated and 9 being obese. Cattle grazed mixed bermudagrass and fescue pastures at stocking rates of 1 cow/0.85 ac (thin BC) or 1 cow/2 ac (good BC). Cows from each BCS were then assigned to either common bermudagrass (CB; n = 3 pastures) or toxic endophyte-infected tall fescue (EI; n = 2 pastures) pastures for a 60-d breeding season. Blood samples were collected, buffy coat harvested, and stored at -112 °F for

later DNA analysis. Body weight and BCS of cows were recorded on d 0, 30, and 60 of the breeding season. At weaning, body weights of calves were recorded and adjusted for 205 d of age.

Genomic DNA was harvested using a commercially available QIAmp kit provided by QIAGEN. Primers (5′-GTACAGTCCTGCC TGCATCA -3′ and 5′-CCATTGTTGACACTGGGTGA -3′) were designed to amplify a 452-base pair fragment (bases 489 to 940 of accession number aj401268) of the bovine LDH-B gene. Products were sequenced, SNP sites determined, and haplotypes assigned.

Genotype (with or without SNPs) served as the main effect in the ANOVA with PROC MIXED for the following: Julian date of calving, calf birth weight, calf adjusted 205-d weaning weight, cow BW, BCS, BW change and BCS change. Effects of LDH-B gene SNPs on calving rate was analyzed using Chi-square analysis.

Results and Discussion

Five SNP sites were determined: C541A, A606G, A618G, C652T, and C669T (Table 1). Of the 90 cows, 47 were homozygous for the primary alleles, 42 were heterozygous for the minor allele, and 1 cow was homozygous for the minor allele (Table 1). The single cow that was homozygous for the minor allele was used with the heterozygous cows for statistical analyses.

Genotype did not influence ($P > 0.10$) Julian date of calving. Julian date of calving was effected by sex of calf ($P < 0.02$); mean Julian date for male calves was d 70 ± 2, and female calves had an average Julian date of birth at d 62 ± 2. Thin-BC cows had earlier ($P < 0.01$) calving dates (d 61 ± 2) than cows in good BC (d 70 ± 2).

Calf birth weight was affected by genotype of cow ($P < 0.01$), BC of cow ($P < 0.0001$), and sex of calf ($P < 0.04$). Cows without a SNP in the LDH-B gene had calves that were heavier at birth (79 ± 1 lb) compared with calves from cows that had SNPs in the LDH-B gene

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(75 ± 1 lb). Recently, Looper et al., (2008) reported LDHr activity of the cow was inversely related to calf hip height and weaning weights; however, birth weights of calves were not recorded in that study. Thin-BC cows had lighter calves at birth (74 ± 1 lb) than good-BC cows (80 ± 1 lb). Male calves averaged 79 ± 2 lb at birth while female calves where lighter and averaged 75 ± 2 lb.

There was no forage × genotype interaction on adjusted 205-d weaning weights (P > 0.10) with adjusted 205-d weaning weights ranging from 488 to 528 lb ± 15. Adjusted 205-d weaning weight was affected by BC (P < 0.03) with calves from good-BC cows being heavier at weaning (530 ± 11) than thin-BC cows (497 ± 11). Cows in a thin BC tend to wean lighter calves, and Selk et al. (1988) reported that an adequate body condition is necessary for optimal reproductive efficiency.

Thin-BC cows grazing CB gained 161 ± 14 lb during the 60-d breeding season while thin-BC cows grazing EI gained only 35 ± 17 lbs (P < 0.02; Table 2). Good-BC cows grazing CB gained 81 ± 14 lb and good-BC cows grazing EI lost -18 ± 16 lb during the breeding season (P < 0.02). These results were consistent with the BC changes that were recorded during this time. Thin-BC cows grazing CB gained 0.7 ± 0.1 BCS units while thin-BC cows grazing EI lost -0.2 ± 0.2 BCS units (P < 0.01; Table 3). Cows in good BC grazing CB gained 0.2 ± 0.1 BCS units while the good-BC cows grazing EI lost -0.5 ± 0.1 BCS units (P < 0.01).

Calving rates were affected (P < 0.05) by a forage × genotype interaction with cows that had SNPs in the LDH-B gene (heterozygous) and grazed EI had a 100% calving rate while cows without SNPs in the LDH-B gene (homozygous) grazing EI had a 65% calving rate (Fig. 1). Cows grazing CB had calving rates of 70 and 76% with (heterozygous) and without (homozygous) SNPs in the LDH-B gene, respectively.

Implications

Cows with SNPs in the LDH-B gene had smaller calves at birth than calves from cows without a LDH-B gene SNP. Thin cows calved earlier in the calving season, but had lighter calves at birth and at 205-d weaning. These findings may aid in improving the accuracies of a birth weight molecular breeding value as well as aid beef producers in selecting cattle that may compliment their available forge and labor resources.

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Table 1. Genotypic distributions of five single nucleotide polymorphisms of the lactate dehydrogenase-B gene in beef cattle.

Polymorphism	Homo[1]	hetero[2]	homo[3]	MAF[c]
C541A	47	42	1	24.4
A606G	47	42	1	24.4
A618G	47	42	1	24.4
C652T	49	40	1	23.3
C669T	47	42	1	24.4

[c] Minor allele frequency.
[1] Homozygous for the primary allele.
[2] Heterozygous for the minor allele.
[3] Homozygous for the minor allele.

Table 2. Body weight and BW change of beef cows in either thin (4.3 ± 0.1) or good (6.4 ± 0.1) body condition (BC) grazing either common bermudagrass (CB) or toxic endophyte-infected tall fescue (EI) during a 60-d breeding season.

| | Forage | | | | P value | | |
| | CB | | EI | | | | |
Item	Thin BC	Good BC	Thin BC	Good BC	F[1]	BC	F[1] × BC
No. of cows	23	25	20	22	-	-	-
BW, lb							
d 0	989 ± 27	1354 ± 26	1043 ± 29	1318 ± 28	0.74	0.01	0.10
d 30	1097 ± 29	1416 ± 27	1058 ± 32	1304 ± 30	0.01	0.01	0.22
d 60	1149 ± 29	1435 ± 27	1085 ± 32	1300 ± 30	0.01	0.01	0.23
BW change, lb	161 ± 14	81 ± 14	35 ± 17	-18 ± 16	0.02	0.01	0.33

[1]Forage.

Table 3. Body condition score and BCS change of beef cows in either thin (4.3 ± 0.1) or good (6.4 ± 0.1) body condition (BC) grazing either common bermudagrass (CB) or toxic endophyte-infected tall fescue (EI) during a 60-d breeding season.

| | Forage | | | | P value | | |
| | CB | | EI | | | | |
Item	Thin BC	Good BC	Thin BC	Good BC	F[1]	BC	F[1] × BC
No. of cows	23	25	20	22	-	-	-
BCS							
d 0	4.2 ± 0.2	6.4 ± 0.2	4.5 ± 0.2	6.3 ± 0.2	0.68	0.01	0.33
d 30	4.3 ± 0.2	6.8 ± 0.2	4.4 ± 0.2	5.9 ± 0.2	0.03	0.01	0.01
d 60	5.0 ± 0.2	6.6 ± 0.2	4.8 ± 0.2	5.8 ± 0.2	0.01	0.01	0.46
BCS change	0.7 ± 0.1	0.2 ± 0.1	-0.2 ± 0.2	-0.5 ± 0.1	0.01	0.01	0.58

[1]Forage.
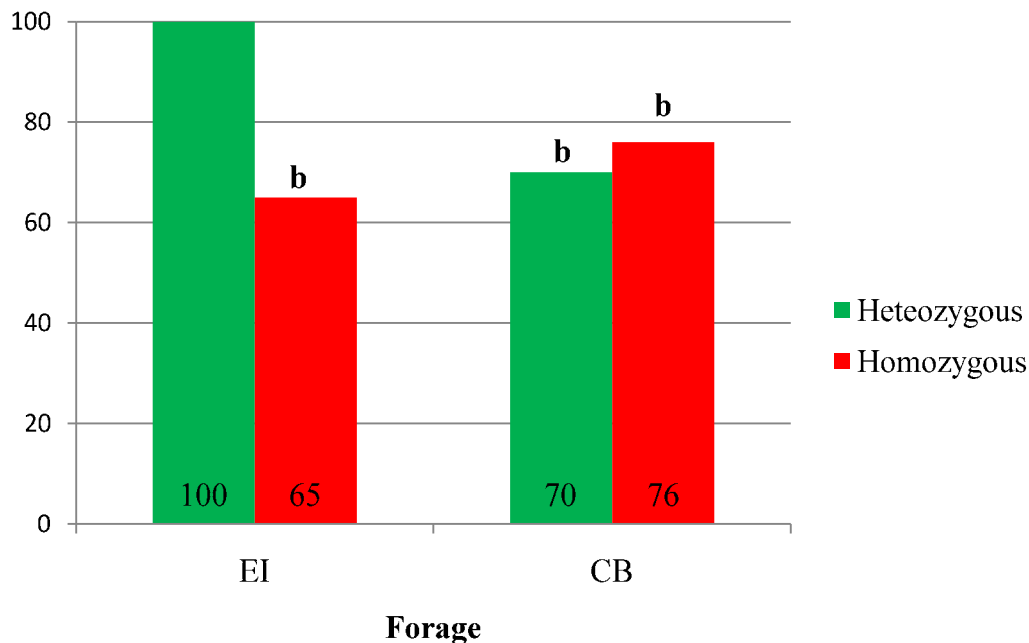
Fig. 1. Calving rate of beef cows with (heterozygous) or without (homozygous) single nucleotide polymorphisms in the lactate dehydrogenase-B gene grazing either toxic endophyte-infected tall fescue (EI) or common bermudagrass (CB) during a 60-d breeding season; forage x genotype interaction, [a,b]*P* < 0.05.

Effects of Heat Shock Protein 70 Haplotype and Tall Fescue Variety on Bull Sperm Characteristics

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Story in Brief

Our objective was to determine the effects of Hsp70 single nucleotide polymorphisms and tall fescue variety on bull sperm characteristics. Angus-sired crossbred (≤3/16 Brahman) yearling bulls (n = 15) were assigned to graze either toxic Kentucky 31 tall fescue (K31) or nontoxic MaxQ tall fescue (MaxQ). Semen was collected via electroejaculation monthly from April through August. Spermatozoa were analyzed using an integrated visual optical system. Bulls were haplotyped based on sequences of the promoter region of the heat shock protein 70 gene, and assigned to one of three categories (No = no mutations; Del = bulls had a cytosine deletion; and Yes = bulls had a mutation other than the cytosine deletion). Experimental treatments were: 1) MaxQ-No, 2) MaxQ-Yes, 3) K31-No, 4) K31-Yes, and 5) K31-Del. Data were analyzed using a one-way analysis of variance with pasture as the experimental unit and included time and treatment as main effects. Number and percentage of live spermatozoa in April was less ($P < 0.05$) than in subsequent months. Computer-assisted sperm analysis indicated that our treatments affected ($P < 0.05$) sperm velocity parameters (path velocity, progressive velocity, and track speed) and sperm beat frequency. The K31-Del bulls generally had the least desirable sperm characteristics during the 5-mo trial. Based on our assessments, all bulls in this trial would have passed the standard breeding soundness examination. Additional research with a larger number of haplotyped bulls grazing toxic tall fescue will be required for conclusive recommendations.

Introduction

Ergot alkaloids are toxins produced by an endophytic fungus (*Neotyphoidium coenophialum*) that commonly exists in tall fescue grasses (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire). Those toxins are known to have negative effects on reproductive efficiency of cows, ewes, mares, and does. Effects of toxic tall fescue on bull sperm characteristics were not known until recently when we demonstrated that bulls grazing toxic fescue in the summer had reduced sperm quality (Looper et al., 2009).

Heat stress has a major effect on livestock productivity and can cost producers billions of dollars each year; furthermore, it exacerbates the negative effects of ergot alkaloids found in toxic endophyte-infected tall fescue. Heat shock protein 70, or Hsp70, has many functions, but one of its main functions is to protect animals that are exposed to environmental or pathological conditions. Genetic mutations, polymorphisms, found in the 5' flanking region of the Hsp70 gene have been linked to diminished semen quality and decreased pregnancy rates in swine (Huang et al., 2002) and lower calving rates in cattle (Rosenkrans et al., 2010). Our objective was to evaluate the effects of Hsp70 single nucleotide polymorphisms (SNP) and tall fescue variety on bull sperm characteristics.

Materials and Methods

Animal Management. The committee for animal welfare at the USDA-ARS, Dale Bumpers Small Farms Research Center in Booneville, Ark., and the University of Arkansas IACUC approved the animal procedures used in this study. For 11 months prior to this study, Brahman-influenced bulls (1/8 to 3/16 Brahman) were maintained on bermudagrass pastures [*Cynodon dactylon* (L.) Pers.] overseeded with Elbon rye (*Secule cereale* L.). At the beginning of this trial, bulls (n = 15) were 1.1 ± 0.1 years of age and had a mean body weight of 1052 ± 75 pounds. They were blocked according to weaning

weight, scrotal circumference, percentage motile and progressive spermatozoa, and total spermatozoa (millions of cells) across three previous collection dates. Bulls were assigned to graze a commercial non-toxic tall fescue (MaxQ, Pennington Seed, Atlanta, Ga.) or toxic endophyte-infected tall fescue pasture (Kentucky 31) for 121 days (April 17 to August 16).

Semen Collection and Evaluation. Semen was collected monthly between 0900 and 1030 hrs by electroejaculation using an Electroejac IV (Ideal Instruments/Neogen Corp., Lansing, Mich.). Ejaculates were placed in a 15-mL conical centrifuge tube and held in a 96 °F water bath. All samples were evaluated within 30 minutes of collection and diluted 20:1 in warm buffer, Dulbecco's PBS, immediately before evaluation.

A Hamilton Thorne IVOS computerized sperm analysis system (CASA; Hamilton-Thorne Biosciences, Beverly, Mass.) was used to evaluate the spermatozoa for motility and morphology variables listed in Table 1 using Animal Motility Software, version 12.1. Ten microscope fields were evaluated and means calculated for all sperm variables. Within each field, 30 video frames were captured for the analysis of sperm motility.

Semen samples were fixed using an eosin-nigrosin-based live-dead stain (Jorvet Stain, Jorgensen Laboratories, Loveland, Colo.) for evaluation of morphology. Approximately 100 spermatozoa per slide were evaluated for percentage live (dye exclusion) and dead.

DNA Isolation, Amplification, and Analysis. Bulls were genotyped based on analysis of blood cells. Blood tubes containing EDTA were cooled to 39 °F and centrifuged at 1500 × g for 25 minutes. The plasma was decanted and buffy coats were harvested and stored at -112 °F until DNA was extracted.

Genomic DNA was extracted from buffy coats using the QIAamp blood and body fluid spin protocol (QIAGEN Inc., Valencia, Calif.). A Hoefer DyNA Quant 200 fluorometer (Amersham Biosciences Corp., Piscataway, N.J.) was used to quantify DNA following purification.

Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) based on the bovine Hsp70 gene sequence (GenBank

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accession # M98823). Specific primers, both forward (HSP-Pro-749F; 5′-GCCAGGAAACCAGAGACAGA-3′) and reverse (HSP-Pro-1268R; 5′-CCTACGCAGGAGTAGGTGGT-3′), were commercially synthesized (Invitrogen, Carlsbad, Calif.) and used for DNA amplification via polymerase chain reaction (PCR). A Peltier thermal cycler (MJ Research, Waltham, Mass.) was used for PCR. Each PCR began with an initial 2 minute heating at 201 °F, followed by 35 cycles at 201 °F for 30 seconds, 1 minute at 131 °F, and 1 minute at 154 °F. A final elongation step consisted of 10 minutes at 154 °F. Included in each PCR was 100 ng genomic DNA, 0.20 μM of each primer, and 45 μl of Platinum PCR Supermix (Invitrogen, Carlsbad, Calif.) for a total volume of 50 μl. Amplification products were visualized by electrophoresis in 1.2% agarose gels stained with ethidium bromide in $1.0 \times$ Tris/Boric Acid/EDTA buffer.

Purification of amplification products was accomplished using the QIAquick 96 PCR purification kit (Qiagen, Valencia, Calif.). Purified PCR products were then sequenced at the University of Arkansas DNA Core Lab using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif.). Comparative analysis of the sequences was carried out using the web-based software package ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html; European Bioinformatics Institute, Cambridge, U.K.).

Statistical Analyses. Sperm characteristics were analyzed using a mixed model analysis of variance. Pasture was considered the experimental unit with the whole plot consisting of forage type (MaxQ vs. Kentucky 31 (K31)). The subplot was bull, and monthly semen collections (Time) were analyzed as a repeated measure. Due to the incomplete treatment structure, a one-way model with time and treatment were used to determine effects. The five treatments were based on forage type bull haplotypes which were: No (no mutations), Del (bulls had a cytosine deletion), and Yes (bulls had a mutation other than the cytosine deletion). Experimental treatments were: 1) MaxQ-No, 2) MaxQ-Yes, 3) K31-No, 4) K31-Yes, and 5) K31-Del. When main effects F-tests were significant ($P < 0.05$) least squares means were separated using multiple t-tests. Two separate analyses were conducted: 1) contained the data from all five months, and 2) only included data from July and August.

Results

Polymorphisms and Haplotypes. Seven polymorphisms were found in the 539-bp segment of the bovine Hsp70 promoter region (Table 2). Of these seven SNP, there was one deletion [C895D (cytosine was deleted and not replaced with a base; represented by a D)], four transitions [G1013A (guanine replaced with adenine), C1069T (cytosine replaced with thiamine), G1117A (guanine replaced with adenine), and T1204C (thymine replaced with cytosine)], and two transversions [A1125C (adenine replaced with cytosine) and G1128T (guanine replaced with thymine)]. Based on published literature, alleles were noted as minor or not and minor allele frequencies were calculated. The SNP A1125C and T1204C were the most prevalent at 63.3 percent each (Table 2). Five unique haplotypes were identified in the 15 bulls used in this study (Table 3).

Sperm Characteristics: April through August. The percentage of slow progressive spermatozoa was greater ($P < 0.05$) in the bulls that contained some SNP other than the C895D deletion and consumed toxic fescue when compared with other treatments (Table 4). Average velocity of the smoothed sperm path (VAP), progressive velocity in a straight line from the beginning to the end of the sperm track (VSL), and track velocity of sperm over the actual point-to-point track (VCL) were decreased ($P < 0.07$) in the bulls that consumed toxic fescue (Table 4). Those traits also changed over time; VAP and VSL

were less ($P < 0.05$) in August and VCL was greater ($P < 0.05$) in July than the other months (Fig. 1). Lateral amplitude of head oscillation as the spermatozoa swims was less ($P < 0.05$) in toxic tall fescue bulls with the deletion when compared with non-toxic bulls in April through August (Table 4). Beat frequency, which is the frequency of the sperm head crossing the sperm average path in either direction, was less ($P < 0.05$) in bulls with the deletion that consumed toxic tall fescue than bulls on non-toxic fescue and bulls with a SNP other than the deletion on toxic fescue (Table 4). Number of spermatozoa in April was less ($P < 0.05$) than the other months (Fig. 2). Percent live spermatozoa in April was lower ($P < 0.05$) than in May, June, July, and August (Fig. 3). Percentage of medium progressive spermatozoa was greater ($P < 0.05$) in bulls grazing toxic fescue with some SNP other than the deletion (Table 4).

Sperm Characteristics: July and August. Percentage of live spermatozoa was less ($P < 0.05$) in the toxic fescue bulls with the deletion while the percentage of dead spermatozoa was greater ($P < 0.06$; Table 5) than other bulls. Month effects were observed on path velocity (VAP), progressive velocity (VSL), and track speed (VCL), which were greater ($P < 0.05$) in July than August (Fig. 4). Number of spermatozoa, percentage of motile spermatozoa, and percentage of slow progressive spermatozoa tended ($P < 0.1$) to be affected by treatment (Table 5).

Discussion

The effect of ergot alkaloids and tall fescue variety on bull fertility using a CASA to measure sperm variables is an open area for research. In this study, several of the sperm characteristics measured by CASA were affected by time and treatment, which was consistent with our previous report (Looper et al., 2009). However, our results are not consistent with other studies that used purified ergot alkaloid (Schuenemann et al., 2005) or stored tall fescue hay (Evans et al., 1988) to determine effects on bull sperm motility and morphology. Subjective laboratory methods may explain those differences in results. The CASA method has been reported to be highly repeatable and provide a better estimate of sperm motility than traditional procedures.

Previously, we reported that the same upstream elements of the bovine Hsp70 gene were polymorphic and were associated with calving percentages of cows (Rosenkrans et al., 2010). Cows with the deletion described in this paper had lower calving rates when compared with other cows. Therefore, that work coupled with the work presented in this report suggests that cattle with deletion mutation will likely have reduced fertility.

Implications

Heat stress exacerbates the negative effects of ergot alkaloids on cattle fertility. We demonstrate in this report that Hsp70 SNP may be useful in selecting cattle that will have increased fertility in general and on toxic tall fescue in particular.

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Table 1. Sperm variables measured by the Hamilton-Thorne Sperm Analyzer (Hamilton-Thorne Biosciences, Beverly, Mass.).

Variable	Description
Motile	% of total sperm moving at path velocity ≥30 μm/sec and progressive velocity ≥15 μm/sec
Progressive	% of total sperm moving at path velocity ≥50 μm/sec and straightness ≥70%
Rapid	Progressive % with path velocity >50 μm/sec
Medium	Progressive % with path velocity <50 μm/sec but >30 μm/sec
Slow	% of total sperm moving at path velocity <30 μm/sec and progressive velocity <15 μm/sec
Static	Sperm not moving at all
Path velocity (VAP)	Average velocity of the smoothed cell path (μm/sec)
Progressive velocity (VSL)	Average velocity measured in a straight line from the beginning to the end of track
Track speed (VCL)	Average velocity measured over the actual point-to-point track
Lateral amplitude (ALH)	Mean width of the head oscillation as the sperm swims
Beat frequency (BCF)	Frequency of sperm head crossing the sperm average path in either direction
Straightness	Measures departure of average sperm path from straight line (ratio of VSL/VAP)
Linearity	Measures departure of actual sperm track from straight line (ratio of VSL/VCL)
Elongation	Ratio (%) of head width to head length
Area	Average size in square microns of all sperm heads

Table 2. Distribution of SNP of the bovine heat shock protein 70 promoter.

Polymorphism[1]	Genotype distribution[2]			MAF[3]
	Homo	Hetero	homo	
C895D	12	0	3	20.0
G1013A	10	3	2	23.3
C1069T	9	0	6	40.0
G1117A	9	0	6	40.0
A1125C	5	1	9	63.3[1]
G1128T	11	1	3	23.3
T1204C	5	1	9	63.3[1]

[1]Single nucleotide polymorphism occurred at the number indicated. First letter indicates the primary allele and the letter following the digits is the minor allele (D represents deletion of cytosine).
[2]Number of bulls that were homozygous for the primary allele (Homo), heterozygous (Hetero), and homozygous for the minor allele (homo).
[3]Minor allele frequency expressed as a percent. Based on published literature, alleles were noted as minor or not.

Table 3. Haplotype frequency of the bovine heat shock protein 70 promoter.

Haplotype[1]	Bull[2]	Hsp70 SNP[3]	Sequence
1	89	N	CGCGAGT
	118	N	CGCGAGT
	133	N	CGCGAGT
	135	N	CGCGAGT
	167	N	CGCGAGT
2	116	Y	CATACGC
	127	Y	CATACGC
	155	Y	CATACGC
	162	Y	CATACGC
	179	Y	CATACGC
3	149	Y	CGCGCTC
4	65	Y	CGTACGC
5	119	D	DGCGCTC
	158	D	DGCGCTC
	159	D	DGCGCTC

[1]Order of SNP in these haplotypes was C895D, G1013A, C1069T, G1117A, A1125C, G1128T, and T1204C; deletion of a cytosine is presented as D; haplotype 1 represents the published sequence (GenBank accession # M98823).
[2]Bull = individual bull number.
[3]Hsp70 SNP were N = no SNP, Y = some type of SNP other than the deletion at 895, and D = deletion of cytosine at base position 895.

Table 4. Treatment effects on sperm characteristics.[1]

Sperm Variable	Non-toxic[2]		Toxic			P-value		SE[3]
	Y	N	D	Y	N	Time	Treatment	
No. of bulls	4	4	3	3	1	-	-	-
No. of sperm	570.3	377.4	289.3	775.1	508.8	0.0427	0.0809	3.8
Motile, %	63.0	63.6	47.2	53.2	62.4	0.3806	0.3307	7.1
Progressive, %	51.4	52.1	34.9	36.4	46.2	0.6575	0.1455	6.3
Rapid, %	58.3	58.6	41.6	43.6	52.8	0.4589	0.2155	7.0
Medium, %	5.0	4.7	5.7	16.5	9.6	0.0390	0.0894	3.4
Slow, %	8.0[b]	7.7[b]	9.9[b]	16.3[a]	8.0[b]	0.6455	0.0097	1.6
VAP	129.1[c]	124.6[c]	99.6[d]	92.3[d]	97.9[d]	0.0455	0.0039	6.8
VSL	113.9[e]	109.9[e]	86.0[f]	77.4[f]	85.7[f]	0.0241	0.0034	6.4
VCL	202.5[c]	196.2[c]	153.3[d]	153.7[d]	156.6[d]	0.0254	0.0054	10.4
ALH	7.2[a]	7.0[a]	5.7[b]	6.4[ab]	6.3[ab]	0.0068	0.0473	0.4
BCF	28.2[a]	27.8[a]	21.9[b]	27.6[a]	25.9[ab]	0.1431	0.0494	1.6
Straightness	86.9	87.6	79.8	81.9	86.4	0.0128	0.2283	3.0
Linearity	58.3	59.6	55.7	52.1	57.4	<0.0001	0.2534	2.6
Live, %	73.7	78.9	60.5	72.9	73.4	0.0196	0.0723	4.5

[1]Sperm variables were assessed on spermatozoa collected from bulls from April through August. For definition of characteristics see Table 1; path velocity (VAP), progressive velocity (VSL), track speed (VCL), lateral amplitude (ALH), and beat frequency (BCF).
[2]Forages were non-toxic vs. toxic tall fescue, and Hsp70 SNP were Y = some type of SNP other than the deletion at 895, N = no SNP, D = deletion of cytosine at base position 895.
[3]SE = mean of standard errors.
[ab]Means without a common superscript differ (*P* < 0.05).
[cd]Means without a common superscript differ (*P* < 0.06).
[ef]Means without a common superscript differ (*P* < 0.07).

Table 5. Treatment effects on sperm characteristics during July and August.[1]

Sperm Variable	Non-toxic[2]		Toxic			P-value		SE[3]
	Y	N	D	Y	N	Time	Treatment	
No. of bulls	4	4	3	3	1	-	-	-
No. of sperm	751.6	463.1	180.6	884.6	337.5	0.4627	0.0850	186.8
Motile, %	74.1	73.6	40.7	49.8	78.0	0.6950	0.0623	10.0
Progressive, %	57.8	56.3	29.2	31.3	60.0	0.2843	0.0913	9.9
Rapid, %	67.8	66.1	35.3	39.5	66.0	0.3661	0.0810	10.5
Medium, %	6.3	7.2	5.5	27.3	12.0	0.1119	0.2718	8.2
Slow, %	7.8	7.0	7.3	17.5	5.5	0.6207	0.0687	2.9
VAP	125.9	122.0	87.7	87.5	111.9	0.0299	0.0950	13.1
VSL	107.5	103.7	74.2	71.2	97.8	0.0311	0.1092	12.3
VCL	205.6	203.9	145.7	154.2	190.2	0.0154	0.0890	19.5
ALH	7.7	7.8	5.5	6.8	7.8	0.0540	0.1014	0.7
BCF	28.6	28.9	23.5	29.2	28.9	0.0804	0.5206	2.8
Straightness	83.5	84.0	69.6	79.3	87.0	0.1711	0.4753	7.0
Linearity	53.5	53.1	45.0	47.0	52.5	0.2416	0.6402	5.4
Live, %	80.4	79.8	52.5	71.2	86.5	0.7915	0.0305	6.6

[1]Sperm variables were assessed on spermatozoa collected from bulls in July and August. For definition of characteristics see Table 1; path velocity (VAP), progressive velocity (VSL), track speed (VCL), lateral amplitude (ALH), and beat frequency (BCF).
[2]Forages were non-toxic vs. toxic tall fescue, and Hsp70 SNP were Y = some type of SNP other than the deletion at 895, N = no SNP, D = deletion of cytosine at base position 895.
[3]SE = mean of standard errors.
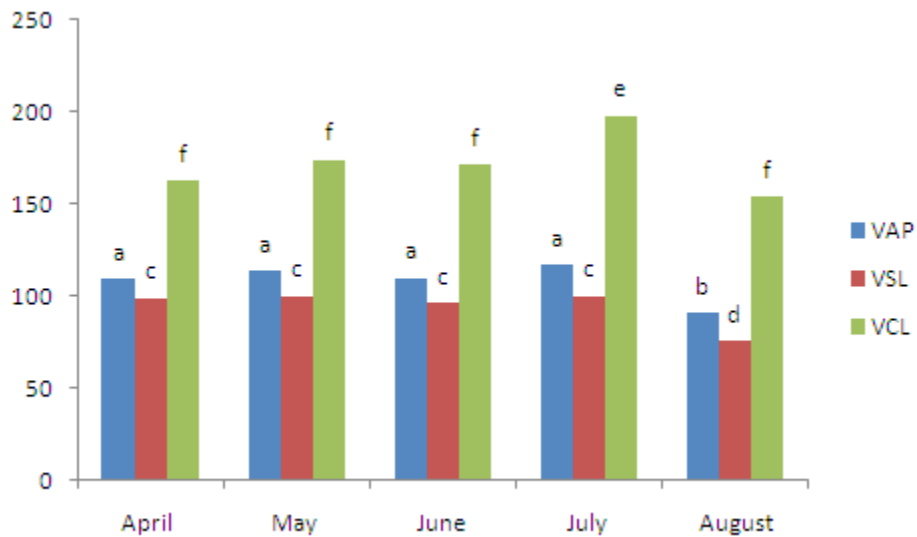
Fig. 1. Change in path velocity (VAP), progressive velocity (VSL), and track speed (VCL) over time. Means without a common superscript differ ($P < 0.05$).
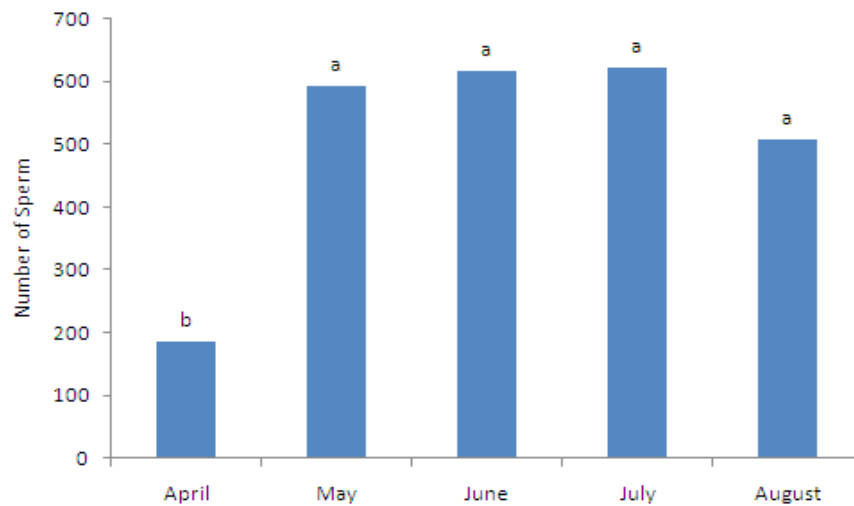
Fig. 2. Change in number of spermatozoa over time. Means without a common superscript differ ($P < 0.05$).

Fig. 3. Change in the percentage of live spermatozoa over time. Means without a common superscript differ ($P < 0.05$).

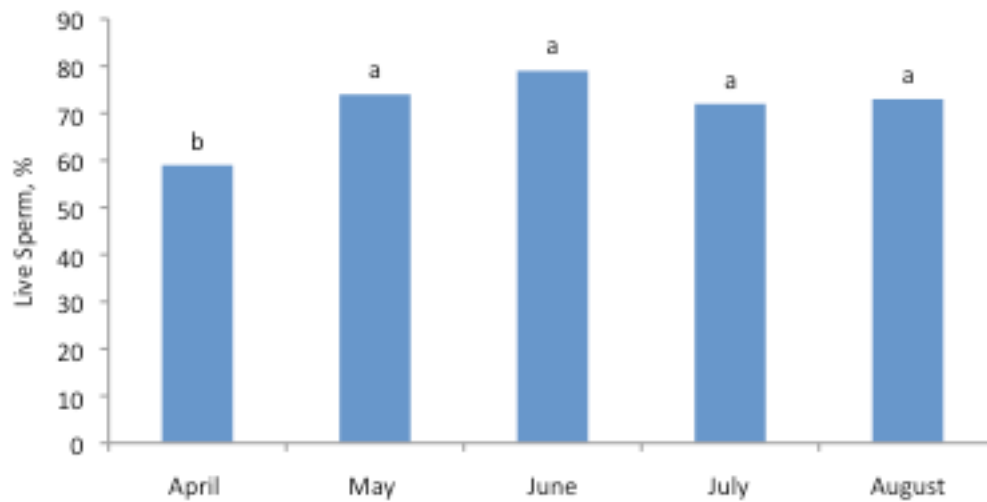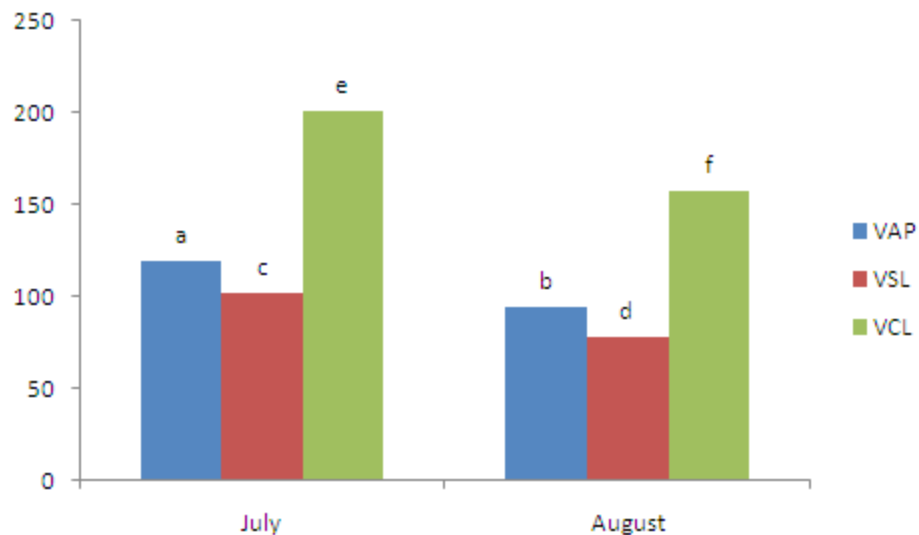**Fig. 4. Change in path velocity (VAP), progressive velocity (VSL), and track speed (VCL) in the hottest months, July and August. Means without a common superscript differ ($P < 0.05$).**

Relationship of Fecal Egg Counts to Prolactin Promoter Polymorphisms in Angus Calves

A. R. Starnes[1], A. H. Brown[1], Jr., Z. B. Johnson[1], J. G. Powell[1], J. L. Reynolds[1], and C. F. Rosenkrans, Jr.[1]

Story in Brief

The objective of this study was to determine the relationship between single nucleotide polymorphisms (SNPs) of the prolactin promoter fecal egg counts of internal parasites in purebred Angus calves (n = 110). Data was collected over a four year period (2005 - 2008) and includes fecal egg counts on d 0, 21, 66, 111, 156, 201, and 246. All calves used were spring born and treated with anthelmintic at weaning in the fall. Calves were genotyped using genomic DNA prepared from buffy coat and our previous published primers for the prolactin gene. Genotypes were homozygous cytosine (CC; n = 9), heterozygous (CT; n = 80), and homozygous thymine (TT; n = 21). Prolactin genotype was related ($P < 0.05$) to nematodirus egg counts at weaning (9 vs 1, and 7 eggs/g for CC, CT, and TT, respectively). Age of the calf at the time of fecal sampling did show a relationship to strongyle egg counts on d 0 ($P = 0.05$), d 66 ($P < 0.05$), and d 111 ($P < 0.05$). A larger study with increased sample size is needed to further test the relationships between prolactin genotypes and fecal egg counts in Angus calves.

Introduction

Internal parasites are prevalent in the Southern region of the United States and can result in an economic loss estimated to be between $25 to $200/animal (Williams and Loyacano, 2001). The development of resistance to the drugs used to control internal parasites has been reported in every livestock host and to every class of anthelmintic (Kaplan, 2004). Examples of this resistance include resistance of nematodes to benzimidazole drugs and endectocides in New Zealand (Williams and Loyacano, 2001). These reports of developed resistance to anthelmintics suggest the need to consider other methods for the control of parasites.

There are previous reports of natural, inherited resistance to internal parasites. A positive correlation between prolactin concentrations and fecal egg counts was determined by Diaz-Torga et al. (2001). In our laboratory, we have previously reported polymorphisms in the prolactin gene that may be useful as genetic markers. Furthermore, development of genetic markers associated with natural resistance to internal parasites would be a valuable tool for producers to use in the selection of animals with natural resistance to parasitic infection. The objective of this study was to determine the relationships between single nucleotide polymorphisms (SNP) in the prolactin gene and fecal egg counts for internal parasites.

Experimental Procedures

Purebred Angus calves (n = 110) were used in this study. All calves were spring born and weaned in the fall of each year (2005-2008). Both sexes were included and all calves are registered with the American Angus Association. At weaning fecal samples were taken and each calf received fenbendazole at the rate of 10 mg/kg body weight (BW). Fecal samples were obtained at d 21 to determine efficacy of the fenbendazole treatment. Subsequent fecal samples were collected at d 66, 111, 156, 201, and 246. Growth implants were not used and calves received no creep feed. Sires were selected with a balanced approach to EPDs. Traits of parasite resistance/susceptibility were not considered in sire selection.

Cow herds were maintained on primarily toxic endophyte-infected fescue. Nematode eggs/g (EPG) were determined by homogenizing 1 g of feces in saturated $MgSO_4$. This solution was placed into a 15 ml centrifuge tube, filled to form a slight emiscus, capped with a 22 mm^2 cover slip and centrifuged for 3 minutes. The cover slip was removed and placed on slide. All "strongyles" and *Nematodirus* eggs were counted, and EPG's calculated. Fecal egg counts were normalized with a log 10(x + 1) transformation. Genomic DNA was prepared from buffy coat. Calves were haplotyped using our previously published primers for bovine prolactin promoter (Looper, 2010). Haplotypes were CC (n = 9), CT (n = 80), and TT (n = 21). Data were analyzed with mixed model procedures. Fixed effects included haplotype, age of calf, and age of dam was used as a covariant. Random effects included in the model were the animals.

Results and Discussion

Figure 1 shows the relationship of prolactin genotype to *Nematodirus* egg count. The CC and TT haplotype had greater ($P < 0.05$) fecal egg counts for *Nematodirus* when compared to CT (9 and 7 vs. 1, respectively) at weaning. Age of calf at the time of fecal sampling was related to strongyle egg counts at d 0 ($P < 0.05$), d 66 ($P < 0.05$), and d 111 ($P < 0.05$). The prolactin genotype showed a relationship ($P < 0.05$) to *Nematodirus* egg count indicating the need to use chemical control could be reduced. There is a need further studies on the use of molecular markers to select for natural parasite resistance and thus reduced dependency on chemical treatments.

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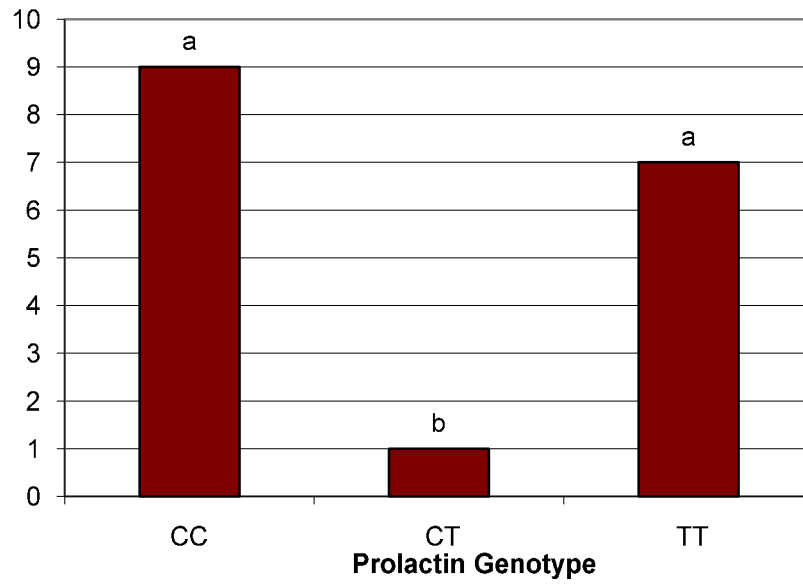[1] University of Arkansas, Department of Animal Science, Fayetteville, Ark.

Fig. 1. Nematodirus fecal egg count (eggs per gram; EPG) for each prolactin genotype (CC, CT and TT) from Angus calves at weaning. Bars with unlike letters differ ($P < 0.05$).

Pre-Arrival Management of Newly Received Beef Calves with or without Continuous Exposure to a Persistently Infected Bovine Viral Diarrhea Virus Type 1 Calf Affects Health, Performance, Bovine Viral Diarrhea Virus Type 1a Titers, and Circulating Leukocytes

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Story in Brief

Calves persistently infected (PI) with bovine viral diarrhea virus (BVDV) are a major source of the virus; however, consequences of exposure to a PI-BVDV calf in preconditioned (PC) vs. auction market (AM) cattle may differ. Our objective was to compare treatments of PC or AM origin, with (PI) or without (CON) exposure to a PI-BVDV calf. Four sets of PC steers (n = 236) from 3 ranches were selected randomly, weaned, vaccinated, tested for PI-BVDV status, and kept on the ranch for ≥ 42 d. Subsequently, PC calves were transported to a receiving unit (RU), weighed (552 ± 4.4 lb), bled, and assigned randomly to treatment (PCPI or PCCON). Simultaneously, 4 sets of AM calves (n = 292) were assembled for delivery to the RU. The AM calves were weighed (539 ± 2.9 lb) and administered the same processing as PC; however, bull calves were castrated, stratified by gender, and AM calves were assigned randomly to treatment (AMPI or AMCON). Daily gain for the entire 42 d was greater ($P < 0.001$) for PC (2.6 lb) than AM (1.9 lb). There was an exposure effect ($P = 0.002$) on ADG from d 28 to 42; CON gained 2.4 lb vs. 2.0 lb for PI. Morbidity rate was greater ($P < 0.001$) in AM (70%) than PC (7%). Treatment with a third antibiotic occurred more often ($P = 0.04$) for PI, likewise the greatest number of chronic cattle were AMPI ($P = 0.06$). Results suggest that PC calves gain faster and require fewer antibiotic treatments; whereas, PI-BVDV exposure reduced gain and increased antibiotic cost, particularly in AM.

Introduction

Bovine respiratory disease (BRD) is a multifaceted disease involving stress, commingling, and several viral and bacterial pathogens. Preconditioning is a management practice first identified in the 1960s to prepare young calves for stocker or feedlot entry by reducing marketing stress and providing disease protection through pre-arrival vaccination against BRD pathogens. Preconditioned calves have reduced morbidity and improved gain performance compared to high-risk calves originating from auction markets.

Bovine viral diarrhea virus (BVDV) is a major culprit in the development of BRD either directly via acute clinical disease or through indirect effects of immunosuppression. Furthermore, calves born persistently infected (PI) with BVDV are a key source of the virus, and although prevalence of PI-BVDV calves in the feedlot is thought to be low, a single PI-BVDV animal has the potential to expose an entire pen and adjoining pens to the virus. Recent research on effects of exposure to PI-BVDV calves in the feedlot is conflicting. One inconsistency among the literature is the use of cattle with varied management and health history, and research evaluating effects of PI-BVDV exposure in single source, preconditioned versus commingled, auction market calves is needed.

Experimental Procedures

Two different cattle source groups were utilized for the receiving trial; 1) a single-source, preconditioned (PC) group containing 236 crossbred steer calves (initial BW = 552 ± 4.4 lb) which arrived in 4 shipment blocks from 3 Arkansas ranches and 2) a commingled, auction market (AM) group of 292 crossbred bull (n = 210) and steer (n = 82) calves (initial BW = 539 ± 2.9 lb) arriving in 4 shipment

blocks acquired from multiple Arkansas auction markets. Within source, calves were stratified by gender (AM only) and d -1 BW, then assigned randomly to 1 of 4 or 1 of 8 pens depending on block (8 to 11 calves/pen). The main effects of cattle source and PI-BVDV exposure were tested resulting in 4 treatments in a 2 × 2 factorial arrangement. For PC treatments, ≥42 d prior to trial initiation, randomly selected steers were weaned, ear-notched to test for PI-BVDV status (Cattle Stats, LLC, Oklahoma City, Okla.) administered a 5-way modified-live virus (MLV) respiratory vaccine [Express® 5, Boehringer Ingelheim Vetmedica (BIVI), St. Joseph, Mo.], Manheimia haemolytica-Pasteurella multocida bacterin-toxoid (Pulmo-guard® PHM-1, BIVI), 7-way clostridial bacterin-toxoid (Alpha® 7, BIVI) and pour-on or injectable anthelmintic (Cydectin®, BIVI). Preconditioned calves were isolated from other cattle, fed hay or pasture along with a supplement, and remained on their origin ranch during the preconditioning period until approximately d -2 when they were shipped to the University of Arkansas Agricultural Experiment Station located near Savoy (RU). On d 0 of the trial PC calves were weighed, bled via jugular venipuncture into evacuated tubes (Vacutainer®; BD Inc, Franklin Lakes, N.J.) for total and differential leukocyte analysis (EDTA tube) and BVDV type 1a antibody titers (plain tube), and assigned randomly to treatment (PCCON or PCPI). To coincide with PC groups, AM calves were assembled and delivered to the RU within 24 h of PC arrival. Processing for AM occurred upon arrival to the RU (d 0) rather than on an origin ranch prior to stocker receiving as for PC. The AM cattle received the same processing procedures described for PC; however, bull calves were surgically castrated, stratified by gender, then AM calves were assigned randomly to treatment (AMCON or AMPI). Cattle were then moved to their assigned 1.1-acre pens and provided 2 lb/d (as-fed basis) of an identical receiving supplement (15.3% CP, DM basis)

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and free-choice access to bermudagrass hay (13.1% CP, 64% NDF, 42% ADF, DM basis) and water. Supplement offered was step-wise increased to a maximum of 6 lb/d as calves began consuming the supplement.

For AM calves only, a booster vaccination of the 5-way MLV respiratory vaccine (Express® 5, BIVI) occurred on d 14 in addition to a 7-way clostridial bacterin-toxoid (Alpha® 7, BIVI). All calves were weighed at 14-d intervals during the trial (d 14, 28, and 42) to determine interim and overall differences in gain performance. All calves were bled on d 14 and 28 as described for d 0. Anticoagulated whole blood collected into EDTA tubes was kept refrigerated and utilized within 24 h to determine concentrations (n cells/µL) of total leukocytes, and differential leukocytes (lymphocytes, neutrophils, monocytes, eosinophils, and basophils) with an automated hematology analyzer (Cell-Dyn 3500 system, Abbott Laboratories, Abbot Park, Ill.) standardized for analysis of bovine blood. Serum collected from plain tubes was kept frozen until subsequent overnight shipment on ice to the Iowa State University Veterinary Diagnostic Laboratory (Ames, Iowa) for determination of serum neutralizing antibodies against BVDV type 1a (Singer strain).

Calves previously identified (Cattle Stats) as PI-BVDV were acquired from a stocker cattle operation in NE Oklahoma and utilized as PI-BVDV exposure sources. Upon arrival to the RU, each PI-BVDV calf was ear-notched a second time, and samples were sent to a different laboratory [Oklahoma Animal Disease Diagnostic Lab (OADDL), Stillwater, Okla. or USDA, National Animal Disease Center (NADC), Ames, Iowa] for rapid affirmation of positive PI-BVDV status. Additionally, anticoagulated blood was collected from each PI-BVDV animal and shipped to NADC to determine the subgenotype strain of PI-BVDV infection using reverse-transcriptase PCR procedure. All subgenotyped PI-BVDV animals were BVDV subgenotype 1b, except one being BVDV subgenotype 1a.

Calves were observed daily for signs of BRD. If 2 or more visual signs (ie. depression, nasal discharge, ocular discharge, cough, gaunt appearance) existed, calves were brought to a restraining chute and rectal temperature was recorded. If rectal temperature was ≥104 °F, cattle were considered morbid, administered antibiotic therapy following a pre-determined antibiotic treatment protocol, and immediately returned to their home pen. Temperature was taken 48 h following initial treatment with enrofloxacin (Baytril®, Bayer Animal Health, Shawnee Mission, Kan.). If the second temperature was ≥104 °F, a second antibiotic treatment with florfenicol (Nuflor®, Schering-Plough Animal Health, Summit, N.J.) was administered. A 48 h post-treatment interval (PTI) was implemented for cattle administered florfenicol, and rectal temperature was evaluated upon expiration of the PTI. If the temperature was ≥104 °F, a third and final antibiotic treatment with ceftiofur hydrochloride (Excenel®, Pfizer Animal Health, New York, N.Y.) was administered and repeated for 2 consecutive days following the initial injection of ceftiofur hydrochloride. If at any time a temperature was <104 °F, the animal was left untreated and returned to the home pen until further symptoms warranted re-examination. Treatment data were recorded for individual animal including treatment date, rectal temperature, and the amount (ml) of each antibiotic administered.

Statistical Analysis. Performance and morbidity data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, N.C.). Pen was considered the experimental unit. Block and block × replicate × treatment were considered as random effects in the statistical model. Orthogonal contrasts evaluating effects of source (PC or AM) and PI-BVDV exposure (PI or CON) and their interaction were used. If the interaction was significant ($P \leq 0.10$), treatment means were separated

with a t-test using the PDIFF option in SAS. Total and differential leukocytes, and BVDV type 1a titer data were analyzed using the MIXED procedure with repeated measures. Pen was considered the experimental unit for these data. Contrasts for the repeated measures data included source, exposure, and treatment × day interaction.

Results and Discussion

Performance. No treatment interactions were observed for animal performance; therefore, only main effects of source and exposure are reported (Table 1). Preconditioned calves had greater ($P < 0.001$) ADG than AM calves from d 0 to 14 (3.6 vs. 1.4 lb/d), d 0 to 28 (2.8 vs. 1.7 lb/d) and d 0 to 42 (2.6 vs. 1.9 lb/d). These results would clearly suggest that prior vaccination against BRD pathogens coupled with pre-arrival management that reduces physical and psychological stress in beef calves results in greater gain performance during the receiving period. Exposure to a PI-BVDV calf in the pen resulted in conflicting, yet interesting differences in performance during the receiving trial. From d 0 to 28, PI-BVDV exposure tended ($P = 0.09$) to increase performance with exposed calves averaging 2.34 lb/d versus non-exposed calves averaging 2.19 lb/d. However, during the final interim period (d 28 to 42) of the receiving trial, PI-BVDV exposure resulted in a decrease ($P = 0.002$) in ADG (1.97 vs. 2.45 lb/d). This would suggest that negative performance consequences of PI-BVDV exposure in newly received beef calves may be delayed for several weeks. Although the exact reason for our observation of an early increase, followed by a subsequent decrease in performance from PI-BVDV exposure is not known, it may be due to one or more complex epidemiological factors that occurred during the receiving trial. One factor may be that the PI-BVDV calves were shedding a greater amount of BVDV particles during the last 2 weeks of the receiving period, perhaps due to physiological stress occurring in the PI-BVDV animals from previous acute viral or bacterial shedding of non-PI penmates. Furthermore, an additive effect on host immune activity may have resulted in the performance loss observed for d 28 to 42 because repeated immune stimulation results in nutrients being preferentially utilized for immune and homeostatic pathways rather than tissue deposition (Spurlock, 1997).

Health. Morbidity rate was markedly greater ($P < 0.001$) for AM than PC with 70 and 7% of calves, respectively, requiring treatment at least one time for BRD (Table 2). Furthermore, a greater number of AM calves required treatment with a second ($P < 0.001$), and third ($P = 0.001$) antibiotic. Although the overall BRD morbidity rate was not affected ($P = 0.41$) by PI-BVDV exposure, treatment with a third antibiotic occurred more often ($P = 0.04$) when a PI-BVDV calf was present in the pen. A treatment interaction ($P = 0.06$) was observed for the percentage of chronically ill animals; AMPI had the greatest number of chronically ill calves (7.6%), AMCON was intermediate (1.1%), and PCCON and PCPI were least (both 0.4%). A trend ($P = 0.07$) was observed for PI-exposed calves having an increased antibiotic treatment cost which averaged $12.75 and $10.39/animal for PI and CON treatments, respectively. Within AM calves only, PI exposure resulted in an antibiotic treatment cost of $4.07/animal more than CON; this numerical difference being similar to PI-BVDV testing cost/animal. The large differences observed for health among cattle sources suggest that single-source PC calves have fewer BRD-related health problems, improved animal well-being, and reduced antibiotic usage compared to commingled AM calves. Furthermore, the number of chronically ill calves and antibiotic treatment cost was greatest for AMPI with fewer adverse health consequences observed for PCPI, suggesting that pre-arrival vaccination against BVDV may have provided protection against PI-BVDV exposure in the PC calves.

BVDV Type 1a Antibody Titers. On d 0, BVDV type 1a antibody titers were greater (treatment × day, $P < 0.001$) for PC, and seroconversion to BVDV type 1a on d 0 was 100% for PC vs. 23% in AM (Fig. 1). The difference in antibody titers against BVDV type 1a on arrival, supported by large differences in BRD morbidity observed for the 2 cattle source groups, would suggest that AM calves were relatively naïve and PC calves had adequate protection against either acute infection or PI-BVDV exposure during the receiving period. By d 28, BVDV type 1a titers levels were increased for all treatments; however, effects of PI-BVDV exposure were not observed ($P = 0.95$) for BVDV type 1a titer levels. This would indicate that exposure to a single PI-BVDV calf in the pen did not result in greater BVDV type 1a antibody titers by d 28; however, only 1 of the PI-BVDV calves used as an exposure source for the trial was BVDV subgenotype 1a, the remainder were BVDV subgenotype 1b.

Total and Differential Leukocyte Count. Both source ($P < 0.001$) and day ($P < 0.001$) effects were observed for the total number of circulating leukocytes (Fig. 2). Total leukocytes averaged 11.0, 10.8, 8.8, and $8.7 \times 10^3/\mu L$ for PCPI, PCCON, AMPI and AMCON, respectively and counts increased (day effect, $P < 0.001$) overall with time. The neutrophil:lymphocyte ratio (N:L) was greater (source effect, $P < 0.001$) for AM (Fig. 3). As the trial progressed, N:L increased in AM, but decreased for PC (trial × day, $P < 0.001$). The greater N:L observed for AM is indicative of increased stress, disease challenge, or both for that particular source group. Furthermore, a source effect ($P < 0.001$) was observed for platelet concentration; AM had greater platelets on d 14 and 28, indicative of a more stimulated cell-mediated immune response (Fig. 4).

Implications

Single-source, preconditioned calves gained faster, were much healthier, and antibiotic treatment cost was greatly reduced compared to commingled, auction market calves. Pen exposure to a persistently infected bovine viral diarrhea virus type 1 calf resulted in more calves requiring treatment with a third antibiotic and reduced gain during the final interim-weight period.

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Table 1. Effect of pre-arrival management and PI-BVDV exposure on performance of newly received calves.

| | AMCON[2] | AMPI[3] | PCCON[4] | PCPI[5] | SEM | Contrasts[1], $P =$ | | |
						Source	Exposure	Interaction
BW, lb								
Initial	548	546	550	550	20.3	0.37	0.69	0.68
d 14	565	568	601	603	21.1	0.0001	0.62	0.82
d 28	594	594	627	631	19.6	0.0001	0.60	0.73
Final	629	623	662	660	16.5	0.0001	0.39	0.75
ADG, lb								
d 0 to 14	1.19	1.69	3.63	3.63	0.42	0.0001	0.15	0.18
d 0 to 28	1.63	1.78	2.75	2.90	0.33	0.0001	0.09	0.97
d 28 to 42	2.42	1.96	2.49	1.98	0.33	0.78	0.002	0.87
d 0 to 42	1.89	1.85	2.66	2.60	0.22	0.0001	0.28	0.98

[1]Source = main effect of source origin (PC or AM), Exposure = main effect of PI-BVDV exposure
(PI or CON), Interaction = source × exposure.
[2]AMCON = Auction Market, Control.
[3]AMPI = Auction Market, Exposed to PI-BVDV calf.
[4]PCCON = Preconditioned, Control.
[5]PCPI = Preconditioned, Exposed to PI-BVDV calf.

Table 2. Effect of pre-arrival management and PI-BVDV exposure on health of newly received calves.

	AMCON[2]	AMPI[3]	PCCON[4]	PCPI[5]	SEM	Source	Exposure	Interaction
						Contrasts[1], *P* =		
Morbidity, %	67.2	73.7	7.7	6.7	4.22	0.0001	0.41	0.27
Treated with 2nd antibiotic, %	34.2	43.6	4.2	6.4	4.82	0.0001	0.13	0.35
Treated with 3rd antibiotic, %	8.0	17.5	3.2	4.5	2.96	0.001	0.04	0.12
Relapse, %	50.6	58.5	60.3	100	17.96	0.05	0.07	0.21
Chronic, %	1.1[b]	7.6[a]	0.4[b]	0.4[b]	2.17	0.03	0.06	0.06
Antibiotic treatment cost, $/hd	18.02[b]	22.70[a]	3.19[c]	2.60[c]	1.95	0.0001	0.12	0.05

[a,b]Means within a row without a common superscript differ ($P < 0.05$)
[1]Source = main effect of source origin (PC or AM), Exposure = main effect of PI-BVDV exposure (PI or CON),
Interaction = source × exposure.
[2]AMCON = Auction Market, Control.
[3]AMPI = Auction Market, Exposed to PI-BVDV calf.
[4]PCCON = Preconditioned, Control.
[5]PCPI = Preconditioned, Exposed to PI-BVDV calf.
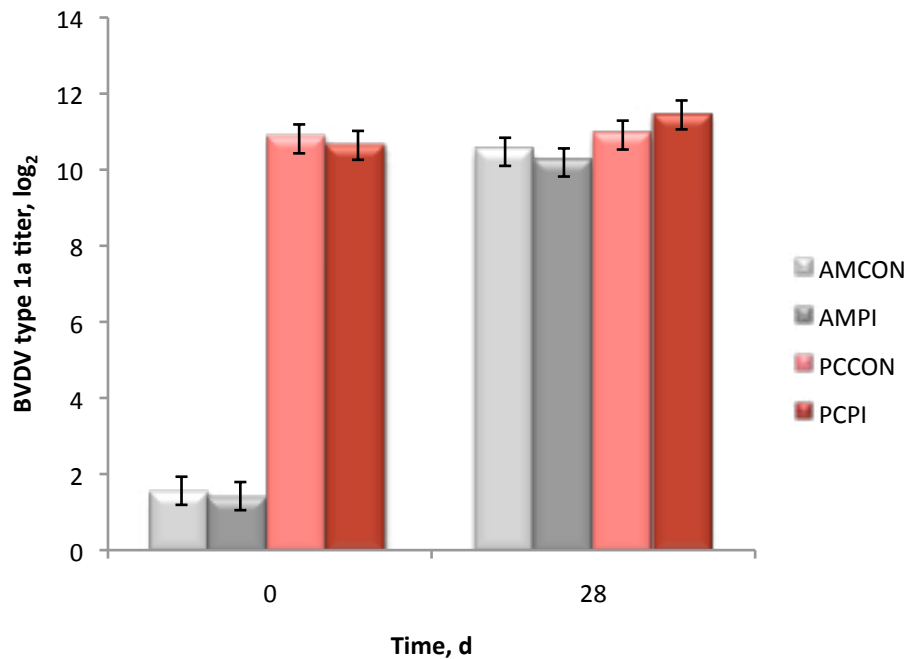
Fig. 1. Effect of pre-arrival management and PI-BVDV exposure on bovine viral diarrhea virus type Ia antibody titer level. AMCON = Auction Market, Control, AMPI = Auction Market, Exposed to PI-BVDV calf, PCCON = Preconditioned, Control, and PCPI = Preconditioned, Exposed to PI-BVDV calf. Source, *P* < 0.001; Day, *P* < 0.001; Treatment × Day, *P* < 0.001.
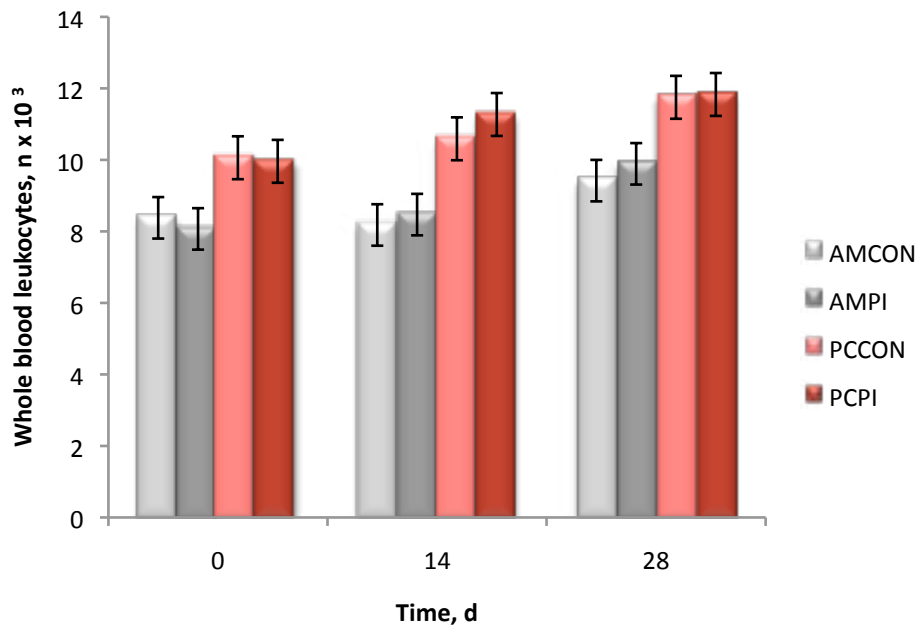
Fig. 2. Effect of pre-arrival management and PI-BVDV exposure on the total concentration of circulating leukocytes. Source, *P* < 0.001; Day, *P* < 0.001.
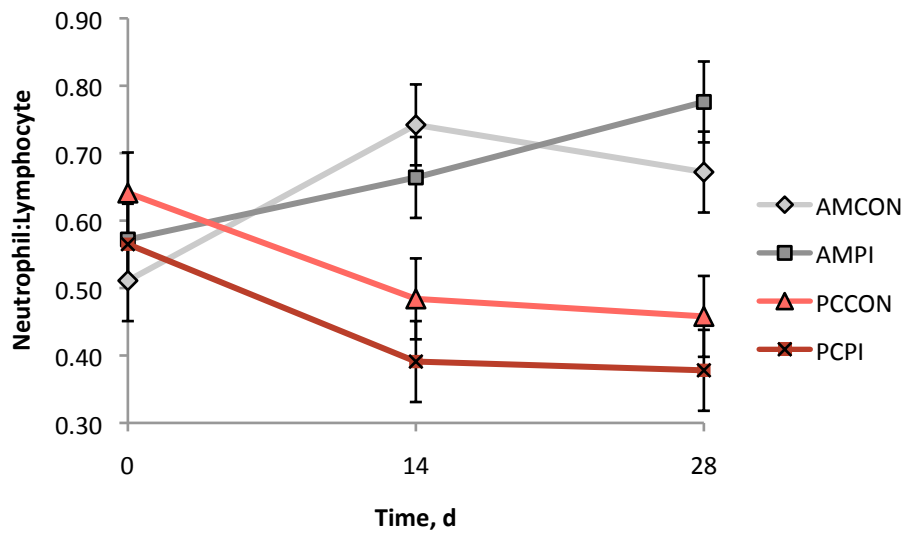
Fig. 3. Effect of pre-arrival management and PI-BVDV exposure on the ratio of circulating neutrophil and lymphocyte concentrations. Source, *P* < 0.001; Treatment × Day, *P* < 0.001.
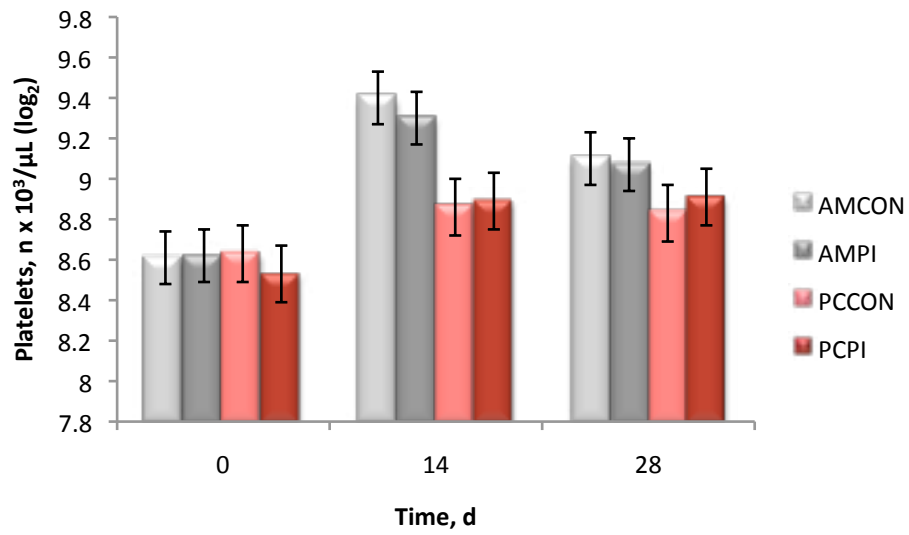
Fig. 4. Effect of pre-arrival management and PI-BVDV exposure on the concentration of circulating platelets. Source, *P* < 0.001; Day, *P* < 0.001.

Influence of Supplemental Calcium and Feeding Fish Oil on Fatty Acid Composition in Muscle of Grazing Calves

J. T. Richeson[1], E. B. Kegley[1], D. L. Galloway[1], and T. J. Wistuba[2]

Story in Brief

To determine the effects of supplemental Ca on the absorption of long-chain fatty acids from fish oil fed to growing cattle, crossbred beef calves (n = 64, initial BW = 548 ± 6.6 lb) were stratified by source, previous vaccination treatment, and sex before being assigned randomly to 16 pens. Pens were assigned randomly to receive 1 of 4 supplements: 2 levels of fish oil (0 or 3%) and 2 levels of Ca (0.5 or 2%) in a 2 × 2 factorial arrangement of treatments. Data were analyzed with pen as the experimental unit and preplanned contrasts were the main effects of fish oil and Ca, and their interaction. Calves grazed mixed-grass pastures during the 84-d study and were offered 4 lb/d of the appropriate grain supplement. Longissimus muscle biopsies were obtained at the conclusion of the trial to determine differences in fatty acid composition. Although, calves receiving fish oil tended ($P = 0.10$) to weigh less on d 28, there were no effects of fish oil or Ca on performance ($P \geq 0.15$). Concentrations of some individual fatty acids in the muscle were greater in fish oil supplemented calves; yet, the ratio of omega-6:omega-3 fatty acids was not improved, nor were the percentages of total saturated, monounsaturated, and polyunsaturated fatty acids altered by any treatment. Results suggest that dietary fish oil supplementation of grazing calves may increase several muscle fatty acid concentrations with minimal impact on cattle performance; however, dietary Ca concentration had little to no impact on muscle fatty acid composition.

Introduction

Fat supplementation in beef cattle diets is used primarily as a method to increase dietary energy level because of the high caloric density associated with fats. Previous research on fat supplemented diets demonstrated positive associative effects with the addition of Ca (Grainger et al., 1961; Henderson, 1973), perhaps due to the formation of Ca soaps within the rumen. Conversely, Zinn and Shen (1996) reported supplemental Ca had no affect on digestibility, whereas Bock et al. (1991) actually observed negative associative effects of increasing Ca level.

Lipids can be classified as saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), and monounsaturated fatty acids (MUFA) with SFA being abundant in beef because of extensive biohydrogenation of lipids occuring in the rumen. Recommendations by the American Heart Association encourage humans to reduce their consumption of SFA while increasing the amount of PUFA and MUFA. Thus, an opportunity exists to modify the fatty acid composition of beef to have greater levels of unsaturated fats, specifically the omega-3 (n3) fatty acids which are abundant in fish oil. Therefore, the objective of this study was to determine the effects of supplemental Ca on the absorption of fatty acids from fish oil fed to grazing beef cattle.

Experimental Procedures

Sixty-four crossbred beef calves (36 heifers and 28 steers, average initial BW = 548 ± 6.6 lb) were stratified by source, gender, and vaccination treatment on a previous study, and assigned randomly to 1 of 16 grass paddocks (4 calves/pen). Pens were assigned randomly to 1 of 4 supplementation treatments (4 pens/treatment) of a 2 × 2 factorial arrangement, containing 0 or 3% fish oil and 0.5 or 2% Ca (Table 1). Calves grazed 1.1-acre, mixed-grass paddocks and were offered 4 lb/d of the appropriate supplement for the 84-d study. Grab samples of the pastures were taken monthly and the forage averaged, on a DM basis, 10.4% CP, 41.3% ADF, 67.9% NDF, and 8.1% ash.

Calves were weighed on 2 consecutive days, beginning at 0800, at the start (July 1) and end of the study, and these weights were averaged to obtain the initial and final weights. Interim weights were also taken on d 28 and 56. All cattle were treated with an anthelmintic (Ivomec Eprinex Pour on, Merial, Duluth, Ga.) on d 0.

Muscle biopsies from all calves were obtained at the conclusion of the study to determine fatty acid concentrations. For the muscle biopsies, cattle were administered 2 mL of a local anesthetic (lidocaine) near the 1-in incision site at the intersection of the 12th and 13th rib. A core sampler was used to extract muscle tissue from the longissimus muscle. Each sample was immediately flash frozen in liquid N_2 and stored for subsequent analysis.

Samples were freeze dried before 80-mg subsamples (duplicate analyses) were subjected to transesterification. An internal standard (glyceryl tritridecanotate [13:0]) was prepared in hexane, and 1 mL was added to each 16 × 125-mm screw-cap tube. Hexane was evaporated leaving the internal standard before samples were added to tubes and incubated in 2.0 mL of 0.2 M methanolic KOH at 122 °F for 30 min with vortex-mixing 2 to 3 times/min until samples were dissolved (Murrieta et al., 2003). Tubes were allowed to cool to room temperature, and 1 mL of saturated NaCl was added to each tube. Two milliliters of hexane were added to the tubes, and tubes were vortexed and centrifuged for 5 min at $1100 \times g$ to separate the phases.

Fatty-acid methyl esters were transferred to gas-liquid chromatography vials that contained a 1.0-mm bed of anhydrous sodium sulfate. Separation of fatty-acid methyl esters was achieved by gas-liquid chromatography (HP model 6890 Series II, with HP 7673 injector [Agilent Technologies, Inc., Wilmington, Del.] and an automatic sample injector) with a 100-m capillary column (Supelco 2560 Fused Silica Capillary column; Supelco Park, Bellefonte, Pa.) and He as a carrier gas at 0.5 mL/min. Oven temperature was maintained at 347 °F for 40 min and then ramped at 18 °F/min to 464 °F. Injector and detector temperatures were 482 °F, and identification of fatty-acid methyl esters peaks was accomplished using purified standards (Supelco; Nu-Chek Prep, Elysian, Minn.; and Matreya, Pleasant Gap, Pa.).

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Data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS Inst., Inc., Cary, N.C.), with pen as the experimental unit. Pre-planned orthogonal contrasts evaluated main effects of Ca, fish oil, and their interaction

Results and Discussion

Calves receiving fish oil tended ($P = 0.10$) to weigh less on d 28 (Table 2), but no other performance differences ($P ≥ 0.15$) were observed among supplementation treatments. In this study, supplements were formulated to be isonitrogenous and isocaloric (through the addition of cottonseed hulls to the fish oil supplemented diets); therefore, it was not anticipated that there would be any treatment effects on growth. Because there were no supplement refusals observed throughout the study, the tendency for a negative effect of fish oil supplementation on BW at d 28 could reflect a decrease in forage intake by the fish oil-fed cattle. However, this effect was transitory and cattle on all treatments weighed the same at the conclusion of the 84-d trial.

Fish oil supplementation increased the concentrations (g/100 g total fatty acid) of the SFA, palmitic (C16:0; $P = 0.001$) and arachidic (C20:0; $P = 0.03$), but concentrations of stearic acid (C18:0; $P = 0.07$) tended to be decreased by fish oil supplementation. There was no effect of fish oil supplementation on the percentage of total SFA ($P = 0.42$), or the sum of all unsaturated fatty acids ($P = 0.86$). Although the percentages of individual unsaturated fatty acids were impacted, palmitelaidic acid (C16:1*trans*; $P < 0.001$), total C18:1*trans* ($P < 0.001$), 11-octadecenoic acid (C18:1*cis*11; $P = 0.01$), eicosenoic acid (C20:1*cis*11; $P = 0.01$), and octadecadienoic acid (CLA*trans*10,*cis*12; $P < 0.001$) concentrations were increased in the fish oil-supplemented cattle. Conversely, the proportion of oleic acid (C18:1*cis*9; $P = 0.08$) tended to be reduced by fish oil supplementation. Neither the total omega-6 or omega-3 fatty acid concentrations, nor the omega-6:omega-3 ratio were affected ($P ≥ 0.14$) by fish oil supplementation. However, the total *trans* fatty acids were increased ($P < 0.001$) in muscle of the fish oil-supplemented cattle. Fish oil not only contains longer chain (20+) fatty acids, but

also contains saturated and unsaturated fatty acids of 16 and 18 chain length. The addition of this fish oil to the diet could explain the increase in concentrations of those fatty acids in the longissimus muscle. Even though the fish oil would not be expected to have those fatty acids in the *trans* form, the initial step in rumen biohydrogenation of an unsaturated fatty acid is the conversion of cis isomers to *trans* isomers.

The only effect of Ca level ($P = 0.02$) and a tendency for a Ca × fish oil interaction ($P = 0.08$) were observed for palmitic acid (C16:0) with the greatest concentration of C16:0 found in the cattle fed fish oil with 0.5% Ca. Calculated total dietary Ca concentrations, using an estimated forage intake, were 0.5 vs. 0.9% for the low- and high-Ca diets, respectively. Results indicate that altering the Ca concentration of the diet of cattle fed fish oil within this range had a minimal impact on biohydrogenation activity within the rumen. The formation of Ca soaps apparently did not occur to a degree which inhibited the biohydrogenation of the unsaturated fatty acids found in fish oil, and thus, did not alter muscle fatty acid concentrations.

Implications

Dietary fish oil supplementation of grazing calves increased several fatty acid concentrations; however, the ratio of omega-6:omega-3 was not altered, nor were the percentages of total saturated, monounsaturated, or polyunsaturated fatty acids affected. Moreover, dietary Ca level had little impact on percentages of fatty acids in the longissimus muscle of grazing calves.

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Table 1. Ingredient composition of supplements fed (4 lb/d) to cattle (as fed basis).

	Fish oil, %			
	0		3	
Ca, %	0.5	2	0.5	2
	----------------------------------%----------------------------------			
Corn, cracked	73.79	74.53	64.07	64.65
Soybean meal	15.9	16.2	17.3	17.6
Molasses	3.0	3.0	3.0	3.0
Dicalcium phosphate	0.32	0.31	0.39	0.39
Limestone	0.73	4.2	0.68	4.1
Salt, white	1.5	1.5	1.5	1.5
Rumensin 80[a]	0.06	0.06	0.06	0.06
Trace mineral premix[b]	0.1	0.1	0.1	0.1
Vitamin premix[c]	0.1	0.1	0.1	0.1
Cottonseed hulls	4.5	0	9.8	5.5
Fish oil	0	0	3.0	3.0

[a]Provided 200 mg monensin/d.
[b]Provided 216 mg Zn (as Zn sulfate), 144 mg Mn (as Mn sulfate), 72 mg Cu (as Cu sulfate), 0.9 mg Co (as Co carbonate), 3.6 mg I (as Ca iodate), and 1.1 mg Se (as Na selenite)/d.
[c]Provided 11,200 IU vitamin A, 2,240 IU vitamin D, and 29 IU vitamin E/d.

Table 2. Effect of supplemental fish oil with low vs. high calcium levels on growth performance of grazing cattle.

	Fish oil, %[a]					P-values		
	0		3					
Ca, %[b]	0.5	2	0.5	2	SEM	Fish oil	Ca	Interaction
Body weight, lb								
Initial	551	550	549	542	4.9	0.33	0.52	0.53
D 28	594	602	590	583	6.3	0.10	0.94	0.27
D 56	636	642	634	627	6.5	0.22	0.87	0.33
Final	681	696	685	678	8.1	0.41	0.64	0.19
ADG, lb								
D 0 to 28	1.6	1.8	1.5	1.5	0.21	0.30	0.54	0.49
D 0 to 56	1.5	1.6	1.5	1.5	0.11	0.58	0.73	0.59
D 0 to 84	1.6	1.7	1.6	1.6	0.08	0.79	0.33	0.28

[a]Fish oil supplemented at 0 or 3% of a grain supplement that was fed at 4 lb/d.
[b]Ca supplemented at 0.5 or 2% of a grain supplement that was fed at 4 lb/d.

Table 3. Effect of supplemental fish oil with low vs. high calcium levels on concentrations of fatty acids (g/100 g total fatty acid) in muscle of grazing cattle.

| | Fish oil, %[a] | | | | | P-values | | |
| | 0 | | 3 | | | | | |
Ca, %[b]	0.5	2	0.5	2	SEM	Fish oil	Ca	Interaction
Saturated fatty acids								
Total	51.6	52.8	51.8	50.5	1.20	0.42	0.94	0.33
C10:0	0.06	0.06	0.05	0.06	0.006	0.27	0.51	0.71
C12:0	0.19	0.19	0.15	0.19	0.021	0.44	0.27	0.46
C14:0	4.7	4.7	4.6	4.6	0.21	0.55	0.95	0.88
C15:0	1.04	1.09	1.04	1.14	0.067	0.73	0.29	0.65
C16:0	25.2^y	25.0^y	27.3^x	25.8^y	0.34	0.001	0.02	0.08
C17:0	1.41	1.47	1.4	1.42	0.034	0.38	0.26	0.57
C18:0	18.8	20.1	17.1	17.1	1.20	0.07	0.59	0.62
C20:0	0.14	0.15	0.20	0.21	0.025	0.03	0.80	0.97
Monounsaturated fatty acids								
Total	40.6	39.3	39.7	40.2	1.22	0.98	0.74	0.47
C14:1	0.8	0.8	0.7	0.8	0.11	0.43	0.76	0.46
C16:1*trans*	0.75	0.78	0.86	0.89	0.024	<0.001	0.27	0.98
C16:1*cis*	3.3	3	3.4	3.5	0.31	0.36	0.75	0.65
C17:1*trans*	0.17	0.17	0.15	0.17	0.012	0.53	0.53	0.35
C18:1*trans*	5.9	6	7.5	7.8	0.31	<0.001	0.57	0.69
C18:1*cis*9	28.8	27.7	26.1	26.1	1.13	0.08	0.64	0.65
C18:1*cis*11	0.81	0.79	0.90	0.88	0.032	0.01	0.63	0.91
C20:1	0.08	0.06	0.11	0.11	0.015	0.01	0.58	0.47
Polyunsaturated fatty acids								
Total	2.8	2.8	2.9	3.1	0.15	0.19	0.31	0.62
C18:2n6	0.98	1.06	1.19	1.07	0.078	0.20	0.74	0.24
C18:2*cis*9*trans*11	1.3	1.3	1.1	1.4	0.12	0.80	0.34	0.28
C18:2*trans*10*cis*12	0.01	0.02	0.08	0.09	0.009	<0.001	0.52	0.90
C18:3	0.42	0.46	0.46	0.46	0.019	0.32	0.40	0.32
C20:4n6	0.01	0.02	0.01	0.05	0.020	0.44	0.34	0.33
C20:5	ND^c	ND	ND	0.02	0.008	0.12	0.12	0.12
C22:5	0.03	0.02	0.03	0.06	0.016	0.14	0.45	0.22
Total unsaturated	43.4	42.1	42.6	43.3	1.31	0.86	0.85	0.46
Total omega3	0.45	0.48	0.49	0.55	0.036	0.14	0.27	0.71
Total omega6	1.01	1.07	1.2	1.12	0.087	0.19	0.94	0.41
Omega6:omega3	2.3	2.3	2.4	2.1	0.16	0.96	0.26	0.32
Total trans	8.2	8.2	9.7	10.4	0.23	<0.001	0.16	0.24

[a]Fish oil supplemented at 0 or 3% of a grain supplement that was fed at 4 lb/d.
[b]Ca supplemented at 0.5 or 2% of a grain supplement that was fed at 4 lb/d.
[c]ND = none detected.
^{xy}Means, in the same row, without a common superscript differ ($P < 0.08$).

Benefits of Natural Branded vs. Conventionally-Fed, Commodity Beef

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Story in Brief

Beef ribeye rolls (IMPS#112A) were purchased from 5 natural-branded programs and 2 commodity beef programs to discern differences in the fresh and cooked quality attributes. Ribeye rolls were shipped to the University of Arkansas Red Meat Abattoir, where they were aged at 36 °F for 14 d from the box date. Ribeye rolls were cut into 1-in.-thick steaks, and steaks from each ribeye roll were allowed to bloom for 30 min before measuring instrumental color. A total of 6 steaks were cooked to an endpoint temperature of 162 °F, with 3 steaks used to measure cooking loss and Warner-Bratzler shear force (WBSF), whereas the other 3 were cut into 0.5-in × 0.5-in × one inch cubes and served warm to consumers who ate beef at least 3 times weekly. Neither muscle pH, moisture content, intramuscular fat content, instrumental color, nor fatty acid composition differed ($P \geq 0.15$) between steaks from commodity beef and steaks from natural beef programs. Steaks from the commodity programs had greater ($P = 0.002$) cooking losses and received higher ($P = 0.050$) juiciness ratings than natural steaks, but there were no ($P \geq 0.272$) differences in WBSF or consumer ratings for tenderness, texture, flavor intensity, beef flavor and overall impression between the two beef programs. The results from this study indicated that, with the exception of price, there are little to no differences between commodity beef and natural beef programs.

Introduction

Growing concerns about animal welfare, food safety and nutrition have increasingly focused public interest on food production methods. This is particularly important when applied to common beef production practices, such as the use of antibiotics, growth-promoting hormones, and animal by-products used in feed, leading some to advocate purchase and consumption of "natural" beef products. For many, "safer" and "healthier" are synonymous with "natural" and "organic." For the average consumer, there may be little distinction between "organic" and "natural" beef. According to an article in the Chicago Tribune, Eng (2009) reported that "the U.S. natural food market grew by 10% to 12.9 billion from 2007 to 2008" and that "all-natural was the second most common claim on food products launched in 2008.".

The term "natural" is not clearly defined or regulated; therefore, "natural" beef will often provide information on the label, such as free of antibiotics, growth hormones/anabolic steroid implants, and an all-vegetarian diet. Organic beef, on the other hand, is strictly regulated by the USDA and cattle must be raised on certified organic pastures and fed only certified organic grains and grasses. So, the objective of this study was to compare the quality characteristics of fresh and cooked steaks from natural-branded beef to that of commodity beef.

Experimental Procedures

Beef ribeye rolls (IMPS#112A) were purchased from 5 natural-branded programs (designated N1, N2, N3, N4, and N5) and 2 beef packers (designated C1 and C2), and shipped under refrigeration to the University of Arkansas Red Meat Abattoir. The natural-fed beef requirements were all vegetarian diet, no hormone implants, and no dietary antibiotics. They were aged at 36 °F for 14 d from the box date and at completion of aging period, ribeye rolls were removed from vacuum-packages and the pH was measured with the Testo hand-held, stab pH meter. Ribeye rolls were trimmed free of external fat, faced, and, beginning at the posterior end, nine 1-in-thick steaks were cut perpendicular to the length of the ribeye roll. The 8 steaks were assigned randomly to: 1) fresh instrumental color and subsequent Warner-Bratzler shear force (n = 3/ribeye); 2) consumer sensory panels (n = 3/ribeye); and 3) freeze-dried for fatty acid analysis (n = 2/ribeye).

A timer was started immediately after each steak was cut and 3 randomly selected steaks were allowed to bloom for 30 min at 39 °F before measuring instrumental color (L⋆, a⋆, b⋆, C⋆, hue angle) with a Hunter MiniScan XE (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, Va., USA). Then, 3 additional randomly assigned steaks were vacuum-packaged and frozen at -4 °F for WBSF.

Steaks were thawed at 39 °F for 16 h, removed from the vacuum-packages, and surface moisture was blotted dry before recording the pre-cooked steaks weight. Then, steaks were cooked on a Presto electric, flat-top griddle to an internal temperature of 162 °F, and steaks were turned every 4 min until the internal temperature was reached. Steaks were then blotted dry on paper towels and re-weighed to calculate cooking loss percentages. After cooking, steaks were allowed to equilibrate to room temperature (70 °F) before six 0.5-in-diameter cores were removed parallel to the muscle fiber orientation. Each core was sheared once with a Warner-Bratzler compression-type shear attachment on an Instron Universal testing machine (Instron Corp., Canton, Mass., USA), equipped with a 110-lb load cell and a cross-head speed of 200 mm/min for determination of instrumental tenderness.

Steaks for consumer panels were thawed overnight at 34 °F and cooked as described previously for WBSF determination. Immediately after cooking, the spinalis dorsi was removed from each steak, the longissimus muscle (LM) was cut into uniform, bite-size pieces (approximately 0.5 in × 0.5 in × one inch), and steak pieces were held in food warmers until served to consumers (*n* = 125). Consumers were selected from a pool of over 4,000 based on an e-mail questionnaire requesting consumers that ate beef 3, or more, times each week. Consumers were scheduled for 20-min sessions, in which 8 consumers were seated in climate-controlled booths with lighting adjusted so that the degree of doneness of the steak-pieces could not be seen. Eight steak-pieces were presented one at a time on small, clean, paper plates to the consumers in random order. Consumers rated each piece on nine-point hedonic scale, with 1 =

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extremely dislike and 9 = extremely like. In addition, consumers were asked to rate beef flavor and juiciness on a "just-about-right" scale, with 1 = too little, 2 = just about right, and 3 = too much.

Approximately 5g of LM from additional steaks were weighed and placed in 30-mL beakers, and beakers were freeze-dried for 60 h. Duplicate 30-mg freeze-dried samples were subjected to direct transesterification by incubating in 2.0 mL of 0.2 *M* methanolic potassium hydroxide in 16 × 125-mm screw-capped tubes at 122 °F for 30 min with vortex mixing 2 to 3 times/min until tissues were dissolved (Murrieta et al., 2003). Tubes were allowed to cool to room temperature, and 1 mL of saturated sodium chloride was added to each tube. A 1-mL quantity of a hexane solution containing an internal standard [glyceryl tridecanoic acid (13:0)] was added to each tube, and the hexane was evaporated before tubes were vortexed and subsequently centrifuged for 5 min at $1100 \times g$ and 68 °F to separate phases. A portion of the hexane layer containing the fatty acid methyl esters was transferred to gas-liquid chromatography (GLC) vials that contained a 1.0-mm bed of anhydrous sodium sulfate. Separation of fatty acid methyl esters was achieved by GLC and He as the carrier gas. Oven temperature was maintained at 347 °F for 35 min, increased at 41 °F/min to 419 °F, and then increased at 50 °F/min to 455°F, whereas injector and detector temperatures were maintained at 482°F. Identification of peaks was accomplished by using purified standards obtained from Nu-Chek Prep (Elysian, Minn.), Matreya (Pleasant Gap, Pa.), and Supelco (Bellefonte, Pa.).

Data were analyzed as a completely randomized design, with ribeye roll as the experimental unit. The ANOVA was generated using the mixed model procedure of SAS (SAS Institute, Inc., Cary, N.C.), with the "brand" as the fixed effect in the model. Least squares means were calculated and separated using pair-wise t-tests (PDIFF option) when a significant ($P \leq 0.05$) *F*-test was noted. Additionally, a contrast statement was included to determine the difference between natural-branded and commodity beef.

Results and Discussion

Commodity vs. Natural Differences. There were no ($P \geq 0.15$) differences in LM pH, moisture content, intramuscular fat content, fresh instrumental color, and fatty acid composition between steaks from commodity or natural-branded programs (Table 1). Steaks from commodity ribeye rolls had greater ($P = 0.002$) cooking losses than steaks from natural programs and consumers rated steaks from commodity ribeye rolls juicier ($P = 0.050$) than steaks from natural-branded ribeye rolls (Table 2). In contrast, WBSF values and consumer panel ratings for tenderness, texture, flavor intensity, beef flavor, and overall impression were similar ($P \geq 0.27$) between the 2 beef purchasing programs.

Brand Differences. The pH of ribeye rolls from C1 and N3 was greater ($P < 0.05$) than the other commodity and natural programs (Table 1). Steaks from N1 and N5 had greater ($P < 0.05$) moisture contents than steaks from N2, N3, and N4, whereas C1 and C2

steaks had greater ($P < 0.05$) moisture percentages than N4 steaks. Furthermore, the intramuscular fat (IMF) content of N5 steaks was greater ($P < 0.05$) than that of C1, N2, N3, and N4 steaks.

Steaks from N1 and N5 were lighter (higher L* values, $P < 0.05$) than steaks from C2 and N3 (Table 1). In addition, steaks from N2 and N5 were redder (higher a* values, $P < 0.05$) than steaks from C1, C2, and N3, whereas steaks from C2, N1, N2, N4, and N5 were more yellow (higher b* values, $P < 0.05$) than steaks from C1 and N3.

The steaks cut from N1 ribeye rolls had a greater ($P < 0.05$) proportion of saturated fatty acids (SFA) than steaks cut from C1, C2, N3, N4, and N5 ribeye rolls (Table 1). Steaks from C2 had greater (P < 0.05) proportions of monounsaturated fatty acids (MUFA) than steaks from C1, N1, and N2, and the proportion of MUFA was greater (P < 0.05) in N5-steaks than C1- and N1-steaks. Interestingly, the proportion of polyunsaturated fatty acids (PUFA) was greater (P < 0.05) in steaks from C1 and N3 than steaks from either N1 or N5.

Steaks from C1 and C2 had greater ($P < 0.05$) cooking loss percentages than steaks from N2 and N5 (Table 2). Steaks from N1, N2, N5, and C2 had lower ($P < 0.05$) WBSF values than steaks from C1; however, consumers rated steaks from N5 to be more ($P < 0.05$) tender than steaks from C1, C2, N1, and N3, and rated N4-steaks the toughest ($P < 0.05$) of all steaks tested. Consumers also rated the texture and flavor intensity of N5-steaks higher ($P < 0.05$) than C1, C2, N1, and N3, and steaks from N4 ribeye rolls received the lowest ($P < 0.05$) consumer ratings for texture and flavor intensity. Even though consumers failed ($P = 0.06$) to note differences among steaks for beef flavor on the just-about-right scale, they did give N5-steaks higher ($P < 0.05$) juiciness scores than N2-, N3-, and N4-steaks. Consumers' scores for overall impression were greater ($P < 0.05$) for steaks cut from N5 ribeye rolls than steaks from C2, N1, and N4 ribeye rolls, and steaks from C1, N2, and N3 received greater ($P < 0.05$) overall impression scores than N4-steaks.

While research on natural-branded beef is limited, consumers have the perception that natural and organic equate certain health benefits. These perceived benefits include lower amounts of SFA and higher amounts of PUFA (Nielsen and Thamsborg, 2005). The current study shows that natural-branded beef was higher in SFA which conflicts with current consumer perceptions of the health benefits of consuming all-natural products. The results also showed that consumers could not distinguish natural beef from commodity beef based solely on palatability. In conclusion, the results of the current study would indicate a greater amount of variation in the fresh and cooked quality attributes among purchasing brands than between natural and commodity beef.

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Table 1. Differences in fresh beef quality characteristics between conventionally-produced, commodity beef and naturally-produced, branded beef.

	Commodity beef (C)		Natural beef (N)					SE	C vs.N[1]
	C1	C2	N1	N2	N3	N4	N5		
No. of ribeye rolls	10	10	10	10	10	10	10		
pH	5.6^a	5.4^b	5.4^b	5.5^b	5.6^a	5.5^b	5.5^b	0.04	0.505
Moisture content, %	78.3^{ab}	78.3^{ab}	78.8^a	77.7^{bc}	77.4^{bc}	77.0^c	78.9^a	0.40	0.223
IMF, %[2]	4.37^{bc}	4.84^{abc}	4.94^{ab}	4.63^{bc}	4.14^c	4.33^{bc}	5.46^a	0.32	0.672
Lightness (L*)[3]	44.7^{ab}	43.6^b	46.7^a	45.8^{ab}	44.1^b	45.6^{ab}	46.4^a	0.97	0.162
Redness (a*)[3]	27.6^b	28.4^b	28.5^{ab}	28.5^a	27.8^b	28.5^{ab}	28.8^a	0.41	0.463
Yellowness (b*)[3]	19.6^b	20.6^a	20.8^a	20.7^a	19.7^b	20.6^a	21.0^a	0.69	0.149
Total SFA, %[4]	45.31^b	44.13^b	47.55^a	45.89^{ab}	44.99^b	44.25^b	44.73^b	0.758	0.217
Total MUFA, %[4]	44.79^{cd}	46.90^a	44.32^d	45.06^{bcd}	45.29^{abcd}	46.12^{abc}	46.59^{ab}	0.671	0.494
Total PUFA, %[4]	6.20^a	5.26^{ab}	4.55^c	5.27^{ab}	6.19^a	5.82^{ab}	4.94^{bc}	0.373	0.235

[1]Probability value for the comparison of conventionally-produced, commodity beef to naturally-produced, branded beef.
[2]Intramuscular fat content.
[3]L* values are a measure of darkness to lightness (a greater L* value indicates a lighter color); a* values measure the green-to-red color axis (a greater positive a* value indicates a redder color); and b* values measure the blue-to-yellow color axis (a greater positive b* value indicates a more yellow color).
[4]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; and PUFA = polyunsaturated fatty acids.
[a-d]Within a row, least squares means lacking a common superscript letter differ, $P < 0.05$.

Table 2. Differences in Warner-Bratzler shear force and consumer panel ratings between conventionally-produced, commodity beef and naturally-produced, branded beef.

	Commodity beef (C)		Natural beef (N)					SE	C vs.N[1]
	C1	C2	N1	N2	N3	N4	N5		
No. of steaks	10	10	10	10	10	10	10		
Cooking loss, %	21.2^a	20.8^a	19.2^{ab}	17.7^b	18.6^{ab}	19.3^{ab}	17.3^b	1.02	0.002
Shear force, kg	2.96^a	2.34^b	2.31^b	2.21^b	2.77^{ab}	2.71^{ab}	2.35^b	1.696	0.565
Tenderness[2]	6.2^{bc}	6.0^c	6.1^{bc}	6.4^{ab}	6.2^{bc}	5.4^d	6.9^a	0.17	0.352
Texture[2]	6.5^b	6.3^b	6.4^b	6.7^{ab}	6.4^b	5.8^c	6.9^a	0.16	0.709
Flavor intensity[2]	6.4^{ab}	6.2^b	6.2^b	6.6^a	6.5^{ab}	6.2^b	6.8^a	0.15	0.272
Beef flavor[3]	2.7	2.7	2.6	2.7	2.7	2.7	2.8	0.06	0.811
Juiciness[3]	2.7^{ab}	2.7^{abc}	2.6^{abcd}	2.6^{bcd}	2.5^{cd}	2.5^d	2.8^a	0.06	0.050
Overall impression[2]	6.6^{abc}	6.3^{cd}	6.4^{bcd}	6.8^{ab}	6.5^{abc}	6.1^d	7.0^a	0.16	0.318

[1]Probability value for the comparison of conventionally-produced, commodity beef to naturally-produced, branded beef.
[2]Hedonic, 9-point scale, with 1 = extremely dislike to 9 = extremely like.
[3]Just-about-right scale, with 1 = too little, 2 = just about right, and 3 = too much.
[a-d]Within a row, least squares means lacking a common superscript letter differ, $P < 0.05$.

The Effects of Cetylpyridinium Chloride, Trisodium Phosphate, Potassium Lactate, Sodium Metasilicate, or Water as Multiple Application Antimicrobial Interventions on Microbiological Characteristics of Beef B*iceps Femoris* Muscles

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Story in Brief

Biceps femoris muscles (n = 12) were cut into three subsections (n = 36 subsections) and each subsection was brush inoculated with *Escherichia coli* (EC; ATCC # 11775) and *Salmonella* Typhimurium (ST; ATCC # 1769NR) at 10^7 log CFU/mL. Subsections were spray treated with either: (1) municipal purified water; (2) 3% potassium lactate (KL); (3) 4% sodium metasilicate (NMS); (4) 0.5% cetylpyridinium chloride (CPC); (5) 10% trisodium phosphate (TSP); or (6) untreated inoculated control. Each subsection was cut into three individual steaks (n = 105) allowing fifteen steaks per treatment per day. The individual steaks were spray treated again with the same treatments allotted at the sub-primal level for a multiple intervention application. Steaks were placed on foam trays with absorbent pads and overwrapped with polyvinyl chloride film. Steaks were sampled for EC, ST, coliforms (CO), and aerobic plate count (APC) on days 1, 2, 3, 5, and 7 of display. On day 2, APC counts were reduced ($P < 0.05$) by CPC and NMS vs. inoculated control, water, KL, and TSP. On day 7, ST counts were reduced ($P < 0.05$) by CPC vs. inoculated control, water, KL, NMS, and TSP. Results suggest the spray treatment of *biceps femoris* muscles at the sub-primal level, and again at the individual steak level with the same allotted treatment may enhance shelf life initially, and these treatment effects may or may not persist through 7 days of display.

Introduction

Consumer demand in the food industry is for fresh product, yet fresh products have a short shelf life. Within the meat industry, challenges continue to emerge to control traditional as well as new, emerging, or evolving pathogenic microorganisms. Meat safety regulatory systems are continually looking for new and improved techniques to minimize the effects of pathogenic bacteria growth in final meat products. Throughout the processing and handling steps, meat provides a good source of nutrients for microbial growth and further contamination. Currently, the meat industry is investigating the use of antimicrobials as ingredients at the end of the production line to enhance the product microbial safety. Many processes in the industry use a single-step method of application of antimicrobials. Although antimicrobials may reduce the microbial load of a meat product, the ingredient may pose a negative impact on quality characteristics such as color or aroma. Characteristics leading to consumer rejection of fresh meat products begin with appearance and aroma deterioration. The objective of this study was to evaluate the use of cetylpyridinium chloride, trisodium phosphate, potassium lactate, sodium metasilicate, or water as multiple antimicrobial interventions on reducing *Escherichia coli* and *Salmonella* Typhimurium populations in pre-inoculated beef steaks.

Experimental Procedures

Inoculation Process. The first step in constructing the bacterial cocktail was to use 0.1 mL *Escherichia coli* (EC; ATCC # 11775) and add it to 40 ml of Brain Heart Infusion (BHI) (n = 40 total tubes). Following that, 0.1 mL *Salmonella* Typhimurium (ST; ATCC # 1769NR) was added to 40 mL of BHI with nalidixic acid (n = 40 total tubes). The tubes were then incubated for 18 hours at 37 °C. Following the incubation period, the tubes were then centrifuged at 3649 × g for 20 minutes at 37 °C. Following centrifugation, the cocktail was resuspended in 0.1% buffered peptone water, and then the bacterial cocktail (EC and ST log 10^7 CFU, 3600 ml) was stored at 4 °C. Beef *biceps femoris* muscles (n = 12) were cut into three subsections (n = 36 subsections), and each subsection was the placed in a sterile bag and left overnight for further bacterial attachment.

Antimicrobial Treatment Preparation. The antimicrobial treatments of 0.5% cetylpyrinidium chloride (Cecure®, Safe Foods Cooperation, Little Rock, Ark.), 10% (w/v) trisodium phosphate (Trisodium phosphate anhydrous (FG), ICL performance products, St. Louis, Mo.), 3% potassium lactate (UltraLac KL – 60, Hawkins Inc., Minneapolis, Minn.), and 4% sodium metasilicate (Metso Pentabead® 20, PQ Corporation, Valley Forge, Pa.) were prepared by mixing appropriate amounts of municipal purified water.

Treatment Application. Inoculated subsections (n = 5 subsections per treatment) (subsections were approximately 4-5 in. by 11-12 in.) were spray (SureSpray™ Sprayer Deluxe, 10 sec for 50 mL per side, therefore speed was 5 mL/sec) treated with either: (1) municipal purified water; (2) 3% potassium lactate (KL); (3) 4% sodium metasilicate (NMS); (4) 0.5% cetylpyridinium chloride (CPC); (5) 10% trisodium phosphate (TSP); or (6) untreated inoculated control. Each subsection was cut into three individual steaks (n = 105) allowing fifteen steaks per treatment per day. The individual steaks were spray treated again with the same treatments allotted at the sub-primal level for a multiple intervention application. Steaks were placed on styrofoam trays with absorbent pads and overwrapped with polyvinyl chloride film (O_2 transmission rate = 14,000 cc/mm²/24h/1 atm; Koch Supplies, Inc., Kansas City, Mo.). The steaks were stored at 4 °F under 1630 lux of deluxe warm white fluorescent lighting (Phillips Inc., Somerset, N.J.).

Microbiological Analysis. On days 1, 2, 3, 5, and 7 of simulated retail display, the microbial enumeration for each steak was carried out by aseptically removing 2.5 g from the surface using a sterile scalpel and forceps as described by Venturini et al. (2006). The 2.5 g samples were placed in sterile whirl pack bags (Nasco, Ft Atkinson, Wis.) separately and 22.5 mL of 0.1% buffered peptone water was added, and the samples were homogenized for 2 minutes at normal

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Table 2. Effect of antimicrobial sequential treatment at sub-primal and steak levels by day of display interaction on least squares means (±SE) log CFU*/g *E. coli* (EC) counts of *biceps femoris* beef steaks.

Treatment**	Days of Display				
	1	2	3	5	7
IN	5.42 ± 0.52^{ab}	9.33 ± 0.43^{c}	8.99 ± 0.49^{b}	9.34 ± 0.05	9.31 ± 0.05
W	5.53 ± 0.52^{ab}	9.34 ± 0.43^{c}	8.27 ± 0.49^{ab}	9.34 ± 0.05	9.31 ± 0.05
CPC	4.65 ± 0.52^{a}	5.94 ± 0.43^{a}	7.51 ± 0.49^{a}	9.18 ± 0.05	8.72 ± 0.05
KL	6.04 ± 0.52^{b}	9.33 ± 0.43^{c}	8.33 ± 0.49^{ab}	9.34 ± 0.05	9.21 ± 0.05
NMS	5.22 ± 0.52^{ab}	7.35 ± 0.43^{b}	7.57 ± 0.49^{a}	9.34 ± 0.05	9.34 ± 0.05
TSP	5.24 ± 0.52^{ab}	8.48 ± 0.43^{c}	8.11 ± 0.49^{ab}	9.34 ± 0.05	9.31 ± 0.05

* Colony forming units.
** Treatment: IN = untreated inoculated control, W = water, CPC = 0.5% cetylpyridinium chloride, KL = 3% potassium lactate, NMS = 4% sodium metasilicate, TSP = 10% trisodium phosphate.
[a-c] Least squares means within a column with different superscripts are different ($P < 0.05$).

Table 3. Effect of antimicrobial sequential treatment at sub-primal and steak levels by day of display interaction on least squares means (±SE) log CFU*/g aerobic plate counts (APC) of *biceps femoris* beef steaks.

Treatment**	Days of Display				
	1	2	3	5	7
IN	5.48 ± 0.49	9.34 ± 0.43^{c}	9.10 ± 0.50^{b}	9.34 ± 0.04	9.31 ± 0.01
W	5.49 ± 0.49	9.33 ± 0.43^{c}	8.67 ± 0.50^{ab}	9.34 ± 0.04	9.31 ± 0.01
CPC	5.05 ± 0.49	6.12 ± 0.43^{a}	7.65 ± 0.50^{a}	9.23 ± 0.04	8.94 ± 0.01
KL	6.06 ± 0.49	9.30 ± 0.43^{c}	8.64 ± 0.50^{ab}	9.34 ± 0.04	9.31 ± 0.01
NMS	5.55 ± 0.49	7.43 ± 0.43^{b}	7.73 ± 0.50^{a}	9.34 ± 0.04	9.34 ± 0.01
TSP	5.33 ± 0.49	8.77 ± 0.43^{c}	8.24 ± 0.50^{ab}	9.34 ± 0.04	9.34 ± 0.01

* Colony forming units.
**Treatment: IN = untreated inoculated control, W = water, CPC = 0.5% cetylpyridinium chloride, KL = 3% potassium lactate, NMS = 4% sodium metasilicate, TSP = 10% trisodium phosphate.
[a-c] Least squares means within a column with different superscripts are different ($P < 0.05$).

Table 4. Effect of antimicrobial sequential treatment at sub-primal and steak levels by day of display interaction on least squares means (±SE) log CFU*/g *Salmonella* (ST) counts of *biceps femoris* beef steaks.

Treatment**	Days of Display				
	1	2	3	5	7
IN	5.16 ± 0.22	9.01 ± 0.49^{c}	4.97 ± 0.22^{b}	5.38 ± 0.15	6.64 ± 0.05^{c}
W	4.88 ± 0.22	9.45 ± 0.49^{c}	4.68 ± 0.22^{b}	5.36 ± 0.15	6.82 ± 0.05^{c}
CPC	4.86 ± 0.22	5.36 ± 0.49^{a}	3.84 ± 0.22^{a}	5.61 ± 0.15	5.00 ± 0.05^{a}
KL	4.98 ± 0.22	9.17 ± 0.49^{c}	4.46 ± 0.22^{ab}	5.25 ± 0.15	5.83 ± 0.05^{b}
NMS	4.61 ± 0.22	5.82 ± 0.49^{a}	4.54 ± 0.22^{ab}	4.92 ± 0.15	6.46 ± 0.05^{bc}
TSP	4.72 ± 0.22	6.69 ± 0.49^{b}	4.59 ± 0.22^{ab}	5.16 ± 0.15	6.65 ± 0.05^{c}

* Colony forming units.
**Treatment: IN = untreated inoculated control, W = water, CPC = 0.5% cetylpyridinium chloride, KL = 3% potassium lactate, NMS = 4% sodium metasilicate, TSP = 10% trisodium phosphate.
[a-c] Least squares means within a column with different superscripts are different ($P < 0.05$).

Influence of Hydrochloric/Citric Acid Mixture Alone
or in Combination with Cetylpyrinidium Chloride and Trisodium Phosphate
in Reducing E. *coli*, Coliform and Aerobic Plate Counts in Inoculated Beef

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Story in Brief

Biceps femoris muscles (n = 3) were cut into steaks and each steak was inoculated by dipping into a bacterial cocktail containing 10^7 log CFU/mL of *Escherichia coli* (EC; ATCC # 11775). The steaks were assigned antimicrobial treatments of hydrochloric/citric acid blend (H) alone or followed by cytylpyrinidium chloride (HCP) or trisodium phosphate (HSP) or both (HCPTP). After treatment application, each steak was placed on styrofoam tray with absorbent pad and overwrapped with polyvinyl chloride film. Steaks from each treatment (n = 3/treatment) were removed from display for bacterial enumeration on day 1, 2, 3, 4, and 7 and sampled for EC, coliforms (CO), and aerobic plate count (APC). All treatments achieved ~1 log CFU reduction for CO, EC and APC counts on day 2, 3 and 4 of display. The H treatments was more effective ($P < 0.05$) than all the other treatments in reducing CO and EC counts on day 1 and 2. However, the HTP and HCP treatments had lowest ($P < 0.05$) CO and EC counts on day 3 and 4, respectively. Results suggest that decontamination of *biceps femoris* steaks using hydrochloric / citric acid alone or as a sequential treatment with TSP and CPC or both showed promising results in decontamination of beef steaks. The outcome of this research will provide guidance on selection of antimicrobial agents that will enhance product safety of meat cuts.

Introduction

Since microbial contamination of meat during processing and handling remains a challenging task, an application of antimicrobial decontamination technique towards the end of the production line will enhance the product microbial safety. Several studies have indicated that cetylpyridinium chloride (Cutter et al., 2000; Ransom et al., 2003) and trisodium phosphate (Dickson et al. 1994; Dorsa et al., 1997; Pohlman 2002a, 2002b) are effective in reducing microbial populations in meat products. Further, an advantage of using cetylpyridinium chloride (CPC) and trisodium phosphate (TSP) in meat decontamination is that they may enhance redness ($a^\star$) and oxymyoglobin redox stability (630 nm/580 nm) without affecting the odor characteristics of ground beef (Pohlman et al., 2002a; Jimenez-Villarreal et al., 2003). The Safe Foods Corporation (Little Rock, Ark.) recently introduced a novel cost effective, generally recognized as safe (GRAS) hydrochloric/citric acid blend (Citrilow™) which sustain a good pH range that is effective in controlling pathogenic bacterial growth. Therefore, our objective of this study was to evaluate the efficacy of 20% hydrochloric/citric acid blend as a single antimicrobial intervention or as a multiple antimicrobial intervention with 0.4% cetylpyridinium chloride and 10% trisodium phosphate on reducing *Escherichia coli* population in pre-inoculated beef steaks.

Experimental Procedures

Inoculation Process. The bacterial cocktail (4 °C) containing 10^7 CFU *E. coli* O157:H7 (EC; ATCC# 43888) was prepared from frozen (-80 °C) pure cultures according to the procedures described by Pohlman et al. (2002a and 2002b). *Biceps femoris* muscles (n = 3) were cut into individual steaks (n = 75) and immersed in the inoculums for 5 s. Inoculated steaks were then placed in a sterile biohazard bag and kept overnight for further bacterial attachment.

Antimicrobial Treatment Preparation. The antimicrobial treatments of 20% (v/v) hydrochloric / citric acid blend (Citrilow™, Safe Foods Cooperation, Little Rock, Ark.), 0.4% cetylpyrinidium chloride (Cecure®, Safe Foods Cooperation, Little Rock, Ark.), and 10% (w/v) trisoidum phosphate (anhydrous (Food Grade), ICL Performance products LP Co., St. Louis, Mo.) was prepared by mixing appropriate amounts of purified water.

Treatment Application. Inoculated beef steaks (5 steaks/treatment/replicate) were dipped in 1 L of 20% H as a single antimicrobial intervention for 30 s and allowed to drip for 10 min prior to packaging. For sequential treatment application, inoculated steaks were first immersed in 20% H. Then the steaks were dipped in 0.4% CPC (HCP), 10% TSP (HTP) or 0.4% CPC followed by 10% TSP (HCPTP). The steaks were dipped in each treatment for 30 s and allowed to stand 10 min prior to the assigned sequential treatments. Next, treated steaks and untreated inoculated controls were placed on styrofoam trays with absorbent pads and overwrapped with polyvinyl chloride film (O_2 transmission rate = 14,000 cc/mm2/24 h/1 atm; Koch Supplies, Inc., Kansas City, Mo.). The steaks were stored at 4 °C under 1630 lux of deluxe warm white fluorescent lighting (Phillips Inc., Somerset, N.J.).

Microbiological Analysis. On day 1, 2, 3, 4 and 7 of simulated retail display, the microbial enumeration for each steak was carried out by aseptically removing 2.5g from the surface using a sterile scalpel and forceps as described by Venturini et al. (2006). The 2.5g samples were placed in sterile whirlpack bags (Nasco, Ft Atkinson, Wis.) separately and 22.5 ml of 0.1% buffered peptone water was added and samples were homogenized for 2 min in a stomacher (Model 400 Lab Stomacher; Seward, London, U.K.). Subsequently, serial 10-fold dilutions were made and spread plating was done in duplicates on aerobic plate count (APC), and *E. coli* (EC) / coliform (CO) counts on Petrifilm® (3M Corporation, St. Paul, Minn.). The EC and APC counts were read after 48 h, whereas coliform plates were read after 24 h. All the counts were recorded as colony forming units per gram (CFU/g).

Analysis of Data. The bacterial values for each treatment (1steak/treatment/day; 3 replicates) were transformed to log values and then

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analyzed for the main effects of antimicrobial treatment and days of display and their interaction using the GLM procedure of SAS (SAS Inst., Inc., Cary, N.C.). Least square means were generated for all variables and were separated using the PDIFF option of SAS.

Results and Discussion

The antimicrobial treatment by day interaction was significant, therefore, only interaction effects are presented. The antimicrobial treatment by day of display interaction for CO, EC and APC counts are presented on Table 1, 2 and 3, respectively. On 1 through 7 d, all treated samples showed significant reduction for CO, EC, and APC ($P < 0.05$) compared with the control. The H treatment showed the highest EC and CO reduction ($P < 0.05$) compared to other treatments on days 1 and 2. Nevertheless, all treatment achieved more than 1.4 log CFU/g for APC on day 1. The HTP, and HCP treated steaks showed the largest reduction for EC, CO and APC on 3 and 4 d respectively ($P < 0.05$), compared to all other treatments.

It is evident from the results that applications of hydrochloric acid/citric acid blend (H) alone or followed by CPC or TSP treatments were more successful in controlling CO, EC and APC counts ($P < 0.05$) compared to H followed by sequential applications of CPC and TSP (HCPTP). However, HCPTP had a similar reduction for EC and CO compared with H and HCP treatments on day 3.

These results are in agreement with the previous studies that recognized successful application of 0.5 % CPC, 10% TSP (Pohlman et al., 2002a) on beef trimmings to reduce bacterial counts of ground

beef under retail display. However, no previous published data is available to compare the antimicrobial effectiveness of hydrochloric acid /citric acid blend. Since hydrochloric /citric acid blend treatment alone had tendency to reduce lightnes, it may be advantageous to use this treatment in combination with CPC and TSP to achieve microbial reduction without negative color impact on the final steaks. Therefore, further studies are required to evaluate the impact of hydrochloric acid /citric acid blend alone or in combination with CPC and TSP on meat color and other quality characteristics.

Implications

The hydrochloric acid /citric acid blend alone or in combination with CPC and TSP as a multiple approach can be utilized as a novel decontamination technique in reducing pathogenic bacteria in beef products. However, further evaluations of its impact on chemical, physical and sensory properties of beef products are required

Literature Cited

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Table 1. Effect of antimicrobial treatment by day of display interaction on log Colony Forming Units/g coliform, *E. coli* and Aerobic Plate Counts (APC) of beef steaks.

	Treatment	Day of Display				
		1	2	3	4	7
Coliform						
	INCON	8.20[a]	8.22[a]	8.28[a]	8.10[a]	8.72[a]
	H	6.29[d]	5.99[e]	6.89[c]	6.87[d]	7.76[c]
	HCP	7.02[c]	7.04[d]	7.03[b]	6.71[e]	8.04[b]
	HCPTP	7.58[b]	7.24[b]	6.96[bc]	7.05[b]	8.76[a]
	HTP	6.93[c]	7.10[c]	6.06[d]	6.94[c]	8.06[b]
SE		0.14	0.01	0.05	0.02	0.05
E. coli						
	INCON	8.21[a]	8.22[a]	8.28[a]	8.09[a]	8.87[a]
	H	6.54[d]	5.99[e]	6.87[c]	6.87[d]	7.77[d]
	HCP	7.01[c]	7.04[d]	7.03[b]	6.72[e]	8.04[c]
	HCPTP	7.57[b]	7.23[b]	6.96[bc]	7.05[b]	8.77[b]
	HTP	6.93[c]	7.10[c]	6.06[d]	6.94[c]	8.06[c]
SE		0.11	0.01	0.05	0.02	0.05
APC						
	INCON	8.51[a]	8.22[a]	8.42[a]	8.20[a]	9.69[a]
	H	6.99[bc]	6.07[d]	6.98[c]	7.18[b]	7.98[c]
	HCP	7.07[b]	7.13[c]	7.16[b]	6.89[b]	8.13[bc]
	HCPTP	6.95[c]	7.28[b]	7.04[bc]	7.11[b]	8.93[b]
	HTP	6.99[bc]	7.17[c]	6.16[d]	6.94[b]	8.15[bc]
SE		0.14	0.02	0.05	0.10	0.03

Treatments: INCON = untreated inoculated control, H = 20% hydrochloric /citric acid mixture HCP = 20% hydrochloric /citric acid mixture HCP followed by 0.5% ctylpyridinium chloride, HCPTP = 20% hydrochloric /citric acid mixture followed by 0.5% ctylpyridinium chloride and 10 % trisodium phosphate,
HTP = 20% hydrochloric /citric acid mixture followed by10% trisodium phosphate
[a,b,c,d,e] Least squares means within a column with different superscripts are different ($P < 0.05$).

Citric Acid Enhancement at Solution pH Values Between 3.5 and 5.0 Does Not Alter the Fresh or Cooked Color of Dark-Cutting Beef

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Story in Brief

Dark-cutting (DC; n = 41) beef strip loins were used to test the effects of citric acid-marination pH on visual and instrumental fresh and cooked color. Both DC (mean pH = 6.65) and normal pH low Choice (CH; mean pH = 5.48) strip loins were cut into 2 equal-length portions, and DC sections were injected to 111% of raw product weight with pH 3.5, 4.0, 4.5 or 5.0 solutions made by mixing citric acid (CA) in either a 0.5% phosphate solution (PO4) or tap water (H_2O). After injection and vacuum-tumbling, steaks (1.0-in-thick) were cut from each section, placed on to foam trays, and over-wrapped with a polyvinyl chloride film or cooked to 160 °F for cooked color analyses. Post-enhancement muscle pH for enhanced DC steaks did not ($P > 0.05$) differ from that of the non-enhanced DC steaks regardless of solution pH. Initial fresh visual color scores of enhanced DC steaks were greater ($P \le 0.05$) than untreated DC, but less ($P \le 0.05$) than CH. Degree of doneness scores increased linearly ($P = 0.03$) as solution pH increased from 3.5 to 5.0, but none of the enhanced DC steaks had degree of doneness scores comparable to CH steaks ($P > 0.05$). Cooked color redness (a∗) and chroma values were similar ($P > 0.05$) among all steaks, and lightness (L*) values for steaks enhanced with the 5.0 pH solution were greater ($P < 0.05$) than only CH steaks. Results from this study showed that pH values of the enhancement solutions, regardless of base solution, were insufficient to improve of the fresh or cooked color of DC beef comparable to that of CH.

Introduction

Dark-cutting beef is characterized by abnormally high muscle pH, increased water-binding, sticky texture, and dark color, which are all due to preslaughter stress, weather, genetics, disposition, and handling practices. The increased water-binding causes the DC beef to have a tight structure; thereby, causing myoglobin to be trapped within the interior portion of the muscle. Trapped myoglobin fails to reach the cut surface and prevents the formation of oxymoglobin. This also causes a persistent pinking color in cooked DC beef. Both the fresh and cooked color defects cost the industry millions of dollars every year.

The use of organic acid marination to improve the fresh and cooked color of DC beef is limited. However, recent research from this laboratory (Sawyer et al., 2008, Sawyer et al., 2009) showed that enhancing longissimus muscle sections from DC carcass with 0 to 1% lactic acid altered visual and instrumental measurements of internal color of DC beef when cooked to a medium degree of doneness and fresh beef color, equivalent to that with normal ultimate pH values. Therefore, the objectives of this study were to test the effects of citric acid marination solution pH on fresh and cooked color of DC beef.

Experimental Procedures

Normal pH, low Choice (CH; n = 5) and dark-cutting (DC; n = 41) beef strip loins were selected based on 24-hour postmortem pH (average pH of 5.48 and 6.65, respectively) and purchased from a large commercial slaughter facility. Upon arrival at the University of Arkansas Red Meat Abattoir, strip loins were removed from the vacuum-package, trimmed free of fat and adjacent muscles, and pH was collected for each strip loin before being cut into two equal sections.

The DC muscle sections (n = 9/treatment) were assigned randomly to 1 of 8 enhancement treatments of pH 3.5, 4.0, 4.5, or 5.0 solutions, made by mixing citric acid (CA) in either a 0.5% orthophosphate solution (PO4) or tap water (H_2O), and a non-injected negative

control, whereas the CH sections served as a non-injected positive control. Enhancement solutions were prepared in 39 °F tap water and agitated continuously until injection. Strip loins were then injected to a targeted 111% of raw weight with their respective enhancement solution using a Fomaco 20/40 injector, vacuum-tumbled, allowed to equilibrate for 30 min, and pH was collected for enhanced pH. Sections were then cut into 1.0-in.-thick steaks and over-wrapped with an oxygen-permeable, polyvinyl chloride film for fresh color analysis or vacuum-packed and stored at -4 °F prior to cooked color analysis. Steaks designated for fresh instrumental and visual color analyses were placed in coffin-style retail display cases maintained at 39.2 °F under 1600 lux warm white fluorescent lighting.

Instrumental color reading of steaks were measured using a Hunter MiniScan XE calibrated against black and white tiles. The lightness (L*), redness (a∗), and yellowness (b*) values were determined from the mean of 3 readings on the surface of each steak using illuminant A, a 10° standard observer, and a 1.0-in. aperture. Additionally, hue angle was calculated as: $\tan^{-1}(b*/a*)$, whereas chroma was calculated as: $\sqrt{a^{*2}+b^{*2}}$ (AMSA, 1991). A 5-member, trained sensory panel was used to evaluate sensory color of steaks during retail display. Panelists evaluated each steak under display for initial beef color (8 = extremely bright cherry-red, 7 = bright cherry-red, 6 = moderately bright cherry-red, 5 = slightly bright-cherry red, 4 = slightly dark cherry-red, 3 = moderately dark red, 2 = dark red, and 1 = extremely dark red), percent discoloration (8 = 0 to 5% discoloration, 7 = 6 to 20% discoloration, 6 = 21 to 35% discoloration, 5 = 36 to 50% discoloration, 4 = 51 to 65% discoloration, 3 = 66 to 80% discolored, 2 = 81 to 95% discolored, and 1 = 96-100% discolored), and overall acceptability (7 = extremely desirable, 6 = desirable, 5 = slightly desirable, 4 = acceptable, 3 = slightly undesirable, 2 = undesirable, 1 = extremely undesirable).

Steaks (1.0-in. thick) were thawed for approximately 16 h at 34 °F before being cooked on a gas-fired, open-hearth grill. Steaks were turned every 3 min until the internal temperature of the steak reached 160 °F, then immediately removed from the grill and allowed to cool

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at room temperature for approximately 5 min prior to slicing for visual and instrumental cooked color analysis. Steaks were cut just off the center (perpendicular to the steak flat surface) and cooked color differences were evaluated on the cut surface. Within 20 seconds of cutting cooked steaks, a 10-member, trained panel evaluated each cut surface (to the nearest 0.5) for internal cooked color (7 = brown, 6 = gray brown, 5 = pinkish gray, 4 = slightly pink, 3 = pink, 2 = medium red, and 1 = very red) and internal doneness (6 = very well, 5 = well done, 4 = medium, 3 = medium rare, 2 = rare, and 1 = very rare). Instrumental cooked color readings of steaks were measured concurrently with visual analysis on one-half of the steak wrapped immediately after cutting with a PVC film to minimize blooming (oxidation of myoglobin). Values were measured using a Hunter MiniScan XE calibrated before every session against black and white tiles. The L*, a*, and b* values, were determined from the mean of three readings on the cut surface of each steak using Illuminant A, a 10° standard observer, and a 0.5-in. aperture

Data were analyzed as a completely randomized design, with treatments arranged in a 2 × 4 factorial structure and loin sections as the experimental unit. The ANOVA was generated using the mixed model procedure of SAS (SAS Inst., Inc., Cary, N.C.), and the statistical model included enhancement treatment as the lone fixed effect. Least squares means were calculated for all treatments, and, because of the unique treatment structure, preplanned contrasts were used to test: 1) based solution differences (PO4 vs. TSP); 2) differences between each enhancement solution pH and untreated, low Choice; and 3) differences between each enhancement solution pH and non-enhanced, DC control.

Results and Discussion

The post enhancement muscle pH values of all enhanced DC section, regardless the base solution or solution pH, were different ($P < 0.05$) than the CH control, but were comparable ($P > 0.05$) to that of the non-enhanced DC sections.

Even though CH steaks had greater ($P < 0.05$) L* values than non-enhanced and enhanced DC steaks, L* values of DC steaks enhanced with 4.5 and 5.0 pH solutions were greater ($P < 0.05$) than the non-enhanced DC steaks (Table 1). However, citric acid enhancement failed ($P > 0.05$) to alter other instrumental measures (a*, b*, hue and

chroma) of fresh color from that of the non-enhanced DC steaks. Visual initial fresh color scores for all enhanced DC steaks, regardless of base solution or solution pH, were greater ($P < 0.05$) than the non-enhanced DC steaks, but less ($P < 0.05$) than the scores for CH steaks. Visual fresh discoloration and overall acceptability scores of all enhanced DC sections differed ($P < 0.05$) from CH steaks, but were comparable ($P > 0.05$) to the non-enhanced DC sections.

Cooked redness (a*) and chroma values were similar ($P > 0.05$) among all steaks, and L* values for DC steaks enhanced with 5.0 solution pH, regardless of base solution, were greater ($P < 0.05$) than CH steaks (Table 2). Interestingly, visual cooked beef color and degree of doneness scores for all enhanced DC steaks were different ($P < 0.05$) from the non-enhanced CH steaks.

Implications

Results obtained from this study indicate that the addition of citric acid at low levels to dark-cutting (DC) beef cannot alter the postmortem muscle pH, fresh color, or cooked color equivalent to that of beef from CH sections. However, low levels of citric acid were able to alter some characteristics from non-enhanced DC sections. Therefore, additional research is needed to investigate how higher levels of citric acid than was used in this study, affect the fresh and cooked color of DC beef.

Acknowledgments

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Table 1. Effects of citric acid marination pH on muscle pH and fresh color[a] of dark-cutting (DC) strip steaks.

	CH[b]	DC[c]	OrthoPhoshpate-base solution				Water-base solution				SEM
			3.5	4.0	4.5	5.0	3.5	4.0	4.5	5.0	
Post enhancment pH[d]	5.48	6.65	6.32[†]	6.64[†]	6.55[†]	6.47[†]	6.73[†]	6.72[†]	6.60[†]	6.75[†]	0.079
Instrumental											
Lightness (L*)	45.47	31.81	35.50[†]	32.91[†]	34.33[†‡]	34.42[†‡]	32.70[†]	34.10[†]	36.37[†‡]	34.89[†‡]	1.023
Redness (a*)	32.34	20.54	21.00[†]	20.81[†]	21.76[†]	21.56[†]	20.61[†]	21.57[†]	21.03[†]	21.16[†]	0.489
Yellowness (b*)	24.59	12.60	13.01[†]	12.35[†]	13.79[†]	13.60[†]	12.42[†]	13.48[†]	13.46[†]	13.53[†]	0.552
Hue	37.25	31.41	31.59[†]	30.56[†]	32.22[†]	32.19[†]	30.94[†]	31.93[†]	32.46[†]	32.48[†]	0.580
Chroma	40.62	24.11	24.72[†]	24.20[†]	25.78[†]	25.50[†]	24.08[†]	25.44[†]	24.98[†]	25.13[†]	0.695
Visual											
Initial Color	6.5	2.1	3.1[†‡]	2.8[†‡]	2.9[†‡]	3.1[†‡]	2.8[†‡]	3.1[†‡]	3.1[†‡]	2.9[†‡]	0.23
Discoloration	7.5	6.0	5.5[†]	5.9[†]	5.8[†]	6.1[†]	5.9[†]	6.1[†]	5.8[†]	5.8[†]	0.95
Acceptabilty	5.4	3.3	3.1[†]	3.3[†]	3.3[†]	3.4[†]	3.4[†]	3.7[†]	3.6[†]	3.5[†]	0.50

[a]L* values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color); Hue angle represents the change from the true red axis (greater angle indicates a greater shift from the true red axis); Chroma is a measure of total color (larger value indicates a more vivid color).
[b]CH = non-enhanced low U.S.D.A. Choice normal pH beef steaks.
[c]DC = non-enhanced dark-cutting beef steaks.
[d]pH measured after 30 min equilibration.
[†]Within a row, contrast indicates mean differs ($P < 0.05$) from CH.
[‡]Within a row, contrast indicates mean differs ($P < 0.05$) from DC.

Table 2. Effects of citric acid marination pH on beef quality characteristics of cooked dark-cutting (DC) strip loin steaks.

Item	CH[a]	DC[b]	OrthoPhoshpate-base solution				Water-base solution				SEM
			3.5	4.0	4.5	5.0	3.5	4.0	4.5	5.0	
Instrumental											
Lightness (L*)[c]	49.64	52.20	52.16	53.00	51.05	52.26†	50.98	50.42	51.45	53.67†	1.277
Redness (a*)[c]	18.68	21.67	20.71	21.91	19.47	22.41	18.74	21.74	21.08	20.97	1.376
Yellowness (b*)[c]	19.79	19.73	18.90‡	18.87	17.59‡	18.83	16.81‡	19.10	18.65‡	18.66	0.886
Hue angle (°)[d]	45.58	41.58	42.08†	40.40†	41.76†	39.25†	41.76†	40.68†	40.87†	41.07†	1.404
Chroma[e]	27.09	29.21	27.94	28.81	26.05	29.01	25.05	28.78	27.99	27.91	1.303
Visual											
Beef color	4.7	3.8	3.4†	3.6†‡	3.6†‡	3.3†	3.8†	3.0†‡	3.1†‡	3.8†	0.14
Doneness	4.3	3.5	3.0†‡	3.3†‡	3.4†	3.1†	3.4†‡	2.9†‡	3.1†	3.7†	0.13

[a]CH = non-enhanced low U.S.D.A. Choice normal pH beef steaks.
[b]DC = non-enhanced dark-cutting beef steaks.
[c]L* values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color).
[d]Hue angle represents the change from the true red axis (greater angle indicates a greater shift from the true red axis).
[e]Chroma is a measure of total color (larger value indicates a more vivid color).
†Within a row, contrast indicates mean differs ($P < 0.05$) from CH.
‡Within a row, contrast indicates mean differs ($P < 0.05$) from DC.

Citric Acid Marination Can Improve the Cooked Color, But Not Fresh Color, of Dark-Cutting Beef

R. J. Stackhouse[1], J. K. Apple[1], J. W. S. Yancey[1], C. A. Keys[1], and T. M. Johnson[1]

Story in Brief

Dark-cutting beef strip loins (DC) were used to test the effects of citric acid-marination pH on visual and instrumental fresh and cooked color characteristics. Both DC (mean pH = 6.61) and normal pH low Choice (CH; mean pH = 5.38) strip loins were cut into 2 equal-length portions, and DC sections were injected to 111% of raw product weight with pH 2.0, 2.5, 3.0, and 3.5 solutions made by mixing citric acid (CA) in either a 0.5% orthophosphate solution (PO4) or a 0.5% tripolyphosphate solution (TSP). Steaks (1.0-in. thick) were cut from each section, placed on two foam trays, and over-wrapped with a polyvinyl chloride film or cooked to 160 °F on a gas-fired open-hearth grill for cooked color analyses. Post-enhancement muscle pH decreased linearly ($P < 0.001$) as solution pH decreased from 3.5 to 2.0, with DC steaks enhanced with pH 2.5 solution comparable ($P = 0.14$) to that of CH. Cooked steaks enhanced with pH 2.5, 3.0, and 3.5 solutions had b* values (yellowness) that were comparable ($P \geq 0.85$) to that of CH steaks. Degree of doneness and cooked color scores increased linearly ($P \leq 0.01$) as solution pH decreased from 3.5 to 2.0, with degree of doneness and cooked color scores of pH 2.5 enhanced DC steaks similar ($P = 0.10$) to CH steaks. Enhancing DC beef with CA, at a solution pH of 2.5 to 3.5, effectively reduced muscle pH and eliminated the persistent red cooked color typically associated with DC beef; however, CA-enhancement of DC beef failed to improve the fresh color to that of CH beef.

Introduction

Dark-cutting beef is characterized by abnormally high muscle pH, increased water-binding, sticky texture, and dark color, which is due to preslaughter stress, weather, genetics, disposition, and handling practices. The increased water-binding causes the DC beef to have a tight structure; thereby, causing myoglobin to be trapped within the interior portion of the muscle. Trapped myoglobin fails to reach the cut surface and prevents the formation of oxymoglobin, which causes a persistent pinking color in cooked DC beef. Both the fresh and cooked color defects cost the industry millions of dollars every year.

The use of organic acid marination to improve the fresh and cooked color of DC beef is limited; however, recent research from this laboratory (Sawyer et al., 2009) has shown that enhancing longissimus muscle sections from DC carcass with 0 to 1% lactic acid altered visual and instrumental measurements of internal color of DC beef and fresh beef color, equivalent to that with normal ultimate pH values. Stackhouse et al., (2010) also found that low levels of citric acid altered some characteristics of DC beef but not comparable to normal pH beef. Therefore, the objectives of this study were to test the effects of lower citric acid marination solution pH on fresh and cooked color of DC beef.

Experimental Procedures

Normal pH, low Choice (CH; n = 5) and dark-cutting (DC; n = 41) beef strip loins (IMPS #180) were selected on 24-hour postmortem pH (average pH of 5.38 and 6.74, respectively) and purchased from a large commercial slaughter facility. Upon arrival at the University of Arkansas Red meat abattoir, strip loins were removed from the vacuum-package, trimmed free of fat and adjacent muscles, and pH was collected for each strip loin before being cut into two equal sections.

The DC muscle sections (n = 9/treatment) were assigned randomly to 1 of 9 enhancement treatments of pH 2.0, 2.5, 3.0, or 3.5 solutions, made by mixing citric acid (CA) in either a 0.5%

trisodium polyphosphate solution (TSP) or 0.5% orthophosphate (PO4) solution , and a non-injected negative control, whereas the CH sections served as a non-injected positive control. Enhancement solutions were prepared in 39 °F tap water and agitated continuously until injection. Strip loins were then injected to a targeted 111% of raw weight with their respective enhancement solution using a Fomaco 20/40 injector, vacuum-tumbled, allowed to equilibrate for 30 min, and pH was collected for enhanced pH. Then, sections were cut into 1.0-in.-thick steaks and over-wrapped with a oxygen permeable, polyvinyl chloride film for fresh color analysis or vacuum-packed and stored at -4 °F for cooked color analysis. Steaks designated for fresh instrumental and visual color analyses were placed in coffin-style retail display cases maintained at 39.2 °F under 1600 lux deluxe warm white fluorescent lighting.

Instrumental color reading of steaks were measured using a Hunter MiniScan XE calibrated against black and white tiles. The lightness (L*), redness (a*), and yellowness (b*) values were determined from the mean of 3 readings on the surface of each steak using illuminant A, a 10° standard observer, and a 1.0-in. aperture. Additionally, hue angle was calculated as: $\tan^{-1}(b*/a*)$, whereas chroma was calculated as: $\sqrt{a*^2 + b*^2}$ (AMSA, 1991). A 5-member, trained panel was used to evaluate sensory color of steaks during retail display. Panelists evaluated each steak under display for initial beef color (8 = extremely bright cherry-red, 7 = bright cherry-red, 6 = moderately bright cherry-red, 5 = slightly bright-cherry red, 4 = slightly dark cherry-red, 3 = moderately dark red, 2 = dark red, and 1 = extremely dark red), percent discoloration (8 = 0 to 5% discoloration, 7 = 6 to 20% discoloration, 6 = 21 to 35% discoloration, 5 = 36 to 50% discoloration, 4 = 51 to 65% discoloration, 3 = 66 to 80% discolored, 2 = 81 to 95% discolored, and 1 = 96-100% discolored), and overall acceptability (7 = extremely desirable, 6 = desirable, 5 = slightly desirable, 4 = acceptable, 3 = slightly undesirable, 2 = undesirable, 1 = extremely undesirable).

Steaks (1.0-in. thick) were thawed for approximately 16 h at 34 °F before being cooked on a gas-fired, open-hearth grill. Steaks were

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turned every 3 min until the internal temperature of the steak reached 160 °F, then immediately removed from the grill and allowed to cool at room temperature for approximately 5 min prior to slicing for visual and instrumental cooked color analysis. Steaks were cut just off the center (perpendicular to the steak flat surface) and cooked color differences were evaluated on the cut surface. Within 20 seconds of cutting cooked steaks, a 10-member, trained panel evaluated each cut surface (to the nearest 0.5) for internal cooked color (7 = brown, 6 = gray brown, 5 = pinkish gray, 4 = slightly pink, 3 = pink, 2 = medium red, and 1 = very red) and internal doneness (6 = very well, 5 = well done, 4 = medium, 3 = medium rare, 2 = rare, and 1 = very rare). Instrumental cooked color readings of steaks were measured concurrently with visual analysis on half of the steak wrapped immediately after cutting with a PVC film to minimize blooming. Values were measured using a Hunter MiniScan XE (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, Va., USA) calibrated before every session against black and white tiles. The L*, a*, and b* values, were determined from the mean of 3 readings on the cut surface of each steak using Illuminant A, a 10° standard observer, and a 0.5-in. aperture

Data were analyzed as a completely randomized design, with treatments in a 2 × 4 factorial arrangement and loin sections as the experimental unit. The ANOVA was generated using the mixed model procedure of SAS (SAS Inst., Inc., Cary, N.C.), and the statistical model included enhancement treatment as the lone fixed effect. Least squares means were calculated for all treatments, and, because of the unique treatment structure, preplanned contrasts were used to test: 1) base solution differences (PO4 vs. TSP) (results not shown); 2) linear, quadratic and cubic responses to decreasing enhancement solution pH (results not shown); 3) differences between each enhancement solution pH and untreated, low Choice; and 4) differences between each enhancement solution pH and non-enhanced, DC control.

Results and Discussion

The pH values of DC section enhanced with solution pH of 2.0, 2.5, and 3.0, regardless of the base solution, were lower ($P < 0.05$) than for the DC control; however, only the DC sections enhanced with a solution pH of 2.5 were comparable ($P = 0.14$) to the CH control (Table 1). Dark-cutting sections enhanced with 2.0 solution pH had a muscle pH value lower ($P < 0.05$) than that of the CH.

Fresh DC steaks treated with 2.0 pH solution regardless of base solution were the lightest (highest L* value) least red (lowest a* values and highest hue angles), least yellow (lower b* value), and lowest chroma score when compared to all other treatments and controls (Table 1). DC steaks enhanced with 2.5 pH solution had an intermediate a* value; however, for all other treatments, regardless of base solution, there was not an improvement of redness (increase of a* value). DC steaks enhanced with 2.5 pH solution on day 0 had intermediate b* values and chroma scores.

Initial color scores were intermediate for DC steaks enhanced with 2.0 or 2.5 pH solution on day 0 (Table 1). Moreover, initial color, discoloration and overall acceptability scores of enhanced DC steaks were not comparable ($P < 0.05$) to non-enhanced CH steaks on any day regardless of pH or base solution.

No differences ($P > 0.05$) were found for L* or a* between cooked non-enhanced CH, non-enhanced DC steaks, or DC steaks enhanced

with solution pH 2.5, 3.0, and 3.5; however, DC steaks enhanced with 2.0, regardless the base solution, had lower ($P < 0.05$) L* and a* values than all other steaks (Table 2). Dark-cutting steaks enhanced with solution pH 2.5, 3.0, and 3.5 had higher ($P < 0.05$) b* values than non-enhanced DC steaks and were comparable ($P > 0.05$) to that of non-enhanced CH steaks. Moreover, the DC steaks enhanced with 2.0 solution pH had lower ($P < 0.05$) b* values and higher ($P < 0.05$) hue angle values than that of the non-enhanced CH and DC steaks, whereas hue angles for DC steaks enhanced with 2.5, 3.0, and 3.5 pH solution had comparable hue angle values to non-enhanced CH steaks. No significant differences were found for chroma values between non-enhanced CH, non-enhanced DC steaks, or DC steaks enhanced with solution pH 2.5 and 3.0; however, DC steaks enhanced with 2.0 regardless the base solution had lower ($P < 0.05$) chroma values than non-enhanced CH and DC steaks, and DC steaks enhanced with 3.5 regardless the base solution had higher ($P < 0.05$) chroma values than non-enhanced DC steaks. Decreasing the pH of the enhancement solution increased the visual cooked color (Fig. 1; linear, $P < 0.0001$) and degree of doneness scores (Fig. 2; linear, $P < 0.0001$). DC steaks enhanced with 2.0, 2.5, and 3.0 had visual color and degree of doneness scores significantly higher than non-enhanced DC steaks; however, DC steaks enhanced with 3.0, 2.5, and 2.0 had significantly lower, comparable, and higher visual color and degree of doneness scores than non-enhanced CH steaks respectively.

Our results with citric acid were similar to those of Sawyer et al., (2009) that found the use of lactic acid at 0.25 to 0.50% reduced the postmortem ultimate pH of DC beef sections to a comparable pH level to CH steaks. The use of 2.0 pH solution was also similar to Sawyer et al, (2009) use of 1.0% lactic, as it lowered the ultimate pH below that of the CH sections. Furthermore, results from this study agreed with those of Sawyer et al, (2009) in reducing the persistent red color of cooked DC beef. However, this study was unable to alter the fresh color of DC beef to be comparable to normal pH beef.

Implications

Results obtained from this study indicate that the addition of citric acid to dark-cutting beef can lower the postmortem muscle pH, thereby improving cooked beef color. However, it was not effective in improving fresh beef color to that comparable to normal pH beef.

Acknowledgments

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Table 1. Effects of citric acid marination pH on muscle pH and fresh color[a] of dark-cutting (DC) strip steaks.

	CH[c]	DC[d]	Orthophosphate-base solution				TSP[b]-base solution				SEM
			2.0	2.5	3.0	3.5	2.0	2.5	3.0	3.5	
Post enhancment pH[e]	5.43	6.67	4.06†‡	5.69‡	6.14†‡	6.35†	4.62†‡	5.70‡	6.07†‡	6.54†	0.147
Instrumental											
Lightness (L*)	42.85	31.05	44.56‡	40.02†‡	36.09†‡	35.17†‡	43.18‡	37.45†‡	36.35†‡	32.95†‡	1.259
Redness (a*)	34.66	21.82	14.49†‡	24.20†‡	23.14†	22.80†	15.41†‡	24.85†‡	22.35†	21.82†	0.628
Yellowness (b*)	26.84	13.65	13.86†	18.11†‡	15.49†‡	14.78†	14.73†	17.86†‡	14.99†‡	13.31†	0.523
Hue	37.75	31.91	43.87†‡	36.73†‡	33.66†‡	32.93†	44.04†‡	35.66†‡	33.86†‡	31.36†	0.579
Chroma	43.84	25.75	20.06†‡	30.24†‡	27.85†	27.18†	21.34†‡	30.61†‡	26.93†	25.56†	0.777
Visual											
Initial Color	7.0	1.9	2.6†‡	3.4†‡	2.7†‡	3.0†‡	2.4†‡	3.4†‡	2.9†‡	2.6†‡	0.20
Discoloration	8.0	6.8	2.3†‡	4.6†‡	6.2†‡	6.8†	2.6†‡	4.7†‡	6.4†‡	6.7†	0.49
Acceptabilty	6.0	3.7	1.3†‡	2.4†‡	3.4†‡	4.0†	1.3†‡	2.3†‡	3.4†‡	3.8†	0.30

[a]L* values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color); Hue angle represents the change from the true red axis (greater angle indicates a greater shift from the true red axis); Chroma is a measure of total color (larger value indicates a more vivid color).
[b]TSP = Trisodium polyphosphate.
[c]CH = non-enhanced low U.S.D.A. Choice normal pH beef steaks.
[d]DC = non-enhanced dark-cutting beef steaks.
[e]pH measured after 30 min equilibration.
†Within a row, contrast indicates mean differs ($P < 0.05$) from CH.
‡Within a row, contrast indicates mean differs ($P < 0.05$) from DC.

Table 2. Effects of citric acid marination pH on beef quality characteristics of cooked dark-cutting (DC) strip loin steaks.

Item	CH[b]	DC[c]	Orthophosphate-base solution				TSP[a]-base solution				SEM
			2.0	2.5	3.0	3.5	2.0	2.5	3.0	3.5	
Lightness (L*)[d]	51.45	51.79	45.67†‡	51.63	52.92	53.22	45.07†‡	52.15	50.33	53.48	1.124
Redness (a*)[d]	18.38	17.74	8.20†‡	19.35	18.93	20.23	10.39†‡	18.17	19.40	19.53	1.385
Yellowness (b*)[d]	19.48	16.66	11.52†‡	19.75‡	19.13‡	19.23‡	13.26†‡	19.20‡	19.44‡	19.93‡	0.964
Hue angle (°)[e]	46.78	42.94	54.12†‡	45.79‡	45.43	43.74	51.94†‡	47.10‡	45.78	45.93	1.278
Chroma[f]	26.71	24.31	14.03†‡	27.52	26.86	27.88‡	16.73†‡	26.34	27.42	27.77‡	1.585

[a]TSP = Trisodium polyphosphate.
[b]CH = non-enhanced low U.S.D.A. Choice normal pH beef steaks.
[c]DC = non-enhanced dark-cutting beef steaks.
[d]L* values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color).
[e]Hue angle represents the change from the true red axis (greater angle indicates a greater shift from the true red axis).
[f]Chroma is a measure of total color (larger value indicates a more vivid color).
†Within a row, contrast indicates mean differs ($P < 0.05$) from CH.
‡Within a row, contrast indicates mean differs ($P < 0.05$) from DC.

Fig. 1. Effect of citric acid marination pH with orthophosphate (PO$_4$) or trisodium polyphosphate (TSP) base solution on visually-evaluated cooked beef color (1 = very red; 2 = medium red; 3 = pink; 4 = slightly pink; 5 = pinkish-gray; 6 = gray brown; 7 = brown) of normal pH (CH) and dark-cutting (DC) beef. Bars with † indicate that means differ ($P < 0.05$) from CH and bars with ‡ indicate means differ ($P < 0.05$) from DC.

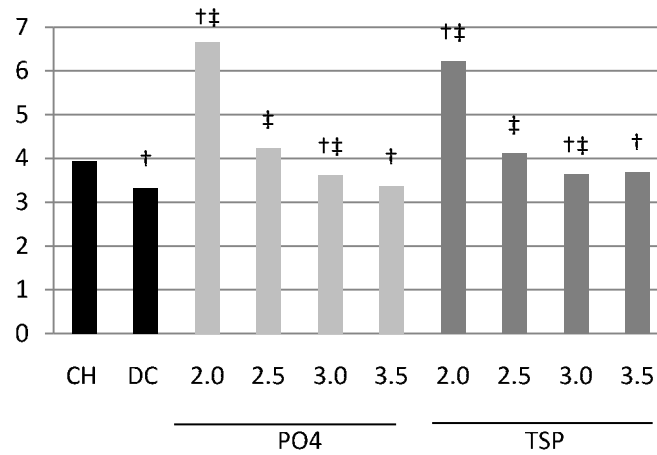**Fig. 2. Effect of citric acid marination pH with orthophosphate (PO$_4$) or trisodium polyphosphate (TSP) base solution on visually-evaluated degree of doneness (1 = very rare; 2 = rare; 3 = medium-rare; 4 = medium; 5 = well done; 6 = very well done) of normal pH (CH) and dark-cutting (DC) beef. Bars with † indicate that means differ ($P < 0.05$) from CH and bars with ‡ indicate means differ ($P < 0.05$) from DC.**
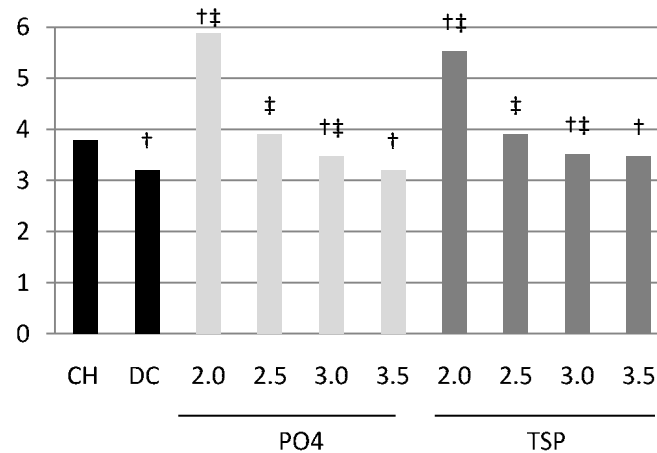
Noni Pulp (*Morinda citrofolia*) Mixed in Beef Patties Enhanced Color Stability

W. Nathan Tapp[1], Janeal W. S. Yancey[1], Jason K. Apple[1], and Richard G. Godbee[2]

Story in Brief

Coarse ground beef was obtained from a local processor and mixed with 0, 2, 4, and 6% Noni (*Morinda citrifolia*) pulp, a natural antioxidant from Southeast Asia. Beef was re-ground, formed into 1/3 lb patties, packaged on foam trays with aerobic overwrap, and placed in retail display for 5 days for visual and instrumental color evaluation. Additional patties were packaged for a measure of oxidation (TBARS; 0, 3, and 5 days) and two types of trained taste panels. Conventional, fresh panels were conducted where patties were cooked to 160 °F and served to panelists warm, and warmed-over flavor panels were conducted where patties were cooked to 160 °F, chilled over night, warmed to 160 °F, and served to panelists. At day 2 and 3 of display, patties with higher concentrations of Noni pulp were perceived as redder and less discolored ($P < 0.05$) by the visual panelists. Furthermore, the instrumental evaluation of the Noni patties also found them to be redder ($P < 0.05$) than controls, even though all patties became less red with increasing display time. After 3 and 5 days of retail display, patties with higher concentrations of Noni pulp also had lower TBARS (were less oxidized; $P < 0.05$). In the fresh panels, as Noni pulp concentration increased, panelists perceived the patties to have less beef flavor, greater incidence of off-flavors, and a less typical beef mouth feel ($P < 0.05$). Similar trends were seen in the warmed-over flavor panels and no treatment developed a significant level of warmed-over flavor. The potential of Noni pulp to improve the color stability and shelf life of fresh ground beef is very promising, but the flavors produced by the Noni in the ground beef may be detrimental to its use.

Introduction

The Noni plant (*Morinda citrifolia*) is grown in Southeast Asia where it has been used as homeopathic remedy for a wide variety of ailments ranging from ulcers and sprains to heart disease and cancer. It has recently been added to the diets of performance horses and feedlot cattle and produced positive changes in performance and efficiency. In human health, Noni products have been shown to have antioxidant and immune stimulating properties. The phytochemicals and antioxidants are largely responsible for Noni's action.

Ground beef in fresh retail display oxidizes at a faster rate than whole muscle products. This oxidation causes a brown discoloration to form on the product and is discriminated by consumers. Oxidation also results in the development of detrimental off-flavors and odors. Prevention of oxidation in ground beef has the potential to lengthen the shelf life and improve the profitability of ground beef products. Several researchers have used natural and synthetic antioxidants to inhibit oxidation in ground beef with varied levels of success. Some natural antioxidants that have been successful at improving the shelf life and color stability of ground beef include rosemary extract, honey, cherries, dried plums, and grapes.

The natural antioxidant properties of Noni pulp have the potential to prevent oxidation in fresh ground beef. Therefore, the objective of the study was to determine the effects of Noni pulp on color stability, oxidation, and shelf life of fresh ground beef in retail display and to determine its effects on the flavor profile.

Experimental Procedures

Coarse ground beef (85% lean) was obtained from a commercial processor and transported to the University of Arkansas and stored at 34 °F. Beef was mixed with Noni pulp at 0, 2, 4, or 6% in 20 lb batches (5 batches per treatment) and ground once through a 3/8 in. plate. Patties (1/3 lb) were formed using a commercial patty-forming machine. From each batch, two patties were packaged on foam trays with overwrap and placed in simulated retail display for 5 d for visual and instrumental color evaluation. Two patties were crust frozen, vacuum packaged, and frozen at -4 °F and two additional packages of patties were prepared and placed in retail display conditions for 3 and 5 d for thiobarbituric acid reactive substances (TBARS; a measure of oxidative rancidity) on days 0, 3, and 5, respectively. Additional patties were crust-frozen in stacks of four and two stacks were vacuum packaged separately for regular trained and warmed-over flavor taste panels.

No less than 8 experienced panelists evaluated the packages of patties daily for five days. Considering both patties, the panelists scored each package for total color, worst-point color, and percent discoloration using the following scales:

Total color and worst-point color scales	Percent discoloration scale
1 = very bright cherry red	1 = 0 to 5% discoloration
2 = bright cherry red	2 = 6 to 20% discoloration
3 = dull red	3 = 21 to 35% discoloration
4 = slightly dark red	4 = 36 to 50% discoloration
5 = moderately dark red to tan	5 = 51 to 65% discoloration
5.5 = borderline panelist acceptable	6 = 66 to 80% discoloration
6 = dark red to brown	7 = 81 to 95% discoloration
7 = very dark red to brown	8 = 96 to 100% discoloration

Panelists were instructed to consider worst-point color as the worst point of discoloration on the two patties, about the size of a dime. To evaluate instrumental color values of L*, a*, and b* (lightness, redness, and yellowness, respectively), each package of patties was scanned three times using the HunterLab MiniScan XE Plus with Illuminant A and the 1 in. aperture size.

For TBARS analysis, patties were removed from the retail display case at their designated time, vacuum packaged and frozen at -4 °F. The TBARS assay was carried out according to the methods described by Witte et al. (1970) with modifications of Apple et al. (2001).

For trained taste panel evaluation, frozen patties were shipped to Kansas State University Meat Science Laboratory for analysis. After arrival, patties were thawed over night at 34 °F and cooked on an electric griddle set to 350 °F. Patties were turned at one minute and

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[2] Tahitian Noni Corp., Provo, Utah.

then every two minutes until they reached an internal temperature of 160 °F, measured by a copper constantan thermocouple (Omega Technologies, Stamford, Conn.) inserted into the geometric center of each patty, and connected to a Doric Trendicator 410A (VAS Engineering, San Francisco, Calif.). Cooked patties were placed in pre-heated, double boilers, cut into 6 equal pieces, returned to the double boilers and served to panelists warm.

Samples for warmed-over flavor (WOF) were cooked as above, wrapped in aluminum foil and held at 34 °F for 24 hour. Patties were re-warmed for evaluation in the aluminum foil wrapping in a forced-air convection oven. A thermocouple wire was inserted in one patty and internal temperature was monitored using the Doric. All patties were removed from the oven when the monitored patty reached an end point of 160 °F. Patties were removed from the foil, cut and placed in double boilers until serving for sensory panel. Warmed-over flavor panels were conducted separately from conventional panels.

Each panelist received a warm-up sample (control), which they discussed as a group, then eight samples in random order. Panelists were asked to cleanse their pallets with an apple slice, cracker and distilled water before beginning and between samples. Panelists were also asked to expectorate all samples, apple and cracker. Panelists scored each sample on 8-point scales for juiciness, beef flavor intensity, warmed-over flavor, mouth feel, and off-flavor intensity where 8 = extremely juicy, intense, no warmed-over flavor, typical beef mouth feel, and no off-flavors and 1 = extremely dry, bland, intense warmed-over flavor, extremely atypical mouth feel, and intense off-flavor.

Data were analyzed in a completely randomized treatment structure with batch as the experimental unit, but analytical design varied. For instrumental color, there was a repeated measure of day. For TBARS, the treatment structure was a split-plot, with Noni treatment as the whole plot and day as the sub-plot. For visual color, there was a repeated measure of day and the data were blocked by panelist. The block of panelist was also used for trained taste panel data. All data was analyzed in the mixed models procedure of SAS using the LSMEANS statement with the PDIFF option to separate the means.

Results and Discussion

There was a display day × Noni treatment interaction ($P < 0.05$) for total color score, worst-point color score, percent discoloration, lightness, redness, and yellowness. This indicated that the differences in the color traits due to Noni treatment differed from day to day. On the first day, all the patties were similar ($P > 0.05$) in total color scores (Fig. 1), but on days 2 and 3, patties with 0% Noni had greater total color scores ($P < 0.05$) than those with higher concentrations of Noni, indicating that the panelists perceived the control patties to be less red, more brown. Patties with 6% Noni had the lowest (most red) color scores at days 2 and 3, and when all the other treatments had mean color scores of 5.5 or greater at day 4, indicating they would be discriminated against, the 6% Noni patties were still perceived as acceptable. A similar trend was observed for the worst-point color scores (Fig. 2), at day 1 all treatments were similar ($P > 0.05$), and at days 2 and 3, patties with higher concentrations of Noni had lower (more red) worst-point color scores ($P < 0.05$). For percent discoloration (Fig. 3), again all treatments were similar at day 1 ($P > 0.05$), but by day 2, the 0% Noni patties had mean discoloration scores greater than 3 (21 to 35% discolored). It is at this 30% discoloration mark that consumers begin to discriminate against fresh retail product. Patties with higher concentrations of Noni had lower percent discoloration scores ($P < 0.05$) at days 2 and 3, and those with 4 and 6% Noni were less than 30% discolored for 3 d.

Although lightness (L*) and yellowness (b*) were evaluated, the differences are less relevant than those of redness (a*), and due to space constraints, only a* will be discussed (Fig. 4). At day 1, patties containing 2% Noni were more red ($P < 0.05$) than those containing 6%, but that difference was small and irrelevant. Although all patties became less red with time ($P < 0.05$), the 0% Noni patties were less red ($P < 0.05$) than those containing Noni at 2, 3, and 4 days of display.

There was a display day × Noni treatment interaction for TBARS ($P < 0.05$; Fig. 5). Although patties with 2% Noni had the lowest ($P < 0.05$) TBARS values at day 0 the differences were small and irrelevant. After 3 days of retail display, TBARS values increased ($P < 0.05$) for all treatments, but patties containing 0 and 2% Noni had higher (P < 0.05) TBARS values (more oxidation) than those with 4 and 6% Noni. After 5 days, patties with 0% Noni had the highest ($P < 0.05$) TBARS values, and patties with 6% Noni had similar values ($P > 0.05$) to those measured at 3 days. The TBARS results indicate that the Noni treatment was inhibiting oxidation, thus improving color stability.

The results for regular trained (cooked and served to panelists) and warmed-over flavor (cooked, chilled overnight, reheated, and served to panelists) taste panels are presented in Table 1. In the regular trained panel, as Noni percentage increased, beef flavor and off-flavor scores decreased ($P < 0.05$). Furthermore, mouth feel scores decreased ($P < 0.05$) with increasing Noni percentage. Also, patties with 2% Noni were perceived as the juiciest ($P < 0.05$). These results indicate that the panelists perceived the Noni-incorporated patties as having less beef-like flavor and more atypical flavors and mouth feel. Some of the off-flavors listed were sweet, chemical, fruity, soapy, and Noni.

In the warmed-over flavor panels, the only differences found were in beef flavor and off-flavor. Again, as Noni percentage increased, beef flavor and off-flavor scores decreased ($P < 0.05$), indicating that increased Noni resulted in patties with a less-typical beef flavor and a higher incidence of off-flavors. Some of the off-flavors listed included fruity, chemical, bitter, sour, and acidic. No differences ($P > 0.05$) were found in warmed-over flavor intensity, juiciness or mouth feel. It was hypothesized that the antioxidant in the Noni pulp would inhibit the development of warmed-over flavor (the cardboard, oxidized flavor associated with left-over meat). When examining the data, the warmed-over flavor scores for the regular trained panels were comparable to the scores for the warmed-over panels. Perhaps the 24 hour chill period was not sufficient for warmed-over flavor development and little oxidation occurred in all the patties, thus not allowing the Noni an environment to have its antioxidant effect.

Conclusions

When Noni pulp was incorporated into fresh ground beef patties, this natural antioxidant was successful at decreasing lipid oxidation and improving the shelf life and color stability. However, the objectionable off-flavors associated with this product may limit its usage in fresh ground beef in a large scale. More research needs to be conducted to improve the flavor profile of beef containing Noni pulp. Perhaps other ingredients or another Noni product would make this combination more acceptable to panelists. It is not yet known how untrained consumers will react to this combination or how they will perceive the incorporation of this natural ingredient into fresh beef.

Literature Cited

Apple et al. (2001) Meat Sci. 57:43
Witte (1970) J. Food Sci. 35:582
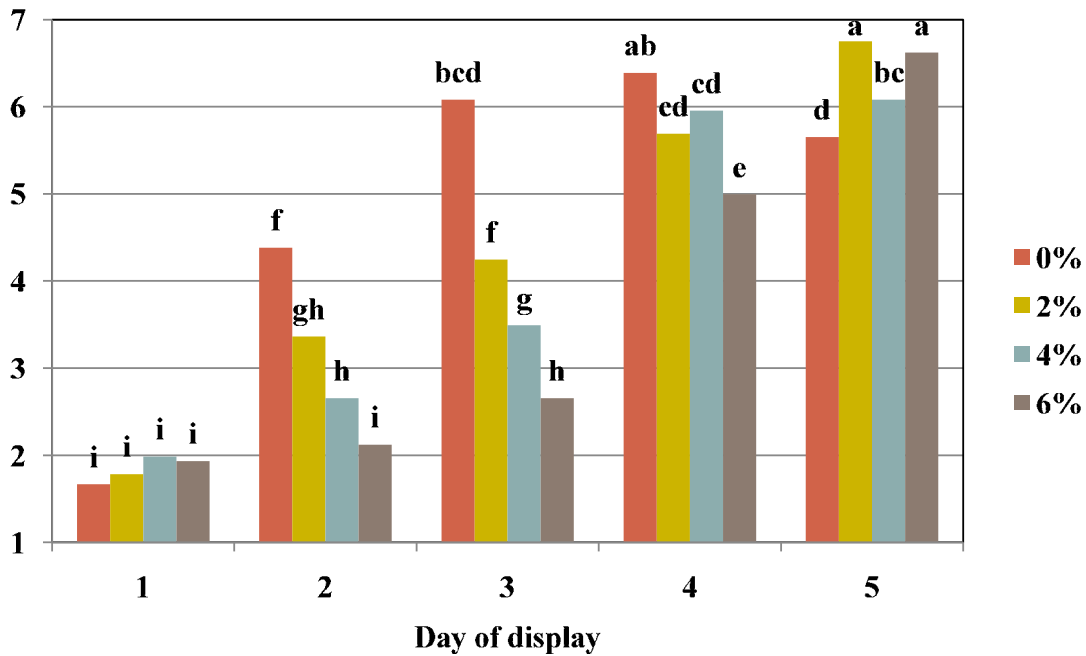
Fig. 1. Mean total color scores[1] for beef patties mixed with 0, 2, 4, or 6% Noni pulp displayed for 5 days.
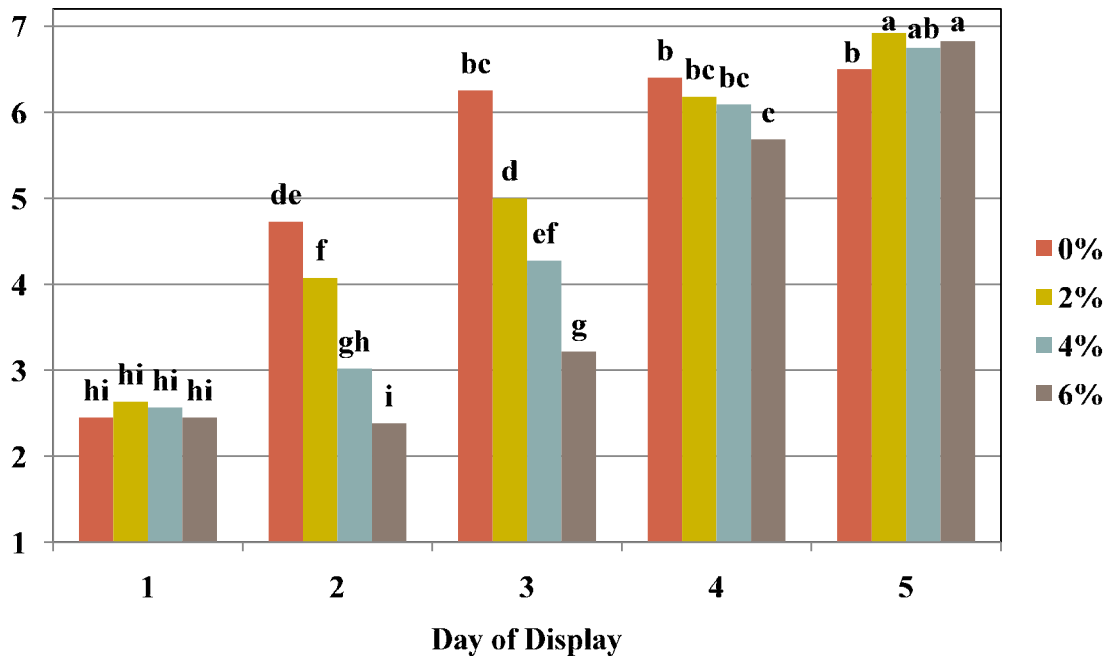
[a–i] Means with different superscript letters differ (*P* < 0.05).
[1] Steaks were evaluated on a 7 point scale where 1 = bright cherry red and 7 = very dark red to brown.
Worst-point color was evaluated as about the size of a dime.

Fig. 2. Mean worst-point color[1] scores for beef patties mixed with 0, 2, 4, or 6% Noni pulp displayed for 5 days.
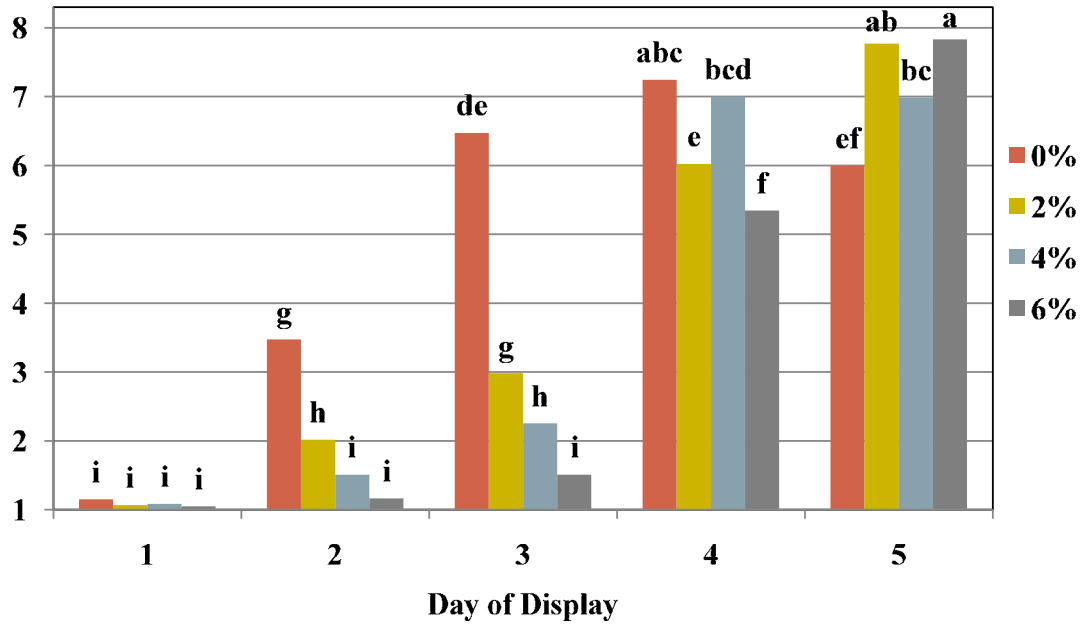
a–i Means with different superscript letters differ (*P* < 0.05).
[1] Steaks were evaluated on an 8 point scale where 1 = 0% to 5% discoloration and 8 = 96% to 100% discoloration.

Fig. 3. Mean percent discoloration[1] scores for beef patties mixed with 0, 2, 4, or 6% Noni pulp displayed for 5 days.
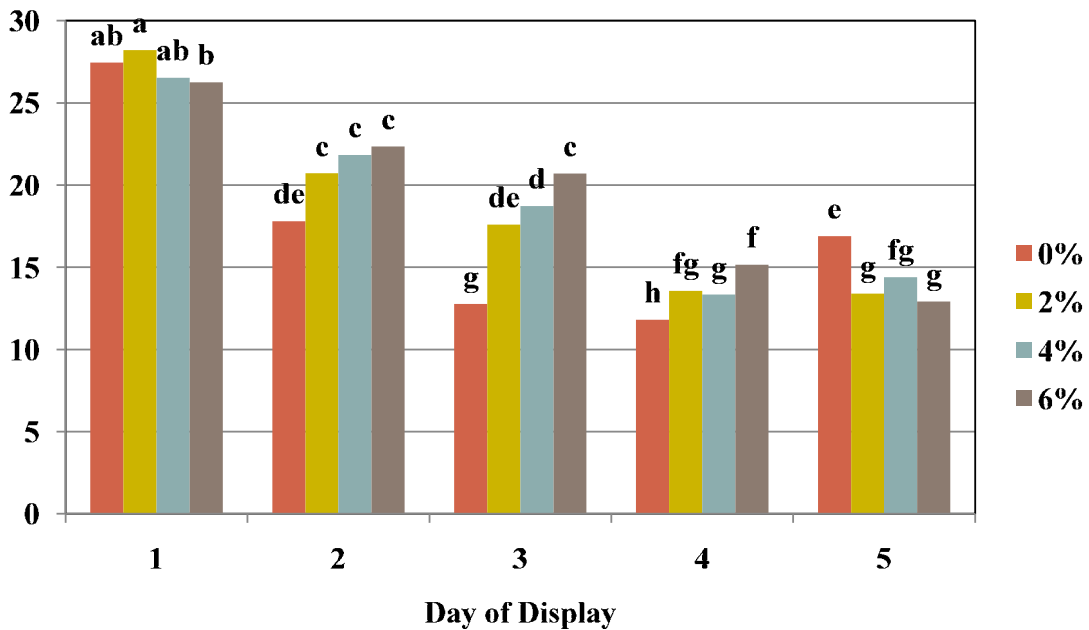
a–g Means with different superscript letters differ (*P* < 0.05).

Fig. 4. Mean values for redness (a*) for beef patties mixed with 0, 2, 4, or 6% Noni pulp displayed for 5 days.
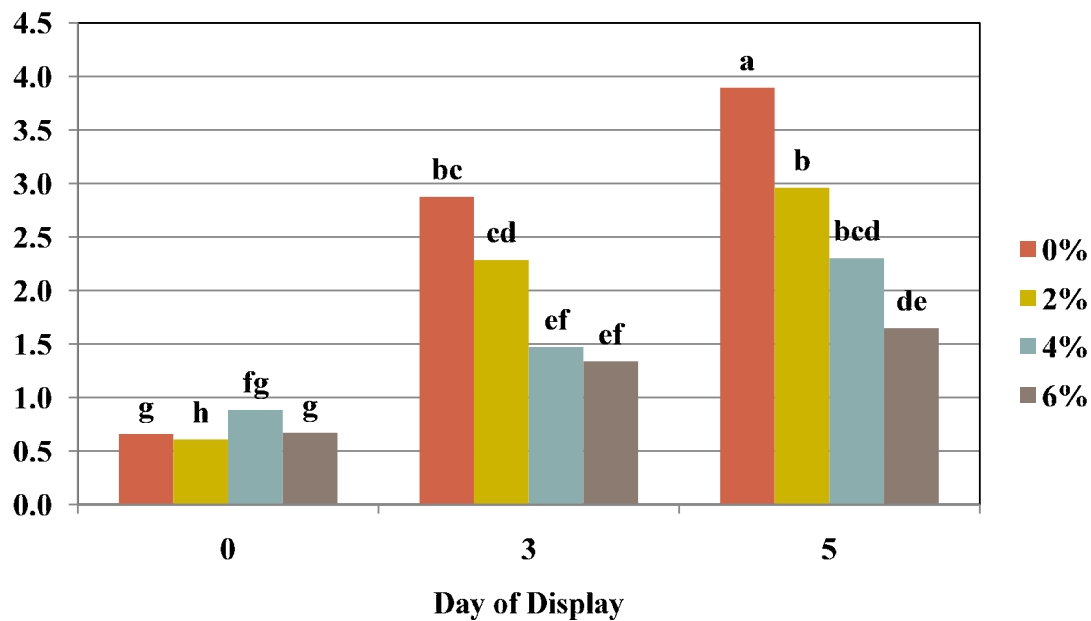
$^{a-g}$ Means with different superscript letters differ ($P < 0.05$).

Fig. 5. Mean values for thiobarbituric acid reactive substances (TBARS, a measure of oxidative rancidity) for beef patties mixed with 0, 2, 4, or 6% Noni pulp displayed for 5 days.

Table 1. Mean values for trained taste panel scores for beef patties mixed with 0, 2, 4, or 6% Noni pulp.

Sensory attributes[2]	Added noni pulp, %				SE	P-value[1]	
	0	2	4	6		NONI	LIN
Regular panel[3]							
Juiciness	5.5^b	5.8^a	5.5^b	5.5^b	0.17	0.037	0.459
Beef flavor intensity	5.5^a	4.6^b	4.1^c	3.8^c	0.16	<0.001	<0.001
Warmed-over flavor	7.2	7.4	7.4	7.4	0.16	0.401	0.149
Mouth feel	6.3^a	6.0^{ab}	5.9^{bc}	5.7^c	0.15	0.001	<0.001
Off-flavor intensity	7.6^a	5.9^b	4.5^c	3.7^d	0.35	<0.001	<0.001
WOF panel[4]							
Juiciness	5.1	5.0	4.9	5.0	0.13	0.419	0.512
Beef flavor intensity	5.2^a	4.9^b	4.2^c	3.9^c	0.14	<0.001	<0.001
Warmed-over flavor	7.0	6.8	6.8	7.1	0.17	0.280	0.481
Mouth feel	6.5	6.4	6.3	6.1	0.13	0.060	0.008
Off-flavor intensity	7.3^a	6.6^b	5.3^c	4.4^c	0.29	<0.001	<0.001

[1]Probability value of the main effect of Noni pulp inclusion level (NONI), and the linear (LIN) responses to Noni pulp inclusion level.
[2]8 = extremely juicy, intense, no warmed-over flavor, typical beef mouth feel, and no off-flavors and 1 = extremely dry, bland, intense warmed-over flavor, extremely atypical mouth feel, and intense off-flavor.
[3]Served warm after cooking.
[4]Warmed-over flavor patties were cooked, stored 24 hour, and served after reheating.
$^{a-d}$Within a row, least squares means lacking common superscript letters differ ($P < 0.05$).

Clarified Noni Juice Spray Was Not Successful As an Antioxidant in Beef Loin Steaks

J. W. S. Yancey[1], J. K. Apple[1], T. M. Johnson[1], and R. M. Stackhouse[1]

Story in Brief

The objective of the current study was to determine the effects of a clarified Noni juice spray applied as an antioxidant to fresh beef loin steaks on color stability during retail display. Forty beef strip steaks were randomly assigned to 1 of 5 treatments (0, 12.5, 25, 50 or 75% clarified Noni juice), both sides were sprayed, and steaks were packaged and placed in simulated retail display for visual and instrumental color evaluation. The 50% Noni treatment had the greatest ($P < 0.05$) antioxidant solution uptake, and the 75% treatment had greater ($P < 0.05$) uptake than the 0% control. Panelist scores for total color, worst-point color, and percent discoloration increased ($P < 0.05$) as display duration increased, indicating that the panelists observed the steaks becoming less red and more discolored with time. Steaks treated with 75% Noni had the greatest ($P < 0.05$) total color and worst-point color scores, indicating that they were the least red when evaluating the entire steak and the worst point of the steak. No differences were found in percent discoloration due to Noni treatment, and the 75% Noni steaks tended to have the poorest discoloration scores ($P = 0.058$). As display duration increased, instrumental color evaluation indicated that steaks became darker, less red, and less yellow (decreased L*, a*, and b*, respectively; $P < 0.05$). Steaks treated with 75% Noni were darker ($P < 0.05$) than those treated with 12.5, 25, or 50% Noni, but those treated with 0% Noni were intermediate ($P > 0.05$). Furthermore, steaks treated with 75% Noni were the least red and yellow ($P < 0.05$), whereas other treatments were similar ($P > 0.05$). These results indicated that clarified Noni spray was not an effective antioxidant in fresh beef loin steaks, but perhaps different applications of this natural antioxidant could be successful in the meat industry.

Introduction

Finding ways to prolong the shelf-life of fresh beef products would have a large impact on the beef industry. In fresh storage, meat naturally discolors with time. Oxidation of the pigment within the meat is a natural part of respiration in the live animal, but in fresh meat this chemical change causes the color of the product to shift from bright red to dull brown. This discoloration is discriminated by consumers and results in the devaluing and eventual waste of meat products. Natural antioxidants, such as rosemary, cherries, dried plums, and grapes, have been successful at delaying the oxidation process.

Products of the *Morinda citrifolia* plant (commonly called Noni) have been shown to have countless antioxidant, immune-stimulating, and tumor-suppressing properties in medicine and have been used, especially in Southeast Asia, to remedy a wide variety of ailments from muscle sprains and headaches to diabetes and heart disease. Researchers at the University of Arkansas have recently added Noni pulp to ground beef patties as an antioxidant and found improved shelf-life and color during retail display (Tapp et al., 2010). Clarified Noni juice is derived by removing the solids from Noni pulp and, to date, has not been tested in meat products. Therefore, the objective of this study was to determine the effects of clarified Noni juice in a spray as an antioxidant in fresh beef steaks in retail display conditions.

Experimental Procedures

Four USDA Choice, beef strip loins (IMPS #180) were purchased from a commercial processor and transported to the University of Arkansas Meat Laboratory and stored at 34 °F. Loins were cut (anterior to posterior) into 2 sets of 5 steaks, and steaks were assigned randomly within their set of 5 to Noni spray treatments (0, 12.5, 25, 50 or 75% clarified Noni juice). Clarified Noni was mixed (v/v) in distilled water and prepared in conventional, garden-style spray bottles. Steaks were weighed, and both sides were sprayed with three full sprays from the designated treatment. After a 5-min equilibration period on racks, steaks were weighed to calculate uptake. Steaks were packaged on 2S foam trays with PVC overwrap, and placed in simulated retail display (34 °F and 1600 lux warm-white lighting) for 5 d. Visual color evaluation was determined by 8 experienced panelists who scored total color, worst-point color and percent discoloration using the following scales:

Total color and worst-point color scales	Percent discoloration scale
1 = very bright cherry red	1 = 0 to 5% discoloration
2 = bright cherry red	2 = 6 to 20% discoloration
3 = dull red	3 = 21 to 35% discoloration
4 = slightly dark red	4 = 36 to 50% discoloration
5 = moderately dark red to tan	5 = 51 to 65% discoloration
5.5 = borderline panelist acceptable	6 = 66 to 80% discoloration
6 = dark red to brown	7 = 81 to 95% discoloration
7 = very dark red to brown	8 = 96 to 100% discoloration

To determine worst-point discoloration, panelists were asked to determine the worst point on the steak (about the size of a dime). Instrumental color (L*, a*, b*) was evaluated using a Hunter MiniScan XE Plus with Illuminant A and a 1-in. aperture.

Data were analyzed in a randomized complete block design, with set of steaks as the blocking factor. Steak was the experimental unit. The MIXED procedure of SAS was used to analyze the data. The LSMEANS statement was used with the PDIFF option to separate the means ($P < 0.05$). Contrast statements were used to test the linearity of the data.

Results and Discussion

Although the goal was a 1 to 2% uptake of the Noni Juice spray, the steaks only retained from 0.3 to 0.7% of their weight from the

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spray treatments (Table 1). Steaks sprayed with 50% Noni had the greatest ($P < 0.05$) retention of the treatment in the 5 min after spraying. Steaks treated with 75% Noni had greater ($P < 0.05$) treatment uptake than those with 0% Noni (just sprayed with water), whereas those sprayed with 12.5 and 25% Noni were similar to the control ($P > 0.05$).

There was no display day × Noni treatment interaction ($P > 0.05$) for any instrumental or visual color measurement, meaning that treatment differences were not affected by differences in day of display. Expectedly, the visual color scores for total color, worst-point color, and discoloration increased with display time (linear $P < 0.0001$), indicating that the panelists perceived that the steaks became less red, both the entire steak and the worst-point, and more discolored with time in retail display (Fig. 1). For instrumental color, values for L*, a*, and b* decreased with display duration (linear $P < 0.0001$), indicating that the steaks became darker, less red, and less yellow, respectively (Fig. 2).

Visual differences in the steaks due to Noni treatment were not promising. For total color and worst-point color, steaks treated with the 75% Noni had the greatest scores ($P < 0.05$), and all other treatments were similar ($P > 0.05$). This indicated that the panelists perceived the steaks treated with the greatest percentage of Noni juice to have the least red, brownest, least desirable color, and that steaks treated with the lower percentages of Noni juice were not different from the control. Noni treatment did not have an effect on percent discoloration ($P = 0.058$). This indicated that the panelists perceived the Noni steaks to be discolored similarly to those treated with water.

When the instrumental color was evaluated, steaks treated with 75% Noni juice were darker (lower L*; $P < 0.05$) than those treated with 12.5, 25, and 50%, but all of the treatments (12.5 to 75%) had similar ($P > 0.05$) L* values to the control. Steaks treated with 75% Noni juice were also the least red and yellow ($P < 0.05$) of all the treatments, and the other percentages were similar ($P > 0.05$).

In contrast to the positive results found with Noni pulp in ground beef patties, these results indicate that using clarified Noni-juice in a spray was not an effective way to inhibit oxidation on fresh beef steaks. In fact, the highest level of Noni (75%) may have been detrimental to color stability. When used in the beef patties, the Noni pulp was added at much lower levels (2, 4, and 6%), but was very successful at inhibiting lipid oxidation and maintaining fresh beef color (Tapp et al., 2010). In some cases, antioxidants can have an oxidizing effect when applied at high levels. Other components in the Noni may have had an oxidizing or discoloring affect when applied at this high level.

Lower concentrations of Noni-juice (12.5 – 50%), however, had no effect on the shelf life or color of the fresh beef steaks. This is in contrast to very positive results seen in ground beef patties (Tapp et al., 2010). Ground beef patties have a much higher fat concentration than beef strip steaks. Perhaps the antioxidant effects of Noni are only effective on lipid oxidation and thus were of little effect on the leaner, strip steaks.

Conclusions

Spraying solutions of clarified Noni juice in water was not successful at improving the color stability of fresh beef strip steaks in retail display. In fact, the highest concentration of Noni was actually detrimental to beef color. Perhaps another method of application would be more successful for this natural antioxidant in lean, whole muscle beef products.

Literature Cited

Tapp, W. N, J. W. S. Yancey, and J. K. Apple. 2010. J. Anim. Sci. 88: Suppl. 2 (Abstract)

Table 1. Mean values for treatment uptake, visual color scores, and instrumental color values of beef strip steaks treated with 0, 12.5, 25, 50, and 75% clarified Noni juice and held in retail display for 5 d.

	Noni Treatments				
	0%	12.5%	25%	50%	75%
Treatment uptake (%)	0.31[c]	0.43[bc]	0.37[bc]	0.66[a]	0.43[b]
Total color[1]	3.11[b]	3.04[b]	3.35[b]	3.33[b]	3.72[a]
Worst-point color[2]	3.60[b]	3.63[b]	3.99[b]	4.01[b]	4.41[a]
Percent discoloration[3]	2.13	2.19	2.32	2.41	2.98
Lightness (L*)	40.81[ab]	41.37[a]	41.12[a]	40.39[a]	39.91[b]
Redness (a*)	25.95[a]	25.96[a]	25.38[a]	25.54[a]	23.44[b]
Yellowness (b*)	21.43[a]	21.32[a]	21.20[a]	21.50[a]	20.18[b]

[1] Total color was evaluated on a 7 point scale where 1 = bright cherry red and 7 = very dark red to brown.
[2] Worst-point color was evaluated on a 7 point scale where 1 = bright cherry red and 7 = very dark red to brown. Worst-point color was evaluated as about the size of a dime.
[3] Percent discoloration was evaluated on an 8 point scale where 1 = 0 to 5% discoloration and 8 = 96 to 100 % discoloration.
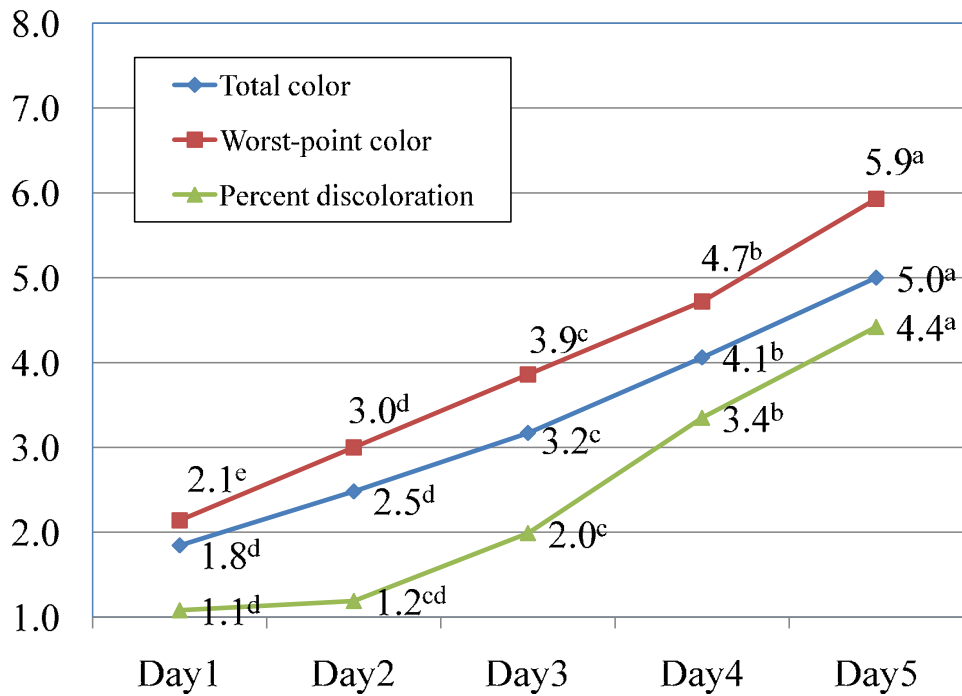
a-e Means, within a color scale, with different superscript letters differ ($P < 0.05$).

[1] Steaks were evaluated on 7 point scales for total color and worst-point color where 1 = bright cherry red and 7 = very dark red to brown and an 8 point scale for percent discoloration where 1 = 0% to 5% discoloration and 8 = 96% to 100% discoloration. Worst-point color was evaluated as about the size of a dime.

Fig. 1. Visual color scores[1] of beef strip steaks treated with 0, 12.5, 25, 50, and 75% clarified Noni juice.
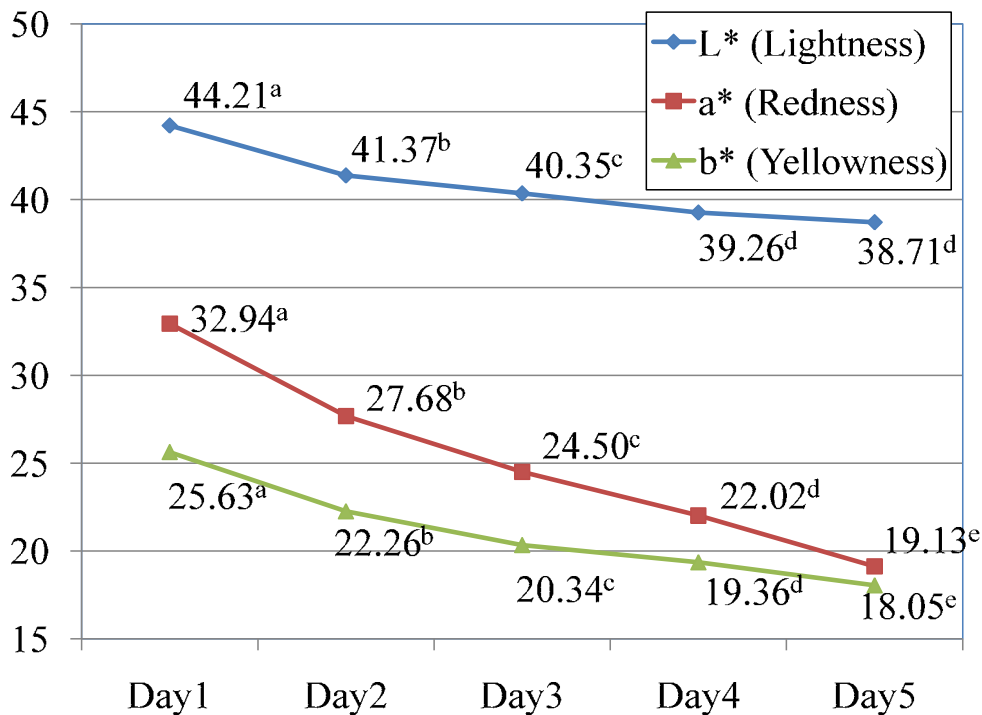
a-e Means, within a color value, with different superscript letters differ ($P < 0.05$).

Fig. 2. Instrumental color means of beef strip steaks treated with 0, 12.5, 25, 50, and 75% clarified Noni juice.

Effect of Dietary Mannanase Supplementation on Pig Growth Performance

B. E. Bass[1], J. W. Frank[1], Z. B. Johnson[1], C. V. Maxwell[1], and J. H. Lee[2]

Story in Brief

A study was conducted to determine the effect of feeding diets supplemented with β-mannanase (CTCZYME, CTCBIO Inc.) on growth performance in growing-finishing pigs. A total of 140 pigs (BW = 66.3 lb) were penned in groups of 5 pigs/pen and fed a 3-phase diet program (phase 1: 65-110 lb; phase 2: 110-200 lb; phase 3: 200-265 lb). Pigs were randomly assigned to one of four treatments: 1) Negative control (NC); 2) 200,000 IU enzyme added to NC (MAN2); 3) 400,000 IU enzyme added to NC (MAN4); and 4) Positive control containing an additional 45.4 kcal/lb ME from fat (PC). During phase 1 of the growing-finishing study, there was a linear decrease in ADFI ($P = 0.05$) and linear improvement in F:G ($P = 0.02$) as the level of enzyme increased. During phase 2, there was also a linear decrease ($P = 0.02$) in ADFI as the level of enzyme increased. Overall ADG (2.31 vs. 2.2 lb/d) and BW (274.5 vs. 265.9 lb) were greater in PC compared to NC ($P < 0.01$). There was an overall linear decrease in ADFI (6.42, 6.28, and 6.00 lb/d for NC, MAN2, and MAN4, respectively; $P = 0.05$) as the level of enzyme increased during the grow-finish study. In conclusion, the addition of β-mannanase to diets improved growth performance in growing-finishing pigs due to improved energy utilization.

Introduction

North American swine diets are commonly corn and soybean meal based, and recently distillers dried grains with solubles from ethanol production has been incorporated. Nutrients from these sources must be broken down to a usable form by the animal in order to support maintenance or production (growth and reproduction). Many plant-based feedstuffs contain non-digestible factors, such as β-mannans that may reduce growth performance, as well as energy and nutrient digestibility. Soybean meal in particular contains 1.26 to 1.61% β-mannans (Hsiao et al., 2006). However, pigs lack enzymes, such as β-mannanase, necessary to more completely digest β-mannan. A previous study performed at the University of Arkansas Swine Research Facility using nursery pigs indicated that improved feed efficiency was achieved in pigs fed reduced energy diets supplemented with β-mannanase, achieving performance similar to pigs fed diets containing an additional 45.4 kcal/lb of metabolizable energy (Frank et al., 2009). Thus, the purpose of this study was to determine the efficacy of CTCzyme, a β-mannanase product, in enhancing performance and energy utilization in growing-finishing pigs.

Experimental Procedures

Animals. A total of 140 pigs with an average initial body weight of 66.3 ± 0.42 lbs were transported to the University of Arkansas Growing-Finishing Swine Research Farm. Pigs were weighed individually, blocked based on initial body weight and randomly assigned into equal subgroups with stratification based on sex and litter. Pigs were penned in groups of 5 pigs/pen and fed a 3-phase diet program (phase 1: 65-110 lb; phase 2: 110-200 lb; phase 3: 200-265 lb) that was formulated to meet or exceed NRC recommendations for growing-finishing pigs. Pens within weight blocks were randomly assigned to one of four treatments: 1) Negative control (NC); 2) 200,000 IU enzyme added to NC (MAN2); 3) 400,000 IU enzyme added to NC (MAN4); and 4) Positive control containing an additional 45.4 kcal/lb ME from fat (PC). The treatments were randomly assigned to 28 pens within weight block such that each dietary treatment had 7 replicates. Dietary treatment for Phase 1, 2, and 3 are shown in Table 1. Pigs were provided feed and water *ad libitum*.

Pen body weights and feed intake were measured at the end of each phase. Pen ADG, ADFI, and F/G were calculated for each of the phases and for overall growing-finishing period.

Statistical Analysis. Preplanned orthogonal contrasts comparing PC vs. NC, as well as linear and quadratic responses of enzyme level (NC, MAN2, and MAN4) were evaluated for each performance parameter measured (SAS Institute Inc., Cary, N.C.).

Results and Discussion

Pigs fed the PC diets had improved growth performance at the end of the study compared to those fed the NC diet (Table 2). This was mainly observed as an increase of 4.1% in overall ADG, and 8.5 lbs in additional BW ($P < 0.01$) at the end of the study in PC pigs. The improvement in overall ADG and final BW was likely due to the additional energy (45.4 kcal/lb) provided by the PC diet compared to the NC diet.

Supplementation with β-mannanase in the NC diet decreased ADFI in phase 1, phase 2, and the overall growing-finishing study (Linear, $P < 0.05$) as the level of enzyme increased (Table 2). Additionally, F:G was improved at each level of β-mannanase addition during phase 1 (Linear, $P < 0.05$) and the overall growing-finishing study (Linear, $P < 0.1$; Table 2).

Implications

The decreased ADFI and similar body weights of NC compared to MAN2 and MAN4, indicate an improvement in energy utilization in pigs fed β-mannanase supplemented diets. Addition of enzymes, such as β-mannanase may allow reduced energy diets to be fed to growing-finishing pigs without detrimental effects on growth performance.

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[1] University of Arkansas, Department of Animal Science, Fayetteville, Ark.

[2] CTCBIO Inc., Seoul, Korea.

Table 1. Composition of Growing-Finishing diets (as-fed basis).

Item, %	Phase 1		Phase 2		Phase 3	
	PC	NC[1]	PC	NC[1]	PC	NC[1]
Yellow corn	62.16	64.87	61.48	64.13	61.84	64.50
DDGS	5.00	5.00	10.00	10.00	15.00	15.00
Soy meal, 48%	25.90	25.90	21.90	21.90	16.60	16.60
Fat, Darling	4.09	1.50	4.08	1.50	4.08	1.50
Lysine	0.20	0.15	0.20	0.15	0.20	0.15
Other	2.65	2.58	2.34	2.32	2.28	2.25
Calculated composition						
Lysine	1.14	1.11	1.06	1.03	0.94	0.91
Threonine	0.74	0.72	0.69	0.69	0.64	0.65
Met + Cys	0.66	0.65	0.65	0.65	0.63	0.64
ME, Mcal/lb	1.579	1.533	1.583	1.537	1.584	1.539

[1]β-mannanase (CTCZYME, CTCBIO) was added to the negative control (NC) diet to provide either 200,000 (MAN2) or 400,000 (MAN4) IU of enzyme per kg of diet. PC = Positive control.

Table 2. Growth performance of growing-finishing pigs supplemented with β-mannanase.[1]

	NC	MAN2	MAN4	PC	SE	Statistic[2]
ADG, lb/d						
Phase 1	1.98	2.00	2.00	2.03	0.04	
Phase 2	2.20	2.20	2.15	2.26	0.06	
Phase 3	2.39	2.39	2.47	2.56	0.08	
Overall	2.21	2.21	2.18	2.30	0.02	C*
ADFI, lb/d						
Phase 1	4.44	4.05	4.05	4.55	0.13	L
Phase 2	6.14	6.18	5.67	6.42	0.13	L
Phase 3	8.18	8.02	7.81	8.16	0.21	
Overall	6.40	6.28	5.99	6.55	0.14	L
F:G						
Phase 1	2.26	2.03	2.03	2.26	0.06	L
Phase 2	2.79	2.83	2.65	2.85	0.10	
Phase 3	3.43	3.37	3.18	3.20	0.12	
Overall	2.90	2.84	2.75	2.85	0.06	L‡
BW, lb						
Initial	66.6	66.2	65.9	66.6	0.4	
Phase 1	106.2	106.2	105.9	107.2	0.9	
Phase 2	198.4	198.4	196.5	202.1	2.2	
Phase 3	265.3	265.2	265.7	273.8	1.8	C*

[1] Phase 1 = 65-110 lb; Phase 2 = 110-200 lb; Phase 3 = 200-265 lb
[2] L‡ = Linear response to enzyme addition (Negative Control, 200,000 IU Mannanase, and 400,000 IU Mannanase; $P < 0.10$); L = Linear response to enzyme addition (Negative Control, 200,000 IU Mannanase, and 400,000 IU Mannanase; $P \leq 0.05$); C* = Positive Control vs. Negative control ($P \leq 0.01$).

Effect of Replacing Fish Meal with Synthetic Amino Acids or Soy Protein Concentrate in Nursery Diets Containing Distillers Dried Grains with Solubles

C. L. Bradley[1], C. V. Maxwell[1], Z. B. Johnson[1], J. L. Usry[2], and J. W. Frank[1]

Story in Brief

In today's swine industry, producers are faced with rising feed costs for their livestock. Protein sources in swine nursery diets, such as fish meal, represent one such example of fluctuation of an ingredient with large input costs. Therefore, the objective of this study was to determine if fish meal (FM) could be replaced with soy protein concentrate (SPC), synthetic amino acids (SAA), or SAA plus omega-3 fatty acids, with an omega-3: omega-6 ratio equaled the FM treatment (SAA+FO) in Phase 1 and 2 nursery diets containing good quality distillers dried grains with solubles (DDGS; 20% of diet). A positive control diet (PC) containing FM but no DDGS was also evaluated in all 3 phases. Weaned pigs, (21 d of age; 15.67 ± 0.02 lb. BW) were used in the 33-d growth study (6 pigs/ pen and 7 pens/treatment). During Phase 1 (d 1 to 7) and Phase 2 (d 8 to 17) pigs were fed 1 of the following treatments: (1) a positive control diet that contained FM, but with no DDGS (PC); (2) a diet containing DDGS + FM (DDGS+FM); (3) a diet containing DDGS + soy protein concentrate (SPC); (4) a diet containing DDGS + synthetic amino acids (SAA); and a diet containing DDGS + SAA + fish oil (SAA+FO). During Phase 3 (18 to 33 d), the PC diet continued, with all other treatment groups being fed a common corn-soy-DDGS diet (DDSG+FM, SPC, SAA, and SAA+FO). In general, pigs that were fed DDGS grew faster ($P = 0.03$) during Phase 1 and were heavier ($P = 0.03$) at the end of Phase 1 (7 d) and the end of the study (d 33) than pigs fed the PC treatment. Furthermore, replacing FM with different protein or amino acid sources did not impact growth during the nursery period; thus, FM can be replaced with cheaper, alternative feedstuffs during the nursery period.

Introduction

Rising feed costs are forcing swine producers and nutritionists to consider alternative feed ingredients for swine rations. During the initial part of the nursery period pigs are fed complex diets with expensive protein sources, such as fish meal and/or spray dried plasma. Synthetic amino acids are becoming more widely available and used as alternatives for proteins in swine diets. Using synthetic amino acids in swine diet formulations allow the diet to more precisely meet the amino acid needs of the animals without providing unneeded excess protein. It was previously demonstrated that fish meal could be replaced with synthetic amino acids without negatively impacting growth performance during the nursery period (Bradley et al., 2008). Therefore, the main objective of this study was to re-evaluate fish meal replacement with soy protein concentrate and synthetic amino acids, and synthetic amino acids plus omega-3 fatty acids. A secondary objective was to test the efficacy of DDGS in nursery diets.

Experimental Procedures

At weaning, 210 pigs (21 d of age and 15.67 ± 0.02 lb. BW) from the mating of GPK35 dams to EBU boars (Newsham Choice Genetics, West Des Moines, IA) were used in a 33 d growth study. Pigs were blocked by initial BW and assigned to pens that consisted of 6 pigs per pen. Pigs were fed 1 of 5 dietary treatments during Phase 1 (d 1 to 7; 1.44% TID Lys) and Phase 2 (d 8 to 17; 1.39% TID Lys): (1) a positive control diet (PC) that contained fishmeal (FM), but with no distiller dried grains with solubles (DDGS); (2) a diet containing DDGS + FM (DDGS+FM); (3) a diet containing DDGS + soy protein concentrate (SPC); (4) a diet containing DDGS + synthetic amino acids (SAA); and (5) a diet containing DDGS + SAA + fish oil (SAA+FO). In

Phase 3 (18 to 33 d; 1.26% TID Lys), pigs were fed PC, DDSG+FM, SAA, SPC, or SAA+FO supplemented with 0.40% L-Lys. All diets maintained ideal ratios of TID Methionine + Cysteine, Threonine, Tryptophan, and Valine at minimums of 0.58, 0.60, 0.165, and 0.65 to Lys, respectively. Phase 1, 2, and 3 diet compositions for ingredients, calculated compositions, and analyzed amino acid compositions are in Tables 1, 2, and 3, respectively.

Pigs were housed in wire-floored pens (4 ft × 5 ft) with ad libitum access to feed and water throughout the study. Pigs were individually weighed and pen feed intake was measured for each phase to calculate ADG, ADFI, and F: G for each phase and the overall nursery period.

Data was analyzed as a randomized complete block design using the GLM procedure of SAS. Dietary treatment was the main effect included in the model, and pen was the experimental unit. A contrast statement was included in the model to test the effect of 20% DDGS inclusion (PC vs. DDGS+FM, SPC, SAA, and SAA+FO).

Results and Discussion

Pigs consuming diets containing DDGS had greater ADG (0.253 vs. 0.185 lbs./d; $P = 0.03$) and BW (17.44 vs. 16.97 lbs.; $P = 0.03$) compared to PC for Phase 1 (Table 4). The final BW on d 33 of PC fed pigs tended to be less ($P = 0.11$) than that of pigs consuming DDGS formulated diets (50.24 vs. 51.17 lbs.). During Phase 1, pigs consuming DDSG+FM had greater ($P < 0.05$) ADG than those fed SPC, with SAA and SAA+FO being intermediate. There were no differences in ADFI ($P = 0.28$) or F: G ($P = 0.18$) during Phase 1, nor was ADG, ADFI, and F: G affected by dietary treatments during Phase 2 ($P \geq 0.15$), Phase 3 ($P \geq 0.17$), or the overall 33-d nursery trial ($P \geq 0.11$).

The results of this experiment indicate that nursery pigs can tolerate being fed high levels of DDGS. In addition, replacing FM with SAA or

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[2] Ajinomoto Heartland LLC, Chicago, Ill.

SAA+FO had no effect on growth performance, similar to results of Bradley et al. (2008).

Implications

Growth performance of nursery pigs is not negatively impacted by inclusion of DDGS at levels of 20% in the diet. More over, fish meal can be effectively replaced with alternative protein sources, such as soy protein concentrate or synthetic amino acids, without reducing growth performance while maintaining least cost feed formulations.

Literature Cited

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Table 1. Phase 1 diet composition[1].

	Dietary Treatment[2]				
		DDGS+			SAA+
Ingredients, %	PC	FM	SPC	SAA	FO
Corn	35.060	18.270	17.520	20.270	19.410
Soybean Meal - 48% CP	18.000	18.000	18.000	18.000	18.000
DDGS	0.000	20.000	20.000	20.000	20.000
Poultry Fat	3.000	3.300	3.350	3.650	3.450
Dicalcium Phosphate	0.000	0.103	0.103	0.660	0.660
Limestone	0.450	0.920	0.920	1.040	1.040
Salt	0.300	0.300	0.300	0.300	0.300
L-Lysine	0.093	0.261	0.296	0.468	0.468
DL-Methionine	0.108	0.114	0.145	0.200	0.200
L-Threonine	0.005	0.008	0.005	0.088	0.088
L-Valine	0.000	0.000	0.000	0.078	0.078
L-Tryptophan	0.000	0.000	0.000	0.015	0.015
Soycomil P	0.000	0.000	3.500	0.000	0.000
Whey	30.000	30.000	30.000	30.000	30.000
Spray Dried Plasma	3.500	3.500	3.500	3.500	3.500
Menhaden Fish Meal	7.750	3.500	0.000	0.000	0.000
Gromega 365	0.000	0.000	0.000	0.000	1.060
Zinc Oxide	0.300	0.300	0.300	0.300	0.300
Other	1.430	1.430	1.430	1.430	1.430
Calculated Composition					
ME, Mcal/lb.	1.55	1.55	1.55	1.55	1.55
Crude Protein, %	22.95	24.49	24.51	22.78	22.81
Total Lysine, %	1.60	1.65	1.64	1.64	1.64
TID Lysine, %	1.44	1.44	1.44	1.44	1.44
TID Met + Cys/Lysine, %	58.02	58.07	58.05	58.08	57.96
TID Threonine/Lysine, %	60.03	60.09	60.24	60.11	60.06
TID Tryptophan/Lysine, %	16.45	16.48	17.12	16.47	16.47
TID Isoleucine/Lysine, %	60.14	60.65	61.71	54.77	54.68
TID Valine/Lysine, %	69.49	70.83	71.12	69.37	69.27
Omega 3 : 6 Ratio	7.09	9.94	29.15	29.81	9.94

[1] Phase 1 was fed from d 0-7 d post-weaning
[2]PC = Positive control diet devoid of fish meal (FM) but devoid of dry distillers grain soluble (DDGS); DDGS+FM = Diet containing DDGS+FM ;SPC = Diet containing soy protein concentrate and DDGS; SAA = Diet containing synthetic amino acids and DDGS; SAA+FO = Diet containing synthetic amino acids and the same Omega 3: 6 ratio as the FM treatment and DDGS.

Table 2. Phase 2 diet composition[1].

Ingredients, %	PC	DDGS+ FM	SPC	SAA	SAA+ FO
Corn	48.170	31.320	30.680	33.200	32.390
Soybean Meal - 48% CP	25.500	25.500	25.500	25.500	25.500
DDGS	0.000	20.000	20.000	20.000	20.000
Poultry Fat	3.000	3.350	3.350	3.650	3.500
Dicalcium Phosphate	0.225	0.325	0.805	0.845	0.845
Limestone	0.350	0.820	0.930	0.935	0.935
Salt	0.300	0.300	0.300	0.300	0.300
L-Lysine	0.120	0.290	0.321	0.482	0.482
DL-Methionine	0.076	0.083	0.111	0.164	0.164
L-Threonine	0.028	0.031	0.025	0.105	0.105
L-Valine	0.000	0.000	0.000	0.074	0.074
L-Tryptophan	0.000	0.000	0.000	0.014	0.014
Soycomil P	0.000	0.000	3.250	0.000	0.000
Whey	12.000	12.000	12.000	12.000	12.000
Spray Dried Plasma	1.000	1.000	1.000	1.000	1.000
Menhaden Fish Meal	7.500	3.250	0.000	0.000	0.000
Gromega 365	0.000	0.000	0.000	0.000	0.970
Zinc Oxide	0.300	0.300	0.300	0.300	0.300
Other	1.700	1.700	1.700	1.700	1.700
Calculated Composition					
ME, Mcal/lb	1.56	1.56	1.56	1.56	1.56
Crude Protein, %	23.34	24.87	24.89	23.29	23.32
Total Lysine, %	1.54	1.59	1.58	1.58	1.58
TID Lysine, %	1.39	1.39	1.39	1.39	1.39
TID Met + Cys/Lysine, %	58.01	58.08	58.02	58.16	58.04
TID Threonine/Lysine, %	60.03	60.03	60.00	60.06	60.01
TID Tryptophan/Lysine, %	16.84	16.85	17.48	16.86	16.85
TID Isoleucine/Lysine, %	63.39	63.86	64.93	58.26	58.17
TID Valine/Lysine, %	71.40	72.71	73.06	71.46	71.36
Omega 3 : 6 Ratio	8.74	13.09	36.47	36.42	13.09

[1] Phase 2 was fed from d 7 – 21 d post-weaning.
[2] PC = Positive control diet devoid of fish meal (FM) but devoid of dry distillers grain soluble (DDGS); DDGS+FM = Diet containing DDGS+FM ;SPC = Diet containing soy protein concentrate and DDGS; SAA = Diet containing synthetic amino acids and DDGS; SAA+FO = Diet containing synthetic amino acids and the same Omega 3: 6 ratio as the DDGS+FM treatment.

Table 3. Phase 3 diet composition[1].

Ingredients, %	Dietary Treatment	
	PC	DDGS
Corn	62.36	36.90
SBM - 48% CP	30.00	26.25
DDGS	0.00	30.00
Poultry Fat	3.00	2.75
Dicalcium Phosphate	1.41	0.57
Limestone	0.61	1.10
Salt	0.40	0.40
L-Lysine	0.40	0.40
DL-Methionine	0.16	0.05
L-Threonine	0.14	0.05
Copper Sulfate	0.10	0.10
Other	1.43	1.43
Calculated Composition		
ME, Mcal/lb	1.55	1.55
Crude Protein, %	19.99	24.22
Total Lysine, %	1.38	1.45
TID Lysine, %	1.26	1.26
TID Met + Cys/Lysine, %	57.95	57.96
TID Threonine/Lysine, %	60.98	61.07
TID Tryptophan/Lysine, %	16.46	17.03
TID Isoleucine/Lysine, %	57.96	65.87
TID Valine/Lysine, %	64.50	75.79
Omega 3 : 6 Ratio	58.50	44.11

[1]Phase 3 was fed from d 21-33 post-weaning.
[2]Positive control diet devoid of DDGS (PC);the four other dietary treatments (DDGS+FM, SPC, SAA, SAA+FO) were continued on a common diet containing DDGS.

Table 4. Growth Performance.

Parameter	Dietary Treatment[1]					Std. Error	P value
	PC	DDSG+FM	SPC	SAA	SAA+ FO		
ADG, lbs.							
Phase 1[2]	0.185[c]	0.279[a]	0.207[bc]	0.253[abc]	0.271[ab]	0.024	0.05
Phase 2	0.871	0.909	0.821	0.898	0.862	0.053	0.78
Phase 3	1.487	1.547	1.569	1.553	1.542	0.033	0.50
Overall	1.025	1.085	1.054	1.060	1.067	0.029	0.67
ADFI, lbs.							
Phase 1	0.341	0.398	0.326	0.389	0.383	0.029	0.28
Phase 2	0.953	1.082	1.003	1.06	1.109	0.053	0.23
Phase 3	2.290	2.449	2.275	2.427	2.468	0.077	0.25
Overall	1.472	1.599	1.476	1.582	1.615	0.048	0.11
F: G							
Phase 1	1.798	1.457	1.703	1.646	1.455	0.12	0.18
Phase 2	1.104	1.212	1.254	1.169	1.291	0.05	0.15
Phase 3	1.536	1.591	1.450	1.600	1.598	0.05	0.17
Overall	1.426	1.476	1.402	1.478	1.512	0.04	0.22
BW, lbs.							
Initial	15.67	15.65	15.70	15.65	15.67	0.02	0.15
Phase 1	16.97	17.61	17.13	17.44	17.57	0.18	0.07
Phase 2	25.73	26.72	25.35	26.43	26.21	0.62	0.52
Phase 3	50.24	51.50	50.51	51.57	50.93	0.86	0.46

[1] PC = Positive control diet devoid of fish meal (FM) but devoid of dry distillers grain soluble (DDGS); DDGS+FM = Diet containing DDGS+FM ;SPC = Diet containing soy protein concentrate and DDGS; SAA = Diet containing synthetic amino acids and DDGS; SAA+FO = Diet containing synthetic amino acids and the same Omega 3: 6 ratio as the DDGS+FM treatment and DDGS
[2] PC differed ($P = 0.03$) from diets formulated with 20% DDGS.
[a, b, c] Within a row, means lacking a common superscripted letter differ ($P < 0.05$).

Clovers in Response to Broadcast vs. No-Till Drill Planting—Second Year Results

D. Philipp[1], B. Briggs[1], K. Coffey[1], John Jennings[1], and R. Rhein[1]

Story in Brief

The objective of this study, initiated in fall of 2008, was to examine the effects of different strategies of crimson clover (*Trifolium incarnatum* L.) and white clover (*T. repens* L.) establishment. The experimental design was a randomized complete block with whole plots representing cattle grazing before and after planting to test for effects of hoof action on clover emergence. Within each whole plot, eight treatments were randomly imposed as a subplot factors; these included planting by no-till drill or by broadcast at high and low seeding rates for both species. During the second year of the study, seedling counts were performed in fall of 2009 and then again in spring of 2010. No-till planting of white clover resulted in higher (*P* < 0.05) seedling counts than the broadcast method at either high or low seeding rates. With crimson clover, no-till planting and broadcast resulted in similar (*P* > 0.05) seedling counts under a low seeding rate. Maximum counts observed for crimson clover and white clover were 20 seedlings / square foot for both crimson and white clover, using no-till planting with a high seeding rate. Seedling numbers in 'grazed after' white clover plots when broadcasted at a high seeding rate where increased (*P* < 0.05) compared with 'grazed before' plots. The number of seedlings decreased during winter, especially when established with the no-till drill procedure. Broadcasting seeds into an existing bermudagrass stands appears to be a low-cost alternative for clover establishment.

Introduction

Legumes have been used for centuries by producers and researchers that led to the adoptation of appropriate legume species for particular requirements. One of the desirable effects of legume production is the biological ability of legume plants to use atmospheric N, but with the development of synthetic N fertilizer (McNeill, 2000) early in the last century, the use of legumes, and especially clovers, has been replaced with easily applicable and inexpensive commercial fertilizer. The recent, immense increase in energy costs may reverse this trend as N fertilizers, produced from natural gas, have become relatively expensive.

Poor legume establishment can be problematic especially on soils with low water holding capacity, low pH, and unfavorable soil texture that can be detrimental to the large taproot system of some legumes. Therefore, the objective of this study was to test the establishment of white and crimson clover by no-till or broadcast seeding into an existing bermudagrass (*Cynodon dactylon* L.) sward at two seeding rates and to determine the effect of canopy removal before or after planting via grazing animals on legume plant persistence. This report summarizes the results from the second year of this study.

Experimental Procedures

The study was conducted at the University of Arkansas-Watershed Research and Education Center (WREC) located in Washington County, Arkansas. The soil at the site was classified as a Captina silt loam soil (fine-silty, siliceous, active, mesic Typic Fragiudults) which is moderately to well drained and slowly permeable. Slopes are 1 to 3% with rolling hills to moderately level land.

Experimental plots were marked at the beginning of October 2009 in an existing 'Greenfield' bermudagrass sward. Whole plots (grazed before/grazed after treatments) were 0.15 acres with three replications of each. Whole plots assigned to the 'grazed before' treatment were grazed between September 28 and October 2, 2009 with 3 non-lactating fistulated cows each, resulting in a theoretical stocking rate of approximately 9 animal units (AU)/acre. During the 5 days of grazing, animals were placed on paddocks at 8 am and removed at 5 pm each day. Canopy height was reduced from approximately 5 inches at day 1 to 2 inches at the end of day 5.

On October 3, 2009, subplots were randomly planted within whole plots and included the following treatment combinations each for crimson and white clovers: a) No-till high seeding rate; b) no-till low seeding rate; c) broadcast high seeding rate; d) broadcast low seeding rate. High and low seeding rates were 16.8 and 8.4 lbs pure live seeds (PLS) for crimson clover, respectively, and 6.2 and 3.1 lbs PLS for white clover, respectively. No-till planting was performed using a 7-foot wide Tye drill with 0.5-foot row spacing and a planting depth of approximately 0.5 inches. Seeds were broadcasted using a hand-held fertilizer spreader. After planting, cattle were stocked on whole plots assigned to 'grazed after' and remained for the same amount of time as in 'grazed before' plots (5 days) between October 5 and 9, 2009.

Seedling counts were performed randomly four times in each plot on October 26 through October 29, 2009, using a metal grid frame (Vogel and Masters, 2001). Seedlings were counted within a total area of 4 square feet at each of the four locations. Seedling counts were repeated on March 30 through April 2, 2010. Data were analyzed as a randomized block design with factorial treatment arrangement at the subplot level using the Proc GLM of SAS (SAS Inst., Cary, N.C.). Species were analyzed separately due to their botanical differences. Statistical differences were considered significant at *P* < 0.05 unless otherwise indicated.

Results and Discussion

Fall 2009. In general, grazing appeared to have little impact on the success of clover establishment (Fig. 1). With the exception of broadcasting crimson clover and white clover at high rates, seedling counts for either "grazed before" or 'grazed after' were similar. It also appeared that broadcasting white clover at either high or low rates resulted in similar seedling counts as no-till planting at a low rate (Fig. 1). Further, broadcast establishment of white clover at a high rate appeared to increase seedling counts in 'grazed after' compared

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with 'grazed before.' However, total seedling count numbers may have been too low to draw a reliable conclusion despite similarities to the previous year. There seemed to be little evidence that cattle hoof action may play a role in clover establishment. Unlike during the previous year, soils at the experimental site were saturated due to strong precipitation events during the month of September 2009, and good seed-soil contact may have been provided by favorable soil moisture conditions.

Spring 2009. Six months after planting, seedling counts were generally reduced. Seeding method and rate interacted in the case of crimson clover; thus, differences among treatments were displayed accordingly (Fig. 2). In white clover, seeding rate and method both affected ($P < 0.05$) seedling counts independently. Winter survival in no-till drilled crimson clover may have been affected by the proximity of plants in drill rows that influenced their survivability. Overall, clover plant density positively affected weed suppression in plots of both species that were established using the no-till drill method.

For both crimson and white clovers, various establishment strategies were evaluated with the objective of providing producers with alternatives tailored to their production system. After the second

year of this study, broadcasting clover seeds still appears to be feasible, but decisions on the establishment method should take production goals, cost, and ease of establishment into consideration.

Implications

Both crimson and white clovers can be established using either the no-till drill or broadcasting method. However, because no-till planting will result in better stand establishment, producers should choose that scenario if financially feasible. Grazing may not have a large effect if soil is relatively wet during planting.

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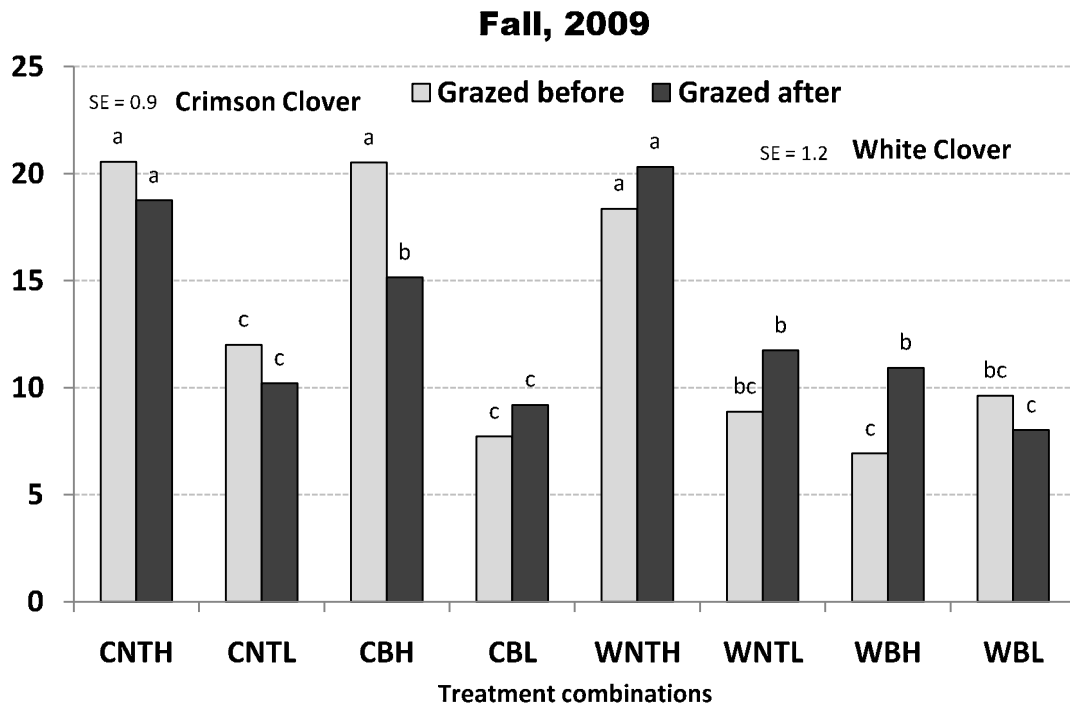
Fig. 1. Effect of no-till drill and broadcast planting methods on seedling counts/square foot in crimson clover and white clover grazed before and after planting in fall of 2009. Treatments were crimson no-till high seeding rate (CNTH), crimson no-till low seeding rate (CNTL), crimson broadcast high seeding rate (CBH), crimson broadcast low seeding rate (CBL), white no-till high seeding rate (WNTH), white no-till low seeding rate (WNTL) white broadcast high seeding rate (WBH), and white broadcast low seeding rate (WBL). Means displaying the same letter within same species are not significant different (*P* < 0.05). Both species were analyzed separately.

Fall, 2010
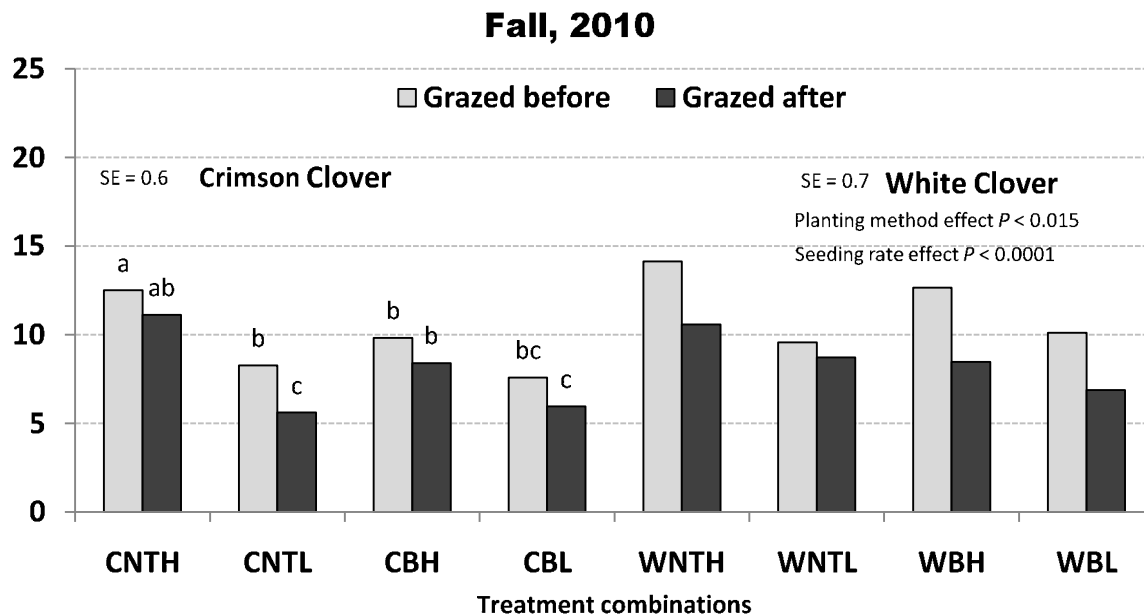
Fig. 2. Effect of no-till drill and broadcast planting methods on seedling counts/square foot in crimson clover and white clover grazed before and after planting in spring of 2010. Treatments were crimson no-till high seeding rate (CNTH), crimson no-till low seeding rate (CNTL), crimson broadcast high seeding rate (CBH), crimson broadcast low seeding rate (CBL), white no-till high seeding rate (WNTH), white no-till low seeding rate (WNTL) white broadcast high seeding rate (WBH), and white broadcast low seeding rate (WBL). Means displaying the same letter within same species are not significant different ($P < 0.05$). Both species were analyzed separately. There was no seeding rate by planting method interaction present with white clover.

Impact of a Starch- or Fiber-Based Creep Feed and Backgrounding Diet on Calf Growth Performance and Carcass Characteristics

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Story in Brief

The objective of this study was to examine the effect of creep feed and backgrounding diet energy source on lifetime performance of fall born calves. Sixty cows with calves were assigned to 1 of 6, 10-acre paddocks 94 d prior to weaning. Ryegrass paddocks were assigned to 1 of 3 creep feed treatments (2 paddocks/treatment): no creep, corn-based creep feed, or soybean hull-based creep feed. Upon weaning, calves were assigned within paddock to either a corn (6 pens) or soybean hull (6 pens) based backgrounding program for 67 d. At the end of the backgrounding phase, calves were sent to Texas Tech University and finished on a common steam flaked corn diet for 133 d. Pre-weaning ADG was not affected by creep feed ($P = 0.22$) or source of creep feed ($P = 0.95$). Backgrounding ADG was similar among creep treatments ($P = 0.15$) and backgrounding treatments ($P = 0.54$). Finishing ADG was similar among creep treatments ($P = 0.88$) and backgrounding treatments ($P = 0.86$). There were no enhancements in carcass yield or quality grade ($P > 0.20$) due to creep feeding or backgrounding diet energy source. Creep feeding does not appear to enhance animal performance or carcass quality when calves are raised on ryegrass pasture pre-weaning.

Introduction

In 2007, the American Angus Association produced its Best Practices Manual (American Angus Association, 2007) as a management guide for cow-calf producers. The guide outlines practices aimed at improving herd health, nutrition, genetics, and marketing. The guide references the benefit of creep feeding and utilization of starch based diets for improving marbling (USDA Quality Grade). These claims are supported by the research of Faulkner et al. (1994) whereby calves consuming a corn-based creep feed had greater quality grades (more marbling) than calves fed a soybean hull based creep, and quality grade increased with increased level of creep feed consumed. Other studies, however, have not observed a significant improvement in quality grade with creep feeding (Rouquette et al., 1983, Lancaster et al., 2007, and Gadberry et al., 2009). Differences among studies could be attributed to the amount of creep feed consumed, type of creep offered (energy versus protein emphasis), basal forage species composition, and forage availability. Therefore, the objective of the current study was to expand the knowledge base of the impact of pre-weaning energy level (no creep versus creep) and source (corn versus soybean hulls) on lifetime performance. In addition, the current study examined the effects of a corn or soybean hull based backgrounding diet and how pre-weaning energy source might interact with post-weaning energy source.

Experimental Procedures

Creep Phase. Sixty cows with fall born calves, Beefmaster and Angus ancestry, were randomly assigned to 1 of 6, 10-acre paddocks at the Southeast Research and Extension Center, Monticello, Ark. The forage type available during the creep-phase was annual ryegrass. Pairs were assigned to paddocks balancing for cow age, cow BW, and sex of calf. Each paddock consisted of pairs with 4 heifer and 6 steer calves. Ninety-four days prior to weaning, each paddock was randomly assigned to 1 of 3 creep feed treatments (2 paddocks/treatment): no creep (NC), corn-based creep feed (CC), or soybean hull-based creep feed (SC). Creep feed was delivered 2×/wk and residue weighed back 1×/wk.

Creep feed intake was targeted at 1% BW, as-fed basis. Creep rations were balanced to contain 15% crude protein (dry matter basis), and a complete mineral (Sunbelt Custom Mineral, Sulfur Spring, Texas) and Rumensin (200 mg/hd, Elanco Animal Health, Greenfield, Ind.) were added to the creep rations, as well. Non-creep fed calves had free choice access to the mineral added to the creep supplements. Calves were weighed at the beginning and end of the creep feed phase and every 28 d throughout. At weaning, calves were vaccinated against clostridial diseases, respiratory diseases, and were treated with an anthelmentic.

Backgrounding Phase. Following weaning, calves were relocated to the Southwest Research and Extension Center (Hope, Ark.) 12 pen drylot facility, and within paddock treatment, calves were randomly assigned to either a corn (CPR, 6 pens/treatment) or soybean hull (SPR, 6 pens/treatment) based 67-d backgrounding program. During the backgrounding phase, calves were fed a 40% roughage diet for the first 19 d, 30% roughage diet for the second 17 d, and 20% roughage diet for the remainder of the backgrounding period. The 20% roughage backgrounding diet was balanced to contain 14.5% crude protein. Upon arrival, calves were weighed and BW continued to be recorded every 14 d.

Finishing Phase. After the backgrounding program, calves were transported to Texas Tech University, Beef Center Feedlot Facility (Lubbock, Texas). Upon arrival, calves were weighed, implanted with Ralgro (Intervet, Millsboro, Del.), and penned based on sex and BW. During the finishing phase, all calves were fed the same steam-flaked corn finishing ration. Calves were re-implanted with either Synovex H or S (Fort Dodge Animal Health, Overland Park, Kan.) on d 60 of the finishing phase. The implant/re-implant program is mild compared to commercial feedlot practices; however, this regimen was chosen to minimize any negative effects that implanting might have on carcass quality grade.

Calves were weighed on 28-d intervals. Two calves died from unpredictable circumstances during this phase of the study. All calves were shipped to the Cargill Packing Facility in Plainview, Texas after 133 days on feed. Postmortem data collected included hot carcass weight, back fat thickness, ribeye area, USDA yield grade, and USDA quality grade.

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Statistical Analysis. Data collected during the creep feeding phase was analyzed as a completely randomized design, and paddock was treated as the experimental unit. Post-weaning data was analyzed with the MIXED procedure (SAS Inst., Cary, N.C.) as a split-plot design and backgrounding pen within paddock was treated as the experimental unit. Creep feed treatment was considered the whole plot and paddock within creep feed treatment was the error term. Backgrounding treatment was considered the sub-plot and the independent variables backgrounding treatment and creep treatment by backgrounding treatment interaction were tested using the residual error. Whole plot treatment differences were computed by contrasting NC versus (CC+SC)/2 and CC versus SC. Sub-plot treatment differences were computed using the PDIFF option of the LSMEANS statement within PROC MIXED (SAS Inst., Cary, N.C.). Choice and Select were converted to a binary response variable and analyzed with PROC MIXED whereby the mean response was equal to the occurrence rate.

Results and Discussion

During the creep phase, calves had access to ryegrass paddock with their dams. Creep feed was weighed back 1×/wk, expecting a daily consumption of 1% BW or approximately 3 lb/d. Creep feed consumption averaged 2.0 lb/d for the CC and 2.7 lb/d for the SC (Table 1). Creep feed intake was near zero for the first 3 wk. Non-creep fed calves gained 2.78 lb/d and creep feeding did not significantly improve ADG ($P = 0.22$). The lack of statistical difference in ADG may be due to limited experimental units. Nevertheless, forage quantity and quality (data not shown) were sufficient to produce gains in excess of 2.5 lb/d with non-supplemented calves and keep creep intake low. The creep may have exhibited more of a substitution response (reduce forage intake) as opposed to a supplementation response (maintain or enhance forage intake) with these calves. Supplementing calves grazing wheat pasture has been used as a means to increase carrying capacity for stockers due to the substitution response.

Initial BW entering the backgrounding phase was similar among creep treatments (Table 2, $P = 0.28$). Average daily gain was not affected by creep feed ($P = 0.15$), backgrounding diet ($P = 0.38$), nor their interaction ($P = 0.54$). In addition the effects of creep feeding or backgrounding diet did not carry-over into feedlot gain performance ($P \geq 0.52$).

Cattle were determined finished at 133 days on feed by feedlot management. The USDA yield and quality grade were not affected by creep treatment ($P \geq 0.32$), backgrounding treatment ($P \geq 0.18$), or their interactions ($P \geq 0.56$).

Although the number of replications per treatment in this study was small, these findings suggest that neither creep feed nor creep feed source (fiber versus starch) had a significant influence on animal performance or carcass grades when cattle had access to high quality pasture pre-weaning. In addition, the use of soybean hulls or corn during a 67-d backgrounding program produced similar gains without carry-over effects during finishing and did not affect carcass quality grade.

Acknowledgements

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Table 1. Effect of creep feed energy source on calf performance beginning 90 d prior to weaning.

	Treatment[ab]				Contrast P-value	
	NC	CC	SC	SE	NC vs. (CC + SC)/2	CC vs. SC
Creep intake, lb/d	0.0	2.0	2.7	0.74	----	0.59
Initial BW, lb	332.3	346.9	330.8	18.5	0.71	0.45
ADG, lb	2.78	3.06	3.04	0.20	0.22	0.95
Final BW, lb	590.4	631.4	613.9	25.8	0.24	0.55
Creep Efficiency, F/G		7.1	10.4			

[a]Creep treatment (n = 2/treatment).
[b]NC = no creep, CC = corn creep, SC = soybean hull creep.

Table 2. Effect of creep feed and backgrounding diet energy source on post-weaning calf performance.

| | Treatment[ab] | | | | | | | Model [c] P-value | | |
| | NC | | CC | | SC | | | | | |
	CPR	SPR	CPR	SPR	CPR	SPR	SE	Creep	Precon	Creep × Precon
Backgrounding										
Initial BW, lb	559.4	568.0	622.8	599.4	564.4	610.0	33.2	0.28	0.63	0.44
ADG, lb	2.62	2.55	2.37	2.52	2.72	3.04	0.22	0.15	0.38	0.54
Final BW, lb	724.3	728.4	772.0	758.4	735.8	801.4	39.7	0.37	0.47	0.44
Finishing										
Initial BW, lb	688.3	701.5	721.2	718.8	688.2	751.5	37.1	0.60	0.34	0.51
ADG, lb	4.02	3.87	4.04	3.91	3.90	3.90	0.21	0.88	0.52	0.86
Final BW, lb	1223.1	1216.5	1258.9	1239.0	1225.6	1270.5	53.3	0.71	0.86	0.71
Carcass Merit										
HCW, lb	736.8	732.4	753.4	745.3	745.2	760.7	32.0	0.71	0.96	0.86
BF, in	0.56	0.55	0.63	0.71	0.57	0.73	0.08	0.27	0.21	0.45
REA, sq. in	12.0	12.3	12.4	11.6	12.0	12.0	0.51	0.88	0.56	0.44
USDA Yield Grade	3.7	3.8	4.0	4.4	3.8	4.4	0.32	0.26	0.18	0.56
USDA Quality Grade										
Choice, %	70.0	60.0	55.4	60.0	43.0	50.0	28.0	0.75	0.97	0.85
Select, %	30.0	40.0	44.6	40.0	57.0	50.0	28.0	0.75	0.97	0.85

[a]Creep treatment n = 6, backgrounding and backgrounding × creep treatment (n = 12).
[b]NC = no creep, CC = corn creep, SC = soybean hull creep, CPR = corn backgrounding, SPR = soybean hull backgrounding.
[c]Independent variable creep = creep feed effect, precon = backgrounding diet effect, and their interaction effect.

300 Day Grazing Demonstration—Year 2

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Story in Brief

In July 2008, the Animal Science faculty began a project to apply research based management practices to demonstrate 300 d of grazing. The goals were to 1) enhance the utilization of forages, 2) demonstrate efficient and targeted fertilizer use, 3) reduce the supplemental hay needs to ≤60 d, 4) maintain a 90 percent net calf crop and 5) wean an average weight of 550 pounds. The project was conducted at the Livestock and Forestry Research Station at Batesville, Ark. and contained 130 acres with 38 fall calving Balancer cows. Pastures consisted of 40 acres of common bermudagrass and 90 acres of fescue. The seasonal forage distribution was approximately 70% cool-season forages and 30% warm-season forages. The concept was to plan forage production in seasonal blocks of summer, fall, winter, and spring to match the fall-calving herd. July 2009 through June 2010 was the second yr for this demonstration. During year 2, 10 cows were replaced because they either were not pregnant, calf died, suffered from fescue foot or poor performance. The average adjusted 205-d weaning weight was 480 lbs and 448 lbs for the steers and heifers, respectively. The overall cow efficiency was 45.0% (calf adjusted 205 d wt/cow wt at weaning). Hay feeding began on January 4, 2010 and was intermittent through March 8 then continued daily until April 2. Hay was fed 54 days, making the grazing season 311 days for the winter of 2009/10. The total pounds of beef sold, income, and costs were higher than projected. This resulted in an actual herd-breakeven of $0.60 which was 15% lower than projected. This production system will be maintained for a number of years to determine the sustainability of the system.

Introduction

Livestock producers continue to suffer from increased input costs. Producers are challenged to determine what management adjustments are necessary for their operation. In order to survive, some producers chose not to make purchases (i.e. fertilizer), reduced livestock numbers, cut expenses at the risk of reducing livestock performance, or a combination of all three. As a result many livestock producers are faced with economic losses in the coming years. In an effort to help livestock producers better manage their "bottom line," the 300 Day Grazing Program was developed (Troxel et al., 2009). The concept was to plan forage production in seasonal blocks of summer, fall, winter, and spring to match the fall-calving herd. The goals of the program were to 1) enhance the utilization of forages, 2) demonstrate efficient and targeted fertilizer use, 3) reduce the supplemental hay needs to 60 d or less, 4) maintain 90 percent net calf crop and 5) wean an average weight of 550 pounds.

Experimental Procedures

On July 1, 2008, the Livestock and Forestry Research Station at Batesville and Animal Science faculty began a project to apply research based management practices to demonstrate 300 d of grazing (Troxel et al., 2009).

Herd Management. The cow herd was predominately Balancer females (38 head) with a September 1 to November 1 calving season and a November 21 to January 2 breeding season. In the fall of 2008, 2 Hereford bulls were leased and fertility tested prior to the breeding season. Table 1 lists the birth weight, weaning weight, yearling weight, milk, scrotal circumference, rib eye area, and marbling EPD's for the Herford bulls used and the American Hereford breed average for 2008 born claves. Both bulls were in the top 5% and 10% in weaning weight and yearling weight EPD's, respectively.

On April 9, 2010 the calves from the 2009 breeding season were administered a 7-way clostridial booster and a killed vaccine containing respiratory viruses, leptospirosis, and vibriosis (Virashield 6 + VL5; Novartis Animal Health, Greensboro, N.C.). In addition, all cows and calves were dewormed with Cydectin Pour-on (Fort Dodge Animal Health, Fort Dodge, Iowa). In March 2010 blood samples were collected from the cows to determine pregnancy rate for the 2009-10 breeding season (SEK Genetics, Inc., Galesburg, Kan.); 37 of the 38 cows (97%) were determined to be pregnant. Cows and calves were weighed and the weights were determined for the cow herd performance program.

On May 12, 2010 calves were administered a Virashield 6 + VL5 booster and all steers and all heifers that were not retained as a replacement were implanted with a Ralgro implant (Intervet/ Schering-Plough Animal Health, Millsboro, Del.). At processing a certified livestock market reporter determined the selling value of the calves. Following processing, the herd was returned to pasture for fenceline weaning. Fenceline weaning was accomplished by placing calves in the pasture labeled Fld12 and their dams were placed in the adjacent pasture labeled Fld11 (Fig. 1).

Pasture Management. Pastures consisted of 40 acres of common bermudagrass (Bm1, Bm2, Bm3 and Bm4), two 22.5-acre pastures of toxic KY-31 tall fescue (Fld10 and Fld11), 23 acres of novel nontoxic tall fescue (Fld12), and 23 acres of novel nontoxic fescue/common crabgrass mix (Fld9) for a total area of approximately 130 acres (Fig. 1). All pastures were soil tested in 2008. The bermudagrass was divided into 4, 10-acre paddocks with a water source in each paddock. Each of the fescue pastures contained ponds for livestock water. All pastures were fenced with electric fences and could be subdivided as necessary for grazing management. The overall stocking rate was 2.9 acres/AU. Animal unit was calculated based on metabolizable energy requirements as described by Gadberry and Troxel (1999).

Plans for seasonal forage management practices were made and revised as needed at approximately monthly intervals during 2009 and early 2010. One Ky-31 fescue field was fertilized with 34 lbs/acre N in February 2009 to provide early spring grazing. Fifty lbs/acre N were applied to 1, 10 acre paddock of bermudagrass in June 2009 for summer forage and 50 lbs/acre N were applied to the 2 KY-31 fescue fields on September 1, 2009 to prepare for stockpiling fescue

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for winter grazing. In total, N fertilizer was applied to 79 acres for the year. Bermudagrass was sprayed with glyphosate in February 2009 and March 2010 to control winter annual weeds and fescue. Red clover was interseeded into the novel nontoxic tall fescue (Fld12) in the fall of 2009 to provide a high quality pasture for weaned calves and for cows prior to breeding. In February 2010, white clover was interseeded into 6 acre blocks of the 2, Ky-31 fescue fields to dilute the effect of toxic tall fescue and reduce N fertilizer requirement.

Financial Management. The budget included herd inventory, number of AU, production information, income, and expenses. Production performance and costs were determined on a fiscal year of July 1, 2009 to June 30, 2010. The herd inventory reflected the number of animals as of July 1, 2009. It included the number of mature cows and the number of AU's. Production information for the mature cows included calf-crop percentage, culling percentage, replacement rate, death loss, and number of females exposed to the bull. Calf-crop percentages were determined by dividing the number of calves weaned by the number of females exposed to the bull.

Income summary included the number of head sold, average BW per head, and average price per lb sold. Included in the income section were calculated values for total lbs sold, total gross income, average selling price, total lbs sold per AU, and income per AU.

The specified expenses included: salt and mineral, supplemental feed, veterinarian costs, growth implants, fly control, sale commission, hauling, pregnancy testing, bull cost or AI, breeding soundness examinations, replacement heifer or cow purchase, fertilizer, lime, purchased hay, herbicide, and miscellaneous (eartags for calves, posts, polywire, gate handles, postage, clover seed, etc.). No overhead items (machinery, depreciation, etc.) were included in the budget. Summarized values included total specified cost per AU, herd break-even (specified cost divided by lb of beef sold) and income over specified cost per AU.

Hay purchased during the winter feeding period was from 3 lots. A non-medicated mineral was provided and contained the following nutrients: 14 to 17% calcium; 8% phosphorus; 16 to 19% salt; 2% magnesium; 0.1% potassium; 3,250 ppm manganese; 25 ppm cobalt; 1,500 ppm copper; 35 ppm iodine; 30 ppm selenium and 3,500 ppm zinc. In early February, chlortetracycline was included in the free choice mineral supplement. This continued for approximately 2 months.

Results and Discussion

Cattle Production. The calving season started on August 27, 2009 and the last calf was born October 12, 2009. Of the 38 cows, one cow did not calve and 1 calf was euthanized due to a broken leg. Therefore, the calf crop percentage was 95%. The cow that lost a calf and the cow that did not calve were sold and cow-calf pairs were purchased for replacements. Eight additional cows were sold due to fescue foot or performance.

The calves averaged 205 d of age on April 9. The average adjusted 205-d weaning weight was 480 and 448 lb for the steers and heifers, respectively. The average frame score was 4.1 for the steers and 4.3 for the heifers. Ninety-seven percent of the calves were medium frame and 3% were scored large framed, and all calves were scored a muscle score 1. The overall cow efficiency was 45% (calf adjusted 205 d wt/cow wt at weaning). The cow efficiency and weaning weights are short of the production goal of 50% and 550 lb, respectively. Eight of these calves were Balancer sired (from replacement cows) and 30 were sired by Hereford bulls. Five of the Hereford sired heifer calves were identified as potential replacement heifers and were not implanted at weaning.

Forage Production. The seasonal forage distribution was approximately 70% cool-season forages and 30% warm-season forages. The February 2009 N application on Fld11 allowed grazing to begin in March thus resulted in an 18 day feeding period for 2008-09. The glyphosate application on the bermudagrass pastures controlled the spring growth of winter annual weeds and fescue that would normally overlap the grazing period of the fescue pastures. Fertilizing only 10 acres of the bermudagrass pasture was enough to provide quality forage for grazing the weaned calves and cows. Weaned calves and dry cows were grazed through the bermudagrass paddocks in a leader/follower system during June and July until the calves were sold. The novel nontoxic fescue/red clover pasture filled a key grazing period during November for the lactating cows, prior to fall breeding, and provided time for growth of the stockpiled fescue to accumulate. Grazing of the stockpiled fescue began on November 25 and continued until March 8. Hay was fed on snow days and to cows affected by fescue foot disorder. Fescue foot can occur in cows grazing endophyte-infected fescue primarily during winter. Affected animals were removed from the stockpiled fescue and were fed bermudagrass hay. The general outline of forage management plans and practices are shown in Table 2 for bermudagrass and fescue pastures.

The 2009 rainfall was 40% greater than the long term average. The average annual rainfall is 48.02 inches but 67.34 inches of rain fell in 2009, which resulted in favorable bermudagrass and fescue growing conditions. This reduced fertilizer requirements, but presented a challenge for maintaining quality forage especially in the fescue pastures. Stockpiling bermudagrass was not necessary due to abundant fall forage growth, but the 2 KY-31 fescue fields (Fld10 and Fld11) were stockpiled for winter pasture. Hay feeding began on January 4, 2010 and was intermittent through March 8 then continued daily until April 2. Hay was fed 54 days, making the grazing season 311 days for the winter of 2009-10.

Economics. The projected and actual budgets are summarized in Tables 3 and 4. A number of cost items exceeded the projected amount. They included veterinary medicine, sales commission, hauling and purchased hay. Veterinary medicine exceeded the budgeted amount by 79% due to a number of cows requiring treatment for fescue foot (LIQUAMYCIN® LA-200®, Pfizer Animal Health). Sales commission was 50% greater than expected because additional cows were sold to replace non-pregnant cows or cows suffering from severe fescue foot symptoms and the selling value of calves was higher than expected. Fly control was $110 more than expected. The actual cost for replacement cows was $5,150 more than projected because 10 bred heifers were purchased. Hauling was $395 greater than budgeted. Hauling included shipping the cows and calves to market plus shipping the 10 bred heifers from Savoy to Batesville. Due to feeding hay longer in 2009-10 (54 days) compared to 2008-09 (18 days), purchased hay was 89% greater than expected. Herbicide was 51% more than expected due to extra spraying in the bermudagrass fields. The following cost items were lower than expected: Salt and mineral, supplemental feed, fertilizer, lime, and miscellaneous. No supplemental feed (corn, cubes, etc.) was purchased. The purchased hay exceeded the February and March nutritional requirement (9% CP and 56% TDN, DM basis) of fall calving cows. Fertilizer was lower than expected because of the abundant amounts of rainfall and the strategic fertilizer application. Other actual cost items were very close to their budgeted amounts. Overall, total expenses were $5,200 or $136.85 per AU (18%) more than budgeted.

Twenty steer calves weighing 660.5 lbs sold for $117.78/cwt. ($15,558.74) and 18 heifer calves weighing 634.0 lbs sold for $105.92 ($12,088.00). Seven cows were sold at auction (995 lbs for $56.00/cwt. ($3,900.40)) and 3 cows were sold for carcass value (900 lbs. for

$18.90/cwt.). The 3 cows sold for carcass value showed signs of severe fescue foot. The total beef sold (pounds of BW) was 53% greater than projected (Table 4). That was due to selling more cows than expected and heavier calves. The additional pounds of beef sold resulted in more actual gross income than projected. Gross income was $11,475 or $292 per AU more than projected. Income over specified cost on a total and AU basis was $6,273 and $204.38, respectively, greater than projected. The herd breakeven for specified costs was $0.61 which was 14% lower than projected.

Implications

Livestock producers are faced with increasing input costs and volatile markets. Developing environmentally and financially sustainable systems to improve forage utilization thus reduce dependency on hay (<60 d), fertilizer and supplemental feed will improve opportunities for success. Not one management practice will reduce hay dependency, fertilizer or supplemental feed to <60 d . It takes an integrated approach using a number of different practices (stockpiling forages, legumes, grazing management, etc.). The 300 day grazing demonstration is a discovery farm to demonstrate management practices. No attempt was made to compare different management practices or systems. Producers can observe management practices before adopting them. Cow-calf and forage efficiencies may be improved, which can reduce cost and increase the opportunity for success.

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Table 1. The birth weight, weaning weight, yearling weight, milk, scrotal circumstance, rib eye area, and marbling EPD's for the Herford bulls used in this demonstration and the American Hereford breed average for 2008 born calves[a].

Bull ID	Birth Wt.	Weaning Wt.	Yearling Wt.	Milk	Scrotal Circ.	Rib Eye Area	Marbling
H 9K Kootenay 4038 (42531143)	+5.5	+56	+83	+7	+0.6	+1.60	+0.18
H 122L Online 4024 (P42531178)	+6.4	+59	+99	+17	+0.9	+0.63	+0.04
Breed Average	+3.7	+42	+70	+16	+0.7	+0.20	+0.03

[a]Source: American Hereford Association (www.herfnet.com/).

Table 2. Management outline of the bermudagrass and fescue pastures.

Bermudagrass pastures	
Date	Management practice
June 2009	Applied 50 lb/acre N to 10 acres to produce enough forage for summer Grazed weaned calves and cows in a leader/follower system until the calves were sold
July/November	Cows rotationally grazed the bermudagrass pastures until November
February/March	Burned bermudagrass pastures in February to remove residue and then sprayed with glyphosate in March 2010 to control remaining fescue

Fescue pastures	
Date	Management practice
February 2009	Fertilize Fld 11 with 34 lbs/acre N to start early spring grazing
May	Calves were fenceline weaned on the novel nontoxic fescue/red clover then moved to the early growth of bermudagrass
August	Fld 10 & 11 were cut for hay to remove excess forage growth prior to initiation of stockpiling fescue
September	Fld 10 & 11 were fertilized with 50 lbs/acre N to promote growth of stockpiled fescue for winter grazing
November	Grazing continued on bermudagrass until November 5 then cows grazed high quality novel nontoxic fescue/red clover prior to breeding Herd was moved to stockpiled fescue on November 25 and grazed until March 8
January 2010	Cows were affected by fescue foot from grazing toxic fescue during winter. Hay feeding began on January 4. Hay was fed a total of 54 days until April 2nd. Grazing season was 311 days for 2009-10

Table 3. Projected and actual expenses for 300 day grazing demonstration. The actual and budgeted amounts are for the July 1, 2009 to June 30, 2010 fiscal year.

Item:	Projected ($)		Actual ($)	
	Total	AU	Total	AU
Expenses:				
Salt and mineral	1,200	31.58	1,054	27.75
Supplemental feed	800	21.05	0	0.00
Vet. medicine	450	11.84	805	21.20
Growth implants	74	1.95	90	2.36
Fly control	80	2.11	190	5.00
Sale commission	975	25.66	1,460	38.41
Hauling	150	3.95	545	14.34
Pregnancy test	110	2.89	103	2.72
Bull lease	700	18.42	600	15.79
Fertility testing bulls	80	2.11	80	2.11
Replacement cows	4,400	115.79	9,550	251.32
Fertilizer	3,500	92.11	2,592	68.20
Lime	680	17.89	0	0.00
Purchased hay	1,000	26.32	1,890	49.74
Herbicide	280	7.37	423	11.12
Miscellaneous	1,400	36.84	155	4.07
Total expenses	15,879	417.87	21,079	554.72

Table 4. Projected and actual production and income for 300 Day grazing demonstration. The actual and budgeted amounts are for the July 1, 2009 to June 30, 2010 fiscal year.

	Projected		Actual	
Item	Total	AU	Total	AU
Total lbs sold	22,375	589	34,288	902
Average price per lb received	$0.92		$0.94	
Income	$20,614	$552	$32,089	$844
Income over specified cost[a]	$4,735	$85.34	$11,009	$289.72
Herd breakeven[b]	$0.71	----	$0.61	----

[a] Gross income minus the specified expenses. The specified expenses included salt and mineral, supplemental feed, veterinarian costs, growth implants, fly control, sales commission, hauling, pregnancy testing, bull cost or AI, breeding soundness examinations, replacement heifer or cow purchase, fertilizer, lime, purchased hay, herbicide, and miscellaneous.
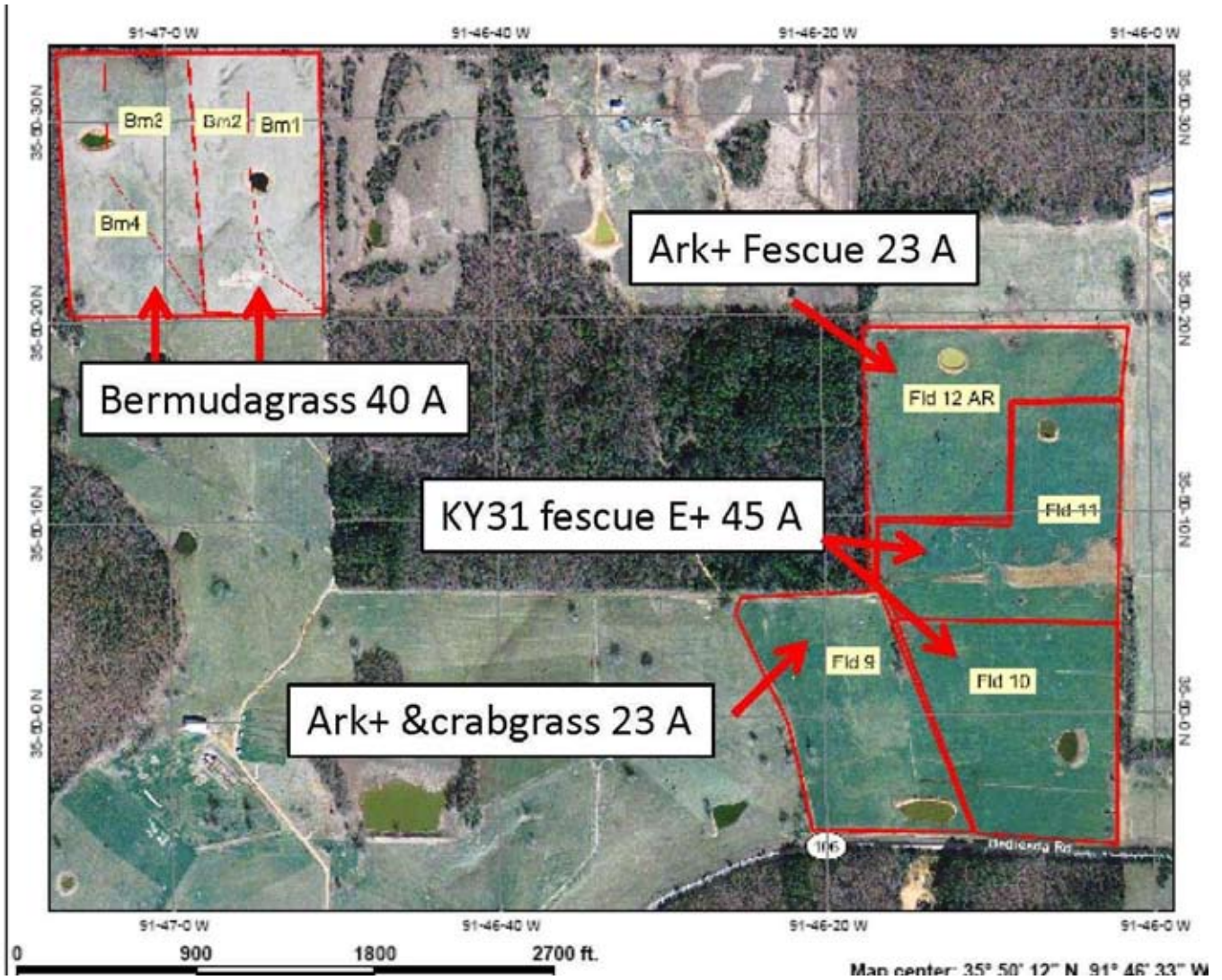[b] Specified cost divided by lb of beef sold.

Fig. 1. Map of the pastures involved in the 300 day grazing demonstration. Pastures contain bermudagrass (Bm1, Bm2, Bm3 and Bm4) and fescue (Fld9, Fld10, Fld11 and Fld12) dominant forages.

Arkansas Steer Feedout Program
2008-2009

Brett Barham[1] and Sammy Cline[1]

Story in Brief

The objective of the Arkansas Steer Feedout Program is to provide cow-calf producers information about the post-weaning feedlot performance and carcass characteristics of their calves. For the 2008-2009 feedout, initial weight entering the feedyard, USDA muscle score, medicine costs, hot carcass weight, and USDA yield grade were factors that affected ($P < 0.05$) the feedlot return over specified costs. Cow calf producers who participated in the program will be able to use the information to evaluate how their cattle breeding programs fit the needs of the beef cattle industry.

Introduction

The University of Arkansas Cooperative Extension Service Steer Feedout Program provides cow-calf producers the opportunity to acquire information about postweaning performance and carcass characteristics of their calves. It also points out factors that influence value beyond the weaned calf phase of beef production. The program is not a contest to compare breeds or breeders or to promote retained ownership. The Feedout Program creates an opportunity for producers to determine how their calf crop fits the needs of the beef industry. The program also provides the information needed to determine if changes in genetics and/or management factors are warranted for producers to be competitive in beef production.

Experimental Procedures

On November 13, 2008, 112 steer calves from 14 Arkansas producers representing 12 counties were placed on feed at Wheeler Brothers Feedyard in Watonga, Oklahoma. Calves were weighed on November 14, 2008. All calves were processed and placed in one pen. Management factors such as processing, medical treatments and rations were the same as the other cattle in the feedyard. The feedyard manager and Extension personnel selected animals for harvest when they reached the weight and condition regarded as acceptable for the industry and market conditions. Cattle were sold on a carcass basis with premiums and discounts for various quality grades, yield grades and carcass weights. Feed, processing and medicine costs were financed by the feedyard. All expenses were deducted from the carcass income, and proceeds were sent to the owners.

Of the 112 steers that started on feed in the fall, 4 died (3.5% death loss). Two calves were sold as railers due to lack of performance or being chronically ill. These 6 calves were not included in the statistical analyses. Therefore, 106 steers were used in the analyses.

Results and Discussion

Table 1 is the overall financial summary. Table 2 is a financial summary of the bottom 25%, top 25% and average for steers based on feedlot net return. A farm break-even value was calculated by dividing the feedlot net return by the in weight. If the feeder calf could have been sold in the fall of 2008 for more than the farm break-even value, financially it would have been better to sell the calf in the fall than to feed it. The steers' farm break-even averaged $0.71 per pound (average in weight was 623 pounds) and ranged from $0.32 to $1.40 per pound. For the week ending November 14, 2008, 500 to 550 pound steers were selling for $0.76 to $0.92 per pound.

The sick pull rate averaged 35% with 39 of 112 calves treated for sickness. This pull rate was very high since all cattle were listed as being preconditioned prior to entering the feedyard. Average medicine cost for the entire pen was $7.60 per steer. The health status of cattle in the feedyard usually has a major impact on performance and profit. Healthy steers had higher ($P < 0.05$) feedlot net returns ($449) than steers that became sick ($414). Steers that did not receive treatment had higher average daily gain, hot carcass weights and lower feed cost of gain and total cost of gain ($P < 0.01$). No differences were noted between healthy and sick steers for dressing percentage, yield grade, ribeye area, and ribeye area per 100 pounds of carcass weight ($P > 0.10$).

Given the past health issues of the cattle in the program, producers need to implement a sound health management plan. By implementing a sound vaccination program at the ranch of origin, predictability and consistency of calves increases along with product value, and calves have the opportunity to express their genetic potential.

The average steer in weight and final weight were 595 pounds (range = 374 to 940 lb.) and 1330 pounds (1070 to 1640 pounds), respectively. Average daily gain was 3.65 pounds and ranged from 2.7 to 5.1 pounds. Overall, 37 percent of the steers graded Choice, compared to the national average of 56.8%. Thirteen steers received a premium for Certified Angus Beef or Angus Pride Choice. Table 2 summarizes the carcass data.

Industry Standards. Carcass standards for the beef cattle industry are Choice quality grade, yield grade of less than 4, and hot carcass weight between 550 and 950 pounds. Thirty-one percent of the steers in this year's program fit these industry standards. Steers that met the industry standards averaged $53 per steer more than those that did not fit the industry standards ($P < 0.05$; Table 3). They had higher carcass values because they graded Choice, and were not discounted for yield grades greater than 4.0 or for carcasses outside the weight range. Of the steers that were in the top 25% based on feedlot net return, 91% met the industry standards, and for those in the bottom 25% based on feedlot net return, 100% did not meet the industry standards.

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Factors Affecting Steers' Feedlot Net Return. Listed below are the significant ($P < 0.05$) factors that affected feedlot net return for steers in the 2008-2009 program. Factors are listed in descending order of importance.

1. *Initial Weight*–The relationship between initial weight and feedlot net return was negative. As initial weight increased feedlot net return decreased. This relationship is slightly misleading. The main reason initial weight shows up as a significant factor was due to the market at the time of harvest. The first group of steers harvested received the lowest carcass price of the 3 harvest groups. This first harvest group of steers was largely made up of the calves with heavier initial weight. Generally, the heavier the calf upon entrance to the feedyard the fewer days it took to reach slaughter weight. With the rising cost of feed, steers that are placed into the feedyard at heavier weights should be at an advantage.

2. *USDA Muscle Score*–Muscle thickness is a major factor that relates to carcass weight. Thickness, depth and fullness of quarter, and width (without excessive fat) of back, loin and rump are indications of muscling. The current USDA Feeder Cattle Grades utilize 4 muscle thickness scores (1 = thick, 2 = slightly thick, 3 = narrow and 4 = very narrow). Thickness is related to muscle-to-bone ratio at a given degree of thickness. Thicker muscled animals will have more lean meat. "Double-muscled" animals are included in the Inferior grade (unthrifty animals). Although such animals have a superior amount of muscle, they are graded U.S. Inferior because of their inability to produce acceptable degrees of meat quality.

 The ideal calf should be Feeder Cattle Grade U.S. 1. Number 1 is thrifty and moderately thick throughout. They are moderately thick and full in the forearm and gaskin, showing a rounded appearance through the back and loin with moderate width between the legs, both front and rear.

3. *Hot Carcass Weight*–The relationship between hot carcass weight and feedlot net return was positive. As hot carcass weight increased, so did feedlot net return (Table 4). The more carcass pounds sold, the greater the gross income and feedlot net return. Table 4 shows the relationship between hot carcass weight, total cost of gain, average daily gain, feedlot net return and calculated return. Factors that affect hot carcass weight include frame size, muscle thickness and backfat.

4. *Medicine Cost*–Cattle that get sick in the feedyard incur additional cost and have lower overall gain. While all sickness in the feedyard cannot be prevented, a solid health program on the farm will give calves the best opportunity to not fall into the sick category in the feedyard.

5. *Yield Grade*–As yield grade increased from 1 to 4, feedlot net return changed very little ($588, $569, $616, $559 per head for yield grades 1, 2, 3, and 4, respectively). A positive note for this year's steers was that no carcasses fell in to the yield grade 5 classification. Yield grade 3 carcasses had higher ($P < 0.05$) returns than grades 1, 2 and 4. There were no differences between grades 1, 2 and 4 for feedlot net return ($P > 0.05$).

 One unique item in this year's program is that USDA Quality Grade was not in the top 5 factors that affected feedlot net return. The Choice-Select spread, or the difference in value between a Choice carcass and a Select carcass remained small, with very little premium being paid to higher quality carcasses.

Summary

The purpose of the Arkansas Steer Feedout Program is to provide the opportunity for cow-calf producers to determine how their cattle fit the needs of the industry. With the traditionally large price spread between Choice and Select, it was very important to the "bottom line" that calves graded Choice. The data from this year's program indicate that cattle that gain well, have a heavy carcass and do not get sick are the ones that have higher returns from the feedyard.

Table 1. Financial results summary, 2008-2009[a].

	Average per steer ($)	Range ($)
Gross Income	1,080.69	711 to 1,403
Expenses		
Feed	548.42	433 to 705
Freight, interest, etc.	87.31	79 to 102
Medicine	7.00	0 to 52
Total	642.73	379 to 625
Feedlot Net Return	437.95	190 to 688
In Value	557.87	320 to 726
Calculated Return	-119.92	-332 to 225

[a] 106 head.

Table 2. Performance summary of the bottom 25%, top 25% and average steers based on feedlot net return.

	Bottom 25%	Top 25%	Average
Number of Steers	26	26	106
Gross Income per head ($)	972[a]	1,150[b]	1,080
Carcass Value Per lb. ($)	1.26[a]	1.34[b]	1.32
In Value per head ($)	587[a]	508[b]	557
Medicine per head ($)	7.36[c]	5.38[d]	7.00
Feed Cost per head ($)	521[a]	563[b]	548
Total Expense per head ($)	617[a]	654[b]	642
Feedlot Net Return per head($)	355[a]	496[b]	437
Calculated Return per head ($)	-232[a]	-12.43[b]	-119.92
Days on Feed	192	194	195
Feed Cost Per lb. of Gain ($)	0.92	0.75	0.84
Total Cost Per lb. of Gain ($)	1.09	0.88	0.99
In Weight (lb.)	652[a]	577[b]	626
Muscle Score	1.38	1.85	1.61
Frame Score			
Large	73%	56%	63%
Medium	27%	44%	37%
Final Weight (lb.)	1,278[a]	1,381[b]	1,339
Average Daily Gain (lb.)	3.28[a]	4.16[b]	3.75
Hot Carcass Weight (lb.)	767[a]	856[b]	817
Carcass Value ($/lb)	1.26[a]	1.34[b]	1.32
Dressing Percentage	62.5%[a]	64.5%[b]	63.4%
Ribeye Area (sq. in.)	12.7	13.0	12.99
Backfat	0.4	0.50	0.46
REA per 100 lb. carcass weight	1.68[a]	1.50[b]	1.60
Quality Grade			
Prime	0%	3%	1%
Choice	27%[a]	59%[b]	41%
Select	34%[a]	37%[b]	47%
No Roll	19%[a]	0%[b]	6%
Over 30 Months	4%	0%	1%
Hard Bone	15%	0%	4%
Yield Grade	2.19	2.44	2.33

[a, b] Values within rows with unlike superscripts are different ($P < 0.0001$).

Table 3. Feedlot net return, average daily gain and carcass value for steers that did or did not meet industry standards[a].

Item	Met Standards	Did Not Meet Standards	Difference
Feedlot Return	$475	$422	$53
Average Daily Gain (lb.)	3.75	3.75	0.0
Carcass Value	$1.35	$1.30	$0.05

[a] USDA Quality Grade Choice, yield grade ≤3.0 and carcass weight of 550 to 950 pounds.

Table 4. Summary of hot carcass weight, total cost of gain, average daily gain, feedlot net return and calculated return.

Hot Carcass Weight (lb.)	Total Cost of Gain ($)	ADG (lb.)	Feedlot Net Return per Head ($)	Calculated Return per Head($)
600-699	1.07	2.9	295	-190
700-799	1.01	3.4	391	-143
800-899	0.95	3.9	465	-95
900+	0.95	4.5	544	-77

Case Study: On-Farm Estimates of Hay Feeding Loss and Economics of Different Hay Feeding Systems

S. Rhoades[1], K. Simon[2], and S. Gadberry[2]

Story in Brief

The objective of this on-farm case study was to estimate hay waste associated with various feeding systems. At location 1, bale processing (PRO) was compared to unprotected (UNP) hay. At location 2, unrolling hay (UNR) was compared to feeding hay in ring feeders (RNG). For location 1, feeding method was the only significant source of variation ($P < 0.001$). Waste for UNP and PRO was 42 and $0.09 \pm 5.8\%$, respectively. At location 2, UNR areas tended to have more waste ($P = 0.07$) than RNG, 23.7 versus $13.0 \pm 3.1\%$, respectively. An economic analysis suggests that reduction's in hay waste associated with PRO is sufficient to cover the annual ownership and operating cost of a grinder-mixer for a 200 cow herd. However, the additional tractor size required to operate a grinder-mixer may result in greater ownership and operating costs than the value of recovered hay alone.

Introduction

The costs associated with hay feeding on a cow/calf operation are significant to a producer's bottom line. These costs are incurred from the actual production of the hay, baling and handling, and losses associated with storage and feeding. This project targets the losses (i.e., waste) from feeding hay by various methods commonly employed by Arkansas cow/calf producers.

Losses are observed in the feeding process by the cows pulling hay from a bale and dropping it on the ground thus allowing it to be trampled into the soil. In order to reduce these losses, producers have utilized various methods to minimize the ability of cows to stomp on hay lying on the ground. Many producers utilize steel rings to place round bales inside and many unroll round bales over pasture ground to allow access to the hay over a large area. In some larger operations, hay is processed (i.e., shredded) in a processor and fed to the cattle either on the ground or large feed troughs.

There are many variations to these feeding methods. Differences from the design of rings, the use of cradles, and wagons that hold round bales are all methods that can be observed on farms across the state. The objective of this project was to compare the waste and economics of feeding systems based on processed hay, ring fed hay, and unrolled hay.

Experimental Procedures

Hay Loss Estimation. Hay loss was estimated from 2 cow-calf production systems in Scott Co, Arkansas. In 2008 and 2009, a system that incorporated processing large round bales in a vertical grinder-mixer (PRO) was compared to unprotected (UNP) hay feeding (location 1). In 2008, the location consisted of 141 beef cows in early lactation, and the cows were managed as 3 separate herds of 17, 45, and 79 cows. Each herd was provided UNP for approximately 1 wk and fed PRO the remainder of the winter. The hay was net-wrapped and stored outside and analyzed 11.5% CP and 59.8 % TDN (DM basis). In 2009, the same herd was used, except the cows were in late gestation at the time of hay waste evaluation. Hay was stored similarly and analyzed 11.7% CP and 61.3% TDN (DM basis). In 2009, the cows were managed as a single group. Due to mechanical problems with the

grinder-mixer, hay was offered UNP for approximately 1 mo. Within year and herd, the producers delivered enough hay to meet the cows' apparent bale consumption over a 24 h period. Three bale feeding sites of each treatment were evaluated.

To estimate hay waste, bales were weighed to determine an average fed-bale weight. Bales averaged 758 ± 77 and 581 ± 25 lb (mean ± sd, DM basis) for yr 1 and 2, respectively. In the PRO system, 2 bales were processed simultaneously and augered into large tire feeders. After a 24 h feeding period, the remaining hay was removed from the tires, weighed and composited for DM determination. For the UNP system, hay loss was determined by measuring the circumference waste area of a bale offered over a 24 h feeding period. Within the area, a 2 ft square was randomly thrown 3 times and the area within the square was collected, weighed, and dried for DM determination and estimation of waste.

At experimental location 2, unrolling hay (UNR) was compared to feeding hay in a round bale ring feeder (RNG). The ring feeder was constructed of pipe and did not have a solid plate skirt at the bottom which is found on some ring feeders in Arkansas. The site consisted of 30 beef cows in early lactation. Hay was stored in a barn and analyzed 10.2% CP and 52.6% TDN (DM basis) and averaged 880 lb (DM basis). The herd was offered hay as a single group, providing enough hay to apparently be consumed over a 24 h feeding period. The UNR and RNG fed methods were not offered as a choice but were offered as independent feeding options. Three UNR sites and 3 RNG sites were evaluated. Day-to-day weather conditions were similar during the week the 6 feeding events occurred. Hay loss from the RNG sites was estimated similar to the UNP sites at location 1. To determine hay loss from the UNR sites, length and width measurements were taken to determine unrolled area. Within each UNR site, a 2 ft square was randomly thrown in 4 different areas; the hay within the square was removed, weighed and dried for DM determination. Areas collected for DM determination were void of fecal contamination.

Economic Analysis. An economic analysis of 3 hay feeding systems was used to determine if the value of hay loss associated with each feeding system would be sufficient to offset increased farm supply and machinery costs. The 3 systems included: processed hay (PRO) using large tires as feeders (1% hay waste), unprotected hay (UNP) with 42% hay waste, and a ring feeder (RNG) based system (13% hay

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waste). The UNR system was not included in the economic analysis because producers often implement the unrolling practice as a means to minimize soil canopy degradation. The systems were based on a 200 head cow-calf operation with a daily, per cow, hay consumption of 27 lb (as-fed) over a 120-d winter feeding period. Bale size was assumed to be 900 lb (as-fed) and valued at $30. Hay waste was factored into meeting the daily intake.

Machinery cost included the operating cost for a 95 horsepower (hp) tractor used in PRO to operate a 540 cu. ft. grinder-mixer, a 75 hp tractor used in all systems, and a 58 hp tractor used in UNP and RNG systems as a second tractor. In the PRO system it was assumed the 95 hp tractor would be required for grinder-mixer operation and a second tractor (75 hp) would be sufficient in size to load the large round bales into the grinder-mixer. Both tractors would be used for haying and other farm activities throughout the year. In the UNP and RNG systems, the 75 hp tractor would be sufficient in size to operate a large round baler. The second tractor of lesser hp would be sufficient for raking and other farm activities that did not require a greater hp at the power-take-off.

The base price of the tractors was determined by collecting tractor price information from http://www.TractorHouse.com with independent variables including year model, hp, loader presences, and drive train (2 or 4 wheel). Modeled tractor costs only differed in hp. Annual tractor cost was determined for a 10-yr period using a Machinery Cost Calculator spreadsheet (www.extension.iastate.edu/agdm/crops/xls/a3-29machcostcalc.xls) with a salvage value equal to 44% of the purchase cost. Annual tractor usage was assumed to be 458 hr. This was determined by surveying 4 Arkansas beef cattle operations and calculating their average annual tractor hours per cow per year (2.29 hr/cow, annually). Annual interest rate was 7%, diesel fuel price was $2.50/gal, and labor during tractor operation was $7.25/h. Annual repair and maintenance, fuel and lubrication consumption was calculated from the spreadsheet based on ASAE standards (Edwards, 2009).

The grinder mixer initial cost was determined by regressing reported mixer price, acquired from http://www.TractorHouse.com, against the independent variables of year model and capacity. Annual cost reflected a 10-yr use and 48% of initial cost, salvage value. Since supplement can be added to the PRO system, the cost of a cake feeder and troughs made from plastic culverts was added to the UNP and RNG system. A truck cost for feed delivery via cake feeder was not added to the UNP and RNG system. Instead, the cake feeder is assumed fitted to the 58 hp tractor.

Statistical Analysis. For this project, bale (3 per treatment within year) was considered the experimental unit. Analysis of variance was examined for location 1 using R (http://www.r-project.org). In 2008 at location 1, waste was analyzed as a randomized complete block design. The herd block effect was not significant and was removed from the model; afterwards, 2008 was combined with 2009 and analyzed for year, treatment, and year by treatment effects. A t-test, assuming equal variance, was used to compare means of unrolled or ring-fed bale waste measured at location 2.

Results and Discussion

Hay Loss Estimate. For location 1, feeding method was the only significant source of variation ($P < 0.001$). Waste for UNP and PRO was 42 and $0.09 \pm 5.8\%$, respectively. In 2008, the hay loss for UNP was high. Initial thought was cattle were adapted to eating processed hay from tire feeders. However, in 2009, cattle were given UNP for a longer period of time, yet wastes were still high and the year by treatment interaction was insignificant ($P > 0.20$). At location 2, UNR areas tended to have more waste ($P = 0.07$) than RNG, 23.7 versus 13.0 ±

3.1%, respectively.

Landblom et al. (2007) reported variable hay wastes by feeding method. In instances of loose hay, it was suggested that waste was not different among feeding methods which included shredding, unrolling, and feeding in a ring+tapered cone feeder. However, Landblom et al. (2007) also indicated dense bales resulted in less waste when fed in a ring feeder with a tapered cone compared to shredding or unrolling. Their bales were augered onto the ground after shredding which could contribute to greater waste; whereas, bales in this on-farm experiment were augered into tire feeders. Buskirk et al. (2003) reported 6.1, 11.4, and 14.6% waste for ring, trailer, and cradle feeders. The greater waste observed with the ring feeder in this study (13%) could be the feeder in their study had a metal skirt around the bottom; whereas, the bottoms were open in the present project. Estimation methods of waste among studies may have contributed to observed differences as well.

Feeding hay in rings produced less waste than unrolling. Visually, unrolling appears to permit less waste. Producers that unroll hay usually prefer this method not for apparent reduction in hay waste but for reduced trampling degradation to soil cover.

Economic Analysis. An economic analysis of a PRO, UNP, and RNG system is presented in Table 1. The value of total hay required was a minimum of $21,816 in the PRO system and a maximum of $30,672 in the UNP system for a 200 cow herd. The hay cost was $24,408 for RNG. The difference in hay cost between PRO and UNP or RNG was $8,856 and $2,592. The value of wasted hay in the UNP system appears more than sufficient to offset the annual ownership and operating cost of a grinder-mixer ($4,344/yr) but not the RNG based system. The greatest economic impact to these systems would be differences in the annual tractor ownership and operating cost. The PRO system was $6,868 greater than the other systems due to operating a 95 hp compared to a 58 hp tractor. The value in hay loss does not appear sufficient enough to offset this cost difference, even when a 42% hay waste was considered. Overall, the RNG system appears most economical. While the grinder-mixer system had the greatest potential to minimize hay waste, producers would have to justify the need for the greater hp and a grinder-mixer with other on-farm enterprises. In addition, the economic analysis of the grinder-mixer system did not account for any potential change in hay intake. Processed hay promotes greater DM intake making more nutrients available to the animal and therefore processed forage consumption by limit-feeding or free-choice feeding should factor into the grinder-mixer purchasing decision as well. While an economic analysis of hay waste was not conducted for unrolling, the value of hay waste of UNR compared to RNG should be considered within farm to determine if the justification for UNR (usually based on maintaining soil canopy and reduced weed emergence in trampled areas around rings) is financially viable.

It should also be kept in mind that hay waste was measured on a small number of experimental units (bales) per location (farm) and per feeding method and further studies may be needed to confirm these on-farm, case study results. The hay waste associated with feeding large round bales unprotected at location 1 was high and may be attributed to the cattle being adapted to processed hay (the default feeding method on this farm) prior to the initiation of this study. In instances where unprotected hay results in less than 42% waste, the value difference of total hay required for UNP versus PRO would diminish along with the economic feasibility of processing.

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Table 1. Partial budget results for processed, unprotected, and ringer feeder based hay feeding systems for a 200 cow herd.

	Annual System Costs[c]		
Cost item[a]	Processed (PRO)	Unprotected (UNP)	Ring Feeder (RNG)
Tractor – 58 hp,MFWD,cab,loader		$11,548	$11,548
Tractor – 75 hp,MFWD,cab,loader	$15,063	$15,063	$15,063
Tractor – 95 hp,MFWD,cab,loader	$18,416		
Mixer – 540 cu. ft.	$4,344		
Hay rings			$386
cake feeder		$214	$214
Feed trough - tire	$28		
Feed trough – plastic culvert		$140	$140
Total	$37,851	$26,965	$27,351
Value of total hay required[b]	$21,816	$30,672	$24,408
Total equipment + hay	$59,667	$57,637	$51,759

[a]Total annual ownership and operating cost, MFWD (modified front wheel drive).
[b]900 lb. bales valued at $30/bale fed.
[c]Systems were based on 1% waste (PRO), 42% waste (UNP), and 13% waste (RNG).

Single Nucleotide Polymorphisms Associated with Ascites in Susceptible and Resistant Broiler Lines

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Story in Brief

Ascites is a disorder in broilers that leads to increased mortality and increased carcass condemnations and costs the worldwide poultry industry $500 billion dollars per year. The condition is characterized by pulmonary hypertension and the accumulation of fluids in the abdominal cavity. Research at the University or Arkansas, has suggested that an altered nitric oxide response is associated with the occurrence of ascites. This study was undertaken to determine if there are base substitutions in the two genes that are primarily responsible for the production of nitric oxide and if there are genetic differences in these genes between ascites resistant and susceptible lines of broilers. Genomic DNA was isolated from 40 randomly selected ascites resistant broilers and 40 randomly selected ascites susceptible broilers. Sections of promoter region of the two genes responsible for the production of nitric oxide were isolated by PCR and base sequences present in each bird's DNA were determined. Comparison of the DNA sequences indicated there were six polymorphisms (SNPs) in the inducible nitric oxide synthase (iNOS) gene and two SNPs in the endothelial nitric oxide synthase (eNOS) gene. Further analysis indicated that frequency of occurrence of 5 of the iNOS SNPs and both of the eNOS SNPs differed significantly ($P < 0.0001$) between the asictes resistant and ascites susceptible broiler lines. Further studies will be required, but these findings suggest that these polymorphisms may be useful as genetic markers for the selection of broiler lines that are resistant to ascites.

Introduction

Ascites in broilers is a disorder in which increased pulmonary blood pressure leads to enlargement of the right ventricle of the heart, increased venous pressure, pulmonary hypertension, liver congestion, the accumulation of fluid in the abdominal cavity (ascites), and high mortality. The syndrome occurs more often in broiler lines that have been selected for high growth rates and feed efficiency and occurs when birds are placed in certain environments and managed for maximum levels of performance. Worldwide industry losses attributed to ascites due to increased mortality and carcass condemnations have been estimated at over $500 billion per year.

Previous research at the University of Arkansas Department of Poultry Science has suggested that nitric oxide (NO) which is a potent vasodilator, may be an important factor in the occurrence of ascites. Nitric oxide in the lungs and circulatory tissues of birds is produced primarily by the action of two enzymes. These enzymes are inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS). It has been suggested that an impaired or altered nitric oxide response to metabolic or environmental stress may contribute to susceptibility of broilers to pulmonary hypertension and ascites.

Production of the endothelial and inducible nitric oxide synthase enzymes is controlled by two specific genes referred to as the eNOS and iNOS genes respectively. The area of focus in this study was the promoter region of these genes which does not affect the coding of amino acids into proteins, but rather controls or alters the degree of transcription (expression) that occurs in these genes. The specific objective of this study was to identify genetic polymorphisms in the eNOS and iNOS genes and to compare the frequency of polymorphisms between genetic lines of broilers selected for either genetic resistance or susceptibility to ascites.

Experimental Procedures

Animals. Animals used in this study were from a line of ascites susceptible (SUS) and ascites resistant (RES) broilers developed at the University of Arkansas. The lines were developed by multiple generations of selection in which birds were determined to be resistant or susceptible to ascites based on mortality rates when exposed to hypobaric chamber conditions. Hypobaric mortality was used to select full sibs of susceptible and resistant birds grown under normal industry standard conditions. After eight generations of selection, the mortality rates due to ascites, in the SUS, RES, and relaxed unselected (REL) lines were 98.5%, 26% and 66%, respectively, when exposed to hypobaric induced hypoxia. Broilers sampled in this study were from the 13th generation of selection and were raised under industry standard practices for broiler breeders. All animal procedures used were approved by the University of Arkansas, Institutional Animal Care and Use Committee (Protocol #06024).

Sampling and DNA Isolation. Genomic DNA was obtained by extraction of total DNA from either blood (n = 20) or liver (n = 60) samples collected from an equal number of randomly selected RES broilers and randomly selected SUS broilers. Working and stock solutions of DNA from individual broilers were stored frozen at -20 °C for further use.

Selection of Primers. The chicken iNOS (Genbank accession number NW_001471508) and eNOS (Genbank accession number NW_001481304) gene sequences were used for the development of primers. Primer 3 software was used to develop primers for the isolation of specific regions of both eNOS and iNOS. Specific primers were designed to isolate a region within the first 600-700 base pairs of the promoter region of both eNOS and iNOS genes (Table 1). Published sequences of the regions targeted by the primers are listed in Table 2.

Polymerase Chain Reaction (PCR). Sample DNA was amplified from the selected promoter region of each gene in 50 µl PCR reactions. Each reaction contained 0.25 µl of 25 nM forward and reverse primer, 4.1 µl of 4 mM dNTPs, 5 µl of 10× NH_4 Buffer, 2 µl 50 mM $MgCl_2$, 25 ng of genomic DNA, 37.4 µl of PCR quality purified water, and 1 unit of Biolase Red DNA polymerase (Bioline, Taunton, Mass.). The amplification was performed in a Perkin Elmer, GeneAMP PCR System 2400 (Perkin Elmer, Waltham, Mass.). The PCR program consisted of an initial denaturization at 95 °C for five minutes, then 31 cycles of:

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[1] University of Arkansas, Department of Animal Science, Fayetteville, Ark..

[2] University of Arkansas, Department of Poultry Science, Fayetteville, Ark.

95 °C for one minute, 56 °C for one and one-half minutes, and to 72 °C for one minute, followed by a final soak at 72 °C for seven minutes. The same protocol and annealing temperature was used for all genes.

Gel electrophoresis (1% agarose) was performed on all individual PCR reactions to confirm the presence of a single band of appropriate size for each gene fragment examined. Following a PCR cleanup procedure, DNA concentrations were determined and samples were submitted to the University of Arkansas Core DNA Lab for sequencing on an ABI 3100 (BigDye v1.1) genetic analyzer.

Identification of Single Nucleotide Polymorphisms (SNPs). Gene sequences published at NCBI were compared to the individual broiler's sequence of the same region using ClustalW2 (www.ebi. ac.uk/Tools/clustalw2/index.html, European Bioinformatics Institute 2006-2008) and by visual examination of the electropherograms of the individual broilers. The region of the published sequence containing the amplified fragment was pasted into ClustalW2 along with the appropriate sequences of the genes obtained from individual broilers. Heterozygosity and homozygosity for each SNP was determined by visual examination of the electropherograms. A SNP was identified when there was at least one broiler with the published base and at least one broiler with a base other than the published base in that position. After SNPs were initially located, the remaining SNP typing (n = 60) was done by visual examination of the appropriate region of electropharograms at each of the identified SNP locations.

Naming of SNPs. When SNPs were identified they were named by the nucleotide base listed in that position in the published NCBI sequence used to generate the primers, then the base location of the SNP in relation to the start of exon 1, and finally the base found to be a substitution at that site. Therefore, a SNP named A-360C refers to a cytosine substitution for an adenine located 360 base pairs upstream of the start of exon 1.

Statistical Analysis. The likelihood that a particular SNP would be more likely to appear in a SUS than a RES bird was examined using Pearson Chi Square analysis in JMP (version 7, 2007).

Results and Discussion

Polymorphisms in the Inducible Nitric Oxide Synthase (iNOS) Gene. Six SNPs were identified in the 681 base pair fragment of the iNOS promoter region (Table 3). The 6 SNPs were: G-347A, C-402T, T-519A, G-546A, T-583C, and C-619T. Of the six SNPs identified, five were more likely to be homozygous for the allele published in NCBI if the broiler was RES ($P = 0.0001$). The G-546A SNP was not different ($P = 0.39$) between SUS and RES broilers in the first 60 birds examined; therefore, examination of this SNP was discontinued. For the remaining SNPs, broilers (n = 80) possessing any deviation from the published homozygous allele at G-347A ($P = 0.0001$), C-402T ($P = 0.0001$), T-519A ($P = 0.0001$), T-583C ($P = 0.0001$), and C-619T ($P = 0.0001$) were more likely to be in the SUS group than in RES.

All iNOS SNPs that showed a difference between RES and SUS broilers appear to be nearly 100% linked. The base combination (meaning heterozygous, homozygous for the published allele, or homozygous for the substitution) at one SNP was consistent throughout all the SNPs, except for one broiler.

In general, 90 % of birds in the RES group were homozygous for the published allele at the -347, -402, -519, or -583 positions of the iNOS gene; 10% were heterozygous; and 0% were homozygous for the SNP at these positions. Conversely, 47.5% of the SUS birds were homozygous for the SNP at these positions, while 40 % were heterozygous for

the SNP and 12.5% were homozygous for the published allele. The relationship was similar at the -346 SNP except for the one bird in 80 that did not exhibit 100% linkage at this SNP location.

Polymorphisms in the Endothelial Nitric Oxide Synthase (eNOS) Gene. Two SNPs were found within the 300 base pair segment sequenced from the eNOS promoter region. Both SNPs, G-108A and G-125C, were related to ascites susceptibility.

Broilers that were homozygous for the NCBI published allele (G) 108 base pairs 5' to the start of exon 1 of eNOS were more likely ($P = 0.0001$) to be susceptible to ascites (SUS) than broilers homozygous or heterozygous for the A substitution at that position (Table 4). Five percent of RES broilers were homozygous for the published allele, while 37.5% were heterozygous for the SNP and 57.5% were homozygous for the SNP. In SUS broilers, 100% of the broilers sampled possessed the published allele, and 0% possessed the SNP in either the heterozygous or homozygous form.

Broilers homozygous for the substitution of cytosine for guanine 125 base pairs upstream of the start of exon 1 were more likely ($P = 0.0001$) to be resistant to ascites (RES) than broilers heterozygous for cytosine and guanine, or homozygous for guanine (the published allele) at that position. In the SUS broilers, 100% were homozygous for the published allele. In RES broilers, 5% possessed the homozygous published allele at -125; 37.5% were heterozygous; and 57.5% possessed the homozygous SNP.

In summary, the frequency of SNPs in the promoter region of both the iNOS and eNOS genes varied significantly between the RES and SUS broiler lines suggesting a strong relationship between the occurrence of these polymorphisms and susceptibility or resistance to ascites in broilers. The promoter region of a gene functions as the control mechanism for the initiation of the transcription of that particular gene. Research in other species has determined that polymorphisms in the promoter region can affect the amount of transcription that occurs, much like a thermostat or rheostat, rather than a simple on off switch. Thus, polymorphisms in the promoter region of a gene have the potential to either increase or decrease the degree of transcription of the gene, depending on whether the polymorphisms alter inhibitory or stimulator regions of the promoter complex. The expression of the proteins (endothelial nitric oxide synthase and inducible nitric oxides synthase enzymes) produced by these genes were not determined in this study, but it is possible the polymorphisms identified in this study could alter the profile of these enzymes in response to stimuli that potentially induce ascites. As discussed previously, earlier research has demonstrated that the nitric oxide response is altered in ascites resistant and ascites susceptible broilers. Further research will be required to determine if these polymorphisms alter the nitric oxide response in different broiler lines.

Implications

Results of this study indicate there are polymorphisms in the promoter regions of the eNOS and iNOS genes and the frequency of occurrence of the polymorphisms differs significantly between ascites resistant and ascites susceptible broiler lines. These findings suggest these polymophisms could potentially be used as genetic markers for the selection of birds resistant to ascites. Further studies in different populations of birds and the use of test matings based on these markers will be needed to confirm their usefulness as tools for genetic selection of ascites resistant broilers.

Table 1. Primers used to isolate and amplify DNA sequences examined for single nucleotide polymorphisms.

Primer	Sequence (5'-3')	Start Distance From Exon 1
iNOS Forward	GGACAGGAGCCAAACTCAAA	-651
iNOS Reverse	TGCTCTGACTTCACTTGATTTGTT	30
eNOS Forward	CTCTGAGGCATCCAGCTTTT	11
eNOS Reverse	CCCTTTCCCTGACCCTAACT	-289

Primers were selected by Primer 3 software (Rozen 2000) using the chicken Genbank NCBI entries of NW_001471508 (iNOS), and NW_001481304 (eNOS).
[a] Original primers used in random broilers to test quality of the gene sequencing results.

Table 2. Actual sequences generated by PCR for the chicken, iNOS and eNOS genes

Chicken iNOS gene, actual region amplified by PCR

>ref|NW_001471508.1|Gga19_WGA256_2:9162050-9162730 Gallus gallus chromosome 19 genomic contig, reference assembly (based on Gallus_gallus-2.1)
GGACAGGAGCCAAACTCAAAATACAACTAGGGCAATATATATTGCTCCAATTGTTATTTTGCATTCTTTAGTTCA
CAGTCCACAAGGCAAGATTTACACCTCTCCGGCAGTAACAAGACTGTATAACTCATCTTAGGCAGGCCACATA
ACTCACAGAGATGAGCTCTTTGCTTAAGGGGAGAGTGCTGGTTGGAGAACACCTCACATCAGCCCTTGGAAG
CAAAGAAACTGCTTCTGAGGGGTTCTTTGCGATACCAGGCAAAGCATCATTAGAAATACCAGTAATCACATACA
GAAAGAAGCTGACTCACCCAACTGGTGATGAGGGTCCTCTGGCTATAAAACACACCTCCTCAAGAAGCAGCC
TTCACCCAAGACACATTCTCCCATCAGAGGCTTGCCCTAAATGAGCCCAGGGAGGGATGGACCCCTCCAGGT
CAGGTGGGAAGTGCAGGAGAATTGCTTGCACCTGTGCTTCCTGGGTCAGCCACCCCTCCCTCCACCAGGTG
CTCAATCATCAGTTCAGGCTGTGTTCTAGCAGTTCCCTGCATCTACCTACCCACCTGCACTTTTTTCAGGCTG
GAGATAATAGGACAAAGAGGTACATTTAAACTACACCAATCTGTTTGTCTTAATATAAAGGTATCTTTTATTACAA
ACAAATCAAGTGAAGTCAGAGCA

Chicken eNOS gene, actual region amplified by PCR

>ref|NW_001481304.1|GgaUn_WGA3052_2:3197-3496 Gallus gallus genomic contig, reference assembly (based on Gallus_gallus-2.1)
CTCTGAGGCATCCAGCTTTTGGGGCCGGGGAACCGGCCTGTGGGATGGGAGGTGGTGGGCATATGGGGGG
GGATGGAGACAGACGTGGGGTGGGGAGGGGATGCGGTAGGTCTGGGGTGGGTTGGGGATAGCGATGAGG
AGGGGGTGGGATGAGGTTAGAATGGGGATGGGAAAGGGGATAGGGAACAGGGAGGGGGATAGGGATGGAT
TGGAGATGTTATACGGAGAGGATGGGAAGGACAAAAGGATGGGGAAGACCATGAGGGAGGGAAAGGGTCG
GAGTTAGGGTCAGGGAAAGGG

Table 3. Distribution of genotypes for single nucleotide polymorphisms[a] in a 681 bp sequence of the 5' region of the iNOS gene of RES and SUS broiler lines.

SNP	Broiler Line	n	Genotype Frequency/(%)			Chi square
G-546 A			GG	AG	AA	
	RES	29	6(20.7)	14(48.3)	9(31.0)	$P = 0.3971$
	SUS	29	4(14.7)	11(37.9)	14(48.3)	
G-347A			GG	GA	AA	
	RES	40	36(90.0)	4(10.0)	0(0)	$P = 0.0001$
	SUS	40	5(12.5)	16(40.0)	19(47.5)	
C-402T			CC	CT	TT	
	RES	40	36(90.0)	4(10.0)	0(0)	$P = 0.0001$
	SUS	40	5(12.5)	16(40.0)	19(47.5)	
T-519A			TT	TA	AA	
	RES	40	36(90.0)	4(10.0)	0(0)	$P = 0.0001$
	SUS	40	5(12.5)	16(40.0)	19(47.5)	
T-583C			TT	TC	CC	
	RES	40	36(90.0)	4(10.0)	0(0)	$P = 0.0001$
	SUS	40	5(12.5)	16(40.0)	19(47.5)	
C-346T			CC	CT	TT	
	RES	40	36(90.0)	4(10.0)	0(0)	$P = 0.0001$
	SUS	39	4(10.2)	16(41.0)	19(48.8)	

RES = Broiler line resistant to ascites, SUS = Broiler line susceptible to ascites.
[a]From comparing to the sequence published in NCBI genebank entry: NW_001471508.
In a single broiler (except one broiler) the base pattern at one significant SNP matched the base patterns at all other significant SNPs.

Table 4. Distribution of genotypes for single nucleotide polymorphisms[a] in a 300 bp sequence of the 5' region of the eNOS gene of RES and SUS broiler lines.

SNP	Broiler line	n	Genotype frequency/(%)			Chi Square
G-108A			GG	AG	AA	
	RES	40	2(5.0)	15(37.5)	23(57.5)	$P = 0.0001$
	SUS	40	40(100)	0(0)	0(0)	
G-125C			GG	GC	CC	
	RES	40	0(0)	0(0)	40(100)	$P = 0.0001$
	SUS	40	3(7.5)	27(67.5)	10(25.0)	

RES = Broiler line resistant to ascites, SUS = Broiler line susceptible to ascites
[a]Based on comparison with the sequence published in NCBI genebank entry: NW_001481304.