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Arkansas Animal Science Department Report 2011

David L. Kreider
University of Arkansas, Fayetteville

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David L. Kreider, Editor

DIVISION OF AGRICULTURE
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ARKANSAS ANIMAL SCIENCE DEPARTMENT REPORT 2011

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INTRODUCTION

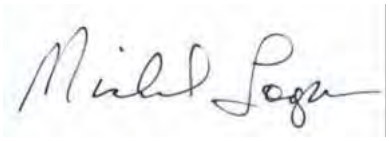
Welcome! While this is my first edition of *Arkansas Animal Science* as Department Head, it is the publication's 14th edition overall. Thanks to the faculty in the Department of Animal Science and especially to Dr. David Kreider who served as editor of this edition. *Arkansas Animal Science* continues to evolve as this Department strives to be a source of on-demand, unbiased, scientific-based knowledge both inside and outside the classroom. With so much misinformation regarding animal production in today's society, and the speed in which it is disseminated, stakeholders, researchers, extension faculty and industry professionals need results as quickly as the data are statistically analyzed and determined ready for use; *Arkansas Animal Science* does just that. Virtually all our extension and research publications are online as well. In order to communicate a clear message to all stakeholders, this year we are releasing a new publication, *Arkansas Animal Science–Research Highlights.* This publication will be distributed in both electronic and printed form and will allow those interested to quickly read, in a few brief statements, the impact of our research and extension programs. A weblink to the entire report will be included within each highlight.

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 Research Station at Batesville. Other valuable research and extension work was conducted at numerous private farms across the state.

Readers are invited to view all programs of the Department of Animal Science at the departmental website at animalscience.uark.edu, the Livestock and Forestry Research Station website at Batesvillestation.uark.edu, the Southwest Research and Extension Center website at swrec.uark.edu, and the Southeast Research and Extension Center website at aaes.uark.edu/serec. 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.

I appreciate your interest in the work that we do to enhance animal production in this state. We hope you find the research, extension and educational programs reported herein to be timely, useful and making a contribution to the field of Animal Science.

Sincerely,



Michael Looper
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)

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Beef Production and Management

Meats and Food Safety

Forages and Forage Management

Influence of organic versus inorganic trace mineral supplementation on bull semen quality

M.P. Rowe[1], J.G. Powell[1], E.B. Kegley[1], T.D. Lester[1], C.L. Williams[1], R.J. Page[1], and R.W. Rorie[1]

Story in Brief

This study evaluated the effect of trace mineral supplementation on bull semen quality during the summer, as measured by computer-assisted sperm analysis. Angus and Balancer bulls were assigned to inorganic (n = 9) and organic (n = 10) mineral treatments, based on initial semen quality, breed, weight, and age. The bulls were maintained in a dry lot and fed mixed grass hay for the 123 d study. Three times a week bulls were individually fed a ration containing either inorganic or organic Zn, Cu, Co and Mn. Starting on d 60, semen was collected by electroejaculation weekly for 9 w. Semen was evaluated by computer-assisted sperm analysis for percent motile, progressive and rapid sperm within 5 min of each collection. Data were analyzed by treatment, week and their interaction, using SAS Proc Mixed for repeated measures. No interaction occurred between week and treatment, nor was week significant ($P > 0.05$). Bulls supplemented with organic trace mineral had more ($P = 0.02$) motile sperm than those supplemented with inorganic trace mineral (65.5 versus 56.1%, respectively). Likewise, progressive sperm was improved ($P = 0.01$) for bulls receiving organic (47.0%) versus inorganic (38.4%) trace mineral. The percentage of motile sperm with rapid motility was also greater ($P = 0.03$) for bulls supplemented with organic as compared with inorganic trace mineral (62.3 versus 52.8%, respectively). These results suggest organic trace mineral supplementation may improve bull semen quality. Additional studies are needed to determine if this treatment results in increased pregnancy rates.

Introduction

Mineral supplements commonly fed to livestock are mainly in inorganic forms (i.e., a molecule that does not contain carbon). Organic minerals consist of a mineral ion bound to an organic molecule (usually an amino acid or carbohydrate). Trace minerals are present in the body almost entirely as organic complexes or chelates and not as free inorganic ions. Thus, it has been suggested that dietary organic trace minerals could be more efficiently utilized. It has been well documented that trace mineral supplementation has an impact on reproductive performance.

It has been well documented that trace mineral supplementation has an impact on reproductive performance. Zinc deficiency has been shown to have a negative impact on spermatogenesis (Hidiroglou, 1979). Supplemental zinc has also been shown to improve the percentage of normal sperm cells (Arthington et al. 1995). Studies indicate that organic forms of trace minerals can improve dairy cow reproductive performance, particularly during times of stress. Limited information has been available on the effects of organic trace mineral supplementation on bull fertility. The objective of this study was to evaluate the effect of source of supplemental trace minerals on bull semen quality as measured by computer-assisted sperm analysis (CASA).

Materials and Methods

This study utilized 19 Angus and Balancer bulls housed at the University of Arkansas Savoy Cow-Calf unit. The bulls averaged 1,819 lb body weight and ~6 yr of age at the start of the study, and were maintained and cared for in accordance to procedures approved by the Institutional Animal Care and Use Committee (protocol 11001). Semen was collected by electroejaculation and evaluated a minimum of 3 times prior to the start of the study to help in assigning bulls to treatments. Bulls were assigned to inorganic (n = 9) or organic (Availa®4, ZinPro Corp., Eden Prairie, Minn.; n = 10) trace mineral treatments based on semen quality, breed, body weight, and age. The treatments consisted of supplemental Zn (450 mg/d), Cu (150 mg/d), Co (12 mg/d), Mn (300 mg/d), Se (3 mg/d), and I (5 mg/d) supplied as either inorganic or organic sources (Table 1).

Bulls were maintained in a dry lot throughout the study and fed mixed grass hay to maintain body condition. Three times each week bulls were individually fed 3 lb of a grain supplement that served as the carrier for treatments containing either inorganic or organic trace mineral treatments for 123 days. Starting on d 60 of the study (July 15), semen was collected by electroejaculation weekly for 9 consecutive weeks. Semen was then evaluated by CASA for percentage motile, progressive, and rapid sperm within 5 min. of each collection. Motile sperm were those with a path velocity greater than 30 μm/sec and progressive velocity greater than 15 μm/sec. Progressive sperm had a path velocity greater than 50 μm/sec and straightness of path over 70%. Rapid sperm were those with a progressive path velocity over 50 μm/sec. Sperm motility data were analyzed by treatment, week and their interaction, using the mixed procedure of SAS for repeated measures. All reported means are least square means ± SE.

Results and Discussion

Sperm production (spermatogenesis) requires about 60 d in bulls. Therefore, bulls were fed the trace mineral supplements for 60 d (mid-May to mid-July) prior to starting the weekly semen collections to ensure adequate time for treatments to influence sperm production or quality. The trial consisted of 9 weekly semen collections during which time the mineral supplementation continued. Nine weeks was chosen to cover the entire length of spermatogenesis. The 9 w trial ran from mid-July to early September when fertility is more likely to be adversely affected by heat stress.

Over the 9 w trial, bulls supplemented with the organic and inorganic trace mineral lost an average of 4 and 7% of their body weight, respectively. Weight loss was more severe ($P = 0.03$) for bulls in the inorganic trace mineral group. During the trial, wet conditions

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developed around the water trough and in the shaded area of the lots. A bull in the inorganic treatment was dropped from the study after the 4th w due to lameness and another bull in the same treatment was removed due to lameness during the 6th week. None of the bulls fed the organic mineral supplement developed lameness problems.

No interaction occurred between week and treatment, nor was week significant ($P > 0.05$); therefore, data were analyzed for the effects of treatment on sperm motility parameters, with weekly collections as repeated measures over time. At the start of the study, all sperm parameters were similar across treatments ($P \geq 0.678$; Table 2). Over the 9 week trial period, bulls supplemented with the organic supplement had a higher ($P = 0.02$) percentage of motile sperm (65.5%) compared with the inorganic treatment at 56.1% (Table 2). The organic mineral treatment also maintained a higher ($P = 0.01$) percentage of progressive motile sperm (47.0 versus 38.4% for organic and inorganic treatments, respectively). The percentage of motile sperm with rapid motility was also greater ($P = 0.03$) for bulls supplemented with organic compared with bulls receiving inorganic trace mineral (62.3 versus 52.8%, respectively).

Comparison of the mean percent motile, progressive, and rapid sperm for bulls in each treatment before and during the trial (Table 2) shows that the organic trace mineral helped to maintain these sperm parameters during the Mid July to early September trail period. Regardless of treatment, these sperm motility parameters declined during the hot weather. Due to the decline in semen quality during the summer, many bull stud facilities avoid as much as possible, collecting semen during this time. Overall, the results of this study suggest organic trace mineral supplementation may improve bull semen quality. Additional studies are needed to determine if this treatment results in increased pregnancy rates.

Implications

Sperm motility is one of the most important semen quality parameters influencing bull fertility. Results of this study suggest organic trace mineral supplementation may improve bull semen quality. Additional studies are needed to determine if this improvement in semen quality contributes to increased pregnancy rates.

Acknowledgements

The authors thank Zinpro Corporation (Eden Prairie, Minn.) for supplying organic mineral (Availa®4) during this experiment and the University of Arkansas Division of Agriculture for providing support for the experiment. The authors also thank B. Lindsey and R. Shofner (both of University of Arkansas Division of Agriculture Savoy cow-calf unit) for providing technical support in animal care and management.

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Table 1. Inorganic and organic trace mineral content of bull supplements.

	Organic diet		Inorganic diet
Mineral	Inorganic portion (mg/d)	Organic portion (mg/d)	Inorganic portion (mg/d)
Zinc	90 as zinc sulfate	360 as zinc amino acid complex	450 as zinc sulfate
Copper	25 as copper sulfate	125 as copper amino acid complex	150 as copper sulfate
Cobalt	none	12 as cobalt glucoheptonate	12 as cobalt carbonate
Manganese	100 manganese sulfate	200 as manganese amino acid complex	300 as manganese sulfate
Selenium	3 as sodium selenite	none	3 as sodium selenite
Iodine	5 as calcium iodate	none	5 as calcium iodate

Table 2. Mean[a] sperm measurements of bulls assigned to inorganic and organic trace mineral treatments at the start and over the nine week study.

	Trace mineral treatment		
CASA sperm parameter	**Inorganic**	**Organic (ZinPro)**	***P* value**
At initiation of trial			
Motile %	72.0 ± 2.0	71.3 ± 1.8	0.801
Progressive %	58.5 ± 2.5	58.1 ± 2.3	0.896
Rapid %	68.2 ± 2.3	66. 9 ± 2.1	0.678
Over the 9 week trail			
Motile %	56.1 ± 2.8	65.5 ± 2.6	0.024
Progressive %	38.4 ± 2.2	47.0 ± 2.0	0.011
Rapid %	52.8 ± 2.9	62.3 ± 2.6	0.027

[a]Least square means.

Effect of progestin source in an estrous synchronization protocol on estrus response and conception rates in beef cows and heifers

J.G. Powell[1], T.D. Lester[1], M.P. Rowe[1], C.L. Williams[1], and R.W. Rorie[1]

Story in Brief

This study evaluated the estrus response and subsequent pregnancy rates of beef cows and heifers after estrous synchronization with two different progestin sources in a progestin-select synch protocol. Beef heifers (n = 64) and beef cows (n = 74) were allocated to two treatment groups based on age, body condition score and body weight. Cows and heifers were managed separately throughout the study but assigned to two similar treatment groups. Cows and heifers assigned to Treatment 1 received a progesterone insert (CIDR) from days 0 to 14, GnRH on day 16, and $PGF_2\alpha$ on day 23. Cows or heifers assigned to a Treatment 2 were fed MGA from day 1 to 14, received GnRH on day 16, and $PGF_2\alpha$ on day 23. Among treatments, heifers exhibited similar ($P > 0.10$) estrus response, mean interval to estrus following $PGF_2\alpha$, AI pregnancy rate, and final pregnancy rate. Mature cows assigned to Treatment 1 exhibited an increased estrus response and AI pregnancy rate ($P < 0.05$) compared to cows in Treatment 2. Final pregnancy rate and mean interval from $PGF_2\alpha$ to estrus in mature cows were similar ($P > 0.10$) among treatments. Results indicate that within the current estrous synchronization protocol, CIDR progesterone inserts and MGA are equally effective for synchronization of beef heifers. Based on estrous response and AI pregnancy rates, CIDR progesterone inserts may be superior to MGA for synchronization of mature beef cows. The CIDR inserts do have the advantage of insuring that individual animal receives adequate progestin over the treatment period compared to oral MGA which is subject to individual animal variation in feed intake.

Introduction

Estrous synchronization and artificial insemination have potential to increase the rate of genetic improvement, calf uniformity and improve calf weaning weight in beef cattle operations. Synchronization programs utilizing exogenous progestins, gonadotropin releasing hormone (GnRH), and prostaglandin ($PGF_2\alpha$) have been shown to be effective for estrous synchronization in beef cows and heifers (Lamb, 2010). The objective of this study was to evaluate the effectiveness of two different progestins [melengestrol acetate (MGA) compared with controlled internal drug-release inserts (CIDR)] when used during a 14-d progestin protocol followed by GnRH 2 d after progestin withdrawal and $PGF_2\alpha$ injection 7 days later.

Materials and Methods

Description of Animals and Treatments. This experiment was conducted at the Savoy Beef Cattle Research Unit near Fayetteville, Arkansas utilizing Angus-cross beef cows (n = 74) and heifers (n = 64). Throughout the study, all animals were maintained and cared for in compliance with the University of Arkansas Animal Care and Use Committee Protocol #11010. All cows utilized for the study were randomly and equally distributed into two treatments groups (Fig. 1) based on BCS (5.9 ± 0.1), age (4.7 yrs ± 0.3) and body weight (1065 ± 17 lbs). Heifers utilized for the study were randomly and equally distributed into two treatments groups based on BCS (6.9 ± 0.1), age (15.9 ± 0.4 mo) and body weight (755 ± 22 lb). Cows and heifers included in the study were managed and maintained separately throughout the study, but they were assigned to similar treatments that were initiated on day 0 (6 November 2010).

Treatment 1 (T1, n = 37 cows and 32 heifers) received a CIDR ((EAZI-Breed CIDR®, Pfizer Animal Health, New York, N.Y.; 1.38g progesterone) and were fed a carrier for 14 days, followed by GnRH (Factrel®, Pfizer Animal Health) and $PGF_2\alpha$ (Lutalyse®, Pfizer Animal Health) treatments on days 16 and 23, respectively. Treatment 2 (T2, n = 37 cows and 32 heifers) animals were fed melengestrol acetate (MGA, 0.5 $mg \cdot head^{-1} \cdot day^{-1}$, Pfizer Animal Health) for 14 days, followed by GnRH and $PGF_2\alpha$ treatments on days 16 and 23, respectively. At the time of $PGF_2\alpha$ dosing, each cow or heifer was equipped with a heat detection patch (Estrotect®, Rockway Inc., Spring Valley, Wis.) to improve detection of standing estrus. During a 96 h period following $PGF_2\alpha$ treatment, all cows and heifers were observed at least twice daily for onset of standing estrus. Time of onset of standing estrus and AI were recorded for each cow or heifer.

Breeding Season and Pregnancy Diagnosis. Artificial insemination was performed by one experienced technician at ~12 hours after standing estrus was observed. Semen from two Angus sires were used for artificial insemination and stratified across treatments. Fertile, Angus bulls were placed into four separate breeding pastures (2 cow pastures, 2 heifer pastures) for natural service 11 d after the 96 h estrus observation period ended and remained there for 56 d. Conception rate to AI was evaluated 70 d after the end of the synchronization period by transrectal ultrasonography (5 MHz transducer, Aloka 500V, Aloka Inc., Wallington, Conn.). Differences in fetal crown-rump length were used to identify pregnancies resulting from AI service or from pasture bulls. A second ultrasonography was conducted 30 to 45 d later to confirm overall seasonal pregnancy rate.

Data and Statistical Analysis. Data from each experiment were analyzed separately, using SAS statistical software (version 8.3) with animal as the experimental unit. Estrous response was defined as the percentage of all females treated that were detected in estrus within the 96 h period following $PGF_2\alpha$ dosing. The AI pregnancy rate was defined as the number of cows or heifers that were determined to be pregnant to AI service divided by the number of cows exhibiting estrus within the 96 h period following $PGF_2\alpha$ dosing and inseminated. Overall pregnancy rate was defined as the percentage of all treated animals that were pregnant at the end of the breeding season. Estrous response, AI pregnancy rate and overall pregnancy

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rate were evaluated using the Chi-Square analysis (Proc Logistic), and all other data were evaluated by general linear model (Proc GLM) of SAS. Initial models for reproductive responses contained fixed effects of treatment, BCS, age, days postpartum, sire, and their interactions. Effects not found significant were removed from the model. All reported means are least square means ± standard error.

Results and Discussion

Initial body condition, body weight, age, and AI sire had no effect on either AI pregnancy rate or overall pregnancy rate ($P \geq 0.41$). During the experiment, one cow was removed from the MGA (T2) group due to a health reason so the remaining 36 animals were evaluated for that treatment.

Results for cow estrus rates, hours to estrus following $PGF_2\alpha$, and pregnancy rates are presented in Table 1. The percentage of cows exhibiting estrus within 96 h of $PGF_2\alpha$ dosing was higher ($P < 0.05$) for T1 (91.8%) than for T2 (72.2%). The mean interval from $PGF_2\alpha$ dosing until estrus detection was similar ($P = 0.55$) at 53.1 h for T1 and 50.0 h for T2. Cows in T1 had a higher ($P < 0.05$) AI pregnancy rate (76.5%) than cows in T2 (50.0%). Overall, seasonal pregnancy rates were similar ($P = 0.12$) for T1 and T2 at 94.6% and 83.3%, respectively.

Based on a previously reported study (Bader et al. 2005), the lower percentage of cows in estrus and AI pregnancy rate for cows assigned to the synchronization treatment utilizing MGA was unexpected. It is possible the difference would be related to progestin delivery. The 37 cows assigned to T2 were managed in one pasture, and even though ample bunk space was provided for MGA supplemented feed, some cows in the group may have received limited feed due to aggressive behavior of others, consequently influencing estrus rates. If that were the case, the estrus response observed (72.2%) would have been primarily due the effects of the GnRH- $PGF_2\alpha$ (select synch) portion of the protocol. The CIDR inserts do have the advantage of insuring each individual animal receives adequate progestin over the treatment period.

Results for heifers evaluated in the experiment are found in Table 2. The percentages of heifers in Experiment 2 that exhibited estrus within the 96 h period following the $PGF_2\alpha$ dosing were similar ($P = 0.77$) at 75% and 78.1% for T1 and T2, respectively. The mean interval from $PGF_2\alpha$ dosing until estrus detection was similar ($P = 0.69$) at 48.3 h for T1 and 49.6 h for T2. The AI pregnancy rate was also similar ($P = 0.31$) at 62.5% for T1 and 76.0% for T2. Overall pregnancy rate (90.6 and 87.5%) for T1 and T2, respectively) did not differ ($P = 0.689$) among treatments. In a similar study, Kojima et al. (2004) substituted then compared the use of CIDR in a MGA-Select Synch protocol for beef heifers. Estrous response was similar, but the pregnancy rate to AI was greater for the protocol utilizing CIDR (63%) than for MGA based (47%) protocol. In the current study, there was a numerical advantage in pregnancy rate for the MGA-Select Synch protocol. Overall, it would appear that either the CIDR-Select Synch or MGA-Select Synch protocols are equally viable options for synchronizing beef heifers.

Implications

In this study, both CIDR inserts and MGA are equally effective options in a 14 d progestin-select synch protocol for beef heifers, where GnRH is given 2 d after progestin withdrawal. Based on estrous response and AI pregnancy rates, CIDR progesterone inserts may be superior to MGA for synchronization of mature beef cows. The CIDR inserts can be legally used in mature beef cows, and may have the advantage of insuring each individual animal receives adequate progestin daily during the treatment period.

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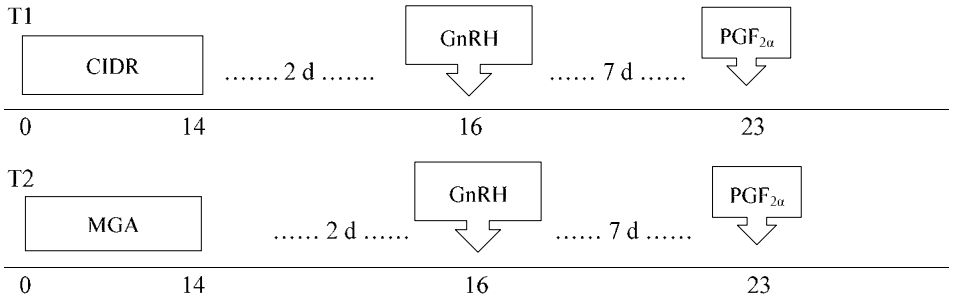

Fig. 1. Treatment schedule for cows assigned to T1 and T2 (Experiment 1).

Table 1. Mature Cows: Effect of progestin source on estrous response and pregnancy rates.

	Estrous synchronization treatment (Cows)*		
Item	**T 1**	**T 2**	**P value**
Cows in estrus	34/37 (91.8%)	26/36 (72.2%)	0.0281
Hours, PG to estrus	53.1 ± 3.4	50.0 ± 3.9	0.5469
AI pregnancy rate	26/34 (76.5%)	13/26 (50.0%)	0.0332
Overall pregnancy rate	35/37 (94.6%)	30/36 (83.3%)	0.1236

*Cows in TRT 1 and 2 received CIDR and MGA as progestin sources, respectively.

Table 2. Heifers: Effect of progestin source on estrous response and pregnancy rates.

	Estrous synchronization treatment (Heifers)*		
Item	**T 1**	**T 2**	**P value**
Heifers in estrus	24/32 (75.0%)	25/32 (78.1%)	0.7679
Hours, PG to estrus	48.3 ± 2.2	49.6 ± 2.1	0.6845
AI pregnancy rate	15/24 (62.5%)	19/25 (76.0%)	0.3054
Overall pregnancy rate	29/32 (90.6%)	28/32 (87.5%)	0.6888

*Heifers in TRT 1 and 2 received CIDR and MGA as progestin sources, respectively.

Effects of osteopontin single nucleotide polymorphisms on bull semen quality

C.L. Williams [1], T.D. Lester [1], M.P. Rowe [1], and R.W. Rorie[1]

Story in Brief

Osteopontin is a fertility-associated protein found in greater concentrations in seminal plasma of bulls with higher proven conception rates. Any single nucleotide polymorphisms that occur within the osteopontin gene might affect fertility and thus, serve as a genetic marker for selection. The objective of this study was to identify, then determine if any single nucleotide polymorphisms in the *Bos Taurus* osteopontin gene promoter region correlated with sperm quality parameters, as determined by computer-assisted sperm analysis. Semen was collected 9 consecutive weeks from 5 Angus and 14 Balancer bulls. Semen samples were evaluated for percentage motile, progressive and rapid sperm. Each bull was genotyped for reported single nucleotide polymorphisms in the gene promoter region by PCR amplification and sequencing of two 700 base pair products. Eight single nucleotide polymorphisms were identified and their affects on percent motile, progressive and rapid sperm were evaluated using SAS mixed procedures for repeated measures. Individual single nucleotide polymorphisms had no affect on ($P \geq 0.1$) percentage motile, progressive or rapid sperm. Haplotypes were constructed based on the individual single nucleotide polymorphism sites, with eleven identified and compared for differences in sperm quality. Sperm motility for bulls with haplotypes 8, 9 and 4 averaged 77% compared to an average of 52% motile sperm for bulls with haplotypes 6, 7, 5 and 1 ($P \leq 0.05$). Bulls with haplotypes 8 and 9 also were higher ($P \leq 0.05$) than those with haplotypes 7, 5 and 1 for progressive (59% vs. 37%) and rapid (77% vs. 46%) motility. Haplotype 1 was the same as the reported normal reference sequence found in the osteopontin gene. Results suggest that polymorphisms within the bovine osteopontin gene promoter region might be useful as a marker in identifying bulls with improved semen quality.

Introduction

Osteopontin (OPN) is a ubiquitous, acidic glycoprotein found throughout the body, is higher concentrations in semen of bulls that have known higher conception rates (Killian et al., 1993). Osteopontin is secreted in the ampulla and seminal vesicles of the bull's reproductive tract where it is believed to bind to ejaculated sperm and then carried to the site of fertilization, where it is thought to play a role in the sperm-egg interaction and fertilization events through signaling of secondary messenger systems. *In vitro* studies have found that pre-treatment of bovine oocytes with purified OPN increases fertilization rates, embryo development, and reduces the occurrence of polyspermy. Osteopontin treated bovine sperm has also shown greater capacitation and viability, as well as increased *in vitro* fertilization and cleavage rates verse non-treated sperm. Recent genetic research has yielded that polymorphisms within the OPN gene promoter region may be positively associated with increased protein concentrations and protein percentages in milk (Schnabel et al., 2005). The objective of this study was to investigate the polymorphic nature of the *Bos Taurus* OPN gene promoter region and determine any association of polymorphisms with bull semen quality variables.

Materials and Methods

Animals and Semen Collection. All practices and techniques utilized in this study were approved by the University of Arkansas Animal Care and Use Committee (Protocol # 11001). A group of 19 bulls, consisting of 5 Angus and 14 Balancer breeds, ranging in age from 5 to 9 years and weight from 1,561 to 2,100 pounds were used for the study. Bulls were placed in two separate groups in dry lots prior and during the project to reduce social (dominance) stress. During this period, bulls were fed 3 lb. of concentrate feed 3 times a week as well as *ad libitum* grass hay to maintain body weight and condition. Prior to the test period, semen was collected from the bulls and evaluated to ensure sperm variables exceeded the minimum acceptable standards for a breeding soundness exam (BSE). The experimental period ran for two months from July 15, 2010 through September 19, 2010 and bulls were retained at the University of Arkansas Beef Research Unit near Savoy, Ark. Bulls were collected via electro-ejaculation weekly for 9 consecutive weeks during the test period.

Semen Analysis. Each semen collection was analyzed using a computer assisted sperm analysis (CASA; Hamilton Thorne Biosciences; Beverly, Mass.) within 5 minutes of ejaculation. The sperm variables measured were percent motile, progressive and rapid sperm (Table 1). Prior to analysis, each semen sample was diluted with Dulbecco's PBS to achieve a concentration of $\sim 25 \times 10^6$ sperm/ml before loading onto a 2X-CEL (Hamilton Thorne Biosciences) slide. The CASA system scanned 8 to 10 areas along the length of the slide and captured 30 video frames per viewing area to construct a composite of the sperm motility variables. A minimum of 400 sperm cells were counted on each slide to achieve a representation of the entire semen collection sample.

DNA Extraction. Blood samples were obtained from each test bull via tail vein in heparinized, 8 ml vacuum tubes. Samples were placed on ice for transport to the lab. The samples were centrifuged at 1,200 g for 20 minutes at room temperature and then buffy coats were extracted and stored at -20 °C. Genomic DNA was extracted from the buff coat using a DNeasy Blood and Tissue kit (QIAGEN; Cat. No. 69504; Valencia, Calif.) with a mammalian whole blood protocol for purifying 300 µl of whole blood. Isolated DNA samples were quantified using a Qubit™ fluorometer (Invitrogen; Eugene, Ore.) and Quant-iT™ dsDNA high sensitivity assay kit (Invitrogen). Following quantification, DNA samples were diluted with sterile PCR water to a concentration of 20 ng/µl. Diluted DNA samples were stored at 4 °C.

Primer Design. Primers for PCR amplification were designed using Primer 3 (v1.1.4) software (Whitehead Institute for Biomedical Research; Cambridge, Mass.). The desired area of amplification was

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copied from the reference sequence of the OPN gene (GenBank accession # AY878328.1) and inserted into Primer 3 to generate forward and reverse primers flanking the desired area of amplification. The selected primers were then checked for uniqueness within the *Bos Taurus* genome using Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information, Bethesda, Md.)

Two separate sets of primers were used to produce two DNA fragments of interest. Primers OPN3307F: 5'- AGC CCA CCA CCA AAT ACC TA-3' and OPN4006R: 5'- TCT GAA GGA CTG GCT TAG ATT TC-3'were used to amplify a 700 bp region between base pairs 3307 and 4006 of the OPN gene promoter region that has reported polymorphisms (Schnabel et al., 2005), including *OPN3907* which is believed to be a polymorphism positively linked to higher protein concentration in milk. Another set of primers, OPN4816F: 5'- TCC CTC CCT CTA CGT TTT CA-3' and OPN5528R: 5'- CAT CCC AAA AGG GCA TAG AA-3', amplified the region between base pair 4816 and 5528 of the OPN gene promoter region that also has reported polymorphisms (Schnabel et al., 2005).

PCR Conditions. Polymerase chain reaction amplification was performed in a 50 µl total reaction volume; which included 5 µl of 10× PCR buffer, 1.5 mM MgCl2, 250 µM dNTP mix, 40 pM of each primer, 100 ng of DNA and 2 units of Biolase DNA polymerase (Bioline USA, Inc.; Taunton, Mass.). Annealing temperature gradients were run to test the uniqueness of the primers and confirm the annealing temperature. The temperature cycles for DNA amplification were as follows: 35 cycles of 94 °C denaturation for 1 minute, 59 °C annealing temperature for 45 seconds, and 72 °C for 1 minute extension time. A 5 µl sample of each PCR product was ran through a 1% agarose gel to confirm the specificity and size of the product. Before genetic sequencing, the PCR product was purified using QIAquick PCR purification kit (QIAGEN; Valencia, Calif.) and PCR purification spin protocol (QIAGEN), designed for the isolation of DNA fragments from PCR reactions.

Genetic Sequencing and Analysis. An ABI 3130xe (AME Bioscience; Toroed, Norway) analyzer was used for automated DNA sequencing by the DNA Technologies Laboratory at the University of Arkansas. Sequencing was performed in both forward and reverse sequences, using samples containing 20 ng of PCR product and 3.4 pmol of the appropriate primer. Electropherograms for both forward and reverse complement DNA sequences were evaluated for polymorphisms using BioEdit Sequence Alignment Editor (Hall, 2005; Ibis Therapeutics, Version 7.0.5.3). Completed sequences were inserted and aligned in ClustalW2 (European Molecular Biology Laboratory Outstation-European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom; Version 2.0) to identify differences in genetic sequences among bulls and the reported normal reference sequence.

Statistical Analysis. Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc.; Cary, N.C.). The model included the individual SNP site and repeated CASA measures for motile, progressive and rapid sperm. Unique haplotypes were also analyzed as the main effect in the PROC MIXED model for repeated measures with the same model as the SNP analyses.

Results and Discussion

Killian et al. (1993) evaluated proteins in the seminal plasma of Holstein bulls for potential markers for fertility. The bulls were of known fertility, based on pregnancy date from at least 1000 inseminations per bull. A 55 kDa protein was found in higher concentrations in the seminal plasma of high fertility bulls when compared with bulls of average or below average fertility. This 55 kDa protein was later identified as osteopontin. Any polymorphisms within the osteopontin gene could positively or negatively influence expression of this protein in seminal plasma and thus, influence fertility. Therefore, the current study was conducted to identify any such polymorphisms and determine their influence on bull fertility.

Through the amplification and sequencing of two separate DNA fragments in the promoter region in the OPN gene, 8 SNP sites were identified. These SNP sites were found at base pairs 3379, 3490, 3492, 4967, 5075, 5205, 5209, and 5263 of the OPN gene. Genotype and allele frequencies are summarized in Table 2. Individual SNP sites were evaluated for their effect on percentage progressive, motile and rapid sperm. These sperm parameters were chosen because sperm motility is the most important sperm trait influencing fertility. None of the individual SNP sites were determined to have an affect ($P \geq 0.1$) on percentage motile, progressive or rapid sperm cells. Therefore, haplotypes were constructed based on the 8 SNP sites to produce 11 unique haplotypes for analysis. Haplotype construction and frequency is summarized in Table 3. Bulls identified with haplotype 1 were the same as the reported normal reference sequence.

Sperm motility for bulls with haplotypes 8, 9 and 4 (79.2%, 78.2% and 72.8% respectively) were significantly higher ($P \leq 0.05$) than that of bulls with haplotypes 6, 7, 5 and 1 (57.3%, 55.1%, 51.7% and 45.4% respectively; Fig. 1). Bulls with haplotypes 8 and 9 were higher ($P \leq 0.05$) than those with haplotypes 7, 5 and 1 for progressive (60.8% and 56.9% respectively vs. 38.8, 38.1and 34.1 % respectively; Fig. 2) and for rapid sperm (76.3% and 77.1% respectively vs. 51.9%, 46.3% and 40.5% respectively; Fig. 3). These data suggest that haplotypes, based on polymorphisms found within the osteopontin gene might be useful as a genetic marker for selecting bulls with higher potential fertility or elimination of bulls with lower fertility. The haplotypes of bulls with superior sperm motility occurred at relatively low frequency (~5%). The low frequency of these haplotypes could limit their use for selection.

Implications

Reproduction is the most important trait affecting profitability of beef operations. Thus, development of genetic markers for identifying bulls with superior potential fertility would be of great economic benefit. The current study indicates that analysis of polymorphisms with the osteopontin gene could be useful as a potential genetic marker for bull fertility.

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Table 1. Description of sperm variables measured by the Hamilton-Thorne Sperm Analyzer.

Variable	Description
Motile	Path velocity ≥ 30 μ/sec and progressive velocity ≥ 15 μm/sec
Progressive	Path velocity ≥ 50 μ/sec and straightness ≥ 70%
Rapid	Progressive % with path velocity > 50 μm/sec

Table 2. Genotype and allele frequencies of single nucleotide polymorphisms found within the promoter region of the OPN gene of Angus and Balancer bulls.

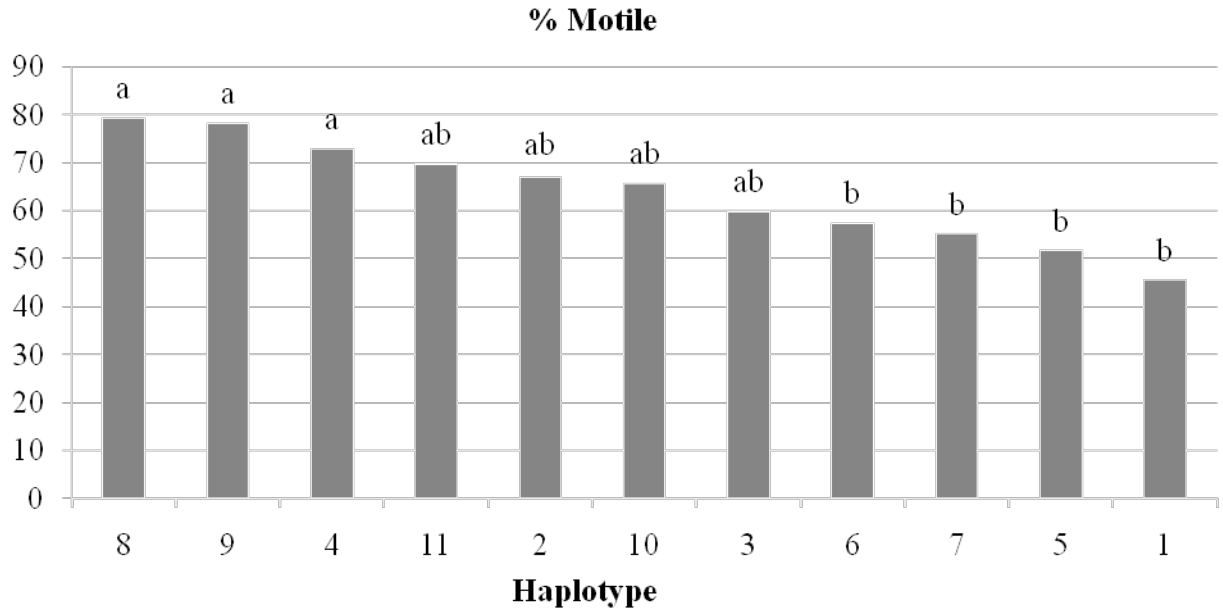
Polymorphism[a]	Homozygous primary allele	Heterozygous	Homozygous minor allele	PAF[b]	MAF[c]
T3379G	0.368	0.421	0.211	0.579	0.421
G3490A	0.526	0.368	0.105	0.711	0.289
A3492G	0.368	0.421	0.211	0.579	0.421
C4967T	0.947	0.053	---------	0.974	0.026
C5075T	0.526	0.368	0.105	0.711	0.289
C5205T	0.789	0.158	0.053	0.868	0.132
G5209A	0.474	0.421	0.053	0.711	0.263
G5209C	0.474	0.053	---------	0.711	0.026
G5263A	0.842	0.158	---------	0.921	0.079

[a] Single nucleotide polymorphism occurred at the base pair number represented, first letter represents primary allele found in the reported normal reference sequence and the letter following the digits represents the resulting minor allele.
[b] Primary Allele Frequency.
[c] Minor Allele Frequency.

Table 3. Haplotype construction and frequency for Angus and Balancers bulls based on the individual SNP sites.

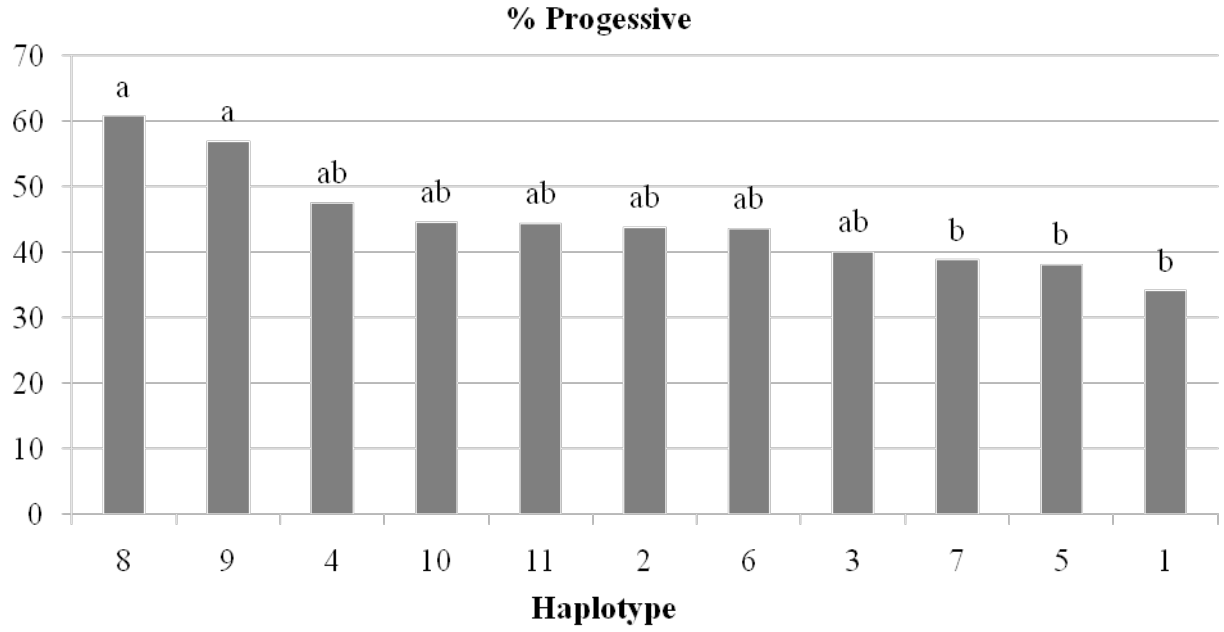
	Base pair of OPN gene where polymorphism occurred								
Haplotype	3379	3490	3492	4967	5075	5205	5209	5263	Haplotype Frequency
1[a]	TT	GG	AA	CC	CC	CC	GG	GG	0.105
2	TT	GG	AA	CC	CC	CC	GA	GG	0.105
3	TT	GG	AA	CC	CC	CT	GA	GG	0.053
4	TT	GG	AA	CC	CC	TT	AA	GA	0.053
5	TT	GG	GG	CC	CC	CT	GA	GA	0.053
6	TG	GA	AG	CC	CT	CC	GA	GG	0.105
7	TG	GA	AG	CC	CT	CC	GG	GG	0.316
8	GG	GA	GG	CT	CT	CC	GG	GG	0.053
9	TG	GG	AA	CC	CC	CC	GA	GG	0.053
10	GG	GG	AG	CC	CC	CC	GC	GG	0.053
11	TG	GG	AG	CC	CC	CT	GA	GA	0.053

[a] Bulls with haplotype 1 were the same as the reported normal reference sequence.



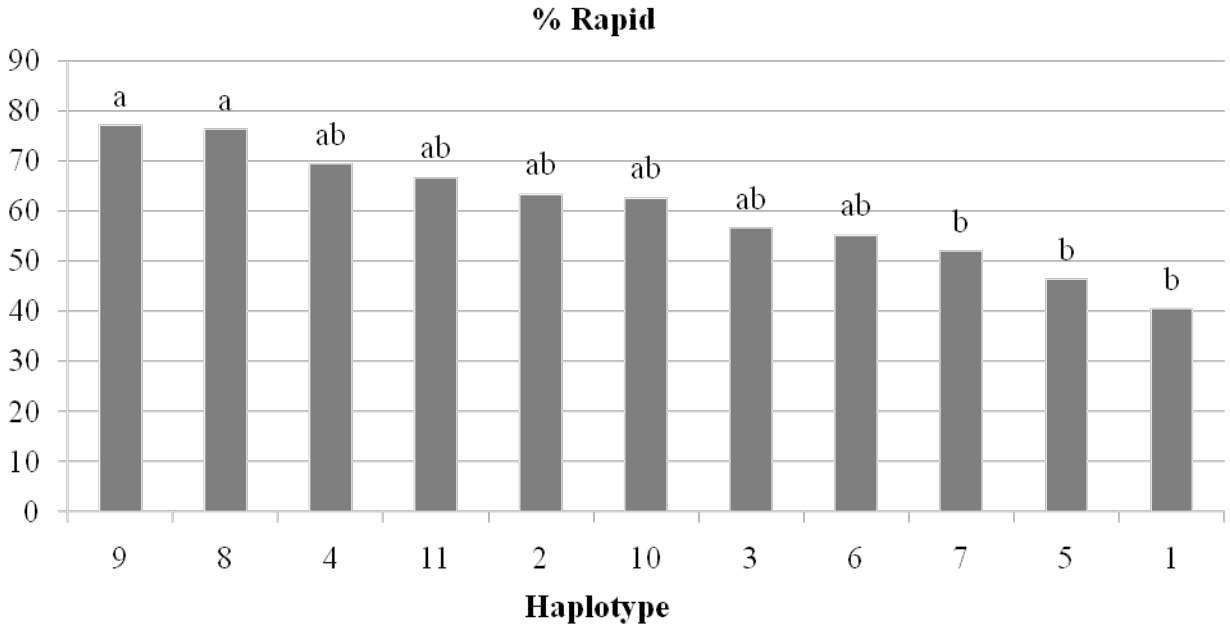
[a,b] Means shown above with different superscripts are significantly different ($P < 0.05$).

Fig. 1. Comparison of bull haplotypes identified and their effect on percentage of motile sperm as measured by CASA.



[a, b] Means shown above with different superscripts are significantly different ($P < 0.05$)

Fig. 2. Comparison of bull haplotypes identified and their effect on percentage of progressive sperm as measured by CASA.



[a, b] Means shown above with different superscripts are significantly different ($P < 0.05$)

Fig. 3. Comparison of bull haplotypes identified and their effect on percentage of rapid sperm as measured by CASA.

Relationships of barometric pressure and environmental temperature with incidence of parturition in beef cows

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Story in Brief

The relationship between barometric pressure and maximum and minimum temperature with the incidence of parturition in beef cows was examined. Spring and fall calving records, over a 5 yr period from the Livestock and Forestry Research Station (Batesville, Ark.) and Savoy Research Unit (Savoy, Ark.) were analyzed. Cows were multiparous, predominately Angus, and naturally bred. During this period, 2,210 calves were born over a cumulative 1,547 d. Barometric pressure and maximum and minimum temperature was obtained from the Southern Regional Climate Center. The combined calving record and climate variables were used to determine differences in barometric pressure and maximum and minimum temperature on d 0 (d of calving) and -1, -2, or -3 (d prior to calving, respectively) calving occurred or did not occur. For fall calving cows, barometric pressure on d 0 and -1, -2 or -3 was not different between days calves were born and days calves were not born ($P > 0.10$). No differences were detected for maximum temperature on d 0 or -2 ($P > 0.10$), however, the maximum temperature was higher on d -1 and -3 for on days calves were born compared to days calves were not born ($P < 0.05$). The minimum temperature was greater on d -1, -2 and -3 on days calves were born compared to days calves were not born ($P < 0.05$). In spring, barometric pressure on d 0, -1, -2 and -3 was higher ($P < 0.05$) on days calves were born compared to days calves were not born. Maximum temperatures on d 0, -1, and -3 were lower on days calves were born compared to days when calves were not born ($P < 0.05$) and minimum temperature for d 0, -1, -2 and -3 was lower ($P < 0.05$) on days calves were born versus days calves were not born. This data indicated for spring, a higher barometric pressure and decrease in environmental temperature was associated with parturition; whereas, for fall an increase in environmental temperature was associated with parturition.

Introduction

A number of studies reported the effect of weather patterns on birth weights and other reproductive traits. Colburn et al. (1997) reported an increase in mean winter temperature over a 3-yr period decreased calf birth weight (10 lb) and calving difficulty 23%. In a 6-yr study using 2 yr old cows, Deutsher et al. (1999) demonstrated the coldest winter was followed by heavier calf birth weights (11 lb) and 29% greater calving difficulty compared with the warmest winter.

Barometric pressure, wind speed and daily rainfall were reported to be negatively correlated with the number of dairy cows presented daily for insemination (Harris, 1985). Gestation period was 4.9 d less in a group of cows that went into labor on, or after, the sixth day of a constant weather situation (Dickie et al., 1994). In addition, Dvorak (1978) reported atmospheric pressure patterns declined 3 and 1 d prepartum, after a rise in pressure for Angus, Hereford and Shorthorn females. Therefore, the objective of this study was to determine the relationship between barometric pressure and maximum and minimum environmental temperatures on the incidence of parturition in beef cows.

Material and Methods

All beef cows were managed in compliance with general husbandry practices and Beef Quality Assurance guidelines. Calving records from the Livestock and Forestry Research Station (Batesville, Ark.) and the Department of Animal Science Savoy Research Unit (Savoy, Ark.) were used. The dates and length of fall (FALL) and spring (SPR) calving seasons and the number of calves born within location and year are summarized (Table 1). The dataset contained 2,210 births over 1,547 d among the calving seasons. All cows were multiparous, predominately Angus, and naturally bred.

Barometric pressure (BARO), maximum (MAX_T), and minimum (MIN_T) environmental temperatures from the Mountain Home and Fayetteville, Ark. weather stations were obtained from the Southern Regional Climate Center, Louisiana State University, Baton Rouge, La. The BARO represents an average daily station pressure. The average daily station pressure was based on 8, 3-h observations/d. Units were expressed in millibars (mbar). Maximum and minimum environmental temperatures were determined with maximum and minimum thermometers or recording instruments and are reported in °F.

Barometric pressure on the day of and 1, 2, and 3 d before calving is designated as BARO 0, -1, -2 and -3, respectively. The maximum environmental temperature on the day of and 1, 2, and 3 d before calving is designated as MAX_T 0, -1, -2 and -3, respectively, and the minimum environmental temperature on the day of calving and 1, 2, and 3 d before calving is designated as MIN_T 0, -1, -2 and -3, respectively.

Since there was not an equal number of cows calving among locations and calving seasons, the proportion of calves born within a location, season, and year was examined to establish an analytical approach. Figure 1. illustrates the relationship between the proportion of observed calving events by the percentage of days representing those events. Among the observed 1,547 d, 46% of those days reported no births. Birthing rates that equaled 1, 2, and 3 or more percent of the calf crop was reported on 20, 16, and 18% of d, respectively. Due to the number of days represented by no births compared to all other birth rates and the diminishing number of days whereby a greater proportion of births occurred, a yes-no response variable was created to represent whether or not calving occurred. Days in which calves were born, regardless of the proportion of the total calf crop born on that date, are represented by CALF and days in which no calves were born are represented as NOCALF.

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Variables of interest were BARO, MAX_T, and MIN_T on d 0, -1,-2, and -3; therefore, these variables were modeled as dependent variables and the full model included the yes-no observed calving response variable, location, season, and 2- and 3-way interactions as fixed effects. Year was modeled as a random effect, and models were reduced by removing non-significant interactions ($P > 0.10$) based on Type III sum of squares. Least-square means separation of dependent variables was conducted for significant interaction terms and pairwise error rates were controlled using TukeyHSD. All data was modeled using the MIXED procedure (SAS Inst., Cary, N.C.).

Results and Discussion

Season by location and season by observed calving date response variable (CALF or NOCALF) were the only significant interactions for BARO, MAX_T, and MIN_T on d 0, -1, -2, and -3, respectively. All climate variables differed by season within location ($P \leq 0.06$). Both the Savoy Research Unit and the Livestock and Forestry Research Station are located in northern Arkansas; however, their longitudinal difference allows for seasonally diverse weather patterns. All climate variables, with the exception of BARO d -3, differed ($P < 0.05$) by calving season for the observed calving date response variable (CALF or NOCALF). The results and discussion of this paper emphasizes the climatic difference between CALF and NOCALF within FALL and SPR calving seasons.

Atmospheric pressure is the force per unit area exerted against a surface by the weight of air above that surface in the earth's atmosphere. Low pressure areas have less atmospheric mass above their location, whereas high pressure areas have more atmospheric mass above their locations. Similarly, as elevation increases there is less overlying atmospheric mass, so that pressure decreases with increasing elevation.

The relationship of BARO on d 0, -1, -2, and -3 for CALF and NOCALF in FALL and SPR is summarized in Table 2. For FALL, BARO on d 0, -1,-2 and -3 was not different between CALF and NOCALF ($P > 0.10$). For SPR, BARO was higher ($P < 0.05$) for CALF compared to NOCALF on d 0 (1017.3 vs. 1015.6), -1 (1017.4 vs. 1015.6), -2 (1017.6 vs. 1015.6) and -3 (1017.4 vs. 1016.0), indicating that in SPR (Jan thru Apr) beef cows calved during times of rising BARO.

These data agree with Dvorak (1978) where it was reported that parturition for Angus, Hereford and Shorthorn cows occurred during a period of rising BARO during the spring (Mar and Apr). Dickie et al. (1994) reported a reduction in the gestation period by almost 5 d, which occurred during long-lasting weather conditions. It seems that cattle make use of a continuous weather situation (increased period of rising BARO) for calving. The fetus triggers the onset of parturition by initiating a number of complex endocrine and biochemical events originating in the hypothalamo-pituitary axis. The hormonal changes associated with parturition are involved with final maturation of the fetus, termination of pregnancy, expansion of the birth canal, initiation of uterine contractions, maternal behavior, synthesis of milk and the ability to eject milk (Bazer and First, 1983). The changes of BARO may be triggering the onset of parturition by stimulating the secretion of glucocorticoids.

Stevenson (1989) reported low BARO appeared 7 to 4 d before calving in Holstein cows and heifers that calves in the fall (Sep thru Dec), with parturition occurring during a period of increasing pressure. It was therefore, hypothesized, the increase in BARO may influence adrenal secretion of glucocorticoids. An exposure to decreasing BARO from 7 to 4 d before calving might be capable of eliciting adrenal secretion of glucocorticoids. The hour of calving was unrelated to barometric pressure and maximum and minimum environmental temperatures during the week preceding parturition. In the current study, a period of increasing BARO (1, 2, and 3 d prior to calving) did not appear to influence parturition in FALL.

The relationship of MAX_T on d 0, -1, -2, and -3 d for CALF and NOCALF in FALL and SPR is summarized in Table 3. The MAX_T was higher on d -1 (75.9° vs. 73.2°) and -3 (76.6° vs. 74.1°) for FALL-CALF compared to NOCALF ($P < 0.05$). There were no differences in MAX_T on d 0 or -2 for CALF compared to NOCALF ($P > 0.10$) in FALL. In SPR, lower MAX_T was associated with parturition. Maximum environmental temperature on d 0 (58.5° vs. 60.8°), -1 (57.9° vs. 60.8°), and -3 (57.2° vs. 60.3°), was lower on CALF compared to NOCALF ($P < 0.05$). No difference was detected on d -2 ($P > 0.10$).

The relationship of MIN_T on d 0, -1, -2, and -3 between CALF and NOCALF in FALL and SPR followed the same pattern detected in MAX_T (Table 4). For FALL, the MIN_T was greater on d -1(55.0° vs. 52.3°), -2 (55.4° vs. 52.5°) and -3 (55.6° vs. 53.1°) for CALF compared to NOCALF ($P < 0.05$). No differences were detected on d 0 ($P > 0.10$). In SPR, CALF temperature for d 0 (36.7° vs. 39.0°), -1(36.5° vs. 38.7°), -2 (35.8° vs. 38.7°) and -3 (35.2° vs. 38.8°) was lower ($P < 0.05$) than NOCALF. This data indicates in SPR, a decrease in environmental temperature may provide a trigger to initiate parturition; whereas, for FALL, an increase in environmental temperature that may provide a trigger to initiate parturition.

Stevenson (1989) reported a small negative correlation of daily temperature with days to calving in Holsteins calving in Sept. to Dec. The net effect of the climatic variables studied indicated that only decreased BARO, increased rainfall, increased humidity, and day length during the last 7 d of gestation were significantly related to the onset of parturition. These effects are not unique events that always precede calving. There are many unexplained factors that account for the onset of calving.

From the fall through early spring (Oct through Apr), a departing storm system will often be followed by a trend toward more tranquil weather and increasing BARO. This may include clearing and colder conditions. In the warmer months and especially summer, changes are usually less noticeable and not as frequent, and pressure tendencies are not as dramatic (John Lewis, National Weather Service, Little Rock, Ark., pers. comm.).

Management factors such as the time of feeding also impact calving patterns. Evening feeding was reported to result in more daylight births in spring calving Hereford and Charolais beef cows (Jaeger et al., 2008) and in dairy cows and heifers (Stevenson, 1989). Jaeger et al. (2008) suggested that providing feed to cattle in the late afternoon before daily temperatures significantly decline might result in an increase in the metabolic heat load. This would possibly offset the daily nighttime decrease in environmental temperature and the precalving maternal body temperature decline, shifting more births to daytime hours. The time of feeding was not documented in the current study.

Implications

Parturition is one of the most important events in the reproductive cycle of a beef cow. Barometric pressure and maximum and minimum environmental temperatures are few of the many factors associated with the initiation of calving in beef cows. Departing storm fronts will often be followed by a trend toward more tranquil weather and increasing barometric pressure during the early spring which may include clearing and colder conditions. This type of weather pattern seems to influence the incidence of beef cows calving in the spring

whereas warmer temperatures may influence calving in the fall. Noting these weather patterns could assist producers in improving timely obstetrical assistance and thus saving more calves at birth.

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Table 1. The dates and length of calving seasons and the number of calves born by season, year and location.

Location[a]	Season	Year	Date of first calf born	Date of last calf born	Calving season duration (d)	Number of calves born
Batesville	Spring	2005	January 27	April 20	84	130
		2006	February 3	April 19	76	129
		2007	February 2	April 19	77	137
		2008	February 6	April 23	78	66
		2009	February 9	April 24	75	180
Savoy	Spring	2005	February 1	April 20	79	52
		2006	January 25	April 28	94	88
		2007	February 9	April 20	71	85
		2008	January 22	April 19	89	82
		2009	February 14	April 260	72	91
Batesville	Fall	2005	August 8	October 20	80	113
		2006	August 28	November 14	79	129
		2007	August 21	November 1	73	121
		2008	August 10	October 26	78	134
		2009	August 10	November 2	85	144
Savoy	Fall	2005	September 7	November 11	66	91
		2006	September 11	November 14	65	92
		2007	September 8	November 19	73	102
		2008	September 12	November 24	74	97
		2009	September 6	November 23	79	147

[a] Livestock and Forestry Research Station - Batesville, Ark., and Department of Animal Science Savoy Research Unit - Savoy, Ark.

Table 2. The relationship of barometric pressure (BARO) on the day of calving and 1, 2, and 3 days prior to calving for a calving date (CALF) and a non-calving date (NOCALF) in fall (FALL) and spring (SPR) calving beef cows.

	FALL		SPR		
BARO[a]	CALF	NOCALF	CALF	NOCALF	SEM
0	1016.8[b]	1017.1[b]	1017.3[b]	1015.6[c]	0.43
-1	1017.0[b]	1016.7[b,c]	1017.4[b]	1015.6[c]	0.41
-2	1017.0[b]	1016.7[b,c]	1017.6[b]	1015.6[c]	0.41
-3	1016.9[c]	1016.7[c]	1017.4[b]	1016.0[c]	0.40

[a] Barometric pressure on the day of calving (0) and -1, -2 and -3, prior to calving. Units expressed in millibars (mbar).
[b,c] Within a row, means that do not have a common superscript differ, $P < 0.05$.

Table 3. The relationship of maximum environmental temperature (MAX_T) on the day of calving and 1, 2, and 3 days prior to calving for a calving date (CALF) and a non-calving date (NOCALF) in fall (FALL) and spring (SPR) calving beef cows.

	FALL		SPR		
MAX_T[a]	CALF	NOCALF	CALF	NOCALF	SEM
0	75.2[b]	73.6[b]	58.5[d]	60.8[c]	1.6
-1	75.9[b]	73.2[c]	57.9[e]	60.8[d]	1.6
-2	75.9[b]	74.1[b]	57.9[c]	59.9[c]	1.6
-3	76.6[b]	74.1[c]	57.2[e]	60.3[d]	1.6

[a] Maximum environmental temperature on the day of (0) and -1, -2 and -3, prior to calving or not calving. Units expressed in degree Fahrenheit.
[b-e] Within a row, means that do not have a common superscript differ, $P < 0.05$.

Table 4. The relationship of minimum environmental temperature (MIN_T) on the day of calving and 1, 2, and 3 days prior to calving for a calving date (CALF) and a non-calving date (NOCALF) in fall (FALL) and spring (SPR) calving beef cows.

	FALL		SPR		
MIN_T[a]	CALF	NOCALF	CALF	NOCALF	SEM
0	54.5[b]	52.3[b]	36.7[d]	39.0[c]	1.1
-1	55.0[b]	52.3[c]	36.5[e]	38.7[d]	1.1
-2	55.4[b]	52.5[c]	35.8[e]	38.7[d]	1.1
-3	55.6[b]	53.1[c]	35.2[e]	38.8[d]	0.9

[a] Minimum environmental temperature on the day of (0) and -1, -2 and -3, prior to calving or not calving. Units expressed in degree Fahrenheit.
[b-e] Within a row, means that do not have a common superscript differ, $P < 0.05$.

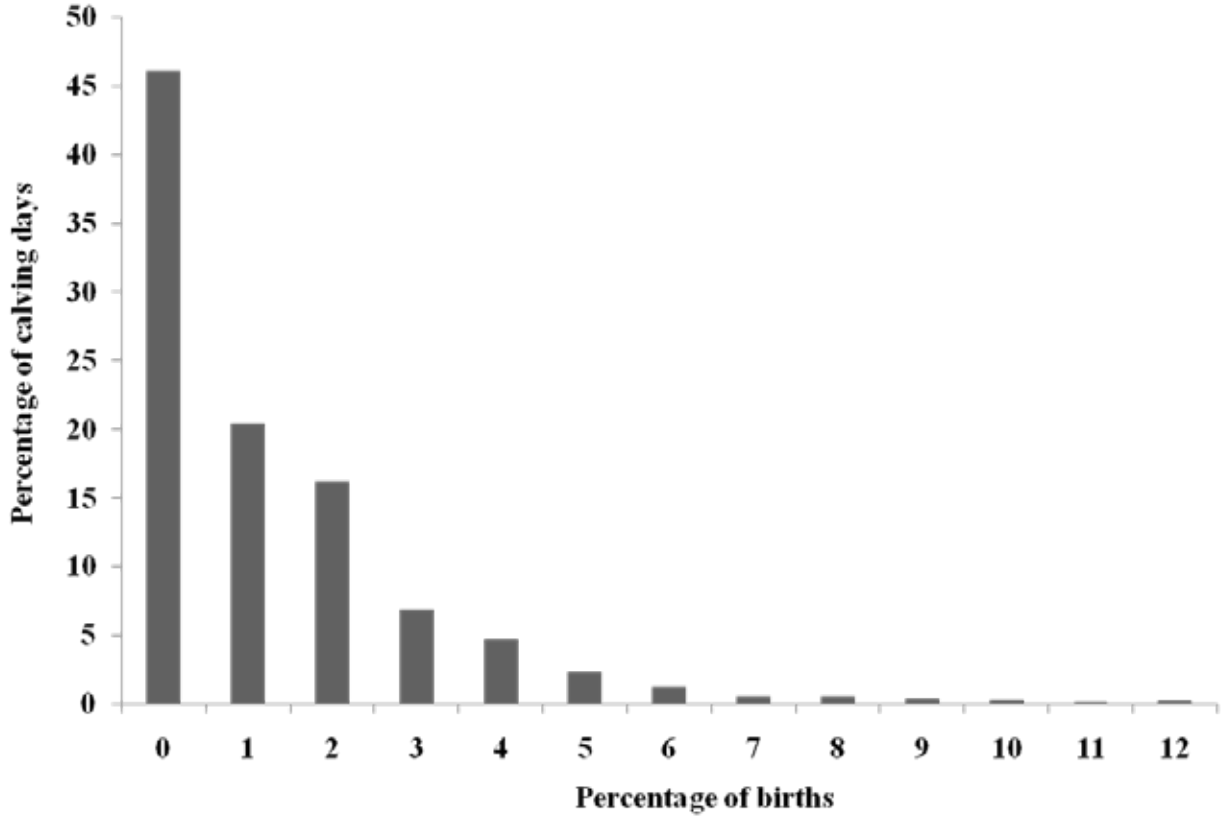

Fig. 1. A frequency distribution illustrating the proportion of births within a location, season, and year by the percentage of calving days the observed birth rates occurred.

Determining effects of two deworming protocols on fecal egg counts and growth performance in beef calves

S.A. O'Berg[1], J.G. Powell[1], K.P. Coffey[1], C.A. Tucker[1], M.P. Rowe[1], and J.L. Reynolds[1]

Story in Brief

This study was undertaken to assess the impact of various regimes of anthelmintic treatment on fecal egg counts and performance of beef calves. Eighty-seven beef calves (body weight = 303.9 lb ± 6.4) carrying natural nematode infections were utilized in a single experiment. Calves were randomly allocated to treatment groups which carried through pre-weaning and post-weaning phases. The three treatment groups included: Treatment 1; calves injected with 1% moxidectin at 85 d prior to weaning (d 0), at weaning (d 85), and mid-point through the stocker phase (d 169), Treatment 2; calves injected at d 85, Treatment 3; and a control group (only treated immediately prior to shipment to feedlot). Statistical analysis was performed using PROC GLM in SAS. Average body and fecal egg count were not statistically different ($P \geq 0.23$) between groups on d 0. Average fecal egg counts were lower ($P < 0.05$) for treated calves on d 14 and tended ($P = 0.07$) to be lower on d 85 compared to untreated calves. Higher ($P < 0.05$) ADG was exhibited by calves treated prior to weaning from d 0 to 85 compared to untreated calves. During the post-weaning phase, fecal egg counts were lower ($P < 0.05$) for Treatment 1 and Treatment 2 compared to Treatment 3 on d 99 and 169. Average fecal egg counts were lower ($P < 0.001$) for Treatment 1 compared to Treatment 2 and Treatment 3 on d 183. Post-weaning ADG was higher ($P < 0.05$) from d 85 to d 197 for Treatment 1 and Treatment 2 compared to Treatment 3. Calves in Treatment 2 made up gain deficits from the pre-weaning phase, and had total ADG similar to those of calves in Treatment 1. Overall, fecal egg counts were reduced with treatment during both the pre-weaning and post-weaning phases. Treatment also improved ADG in beef calves, indicating that routine use of effective anthelmintics would be beneficial to cow-calf operators.

Introduction

Gastrointestinal nematodes have a major negative impact on beef cattle performance and their direct relation to economic loss is well known (Perry and Randolph, 1999). A recent study has placed a value on these losses at $165/head (Ibarbura and Lawrence, 2007). However, according to the USDA NAHMS data (2010), nearly 40% of cow-calf operators do not treat calves with anthelmintics prior to weaning. The objective of the current study was to determine the effects of anthelmintic treatment in the pre- and post-weaning phases of beef cattle production, with regards to growth performance and fecal egg count (FEC) reduction.

Materials and Methods

This study was conducted over 245 d, from March 2010 through November 2010. Cattle were managed at the University of Arkansas Beef Cattle Unit in Savoy, Ark. Study animals were cared for in compliance with the Animal Care Protocol #10030 for cattle experimentation issued by the University of Arkansas Animal Care and Use Committee. Eighty seven beef calves (BW = 303.9 lb ± 6.4) carrying natural nematode infections were utilized in a single experiment. Calves were born between September and November 2009. The animals were managed as cow-calf units on the same pasture prior to weaning, and then separated into three treatment groups on different pastures during the stocker phase. Housing included *ad libitum* pasture, mineral and water throughout the entirety of the trial. A corn gluten ration was fed daily at the rate of 0.5% animal BW, and animals were observed daily for general health and well-being.

The allocation of animals into specific treatment groups was determined through blocking the calves by d -7 FEC, cow age, parity and gender. Within these blocks, animals were then ranked by d -7 BW. Each block contained 29 animals that were bracketed into replicates containing 3 animals. Each of the 3 animals within replicate were randomly assigned to one of three treatments. The three treatment groups were: TRT 1; calves on the aggressive treatment protocol, injected with 1% moxidectin (Cydectin®, Boehringer Ingelheim Vetmedica, Inc, St. Joseph, Mo.) at 85 d prior to weaning (d 0), at weaning (d 85), and mid-point through the stocker phase (d 169). TRT 2; calves injected at d 85 and TRT 3, an untreated negative control group. Data was collected at eight different points throughout the study. Calf body weight (BW) was recorded and fecal samples taken on d -7, 0, 14, 85, 99, 169, 183 and 245. Fecal egg counts (FEC) were determined for each fecal sample using one gram of feces and centrifugation with magnesium sulfate solution. Statistical analysis was performed using PROC GLM in SAS (SAS Institute, Inc. Cary, N.C.).

Results and Discussion

Results indicated similar ($P \geq 0.23$) d 0 average BW and FEC for the treatment groups. Higher ($P < 0.05$) average daily gain (ADG) was exhibited by calves that were treated during the pre-weaning phase from d 0 to 85 when compared to the untreated calves during the same period (1.82 vs. 1.70 lbs/day; Table 1). Post-weaning ADG was higher ($P < 0.05$) from d 85 to 245 for TRT1 and TRT2 compared to TRT3 (Table 2). After d 85 (weaning), calves in TRT2 appeared to make up gain deficits that occurred during the pre-weaning phase, and had total (d 0-d 85) ADG similar to those of calves in TRT1 (Table 3) with both treated groups exhibiting higher gains than untreated calves. Average cost of treatment for calves d 0 was less than $0.90/head. Treated calves were approximately 10 pounds heavier at weaning and current sale prices indicated that treated calves would have been worth approximately $13.00 more per head compared to untreated calves at weaning due to their body weight advantage. This study showed that parasite control improves ADG

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in beef calves during both pre-weaning and post-weaning phases of production, indicating that routine use of effective anthelmintics would be beneficial to cattle producers.

Average FEC were lower ($P < 0.05$) for treated calves on d 14 and tended ($P = 0.07$) to be lower on d 85 compared to untreated calves (Table 4). During the post-weaning phase, FEC were lower ($P < 0.05$) for TRT1 and TRT2 compared to TRT3 on d 99 and 169. Average FEC were lower ($P < 0.001$) for TRT1 compared to TRT2 and TRT3 on d 183. Both treated groups (TRT1 and TRT2) continued to display low FEC through d 169, however, TRT1 received another treatment on d 169 and only they continued to show lower FEC throughout the rest of the study compared to TRT2 and controls. This later (at d 169) treatment did not, however, improve the TRT1 gains compared to TRT2 during the last few weeks of the study. Overall, FEC were reduced with treatment during both the pre-weaning and post-weaning phases, although the efficacy of treatment was not ideal. *Cooperia* larvae have been found in post-treatment copracultures (Yazwinski et. al, 2009), which could account for the average efficacy of moxidectin in this study.

Implications

The results of this study indicate that treatment with 1% moxidectin showed efficacy in reducing fecal egg counts and improving average daily gain in beef calves during both pre-weaning and post-weaning phases. This information is useful in illustrating the significant economic and performance benefits of nematode control in calves.

Acknowledgements

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Table 1. Gain summary during pre-weaning phase (d 0-85). (lb, LSM).

Group	Gain, lb	ADG, lb/d
Calves treated on d0 (TRT1)	154.7[a]	1.82[a]
Calves untreated on d0 (TRT2 & TRT3)	144.5[b]	1.70[b]

Within column, means without a common superscript differ ($P < 0.05$).

Table 2. Gain summary during post-weaning phase (d 85-245). (lb, LSM).

Treatment Group	Gain, lb	ADG, lb/d
TRT1	175.8[a]	1.12[a]
TRT2	179.8[a]	1.14[a]
TRT3	141.9[b]	0.90[b]

Within column, means without a common superscript differ ($P < 0.05$).

Table 3. Gain summary for entire study (d 0-245). (lb, LSM).

Treatment Group	Gain, lb	ADG, lb/d
TRT1	330.8[a]	1.36[a]
TRT2	332.7[a]	1.36[a]
TRT3	290.6[b]	1.19[b]

Within column, means without a common superscript differ ($P < 0.05$).

Table 4. Strongyle fecal egg count for entire study (d 0-245). (GM)

	Study Day						
Treatment Group	**0 TRT1**	**14**	**85 TRT 1 & 2**	**99**	**169 TRT1**	**183**	**245**
TRT1	81.5[a]	9.2[a]	28.6[a]	11.2[a]	101.8[a]	14.8[a]	23.6[a]
TRT2	77.1[a]	103.0[b]	36.2[ab]	9.3[a]	83.6[a]	95.1[b]	69.2[b]
TRT3	75.1[a]	97.2[b]	61.1[b]	106.7[b]	166.9[b]	137.3[b]	45.6[b]

Within column, means without a common superscript differ ($P < 0.05$).

Effects of trace mineral injection 28 days before weaning on calf health, performance and carcass characteristics

E. B. Kegley [1], K. P. Coffey [1], and J. T. Richeson [1]

Story in Brief

The effects of injecting a trace mineral product 28 d before weaning on growth performance, health, and carcass characteristics of beef calves were investigated. Crossbred steers (n = 34) and heifers (n = 39) were allocated to receive no injectable trace mineral or an injection of a trace mineral solution containing 60 mg zinc, 15 mg copper, 10 mg manganese, and 5 mg selenium/mL at a rate of 1 mL/100 lb of body weight. Calves were returned to their dams after treatment. Throughout the study, calves had free-choice access to a dietary mineral supplement containing trace minerals. After 28 d calves were removed from cows, placed in pastures that were adjacent to their dams' pastures, offered corn gluten feed (2 lb/d), and had free choice access to hay. After a 43 d weaning period, cattle were moved to wheat pasture for 155 d. After grazing wheat, a portion of the heifers (n = 24) were removed, then steers and the remainder of the heifers were shipped to a feedlot and finished on a concentrate diet. Injection of trace mineral solution 28 d before weaning did not affect body weight ($P \geq 0.73$), average daily gain ($P \geq 0.47$), or hair coat scores ($P = 0.44$) at any time point during the study. With the exception of yield grade, there were no differences in carcass characteristics ($P \geq 0.23$) due to the injection of trace minerals. Calves that received an injection of trace mineral tended to have a lower ($P = 0.11$) yield grade than negative controls. There was no benefit of using an injectable trace mineral 28 d before weaning in calves that were continuously supplemented with dietary trace minerals and were managed to minimize stress throughout their lifetime.

Introduction

Research indicates that trace minerals including zinc, copper, manganese, and cobalt are necessary for optimal growth performance, and animal health. An injectable trace mineral product administered during critical production events such as weaning may be an effective addition to traditional loose dietary mineral supplementation.

Materials and Methods

Seventy-three Gelbvieh × Angus crossbred steer (n = 34) and heifer (n = 39) calves reared at the University of Arkansas Division of Agriculture Livestock and Forestry Branch Station (LFBS) in Batesville, Ark. were used for this study. Calves were born following a 63 d breeding season, and birthdates ranged from February 12 to April 20, 2009. Calves and their dams were grazed on pastures containing a mixture of bermudagrass and fescue in 3 separate pasture units. From birth to 42-d post-weaning, cattle were offered a free-choice oral mineral supplement (Table 1). On September 14, 2009, cows and calves were individually weighed (d -7). Calves were blocked by pasture group, stratified by gender (heifer or steer), body weight (BW), and birth date and randomly allotted to treatment group. Groups were also balanced for cow weight and age.

Treatments consisted of: 1) no supplemental trace mineral injection (negative control), or 2) supplemental trace mineral injection 28 d before weaning (MULTIMIN 90). The injectable trace mineral product (MULTIMIN® 90, MULTIMIN USA, Inc., Porterville, Calif.) was administered per BQA procedures at a dosage rate of 1 mL/100 lb of BW. The product was formulated to contain at least 60 mg Zn, 15 mg Cu, 10 mg Mn, and 5 mg Se/mL. MULTIMIN 90 was measured to the nearest 0.2 mL, and given subcutaneously on the left side of the neck, prescapula, using a 16 gauge × 1-in needle to designated calves. A new needle was used to inject each calf.

On 23 September 2009 (d 0), all calves were weighed, assigned a hair coat score, and bled via jugular venipuncture into plain evacuated tubes specifically prepared for harvesting serum for trace mineral analyses. Vaccines against respiratory and reproductive infections (ViraShield 6+ VL5; infectious bovine rhinotracheitis virus, bovine viral diarrhea virus type 1 and 2, parainfluenza-3 virus, bovine respiratory syncytial virus, Campylobacter Fetus, Leptospira Canicola-Grippotyphosa-Hardjo-Icterohaemorrhagiae-Pomona Bacterin, Novartis Animal Health, Larchwood, Iowa), and clostridial pathogens (Covexin 8; clostridium Chauvoei-Septicum-Haemolyticum-Novyi-Tetani-Perfringens types C & D bacterin-toxoid; Intervet Schering-Plough Animal Health, Summit, N.J.) were administered subcutaneously on the right side of the neck. After processing, calves and cows were returned to their original pastures until weaning.

At weaning (October 21, 2009, d 28), calves were weighed, assigned a hair coat score, vaccinated with ViraShield 6+VL5, and dewormed with moxidectin pour-on (Cydectin, Fort Dodge Animal Health, Fort Dodge, Iowa). Calves were removed from the cows and placed in 5.4-acre mixed bermudagrass and fescue pastures adjacent to their dams' pastures. Calves were penned by gender resulting in an even distribution of treatments within each weaning pen. Calves were offered corn gluten feed (2 lb/d), had ad libitum access to hay, and continued to have access to free choice mineral (Table 1). During the weaning period, 1 calf from the MULTIMIN 90 treatment severely injured its right front foot. Although this steer was allowed to remain with his cohorts, data were not collected from this calf subsequent to the weaning period.

After a 28 d weaning period (November 18, 2009), calves were weighed, assigned a hair coat score, and returned to their weaning pens because weather conditions had not been favorable for adequate wheat pasture growth. On December 3, 2009, cattle were weighed then moved to wheat pasture for a 155-d wheat grazing phase. Cattle were grouped such that treatment was again evenly represented within each of 9 wheat pastures. Cattle continued to be offered free choice mineral (Table 1), but were not offered supplemental feed during the wheat grazing phase. Supplemental hay was offered to calves when

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available wheat forage was limited. During the wheat grazing phase, interim weights were collected on January 12 and April 19, 2010.

At the conclusion of the wheat grazing phase (May 7, 2010), calves were weighed, and assigned a hair coat score. A portion of the heifers (n = 23) were returned to the LFBS breeding herd, and steers (n = 34) and the remainder of the heifers (n = 15) were shipped approximately 170 mi to a feedlot (Willard Sparks Beef Research Center, Oklahoma State University, Stillwater) and finished on a concentrate diet. Steers were allotted to 4 pens by treatment (2 pens/treatment); whereas, heifers were fed as a single group. Carcass weight, yield and quality data were obtained upon harvesting at a commercial facility (Caviness Packing, Hereford, Texas).

Hair coat scores were assigned by two trained evaluators on d 0, 28, and 56, and one trained evaluator at the conclusion of the wheat pasture phase. The scoring method used was the 1 to 5 scale (Saker et al., 2001) where 1 = smooth, slick, and shiny hair with no evidence of retention of old hair, 2 = less than 25% of the body covered with old, unshed hair, 3 = 25 to 50% of the body covered with old, unshed hair, 4 = 50 to 75% of the body covered with old, unshed hair, and 5 = 75 to 100% rough hair or > 50% of the body covered with old, unshed hair with evidence of having deliberately lain in mud.

To determine serum Zn and Cu concentrations, blood samples were transported on ice, centrifuged at 2,100 × *g* for 20 min and serum was decanted and stored at -20 °C until laboratory analysis. Briefly, serum was thawed at room temperature then deproteinated by mixing 0.5 mL serum with 5 mL of 1 N trace metal grade HNO_3. After 24 h, tubes were centrifuged at 1,700 × *g* and the supernatant was analyzed by ICP-AES (Ciros, Spectro Analytical Instruments, Inc., Mahwah, N.J. 07430) for Zn and Cu concentrations.

Morbidity and mortality data were recorded beginning at d 0, and throughout the grazing and feedlot phases. Through the weaning and grazing phases, cattle were observed daily and assigned a clinical illness score (Table 2), if the score was >2 cattle were brought to the chute and a rectal temperature was taken. If rectal temperature exceeded 104 °F, cattle were treated according to a preplanned antibiotic regimen. The date and amount of medications used were recorded, and cattle were returned to their home pen immediately after treatment. In the feedlot, steer morbidity and mortality records were kept; antibiotic treatments were per the standard routine for the feedyard.

Data were analyzed using the mixed procedure of SAS (Cary, N.C.). Calf was the experimental unit. The model included treatment as a fixed effect. Kenward Rogers was specified as the degrees of freedom selection method. The random statement included sex and calf was identified as the subject. Hair coat scores were analyzed using the mixed procedure with repeated measures. The SP(POW) covariance structure was specified. When 2 people evaluated hair coat scores, scores were averaged prior to statistical analysis. For carcass quality data the frequency procedure was used with Chi-square analyses specified.

Results and Discussion

While at the LFBS, no calves were treated for bovine respiratory disease. A heifer assigned to the negative control treatment, died on January 18, 2010 during the wheat grazing period. No clinical signs had been observed; however, following an ice storm the calf was found dead. Because of inclement weather, a necropsy was not performed and the cause of death was unknown.

Serum trace mineral analyses revealed that d 0 serum Cu concentrations in calves were marginally deficient (0.58 mg/L) and did not differ between treatment groups ($P = 0.62$; Table 3). The d 0 serum Zn concentrations were within a range generally considered adequate and also did not differ ($P = 0.31$). Injection of MULTIMIN 90 28 d before weaning did not affect BW ($P \geq 0.73$), ADG ($P \geq 0.47$), or hair coat scores ($P = 0.44$). All calves had access to a dietary trace mineral product that had adequate concentrations and availability of numerous trace minerals. Because of the role of trace minerals in immune function, additional supplementation with trace minerals is deemed most critical for weaned calves during periods of high stress, such as prior to shipping or exposure to other cattle at an auction facility. In the present study, cattle were weaned using a low-stress weaning program and were not exposed to other cattle during the entire period prior to shipping to the feedlot facility. This may have minimized the need for additional trace minerals in addition to those provided in the free-choice mineral supplement.

Trace mineral injection had little impact ($P \geq 0.23$) on carcass measurements with the exception of yield grade (Table 4). Calves that received an injection of MULTIMIN 90 tended to have a lower ($P = 0.11$) yield grade than negative controls. Quality grade did not differ ($P = 0.66$) due to treatment.

Implications

When beef calves had continual access to dietary trace mineral supplementation, were weaned and grazed on their ranch of origin, and were shipped and fed as a single group at the feedlot; there was no benefit of using an injectable trace mineral product 28 d before weaning on growth performance or health.

Acknowledgments

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Table 1. Ingredient and trace mineral concentrations in free choice mineral supplements (1 oz/d consumption rate) offered from birth to 42 d post-weaning, or during wheat pasture grazing phases.

Ingredient	Supplemental source	Preweaning, %	Wheat pasture, %
Trace mineral premix[a]		10	10
Magnesium oxide		8.9	8.9
Molasses		2	5
Dicalcium phosphate		0	21
Salt, white		79.1	55.1
Trace mineral		ppm[b]	ppm
Cu	Copper sulfate, copper chloride	4,000	4,000
Co	Cobalt carbonate	50	50
Fe	Ferrous sulfate	1,000	1,000
I	Ethylenendiamine dihdriodide, calcium iodate	200	200
Mn	Manganous oxide, manganese sulfate	8,000	8,000
Se	Sodium selenite	60	60
Zn	Zinc sulfate, zinc oxide	12,000	12,000

[a]Ruminant Trace Mineral, NB-8675, Nutra Blend Corp., Neosho, Mo.
[b]Calculated concentrations provided by the free-choice mineral supplement.

Table 2. Clinical illness scores for calves[a].

Score	Description	Appearance
1	Normal	No abnormal signs noted
2	Slightly ill	Mild depression, gaunt, +/- ocular/nasal discharge
3	Moderately ill	Ocular/nasal discharge, gaunt, lags behind other animals in the group, coughing, labored breathing, moderate depression, +/- rough hair coat, weight loss
4	Severely ill	Severe depression, labored breathing, purulent ocular/nasal discharge, not responsive to human approach
5	Moribund	Near death

[a]Modified from clinical assessment score criteria provided by Dianne Hellwig.

Table 3. Body weight, average daily gain, hair coat scores, and baseline serum copper and zinc concentrations for negative control and MULTIMIN 90 treatments.

Variable	Date	Control	MULTIMIN 90	SE	*P*-value
Baseline (d 0) serum					
Cu, mg/L		0.58	0.57	0.02	0.62
Zn, mg/L		1.17	1.23	0.04	0.31
BW, lb					
Initial	Sept 23, 2009	384	388	12	0.82
At weaning	Oct 21, 2009	401	408	13	0.74
28 d after weaning	Nov 18, 2009	443	450	14	0.76
42 d after weaning	Dec 3, 2009	459	465	14	0.73
39 d on wheat	Jan 12, 2010	500	503	15	0.98
136 d on wheat	April 19, 2010	710	712	16	0.89
Final at unit	May 7, 2010	725	728	15	0.93
ADG, lb					
D 0 to weaning		0.66	0.73	0.082	0.49
Weaning to 28 d after weaning		1.50	1.39	0.106	0.47
Weaning to 42 d after weaning		1.32	1.28	0.088	0.67
154 d wheat pasture period		1.72	1.72	0.062	0.92
D 0 to 224 (total at unit)		1.52	1.50	0.042	0.94
Hair coat score[a]					
Initial		2.24	2.38	0.174	
At weaning		1.86	2.03	0.174	
28 d after weaning		2.43	2.47	0.174	
Final date at unit		2.42	2.63	0.176	Note[b]

[a] 1 = smooth, slick, and shiny hair with no evidence of retention of old hair, 2 = less than 25% of the body covered with old, unshed hair, 3 = 25 to 50% of the body covered with old, unshed hair, 4 = 50 to 75% of the body covered with old, unshed hair, and 5 = 75 to 100% rough hair or > 50% of the body covered with old, unshed hair with evidence of having deliberately lain in mud.
[b]Treatment, $P = 0.44$; Day $P < 0.0001$; Treatment x Day, $P = 0.90$.

Table 4. Carcass data for negative control and MULTIMIN 90 treatments.

Carcass Trait	Control	MULTIMIN 90	SE	*P*-value
Hot carcass wt., lb	728	711	18	0.51
Marbling score[a]	42	41	1.1	0.63
Fat thickness (12th rib), in	0.41	0.41	0.028	0.98
Ribeye area, in^2	13.2	13.7	0.31	0.23
Yield grade	2.5	2.3	0.10	0.11
Quality grade				
Choice, %	73	67		0.66
Select, %	27	33		

[a]41 = small10, 42 = small20.

Weaning management of newly received beef calves with or without continuous exposure to a persistently infected bovine viral diarrhea virus pen mate: effects on rectal temperature, peripheral blood leukocytes and serum proinflammatory cytokine and haptoglobin concentrations

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Story in Brief

Exposure to animals persistently infected with bovine viral diarrhea virus (BVDV) results in immunomodulation in cohorts. It is hypothesized that the extent of modulation differs for preconditioned vs. auction market cattle. Our objective was to compare immune responses of preconditioned or auction market calves in presence or absence of a persistently infected-BVDV pen mate using a 2 × 2 factorial arrangement. Crossbred preconditioned steers (n = 27) from a single ranch-origin were weaned, dewormed, vaccinated, tested for BVDV-persistent infection, and kept on the ranch for 61 d. Subsequently, preconditioned steers were transported to a receiving unit, weighed, stratified by d -1 body weight, and assigned randomly to treatment (preconditioned, BVDV-exposed or preconditioned, non-exposed) with no additional processing. Simultaneously, crossbred auction market calves (n = 27) were assembled from regional auction markets and delivered to the receiving unit 24 h before preconditioned arrival. The auction market calves were weighed, stratified by gender and d -1 body weight, processed under the same regimen used for preconditioned steers at their origin ranch except bull calves were castrated, then assigned randomly to treatment (auction market, BVDV-exposed or auction market, non-exposed). Treatment pens were arranged spatially so that BVDV-exposed treatments did not have fence-line contact with non-exposed treatments. In auction market calves, rectal temperature ($P < 0.001$) and haptoglobin ($P < 0.001$) increased sharply on d 1. Exposure to persistently infected-BVDV cohort decreased ($P = 0.01$) the percentage of neutrophils, and increased ($P = 0.02$) percentage lymphocytes resulting in a tendency ($P = 0.07$) for a decreased neutrophil:lymphocyte ratio. Serum concentrations of TNF-α tended to increase ($P = 0.09$) for BVDV-exposed treatments. Interferon-γ concentrations on d 7 and 14, IL-6 concentrations on d 14, and platelets on d 7 were greatest for the auction market, BVDV-exposed treatment ($P \leq 0.05$). Results indicate weaning management and exposure to a persistently infected-BVDV pen mate alter the immune status of newly received calves. These effects may be additive because alterations were greatest for auction market, BVDV-exposed calves.

Introduction

Bovine respiratory disease (BRD) is a multifaceted syndrome involving physiological stress, commingling, and several viral and bacterial pathogens. Physiological alterations in response to individual stressors such as weaning, transportation, castration and commingling have been observed; however, high-risk, newly received stocker cattle are often subjected to these stressors concurrently. Whereas, the negative effects of these stressors are mitigated in cattle preconditioned on their origin ranch before marketing occurs.

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 BVDV transmission and a single PI-BVDV animal has the potential to continuously expose an entire pen and adjoining pens to the virus. We hypothesized that the pro-inflammatory cytokine response would be altered by PI-BVDV exposure; however, the magnitude of physiological alteration would be impacted by previous management. The objective of our study was to determine serum pro-inflammatory cytokine and haptoglobin concentrations, total and differential blood leukocyte concentration, and rectal temperature in commingled auction market vs. single-source preconditioned calves with or without 14-d continuous exposure to PI-BVDV challenge.

Materials and Methods

Cattle. Two different cattle management sources were utilized for this 14-d evaluation period; 1) a low-risk, single-source, preconditioned (PC) group of crossbred steer calves (n = 27; initial BW = 620 ± 3.5 lb) from a single origin ranch located in Izard County, Ark. and 2) a high-risk, commingled, auction market (AM) group of crossbred bull (n = 15) and steer (n = 12) calves (initial BW = 590 ± 5 lb) acquired from an Arkansas auction market. The PC steers arrived at the University of Arkansas Agricultural Experiment Station located near Savoy (receiving unit; RU) on December 6, 2009 and were considered to be low-risk for developing BRD because they had been previously vaccinated against BRD pathogens, were weaned on their origin ranch for 61 d, and were maintained as a single-source without commingling. The AM calves were procured and assembled by an order buyer from a public auction market located in North Central, Ark. and arrived at the RU on December 5, 2009. The order buyer was instructed to purchase AM cattle of similar BW and phenotype as the incoming PC calves. The AM cattle were considered to be high-risk for developing BRD because they had unknown health history and were commingled extensively resulting in greater probability of increased stress and exposure to BRD pathogens.

The main effects of cattle management (AM or PC) and PI-BVDV exposure (not exposed = CON or exposed = PI) were tested in a 2 × 2 factorial arrangement resulting in 4 treatments. For PC

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treatments, 62 d prior to trial initiation, randomly selected steers were weaned and confirmed free of PI-BVDV via ear-notch skin samples tested for the presence of BVDV using the antigen-capture ELISA (ACE) method (Idexx Laboratories, Inc., Westbrook, Maine) at a commercial laboratory (Cattle Stats, LLC, Oklahoma City, Okla.). Also on the day of weaning, PC calves were administered: 1) a 5-way modified-live virus (MLV) respiratory vaccine containing infectious bovine rhinotracheitis, bovine viral diarrhea virus type 1a and 2a, parainfluenza3, and bovine respiratory syncytial virus isolates [Express 5, Boehringer Ingelheim Vetmedica (BIVI), St. Joseph, Mo.], 2) *Manheimia haemolytica-Pastuerella multocida* bacterin-toxoid (Pulmo-guard PHM-1, BIVI), and 3) pour-on anthelmintic (Cydectin, BIVI). Fourteen-d later (d -48), PC calves were administered a 7-way clostridial bacterin-toxoid (Alpha 7, BIVI) and revaccinated with 5-way MLV respiratory vaccine. Preconditioned calves were isolated from other cattle, fed hay or pasture along with a supplement, and remained on their origin ranch during the preconditioning phase until d -1 when they were shipped to the RU.

Upon arrival to the RU, PC calves were maintained in an isolated holding pen with ad libitum access to hay and water until treatment allocation on d 0. On d -1, PC calves were weighed and returned to their isolated holding pen. The following d (d 0), PC calves were weighed, bled, stratified by d -1 BW, then assigned randomly to treatment (PCCON or PCPI).

To coincide with PC, AM calves were assembled from a regional market and delivered to the RU on d -2. Upon arrival to the RU, AM calves were maintained in an isolated holding pen with ad libitum access to hay and water until treatment allocation on d 0. On d -1, AM calves were weighed, identified with a unique ear identification tag, ear notched to test for PI-BVDV status at a commercial laboratory as previously described, and returned to their isolated holding pen. On d 0, AM cattle received the same vaccinations and processing procedures as described for PC on their origin ranch; therefore, the first known processing for AM occurred on d 0 rather than 62 d previously for PC. For AM calves only, revaccination of the 5-way MLV respiratory vaccine (Express 5, BIVI) occurred on d 14 in addition to administration of a 7-way clostridial bacterin-toxoid (Alpha 7, BIVI). Additionally on d 0, bull calves were castrated surgically, stratified by gender and d -1 BW, then AM calves were assigned randomly to treatment (AMCON or AMPI). Cattle were then moved to their randomly assigned pens and provided 2 lb/d (as-fed basis) of a receiving supplement (15.1% CP, DM basis) and *ad libitum* access to bermudagrass hay (13.1% CP, 52.3% NDF, 40.6% ADF, DM basis) and water. Supplement offered was step-wise increased to a maximum of 6 lb/d as each pen completely consumed the supplement offered.

Calves that had been previously ear-notched, tested at a commercial laboratory (Cattle Stats), and identified as PI-BVDV according to ACE method were acquired from a stocker cattle operation in Washington County, Okla. to be utilized as PI-BVDV exposure sources. The PI-BVDV challenge calves were assigned randomly to 6 PI-designated pens.

Receiving pens were arranged spatially prior to treatment allocation to avoid unwanted PI-BVDV fence-line contact with CON. Pens measured 12 × 95 ft, contained 10 ft of feed bunk-line and a fence-line water source shared with an adjacent pen of the same treatment. Treatment pens were configured so that unlike treatment pens were separated by at least 1 unoccupied pen. For PI treatments, a PI-BVDV challenge animal was assigned randomly to each PI-designated treatment pen such that pens had an equivalent number of cattle.

Rectal Temperature Evaluation and Treatment of BRD. Rectal temperature (RT) was recorded concurrent with blood sampling via a digital thermometer (GLA Agricultural Electronics; San Luis Obispo, Calif.; readability = ± 0.1 °F). Calves were considered morbid and treated with an antibiotic based solely on RT. If RT was ≥104 °F, calves were administered antibiotic therapy with enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, Kan.), and immediately returned to their home pen. A 48-h post-treatment interval (PTI) was implemented following administration of enrofloxacin, and a second RT was recorded upon expiration of the initial antibiotic PTI. If the second RT was ≥104 °F, a second antibiotic treatment with florfenicol (Nuflor, Schering-Plough Animal Health, Summit, N.J.) was administered. A 48-h PTI was also implemented for cattle administered florfenicol, and RT was evaluated upon expiration of the second antibiotic PTI. If the temperature was ≥104 °F, a third and final antibiotic treatment with ceftiofur HCl (Excenel RTU, Pfizer Animal Health, New York, N.Y.) was administered and repeated for 2 consecutive days following the initial injection of ceftiofur HCl.

Hemagram, Cytokine and Haptoglobin Analyses. Blood samples were collected on d 0, 1, 3, 5, 7, and 14 to determine interim total and differential leukocytes and serum samples were collected on the same days and stored frozen at -20 °C. Subsequently, 5 serum aliquots from d 0, 1, 3, 7, and 14 were selected randomly from each treatment pen and transported on ice to the USDA, ARS, National Animal Disease Center (NADC) in Ames, Iowa for determination of serum pro-inflammatory cytokines TNF-α, IFN-γ, IL-4, and IL-6. A second aliquot of each sample was used to determine serum haptoglobin concentrations.

Statistical Analyses. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, N.C.). Single degree of freedom orthogonal contrasts evaluating effects of management (PC or AM), exposure (PI or CON), and their interaction were used. If a treatment × day interaction was evident ($P \leq 0.10$), differences of least square means within a day were reported if the resulting t-test was statistically significant ($P \leq 0.05$). For repeated measures data, the model included treatment, day, and treatment × day.

Results and Discussion

Rectal Temperature. Mean RT for AM calves increased sharply (treatment × day interaction; $P < 0.001$) upon trial initiation and remained elevated until reaching baseline on d 14 such that a main effect of management ($P < 0.001$) was evident (Table 1). Febrile RT responses have been consistently reported in experimental disease challenge models. Furthermore, RT ≥104 °F is a standard objective index used to determine clinical BRD morbidity of cattle both in the production setting and in the current study. Peak RT was observed on d 1. The elevated RT in AM corresponded with markedly greater (management effect; $P < 0.001$) morbidity for AM (86%) vs. PC (4%) during the 14-d evaluation period; consequently, antibiotic treatment cost was $23.25 vs. $1.53/animal for AM and PC, respectively.

The greater RT observed for AM may be in response to physiological stress, respiratory pathogens, or both; however differentiation of the febrile response is difficult to determine. Nevertheless, PI-BVDV challenge did not affect ($P = 0.95$) RT in our study; thus, the increased rectal temperature observed for AM but not PC treatments was likely due to greater physiological stress or acute effects of viral or bacterial pathogens encountered by AM cattle during the marketing process.

Hemagram. A main effect of management ($P = 0.01$) and a treatment × day interaction ($P < 0.001$) was evident for total leukocytes (Fig. 1). Total leukocytes were less for AM cattle (management effect; $P = 0.01$) whereas, AMCON had the lowest

concentration of circulating leukocytes on d 5, 7, and 14 (trt × day; $P \leq 0.05$). Total leukocytes did not differ ($P = 0.17$) for PI compared to CON. In a previous study, total leukocytes were reduced in steers exposed to PI-BVDV challenge only, yet total leukocytes were increased when concurrently challenged with *M. haemolytica* (Burciaga-Robles et al., 2010). A frequent outcome of stress- or viral-induced immunosuppression is severe or fatal bacterial pneumonias. However, the degree to which experimental animals in the current study were infected with pathogenic bacteria such as *M. haemolytica*, and potentially confounding effects on leukocyte concentrations was unknown.

The percentage of neutrophils were less ($P = 0.01$; Fig. 1), and the percentage of lymphocytes greater ($P = 0.02$; Fig. 1) in calves exposed to PI-BVDV challenge than CON. Consequently, a trend ($P = 0.07$) was observed for neutrophil:lymphocyte (N:L) ratio (Fig. 1) being less in calves exposed to a PI-BVDV pen mate. Cattle management also affected ($P = 0.02$) N:L ratio; AM calves had greater N:L ratio than PC. Previous studies reported transient increases in N:L for cattle experiencing various stressors. Therefore, the increased N:L ratio would indicate greater stress for AM compared to PC.

A treatment × day interaction ($P < 0.001$) was observed for platelet concentrations. On d 1, platelets were greatest for PCPI, intermediate for PCCON and AMPI, and least for AMCON ($P \leq 0.05$; Fig. 1). However, on d 7 this pattern was altered; AMPI had the greatest platelets, AMCON was intermediate, and PCPI and PCCON were least ($P \leq 0.05$). Platelets are well known for their role in inflammation and hemostasis; however, their function and response to disease challenge or physiological stress are less clear. Further investigation on the platelet response to physiological stress and disease in beef cattle seems warranted.

Serum Cytokine and Haptoglobin Concentrations. Serum TNF-α concentration tended ($P = 0.09$) to be greater in calves exposed to PI-BVDV challenge (Fig. 2). There were no main effects of cattle management on serum proinflammatory cytokine concentrations in the current study. Day effects ($P \leq 0.001$) were observed for TNF-α, IFN-γ, IL-4 and IL-6; concentrations increased transiently with time. Other than a day effect, no differences were observed for serum IL-4 concentration (Fig. 2).

A treatment × day interaction was observed for concentrations of IFN-γ ($P = 0.05$) and IL-6 ($P = 0.006$; Fig. 2). Peak IFN-γ concentrations occurred on d 7 and were 3.3-fold greater in AMPI than PCCON ($P = 0.004$). By d 14, overall IFN-γ concentrations had decreased (day effect; $P = 0.001$); nevertheless, AMPI was greatest, PCPI was intermediate, and AMCON and PCCON were least.

Peak concentrations of IL-6 were observed on d 1 and rapidly returned to near baseline by d 3; nevertheless, a treatment × day interaction ($P = 0.006$) was evident. On d 1, IL-6 concentrations were greatest for AMCON, intermediate for AMPI and PCPI, and least in PCCON. By d 14, AMPI had greater serum concentrations of IL-6 compared to AMCON.

Similar to our results of increased TNF-α, IFN-γ and IL-6 for PI-exposed treatments, Burciaga-Robles et al. (2010) observed increases in TNF-α, IFN-γ, and IL-6 when steers were exposed 72 h to 2 PI-BVDV calves. Proinflammatory cytokine concentrations being greater for AMPI and PCPI suggests a more stimulated immune response via continuous PI-BVDV exposure that may consequently affect growth performance (Spurlock, 1997). Although differences in growth were not observed in this 14-d trial, evidence from a companion study (Richeson et al., 2011) suggests that effects of PI-BVDV exposure on growth performance may be delayed for several weeks, beyond the 14-d evaluation period in this study.

A management effect ($P < 0.001$) was observed for serum haptoglobin concentrations; AM calves had markedly greater concentrations of serum haptoglobin compared to PC (Table 1). For AM, haptoglobin concentrations increased transiently (day effect; $P < 0.001$), and corresponded with increased RT and BRD treatment.

Implications

Continuous exposure of calves to persistently infected-BVDV cohorts may decrease growth performance because the repeated immune stimulation increased proinflammatory cytokines IFN-γ, TNF-α, and IL-6 that can impair growth either directly by acting on tissues, or indirectly through their effects on the endocrine system. Differences in the neutophil:lymphocyte ratio and haptoglobin concentration of newly received beef calves indicate differences in physiological stress existed for the 2 management systems, and these parameters may have potential as biomarkers for predicting or diagnosing bovine respiratory disease.

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Table 1. Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on rectal temperature and haptoglobin concentrations of newly received beef calves.

	Auction market		Preconditioned			Contrasts,[1] *P* =		
Item	**Control**	**PI-BVDV**	**Control**	**PI-BVDV**	**SEM**	**Management**	**Exposure**	**Interaction**
Rectal temperature[a], °F								
d 0	102.4	102.2	102.3	102.4	0.2			
d 1	104.6^{y}	104.4^{y}	102.2^{z}	102.7^{z}				
d 3	102.6^{y}	102.8^{y}	101.5^{z}	101.7^{z}				
d 7	102.8^{x}	102.2^{y}	101.6^{z}	101.5^{z}				
d 14	102.2	101.7	101.9	101.9				
Average	103	102.8	101.9	102.1	0.1	0.001	0.95	0.04
Haptoglobin[ab], mg/dL								
d 0	9.00	6.35	0.14	0.11				
d 1	148.54	107.69	0.25	0.19				
d 3	329.53	268.93	0.10	0.09				
d 7	19.60	42.77	0.19	0.15				
d 14	2.37	1.38	0.04	0.06				
Average	28.99	25.51	0.12	0.11		0.001	0.79	0.96

[a]Treatment x day, $P < 0.001$.

[b]Statistical analyses done on log transformed data, geometric means are reported.

[xyz]Means within a day without a common superscript differ ($P \leq 0.05$).

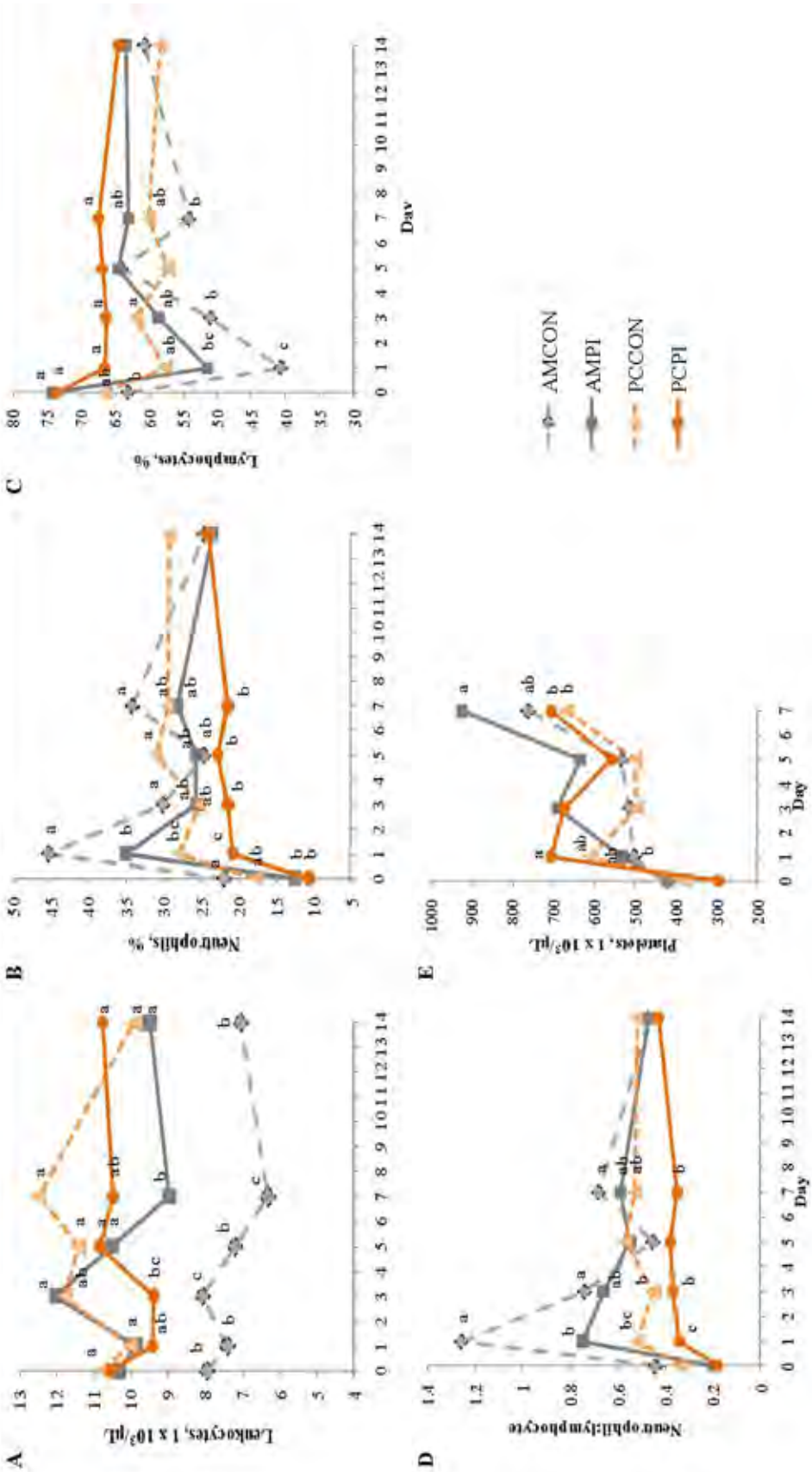

Figure 1. Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on leukocyte concentrations (A; Day, $P = 0.005$; Treatment × Day, $P < 0.001$; Source, $P = 0.01$, SE = 0.88), percentage of neutrophils (B; Day, $P < 0.001$; Treatment × Day, $P < 0.001$; Source, $P = 0.07$; Exposure, $P = 0.01$, SE = 3), and lymphocytes (C; Day, $P < 0.001$; Treatment × Day, $P < 0.001$; Source, $P = 0.10$; Exposure, $P = 0.02$, SE = 3.8), neutrophil:lymphocyte ratio (D; Day, $P < 0.001$; Treatment × Day, $P < 0.001$; Source, $P = 0.02$; Exposure, $P = 0.07$; SE = 0.11), and platelet concentrations (E; Day, $P < 0.001$; Treatment × Day, $P < 0.001$, SE = 73) of newly received beef calves. Means within a day without a common superscript are different ($P \leq 0.05$).

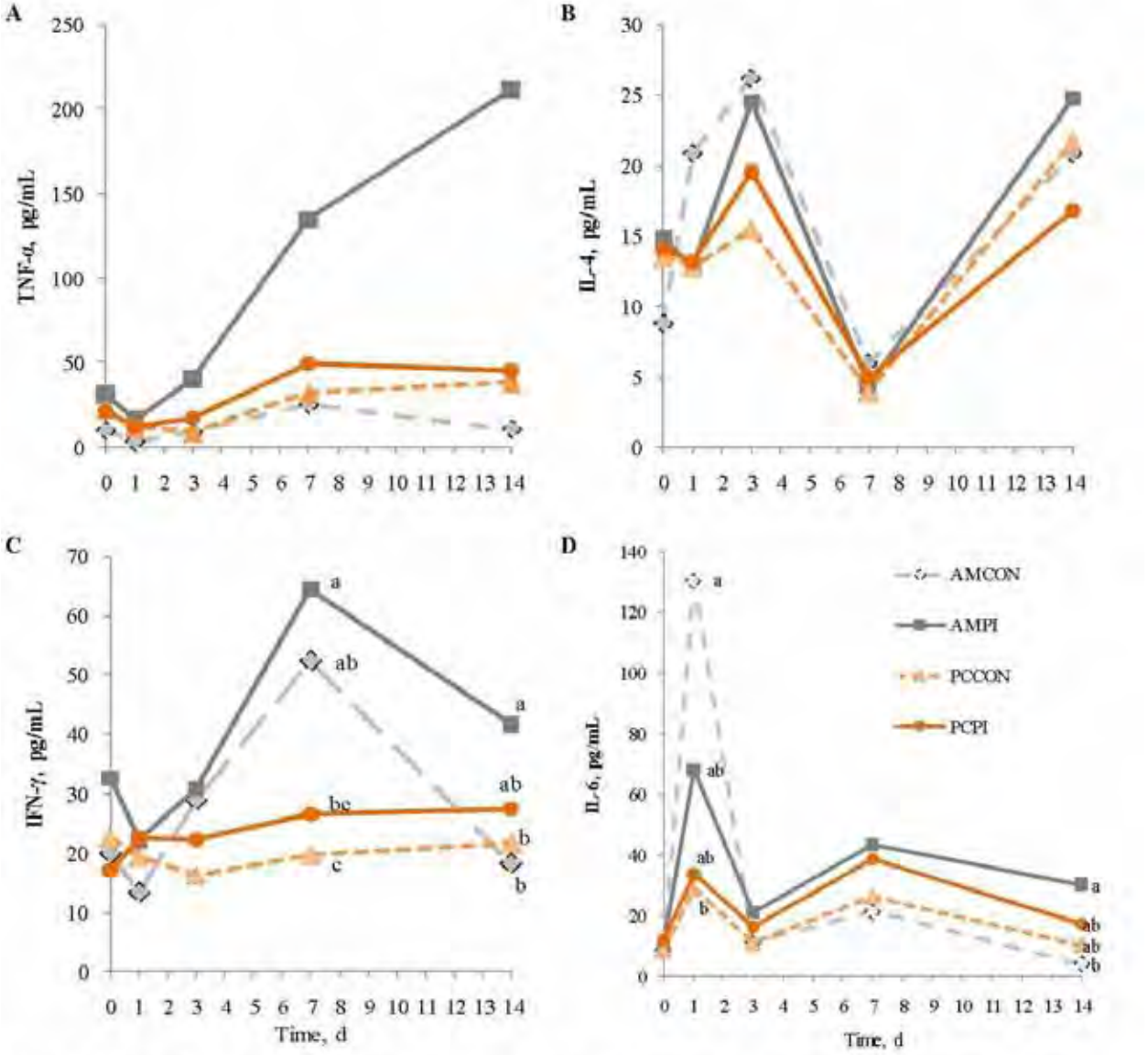

Figure 2. Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum tumor necrosis factor-α (A; Day, $P < 0.001$), interleukin-4 (B), interferon-γ (C; Day, $P = 0.001$; Treatment × Day, $P = 0.05$), and interleukin-6 (D; Day, $P < 0.001$; Treatment × Day, $P = 0.01$) concentrations of newly received beef calves. Values graphed are geometric means of log transformed data. Means within a day without a common superscript are different ($P \leq 0.05$).

Evaluation of an ear-mounted tympanic thermometer device for bovine respiratory disease diagnosis

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Story in Brief

High-risk beef calves (n = 152) from Arkansas auction markets were used to investigate 2 bovine respiratory disease diagnosis procedures and metaphylaxis with tilmicosin phosphate. The 2 bovine respiratory disease diagnosis procedures consisted of 1) calves affixed with an ear-mounted tympanic thermometer device (FeverTag, FeverTags, LLC, Amarillo, Texas) that flashed an indicator light when tympanic temperature was ≥103.6 °F and treated for bovine respiratory disease based solely on the signal status of the device, or 2) pull and treat method based on ≥2 signs of bovine respiratory disease evident and rectal temperature ≥103.6 °F. Within each diagnosis procedure, cattle either received 1.5 mL/100 lb body weight of tilmicosin phosphate on-arrival with a 72 h post-treatment evaluation period or no metaphylaxis and immediately eligible for bovine respiratory disease therapy. During the 32-d receiving trial, no differences ($P \geq 0.53$) in average daily gain were observed. The bovine respiratory disease morbidity rate was the same ($P = 1.0$) for either diagnosis procedure; however, metaphylaxis reduced bovine respiratory disease morbidity (13 vs. 39%; $P = 0.03$) and bovine respiratory disease antibiotic therapy cost/animal ($3.46 vs. $11.42; $P = 0.04$). When cattle were monitored for bovine respiratory disease by experienced personnel, there was no difference in performance or bovine respiratory disease morbidity using either diagnosis procedure; therefore, FeverTag may be an effective alternative for identifying bovine respiratory disease if labor or expertise is limited but additional cost of the device must be considered. Metaphylaxis reduced bovine respiratory disease morbidity but no differences in gain performance were observed in this study.

Introduction

Bovine respiratory disease (BRD) remains the leading cause of morbidity and mortality of stocker and feedlot cattle in the United States resulting in considerable economic costs through death loss, antibiotic treatment cost, reduced performance and increased labor. The most effective strategy available to mitigate the effects of BRD in high-risk, newly received cattle is to administer antibiotic metaphylaxis during initial processing (Smith, 2010). Use of this practice with tilmicosin phosphate has demonstrated favorable therapeutic responses over non-medicated controls in multiple studies. Another critical factor of effective BRD control is the timely and accurate identification of clinically ill cattle; however, experienced personnel with this ability are becoming more difficult to hire and retain. Furthermore, cattle are prey animals with an inherent instinct to conceal disease signs making BRD diagnosis difficult even for experienced personnel.

Rectal temperature is a chute-side, objective diagnostic method available to signify respiratory illness; yet, the practice is labor intensive, resulting in handling stress for people and animals because it requires proper animal restraint to verify. Use of an ear-mounted tympanic thermometer device that continuously monitors tympanic temperature and indicates via flashing light signal when a pre-determined temperature is breached may be an innovative and objective method to assist in pen or pasture diagnosis of BRD. Furthermore, these new technologies could aid feedlot personnel in the detection of cattle with subclinical BRD; thereby, reducing the negative consequences associated with untreated infection. Therefore, the objective of this study was to evaluate 2 different antibiotic treatment strategies using a tympanic temperature detection device or traditional pull and treat method to identify cattle requiring treatment for BRD, with or without antibiotic metaphylaxis on arrival.

Materials and Methods

High-risk beef calves (n = 152) of English-Continental breed type weighing 546 lb were acquired from 3 auction markets in Arkansas and delivered in 2 truck-load blocks on March 12 (block 1; n = 39 bulls, 33 steers) and April 11 (block 2; n = 80 heifers), 2010 to the University of Arkansas Stocker and Receiving Unit located near Savoy.

Upon arrival (d -1), calves were unloaded, weighed, gender was determined, and offered ad libitum access to bermudagrass hay and water overnight. The following day (d 0), calves were weighed, identified individually using a uniquely numbered ear tag, and ear-notched to test for persistently infected (PI) bovine viral diarrhea virus (BVDV). Calves were administered a pentavalent modified-live virus respiratory vaccine (Pyramid 5, Boehringer Ingelheim Vetmedica, St. Joseph, Mo.), a multivalent clostridial/tetanus bacterin-toxoid (Covexin 8, Schering Plough Animal Health, Summit, N.J.), and injectable moxidectin (Cydectin, Boehringer Ingelheim Vetmedica) at a dosage of 1.1 mL/100 lb BW. Additionally, calves on appropriate treatment were administered tilmicosin phosphate (Micotil, Elanco Animal Health, Greenfield, Ind.) at 1.5 mL/100 lb BW and/or were affixed with a tympanic temperature monitoring device in the right ear. The bull calves in block 1 were banded on d 0, and all calves were revaccinated on d 16 of the study.

For block 1, calves were stratified by initial BW and gender (bull or steer) and for block 2, heifers were stratified by initial BW and allocated randomly to 1-acre mixed-grass treatment pens. Cattle were then assigned randomly to 1 of 2 BRD diagnosis procedures with or without on-arrival metaphylaxis with tilmicosin phosphate. The 2 BRD diagnosis procedures consisted of calves affixed with an ear-mounted thermometer device (FeverTag [FT], FeverTags, LLC, Amarillo, Texas; Fig. 1) that flashed a flashing indicator light when tympanic temperature was ≥103.6 °F and treated for BRD based

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solely on the signal status of the FT, or pull and treat method based on ≥2 signs of BRD evident and rectal temperature ≥103.6 °F (PT). Furthermore, within each BRD diagnosis procedure, cattle either received metaphylaxis with 1.5 mL/100 lb BW of tilmicosin phosphate solution on-arrival (d 0) (META) or no metaphylaxis (CON). A 2 × 2 factorial arrangement of treatments was used to evaluate BRD diagnostic procedure, metaphylaxis with tilmicosin phosphate, and their interaction resulting in 4 treatments consisting of 1) FTMETA, 2) FTCON, 3) PTMETA, and 4) PTCON. The post-evaluation interval (PEI) following metaphylaxis was 72 h; thus, immediately for CON calves, and once the PEI had expired for META, PT calves were evaluated daily for clinical signs of BRD by experienced personnel.

BRD Diagnosis. The PT calves were observed once daily (0800) for clinical signs of BRD (depression, nasal discharge, ocular discharge, cough, gaunt appearance, inappetence) by 2 experiment station personnel with a combined 35-yr experience evaluating cattle with BRD. If ≥2 visual signs existed, calves were brought to the restraining chute, weighed, and rectal temperature was recorded via a digital thermometer. If rectal temperature was ≥103.6 °F, cattle were considered morbid, administered antibiotic therapy with enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, Kan.), and immediately returned to their home pen. A 48-h post-treatment interval (PTI) was implemented after administration of enrofloxacin, and a second temperature was recorded upon expiration of the initial antibiotic PTI. If the second temperature was ≥103.6 °F, a second antibiotic treatment with florfenicol (Nuflor, Schering-Plough Animal Health) was administered. A 48-h PTI was also implemented for cattle administered florfenicol, and rectal temperature was evaluated upon expiration of the second antibiotic PTI. If the temperature was ≥103.6 °F, a third and final antibiotic treatment with ceftiofur HCl (Excenel RTU, Pfizer Animal Health, New York, N.Y.) was administered for 3 consecutive days. If at any time rectal temperature was <103.6 °F, the animal was left untreated and returned immediately to the treatment pen until ≥2 subsequent signs warranted re-examination. Treatment data were recorded for individual animal including treatment date, rectal temperature, and the amount (mL) of each antibiotic administered.

The FT device was affixed to the ear of cattle and monitored tympanic temperature in 15 minute intervals. When the tympanic temperature exceeded the factory pre-set temperature of 103.6 °F, an indicator light flashed every 3 seconds enabling straightforward, objective identification of an animal experiencing fever. The FT calves received the same antibiotic regimen and PTI as PT calves; however, BRD morbidity was determined based solely upon status of FT indicator light. The FT calves were observed once daily (0800) for indicator light status; if a treatment eligible calf had a flashing FT device it was removed from the pen and treated with the same antibiotic treatment regimen described for PT.

Statistical Analyses. Animal performance data was analyzed with pen as the experimental unit. A randomized complete design with a 2 × 2 factorial arrangement of treatments was utilized, and computations were made using the mixed models procedure of SAS (SAS Inst. Inc., Cary, N.C.).

Results and Discussion

During the 32-d receiving trial, no differences ($P > 0.05$) in ADG were observed (data not shown). Furthermore, no treatment interactions were evident; therefore, only main effects of BRD diagnosis method and metaphylaxis are reported. Treatment protocol for diagnosing BRD (FT vs. PT) resulted in the same ($P = 1.0$) morbidity rate (26%) for the 2 procedures (Table 1). Therefore, FT may be an effective alternative for identifying BRD in newly received calves if labor or expertise is limited, but the additional cost of the FT device should be considered. The cost of a FT at the time the current study was conducted was $17.50/tag. The manufacturer states that the battery life of the FT is approximately 180 d and can be used for up to 3 60-d receiving periods. Therefore, the estimated cost of a FT when used for 3 animals during 3 separate 60-d receiving periods is $5.83/animal. Application of the FT did not seem to significantly reduce processing speed; however, processing time with and without FT application was not measured in the current study. It is important to note that significant design improvements to the FT were made after the current study was conducted. Regarding the original FT design (Fig. 1), several anecdotal observations are worth mentioning: 1) the FT indicator light was difficult to see on bright sunny days, 2) the failure rate (tag was not retained or was not working properly by the end of the 32-d receiving period) of the original design was relatively high, and 3) the FT caused "ear drop" which could be mistaken for signs of clinical depression. However, the updated FT was modified to address each of these concerns. Further research on the effectiveness of the new FT design for diagnosing BRD in cattle is needed.

Morbidity was reduced (13 vs. 39%; $P = 0.03$) for META compared to CON (Table 1). The reduction in BRD morbidity rate for META resulted in a lower ($P = 0.03$) BRD antibiotic therapy cost/animal than CON ($3.46 vs. $11.42). The additional cost of on-arrival metaphylatic therapy ($9.92/animal) was greater than the subsequent reduction in antibiotic therapy cost/animal for META ($7.96). However, additional time and labor costs associated with pulling sick animals were not included in the antibiotic therapy cost. Therefore, even when BRD morbidity is relatively low, on arrival metaphylaxis with tilmicosin phosphate was cost-effective.

Implications

The morbidity rate between treatment methods (FeverTag vs. Traditional pull and treat) was the same, which suggests that FeverTags may be an effective alternative to traditional "pull and treat" method, particularly when experienced personnel or labor is limited. Morbidity rate was reduced for calves receiving on-arrival antibiotic metaphylaxis compared to the control. No differences in ADG were observed in this study.

Literature Cited

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Acknowledgements

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Table 1. Effects of metaphylaxis and bovine respiratory disease (BRD) diagnosis procedure on health of stocker calves.

Item	Metaphylaxis		No Metaphylaxis		SEM	Contrasts, *P* =		
	FT	PT	FT	PT		BRD Regimen[1]	Metaphylaxis	Interaction
Morbidity, %	16.4	10.0	35.8	42.2	10.6	1.0	0.03	0.55
Treated once, %	10.8	5.0	16.4	21.1	8.8	0.95	0.24	0.56
Treated twice, %	5.6	2.5	19.4	13.6	8.0	0.59	0.15	0.87
Treated thrice, %	0.0	2.5	0.0	7.5	3.9	0.23	0.54	0.54
BRD treatment cost, $/calf	4.11	2.79	10.64	12.19	3.42	0.97	0.03	0.68
Total cost[2], $/calf	20.03	12.71	16.64	12.19	3.42	0.11	0.58	0.68

[1]BRD diagnosis procedure = FeverTag or Pull and Treat.
[2]Total cost includes metaphylaxis ($9.92) and FeverTag ($5.83) if relevant.

Fig. 1. A) FeverTag before (with applicator), B) during, and C) and D) after application.

Post-weaning performance by spring and fall-born calves weaned from full access, limited access, or no access to 'wild-type' endophyte-infected tall fescue pastures – 3 year summary [1]

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Story in Brief

Replacing 'wild-type' endophyte-infected tall fescue with non-toxic endophyte-infected fescue may improve calf BW at weaning, but information about impacts on post-weaning performance are limited. Our objective was to determine the extent having limited access to non-toxic endophyte-infected fescue prior to weaning will affect post-weaning performance by spring and fall-born calves. Gelbvieh × Angus crossbred cows were used in a study that resulted in 258 steers and 245 heifers representing 5 treatments: 1) fall on 100% 'wild-type' endophyte-infected tall fescue; 2) spring on 100% 'wild-type' endophyte-infected tall fescue; 3) fall on 75% 'wild-type' endophyte-infected tall fescue and 25% non-toxic endophyte-infected fescue; 4) spring on 75% 'wild-type' endophyte-infected tall fescue and 25% non-toxic endophyte-infected fescue; and 5) spring on 100% non-toxic endophyte-infected fescue. Steer actual and adjusted weaning BW, feedlot gain, age at harvest, and marbling scores, and percentage USDA Choice were greater ($P < 0.05$) from fall vs. spring, but BW gain between weaning and shipping, BW at shipping to the feedlot, ribeye area, and total post-weaning gain were greater ($P < 0.05$) from spring vs. fall. Steer actual and adjusted weaning BW, harvest BW, and hot carcass wt. were greater ($P < 0.05$) from spring on 100% non-toxic endophyte-infected fescue vs. spring on 75% 'wild-type' endophyte-infected tall fescue and 25% non-toxic endophyte-infected fescue. Heifer BW at weaning was greater ($P < 0.05$) from fall vs. spring, but BW gain and daily gain to breeding, BW at breeding, and subsequent calving rates were greater ($P < 0.05$) from spring vs. fall. Heifers weaned from spring on 100% non-toxic endophyte-infected fescue had greater ($P < 0.05$) weaning and adjusted weaning BW, and tended ($P < 0.10$) to have greater BW at breeding than heifers weaned from spring on 75% 'wild-type' endophyte-infected tall fescue and 25% non-toxic endophyte-infected fescue. Therefore, after three years of post-weaning measurements, fall calving may benefit calf BW through weaning, but post-weaning management may impact these differences during the post-weaning period. Limited use of non-toxic endophyte-infected fescue may benefit steer weaning weights, but have little impact on long-term post-weaning performance.

Introduction

It is well documented that the'wild-type' endophye-infected tall fescue (E+) produces toxins that reduce grazing animal performance, but the impact of these toxins on cattle after removal from E+ have been highly variable both in length and severity. One alternative to grazing E+ is to graze a non-toxic novel endophyte-infected fescue (NE+) that has improved spring-calving cow performance. However, data addressing those impacts on post-weaning performance 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.

Materials and Methods

Gelbvieh × Angus crossbred spring and fall-calving cows (n = 178; 1128 ± 11.3 lb initial BW) 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 26 January 2007. The cows were from the cowherd at the University of Arkansas Livestock and Forestry Research Station (LFRS) near Batesville, Ark. A total of 258 steers and 245 heifers were weaned from the study over the 3-yr period. Cow-calf pairs assigned to SNE100 grazed NE+ fescue throughout the year whereas those assigned to F100 and S100 grazed E+ pastures throughout the year. Cows assigned to S75 and F75 treatments grazed E+ pastures for most of the year, but were moved to NE+ at approximately 28 d prior to the start of the breeding season and 28 d prior to weaning [October 18, 2007; October 23, 2008; October 22, 2009 (spring); May 9, 2007; May 14, 2008; May 12, 2009 (fall)] to mitigate the negative effects from grazing E+ prior to these critical production times.

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 , bovine viral diarrhea virus type 1, parainfluenza-3, 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 pour-on, 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,

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calves had *ad libitum* access to bermudagrass hay (41% IVDMD; 15% CP) and water, and were offered 2 lb/hd daily of corn gluten pellets. Following the 14-d weaning period F groups were moved to bermudagrass pastures and the S groups were moved to winter annual pastures. Heifers were weighed at the start of the breeding season [November 26, 2007; November 20, 2008; and November 24, 2009 (fall); May 8, 2008; May 11, 2009; and May 11, 2010 (spring)] and placed with a bull for a 63-d breeding season. Heifers did not have access to E+ prior to the start of the breeding season or during the breeding season. The F and S steers remained on either bermudagrass or winter annual pastures, respectively, until they were shipped to the Willard Sparks Research Center in Stillwater, Okla. and fed a corn-based, high-concentrate finishing diet. No supplemental concentrate was offered to any post-weaning 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 at a commercial slaughter facility and carcass data were collected following a 24 to 48-h chill. The decision of when to ship the cattle was based on visual estimation of backfat thickness to ensure a common endpoint for the steers.

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) 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. Heifer calving rates and percent USDA Choice were analyzed with the Chi-square procedure of SAS.

Results and Discussion

Steer actual and adjusted weaning BW, feedlot gain, age at harvest, and marbling score, and percentage USDA Choice were greater ($P < 0.05$) from F compared with S, but BW at feedlot shipment, total and daily gain between weaning and feedlot shipment, total post-weaning gain, and ribeye area were greater ($P < 0.01$) from S compared with F (Table 1). Fall-born steers required more ($P < 0.05$) total feed to reach market conditions, but ADG and feed conversions were not different ($P = 0.11$ and 0.92, respectively) between F and S steers. Differences in post-weaning gains between weaning and shipping are a direct reflection of differences in post-weaning forage quality and environmental conditions between grazing bermudagrass during the summer (F steers) compared with winter annual forages during the winter (S steers).

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. However, steers weaned from F100 and S100 tended ($P < 0.10$) to require less feed per pound of gain in the feedlot than steers weaned from F75 and S75. This means that calves weaned from systems where they grazed only E+ prior to weaning did exhibit some degree of compensatory gain during subsequent backgrounding and feedlot periods.

Actual and adjusted BW at weaning, harvest BW, and hot carcass wt. were greater ($P < 0.05$) and BW at shipping to the feedlot tended ($P < 0.10$) to be greater from SNE100 compared with S100. Backfat thickness did not differ ($P \geq 0.30$) across treatments, indicating that the groups were slaughtered at a common endpoint.

Heifer actual BW at weaning was greater ($P < 0.05$) from F vs. S, but total and daily gain from weaning to breeding was greater ($P < 0.05$) from S vs. F, resulting in greater ($P < 0.05$) BW at breeding from S heifers. Spring-born heifers also had 67% greater ($P < 0.05$) calving rates than F on comparable treatments. As was the case with steers, these differences in post-weaning performance between S and F are likely the result of the differences in energy value of winter annual forages utilized by S (higher energy) vs. bermudagrass utilized by F (lower energy) for growing and developing heifers. The lower BW by F heifers likely resulted in fewer of those heifers reaching reproductive maturity during the breeding season, thereby resulting in lower calving rates. It is likely that these calving rates could have been improved had supplementation been offered to increase heifer gains. This could likely have been done in a cost-effective manner, considering the number of open F heifers and the value of a pregnant vs. an open heifer. However, one of the long-term objectives of our program is to develop breeding animals that match the forage resources available to them during their development and to reduce the need for off-farm feed inputs.

Therefore, it appears that fall calving may benefit steer and heifer BW at weaning and may improve the number of steers grading choice at harvest. Furthermore, weaning spring-born steers that have limited access to NE+ during the grazing season may improve steer BW at weaning but those benefits may not persist through subsequent post-weaning and feedlot periods because of partial compensatory gain by calves that grazed E+ only.

Implications

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, feed costs, and other marketing situations should be considered if producers are interested in retained ownership of weaned calves. This may be particularly important to those developing replacement heifers, as options for rapid development of fall-born heifers to calve at 2 yr of age are somewhat limited in Arkansas.

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]**
Steer BW, lb							
At weaning	555	585	611	489	517	23.3	W,X,Y
Adj. weaning[c]	498	526	552	446	480	15.4	W,X,Y
At shipping[d]	663	678	870	774	790	45.3	W,y
Harvest weight	1309	1331	1419	1319	1322	36.9	Y
Steer gain, lb							
Wean to feedlot	112	103	257	281	267	45.6	W
Feedlot	641	647	550	541	525	47.7	W
Total post-weaning	750	752	803	829	804	52.5	W
Steer ADG, lb							
Wean to feedlot	0.62	0.57	1.33	1.46	1.38	0.264	W
Feedlot	3.7	3.8	4.1	4.0	3.9	0.13	W
Feedlot DM intake, lb	3691	3814	3360	3140	3237	246.7	W
F/G, lb/lb	6.12	6.20	6.40	5.93	6.42	0.185	x
Age at harvest, d[e]	586	585	563	561	558	12.5	W
Carcass measurements							
HCW, lb[f]	795	805	865	792	793	21.4	Y
Dressing, %[g]	63.3	63.0	63.6	62.5	62.8	0.47	ns
Ribeye area, in^2	12.9	13.2	14.6	13.9	14.1	0.36	W
Backfat, in	0.45	0.45	0.51	0.48	0.48	0.030	ns
Yield grade	2.7	2.6	3.1	2.8	2.9	0.20	w
Marbling score[h]	458	432	407	388	386	16.6	W
% Choice	82	71	59	53	61		W

[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 response to having 25% of their pasture area as NE+ ($P < 0.05$); lowercase letters represent statistical tendency ($P \leq 0.10$); ns = no significant difference.

[c] Weaning weights were adjusted for age of calf, but additive factors for age of dam were not used.

[d] Shipping weight was the weight measured prior to calves being shipped to the OSU feedlot.

[e] Age from birth to harvest.

[f] HCW = Hot carcass weight.

[g] Dressing percentage was calculated using shrunk final weights.

[h] $300 = Slight^0$, $400 = Small^0$.

Table 2. Post-weaning performance and calving rates by spring (S) and fall-born (F) heifers 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]**	**Contrast[b]**
Heifer BW, lb							
At weaning	512	536	580	490	492	30.0	W,Y
Adj. weaning[c]	460	478	522	448	448	23.4	w,Y
At breeding	646	662	792	745	736	26.5	W,y
Heifer gain, lb							
Wean to breed	135	123	225	251	240	16.8	W
Heifer ADG							
Wean to breed	0.7	0.6	1.1	1.3	1.2	0.09	W
Calving rate, %[d]	40	44	82	70	70		W
Day of conception[e]	13	12	12	16	14	3.2	ns

[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 response to having 25% of their pasture area as NE+ ($P < 0.05$); lowercase letters represent statistical tendency ($P \leq 0.10$); ns = no significant difference.

[c] Weaning weights were adjusted for age of calf, but additive factors for age of dam were not used.

[d] Analyzed using the Chi-square procedure of SAS.

[e] Estimated day of the breeding season the heifers conceived based on actual calving dates and an assumed 285-day gestation interval.

Arkansas Steer Feedout Program 2009-2010

B. Barham[1] and S. 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 2009-2010 feedout, hot carcass weight, initial weight entering the feedyard, medicine costs, USDA muscle score, 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.

Materials and Methods

On November 18, 2009, 158 steer calves from 14 Arkansas producers representing 7 counties were placed on feed at Wheeler Brothers Feedyard in Watonga, Okla. Calves were weighed on November 19, 2009. All calves were processed and placed in one pen. Management factors such as processing, medical treatments and rations were the same as 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 157 steers that started on feed in the fall, 2 died (1.2% death loss). One calf was sold as a railer due to being chronically ill. These 3 calves were not included in the statistical analyses. Therefore, 154 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 start weight. If the feeder calf could have been sold in the fall of 2009 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 $1.10 per pound (average in weight was 659 pounds) and ranged from $0.71 to $1.45 per pound.

The sick pull rate averaged 20% with 31 of 154 calves treated for sickness. This pull rate was an improvement over previous years, but was still high since all cattle were listed as being preconditioned prior to entering the feedyard. Average medicine cost for the entire pen was $5.59 per steer. The health status of cattle in the feedyard usually has a major impact on performance and profit. Healthy steers had higher ($P < 0.05$) feedlot net returns ($741) than steers that became sick ($644). Steers that did not receive treatment had higher average daily gain, hot carcass weights and lower feed cost of gain and total cost of gain ($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 start weight and final weights were 659 pounds (range = 415 to 863 lb.) and 1,290 pounds (1,021 to 1,633 pounds), respectively. Average daily gain was 3.32 pounds and ranged from 2.1 to 4.4 pounds. Overall, 57% of the steers graded Choice, compared to the national average of 56.8%. 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 1000 pounds. Forty five percent of the steers in this year's program fit these industry standards. Steers that met the industry standards averaged $66 per steer more than those that did not fit the industry standards ($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, 77% met the industry standards, and for those in the bottom 25% based on feedlot net return, 74% did not meet the industry standards.

Factors Affecting Steers' Feedlot Net Return. Listed below are the significant ($P < 0.05$) factors that affected feedlot net return for steers in the 2009-2010 program. Factors are listed in descending order of importance.

1. Hot Carcass Weight—The relationship between hot carcass weight and feedlot net return was positive. As hot carcass weight

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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.

2. 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. This largely has to do with the initial value of the calf coming into the feedyard. Larger calves also have a higher initial value and must overcome this difference with higher feedyard returns. The increased costs of feed grains may have caused cattle buyers to seek heavier calves to reduce the amount of grain needed to finish the cattle. This negative relationship was present in our analysis for three years and possibly indicates that the current trend of placing heavier cattle in the feedyard might have pushed the price slide too far, making lighter cattle a better bargain to feed.

3. 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.

4. 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 grades.

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.

5. Yield Grade—As yield grade increased from 1 to 5, feedyard returns increased until values peak for a YG 3 carcass and then decline as YG continues to increase ($655, $707, $771, $758, $736 per head for yield grades 1, 2, 3, 4 and 5, respectively). Yield grade 3 carcasses had higher ($P > 0.05$) feedyard returns than grades 1, 2, 4 and 5. It is interesting to note that even carcasses that were YG 4 & 5 are considered “fall outs” or carcasses that do not fit industry standards; they still had higher numerical feedyard returns than YG 1 & 2 carcasses, even though the difference was not significant ($P > 0.05$).

Implications

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, 2009-2010.

	Average per steer ($)	Range ($)
Gross Income	1,223.47	983 to 1,546
Expenses		
Feed	414.27	310 to 534
Freight, interest, etc.	80.44	69 to 120
Medicine	5.59	0 to 63
Total	500.30	379 to 717
Feedlot Net Return	723.18	499 to 986
Start Value	557.87	311 to 724
Calculated Return	165.44	-152 to 309

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 (n = 154)	38	38	
Gross Income per head ($)	1,157[a]	1,300[b]	1,223
In Value per head ($)	591[a]	537[b]	557
Medicine per head ($)	10.11[c]	4.70[d]	5.59
Feed Cost per head ($)	399[a]	429[b]	414
Total Expense per head ($)	492[a]	514[b]	500
Feedlot Net Return per head($)	664[a]	785[b]	723
Calculated Return per head ($)	74[a]	247[b]	165
Days on Feed	185	195	190
Feed Cost Per Lb. of Gain ($)	0.72	0.62	0.66
Total Cost Per Lb. of Gain ($)	0.89	0.74	0.80
Start Weight (lb.)	695[a]	638[b]	659
Muscle Score	1.76	1.89	1.8
Frame Score			
Large	90%	77%	81%
Medium	10%	23%	19%
Final Weight (lb.)	1,249[a]	1,336[b]	1,290
Average Daily Gain (lb.)	3.00[a]	3.58[b]	3.32
Hot Carcass Weight (lb.)	765[a]	834[b]	798
Carcass Value ($/lb)	1.51[a]	1.55[b]	1.53
Dressing Percentage	61.3%[a]	62.4%[b]	61.8%
Ribeye Area (sq. in.)	12.4	13.0	12.7
Backfat	0.43	0.45	0.45
REA per 100 lb. carcass weight	1.62[a]	1.56[b]	1.61
Quality Grade			
Prime	0%	3%	1%
Choice	26%[a]	77%[b]	57%
Select	71%[a]	21%[b]	42%
No Roll	0%	0%[b]	0%
Hard Bone	3%	0%	1%
Yield Grade	2.4	2.3	2.4

[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	$986	$920	$66
Average Daily Gain (lb)	3.32	3.31	0.01
Carcass Value	$1.57	$1.50	$0.07

[a] USDA Quality Grade Choice, yield grade ≤ 3.0 and carcass weight of 550 to 1,000 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	0.80	2.8	591	135
700-799	0.80	3.2	680	162
800-899	0.82	3.5	776	174
900+	0.79	3.8	844	185

Factors affecting the selling price of feeder cattle sold at Arkansas livestock auctions in 2010

T. R. Troxel[1], B. L. Barham[1], S. Cline[1], J. Foley[1], D. Hardgrave[1], W. Wiedower[1], and R. Wiedower[1]

Story in Brief

Data were collected from 14 Arkansas livestock auctions to determine factors affecting selling price in 2010. Data included selling in groups, gender, breed or breed type, color, muscle thickness, horn status, frame score, fill, body condition, age, health, and weight. Data were randomly collected on 38,346 lots. The selling prices for steers ($116.16 ± 0.16), bulls ($109.85 ± 0.19) and heifers ($102.71 ± 0.13) were significantly different (P < 0.001). Angus × Brahman ($111.82 ± 0.33), Angus × Hereford ($111.70 ± 0.33), Angus ($111.36 ± 0.22), Hereford × Charolais ($110.48 ± 0.82) and Angus × Hereford × ¼ Brahman ($110.22 ± 0.62) feeder cattle sold for the highest price and were not significantly different ($P > 0.10$). The breed or breed types that sold for the lowest price ($P < 0.01$) were Simmental ($99.90 ± 1.29), Brahman ($94.34 ± 1.37), and Longhorn ($71.75 ± 0.96). Black-white faced color sold for the highest price ($111.74 ± 0.29; $P < 0.001$) followed by black ($110.23 ± 0.14), yellow ($110.09 ± 0.29) and yellow-white faced ($109.81 ± 0.61) but were not statistically different ($P > 0.10$). Spotted or striped feeder cattle received the lowest ($82.16 ± 0.81) price. Selling in groups, muscle score, horn status, frame score, fill and body condition impacted selling price ($P < 0.001$). A number of management and genetic factors affected the selling price of feeder cattle.

Introduction

Cow-calf producers are often referred to as price takers. Throughout the country, cattle producers sell their cattle at local livestock auction by putting their cattle on public display and asking buyers to determine the selling price. When buyers at a livestock auction view feeder calves, they must appraise individual characteristics (muscle thickness, frame score, breed composition, etc.) as predictors of quality and animal performance and adjust their bids accordingly. Many of these factors such as breed or breed type are very subjective. Therefore, many cow-calf producers believe that feeder cattle are priced inconsistently. Producers do not understand why some phenotypic characteristics are discounted and others are not. Most feeder calf market reports list the selling prices of steers and heifers, by weight groups, and frame and muscle score. Other reports have indicated that breed or breed type, health, gender, frame and muscle score, and other noticeable factors do affect feeder calf selling price (Barham and Troxel, 2007; Leupp et. al., 2008; Schulz et. al., 2009). Therefore, the objective was to determine the factors that affect the selling price of feeder cattle in Arkansas weekly livestock auctions.

Materials and Methods

Five USDA certified livestock market reporters collected data from 14 weekly livestock auctions in Arkansas from January 1, 2010 to December 31, 2010. The livestock auctions were located in Ash Flat, Charlotte, Conway, Fort Smith, Glenwood, Green Forest, Heber Springs, Hope, Ola, Ozark, Pocahontas, Ratcliff, Siloam Springs and Springdale. The data collected included selling in groups (singles, 2 to 5 hd or 6 hd or more) calf gender (bull, steer, or heifer), breed or breed type, color, muscle thickness (USDA, 2000), horn status (polled (dehorned) or horned), frame score (large, medium or small), fill (gaunt, shrunk, average, full or tanked), condition (very thin, thin, average, fleshy, or fat), age (calf or yearling), health (dead hair, stale, sick, bad eye(s), lame, healthy or preconditioned), and weight. A total of 426,405 feeder cattle were sold through these livestock auctions, and data were randomly collected (every 5th to 6th calf) on 38,346 lots consisting of 79,822 head (19.0%). Frame and muscle scores were determined based on the U.S. Standards for Grades of Feeder Cattle (USDA, 2000).

Data Analyses. The percent of calves within marketing group size, age, gender, breed or breed type, color, horn status, frame score, muscle score, fill, condition, weight group and health were determined by the frequency procedure of SAS. Analysis was based on 38,346 lots (n = 38,346). Due to the unbalanced nature of the data, calf characteristics were analyzed individually as independent variables in which the model included month and weight as covariates. Sale price was the dependent variable. All other variables contributed to the error sum of squares. The analysis of variance was performed with the Generalized Linear Model procedure of SAS. Least-squares means were generated, separated based on predicted differences, and both are reported throughout. Since all colors are not represented within each breed or breed type, color and breed or breed type data are inherently confounded. All selling prices reported are in U.S. dollars/100 lb.

Results and Discussion

The mean selling price was $108.58 ± 18.96 (mean ± SD) and all main effects reported were significant sources of variation ($P < 0.001$). Over 68% of the feeder cattle were classified as calves and 31.4% were classified as yearlings. The selling price of calves ($110.29 ± 0.12) was greater ($P < 0.001$) than the selling price of yearlings ($104.81 ± 0.19). In 2010, the month with the lowest and highest selling price was January and December ($94.85 ± 0.32 and $117.02 ± 0.60, respective; $P < 0.001$). Selling price varied by month with greatest selling prices recorded in the spring (March, April and May) and lesser prices in the summer and early fall (September and October; P < 0.001; Fig. 1.) This seasonal trend followed the 5-, 10- and 20- year average seasonal trend (Cheney, 2011). Over 71% of the cattle sold weighed less than 550 lb (Fig. 2). As selling weight increased, price per cwt. decreased.

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The selling price for feeder cattle sold in groups of 2 to 5 calves was greater than that of feeder cattle sold as singles ($110.52 ± 0.22 vs. $107.81 ± 0.11; $P < 0.0001$). The selling price of singles was lower than the selling price for those sold in groups of 6 or more ($107.81 ± 0.11vs. $112.60 ± 0.42; $P < 0.0001$). The selling price of feeder cattle sold in groups of 2 to 5 calves compared with groups of 6 or more was also significantly different ($110.52 ± 0.22 vs. $112.60 ± 0.42; $P > 0.001$). These results show a financial advantage for marketing calves in groups as opposed to marketing them as individuals.

Heifers made up 45.8% of the cattle sold whereas steers and bulls made up 32.0% and 22.2%, respectively (Table 1). The selling prices for steers ($116.16 ± 0.16), bulls ($109.85 ± 0.19) and heifers ($102.71 ± 0.13) were all significantly different ($P < 0.001$). Castration is a common practice to reduce management problems associated with aggressive and sexual behavior associated with commingling bull calves. The prices received for bulls were lower due to the expected reduction in animal performance experienced with these animals subsequent to castration as well as the costs associated with the actual procedure.

Table 1 summarizes the percentage of the population sampled and selling price based on muscle score, horn status, health status, frame score, body fill and body condition. All factors affected the selling price. Buyers discounted feeder calves that were light muscled, horned, unhealthy, small framed, appeared to have the potential for excessive shrinkage and over-conditioned.

Twenty breeds or breed types represented 97.7% of the total feeder cattle. The breed or breed type was based upon common industry perception rather than actually knowing the breed composition. This, however, is what a buyer must do before a bid price can be offered. The main effect of cattle breed or breed type on the selling price of feeder cattle was significant ($P < 0.001$; Table 2). Angus × Brahman ($111.82 ± 0.33), Angus × Hereford ($111.70 ± 0.33), Angus ($111.36 ± 0.22), Hereford x Charolais ($110.48 ± 0.82) and Angus × Hereford × ¼ Brahman ($110.22 ± 0.62) feeder cattle sold for the highest price and were not different from each other ($P > 0.10$). The breed or breed types that sold for the lowest price were Simmental ($99.90 ± 1.29), Brahman ($94.34 ± 1.37), and Longhorn ($71.75 ± 0.96) and were different from each other ($P < 0.01$). There was an approximate $40.00 difference between the breed or breed types selling for the highest price and Longhorn feeder cattle, which sold for the least price.

When reviewing the breeds or breed combinations above the average, many breeds or breed combinations were not different from each other. The same was true with the breeds or breed combinations below the average. The discounts on the breeds or breed types listed on the bottom are greater than the premium for the breeds or breed types listed at the top.

Breeds or breed types do affect the selling price of feeder cattle. This is due to the perception by order buyers as to how different breeds or breed types perform (gain, sick rate, quality grade, etc.). For many years, a perception existed that if cattle were black they had some degree of Angus breeding. Today that may or may not be true. Many beef breeds have animals that are black, such as Limousin, Simmental and Gelbvieh, to name a few. The perceptions regarding certain breeds and subsequent performance may be right or wrong, but they exist. With a high percentage of feeder cattle sold in livestock auctions weighing less than 550 pounds, the majority of these cattle are purchased for placement in a backgrounding grazing program. Backgrounding programs are forage based (native pasture, wheat, etc.), and buyers are looking for the breeds or breed combinations that perform best under those conditions. Cow-calf producers should be aware that the breeds or breed types that perform best under backgrounding programs might not be the breeds or breed types that make good replacements. Cow-calf producers must be attentive of this and design an appropriate breeding program.

Ten colors represented 94.2% of the total population (Table 3). Black-white faced color sold for the highest price ($111.74 ± 0.29; $P < 0.001$) followed by black ($110.23 ± 0.14), yellow ($110.09 ± 0.29) and yellow-white faced ($109.81 ± 0.61) but were not different from each other ($P > 0.10$). Grey calves sold for ($106.88 ± 0.34). White, red-white faced, grey-white faced and red calves sold for similar prices ($P > 0.10$). Spotted or striped feeder cattle received the lowest ($82.16 ± 0.81). Unlike breed or breed combinations, most colors were different from each other.

Implications

The majority of cow-calf producers in Arkansas sell feeder cattle at local livestock auctions. The major factors affecting selling prices of feeder cattle were calf health, perceived breed or breed type, muscle thickness, frame score, fill, color and body condition, calf gender, and horn status. The combination of all these factors determines the final selling price. Most of the major factors affecting selling price can be addressed through genetic selection and management. Once the impact of these factors are identified and understood, cow-calf producers can make cost effective management changes that can improve feeder calf value and total returns.

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Table 1. The percentage of the sampled population and 2010 Arkansas selling prices (mean ± SE) due to calf gender, muscle score, health, frame score, body condition, horn status and body fill.

Item	Percentage of the sampled population	Selling price ($/cwt.)
Calf gender:[a]		
Bulls	22.2	$109.85 ± 0.19
Steers	32.0	$116.16 ± 0.16
Heifers	45.8	$102.71 ± 0.13
Muscle score:[a]		
1	78.5	$110.82 ± 0.10
2	20.1	$101.88 ± 0.21
3	1.2	$78.41 ± 0.84
4	0.2	$53.64 ± 2.23
Health status:[b]		
Sick	0.02	$62.48 ± 6.10 [a]
Lame	0.2	$68.57 ± 2.07 [a]
Stale	0.2	$87.21 ± 1.98 [b]
Bad eyes	0.4	$95.38 ± 1.56 [c]
Dead hair	0.3	$98.43 ± 1.85 [c]
Healthy	95.5	$108.69 ± 0.10 [d]
Preconditioned	3.2	$113.57 ± 0.54 [e]

Item	Percentage of the sampled population	Selling price ($/cwt.)
Frame score:[b]		
Large	59.0	$108.81 ± 0.13[a]
Medium	40.0	$108.67 ± 0.15[a]
Small	0.8	$86.71 ± 1.06[b]
Body condition:[b]		
Fat	0.05	$94.40 ± 4.02 [a]
Very thin	0.8	$98.05 ± 1.08 [a,b]
Fleshy	4.4	$102.23 ± 0.46 [b]
Average	62.4	$108.36 ± 0.12 [c]
Thin	32.4	$110.11 ± 0.17 [d]
Horned status:[a]		
Polled/ dehorned	89.4	$109.36 ± 0.10
Horned	9.6	$101.33 ± 0.31
Body fill:[a]		
Gaunt	16.1	$114.40 ± 0.24
Shrunk	36.1	$109.65 ± 0.16
Average	44.7	$106.28 ± 0.14
Full	3	$99.41 ± 0.54
Tanked	0.1	$90.33 ± 2.95

[a] All least-square means within an item are different from each other ($P < 0.001$).
[b] Least-squares means without a common superscript differ ($P < 0.01$).

Table 2. The percentage of the sampled population and 2010 Arkansas selling prices (mean ± SE) of feeder calves sold based on breed or breed type[a].

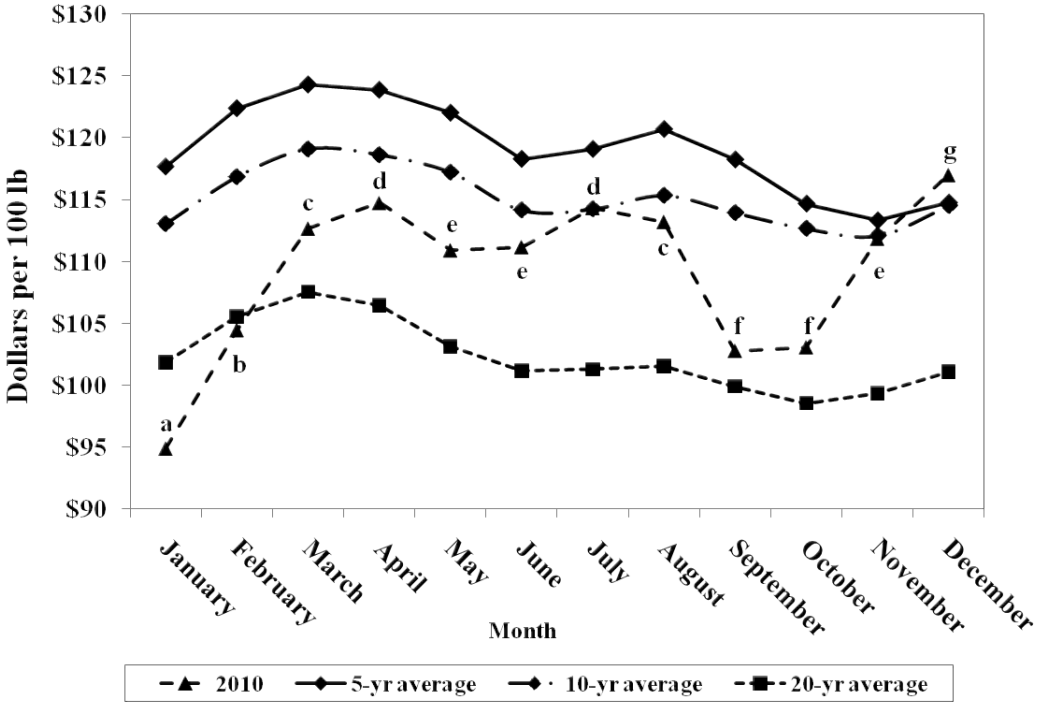
Breed or breed type	Percentage of the sampled population	Selling price ($/cwt.)[b]
Angus × Brahman	8.2%	$111.82 ± 0.33 [c]
Angus × Hereford	7.8%	$111.70 ± 0.33 [c]
Angus	18.0%	$111.36 ± 0.22 [c]
Hereford × Charolais	1.3%	$110.48 ± 0.82 [c, d]
Angus × Hereford × ¼ Brahman	2.3%	$110.22 ± 0.62 [c, d]
Hereford	4.6%	$109.82 ± 0.44 [d]
Angus × Charolais	3.8%	$109.27 ± 0.48 [d,e]
Hereford × Brahman x Angus	0.3%	$108.95 ± 1.70 [d,e]
Charolais	15.0%	$108.65 ± 0.24 [d,e]
Charolais × Limousin	1.4%	$108.48 ± 0.78 [d,e,f]
Limousin	6.7%	$108.02 ± 0.36 [e,f]
Hereford × Limousin	0.9%	$107.74 ± 1.01 [e,f,g]
Brangus	11.0%	$107.18 ± 0.28 [f, g]
Charolais × ¼ Brahman	2.7%	$105.60 ± 0.57 [g, h]
½ Brahman × other crosses	1.4%	$105.31 ± 0.81 [g, h]
¼ Brahman × other crosses	8.2%	$105.23 ± 0.31 [e,h]
Hereford × ¼ Brahman	1.1%	$104.16 ± 0.32 [h]
Simmental	0.5%	$99.90 ± 0.89 [i]
Brahman	0.5%	$94.34 ± 1.29 [j]
Longhorn	1.0%	$71.75 ± 0.96 [k]

[a] Main effect of breed or breed type on selling price ($P < 0.0001$).
[b] Least-squares mean ± SE ($/cwt.).
[c…k] Least square means without a common superscript differ ($P < 0.001$).

Table 3. The percentage of the sampled population and 2010 Arkansas selling prices (mean ± SE) of feeder calves sold based on calf color[a].

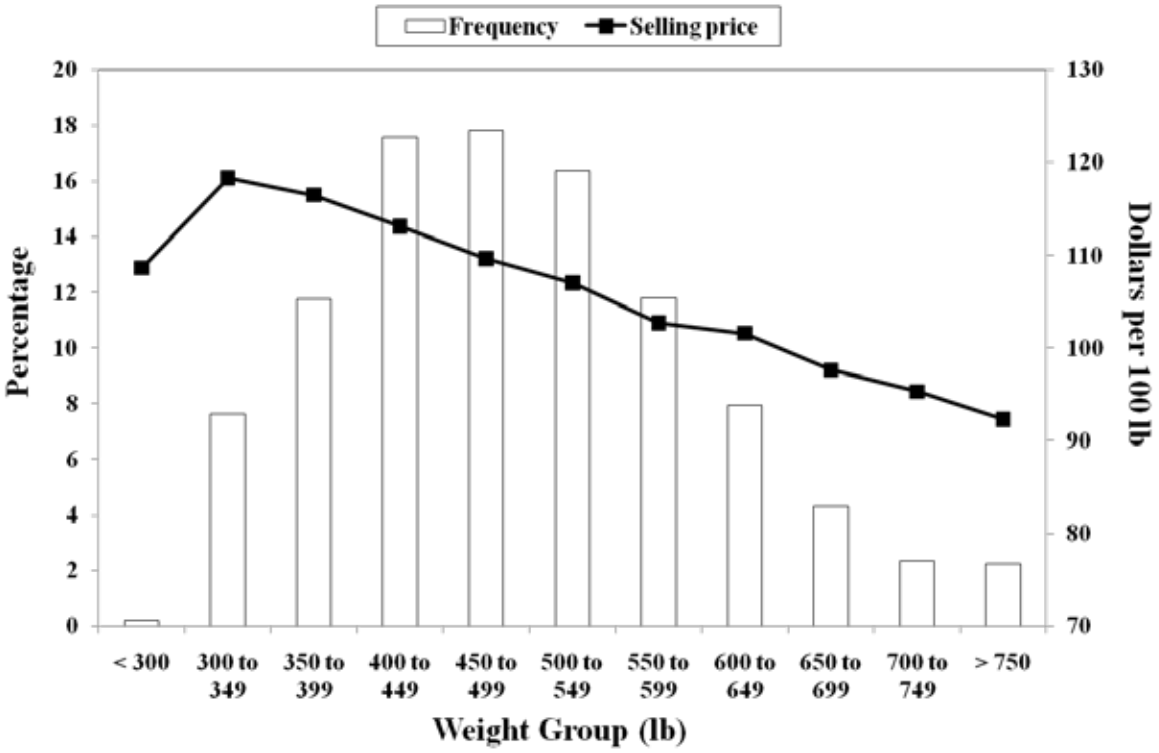
Calf color	Percentage of the sampled population	Selling price ($/cwt.)[b]
Black-white Face	10.9%	$111.74 ± 0.29 [c]
Black	42.1%	$110.23 ± 0.14 [d]
Yellow	10.3%	$110.09 ± 0.29 [d]
Yellow-white Face	2.5%	$109.81 ± 0.61 [d]
Grey	7.5%	$106.88 ± 0.34 [e]
White	5.0%	$104.55 ± 0.41 [f]
Red-white Face	5.2%	$104.45 ± 0.42 [f]
Grey-white Face	0.7%	$103.99 ± 1.18 [f]
Red	8.6%	$103.76 ± 0.32 [f]
Spotted or Stripped	1.4%	$82.16 ± 0.81 [g]

[a] Main effect of calf color on selling price ($P < 0.0001$).
[b] Least-squares mean ± SE ($/cwt.).
[c…g] Least square means without a common superscript differ ($P < 0.001$).



[a] Main effect of month on selling price ($P < 0.0001$).
[b] Unlike superscripts for the months of 2010 are different ($P < 0.01$).

Fig. 1. The mean selling price for year 2010 and the 5-, 10- and 20-yr averages for 400 to 500 lb feeder cattle by month[a, b].



[a] Main effect of weight group on selling price ($P < 0.0001$).
[b] Least-squares mean (dollars/100 lb).

Fig. 2. The percentage of the sampled population and mean selling price of calves by weight groups[a, b].

Relationship of temperament scores and prolactin promoter polymorphisms in Angus calves

A. R. Starnes[1], A. H. Brown[1], Jr., Z. B. Johnson[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 and temperament scores in Angus calves. Data was collected over a four-year period (2005 through 2008) on spring-born calves (n = 110) weaned in the fall of each year. Temperament was determined using a chute score of 1 to 5, where 1 was calm and 5 was berserk frenzy. In addition, calves were genotyped using genomic DNA prepared from buffy coat and previously published primers for the prolactin gene. Genotypes were homozygous cytosine (CC; n = 9), heterozygous (CT; n = 80), and homozygous thymine (TT; n = 21). Chute scores were affected by prolaction genotype only in December. Average daily gain and genotype also have a genotype interaction ($P < 0.05$).

Introduction

The behavioral reaction that cattle have to stressful situations is considered temperament, and temperament is of importance to the cattle industry because it affects production and cost. Cattle with excitable temperament ratings have reduced growth rates and greater incidences of dark-cutter carcasses (Voisinet et al., 1997), and bruising, which cost the beef industry $3.91/head marketed or approximately $30 million annually (Grandin, 1994). Reports of cattle with high excitability that become easily agitated when handled or restrained have increased over the years (Grandin, 1994). Temperament is a heritable trait and can be changed through genetic selection; however, over-selection may have detrimental effects on some economically important traits. Therefore, the objective of this study was to determine the relationship between prolactin promoter single nucleotide polymorphisms (SNPs) and temperament scores in Angus calves.

Materials and Methods

Purebred Angus calves (n = 110) were spring-born and weaned in the fall of each year of this four year study (2005 through 2008). Both sexes were included in this study and all calves were registered with the American Angus Association. Sires were selected with a balanced approach to expected progeny differences (EPDs), but temperament was not considered during sire selection. Calf temperament was determined using a five-point chute score (1 = calm and 5 = berserk frenzy). Data were collected in December, February, March, May, and June of each year. Weaning weight, yearling weight, and average daily gain (ADG) were also considered in the analysis.

Calves were genotyped using genomic DNA prepared from buffy coat and previously published primers for the prolactin gene (Looper, 2010). Genotypes were homozygous cytosine (CC; n = 9), heterozygous (CT; n = 80), and homozygous thymine (TT; n = 21). Estimates were obtained using Proc Mixed procedure and correlations were estimated using the Proc Corr procedure (SAS Inst. Inc, Cary, N.C.). Genotype and year were the fixed and random effects, respectively, included in the model.

Results and Discussion

The genotypes CC and TT were more excitable ($P < 0.05$) than the CT genotype in December. However, there are no other months x genotype interactions for chute scores. These results could be attributed to a lack of human handling at this age. Furthermore, genotype TT had the lowest ($P < 0.05$) ADG when compared to CC and CT genotypes, but weaning and yearling weights did not differ among genotypes.

Correlations for chute scores for the months of November, December, February, March, May, and June along with the correlations for weaning weight and yearling weight are presented in Table 1. November had a positive correlation for February chute scores ($r = 0.075$), March chute scores ($r = 0.084$), May chute scores ($r = 0.107$), and June chute scores ($r = 0.022$). December chute scores had a positive correlation to February chute scores ($r = 0.061$), March chute scores ($r = 0.002$), May chute scores ($r = 0.002$), and June chute scores ($r = 0.001$). February chute scores had a positive correlation with March chute scores ($r = 0.0008$), May chute scores ($r = 0.001$), and June chute scores ($r = 0.001$). March chute scores had a positive correlation with May chute scores ($r = 0.01$). May chute scores had a positive correlation with June chute scores ($r = 0.05$). Weaning weight was a positively correlated with yearling weight ($r = 0.555$) and March chute scores ($r = 0.021$). Yearling weight had a positive correlation with weaning weight ($r = 0.555$), March chute scores ($r = 0.022$), and June chute scores ($r = 0.186$).

Implications

Temperament can be controlled through selection for docility. By selection for less excitable cattle, productivity and manageability can be improved. In having calmer cattle, handlers have less chance of injury. According to research, ADG is also improved through selection for docile cattle. Not only will selection for docility improve cattle herds, also an increase in human contact with cattle may improve temperament scores. Our study showed that chute scores were affected by prolactin genotype only in December, which was the second time after weaning they were handled. With more

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time and handling, the effect of genotype on chute scores was no longer significant, suggesting selection and handling can improve temperament scores.

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Table 1. Pearson correlations for Weaning weight (WnWt), yearling weight (YearWt), November (NovCS), December (DecCS), February (FebCS), March (MarCS), May (MayCS), and June (JuneCS) chute scores.

	WnWt	YearWt	NovCS	DecCS	FebCS	MarCS	MayCS	JuneCS
WnWt	1.00	0.555*	-0.049	-0.089	-0.091	0.021	-0.096	-0.023
YearWt		1.00	-0.055	-0.039	-0.168	0.022	-0.095	0.186
NovCS			1.00	-0.149	0.075	0.084	0.107	0.022
DecCS				1.00	0.061	0.359**	0.357**	0.511*
FebCS					1.00	0.315*	0.302*	0.429**
MarCS						1.00	0.244**	0.173
MayCS							1.00	0.319*
JuneCS								1.00

*$P < 0.001$
**$P < 0.05$

Effects of fescue cultivar and heat shock protein haplotype on growth and fertility of crossbred beef heifers

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Story in Brief

Thirty-six Angus-sired heifers were weighed and assigned to replicated pastures of toxic (E+) or non-toxic (Novel) endophyte-infected tall fescue for 190 d. Data were collected at weaning (7 to 8 mo), yearling (10 to11 mo), and prebreeding (13 to 14 mo), and included: body weight, hip height, hip width, pelvic height, pelvic width, pelvic area and exit velocity; sec/m). Heifers were haplotyped based on heat shock protein 70 sequences. Radio-telemetry was used to monitor estrus during the first 30 d of the breeding season. Antral follicle count was determined via ultrasound at yearling (d 75 and d 85) and prebreeding (d 183). Overall ADG tended to be greater ($P = 0.08$) for heifers grazing novel endophyte fescue (1.32 ± 0.22 lbs) versus heifers grazing toxic endophyte fescue (0.88 ± 0.22 lbs). Hip width increased ($P < 0.001$) over time. Novel endophyte fescue heifers (14.4 ± 0.1 in) had larger ($P < 0.05$) hip width compared with toxic endophyte fescue heifers (14.1 ± 0.1 in). Similarly, novel endophyte fescue heifers (5.5 ± 0.1 in) pelvic height tended ($P < 0.06$) to be greater than in toxic endophyte heifers (5.3 ± 0.1 in); consequently, pelvic area tended ($P < 0.06$) to be larger in novel endophyte fescue heifers (61.6 ± 1.2 in2) than toxic endophyte fescue heifers (58.6 ± 1.2 in2). Heifers grazing toxic endophyte fescue (0.6 ± 0.1 sec/m) tended ($P < 0.08$) to exit the chute faster than novel endophyte fescue heifers (0.8 ± 0.1 sec/m). Day of scan affected ($P < 0.007$) antral follicle count (9.0, 13.6, and 10.2; d 75, d 85, and d 183, respectively). Fescue cultivar affected ($P < 0.002$) antral follicle count (8.4 vs. 13.5; novel endophyte fescue vs. toxic endophyte fescue) and pregnancy rates (81.0% vs. 36.0%; novel endophypte fescue vs. toxic endophyte fescue). Haplotypes of heat shock protein 70 promoter ($P < 0.08$) and coding sequences ($P < 0.003$) affected antral follicle count. Heifers grazing toxic endophyte fecue had higher antral follicle counts but lower pregnancy rates compared to novel endophyte fescue heifers, suggesting that antral follicle count may not be a useful predictor of fertility in heifers grazing toxic tall fescue.

Introduction

Fescue toxicosis in cattle is characterized by elevated body temperature, reduced feed intake, lowered average daily gain (ADG) and reduced conception rates (Burke et al., 2001). Studies have shown heifers grazing toxic endophyte infected fescue (E+) have suppressed growth which could be detrimental to reproduction (Paterson et al., 1995). Burke et al. (2004) found that heat stress combined with E+ consumption caused reduced preovulatory follicle diameter and fewer antral follicles during heifer estrous cycles. Pregnancy rate and embryonic loss tended to be different among cows between 30 and 60 d of gestation after temperature elevation for 3 weeks when grazing E+ or novel endophyte infected fescue (Novel; Burke et al., 2001).

Stress proteins such as heat shock proteins are front-line defense molecules expressed when cells are exposed to stressors. Heat shock protein 70 (Hsp70) possesses numerous functions including acting as a molecular chaperone. As a molecular chaperone, Hsp70 provides thermo-tolerance to stressed cells and assists in protein folding. Rosenkrans et al. (2010) documented reduced calving rates for cows with a deletion in the promoter region of Hsp70. Our objective was to determine the influence of fescue cultivar and Hsp70 haplotype of beef heifer on growth and fertility.

Materials and Methods

Test Animals. Thirty-six Angus-sired (1/4 to 3/8 Brahman) heifers were weighed and assigned to replicated pastures of toxic (E+) or non-toxic (Novel) endophyte-infected tall fescue for 190 d. Throughout the study, heifer husbandry was in accordance with guidelines recommended by The Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, Consortium (1988).

Growth Parameters. Data were obtained at weaning (7 to 8 mo), yearling (10 to 11 mo), and prior to the 60 d breeding season (13 to 14 mo). The following growth parameters were collected: body weight (BW), hip height (HH), hip width (HW), pelvic height (PH), pelvic width (PW), pelvic area (PA), and chute exit velocity (EV; s/m). To measure HH and HW, a sliding caliper which was developed specifically to measure external body dimensions in beef cattle was used (Altitude Stick, NASCO, Fort Atkinson, Wis.). Measurements for PH and PW were obtained per rectum using a Rice Pelvimeter (Lane Manufacturing, Denver, Colo.). Exit velocity was recorded using two infrared sensors (FarmTek, Inc., North Wiley, Texas) to determine the rate at which the heifers exited the squeeze chute and traversed 1.83 m. Exit velocity was recorded as time (sec)/distance (m).

Reproductive Traits. Antral follicle count (AFC) is the total number of follicles > 3 mm in diameter on both ovaries as described by Ireland et al. (2008). Antral follicle count was determined via ultrasound scan by an individual technician using real-time ultrasonography (Aloka 500 V®; Corometrics, Wallingford, Conn., equipped with a 5.0-MHz transducer) at yearling (d 75 and d 85) and prebreeding (d 183). Heifers received a gonadotropin releasing hormone injection (GnRH) along with a controlled internal drug-releasing device (CIDR) for 7 d prior to the 60 d breeding season, and were administered prostaglandin $F_2\alpha$ at removal of CIDR. Estrus behavior was monitored by radio-telemetry (HeatWatch, Cow Chips LLC, Denver, Colo.) during the first 30 d of the breeding season. Pregnancy diagnostics were conducted at 60 d after bull removal by ultrasound.

Genotypes and Haplotypes. Blood was collected from heifers by venipuncture of the median caudal vein using EDTA-containing Vacutainers (Becton Dickinson, Frankling Lakes, N.J.). Samples were immediately placed on ice following collection and centrifuged at

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1,200 × g for 20 min (Marathon 22KBR, Fisher Scientific, Hermle-Labortechnik, Germany) to isolate the buffy coat. Genomic DNA was obtained from the buffy coat (QIAGEN Inc., Valencia, Calif.) and diluted to 20 ng/μL.

Heifers were haplotyped based on Hsp70 promoter sequence as described by Rosenkrans et al. (2010); No = no SNP; Deletion = cytosine deletion at nucleotide base 895; Yes = a SNP other than a deletion. Heifers also were haplotyped for Hsp70 coding sequences; Haplotype 1 = same sequence as the National Center for Biotechnology Information (NCBI); Haplotype 2 = cytosine to guanine base change at base 2087; and Haplotype 3 = guanine to cytosine base change at base 2033. Polymerase chain reaction (PCR) primers for Hsp70 promoter sequencing [forward (5'-GCCAGGAAACCAGAGACAGA-3') and reverse (5'-CCTACGCAGGAGTAGGTGGT-3')] were utilized to amplify a 539-base sequence that spanned a conserved region within the Hsp70 promoter region. Coding sequence primers for Hsp70 [forward (5'-GAAGAGCGCCGTGGAGGATG -3') and reverse (5'-CTTGGAAGTAAACAGAAACGGG -3')] were utilized to amplify a 570-base sequence for PCR. A Peltier thermal cycler (MJ Research, Waltham, Mass.) was used for PCR. Each PCR began with an initial 2-min heating at 94 °C, followed by 35 cycles at 94 °C for 30 s, 1 min at 55 °C, and 1 min at 68 °C. A final elongation step consisted of 10 min at 68 °C. Approximately 5 μL of 20 ng/μL genomic DNA, 1 μL of 10 μM forward primer, 1 μL of 10 μM of reverse primer and 43 μL of Platinum PCR Supermix (Invitrogen, Carlsbad, Calif.) for a total volume of 50 μL in each PCR. Amplicons were visualized via electrophoresis in 1.2% agarose gels stained with ethidium bromide in 1.0X Tris/Boric Acid/EDTA.

Amplification products were purified using QIAquick PCR purification kit (QIAGEN Inc., Valencia, Calif.). Purified PCR products were sequenced at the University of Arkansas DNA Core Lab using ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif.). Sequences were compared using the web-based software package ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html; European Bioinformatics Institute, Cambridge, UK). Homozygous and heterozygous alleles were identified by assessing sequence chromatograms using BioEdit.

Statistical Analyses. The experimental design was randomized complete block and pasture was the experimental unit in all statistical analyses. Haplotypes, growth measurements and AFC were analyzed by ANOVA utilizing the MIXED procedure (SAS Inst., Cary, N.C.). Pregnancy rate was analyzed by chi-squared analysis of SAS.

Results and Discussion

Heifers grazing E+ tall fescue had reduced overall growth compared to heifers grazing Novel fescue which reiterates previous research (Paterson et al., 1995). Overall ADG tended ($P = 0.08$) to be greater for heifers grazing novel (1.32 ± 0.22 lbs) than E+ heifers (0.88 ± 0.22 lbs). Heifers grazing E+ tall fescue had smaller pelvic height, pelvic width and pelvic area. Hip width increased (day effect; ($P < 0.001$) over time. Novel heifers (14.4 ± 0.1 in) had larger ($P < 0.05$) HW compared with E+ heifers (14.1 ± 0.1 in). Similarly, PH tended ($P < 0.06$) to be greater in Novel heifers (5.5 ± 0.1 in) than E+ heifers (5.3 ± 0.1 in); consequently, PA tended ($P < 0.06$) to be larger in Novel heifers (61.6 ± 1.2 in^2) than E+ heifers (58.6 ± 1.2 in^2). Heifers grazing E+ (0.6 ± 0.1 sec/m) tended ($P < 0.08$) to exit the chute faster than Novel heifers (0.8 ± 0.1 sec/m) suggesting E+ heifers were more excitable or nervous.

There were no interactions ($P > 0.12$) between haplotypes and fescue or haplotypes and day. Heifers grazing E+ tall fescue had higher AFC compared to Novel heifers; however, Novel heifer pregnancy rates were greater than heifers grazing E+. The day of scan affected ($P < 0.007$) AFC (9.0 antral follicles, 13.6 antral follicles, and 10.2 antral follicles; d 75, 85, and 183, respectively) (Table 2). Fescue cultivar affected ($P < 0.002$) AFC (8.4 antral follicles vs. 13.5 antral follicles; Novel vs.E+) and pregnancy rates (81.0% vs. 36.0%; Novel vs.E+). These data are inconsistent with previous studies that indicate AFC is associated with increased fertility in cattle (Ireland et al., 2008); however, there may be other factors involved. Haplotypes of Hsp70 promoter ($P < 0.08$) and coding sequence ($P < 0.003$) affected AFC.

Implications

Heifers grazing toxic endophyte tall fescue had a reduction in overall growth compared to heifers grazing novel endophyte infected fescue; thus, livestock producers should utilize novel endophyte infected fescue for development of replacement beef heifers. Heifers grazing toxic endophyte infected fescue had higher antral follicle counts compared to novel endophyte heifers; however, pregnancy rate in heifers grazing novel endophyte infected fescue was significantly higher than in heifers grazing toxic endophyte infected fescue suggesting other factors may have impacted antral follicle count. Additionally, heat shock protein 70 promoter and coding sequence haplotypes may be useful indicators of fertility in replacement heifers.

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Table 1. Overall growth and development measurements of beef heifers grazing either toxic endophyte-infected (E+) or non-toxic (Novel) endophyte-infected tall fescue.

Parameter	E+	Novel	P Value
Average Daily Gain, lbs	0.88 ± 0.22	1.32 ± 0.22	0.08
Hip Width, in	14.1 ± 0.1	14.4 ± 0.1	< 0.05
Pelvic Height, in	5.3 ± 0.1	5.5 ± 0.1	< 0.06
Pelvic Area, in^2	58.6 ± 1.2	61.6 ± 1.2	< 0.06
Exit Velocity, s/m	0.6 ± 0.1	0.8 ± 0.1	< 0.08

Table 2. Heifer prebreeding antral follicle count (AFC) and subsequent pregnancy rate of beef heifers grazing either toxic endophyte-infected (E+) or non-toxic (Novel) endophyte-infected tall fescue.

Parameter	E+	Novel	P Value
Antral Follicle Count	13.7	10	< 0.002
Pregnancy Rate, %	36.0	81.0	< 0.05

Relationship between polymorphisms in the prolactin promoter and cytochrome P450 genes and pre-weaning performance by steers weaned from wild-type or non-toxic endophyte-infected tall fescue pastures

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Story in Brief

Fescue toxicosis causes a reduction in cattle performance by reducing feed intake and weight gain. Mutations in specific genes may interact with tall fescue toxicosis and may affect animal growth. The objective of this study was to determine the relationship among single nucleotide polymorphisms in the prolactin promoter and cytochrome P450 genes with pre-weaning performance by steers weaned from toxic wild type or non-toxic endophyte-infected tall fescue. A total of 156 spring or fall-born Gelbvieh × Angus steers were born on and weaned from cows that grazed either wild type tall fescue for the entire year, or from spring or fall calving cows that grazed wild type tall fescue for most of the year but grazed non-toxic endophyte infected tall fescue for 1 mo. prior to weaning. Additional spring born steers grazed non-toxic endophyte infected tall fescue continuously from birth to weaning. Genomic DNA was collected from white blood cells and genotypes were determined. Genotypes were homozygous cytosine (CC for both genes), homozygous thymine and guanine (TT for prolactin promoter, and GG for cytochrome P450, respectively), and heterozygous (CT for prolactin promoter, and CG for cytochrome P450 gene). Weaning weight, adjusted weaning weight, pre-weaning gain, and pre-weaning average daily gain (ADG) were affected ($P < 0.05$) but not by prolactin and cytochrome P450 genotypes or their interaction with treatments. Weaning weight, pre-weaning gain, and pre-weaning ADG were greater ($P < 0.05$) by steers weaned from cows grazing non-toxic endophyte infected tall fescue year round, steers weaned from fall calving cows grazing non-toxic endophyte infected tall fescue for one month prior to weaning, and steers weaned from fall calving cows grazing wild type tall fescue year round than for steers weaned from spring calving cows grazing wild type tall fescue year round and steers weaned from spring calving cow sgrazing non-toxic endophyte infected tall fescue one month prior to weaning. Therefore, grazing spring-born calves on non-toxic endophyte-infected tall fescue, or grazing fall-born calves on wild-type tall fescue may impact steer pre-weaning performance measurements positively, but variations in prolactin promoter and cytochrome P450 genes may not affect pre-weaning performance by steers managed under the conditions of this experiment.

Introduction

Endophyte-infected tall fescue (E+) is a commonly-used forage in grazing systems in the southeastern U.S. It is infected with a fungus which produces ergot alkaloids that when consumed by cattle, impacts their performance negatively (Porter, 1995). Furthermore, the interaction between ergot alkaloids and specific gene mutations (polymorphisms) may also impact cattle performance and productivity (Rosenkrans, 2007). Therefore, the objective of this study was to determine the relationship among single nucleotide polymorphisms (SNP) in prolactin promoter and cytochrome P450 genes and pre-weaning performance from steers weaned from toxic wild type (E+) or non-toxic (NE+) endophyte-infected tall fescue.

Materials and Methods

Treatments and Steer Management. A total of 156 Gelbvieh × Angus crossbreed spring (S) and fall-born (F) steers at the University of Arkansas Livestock and Forestry Research Station near Batesville, Ark., were born on, and weaned from 1 of 5 cow-calf grazing treatments: 1) F100; F grazing 100% E+, 2) S100; S grazing 100% E+, 3) F75; F grazing 75% E+ and 25% NE+, 4) S75; S grazing 75% E+ and 25% NE+, and 5) SNE100; S grazing 100% NE+. Steers that were assigned to the F100 and S100 groups grazed E+ from birth to weaning and those assigned to the SNE100 groups grazed NE+ from birth to weaning. Steers that were assigned to S75 and F75 groups grazed E+ most of the time prior to weaning, but were moved to NE+ 28 days prior to weaning to reduce the negative effects of E+. All pastures were grazed using a rotational grazing system.

Sample Collection and Analysis. Blood samples were collected into vacuum tubes and Buffy coats (white blood cells) were collected. All steers were genotyped for SNPs in the prolactin promoter and cytochrome P450 genes resulting in CC, CT, and TT genotypes for the prolactin promoter gene and CC, CG, and GG genotypes for the cytochrome P450 gene.

Statistical Analysis. Data were analyzed using PROC MIXED of SAS (version 9.1, SAS Institute, Inc. Cary, N.C.) with steer considered the experimental unit for the genotype and genotype × treatment effects and group of steers within a pasture were considered the experimental unit for the treatment effects. Treatments within pastures were considered as random effects. The mean separation was performed using an F-protected t-test.

Results and Discussions

Weaning weight, adjusted weaning weight, pre-weaning gain, and pre-weaning average daily gain (ADG) were affected ($P < 0.05$) by treatments but not by prolactin promoter genotype or the treatment × prolactin promoter genotype interaction (Table 1). Birth weight was not affected ($P = 0.63$) by either main effect or interaction among main effects. However, others reported that SNPs in prolactin promoter genes affected calf birth and weaning weights (Rosenkrans,

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2009). Steers weaned from SNE100, F75, and F100 had greater ($P < 0.05$) weaning weight, pre-weaning gain, and pre-weaning ADG than steers weaned from S100 and S75. Adjusted weaning weight was greater from steers weaned from SNE100 than those weaned from S100 and S75.

Pre-weaning performance measurements were not affected by the cytochrome P450 genotypes or their interactions with pre-weaning treatments (Table 2). Therefore, only effects of pre-weaning treatments were observed. Weaning weight and pre-weaning gain were greater ($P < 0.05$) for steers weaned from SNE100 than steers weaned from S100 and S75. Polymorphisms in the cytochrome P450 polynucleotide were predictive of calf weight at weaning among cattle that grazed E+ in a previous study (Rosenkrans, 2009).

Pre-weaning ADG was greater ($P < 0.05$) by steers weaned from SNE100 and F75 than by steers weaned from S100 and S75. These data are in agreement with other who reported reduced gains by calves weaned from E+ (Paterson et al., 1995).

Therefore, grazing novel endophyte-infected tall fescue before weaning affected steer weaning weight, adjusted weaning weight, pre-weaning gain, and pre-weaning ADG positively. Polymorphisms in prolactin promoter and cytochrome P450 genes had no effect on steers pre-weaning performance, and the interaction between treatments and genotypes did not impact these pre-weaning performance measurements.

Implications

Changing the calving season from spring to fall and grazing spring-born calves on NE+ prior to weaning may impact steer pre-weaning performance measurements positively. However, variations in prolactin promoter and cytochrome P450 genes may not affect pre-weaning performance by steers managed under the conditions of this experiment.

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Table 1. Relationship among the single nucleotide polymorphisms in the prolactin promoter gene and grazing treatments, for pre-weaning performance by steers weaned from E+ and (or) NE+.

		Treatments[1]						
Item	Genotype[2]	F100	F75	SNE100	S100	S75	Effects[3]	SEM[4]
Birth Weight	CC	85	83	92	85	97	ns	5.1
	CT	80	89	85	81	85		
	TT	84	82	73	92	78		
Actual Weaning Wt.	CC	570	611	627	556	510	Tr[a]	27.3
	CT	572	629	622	478	528		
	TT	597	591	670	535	532		
Adjusted Weaning Wt.	CC	500	536	578	501	480	Tr[b]	24.9
	CT	513	547	562	438	491		
	TT	530	514	579	485	468		
Pre-weaning Gain	CC	485	528	535	471	413	Tr[a]	25.2
	CT	492	540	537	397	443		
	TT	514	509	597	443	453		
Pre-weaning ADG	CC	2.0	2.2	2.4	2.0	1.9	Tr[a]	0.10
	CT	2.1	2.2	2.3	1.7	2.0		
	TT	2.2	2.1	2.5	1.9	1.9		

[1] F100 = fall born steers weaned from wild-type endophyte infected tall fescue (E+), F75 = fall born steers weaned from 75% E+ and 25% NE+, SNE100 = spring born steers weaned from non-toxic novel endophyte-infected tall fescue (NE+), S100 = spring born steers weaned from100% E+, S75 = spring born steers weaned from 75% E+ and 25% NE+.

[2] Prolactin gene allele CC represents steers that were homozygous cytosine, TT allele represents steers that were homozygous thymine, and CT represents steers that were heterozygous cytosine and thymine.

[3] Main effects of treatments (Tr, $P < 0.05$), on pre-weaning performance.

[4] SEM = pooled standard error of the mean.

[a] Means by steers weaned from SNE100, F75, and F100 were greater ($P < 0.05$) than those weaned from S100 and S75.

[b] Means by steers weaned from SNE100 were greater ($P < 0.05$) than those weaned from S100 and S75; means by steers weaned from F75 and F100 were intermediate and did not differ ($P > 0.05$) from the other treatments.

Table 2. Relationship among single nucleotide polymorphisms in the cytochrome P450 gene and grazing treatments for pre-weaning performance by steers weaned from E+ and (or) NE+.

		Treatments[1]						
Item	**Genotype[2]**	**F100**	**F75**	**SNE100**	**S100**	**S75**	**Effects[3]**	**SEM[4]**
Birth Weight	CC	86	79	89	87	93	ns	5.5
	CG	82	86	90	91	89		
	GG	81	88	84	78	82		
Actual Weaning Wt.	CC	560	598	649	599	530	Tr[a]	28.5
	CG	577	619	625	535	520		
	GG	568	617	624	470	527		
Adjusted Weaning Wt.	CC	532	522	561	531	509	tr	26.7
	CG	506	541	572	488	480		
	GG	509	537	566	430	481		
Pre-weaning Gain	CC	474	519	560	512	437	Tr[a]	26.7
	CG	495	532	535	444	431		
	GG	487	529	540	392	446		
Pre-weaning ADG	CC	2.2	2.2	2.3	2.2	2.0	Tr[b]	0.10
	CG	2.1	2.2	2.4	1.9	1.9		
	GG	2.1	2.2	2.4	1.7	2.0		

[1] F100 = fall born steers weaned from wild type endophyte infected tall fescue (E+), F75 = fall born steers weaned from 75% E+ and 25% NE+, SNE100= spring born steers weaned from non-toxic novel endophyte-infected tall fescue (NE+), S100 = spring born steers weaned from100% E+, S75 = spring born steers weaned from 75% E+ and 25% NE+.

[2] Cytochrome P450 gene allele CC represents steers that were homozygous cytosine, GG allele represents steers that were homozygous guanine, and CG represents steers that were heterozygous cytosine and guanine.

[3] Main effects of treatments (Tr and tr, $P < 0.05$ and $P < 0.10$, respectively) on pre-weaning performance.

[a] Means by steers weaned from SNE100 were greater ($P < 0.05$) than those weaned from S100 and S75; means by steers weaned from F75and F100 were intermediate and did not differ ($P > 0.05$) from the other treatments.

[b] Means by steers weaned from SNE100 and F75 were greater ($P < 0.05$) than those weaned from S100 and S75; means by steers weaned from F100 were intermediate and did not differ ($P > 0.05$) from the other treatments.

Relationship between polymorphisms in the prolactin promoter and cytochrome P450 genes and post-weaning performance by steers weaned from wild-type or non-toxic endophyte-infected tall fescue pastures

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Story in Brief

The objective of this study was to determine the relationship among single nucleotide polymorphisms in prolactin promoter and cytochrome P450 genes with post-weaning performance of steers weaned from toxic wild type or non-toxic endophyte-infected tall fescue. A total of 156 spring or fall-born Gelbvieh × Angus steers (542.7 ± 82.0lb) were weaned from calving systems where they grazed either toxic wild type tall fescue for the entire year, or from calving systems that grazed toxic wild type tall fescue for most of the year, but grazed non-toxic endophyte-infected tall fescue for 1 mo. prior to weaning in either spring or fall. Additional spring born steers grazed non-toxic endophyte-infected tall fescue continuously prior to weaning. Genotypes of homozygous cytosine (CC for both genes), homozygous thymine and guanine (TT for prolactin promoter, and GG for cytochrome P450, respectively), and heterozygous (CT for prolactin promoter, and CG for cytochrome P450 gene) were determined from collected white blood cells. Post-weaning gain was greater ($P < 0.05$) for steers with TT prolactin genotype and weaned from spring calving cows grazing wild type tall fescue year round than those weaned from fall calving cows grazing wild type tall fescue years round or from fall calving cows that grazed non-toxic endophyte infected fescue for one month prior to weaning. Feedlot gains were greater ($P < 0.05$) for steers with TT genotype compared with CC within the group weaned from fall calving cows grazing non-toxic endophyte infected fescue one month prior to weaning, and greater ($P < 0.05$) for CC compared with TT genotype within steers weaned from spring calving cows grazing non-toxic endophyte fescue for one month prior to weaning. Steers weaned from spring calving cows grazing non-toxic endophyte infected tall fescue year round, from spring calving cows grazing wild type tall fescue year round, and from spring calving cows grazing non-toxic endophyte infected tall fescue for one month prior to weaning had larger ($P < 0.05$) longissimus area than steers weaned from fall calving cows grazing wild-type tall fescue year round or from fall calving cows grazing non-toxic endophyte infected fescue for one month prior to weaning. Results from this study indicate that the interaction between fescue grazing and specific polymorphic genotypes may impact post-weaning performance for steers weaned from non-toxic endophyte-infected tall fescue or toxic wild type tall fescue.

Introduction

Grazing endophyte-infected tall fescue (E+) negatively impacts cattle feed intake and growth negatively (Crawford et al., 1989). However, the interaction between environmental factors and animal genetics may influence important cattle growth measurements before and after the weaning process (Phillips et al, 2001). For example, polymorphisms (mutations) in the prolactin and cytochrome P450 genes were related to cattle productivity and profitability (Rosenkrans, 2007), and may interact with ergot alkaloids produced by the endophyte in toxic tall fescue which affect animal performance. Furthermore, selection of cattle with greater carcass composition, such as heavier carcass weight, is important for both producers and consumers (Zhao et al., 2004). Therefore, the objective of this study was to determine the relationship among single nucleotide polymorphisms (SNP) in prolactin promoter and cytochrome P450 genes and post-weaning performance and carcass measurements from steers weaned from toxic wild type (E+) or non-toxic (NE+) endophyte-infected tall fescue.

Materials and Methods

Treatments and Steer Management. Spring (S) and fall-born (F) Gelbvieh × Angus crossbreed steers (n = 155; 542.7 ± 82.0 lb; average body weight (BW) at weaning) from the University of Arkansas Livestock and Forestry Research Station near Batesville, Ark., were weaned from 1 of 5 cow-calf grazing treatments: 1) F100; F grazing 100% E+, 2) S100; S grazing 100% E+, 3) F75; F grazing 75% E+ and 25% NE+, 4) S75; S grazing 75% E+ and 25% NE+, and 5) SNE100; S grazing 100% NE+. Steers assigned to the F100 and S100 groups grazed E+ from birth to weaning, whereas those assigned to the SNE100 groups grazed NE+ from birth to weaning. Steers assigned to S75 and F75 groups grazed E+ most of the time prior to weaning, but were moved to NE+ 28 d prior to weaning to reduce the negative effects of E+. All pastures were grazed using a rotational grazing system. The weaning dates were May 14, 2008, and May 12, 2009 for F and October 23, 2008, and October 22, 2009 for S.

Steers were gathered 28 d prior to weaning, weighed, and vaccinated against 7 clostridial strains, infectious bovine rhinotraceitis (IBR), bovine virus diarrhea (BVD), parainfluenza (PI3), bovine respiratory syncytial virus (BRSV), *Haemophilus somnus*, and 5 strains of *Leptospira*. At weaning, steers were re-vaccinated and were treated for internal parasites. During the 14-d weaning period, steers had access to bermudagrass hay, water, and trace mineral salt. After the 14-d weaning period, S were moved to winter annual pastures while F were moved to bermudagrass pastures and grazed those pastures until they were shipped to the Oklahoma State University feedlot facility and finished on a high-concentrate diet. Steers were harvested at a commercial slaughter facility at a constant 12th rib of 0.4-in fat thickness, and the carcass data were collected by experienced personnel from Garden City Community College (Garden City, Kan.) following a 24-h chill.

[1] University of Arkansas System Division of Agriculture, Department of Animal Science, Fayetteville, Ark.
[2] University of Arkansas System Division of Agriculture, Livestock and Forestry Research Station, Batesville.

Sample Collection and Analysis. Blood samples were collected into vacuum tubes and Buffy coats (white blood cells) were collected. Steers were genotyped for SNPs in the prolactin promoter and cytochrome P450 genes resulting in CC, CT, and TT genotypes for the prolactin promoter gene and CC, CG, and GG genotypes for the cytochrome P450 gene.

Statistical Analysis. Data were analyzed using PROC MIXED of SAS (version 9.1, SAS Institute, Inc. Cary, N.C.) with steer considered the experimental unit (n = 155) for the genotype and genotype × pre-weaning grazing treatment effects and group of steers within a pasture (n = 14 pastures) were considered the experimental unit for the pre weaning grazing treatment effects. Mean separation was performed using an F-protected t-test.

Results and Discussions

Post-weaning weight gain (average of 200 d period post-weaning for both S and F groups) was greater ($P < 0.05$) for steers with TT genotype and weaned from S100 than TT and weaned from F100 (Table1). Shipping weight was greater ($P < 0.05$) across treatments by TT and CT steers than by CC steers. There was also a tendency for the treatment × genotype interaction for shipping weight and was greater ($P < 0.10$) for steers with TT genotype and weaned from SNE100 than from steers with CC and weaned from S75 (Table 1). Feedlot gain was affected by the genotype by treatment interaction. For steers with the CC and CT genotypes, feedlot gain did not differ ($P < 0.10$) among pre-weaning grazing treatments. However, feedlot gain was greater for steers from F75 and F100 than S75 within the TT genotype (Table 1). Feedlot average daily gain was affected by genotype, but not by treatment × genotype interaction ($P > 0.10$). Steers with CC genotype had greater ($P < 0.05$) feedlot ADG than CT steers when averaged across previous pre-weaning treatments (Table 1). Sales et al. (2010) reported that prolactin genotypes tended to affect the average daily gain of stocker steers that grazed E+ and it was lower from TT compared with CC and CT.

When comparisons were made in relation to the cytochrome P450 genotypes, post-weaning weight gain was greater ($P < 0.05$) for steers weaned from SNE100, S100, and S75 than those weaned from F100 and F75 (Table 2). Final weight, feedlot gain, and feedlot ADG were not affected by treatment, genotype, or the treatment × cytochrome P450 genotype interaction (Table 2). Cole et al. (2001) reported that steer feedlot body weight gain during the first 56 d in feedlot was not affected by previous grazing of endophyte-infected fescue. Furthermore, subsequent feedlot ADG tended to be greater from steers that grazed high- endophyte infected pastures than from those that grazed low and moderate endophyte pastures prior to feedlot placement.

Hot carcass weight, dressing percentage, 12th rib fat thickness, yield grade, longissimus thickness, and marbling scores were not affected by prolactin genotype, pre-weaning treatment, or the grazing treatment × prolactin genotype interaction (Table 3). Furthermore, the longissimus area of steers weaned from SNE100, S100, and S75 was larger ($P < 0.05$) than those weaned from F100 and F75, but other carcass measurements did not differ among grazing treatments, genotypes, or the grazing treatment × cytochrome P450 genotype interactions (Table 4). Realini et al. (2005) reported that hot carcass weight was greater for steers that grazed NE+ than steers grazed E+, but there was no effect from the different treatments on the other carcass measurements.

Therefore, grazing season appeared to have the greatest impact on post-weaning grazing performance, primarily due to the type of forage that was available during the immediate post-weaning period (S groups were moved to winter annual pastures while F groups were moved to bermudagrass pastures after weaning). Grazing novel endophyte-infected fescue before weaning had a little effect on steer post-weaning weight gain, feedlot ADG, and longissimus area. On the other hand, polymorphism in prolactin promoter gene affected steer post-weaning gain, shipping weight, feedlot gain, and feedlot ADG. Interaction between treatments and genotypes showed an impact on some of the measurements of feedlot performance but not on carcass measurements.

Implications

The combination of grazing strategies and specific genotypes polymorphisms may impacts a limited number of post-weaning measurements that may help in enhancing cattle profitability. Allowing calves access to non-toxic, novel-endophyte infected fescue prior to weaning may have positive benefits on weaning performance, but these benefits may not persist through subsequent production stages.

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Table 1. Relationship among the single nucleotide polymorphisms in prolactin promoter gene and grazing treatments, for feedlot performance by steers weaned from E+ and (or) NE+.

		Treatments[1]						
Item[2]	**Genotype[3]**	**F100**	**F75**	**SNE100**	**S100**	**S75**	**Effects[4]**	**SEM[5]**
Post-weaning Gain, lb	CC	94 ^{bc}	84 ^{c}	89 ^{c}	207 ^{ab}	141 ^{bc}	Tr,G,Tr*G	33.4
	CT	94 ^{bc}	83 ^{c}	245 ^{ab}	217 ^{ab}	222 ^{ab}		
	TT	67 ^{c}	76 ^{c}	244 ^{ab}	288 ^{a}	229 ^{ab}		
Shipping Weight, lb	CC	667	674	714	758	643	tr, G[d], tr*g	47.9
	CT	664	711	864	696	751		
	TT	658	670	921	829	758		
Final Weight, lb	CC	1257	1225	1296	1267	1216	ns	52.5
	CT	1216	1272	1322	1187	1213		
	TT	1267	1298	1379	1294	1172		
Feedlot Gain, lb	CC	586 ^{ab}	550 ^{b}	583 ^{ab}	514 ^{bc}	568 ^{ab}	tr, G, Tr*G	42.2
	CT	553 ^{ab}	563 ^{ab}	460 ^{bc}	490 ^{bc}	460 ^{bc}		
	TT	617 ^{ab}	625 ^{a}	452 ^{bc}	457 ^{bc}	418 ^{c}		
Feedlot ADG	CC	3.7	3.5	4.3	4.0	4.1	tr, G[e]	0.25
	CT	3.5	3.6	3.7	3.8	3.6		
	TT	3.9	4.0	4.0	3.6	3.3		

[1] F100 = fall born steers weaned from wild-type endophyte infected tall fescue (E+), F75 = fall born steers weaned from 75% E+ and 25% NE+, SNE100 = spring born steers weaned from non-toxic novel endophyte-infected tall fescue (NE+), S100 = spring born steers weaned from100% E+, S75 = spring born steers weaned from 75% E+ and 25% NE+.

[2] Post-weaning gain = gain between weaning and shipping; Shipping and final weight were calculated as shrunk weight.

[3] Prolactin gene allele CC represents steers that were homozygous, CT represents the heterozygous, and TT allele represents steers that were homozygous.

[4]Main effects of treatments (Tr and tr, $P < 0.05$ and $P < 0.10$, respectively), genotype (G, $P < 0.05$), and interactive effects of treatments and genotype (Tr*G and tr*g, $P < 0.05$ and $P < 0.10$, respectively) on performance.

[5]SEM = pooled standard error of the mean.

[a,b,c] = means within items without a common subscript differ, ($P < 0.05$).

[d]Means for steers with TT and CT prolactin genotypes were greater ($P < 0.05$) than those with CC genotypes.

[e]Means for steers with CC prolactin promoter genotype were greater ($P < 0.05$) than those with CT genotype; means by steers with TT genotype were intermediate and did not differ ($P > 0.05$) from other genotypes.

Table 2. Relationship among the single nucleotide polymorphisms in the cytochrome P450 gene and grazing treatments, for feedlot performance of steers weaned from E+ and (or) NE+.

		Treatments[1]						
Item[2]	**Genotype[3]**	**F100**	**F75**	**SNE100**	**S100**	**S75**	**Effects[4]**	**SEM[5]**
Post-weaning Gain, lb	CC	66	85	331	182	168	Tr[a]	37.1
	CG	95	79	141	245	208		
	GG	89	89	211	224	218		
Shipping Weight, lb	CC	632	684	986	784	710	tr	53.3
	CG	670	685	766	778	722		
	GG	658	707	833	692	744		
Final Weight, lb	CC	1177	1234	1448	1287	1261	ns	53.5
	CG	1254	1268	1310	1270	1193		
	GG	1201	1256	1306	1179	1204		
Feedlot Gain, lb	CC	533	546	455	508	534	ns	43.1
	CG	585	583	546	490	469		
	GG	539	546	476	486	465		
Feedlot ADG	CC	3.4	3.5	4.0	3.9	4.0	ns	0.24
	CG	3.7	3.7	4.1	3.8	3.7		
	GG	3.4	3.4	3.8	3.8	3.7		

[1] F100 = fall born steers weaned from wild type endophyte infected tall fescue (E+), F75 = fall born steers weaned from 75% E+ and 25% NE+, SNE100 = spring born steers weaned from non-toxic novel endophyte-infected tall fescue (NE+), S100 = spring born steers weaned from100% E+, S75= spring born steers weaned from 75% E+ and 25% NE+.

[2] Post-weaning gain = gain between weaning and shipping; Shipping and final weight were calculated as shrunk weight.

[3] Cytochrome P450 gene allele CC represents steers that were homozygous, CG represents the heterozygous, and GG allele represents steers that were homozygous.

[4]Main effects of treatments (Tr and tr, $P < 0.05$ and $P < 0.10$, respectively) on feedlot performance.

[5]SEM = pooled standard error of the mean.

[a]Means for steers weaned from SNE100, S100, and S75 were greater ($P < 0.05$) than those weaned from F100 and F75.

Table 3. Relationship among the single nucleotide polymorphisms in the prolactin promoter gene and grazing treatments, for carcass measurements by steers weaned from E+ and (or) NE+.

		Treatments[1]						
Item[2]	**Genotype[3]**	**F100**	**F75**	**SNE100**	**S100**	**S75**	**Effects[4]**	**SEM[5]**
	CC	785	765	844	800	772		
HCW, lb	CT	764	788	837	760	764	ns	36.3
	TT	789	818	777	811	741		
	CC	62	62	65	63	63		
Dressing %	CT	63	62	64	63	63	ns	0.3
	TT	62	63	63	62	64		
	CC	0.4	0.4	0.6	0.5	0.5		
Rib Fat, in	CT	0.5	0.4	0.5	0.5	0.4	ns	0.05
	TT	0.4	0.4	0.4	0.4	0.4		
	CC	2.7	2.6	2.9	3.0	3.0		
Yield Grade	CT	2.8	2.7	3.2	2.8	2.7	ns	0.25
	TT	2.7	2.8	3.1	2.7	2.7		
	CC	13.3	13.3	14.7	14.4	13.4		
Longissimus, in^2	CT	12.7	13.1	15.3	14.2	14.6	tr	0.56
	TT	13.2	13.3	13.5	13.7	14.6		
	CC	426	401	418	457	430		
Marbling	CT	440	432	482	427	438	ns	30.7
	TT	510	431	460	474	356		

[1] F100 = fall born steers weaned from wild-type endophyte infected tall fescue (E+), F75 = fall born steers weaned from 75% E+ and 25% NE+, SNE100 = spring born steers weaned from non-toxic novel endophyte-infected tall fescue (NE+), S100 = spring born steers weaned from 100% E+, S75 = spring born steers weaned from 75% E+ and 25% NE+.

[2] HCW = Hot carcass weight. Dressing% = (HCW / Shrunk Final weight) *100. Yield grade: 1 to 5 scale. Marbling score: 300-399 = slight, 400-499 = small, 500-599 = modest.

[3] Prolactin gene allele CC represents steers that were homozygous, CT represents the heterozygous, and TT allele represents steers that were homozygous.

[4] Main effects of treatments (tr, $P < 0.10$) on carcass measurements.

[5] SEM = pooled standard error of the mean.

Table 4. Relationship among the single nucleotide polymorphisms in the cytochrome P450 gene and grazing treatments, for carcass measurements by steers weaned from E+ and (or) NE+.

		Treatments[1]						
Item[2]	**Genotype[3]**	**F100**	**F75**	**SNE100**	**S100**	**S75**	**Effects[4]**	**SEM[5]**
	CC	821	791	911	804	797		
HCW, lb	CG	765	798	850	799	757	g	35.7
	GG	782	736	834	754	761		
	CC	62	62	63	62	63		
Dressing%	CG	62	62	65	63	63	ns	0.7
	GG	63	62	64	64	63		
	CC	0.4	0.4	0.5	0.5	0.4		
Rib Fat, in	CG	0.4	0.4	0.6	0.5	0.5	ns	0.06
	GG	0.5	0.4	0.5	0.5	0.4		
	CC	2.8	2.6	3.2	3.0	2.3		
Yield Grade	CG	2.7	2.8	3.0	2.8	2.9	ns	0.26
	GG	2.8	2.5	3.1	2.8	2.9		
	CC	13.2	13.4	16.3	14.0	15.2		
Longissimus, in^2	CG	13.0	13.2	14.9	14.5	14.2	Tr[a]	0.64
	GG	12.8	13.2	14.8	14.0	14.2		
	CC	410	414	640	513	406		
MARB	CG	446	413	449	404	408	g	33.3
	GG	439	458	442	461	449		

[1] F100 = fall born steers weaned from wild type endophyte infected tall fescue (E+), F75 = fall born steers weaned from 75% E+ and 25% NE+, SNE100 = spring born steers weaned from non-toxic novel endophyte-infected tall fescue (NE+), S100 = spring born steers weaned from 100% E+, S75 = spring born steers weaned from 75% E+ and 25% NE+.

[2] HCW = Hot carcass weight. Dressing% = (HCW / Shrunk final weight) *100. Yield grade: 1 to 5 scale. Marbling score: 300-399 = slight, 400-499 = small, 500-599 = modest.

[3] Cytochrome P450 gene allele CC represents steers that were homozygous, CG represents the heterozygous, and GG allele represents steers that were homozygous.

[4] Main effects of treatments (Tr, $P < 0.05$), genotype (g, $P < 0.10$) on carcass measurements.

[5] SEM = pooled standard error of the mean [a]. Means for steers weaned from SNE100, S100, and S75 were greater ($P < 0.05$) than those weaned from F100 and F75.

Effects of genotype and transportation stress on cytokine gene expression in steers

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Story in Brief

Cytokines are proteins that produce an immune response to stressors. We aimed to determine effects of transportation stress and DNA sequence changes (polymorphisms) of the cytochrome P450 gene (C994G) and prolactin gene promoter region (C1286T) on gene expression of prolactin receptor and three cytokines: monocyte chemoattractant protein, interleukin-8, and tumor necrosis factor α. Cytochrome P450 and prolactin gene expression were also assessed. Gene expression in buffy coat cells of Gelbvieh × Angus crossbred steers (n = 47; mean BW = 668 ± 68 lb; mean age = 294± 19 d) was evaluated at three time points relative to transport: 27 h before, and 6 and 20 h after transport from Booneville, Ark. to Stillwater, Okla. Statistical analyses indicated that time, genotype, and their interaction affected gene expression. Both polymorphisms affected prolactin expression. The minor alleles (GG and TT) either tended to enhance ($P < 0.2$, C994G) or increase ($P < 0.01$, C1286T) expression. Time influenced ($P < 0.01$) cytochrome P450 and prolactin expression. Lowest expression of cytochrome P450 and prolactin occurred at 6 h after transport. A C1286T × time interaction affected ($P < 0.01$) monocyte chemoattractant protein expression. The lowest monocyte chemoattractant protein expression was in CT steers at 6 h after transport. Time affected ($P < 0.01$) prolactin receptor and tumor necrosis factor α expression in C994G and C1286T genotypes. Highest expression occurred at 20 hours after transport. Genotype affected ($P < 0.01$) monocyte chemoattractant protein, with lowest expression when steers were CT at C1286T or CC at C994G. Genotype effects on interleukin 8 and tumor necrosis factor α were evident only in C1286T genotypes with greatest ($P < 0.01$) expression in CC steers. Our findings may lead to the identification of genetic markers related to stress response or to development of therapeutic measures to reduce transport stress.

Introduction

Plasma cortisol concentrations in cattle have been documented to increase during truck transport; neutrophils extracted from buffy coat respond to cortisol levels with changes in expression of genes involved in immune response, among others (Buckham Sporer et al., 2008). In this study, we associated single nucleotide polymorphisms (SNP) in bovine cytochrome (P450; C994G) and prolactin (PRL) promoter (C1286T) at three time points relative to transport (T)—T-27, which would represent the least stressful condition; 6 h (T+6) after arrival as the most stressful; and 20 h after arrival (T+20) as a recovery period—with differences in expression of prolactin receptor (PRLR) and three cytokines: chemokine (C-C motif)) ligand 2 (CCL2); interleukin 8 (IL8); and tumor necrosis factor (TNF); PRLR is a cytokine receptor that interacts with the prolactin molecule (Corbacho et al., 2003), whereas CCL2, IL8, and TNF are inflammatory cytokines that have been documented to play critical roles in the pathogenesis of respiratory diseases in cattle (Yoo et al., 1995). Since P450 and PRL have been associated with stress tolerance in cattle, particularly in relation to fescue toxicosis, their gene expression levels also were assessed to evaluate effects of SNP present in their coding or promoter regions. Stress does not always result in clinical diseases but does decrease tolerance to disease challenge.

Materials and Methods

Procedures used in this study were approved by local institutional committees on animal use and welfare. Blood samples were collected by venipuncture from Gelbvieh × Angus crossbred steers (n = 47; mean BW = 668 ± 86 lb; mean age = 294± 19 d) at three time points relative to transport (T) in May: 27 h before transport (T-27), 6 h after transport (T+6), and 20 h after transport (T+20). The steers were loaded in a double-deck livestock trailer for a 5-h transport from Booneville, Ark. to Stillwater, Okla. (approx. 250 miles). Buffy coat was extracted and stored in RNALater (Ambion, Life Technologies, Carlsbad, Calif.) until RNA extraction. Buffy coat contains peripheral blood mononuclear cells, the blood cells which are critical components of the immune system. Total RNA was extracted from buffy coat and was used to produce cDNA using a commercially available reverse transcription kit (Applied Biosystems [AB], Life Technologies, Carlsbad, Calif.), following manufacturer's instructions. Assays for the target genes of interest—CCL2, IL8, TNF, P450, PRL, PRLR, and cyclophilin (control) were custom-made by Applied Biosystems (Table 1). A real-time PCR machine (AB StepOnePlus, Life Technologies, Carlsbad, Calif.) was used to quantify gene expression which was expressed as a fold change relative to a reference sample assigned a score of 1.0; values were either lesser or greater than 1.0 (Tusher et al., 2001); steers with the CC genotype in both C994G and C1286T was the reference sample used. Fold changes in expression were analyzed using ANOVA with the MIXED procedure for repeated measures in SAS (SAS Institute, Cary, N.C.). Steer was the experimental unit and fold change was tested against genotype, time, and genotype × time. When F-tests were significant, means were separated using multiple *t*-tests. For graphical illustration, fold change <1.0 was plotted using its negative reciprocal (-1/fold change), to indicate that fold change in that sample decreased compared to the reference.

Results and Discussion

Genotype and Time Effects on P450 and PRL Expression. Both C994G and C1286T SNP influenced PRL but not P450 gene expression: a GG genotype for C994G tended ($P < 0.2$) to have the greatest PRL expression, whereas a TT genotype for C1286T had the greatest ($P < 0.01$) mRNA levels of PRL (Fig. 1). These findings suggest that minor alleles in C994G and C1286T (GG and TT, respectively) enhance expression of PRL. Time also affected P450 ($P < 0.0001$) and PRL ($P < 0.01$) expression: lowest expression levels of P450 in both C994G and C1286T genotypes occurred at T+6, whereas lowest amounts of PRL transcripts occurred at T-27 (Fig. 2).

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Transport Time Effect on TNF and PRLR. Transport time influenced ($P < 0.0001$) expression of TNF and PRLR in genotypes of both C994G and C1286T (Fig. 3): TNF expression numerically increased over time, albeit similar at T-27 and T+6, with the highest level at T+20. The similar expression pattern between both PRLR and TNF agrees with a report that PRLR expression corresponds to TNF expression in pulmonary fibroblasts in rats (Corbacho et al., 2003). In addition, those authors showed that PRLR is induced by cytokines with or without PRL, but PRL activity depends on the presence of PRLR in target tissues. In this study, PRL followed a similar expression pattern to that of PRLR (Fig. 1 and 3) with greatest ($P < 0.01$) expression at T+20. Since the most stressful aspect of transport for cattle is confinement on a moving vehicle (Tarrant, 1990), an expression peak 14 h after transport seem to agree with previous reports showing evidence for peak levels of TNF after a stress challenge on the immune cells. For instance, large amounts of TNF are released in response to lipopolysaccharide (LPS; Walsh et al., 1991); LPS has long been used as a model for the pro-inflammatory or acute phase response (APR), an early physiological response to inflammation. In sheep, TNF mRNA maximum expression was observed at 2 h and disappeared 24 h after LPS-stimulation of immune cells in the lungs (Green and Sargan, 1991).

Genotype Effect on Cytokines: CCL2, IL8 and TNF. Genotype did not affect expression of the cytokine receptor PRLR, but influenced expression of the cytokines. In regard to C994G, CCL2 expression was lowest ($P < 0.01$) in CC steers compared to the CG and GG animals (Fig. 4).

As to C1286T, CCL2 expression was lowest ($P < 0.0001$) in CT steers. In addition, IL8 and TNF expression levels were greatest ($P < 0.01$) in CC steers. The expression patterns for these three cytokines appear to confirm findings in related studies. Although there have not been many reports on CCL2 and TNF gene expression directly associated with transportation stress, both genes have been shown to respond similarly in response to other stressors, e.g. both were suppressed during *Mycobacterium avium* infection in cattle, and TNF mediates CCL2 induction by mycobacterial antigens (Buza et al., 2003). In regard to IL8 and TNF, both were induced by *Pasteurella haemolytica*, the pathogen in shipping fever, in immune cells of bovine lungs, albeit regulated in a different manner (Morsey et al., 1999); Lafleur et al. (1998) suggest that TNF regulates IL8 expression. Lower TNF expression in the CT and TT genotypes of C1286T may be related with prolactin effects on TNF expression. We have shown that PRL expression is greatest in TT steers (Fig. 1). In mice lymphocytes, prolactin administered during an ongoing autoimmune challenge suppressed TNF expression (Lau et al., 2006). Those results suggest a protective regulatory action of prolactin. To be conclusive, however, it is necessary to establish that C1286T changes prolactin activity, particularly since a contradicting effect of the minor allele on stress tolerance has been implied in a related study—lower reproductive efficiencies were observed in TT cows grazing on toxic fescue, suggesting lower tolerance to heat stress accompanying fescue toxicosis (Looper et al., 2010). Complex factors other than genotypic differences are evidently involved.

Time × C1286T interaction on CCL2. Time × C1286T interacted ($P < 0.01$) to influence CCL2 expression (Fig. 5): lowest mRNA level was in CT steers at T+6; although expression level in CT steers increased after 14 h, it did not rise enough to return to a similar level at T-27. Under the same assumption that T+6 would be the most stressful time point in this study, our findings concur with a study reporting suppressed CCL2 levels in cattle infected with *M. avium* (Buza, 2003).

Implications

Genotypes of steers at C994G and C1286T SNP sites and how they influence expression of genes involved in stress response may be useful indicators for the animals' tolerance to shipping stress. Evaluations in more animals are necessary to confirm our findings. Furthermore, additional research to investigate effects of other sources of stress prior to and/or after transport at closer time points on the expression of other genes involved in stress response will help identify critical control points when developing practical and/or therapeutic measures to reduce, if not prevent transportation stress.

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Table 1. Real-time PCR TaqMan gene expression assays.

Gene Name	Assay ID	Context Sequence
Cyclophilin A	Bt03224615_g1	AGACAGCAGAAAACTTTCGTGCTCT
Cytochrome P450 (P450)	Bt03244424_m1	GACAACCATAAAGCTCTGTCTGACA
Chemokine (C-C motif) ligand 2 (CCL2)	Bt03212321_m1	GCTCAGCCAGATGCAATTAACTCCC
Interleukin 8 (IL8)	Bt03211907_g1	TGAAAATTCAGAAATCATTGTTAAG
Prolactin (PRL)	Bt03212031_m1	TATTTGGCCAGGTTATTCCTGGAGC
Prolactin receptor (PRLR)	Bt03207219_m1	TGATTGGGAGACTCATTTTACTCTG
Tumor necrosis factor α (TNF)	Bt03259154_m1	GGCCCCCAGAGGGAAGAGCAGTCCC

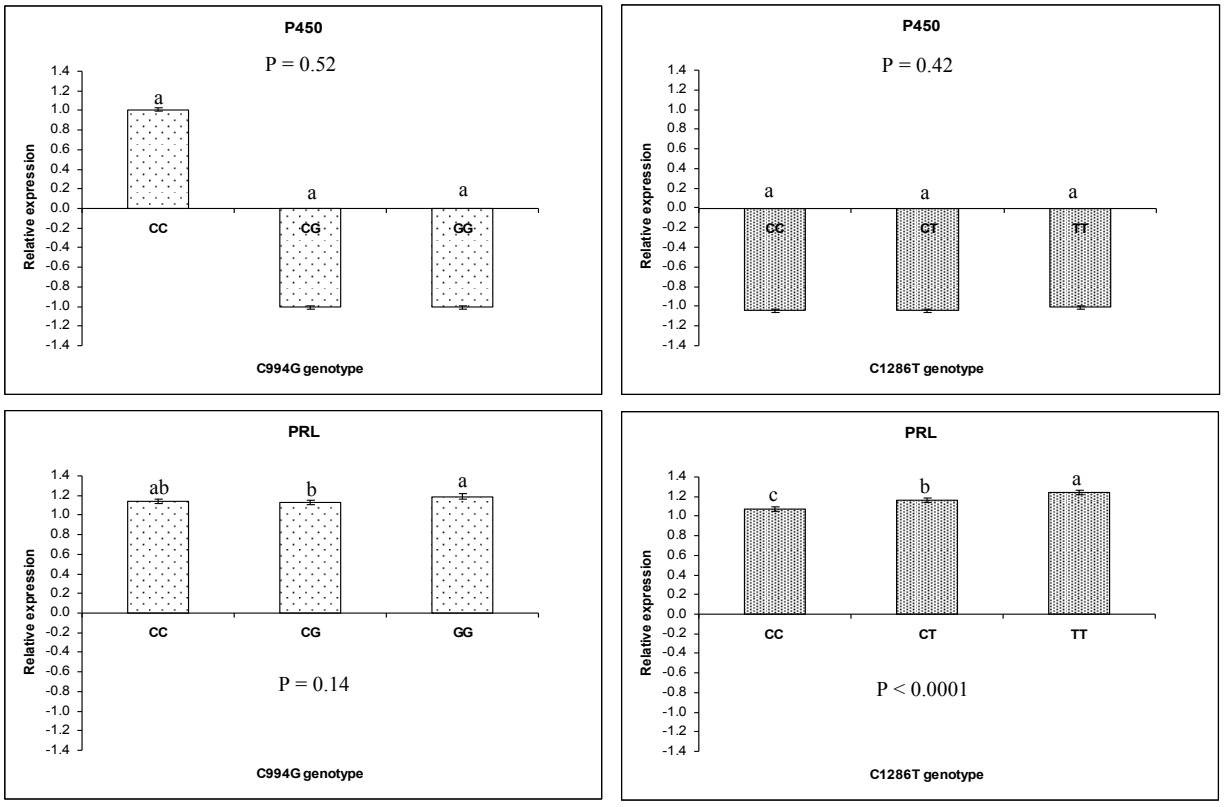

Fig. 1. Gene expression in buffy coat cells of yearling steers. Data shown are an average of n = 47 biological samples ± pooled standard error. Three technical replicates were performed for each biological sample. Means within each gene of interest followed by the same letter are not different at significance level α = 0.05. Genes of interest are P450 (cytochrome P450) and PRL (prolactin); C994G and C1286T are SNP in the P450 coding sequence and PRL promoter region, respectively; genotypes are homozygous cytosine (CC), heterozygous cytosine-guanine (CG), homozygous guanine (GG), heterozygous cytosine-thymine (CT), and homozygous thymine (TT).

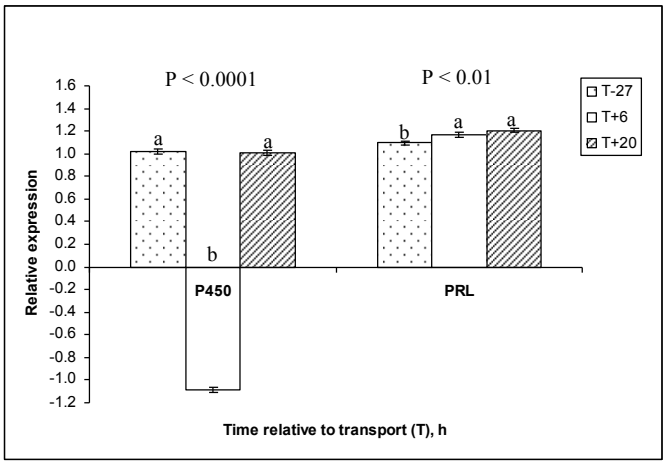

Fig. 2. Gene expression in buffy coat cells of yearling steers. Data shown are an average of n = 47 biological samples ± pooled standard error. Three technical replicates were performed for each biological sample. Means within each gene of interest followed by the same letter are not different at significance level α = 0.05. Genes of interest are P450 (cytochrome P450) and PRL (prolactin); C994G and C1286T are SNP in the P450 coding sequence and PRL promoter region, respectively; time points were 27 h before (T-27), and 6 and 20 h after (T+6 and T+20, respectively) transport (T) from Booneville, Ark., to Stillwater, Okla.

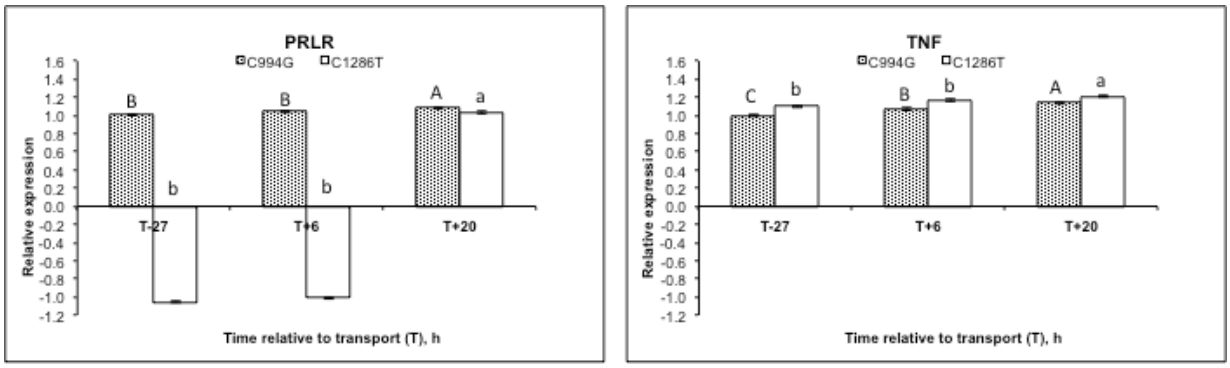

Fig. 3. Gene expression in buffy coat cells of yearling steers. Data shown are an average of n = 47 biological samples ± pooled standard error. Three technical replicates were performed for each biological sample. Means within each gene of interest followed by the same letter are not different: TNF (tumor necrosis factor α; $P < 0.0001$) and PRLR (prolactin receptor; $P < 0.0001$); C994G and C1286T are SNP in the P450 coding sequence and PRL promoter region, respectively; time points were 27 h before (T-27), and 6 and 20 h after (T+6 and T+20, respectively) transport (T) from Booneville, Ark., to Stillwater, Okla.

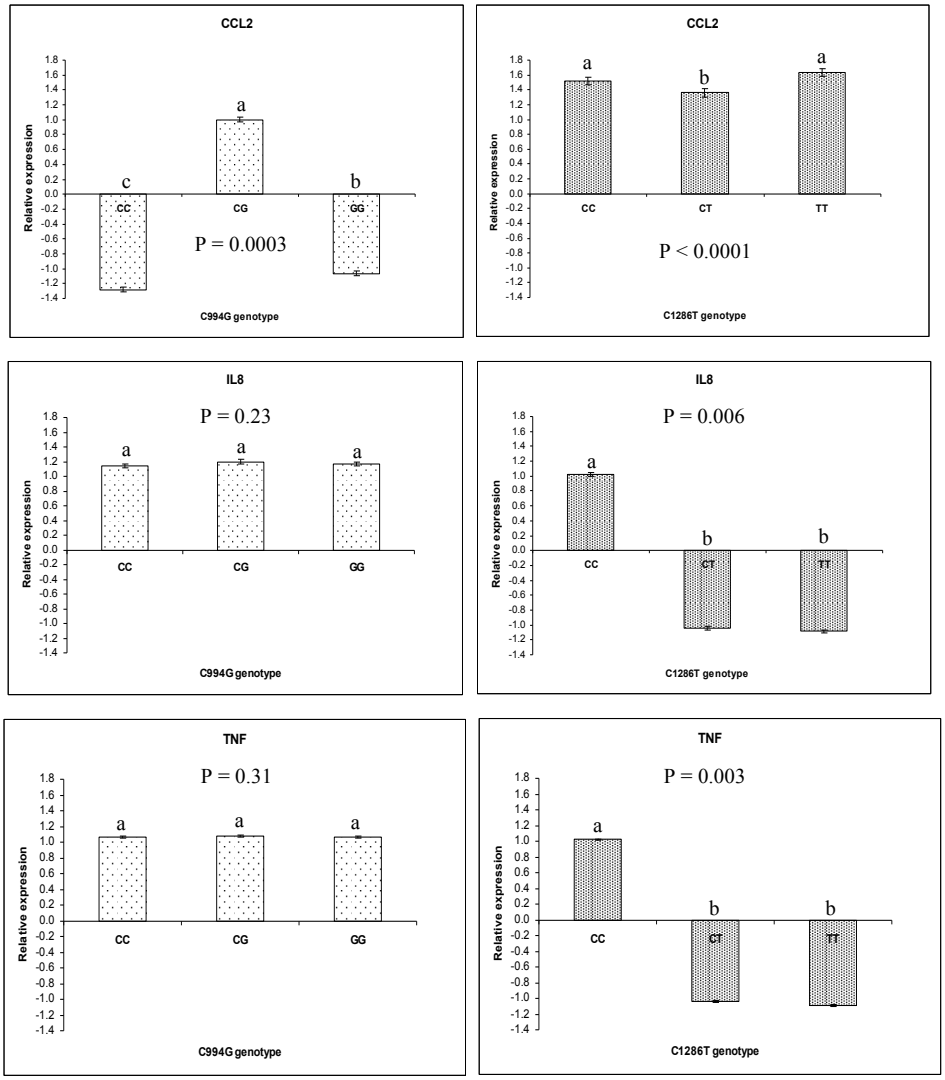

Fig. 4. Gene expression in buffy coat cells from yearling steers. Data shown are an average of n = 47 biological samples ± pooled standard error. Three technical replicates were performed for each biological sample. Means within each gene of interest followed by the same letter are not different at significance level α = 0.05. Genes of interest are CCL2 (chemokine [C-C motif] ligand 2); IL8 (interleukin 8); and TNF (tumor necrosis factor α); C994G and C1286T are SNP in the P450 coding sequence and PRL promoter region, respectively; genotypes are homozygous cytosine (CC), heterozygous cytosine-guanine (CG), homozygous guanine (GG), heterozygous cytosine-thymine (CT), and homozygous thymine (TT).

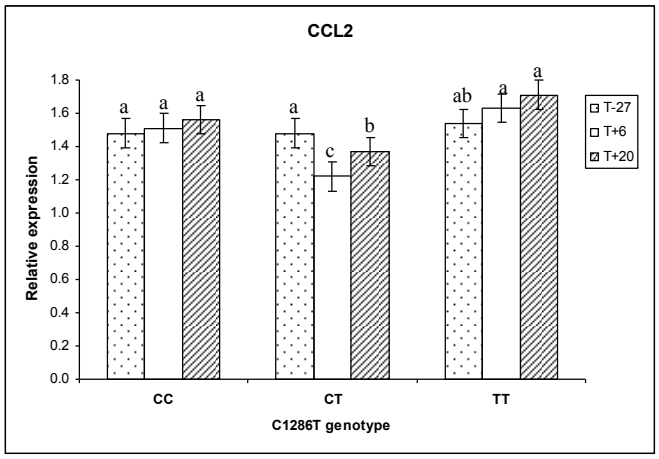

Fig. 5. Gene expression of CCL2 (chemokine [C-C motif] ligand 2) in buffy coat cells from yearling steers with different C1286T genotypes. Data shown are an average of n = 47 biological samples ± pooled standard error. Three technical replicates were performed for each biological sample. Means followed by the same letter are not different ($P < 0.01$). Genotypes are homozygous cytosine (CC), heterozygous cytosine-thymine (CT), and homozygous thymine (TT).

Phase-feeding dietary fat to growing-finishing pigs fed dried distillers grains with solubles I. Performance and carcass characteristics

Nicole A. Browne[1], Jason K. Apple[1], and Janeal W. S. Yancey[1]

Story in Brief

Crossbred pigs (n = 216) were used to test the effects of phase-feeding beef tallow on live performance and carcass characteristics of growing-finishing swine. Pigs were blocked by initial body weight and gender before allotment to pens (6 pigs/pen), and pens (6 pens/block) were allotted randomly to dietary treatments. Grower and finisher dietary treatments consisted of a negative control diet formulated with 4.7% yellow grease for all phases; a positive control with 5% beef tallow for all phases; or treatments including 5% beef tallow fed during phases 1 and 2, 5% beef tallow fed during phases 1, 2, and 3, 5% beef tallow fed during phases 3, 4, and 5, or 5% beef tallow fed during phases 4 and 5. All dietary treatments were formulated with 30% dried distillers grains with solubles (DDGS) during phases 1, 2, and 3, 15% DDGS during phase 4, and no DDGS during phase 5. Live pig performance was not affected by dietary treatments during phase 1 ($P \geq 0.15$), phase 2 ($P \geq 0.44$), phase 3 ($P \geq 0.54$), and phase 5 ($P \geq 0.11$); however, during phase 4, average daily feed intake was reduced ($P < 0.05$) in pigs fed beef tallow during all phases compared to pigs in the treatment fed beef tallow during phases 1 and 2, treatments fed beef tallow during phases 1, 2, and 3, and treatments fed beef tallow during phases 4 and 5. Over the entire trial, pigs fed beef tallow during phases 3, 4, and 5 tended to have greater ($P = 0.10$) average daily gain (ADG) than the pigs fed yellow grease during all phases, pigs fed beef tallow during phases 1, 2, and 3, and pigs fed beef tallow during phases 4 and 5, and ADG of pigs fed beef tallow during phases 4 and 5 was less than that of pigs fed beef tallow during phases 1 and 2 and pigs fed beef tallow during phases 3, 4, and 5. In addition, pigs fed beef tallow during all phases tended to consume less ($P = 0.09$) feed than all other dietary treatments. There was no ($P \geq 0.23$) effect of beef tallow inclusion on dressing percentage, carcass lean percentage, back fat thickness, and *longissimus* muscle depth. Results of this study indicate that phase-feeding beef tallow to pigs fed diets formulated with DDGS had little to no impact on performance and carcass characteristics.

Introduction

Soft pork fat and fresh pork bellies have become a major concern of pork processors. Soft pork bellies have been associated with fabrication difficulties, reduced product yields, unattractive bacon slices, reduced product shelf-life, and subsequent consumer discrimination. The increased incidence of soft pork fat and bellies has been attributed to the elevation of the polyunsaturated fatty acid (PUFA) composition of diets by feeding large amounts of dried distillers grains with soluble (DDGS). There does not appear to be a cost-effective, nutritional method of reversing the negative effects of feeding highly polyunsaturated fat sources, along with DDGS, on pork fat and belly firmness issues.

Previous research indicated that almost 70% of the fatty acid composition of pork fat was established in the first 50 lbs of BW gain (Apple et al., 2009); therefore, it was hypothesized that feeding a more saturated fat source during the growing phases would establish more saturated pork fat depots; so, when large amounts of DDGS and/or polyunsaturated fat sources were fed in later dietary phases, the negative effects of elevating dietary PUFA on belly firmness would be negligible. Thus, pigs were used to test the effects of phase-feeding beef tallow (BT), a saturated fat source, on live performance and carcass quality characteristics from growing-finishing swine.

Materials and Methods

Crossbred pigs (n = 216) were fed a 5-phase diet to test the effects of phase-feeding BT (Table 1) on live performance and carcass characteristics of growing-finishing swine fed diets formulated with DDGS and yellow grease (a polyunsaturated fat source; Table 1). Pigs were blocked by initial body weight (55 lbs) and allotment to pens (6 pigs/pen) with equal numbers of barrows and gilts, and pens (6 pens/block) were allotted randomly to 1 of 6 dietary treatments (Tables 2 and 3). Corn-soybean meal based dietary treatments consisted of: negative control (NC) dietary treatment where diets were formulated with 3.5% yellow grease for all phases; a positive control (PC) dietary treatment where diets were formulated with 5.0% BT for all phases; or treatments including 5% BT during phases 1 and 2 (BT12), during phases 1, 2, and 3 (BT123), during phases 3, 4, and 5 (BT345), or during phases 4 and 5 (BT45). All dietary treatments were formulated to represent standard commercial inclusion levels of DDGS, with 30% DDGS during phases 1, 2, and 3, 15% DDGS during phase 4, and no DDGS during phase 5. Individual pig BW and pen feed disappearance were measured for each feeding phase to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratios (F/G).

At an average body weight of 275 lbs, pigs were slaughtered at a commercial pork packing plant according to industry-accepted standards. Fat and *longissimus* muscle (LM) depths were measured on-line with a Fat-O-Meat'er probe (Carmometec, Denmark), and hot carcass weights (HCW) and estimated fat free lean yields were recorded before carcass chilling.

Data were analyzed as a randomized complete block design, with blocks based on initial BW and pen as the experimental unit. Analysis of variance was done using the mixed model procedure (SAS Inst. Inc., Cary, N.C.). The lone fixed effect in all statistical models was the dietary treatment, whereas initial BW block was considered a random effect in models for live pig performance and carcass composition. Least-squares means were computed and separated statistically using pairwise *t*-tests (PDIFF option) when a significant *F*-test ($P \leq 0.05$) was observed.

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Results

Live pig performance was not affected by dietary treatments during phase 1 ($P \geq 0.15$), phase 2 ($P \geq 0.44$), phase 3 ($P \geq 0.54$), and phase 5 ($P \geq 0.11$); however, ADFI was reduced ($P < 0.05$) in pigs fed the PC diet compared to pigs in the BT12, BT123, and BT45 treatments during phase 4. Average daily gain of the pigs on study ranged from 2.03 to 2.11 lb/day, and, across the entire study, pigs fed BT345 tended to have greater ($P = 0.10$) ADG than pigs fed the NC, BT123, and BT45 treatments, and the ADG of BT45-fed pigs was less than pigs fed BT12 and BT345 (Table 4). Overall ADFI of the pigs ranged between 5.32 and 5.62 lb/day, and PC-fed pigs tended to consume less ($P = 0.09$) feed than all other dietary treatments; however, F/G was not ($P \geq 0.11$) affected by dietary treatment. Additionally, slaughter weight, HCW, dressing percentage, 10th rib fat depth, LM depth, and lean muscle yields were not ($P \geq 0.23$) affected by the dietary treatments imposed on these pigs (Table 5).

Implications

Results of this study indicate that phase-feeding beef tallow in place of yellow grease to pigs fed diets formulated with DDGS had little to no impact on live pig performance and carcass characteristics.

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Table 1. Fatty acid composition (as-fed basis) of dietary fat sources.

	Dietary fat source	
Fatty acid composition,[1] %	**Beef Tallow[2]**	**Yellow Grease[3]**
SFA		
Myristic acid (14:0)	2.70	2.10
Pentadecanoic acid (15:0)	0.50	0.30
Palmitic acid (16:0)	22.70	19.00
Margaric acid (17:0)	1.40	1.00
Stearic acid (18:0)	18.96	10.72
Arachidic acid (20:0)	0.14	0.21
MUFA		
Myristoleic acid (14:1)	0.48	0.50
Palmitelaidic acid (16:1*t*)	0.42	0.25
Palmitoleic acid (16:1*c*)	2.35	2.30
Heptadecenoic acid (17:1*t*)	0.14	0.09
Total 18:1*t* fatty acids	5.35	4.83
Oleic acid (18:1*c*9)	35.21	34.63
Vaccenic acid (18:1*c*11)	1.58	2.06
Gadoleic acid (20:1*c*11)	0.21	0.41
PUFA		
Linoleic acid (18:2n-6)	2.55	14.92
CLA (18:2*c*9*t*11)	0.50	0.27
α-Linolenic acid (18:3n-3)	0.21	1.29
γ-Linolenic acid (18:3n-6)	ND[4]	0.13
Eicosadienoic acid (20:2)	0.03	0.08
Dihomo-γ-linolenic acid (20:3n-6)	0.06	0.05
Arachidonic acid (20:4n-6)	0.03	0.04
Docosapentaenoic acid (22:5n-3)	0.01	0.01
Docosahexaenoic acid (22:6n-3)	ND	ND
Other fatty acid peaks	4.23	4.37

[1]*t = trans; c = cis.*

[2]Values represent averages of 12 beef tallow samples.

[3]Values represent averages of 11 yellow grease samples.

[4]ND = not detectable.

Table 2. Beef tallow (gray area) and yellow grease (white area) inclusion in dietary treatments[1].

Dietary Treatment[2]	Production Phase (pig BW)				
	Grower I (55 to 95 lb)	Grower II (95 to 150 lb)	Finisher I (150 to 175 lb)	Finisher II (175 to 225 lb)	Finisher III (225 to 275 lb)
NC	4.7%	4.7%	4.7%	4.7%	4.7%
BT12	5.0%	5.0%	4.7%	4.7%	4.7%
BT123	5.0%	5.0%	5.0%	4.7%	4.7%
BT345	4.7%	4.7%	5.0%	5.0%	5.0%
BT45	4.7%	4.7%	4.7%	5.0%	5.0%
PC	5.0%	5.0%	5.0%	5.0%	5.0%

[1]All diets within a phase were isocaloric.
[2] NC = negative control (4.7 % yellow grease fed for entire trial); BT12 – 5.0 % beef tallow fed during grower phases I and II; BT123 = 5.0% beef tallow fed during grower phases I and II and finisher phase I; BT345 = 5.0% beef tallow fed during finisher phases I, II, and III; BT45 = 5.0% beef tallow fed during finisher phases II and III; and PC = positive control (5.0% beef tallow fed for entire trial).

Table 3. Composition[1] of grower I, grower II, finisher I, finisher II, and finisher III diets.

Item	Grower I (55 to 95 lb)	Grower II (95 to 150 lb)	Finisher I (150 to 175 lb)	Finisher II (175 to 225 lb)	Finisher III (225 to 275 lb)
Ingredient, %					
Corn	38.41	44.45	49.29	64.95	78.92
Soybean meal	23.55	17.95	13.20	12.55	13.50
DDGS	30.00	30.00	30.00	15.00	0.00
Sand[2]	0.32	0.32	0.32	0.32	0.32
Yellow grease[2]	4.68	4.68	4.68	4.68	4.68
Beef tallow[2]	0.00	0.00	0.00	0.00	0.00
Dicalcium phosphate	0.740	0.44	0.35	0.72	0.96
Limestone	1.200	1.100	1.133	0.885	0.695
Salt	0.400	0.400	0.400	0.400	0.400
L-Lysine	0.300	0.275	0.250	0.213	0.200
L-Threonine	0.017	0.009	0.000	0.013	0.053
Vitamin premix[3]	0.150	0.150	0.150	0.100	0.100
Mineral premix[4]	0.150	0.150	0.150	0.125	0.125
Ethoxiquin	0.030	0.030	0.030	0.030	0.030
Tylan-40	0.050	0.050	0.050	0.025	0.025
Calculated composition, %					
CP	22.56	20.37	18.49	15.51	13.19
Total Lysine	1.314	1.141	0.990	0.849	0.770
TID Lysine	1.120	0.960	0.821	0.723	0.685
TID M+C	0.658	0.607	0.562	0.483	0.419
TID Threonine	0.683	0.600	0.526	0.467	0.457
TID Tryptophan	0.198	0.169	0.143	0.127	0.119
Total P	0.638	0.560	0.525	0.516	0.491
Available P	0.360	0.300	0.280	0.260	0.220
Ca	0.750	0.630	0.610	0.580	0.561
ME, Mcal/kg	3.533	3.549	3.553	3.552	3.550

[1]Ingredients and diet composition reported on an as-fed basis.
[2]Beef tallow (5%) replaced yellow grease (4.68%) and sand (0.32%) resulting in an isocaloric diet within each phase.
[3]Premix supplied 6,614 IU of vitamin A, 827 IU of vitamin D_3, 26 IU of vitamin E, 2.7 mg of vitamin K, 16.5 mg of pantothenic acid, 30 mg of niacin, 5 mg of riboflavin, and 26 µg of vitamin B_{12} per kilogram of feed (Nutra Blend Corp., Neosho, Mo.).
[4]Premix supplied 138 mg/kg of Fe from ferrous sulfate, 138 mg/kg of Zn from zinc sulfate, 33 mg/kg of Mn as manganous sulfate, 13.8 mg/kg of Cu from copper sulfate, 0.25 mg/kg of Se from sodium selenite, and 0.25 mg/kg of I from calcium iodate per kilogram of feed (Nutra Blend Corp., Neosho, Mo.).

Table 4. Effect of dietary treatments on growth performance of growing-finishing swine.

	Dietary Treatment[1]							
Item	**NC**	**BT12**	**BT123**	**BT345**	**BT45**	**PC**	**SEM**	***P > F***
No. of pens	6	6	6	6	6	6		
Grower-I phase (55 to 95 lb)								
ADG, lb	1.88	1.86	1.84	1.84	1.90	1.89	0.065	0.904
ADFI,[2] lb	3.48	3.57	3.49	3.56	3.43	3.53	0.103	0.666
F/G[2]	1.85	1.92	1.90	1.93	1.81	1.87	0.010	0.147
Grower-II phase (95 to 150 lb)								
ADG, lb	2.34	2.38	2.31	2.30	2.27	2.28	0.041	0.438
ADFI,[2] lb	5.31	5.40	5.23	5.33	5.16	5.22	0.122	0.589
F/G[2]	2.27	2.27	2.26	2.32	2.27	2.29	0.009	0.952
Finisher-I phase (150 to 175 lb)								
ADG, lb	2.27	2.24	2.30	2.32	2.20	2.31	0.051	0.537
ADFI,[2] lb	6.12	6.17	6.10	6.11	5.84	6.02	0.136	0.562
F/G[2]	2.70	2.75	2.65	2.63	2.65	2.61	0.009	0.739
Finisher-II phase (175 to 225 lb)								
ADG, lb	2.24	2.16	2.12	2.16	2.30	2.10	0.062	0.191
ADFI,[2] lb	6.09^{abc}	6.29^{a}	5.97^{bc}	6.07^{abc}	6.22^{ab}	5.80^{c}	0.121	0.036
F/G[2]	2.72	2.91	2.82	2.81	2.70	2.76	0.007	0.178
Finisher-III phase (225 to 275 lb)								
ADG, lb	1.73	1.93	1.98	2.00	1.83	1.84	0.081	0.143
ADFI,[2] lb	6.04	6.34	6.32	6.33	6.08	5.82	0.165	0.125
F/G[2]	3.49	3.28	3.19	3.17	3.32	3.16	0.008	0.114
Overall (55 to 275 lb)								
ADG, lb	2.04^{bc}	2.10^{ab}	2.05^{bc}	2.11^{a}	2.03^{c}	2.06^{abc}	0.026	0.103
ADFI,[2] lb	5.45^{ab}	5.62^{a}	5.46^{ab}	5.54^{ab}	5.39^{b}	5.32^{c}	0.083	0.092
F/G[2]	2.67	2.68	2.66	2.63	2.66	2.58	0.005	0.247

[a-c] Within a row, least squares means lacking a common superscript letter differ ($P \leq 0.10$).
[1] NC = negative control (4.7% yellow grease fed for entire trial); BT12 = 5.0% beef tallow fed during grower phases I and II; BT123 = 5.0% beef tallow fed during grower phases I and II and finisher phase I; BT345 = 5.0% beef tallow fed during finisher phases I, II, and III; BT45 = 5.0% beef tallow fed during finisher phases II and III; and PC = positive control (5.0% beef tallow fed for entire trial).
[2]As-fed basis.

Table 5. Effect of dietary treatments on carcass characteristics.

	Dietary Treatment[1]							
Item	**NC**	**BT12**	**BT123**	**BT345**	**BT45**	**PC**	**SEM**	***P > F***
No. of pens	6	6	6	6	6	6		
Slaughter weight, lb	271.3	274.3	274.8	277.7	273.7	271.1	3.49	0.492
Dressing percentage	73.5	73.6	73.1	73.5	73.6	74.0	0.31	0.445
HCW,[2] lb	199.4	202.0	200.7	203.9	201.4	200.6	2.70	0.754
Percent lean/muscle	51.9	50.9	51.2	51.5	51.1	51.6	0.32	0.231
LM[3] depth, in	2.21	2.13	2.18	2.24	2.20	2.23	0.41	0.292
10th-rib fat depth, in	0.80	0.85	0.84	0.87	0.87	0.83	0.29	0.456

[1] NC = negative control (4.7% yellow grease fed for entire trial); BT12 = 5.0% beef tallow fed during grower phases I and II; BT123 = 5.0% beef tallow fed during grower phases I and II and finisher phase I; BT345 = 5.0% beef tallow fed during finisher phases I, II, and III; BT45 = 5.0% beef tallow fed during finisher phases II and III; and PC = positive control (5.0% beef tallow fed for entire trial).
[2] Hot carcass weight.
[3] *Longissimus* muscle.

Phase-feeding dietary fat to growing-finishing pigs fed dried distillers grains with solubles II. Fresh belly quality and yields of commercially-processed bacon

Nicole A. Browne[1], Jason K. Apple[1], and Janeal W. S. Yancey[1]

Story in Brief

Crossbred pigs (n = 216) were used to test the effects of phase-feeding beef tallow (BT) on belly quality characteristics and yields of commercially-processed bacon from growing-finishing swine. Pigs were blocked by BW and gender before allotment to pens (6 pigs/pen), and pens (6 pens/block) were allotted randomly to dietary treatments. Dietary treatments consisted of a negative control formulated with 4.7% yellow grease for all phases; a positive control with 5% beef tallow for all phases; or treatments including 5% beef tallow during phases 1 and 2, during phases 1, 2, and 3, during phases 3, 4, and 5, or during phases 4 and 5. All dietary treatments were formulated with 30% dried distillers grains with solubles (DDGS) during phases 1, 2, and 3, 15% DDGS during phase 4, and no DDGS during phase 5. Fresh belly quality data were collected on the left-sided bellies, whereas bacon from the right-sided bellies was prepared under commercial processing conditions. Weights were recorded at all stages of processing, and yields were calculated as a percentage of the green belly weight. There were no effects ($P \geq 0.14$) of beef tallow inclusion on fresh belly width, length, or average thickness. Bellies from the negative control treatments tended ($P = 0.08$) to be less firm than bellies from the treatment fed 5% beef tallow during phases 3, 4, and 5, the treatment fed 5% beef tallow during phases 4 and 5, and the treatment fed 5% beef tallow for all phases; but instrumental puncture test showed that mechanical firmness of bellies did not differ among treatments ($P \geq 0.38$). Yield of commercially-processed bacon (pumped belly yield, smoked belly yield, and the percentage of number 1 bacon slices) was not ($P \geq 0.14$) affected by the dietary treatments. Results of this study indicated that phase-feeding beef tallow to pigs fed diets formulated with DDGS had a tendency to improve fresh belly firmness but had no effect on yields of commercially-processed bacon.

Introduction

Soft pork fat and pork bellies are a major concern of pork processors. Soft pork bellies are associated with: fabrication difficulties, reduced product yields, unattractive bacon slices, reduced product shelf-life, and subsequent consumer discrimination. The increased incidence of soft pork fat and bellies has been attributed to elevated levels of polyunsaturated fatty acid (PUFA) by feeding large amounts of DDGS. There does not appear to be a cost-effective, nutritional method of reversing the negative effects of feeding highly polyunsaturated fat sources on pork fat and belly firmness.

Previous research indicated that almost 70% of the fatty acid composition of pork fat was established in the first 50 lbs of body weight gain (Apple et al., 2009); therefore, it was hypothesized that feeding a more saturated fat source during the growing phases would establish more saturated pork fat depots, so, when large amounts of DDGS and/or polyunsaturated fat sources were fed in later dietary phases, the negative effects of elevating dietary PUFA on belly firmness would be negligible. Thus, the objective of this study was to test the effects of phase-feeding beef tallow during the growing-finishing phases on belly quality characteristics and yields of commercially-processed bacon.

Materials and Methods

Crossbred pigs (n = 216) were fed a 5-phase diet to test the effects of phase-feeding beef tallow (BT) on fresh belly and bacon quality characteristics of growing-finishing swine fed diets formulated with DDGS and yellow grease (YGr). Pigs were blocked by initial body weight (55 lbs) and allotted to pens (6 pigs/pen) with equal numbers of barrows and gilts, and pens (6 pens/block) were allotted randomly to 1 of 6 dietary treatments (Table 1). Corn-soybean meal-based dietary treatments consisted of: negative control (NC) dietary treatment where diets were formulated with 4.7% yellow grease for all phases; a positive control (PC) dietary treatment where diets were formulated with 5% beef tallow for all phases; or treatments including 5% beef tallow during phases 1 and 2 (BT12), during phases 1, 2, and 3 (BT123), during phases 3, 4, and 5 (BT345), or during phases 4 and 5 (BT45). All dietary treatments were formulated to represent standard commercial inclusion levels of DDGS, with 30% DDGS during phases 1, 2, and 3, 15% DDGS during phase 4, and no DDGS during phase 5.

At an average body weight of 275 lbs, pigs were slaughtered at a commercial pork packing plant according to industry-accepted standards. Before carcass fabrication, bellies were identified with the each pig's individual tattoo number, and both sides were collected during pork carcass fabrication, placed in combos, and transported back to the University of Arkansas for further data collection. Upon arrival, left and right sides were sorted, and fresh belly quality measurements were collected on left-side bellies. The length, width, thickness (mean of cranial, caudal, dorsal, and ventral measurements), and temperature (belly firmness was only measured on bellies with temperatures between 35 °F to 37 °F) were measured on each belly. Subjective belly firmness was measured according to the bar-suspension (flop) method of Theil-Cooper et al. (2001), by measuring the distance between belly ends when the length of the belly was suspended perpendicular (skin-side up and skin-side down) to a 0.75-in diam. bar. Belly firmness angle (the upper angle of the isosceles triangle formed by suspending the belly across the bar) was also calculated using the equation of Whitney et al. (2006): $\cos^{-1}([\{0.5 \times L^2\} - D^2] / \{0.5 \times L^2\})$; where L is the belly length and D is the distance between belly ends when suspended perpendicular to the bar. Additionally, instrumental color of the belly fat was measured with a Hunter Miniscan XE Plus (Hunter Associate Laboratories,

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Reston, Va.) using illuminant C, a 1-inch aperture, and a 10° standard observer to obtain L*, a*, and b* values (lightness, redness, and yellowness, respectively). Color was also evaluated visually by a trained panelist using the Japanese fat color scores where larger numbers indicate a darker color.

Objective belly firmness was measured according to the Instron puncture test of Trusell et al. (2009) by thawing the belly strips at 34 °F for 48 h prior to puncturing. Each belly strip was separated into 4 equal length portions. Temperature of each belly section was measured (belly firmness was only measured on sections with temperatures between 35 °F and 37 °F) before thickness of each section was measured with calipers to determine belly thickness. Subsequently, belly sections were placed skin-side down on a lower flat plate, and were punctured to 65% of their specific thickness with an Instron testing machine (model 4466; Instron Corp., Canton, Mass.) equipped with a 3.9-in-long, 0.5-in-diameter, rounded tip puncturing bar, a 110-lb load cell, and a crosshead speed of 3.9 in/min.

The right-side bellies were then transported to a commercial bacon-manufacturing plant where they were cured, thermal-processed, and sliced according to industry standards. Belly weights were recorded at all stages of bacon processing and yields were calculated as a percentage of the initial belly weight. Then, bellies were sliced, and USDA-certified No. 1 slices were collected.

Data were analyzed as a randomized complete block design, with blocks based on initial BW and pen as the experimental unit. Analysis of variance was done using the mixed model procedure (SAS Inst. Inc., Cary, N.C.). The lone fixed effect in all statistical models was the dietary treatment, whereas initial BW block was considered a random effect in models for fresh belly and bacon yields. Least-squares means were computed and separated statistically using pairwise *t*-tests (PDIFF option) when a significant *F*-test ($P \leq 0.05$) was observed.

Results

Fresh belly width, length, and thickness were not ($P \geq 0.14$) affected by the dietary treatments; yet according to the belly flop evaluations, fresh bellies from pigs fed the PC, BT345, and BT45 tended to be firmer ($P = 0.08$) than bellies from pigs fed the NC diets (Table 2). However, mechanically-measured firmness of fresh bellies, using the puncture probe, did not ($P \geq 0.38$) differ among the treatments, and dietary treatments did not ($P \geq 0.14$) affect bacon processing yields during bacon production or the percentage of no. 1 bacon slices (Table 3). Fat color of bellies did not differ ($P > 0.05$) due to dietary treatment whether measured instrumentally (L*, a*, b*) or evaluated visually.

Implications

Results of this study indicated that phase-feeding beef tallow in place of yellow grease to pigs fed diets formulated with dried distillers grains with solubles had a tendency to improve fresh belly firmness but had no effect on yields of commercially-processed bacon. Even though fatty acid analyses of subcutaneous depots are not complete at this time, it is expected that these dietary treatments will have an effect on fatty acid profiles. These preliminary results would indicate that other factors may contribute as much, if not more, than the fatty acid composition of belly fat to the problem of soft pork bellies.

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Apple, J. K., C. V. Maxwell, D. L. Galloway, C. R. Hamilton, and J. W. S. Yancey. 2009. Interactive effects of dietary fat source and slaughter weight in growing-finishing swine: II. Fatty acid composition of subcutaneous fat. J. Anim. Sci. 87:1423-1440.

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Table 1. Beef tallow (gray) area and yellow grease (white area) inclusion in dietary treatments[1].

Dietary Treatment[2]	Production Phase				
	Grower I (55 to 95 lb)	**Grower II (95 to 150 lb)**	**Finisher I (150 to 175 lb)**	**Finisher II (175 to 225 lb)**	**Finisher III (225 to 275 lb)**
NC	4.7%	4.7%	4.7%	4.7%	4.7%
BT12	5.0%	5.0%	4.7%	4.7%	4.7%
BT123	5.0%	5.0%	5.0%	4.7%	4.7%
BT345	4.7%	4.7%	5.0%	5.0%	5.0%
BT45	4.7%	4.7%	4.7%	5.0%	5.0%
PC	5.0%	5.0%	5.0%	5.0%	5.0%

[1]All diets within a phase were isocaloric.
[2] NC = negative control (4.7% yellow grease fed for entire trial); BT12 = 5.0% beef tallow fed during grower phases I and II; BT123 = 5.0% beef tallow fed during grower phases I and II and finisher phase I; BT345 = 5.0% beef tallow fed during finisher phases I, II, and III; BT45 = 5.0% beef tallow fed during finisher phases II and III; and PC = positive control (5.0% beef tallow fed for entire trial).

Table 2. Effect of dietary treatments on fresh belly characteristics.

Item	Dietary Treatment[1]						SEM	*P > F*
	NC	**BT12**	**BT123**	**BT345**	**BT45**	**PC**		
Width, in	13.19	13.44	13.27	13.31	13.46	13.14	0.168	0.712
Length, in	26.27	26.30	27.00	26.89	26.61	26.65	0.220	0.145
Thickness, in	1.06	1.14	1.12	1.13	1.13	1.14	0.038	0.667
Belly flop, in								
Skin-side up	5.72^{b}	6.59^{ab}	6.38^{ab}	7.26^{a}	6.85^{a}	6.74^{a}	0.363	0.085
Skin-side down	4.79^{b}	5.97^{a}	5.72^{a}	5.93^{a}	6.17^{a}	5.82^{a}	0.332	0.071
Belly angle, °								
Skin-side up	25.24^{b}	28.95^{ab}	27.30^{ab}	31.35^{a}	30.01^{a}	29.47^{a}	1.561	0.090
Skin-side down	21.05^{b}	26.16^{a}	24.39^{ab}	25.51^{a}	26.82^{a}	25.33^{a}	1.386	0.062
Puncture force, lb	17.05	18.72	20.75	18.15	16.06	16.32	1.663	0.380
Fat color[2]								
L*	81.72	81.07	81.60	81.65	81.03	82.20	0.524	0.627
a*	10.42	10.90	10.50	10.12	11.37	10.19	0.445	0.369
b*	13.69	14.02	13.78	13.10	14.86	13.41	0.397	0.075
Visual	2.51	2.49	2.35	2.34	2.46	2.29	0.159	0.855

[a,b] Within a row, least squares means lacking a common superscript letter differ ($P < 0.10$).
[1] NC = negative control (4.7% yellow grease fed for entire trial); BT12 = 5.0% beef tallow fed during grower phases I and II; BT123 = 5.0% beef tallow fed during grower phases I and II and finisher phase I; BT345 = 5.0% beef tallow fed during finisher phases I, II, and III; BT45 = 5.0% beef tallow fed during finisher phases II and III; and PC = positive control (5.0% beef tallow fed for entire trial).
[2] L* indicates lightness where a larger number is lighter, a* indicates redness where a larger number in redder, and b* indicates yellowness where a larger number is more yellow. Visual color was evaluated using the Japanese fat color scores where a larger number indicates a darker color.

Table 3. Effect of dietary treatments on bacon yields.

	Dietary Treatment[1]							
Item	**NC**	**BT12**	**BT123**	**BT345**	**BT45**	**PC**	**SEM**	***P > F***
Bacon yields, %								
Pump	110.5	110.8	111.1	110.2	110.2	111.3	0.35	0.140
Smokehouse	98.2	98.7	98.6	98.0	97.9	98.5	0.37	0.409
No. 1 slices	83.8	84.4	82.5	83.4	82.5	83.1	1.26	0.869

[1] NC = negative control (4.7% yellow grease fed for entire trial); BT12 = 5.0% beef tallow fed during grower phases I and II; BT123 = 5.0% beef tallow fed during grower phases I and II and finisher phase I; BT345 = 5.0% beef tallow fed during finisher phases I, II, and III; BT45 = 5.0% beef tallow fed during finisher phases II and III; and PC = positive control (5.0% beef tallow fed for entire trial).

Phase-feeding dietary fat to growing-finishing pigs fed dried distillers grains with soluble III. Quality characteristics of bacon

Nicole A. Browne[1], Jason K. Apple[1], and Janeal W. S. Yancey[1]

Story in Brief

Crossbred pigs (n = 216) were used to test effect of phase-feeding beef tallow on bacon quality characteristics in growing-finishing swine. Pigs were blocked by initial body weight and gender before allotment to pens (6 pigs/pen), and pens (6 pens/block) were allotted to dietary treatments. Dietary treatments consisted of: a negative control (NC) devoid of beef tallow for all phases; a positive control with 5% beef tallow for all phases; or treatments including 5% beef tallow during phases 1 and 2, during phases 1, 2, and 3, during phases 3, 4, and 5, or during phases 4 and 5. All dietary treatments were formulated with 30% dried distillers grains with solubles (DDGS) during phases 1, 2, and 3, 15% DDGS during phase 4, and no DDGS during phase 5. Bacon was cooked in a commercial convection oven, center sections of the slices were collected, and shear force was measured with an Allo-Kramer device. For each taste panel session (n = 18), bacon was cooked in convection ovens, and each panelist received 6 slices of bacon in random order. There was no ($P \geq 0.23$) effect of beef tallow inclusion on color of the bacon fat, cook loss percentage, or Allo-Kramer shear force. Traits evaluated by the sensory panel (initial crispiness, bacon flavor intensity, saltiness, sustained chewiness, oiliness, and off flavor intensity) were not ($P \geq 0.55$) affected by dietary treatments. Results of this study indicated that phase-feeding beef tallow to pigs fed diets formulated with DDGS had no impact on quality characteristics of bacon.

Introduction

Soft pork fat in fresh pork bellies has become a major concern of pork processors. Soft pork bellies have been associated with: fabrication difficulties, reduced product yields, unattractive bacon slices, reduced product shelf-life, and subsequent consumer discrimination. The increased incidence of soft pork fat and bellies has been attributed to the elevation of the polyunsaturated fatty acid (PUFA) composition of diets containing large amounts of dried distillers grains with soluble (DDGS). There does not appear to be a cost-effective, nutritional method of reversing the negative effects of feeding highly polyunsaturated fat sources, along with DDGS, on pork fat and belly firmness issues.

Previous research indicated that almost 70% of the fatty acid composition of pork fat was established in the first 50 lbs of body weight gain (Apple et al., 2009); therefore, it was hypothesized that feeding a more saturated fat source during the growing phases would establish more saturated pork fat depots, so, when large amounts of DDGS and/or polyunsaturated fat sources were fed in later dietary phases, the negative effects of elevating dietary PUFA on belly firmness would be negligible. Thus, the objective of this study was to test the effects of phase-feeding beef tallow on quality characteristics of bacon from growing-finishing swine.

Materials and Methods

Crossbred pigs (n = 216) were fed a five-phase diet to test effects of phase-feeding beef tallow on bacon quality characteristics of growing-finishing swine fed diets formulated with DDGS and yellow grease. Pigs were blocked by initial body weight (55 lbs) and gender before allotment to pens (six pigs/pen), and pens (six pens/block) were allotted randomly to one of six dietary treatments (Table 1). Corn-soybean meal-based dietary treatments consisted of: negative control (NC) dietary treatment where diets were formulated with 4.7% yellow grease for all phases; a positive control (PC) dietary treatment where diets were formulated with 5% beef tallow for all phases; or treatments including 5% beef tallow during phases 1 and 2 (BT12), during phases 1, 2, and 3 (BT123), during phases 3, 4, and 5 (BT345), or during phases 4 and 5 (BT45). All dietary treatments were formulated to represent standard commercial inclusion levels of DDGS, with 30% DDGS during phases 1, 2, and 3, 15% DDGS during phase 4, and no DDGS during phase 5.

At an average body weight of 275 lbs, pigs were slaughtered at a commercial pork packing plant according to industry-accepted standards. Bellies were identified with each pig's identification number before transportation to a commercial bacon-manufacturing plant, where weights were recorded during each step of curing and thermal processing. Bellies were sliced, and USDA-certified No. 1 slices were collected, individually boxed, and transported under refrigeration to the University of Arkansas for cooking characteristics and sensory panel evaluations.

Upon arrival, sliced bacon from the center of each belly was vacuum packaged and stored at -4 °F. Bacon for cooking characteristic evaluation was thawed at 34 °F for 24 h prior to evaluation. Instrumental color values measuring lightness, redness, and yellowness (L^*, a^*, and b^*, respectively) of the uncooked bacon fat were determined with a Hunter Miniscan XE Plus. Bacon was cooked in a commercial convection oven preheated to 400 °F for 9 min and blotted dry. Precooked and cooked weights were used to calculate cooking loss percentage. A 2.4-in section of bacon was removed from the center of each slice and sheared once with a six-blade Lee-Kramer shear force device attached to an Instron Universal Testing Machine. Shear force values of the cooked bacon were determined from a mean of 4 sections.

For each taste panel session (n = 18), 12 slices of bacon from one pig from each of the dietary treatments were thawed for 48 hours at 34 °F, striated randomly across 8 racks (9 slices/rack), and cooked for 17 minutes in domestic convection ovens preheated to 425 °F for trained sensory panel evaluations. Each panelist received 6 slices (approximately 3 in length) of bacon in random order. Panelists evaluated bacon strips for initial crispiness, bacon flavor intensity,

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saltiness, sustained chewiness, oiliness, off flavor intensity, and off flavor descriptors from 1 (extremely crisp, bland, bland, crumbly, abundant, and abundant, respectively) to 8 (extremely soft, intense, salty, chewy, none, and none, respectively). Panelists were provided unsalted saltine crackers, drinking water, and apple juice or apple slices to cleanse their palates between samples.

Data were analyzed as a randomized complete block design, with blocks based on initial BW and pen as the experimental unit. Analysis of variance was done using the mixed model procedure (SAS Inst. Inc., Cary, N.C.). The lone fixed effect in all statistical models was dietary treatment, whereas initial BW block was considered a random effect in models for bacon characteristics. However, in the analysis of the cooked bacon sensory data, panelist nested within session was also included in the model as a random effect. Least-squares means were computed and separated statistically using pairwise t-tests (PDIFF option) when a significant F-test ($P \leq 0.05$) was observed.

Results

There was no effect ($P \geq 0.23$) of BT inclusion on color (L*, a*, and b* values) of the bacon fat, cook loss percentage, or Lee-Kramer shear force (Table 2). Traits evaluated by the sensory panel (initial crispiness, bacon flavor intensity, saltiness, sustained chewiness, oiliness, and off flavor intensity) were not affected by the dietary treatments ($P \geq 0.55$; Table 2).

Implications

Results of this study indicated that phase-feeding beef tallow to pigs fed diets formulated with dried distillers grains with solubles had no impact on quality characteristics of bacon. Even though fatty acid analyses of subcutaneous depots are not complete at this time, it is expected that these dietary treatments will have an effect on fatty acid profiles. These preliminary results would indicate that other factors may contribute as much, if not more, than the fatty acid composition of belly fat to the problem of soft pork bellies.

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Apple, J. K., C. V. Maxwell, D. L. Galloway, C. R. Hamilton, and J. W. S. Yancey. 2009. Interactive effects of dietary fat source and slaughter weight in growing-finishing swine: II. Fatty acid composition of subcutaneous fat. J. Anim. Sci. 87:1423-1440.

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Table 1. Beef tallow (gray) area and yellow grease (white area) inclusion in dietary treatments[1].

	Production Phase				
Dietary Treatment[2]	**Grower I (55 to 95 lb)**	**Grower II (95 to 150 lb)**	**Finisher I (150 to 175 lb)**	**Finisher II (175 to 225 lb)**	**Finisher III (225 to 275 lb)**
NC	4.7%	4.7%	4.7%	4.7%	4.7%
BT12	5.0%	5.0%	4.7%	4.7%	4.7%
BT123	5.0%	5.0%	5.0%	4.7%	4.7%
BT345	4.7%	4.7%	5.0%	5.0%	5.0%
BT45	4.7%	4.7%	4.7%	5.0%	5.0%
PC	5.0%	5.0%	5.0%	5.0%	5.0%

[1]All diets within a phase were isocaloric.

[2] NC – Negative control (4.7% yellow grease fed for entire trial); BT12 – 5.0% beef tallow fed during grower phases I and II; BT123 – 5.0% beef tallow fed during grower phases I and II and finisher phase I; BT345 – 5.0% beef tallow fed during finisher phases I, II, and III; BT45 – 5.0% beef tallow fed during finisher phases II and III; PC – Positive control (5.0% beef tallow fed for entire trial).

Table 2. Effect of dietary treatments on bacon yields, cooked bacon characteristics, and sensory traits of bacon.

	Dietary Treatment[1]							
Item	**NC**	**BT12**	**BT123**	**BT345**	**BT45**	**PC**	**SEM**	***P > F***
Fat color								
L*	75.38	77.53	75.15	76.48	75.96	76.48	0.969	0.453
a*	2.22	2.07	2.81	2.36	2.25	2.41	0.209	0.228
b*	8.99	8.82	8.99	8.97	8.82	8.93	0.125	0.830
Cook loss, %	73.35	74.01	73.06	74.28	74.51	73.79	0.556	0.351
L-K shear force, lb	192.28	187.90	189.84	190.04	193.78	187.51	8.523	0.994
Sensory traits evaluated								
Initial crispiness[2]	4.06	4.00	4.02	3.99	4.08	3.75	0.192	0.794
Bacon flavor intensity[3]	5.55	5.33	5.44	5.42	5.43	5.56	0.108	0.598
Saltiness[4]	5.29	5.06	5.22	5.14	5.21	5.16	0.163	0.939
Chewiness[5]	4.34	4.28	4.31	4.30	4.27	4.10	0.175	0.930
Oiliness[6]	6.52	6.55	6.42	6.59	6.53	6.60	0.111	0.842
Off flavor intensity[6]	7.75	7.59	7.64	7.61	7.70	7.69	0.067	0.551

[1] NC – Negative control (4.7% yellow grease fed for entire trial); BT12 – 5.0% beef tallow fed during grower phases I and II; BT123 – 5.0% beef tallow fed during grower phases I and II and finisher phase I; BT345 – 5.0% beef tallow fed during finisher phases I, II, and III; BT45 – 5.0% beef tallow fed during finisher phases II and III; PC – Positive control (5.0% beef tallow fed for entire trial).
[2] 1 = extremely crisp, 8 = extremely soft.
[3] 1 = extremely bland, 8 = extremely intense.
[4] 1 = extremely bland, 8 = extremely salty.
[5] 1 = extremely crumbly, 8 = extremely chewy.
[6] 1 = abundant, 8 = none.

Evaluation of product safety enhancement through antimicrobial electrostatic spray applications on *Longissimus lumborum* at the sub-primal level and its impact on meat color characteristics

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Story in Brief

Ensured beef microbial safety is of vital importance to avoid food-borne pathogenic outbreaks. Electrostatic spray systems may allow a novel, efficient utilization of antimicrobials for meat decontamination technology. Therefore, effectiveness of different antimicrobial electrostatic spray applications on loin eye muscle (*Longissimus lumborum*) at the sub-primal level on microbial quality and instrumental color of beef steaks during display was evaluated. Loin eye muscles (n = 11) were cut into 3 subsections (n = 33) and inoculated with *Escherichia coli* (ATCC # 11775) and *Salmonella* Typhimurium (ATCC # 1769NR) at 10^7 CFU/mL. The inoculated subsections (n = 33) were assigned to one of the following treatments: (1) water, (2) 10% trisodium phosphate, (3) 3% potassium lactate, (4) 0.4% cetylpyridinium chloride, (5) 20% hydrochloric/citric acid mixture. Subsequently all treated subsections, including (6) untreated inoculated control, and (7) un-inoculated untreated control (n = 3), were cut into 2.5cm thickness steaks (n = 15/ treatment). The steaks were then placed on foam trays with absorbent pads and overwrapped with polyvinyl chloride film. Steaks from each treatment were removed from display for bacterial enumeration of *Escherichia coli*, *Salmonella* Typhimurium , coliform, aerobic plate count and instrumental color analysis on days 1, 3 and 7. The 20% hydrochloric/cirtic acid mixture treatment had the greatest ($P < 0.05$) reduction in Escherichia coli compared to 0.04% cetylpyridinium chloride, 3% potassium lactate, 10% trisodium lactate, and water (6.02 vs. 7.50, 7.30, 7.93, and 7.63 respectively.) All treatments significantly reduced all bacterial counts when compared to the inoculated control, with the exception of coliform by water and 0.04% cetylpyridinium chloride. The water, 20% hydrochloric/ cirtic acid mixture, 0.04% cetylpyridinium chloride, and 3% potassium lactate treatments had no significant differences ($P < 0.05$) for lightness (L^*), redness (a^*), yellowness (b^*), hue angle and saturation index properties compared to inoculated control steaks. The results suggest that the electrostatic spray application of 3% potassium lactate, 10% trisodium lactate, and 20% hydrochloric/cirtic acid mixture treatments allowed for enhanced microbial quality. Likewise, little impact on color through display was observed due to treatment application.

Introduction

Pathogenic contamination of beef has prompted consumer fear and concern around the globe. It has threatened trade and economic profit and has stimulated ideas for developing new control measures to enhance product safety in beef processes. Even though the beef industry has utilized technologies such as steam pasteurization, and organic acid rinses in carcass decontamination, there is a need for microbial interventions near the end of the production chain to enhance microbial safety. Decontamination technologies have been applied to include: the conventional spraying with water, the spray wash disinfectant, steam pasteurization, and dipping. An alternate form of antimicrobial application is the Electrostatic Spray System. Electrostatic spray application is a novel technology that applies an electrical charge to liquid droplets as they are sprayed through a nozzle allowing uniform coating of the product.

Therefore, the purpose of this study was to evaluate the effectiveness of electrostatic spray application of a hydrochloric/citric acid blend, cetylpyridinium chloride, potassium lactate, trisodium phosphate and water as single antimicrobial interventions at the sub-primal level on reducing *Escherichia coli* and *Salmonella* typhimurium populations on consequent beef steaks and the treatment impact on beef steak instrumental color properties.

Materials and Methods

Inoculation Process. To make the inoculums cocktail, 0.1mL of *E. coli* (ATCC # 11755; EC) and 0.1 mL of S. *typhimurium* (ATCC # 1769NR; ST) were inoculated into 40 separate 40 ml aliquots of Brain Heart Infusion broth (BHI), (n = 80 total tubes) and BHI with Nalidixic acid, respectively. The inoculated tubes were incubated at 37 °C for 18 hours. Following incubation, the tubes were centrifuged (3500 g for 20 minutes at 37 °C). The liquid supernatant was discarded and the bacterial pellets were re-suspended in 40 mL buffered peptone water. Finally, the bacterial cocktail (EC and ST log 10^7 CFU, 3600 mL) was stored in a 4 °C cooler until ready for further use. Longissimus lumborum muscles (n = 11) were cut into 6 × 4 in. sub-sections (n = 33) and each sub-section was brushed inoculated with the bacterial cocktail and placed in a sterile biohazard bag. The inoculated beef subsections were kept in the cooler (4 °C) for 18 hours for further microbial attachment.

Antimicrobial Treatment Preparation. The antimicrobial treatments of 20% hydrochloric/citric acid blend (Citrilow™, Safe Foods Corporation, Little Rock, Ark.), 0.4% cetylpyridinium chloride (Cecure®, Safe Foods Corporation, Little Rock, Ark.), 3% potassium lactate (UltraLac KL-60, Hawkins Inc., Minneapolis, Minn.), and 10% trisodium phosphate (Trisodium phosphate anhydrous (FG), (ICL Performance Products, St. Louis, Mo.)

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were prepared by using appropriate amounts of municipal purified water. Inoculated untreated samples (INCON) and uninoculated untreated samples (C) were used as control treatments of the experiment. The C was not used for microbial analysis, however it was used for instrumental color evaluation.

Treatment Application. *Longissimus lumborum* subsections (n = 3 subsections/ treatment) were spray treated (3 ml/sec) using an electrostatic spray system (ESS; Electrostatic Spraying Systems, Inc. Watkinsville, Ga., USA) with either: (1) water (W), (2) 10% trisodium phosphate, (3) 3% potassium lactate, (4) 0.4% cetylpyridinium chloride, or (5) 20% hydrochloric/citric acid mixture. Control samples were separately run as: untreated inoculated control (INCON); or as uninoculated control (C). Each treated subsection and control subsections were cut into three steaks (n = 105) allowing fifteen steaks per treatment per display day. Steaks were then placed in styrofoam trays with absorbent pads and overwrapped with polyvinyl chloride film (O_2 transmission rate = 14,000 cc/mm^2/24h/1 atm; Koch Supplies, Inc. Kansas City, Mo.). Steaks were stored in retail display condition at 4 °C under 1,630 lx of deluxe warm white fluorescent lighting (Phillips Inc., Somerset, N.J.).

Microbial Sampling. On days 1, 3, and 7 of simulated retail display, bacterial enumeration for each steak was performed aseptically by removing 25 g from the surface using a sterile scalpel and forceps as described by Venturini et al. (2006). Microbial analysis was done for the uninoculated untreated control (C), however this was not reported due to no visible colonies. Samples were placed in sterile whirl pack bag (Nasco, Ft. Atkinson Wis.) separately and 225 mL of 0.1% buffered peptone water was added. The samples were homogenized in a stomacher (Model 400 Lab Stomacher, Seward, London, UK) at normal speed for 2 minutes. Following serial 10-fold dilutions, spread plating was done in duplicates on aerobic plate counts (APC), and *E. coli* (EC)/coliform (CO) Petrifilm® (3M Corporation, St. Paul Minn.). The ST counts were done on *Salmonella* shigella agar (Difco Laboratories, Detroit, Mich.) containing nalidixic acid. Plates were then incubated at 37 °C in an aerobic incubation chamber (either VWR Model 5015 or Model 3015 incubators, VWR Scientific, Cornelius Ore., 97113. USA.) The EC, APC and ST counts were read after 48 hours, whereas coliform plates were read at 24 hours. All counts were recorded as colony forming units per gram (CFU/g).

Instrumental Color. On days 1, 3, and 7 of simulated retail display, instrumental color was measured using a Hunter Lab Miniscan XE Spectrocolorimeter, (Model 4500L, Hunter Associates Laboratory Inc., Reston W. Va.) Samples were analyzed using Illuminant A/10° observer and evaluated for CIE $L^\star$, $a^\star$, $b^\star$, hue angle, and saturation index. In addition, hue angle, which describes the color of the steaks was calculated by the equation: ($\tan^{-1}$ ($b^\star/a^\star$)). The saturation index was calculated using the equation: (($a^{\star 2} + b^{\star 2})^{0.5}$), which describes the brightness or vividness of the red color (AMSA, 1991). Before use, the spectrophotometer was standardized using white tile, black tile and working standards. Three measurements were taken of each sample and averaged for statistical analysis.

Data Analysis. The experiment was replicated three times. A randomized, complete block, six by three factorial arrangement of treatments, was analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, N.C., USA; Version 9.1). Bacterial counts were transformed to log values prior to analysis. Treatments were blocked by replicate and then analyzed for main effects of antimicrobial treatment, day of display, and treatment by day of display interaction. Least square means for variables were generated and separated using the PDIFF option of SAS.

Results and Discussion

The effect of antimicrobial treatment at the sub-primal level by treatment and day of display on microbial populations of *Longissimus lumborum* beef steaks is summarized in Tables 1 and 2, respectively. As previously mentioned, the C treatment was used for color analysis only. Results indicate that all treatments were effective ($P < 0.05$) in reducing the *E. coli* and *Salmonella* populations. The hydrochloric/citric acid blend (CIT) showed the lowest *E. coli* population reduction ($P < 0.05$) compared to CPC, KL, TSP, and water (6.02 vs. 7.50, 7.30, 7.93, and 7.63, respectively. The steaks from CPC, KL, and TSP sub-primals also exhibited similar *Salmonella* population reduction compared to hydrochloric/citric acid blend (CIT) treated samples. Both CIT and KL treatments had similar coliform and APC counts. The CPC and W treatments did not show a reduction ($P < 0.05$) in CO, ST, and APC counts respectively, compared to other treatments; however, these treatments were effective in reducing other tested organism counts. This is similar to results found by Edwards and Fung (2006) which reported CPC has been proven to be a successful sanitizer at many different stages of beef processing, and achieves 4.8 log cfu/cm^2 reductions in *E. coli* (Ransom et al. 2003). The CIT and KL treatments showed no significant differences ($P < 0.05$) from CPC, or TSP.

The ST counts (Table 2) stayed consistent through all seven days of display. Coliform EC, and APC counts were lower ($P < 0.05$) for day 1 compared to day 3. Further, there was significantly lower ($P > 0.05$) EC count on day 3 compared to day 7.

Steaks from *Longissimus lumborum* muscles treated with CIT, CPC, and KL had similar ($P > 0.05$) lightness ($L^\star$), redness ($a^\star$), yellowness ($b^\star$), hue angle, and saturation index compared to that of the untreated inoculated control steaks. TSP and W treated steaks had similar ($P > 0.05$) $L^\star$, hue angle, and saturation index compared with inoculated control, and the yellowness of the product was lower ($P < 0.05$). This agrees with Jiminez-Villarreal, Pohlman, Johnson and Brown Jr. (2003), who found that 10% TSP treatment had similar ($P < 0.05$) yellow ($b^\star$) values when compared to the control in ground beef. It was also reported that steaks were less yellow ($P < 0.05$) across 7 days of display. Steaks also had a less true red color (hue angle) ($P < 0.05$) over 7 days of display (37.86, 44.17 and 54.75). Steak color became less vivid in redness intensity (saturation index) ($P < 0.05$) through day 7 of display (27.49, 20.10, and 15.91) (Table 2).

Implications

The 3% potassium lactate, 10% trisodium phosphate, and 20% hydrochloric/citric acid blend treatments outperform water and 0.4% cetylpyridinium chloride treatments in reducing bacterial counts. Electrostatic spray application of these treatments at the sub-primal level of loin eye (*Longissimus lumborum*) muscle may reduce microorganisms on steaks through three days of display storage without adversely affecting color quality. Further studies are also needed to evaluate the effectiveness of hydrochloric/citric acid blend either alone, or in combination with other antimicrobials on uninoculated meat color and other quality characteristics.

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Table 1. Effect of antimicrobial treatment on least squares means (±SE) log CFU*/g Coliform, *Escherichia coli, Salmonella* Typhimurium, Aerobic Plate Count (APC) and CIE *L*, a and *b** values of beef steaks through simulated retail display.**

	Treatment**						
	INCON	**W**	**CPC**	**KL**	**TSP**	**CIT**	**±SE**
Microorganism							
Escherichia coli	8.97^{d}	7.63^{bc}	7.50^{bc}	7.30^{b}	7.93^{c}	6.02^{a}	0.19
Coliform	8.23^{c}	7.64^{bc}	7.30^{bc}	6.66^{ab}	7.16^{b}	6.13^{a}	0.34
Salmonella Typhimurium	7.86^{c}	5.55^{b}	4.94^{ab}	5.08^{ab}	5.29^{ab}	4.75^{a}	0.26
Aerobic Plate Count (APC)	8.34^{d}	7.67^{cd}	7.38^{bc}	6.69^{ab}	7.26^{bc}	6.15^{a}	0.33
Instrumental color							
*L**	37.19^{ab}	38.98^{b}	36.17^{a}	38.80^{b}	38.67^{ab}	38.60^{ab}	0.91
*a**	15.44^{abc}	13.79^{a}	14.40^{ab}	16.41^{bc}	13.76^{a}	15.15^{abc}	0.89
*b**	15.42^{b}	14.26^{ab}	14.41^{ab}	14.95^{ab}	13.53^{a}	14.40^{ab}	0.61
Hue Angle	47.27^{bc}	48.06^{c}	46.64^{bc}	43.72^{ab}	46.54^{bc}	45.11^{abc}	1.41
Saturation Index (Chroma)	22.07^{ab}	20.03^{ab}	20.56^{ab}	22.34^{b}	19.46^{a}	21.11^{ab}	0.99

*CFU: colony forming unit.

** Treatments: INCON = untreated inoculated control, W = water, CPC = 0.4% cetylpyridinium chloride, KL = 3% potassium lactate, TSP = 10% trisodium phosphate, CIT = 20% hydrochloric/citric acid mixture.

[a-d] Least square means within a row with different superscripts are different ($P < 0.05$).

Table 2. Effect of duration of display, pooled across antimicrobial treatments, on least squares means (±SE) log CFU*/g *Escherichia coli,* coliform, *Salmonella* Typhimuirium, aerobic plate count (APC), *L*, a*,* and *b values through simulated retail display.**

	Display Days			
	1	**3**	**7**	**±SE**
Microorganism				
Escherichia coli	7.01^{a}	7.58^{b}	8.08^{c}	0.14
Coliform	6.34^{a}	7.44^{b}	7.79^{b}	0.24
Salmonella Typhimurium	5.70	5.64	5.40	0.19
Aerobic Plate Count (APC)	6.38^{a}	7.42^{b}	7.94^{b}	0.24
Instrumental color				
*L**	39.69^{b}	36.57^{a}	37.59^{a}	0.60
*a**	21.70^{c}	14.54^{b}	9.18^{a}	0.58
*b**	16.86^{b}	13.79^{a}	12.91^{b}	0.40
Hue Angle	37.86^{a}	44.17^{b}	54.75^{c}	0.92
Saturation Index (Chroma)	27.49^{c}	20.10^{b}	15.91^{a}	0.65

*CFU:colony forming units.

**Treatments: INCON = untreated inoculated control, CIT = 20% hydrochloric/citric acid mixture, CPC = 0.4 % cetylpyridinium chloride, KL = 3% potassium lactate, TSP = 10% trisodium phosphate, W = water.

[a-c] Least square means within a row with different superscripts are different ($P < 0.05$).

Effects of Noni puree vs. clarified Noni juice on quality characteristics of fresh ground beef patties

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Story in Brief

Noni puree and clarified Noni juice are derived from the Noni plant, which has been used for centuries in home remedies to heal and treat ailments ranging from minor sprains to major concerns such as cancer and heart disease. Recently, Noni was fed to cattle and horses to improve growth, health and performance. Previous work conducted at the University of Arkansas found that Noni puree improved fresh color and inhibited lipid oxidation in ground beef patties; therefore, the objective of this study was to determine and compare the effects of Noni puree and clarified Noni juice (derived from filtering and re-pasteurizing Noni puree) on fresh ground beef color and lipid oxidation (TBARS) during 5 days of retail display. Ground beef was mixed with 0, 2.5 or 5% Noni puree or clarified Noni juice and formed into 1/3 lb. patties. Over-wrapped patties were placed in retail display and evaluated by visual panelists and for instrumental color for 5 days. Additional patties were placed in retail display for TBARS measurement (oxidative rancidity). Although visual color scores increased overtime (became less red and more discolored) and instrumentally-measured redness (a* values) decreased, there was no difference between control and Noni-treated patties. Instrumental measurements determined that patties treated with puree were redder than those treated with juice. Lipid oxidation increased with display time in control patties, but Noni-treated patties were less oxidized than controls at days 3 and 5 of display. Furthermore, patties treated with 2.5% juice and both 5% Noni treatments had no significant increase in TBARS values from 0 to 5 days of display. Although Noni addition did not improve color stability of fresh ground beef patties in this study, it did inhibit lipid oxidation and may have potential as a natural antioxidant ingredient in meat products.

Introduction

Noni (*Morinda citrifolia*) is an evergreen plant found in tropical regions of the world, and for hundreds of years this plant has been used in homeopathic remedies to treat ailments ranging from sprains and strains to cancer. Recently, juices from Noni have become popular in nutraceutical drinks for their immune-stimulating and antioxidant properties. Noni supplements have also improved growth and health in growing cattle (Yancey et al., 2011). Previous research has shown beneficial effects of Noni puree when added to ground beef (Tapp et al., 2010), with patties containing Noni puree having greater shelf life in retail display by one to two extra days and were less rancid. Unfortunately, trained taste panels rated the Noni patties lower in beef flavor and off-flavors.

Clarified Noni juice is derived from a filtering and repasteurization process of the Noni puree, and this product has the potential to have a less offensive effect on the flavor of ground beef patties. Thus, the objective of this study was to compare the effects of Noni puree and clarified Noni juice on the fresh color stability and lipid oxidation during simulated retail display.

Materials and Methods

Ground beef (85% lean) was obtained from a commercial processor, transported to the University of Arkansas, and stored at 34 °F. Beef was mixed with either Noni puree or clarified Noni juice at 0, 2.5, or 5% in 20-lb batches (5 treatments with 5 batches / treatment) and ground once through a 3/8-inch plate. Patties (1/3 lb) were formed using a commercial patty-forming machine, and two patties from each batch were packaged on foam trays with overwrap and placed in simulated retail display for 5 d for visual and instrumental color evaluation. One patty was vacuum-packaged and frozen at -4 °F; whereas, two additional patties were prepared on foam trays with PVC overwrap 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 of display, respectively.

No fewer than 8 experienced panelists evaluated the packages of patties daily across the 5 d of display. 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:
1 = very bright cherry red
2 = bright cherry red
3 = dull red
4 = slightly dark red
5 = moderately dark red to tan
5.5 = borderline panelist acceptable
6 = dark red to brown
7 = very dark red to brown

Percent discoloration scale:
1 = 0 to 5% discoloration
2 = 6 to 20% discoloration
3 = 21 to 35% discoloration
4 = 36 to 50% discoloration
5 = 51 to 65% discoloration
6 = 66 to 80% discoloration
7 = 81 to 95% discoloration
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 (Hunter Associates

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Laboratory Inc., Reston, Va.) with Illuminant A and the 1-inch 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).

Data were analyzed in a 2 × 2 factorial, plus a control treatment structure, with batch as the experimental unit, but analytical design varied. For instrumental color, there was a repeated measure of day. For visual color, there was a repeated measure of day and the data were blocked by panelist. For TBARS, the design structure was a split-plot, with Noni treatment as the whole plot and day as the sub-plot. All data was analyzed in the mixed models procedure of SAS using the LSMEANS statement with the PDIFF option to separate the means. Contrast statements were used to specifically compare differences in controls vs. Noni, puree vs. juice, 2.5% vs. 5%, and the interaction of Noni product and inclusion level, excluding the control.

Results and Discussion

Total color scores increased ($P < 0.05$) with increasing time in display (Fig. 1), which indicated that the patties were perceived as less red and browner with extended time in display. Within each day, the Noni-treated patties (puree and juice) were similar ($P > 0.05$) to control patties until day 5, when all the patties received scores above 5.5 (borderline panelist acceptable). Similarly, discoloration scores were greater ($P < 0.05$) with increasing time in display, but, within each day, Noni-treated patties were similar to controls ($P > 0.05$) from day 0 to 4. At day 5, control patties had greater ($P < 0.05$) discoloration scores than those treated with Noni puree, but all the scores were greater than 5, indicating greater than 51% discoloration.

As expected, fresh patties in display became less red (lower a*; $P < 0.05$) with increased time in display; however, the analysis of variance revealed no Noni treatment differences ($P > 0.05$) in instrumental redness values (Fig. 2). Nevertheless, when the contrast statements were analyzed, it was determined that the Noni puree-treated patties were redder ($P = 0.045$) than those treated with Noni juice. On day 1, patties were lightest (greatest L*; $P < 0.05$), but no differences in Noni treatments were detected in lightness ($P > 0.05$). Patties also became less yellow ($P < 0.05$) with increasing time in display, but no differences in yellowness ($P > 0.05$) due to Noni treatment were found (L* and b* data not graphically presented).

Day 0 patties treated with 5% Noni juice had greater ($P < 0.05$) TBARS (a measure of oxidation) than control, 2.5% juice, and 5% puree. It was determined this was a scientific anomaly that self-corrected in day 3 samples. As was expected, TBARS values increased ($P < 0.05$) in the control patties with increasing days in display, and, on days 3 and 5 of display control patties had greater TBARS values ($P < 0.05$) than those treated with Noni. In addition, TBARS of patties containing 2.5% puree also increased ($P < 0.05$) from day 0 to day 3, but values were similar ($P > 0.05$) to patties treated with 2.5% juice and 5% puree, with little change between day 3 and day 5. Moreover, TBARS values did not change during the 5 days of display for patties treated with 2.5 % juice and both 5% treatments, indicating that Noni treatment successfully-inhibited lipid oxidation.

Unlike the previously reported study (Tapp et al., 2010), Noni puree did not significantly improve ground beef color stability in retail display, and findings for Noni juice were similar to that of Noni puree. Several factors affect the fresh color of ground beef and it is not known why this trial was not as successful as that of Tapp et al. (2010). According to the TBARS results, Noni puree and juice were successful at inhibiting lipid oxidation during retail display.

Implications

The TBARS results demonstrated that Noni-incorporations inhibited lipid oxidation in ground beef during retail display. It is possible that Noni products could be used in processed meat products, such as pre-cooked beef patties or sausages, to inhibit oxidation and the development of off-flavors. Furthermore, Noni products may have potential to improve fresh color and inhibit oxidation in meat products that are more susceptible to oxidation, such as forage-fed beef or ground turkey.

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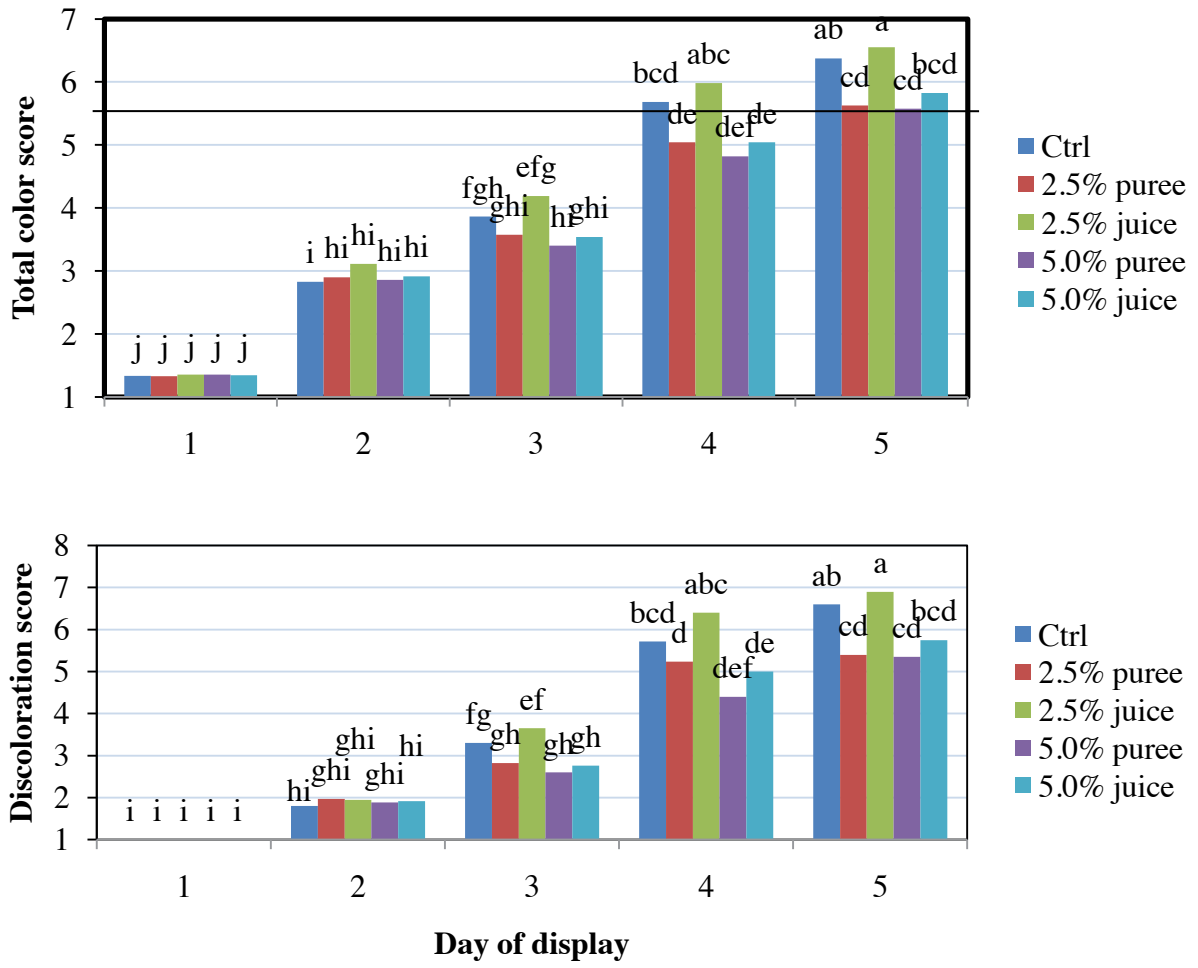

Fig. 1. Mean total color and discoloration scores for ground beef patties treated with 0 (control), 2.5, or 5% Noni puree or clarified Noni juice.

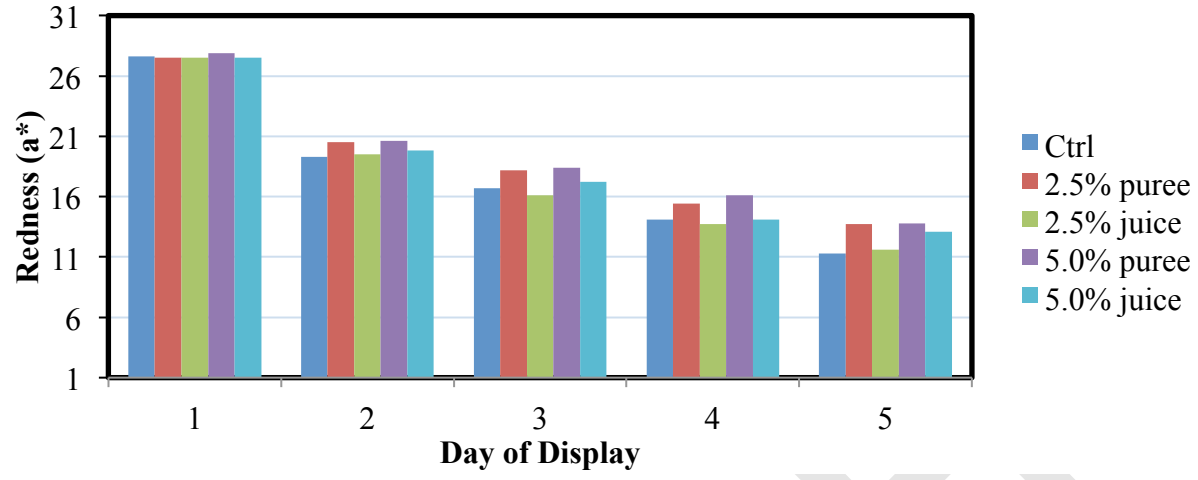

Fig. 2. Redness (*a) of fresh ground beef patties treated with 0 (control), 2.5, or 5% Noni puree or clarified Noni juice and displayed for 5 days.**

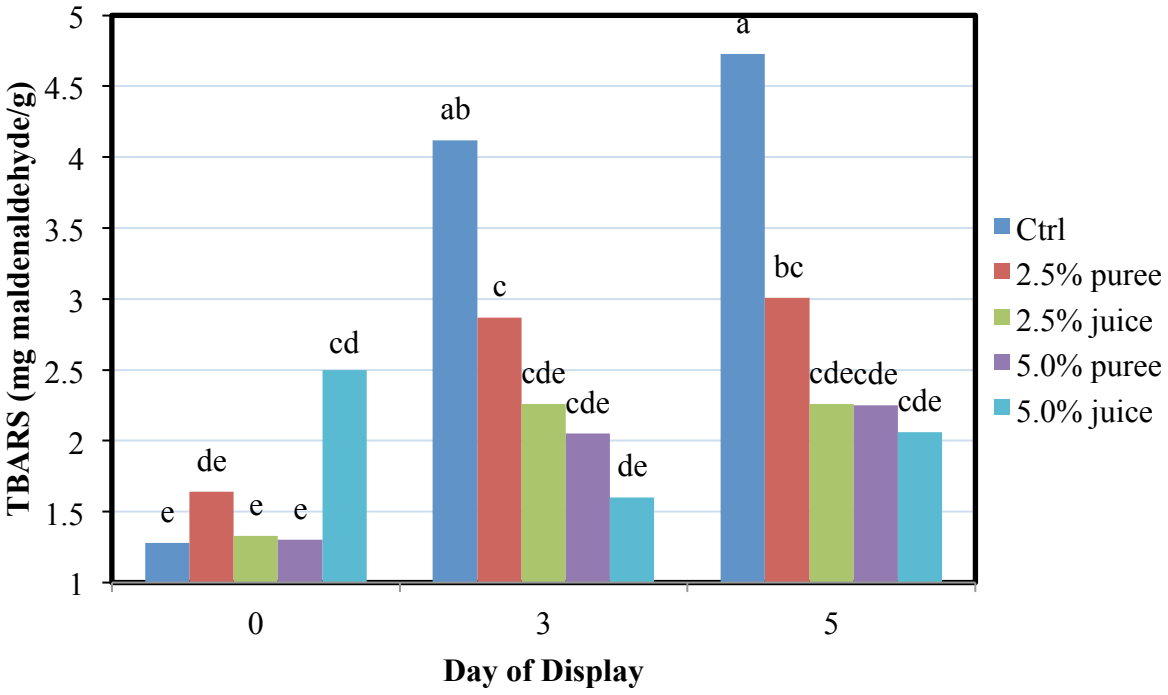

Fig. 3. Mean values for thiobarbituric acid reactive substances (TBARS, a measure of oxidative rancidity) of fresh ground beef patties treated with 0 (control), 2.5, or 5% Noni puree or clarified Noni juice and displayed for 5 days.

Effects of Noni puree vs. clarified Noni juice on quality characteristics of cooked ground beef patties

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Story in Brief

Puree derived from the Noni plant (*Morinda citrifolia*) has recently been incorporated into ground beef to improve color stability and reduce rancidity caused by lipid oxidation. The success of this product has been challenged by the flavor it imparts on the cooked ground beef. Furthermore, little is known about the effects of Noni on the cooked characteristics of ground beef and how consumers will respond to Noni products in ground beef. Clarified Noni juice is produced through filtering and re-pasteurizing of Noni puree, and Noni juice is a less viscous product with a milder aroma. Therefore, the objective of this study was to determine and compare the quality characteristics of cooked ground beef treated with Noni puree or clarified Noni juice, and to determine the responses of consumers to seasoned ground beef patties treated with Noni puree and clarified juice. Ground beef was mixed with 0, 2.5 or 5% Noni puree or clarified Noni juice, and formed into 1/3-lb patties. Patties were frozen and stored prior to thawing and cooking to 160 °F for Lee-Kramer shear force, cooked color measurement (observed by the Hunter MiniScan), and cooking losses. Additional patties were stored for consumer testing (150 consumers who eat beef more than 3 times weekly), and patties (cooked to 160 °F) for consumer testing were seasoned prior to serving. Noni treatments did not differ ($P > 0.05$) from controls in cooked color or cooking loss measurements. Although control patties required more force to shear ($P < 0.05$) than patties treated with Noni, consumers rated the texture of control patties as more acceptable than patties with 5% Noni ($P < 0.05$). Patties with 5% Noni were also rated lower than controls in overall acceptability, flavor, and off-flavor observance ($P < 0.05$). Nonetheless, consumers rated ground beef patties formulated with 2.5% Noni similar ($P > 0.05$) in palatability to control patties.

Introduction

The Noni plant (*Morinda citrifolia*) has long been used in its native Polynesia as medicine to heal and treat several ailments. Compounds in the Noni fruit are believed to act as antioxidants to produce immune-stimulating and health improving effects. These antioxidant properties were also utilized in previous research to improve color stability and inhibit lipid oxidation in fresh ground beef (Tapp et al., 2010). Although Noni-incorporated patties resulted in 1 to 2 d of extra shelf life, trained taste panelists found the flavor of Noni-incorporated patties to be objectionable.

Clarified Noni juice is derived from filtering and pasteurizing the Noni puree, resulting in a liquid product with a milder aroma. As was the case with Noni puree, Noni juice has not been incorporated into ground beef products and neither product has been evaluated for its effects on mechanical texture, cooked color, or consumer acceptability. Thus, the objective of this study was to compare the effects of Noni puree and clarified Noni juice on the cooked texture, cooked color, cooking losses, and consumer responses of ground beef patties.

Materials and Methods

Ground beef (85% lean) was obtained from a commercial processor, transported to the University of Arkansas and stored at 34 °F. Beef was mixed with either Noni puree or clarified Noni juice at 0, 2.5, or 5% in 20-lb batches (5 treatments with 5 batches/treatment) and ground once through a 3/8-inch plate. Patties (1/3 lb) were formed using a commercial patty-forming machine, and individual patties were vacuum-packaged and frozen for cooked color and Lee-Kramer (mechanical texture) evaluations. Additional patties were crust-frozen in stacks of four, and three stacks were vacuum packaged separately for consumer panels.

For mechanical texture analysis, patties were thawed overnight at 34 °F and cooked to 160 °F (medium degree of doneness) on countertop electric griddles set to 375 °F. Patties were turned every 4 minutes, monitored with a hand-held thermometer and allowed to cool to room temperature. A 2.4 × 2.4-inch piece was removed from the center of each patty and that section was sheared once using a 6-blade Lee-Kramer apparatus attached to the Instron Universal Testing Machine (Instron Worldwide Headquarters, Norwood, Mass).

For cooked color analysis, patties were thawed and cooked as described for mechanical texture analysis, but, immediately after cooking, patties were placed in a plastic bag and immersed in an ice bath to immediately halt cooking. After 5 min in the ice bath, patties were removed and sliced parallel to the patty surface to reveal an internal surface. The internal surface was wrapped with PVC overwrap before three scans were taken to measure lightness (L^*), redness (a^*), and yellowness (b^*) values using the HunterLab MiniScan XE Plus (Hunter Associates Laboratory, Inc, Reston, Va.) with Illuminant A and the 1-inch aperture size. Hue angle, a measure of the change from red to brown ($\tan^{-1}(b^* \div a^*)$), and chroma, a measure of total saturation of color ($\sqrt{(a^{*2} + b^{*2})}$), were calculated from a^* and b^* values, and the ratio of reflectance values at 630 over 580 nm was calculated to estimate redness. Cooked color patties were weighed prior to cooking and after ice-bath immersion to measure cooking losses using the following equation:

$$\left(\frac{(\text{Raw weight} - \text{Cooked weight})}{(\text{Raw weight})}\right) \times 100$$

Consumer panelists (n = 150), who ate beef 3 or more times weekly, were selected from a pool of over 10,000. Patties were thawed and cooked on countertop griddles to 160 °F as described for mechanical texture analysis. Immediately after cooking, patties for consumer

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testing were seasoned with 0.014-oz of Grill Mates® Montreal Steak Seasoning (McCormick) prior to cutting into 4 samples. Samples were held in warmers prior to being served to consumers. Panelists were seated in a climate-controlled room in individual booths with lighting adjusted so that degree of patty doneness could not be assessed to bias the panelists. Each panelist was given a sample from each treatment in a random order and asked to evaluate six attributes. Overall acceptability, beef flavor, and texture were evaluated on 9-point scales, where 1 = dislike extremely and 9 = like extremely. Juiciness and beef flavor were also evaluated on 5-point just-about-right scales, where 1 = much too dry/weak beef flavor, 3 = just about right, and 5 = much too juicy/strong beef flavor. Lastly, the presence of non-beef flavor was assessed by a yes/no question, and, if an off-flavor was detected, panelists were asked to describe the flavor.

Data were analyzed in a 2 × 2 factorial + control treatment structure, with batch as the experimental unit, but analytical design varied. For cooked color, Lee-Kramer shear force, and cooking loss, data were analyzed in a completely randomized design. The block of panelist was used for consumer 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. Contrast statements were used to specifically compare differences in controls vs. Noni, puree vs. juice, 2.5 vs. 5%, and the interaction of Noni product and inclusion level, excluding the control.

Results and Discussion

Lee-Kramer shear force measures the force required to shear a piece of cooked patty using six steel blades, and these values are related to the texture or tenderness of the beef patties, with higher values indicative of "tougher" patties. Control patties and those containing 2.5% juice had greater shear force values ($P = 0.04$) than patties with 2.5% Noni puree or either of the 5% Noni treatments (Table 1). Furthermore, contrast statements indicated that patties with 2.5% Noni tended ($P = 0.08$) to have greater shear force values than those with 5% Noni. The Noni products may have extracted the soluble proteins in the patties, tenderizing the beef as would happen in sausage manufacture.

Few differences were found in internal cooked color of the beef patties (Table 1). Noni treatment did not affect ($P > 0.05$) lightness (L^*), redness (a^*), hue angle (change from red to brown), or the red-brown ratio (measured using reflectance values). Contrast statements revealed that patties with Noni juice were more yellow ($P = 0.008$) than those with puree, but all patties were similar to controls in yellowness ($P > 0.05$). Patties with juice also had greater chroma values, a measure of total color intensity (juice vs. puree; $P = 0.02$), but all Noni treatments were similar ($P > 0.05$) to controls for chroma.

All Noni-treated patties were similar ($P > 0.10$) in cooking loss to control patties (Table 1). Patties with 2.5% Noni puree tended ($P = 0.095$) to have less cooking loss than patties containing 5% Noni puree or juice. Furthermore, contrast statements showed that patties with 5% Noni products had greater cooking losses ($P = 0.031$) than those with 2.5% Noni. The weight lost during cookery is largely water, and Noni juice and, to a lesser extent, puree are largely made up of water, thereby contributing to the tendency toward greater cooking losses.

Consumer panelists did not rate the patties incorporated with 5% Noni as high as those without Noni, indicating that they did not find them as acceptable (Table 2). Scores for overall acceptance of 2.5% Noni products were similar ($P > 0.05$) to control patties, but patties with 5% Noni were scored lower ($P < 0.05$). When contrast statements were used to directly compare Noni-incorporated patties to controls, control patties had greater overall acceptability scores ($P = 0.01$) than those treated with Noni.

When texture was analyzed by the consumers, patties with 2.5% Noni juice and puree resulted in similar ($P > 0.05$) scores to control patties, but patties with 2.5% Noni puree patties were also similar ($P > 0.05$) to patties with 5% Noni, which were lower ($P < 0.05$) than controls (Table 2). Evaluation of contrast statements also indicated that the texture of the 5% Noni patties was scored lower (less acceptable; $P = 0.004$) than the 2.5% Noni patties. Although the results from the Lee-Kramer shear force device indicated that the Noni-incorporated patties required less force to shear, consumers did not find their texture to be as acceptable. Consumers were likely evaluating the texture that they typically expect from cooked ground beef, and the Noni products may have deviated from that, causing the consumers to rate it as less acceptable.

Beef patties with 2.5% Noni were rated similarly ($P > 0.05$) to control patties for flavor acceptability and were scored greater ($P < 0.05$) than patties with 5% Noni, indicating that consumers rated the 5% patties as less acceptable in flavor (Table 2). Contrast statements also found that the Noni-incorporated patties were scored lower (less acceptable in flavor) than controls ($P < 0.001$) and that patties with 5% Noni were scored lower ($P < 0.001$) than those with 2.5%.

Just-about-right scales were used to evaluate beef flavor and juiciness, where a score of 3 equates to the flavor and juiciness rating of just-about-right. Although the mean values for all treatments were less than 3.0, control and Noni-treated patties had similar ($P > 0.05$) scores for both beef flavor and juiciness.

For off-flavor evaluation, the answer 'yes' received a score of 2, whereas a 'no' received a score of 1. Control patties and patties with 2.5% Noni received greater scores ($P < 0.05$) for off-flavor presence than patties with 5% Noni. Roughly 50% of the 5% Noni patties were identified as having an off-flavor, whereas about 25% of the 2.5% Noni and control patties were identified with an off-flavor. All patties were seasoned with a commercial seasoning which could contribute to the off-flavors perceived by the consumers. Consumers were also asked to describe the off-flavor if they answered "yes" to the off-flavor question, and several consumers described the off-flavor as 'salty', 'peppery', or 'garlic', but those can likely be attributed to the seasoning. Some of the descriptors for the Noni-treated patties included 'spoiled', 'gone bad', 'sweet', 'tangy', 'chemical', or simply 'non-beef' or 'off-flavor'.

Implications

Previous research has shown that Noni puree and juice can be successfully incorporated into ground beef to inhibit lipid oxidation. In cooked ground beef, Noni did not have a large impact on cooked color or cooking losses, but patties with Noni required less force to shear. Patties with 2.5% Noni were similar to controls in each palatability attribute evaluated by the consumers, but incorporation of 5% Noni into ground beef will continue to be challenged by the flavors and aroma of the finished product.

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Table 1. Mean values of Lee-Kramer shear force measurements, cooked color, and cooking loss of ground beef patties treated with 0, 2.5, or 5% Noni puree or juice.

		2.5%		5%			Contrast Statements[1]			
	Control	Puree	Juice	Puree	Juice	*P* value	C vs.N	J vs. P	Level	Int.
Lee-Kramer shear (N)	175.3[a]	165.3[b]	176.3[a]	164.8[b]	163.8[b]	0.038	0.059	0.163	0.082	0.098
Cooked color										
Lightness (*L**)	61.86	61.10	61.33	60.38	61.21	0.124	0.053	0.167	0.266	0.426
Redness (*a**)	14.47	14.75	15.62	12.35	14.31	0.148	0.829	0.119	0.045	0.535
Yellowness (*b**)	16.29[xy]	16.11[xy]	16.88[x]	15.53[y]	17.23[x]	0.069	0.756	0.008	0.786	0.270
Hue angle[2]	48.68	47.70	47.51	51.66	50.51	0.298	0.712	0.677	0.039	0.765
Chroma[3]	21.85[xy]	21.90[xy]	23.07[x]	19.86[y]	22.44[x]	0.068	0.975	0.021	0.089	0.359
Red-brown ratio[4]	1.77	1.83	1.93	1.55	1.76	0.202	0.964	0.166	0.051	0.661
Cooking loss (%)	33.19[xy]	31.69[y]	34.04[xy]	34.84[x]	35.21[x]	0.095	0.476	0.160	0.031	0.299

[a,b] Means, within a row, with different superscript letters differ ($P < 0.05$).

[x,y] Means, within a row, with different superscript letters differ ($P < 0.10$).

[1] Contrast statements were calculated to determine specific differences between control versus all Noni products (C vs. N), clarified Noni juice versus Noni puree (J vs. P), 2.5% versus 5% inclusion levels (Level), and the interaction of Noni product source and level with the exclusion of the control (Int.).

[2] Hue angle is a measure of the change of color from red to brown, calculated $\tan^{-1}(b^* \div a^*)$. Larger numbers indicate less red, more brown.

[3] Chroma is a measure of total saturation of color, calculated $\sqrt{(a^{*2} + b^{*2})}$. Larger numbers indicate a more saturated, vivid coloration.

[4] Red-brown ratio is the ratio of the reflectance values at 630 nm/580 nm. Larger numbers reflect a redder color.

Table 2. Mean values of consumer panel scores for overall acceptance, texture, flavor, beef flavor, juiciness, and off flavor of ground beef patties treated with 0, 2.5, or 5% Noni puree or juice.

		2.5%		5%			Contrast Statements[1]			
	Control	Puree	Juice	Puree	Juice	*P* value	C vs.N	J vs. P	Level	Int.
Overall acceptance[2]	6.88[a]	6.58[a]	6.65[a]	6.04[b]	6.12[b]	<0.001	0.010	0.591	<0.001	0.979
Texture[2]	6.69[a]	6.44[ab]	6.77[a]	6.23[b]	6.16[b]	0.007	0.071	0.372	0.004	0.159
Flavor[2]	6.83[a]	6.44[a]	6.50[a]	5.76[b]	5.90[b]	<0.001	<0.001	0.521	<0.001	0.797
Beef flavor[3]	2.65	2.57	2.51	2.53	2.58	0.590	0.166	0.875	0.793	0.372
Juiciness[3]	2.77	2.77	2.77	2.79	2.79	0.993	0.810	1.000	0.686	0.893
Off-flavor[4]	1.75[a]	1.72[a]	1.69[a]	1.50[b]	1.55[b]	<0.001	0.001	0.795	<0.001	0.260

[a,b] Means, within a row, with different superscript letter differ ($P < 0.05$).

[1] Contrast statements were calculated to determine specific differences between control versus all Noni products (C vs. N), clarified Noni juice versus Noni puree (J vs. P), 2.5% versus 5% inclusion levels (Level), and the interaction of Noni product source and level with the exclusion of the control (Int.).

[2] Overall acceptance, texture, and flavor were evaluated on a 9-point scale, where 1 = dislike extremely and 9 = like extremely

[3] Beef flavor and juiciness were evaluated on a 5 point, just-about-right scale where 1 = much too dry/weak beef flavor, 3 = just about right, 5 = much too juicy/strong beef flavor.

[4] Off-flavor was evaluated as a yes or no question, for analysis 1 = yes and 2 = no.

Instrumental color properties of ground beef processed from beef trims pre-treated with hydrochloric/citric acid based decontamination interventions

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Story in Brief

This study was conducted to evaluate the impact of hydrochloric acid/citric acid based pre-grinding decontamination interventions on ground beef instrumental color properties. Inoculated (*Escherichia coli* and *Salmonella* Typhimurium, 10^7 CFU/ml) beef trimmings (5 lb/treatment/replicate) were vacuum tumbled for 20s with 500ml of selected antimicrobial treatments. The treatments involved 15% hydrochloric/citric acid blend (Citrilow™) alone or 15% hydrochloric/citric acid blend followed by 4% sodium metasilicate, 0.4% cetylpirinidium chloride, 10% trisodium phosphate or water. The un-treated un-inoculated control and inoculated untreated controls along with treated beef trimmings were ground twice and 200 g from each treatment were placed on individual plastic foam trays with absorbent pads. The packages were over wrapped with polyvinyl chloride film and stored under simulated retail conditions (4C). Subsequently, the samples were evaluated for instrumental color (CIE) L^*, a^* and b^* values on days 0, 1, 2, 3, 4 and 5 of display using illuminant A/10° observer. On day 0 of display, 10% trisodium phosphate ground beef was more red ($P < 0.05$) compared to inoculated control and un-treated un-inoculated control samples. Ten percent trisodium phosphate and 15% hydrochloric/citric acid blend followed by 4% sodium metasilicate treatments maintained a similar redness ($P > 0.05$) compared to inoculated control and un-treated un-inoculated control, respectively on days 1 through 5 of display. Findings from this study indicate that use of hydrochloric/citric acid blend along with trisodium phosphate or sodium metasilicate may improve the ground beef redness and extend shelf-life. Therefore, the antimicrobial properties of these agents be may used as potential multiple pre-grinding interventions to enhance ground beef safety without adverse effects on ground beef color.

Introduction

Despite advanced decontamination technologies employed in meat processing, ground beef safety concerns continue with frequent safety recalls related to possible contamination with pathogenic bacteria. Single and multiple antimicrobial interventions have shown promising results in decontaminating meat products; however, any intervention that has a negative impact on meat color characteristics leads to severe economic losses. Application of cetylpirinidium chloride (CPC) and trisodium phosphate (TSP) as an antimicrobial intervention, as reported by Pohlman et al. (2002) and Jimenez-Villarreal et al. (2003), may enhance redness (a^*) and oxymyoglobin stability (630 nm/580 nm) without affecting the odor characteristics in ground beef. A novel hydrochloric/citric acid blend has showed promise as a potential decontamination technique to enhance microbial quality of meat products. However, impact of such intervention on ground beef color properties is under-investigated. Jimenez-Villarreal et al. (2003) and Pohlman et al. (2009) found out the use of CPC, TSP and sodium metasilicate (NMS) may not adversely affect the color characteristics when used in a ground beef system. Therefore, the objective of this study was to evaluate the impact of hydrochloric/citric acid blend alone or followed by CPC, TSP, NMS or water when used as a pre-grinding intervention on instrumental color properties of ground beef.

Materials and Methods

Antimicrobial Treatment and Processing. The treatments involved 15% hydrochloric/citric acid blend (Citrilow™ Safe Foods Corporation, Little Rock Ark.; CIT) alone or CIT followed by 4% sodium metasilicate (Avgard®, Rhodia Inc., Cranbury, N.J.; CITNMS), 0.4% cetylpirinidium chloride (CECURE™ Safe Foods Corporation; CITCPC), 10% trisodium phosphate (Trisodium phosphate anhydrous (FG), ICL performance products, St. Louis, Mo.; CITTSP) or water (CITW). For treatment application, 5 lb batches of beef trims were placed into a meat tumbler (Model 4Q, Lyco Inc. Janesville, Wis.) and 500 ml of hydrochloric/citric acid blend was added and tumbled at 60 rpm for 2 min. The CIT-treated trims designated for secondary antimicrobial treatment were allowed to drip for 30 min prior to vacuum tumbling for 2 min at 60 rpm with (1) sodium metasilicate (CITNMS), trisodium phosphate (CITTSP), cetylpyrinidium chloride (CITCPC) or water (CITW). Each antimicrobial treatment was repeated three times. Next, pre-treated trims as well as untreated un-inoculated (CON) and inoculated (INCON) control trims were ground twice. Samples of 200 g of ground beef processed from each treatment (n = 5 per treatment per replicate) were placed on styrofoam trays with absorbent pads and overwrapped with polyvinyl chloride film with an oxygen transmission rate of 14,020 $cm^3O_2/m^2/24$ h/atm^{-6} (Koch Supplies, Inc., Kansas City, Mo.). The ground beef samples were stored under simulated retail conditions (4C; deluxe warm white fluorescent lighting, 1630 lux, Phillips Inc., Somerset, N.J.

Instrumental Color. Instrumental color measurements of ground beef processed from treated and untreated beef trim were taken on days 0, 1, 2, 3, 4 and 5 of simulated display using a Hunter-Lab MiniScan XE Spectrocolorimeter, Model 4500L (Hunter Associates Laboratory, Reston, W.Va.). The samples were assessed for CIE (L^*, a^*, and b^*) color values, hue angle ($\tan^{-1}(b^*/a^*)$, which describes the hue or color of ground beef, and saturation index $(a^{*2}+ b^{*2})^{0.5}$, which describes the brightness or vividness of color (Hunt, et al., 1991). Reflectance measurements were also taken in the visible spectrum from 580 to 630 nm and reflectance ratio (630/580 nm) was calculated to estimate the oxymyoglobin proportion of the myoglobin pigment (Hunt et al., 1991). Each sample was measured three times using Illuminant A/10° observer and the spectrocolorimeter was standardized using white tile, black tile and working standards before used in measurements.

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Analysis of Data. Lightness (L^*), redness (a^*), yellowness (b^*), hue angle, reflectance ratio and saturation index were arranged in a completely randomized 5 × 5 factorial design. The experiment was replicated three times. Treatments were analyzed for the main effects of antimicrobial treatment, day of display and treatment by day interactions using the GLM procedure of SAS (SAS Inst. Inc., Cary, N.C.). Means were generated using LSMEANS and separated with the PDIFF option of SAS.

Results and Discussion

The results (Figs. 1 and 2) indicate that CITTSP-treated ground beef was more red ($P < 0.05$) compared to all other treatments on day 0 of simulated display. Ground beef processed from CITTSP maintained a similar a^* ($P > 0.05$) compared to INCON, respectively on days 1 through 5 of display. Similarly, CITNMS treatment showed similar a^* ($P > 0.05$) compared to CON on day 1 through 5 of display. The hue angle value for CITTSP and CITNMS- treated ground beef was lower compared to other treatments on day 0 indicating more redness in color than the rest of the treatments. In addition CITTSP and CITNMS treatments continued to maintain lower hue angle compared to CIT, CITCPC, and CITW treatments on day 2 through 5 of display. Similarly, CITTSP and CITNMS treated samples had more ($P < 0.05$) vivid color compared to all the treatments on day 0 and had similar vivid color to CON and INCON on days 2, 3 and 4 of display. Additionally, the oxymyoglobin proportions (630 nm/580 nm ratio) were highest ($P < 0.05$) in CITTSP compared to all the treatments on day 1 and 2 of display. The ground beef samples were lighter ($P < 0.05$) and more yellowish ($P < 0.05$) on day 0 compared to other days of display. There was no significant ($P > 0.05$) difference in L^* during day 1 through 5 of display, however, the intensity of b^* reduced ($P < 0.05$) with the time. These results were in agreement with Pohlman et al. (2009) and Jimenez-Villarreal et al. (2003) who reported TSP and NMS may enhance redness (a^*) and oxymyoglobin stability (630 nm/580 nm) when used as a pre-grinding treatment.

Implications

Findings from this study indicate that use of hydrochloric/citric acid blend along with trisodium phosphate or sodium metasilicate may improve the ground beef redness and extend shelf-life. Therefore, the antimicrobial properties of these agents be may used as potential multiple pre-grinding interventions to enhance ground beef safety without adverse effects on ground beef color.

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Effect of antimicrobial treatment and day of display interaction on redness (*a), Hue angle[1], saturation index and oxymygolobin proportion[2] of the myoglobin pigment (630nm/580nm) values ground beef.**

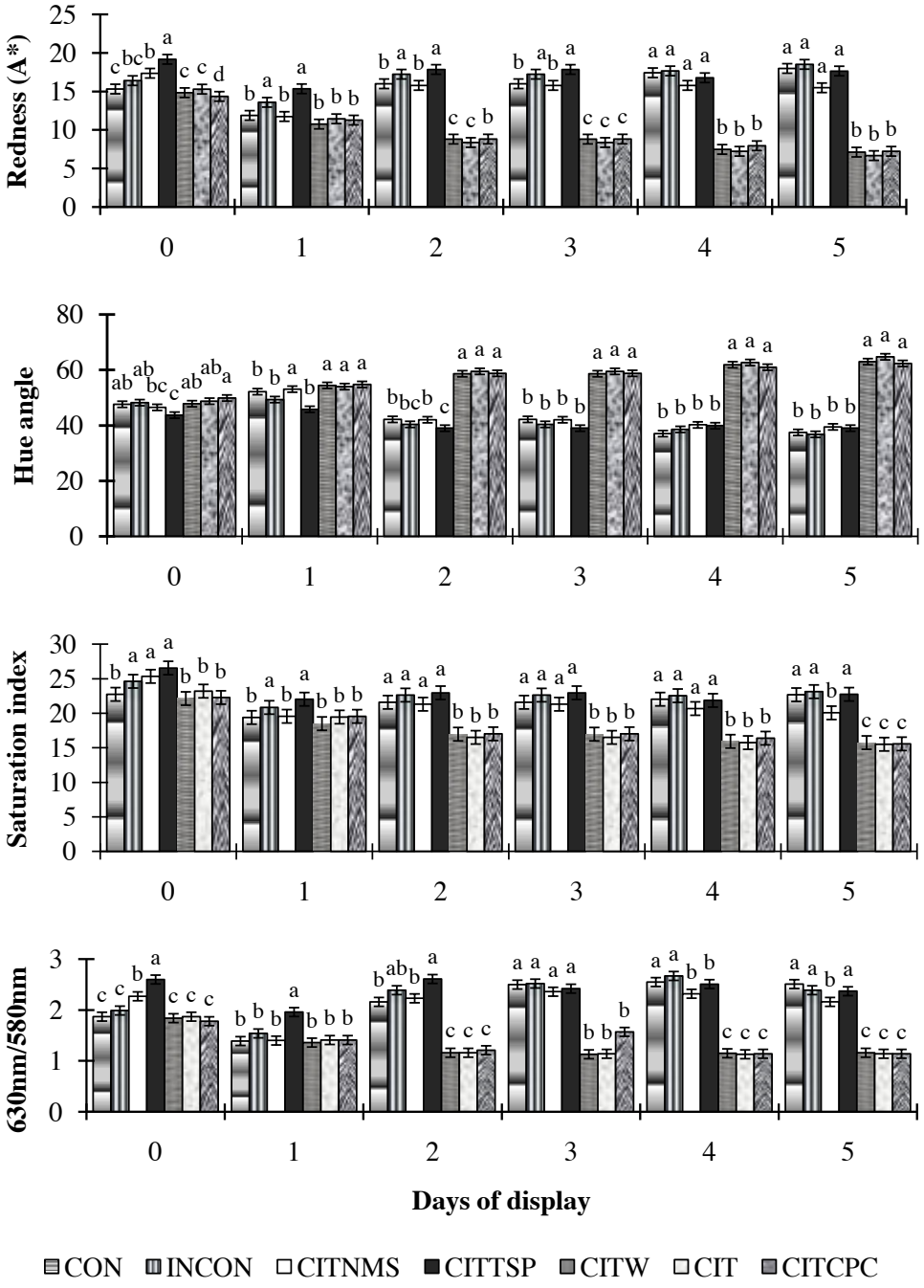

Fig. 1. Data sharing a common letter (a-c) within a day are significantly not different (*P* > 0.05). Estimates (n = 3/treatment/replicate) for redness (a*), Hue angle, saturation index and oxymygolobin proportion of the myoglobin pigment (630 nm/580 nm) with ±SE represented on the vertical bars. CON- un-inoculated untreated control; INCON- inoculated untreated control; CITNMS-15% hydrochloric/citric acid followed by 4 % sodium metasilicate, CITTSP-15% hydrochloric/citric acid followed by 10% trisodium phosphate; CITW-15% hydrochloric/citric acid followed by water; CIT-15% hydrochloric/citric acid alone; CITCPC-15% hydrochloric/citric acid followed by 0.4% cetylpyrinidium chloride. Hue angle[1] - ($\tan^{-1}(b^*/a^*)$), saturation index[2] ($(a^{*2}+b^{*2})^{0.5}$).

Effect of antimicrobial treatment and day of display effects on lightness (L*) and effect of day of display on least squares means (± SE) yellowness (b*) values of ground beef

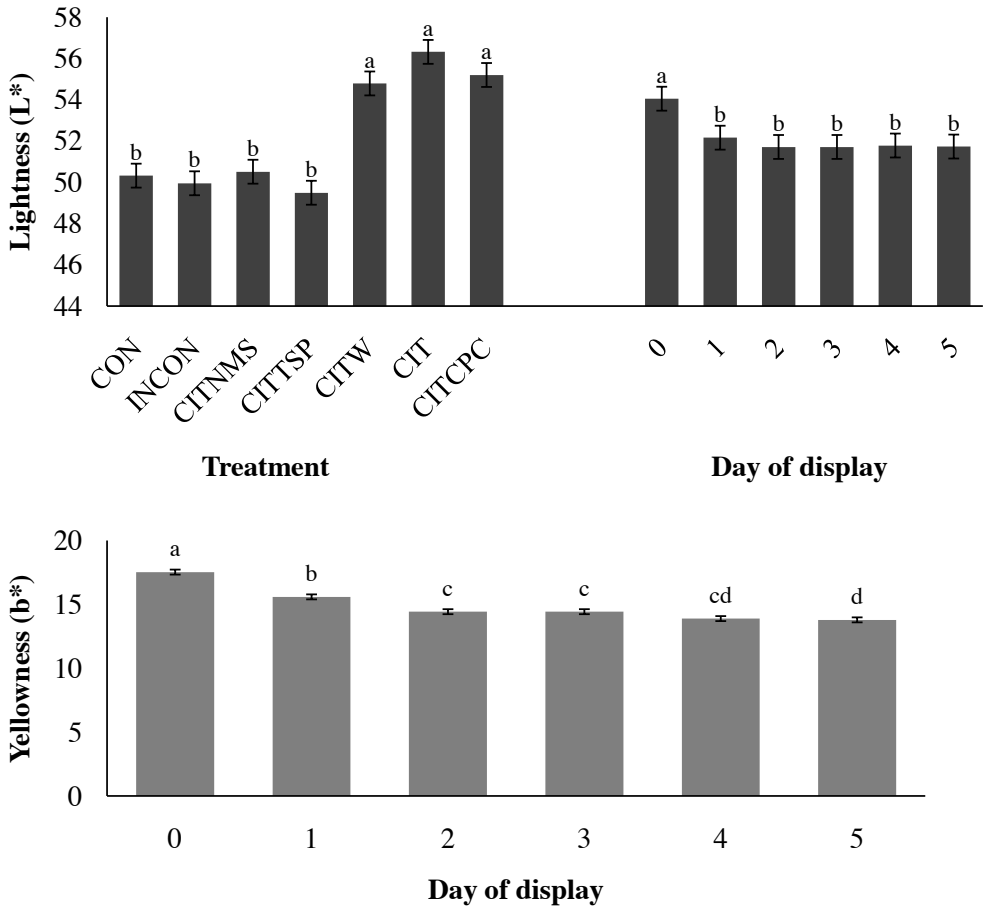

Fig. 2. Data sharing a common letter (a-d) are significantly not different (*P* > 0.05). Estimates (n = 3/ treatment/replicate) for Lightness (*L) and yellowness (*b**) with ± SE are represented on the vertical bars. CON- un-inoculated untreated control; INCON- inoculated untreated control; CITNMS-15% hydrochloric/ citric acid followed by 4 % sodium metasilicate, CITTSP-15% hydrochloric/citric acid followed by 10% trisodium phosphate; CITW-15% hydrochloric/citric acid followed by water; CIT-15% hydrochloric/citric acid alone; CITCPC- 15% hydrochloric/citric acid followed by 0.4% cetylpyrinidium chloride.**

The impact of cetylpyridinium chloride, trisodium phosphate, potassium lactate, sodium metasilicate, or water as antimicrobial interventions on microbiological characteristics of beef *biceps femoris* muscles

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Story in Brief

Bottom outside round (*Biceps femoris*) muscles (n = 12) were brush inoculated with *Escherichia coli* and *Salmonella* Typhimurium at 10^7 colony forming units/ml, after being cut into three subsections per muscle (n = 36 total subsections). Each subsection was spray treated with either: (1) water; (2) 3% potassium lactate ; (3) 4% sodium metasilicate ; (4) 0.5% cetylpyridunium chloride ; (5) 10% trisodium phosphate ; (6) inoculated untreated control ; or (7) uninoculated untreated control . Following spray treatments, each subsection was cut into three individual steaks (n = 105) allowing fifteen steaks per display day per treatment. Steaks were placed on foam trays with absorbent pads, overwrapped with polyvinyl chloride film, and sampled for *Escherichia coli*, *Salmonella* Typhimurium, coliforms, and aerobic plate count on day 0, 1, 2, 3, 5, and 7 of display. The 3% potassium lactate treatment effectively reduced ($P < 0.05$) coliforms, *Escherichia coli*, and aerobic plate counts compared to the inoculated control on day 0. The 4% sodium metasilicate treated samples had 1.57 and 1.56 ($P < 0.05$) log reduction in coliforms and *Escherichia coli* counts, respectively, compared to the inoculated control on day 1. The 0.5% cetylpyridunium chloride and 4% sodium metasilicate treatments outperformed other treatments in reducing CO, *Escherichia coli* and aerobic plate counts on day 3 of display. Therefore spray treating beef bottom outside round sub–primals with antimicrobials may be beneficial for reducing bacteria on steaks.

Introduction

Within the meat industry, new and emerging pathogens continue to provide an obstacle for producers and consumers concerning meat safety. Adam and Bulisauer (2010) have stated that the production of safe red meat depends on effective control of pathogenic microorganisms at all stages of the "farm-to-fork" chain. The most serious meat safety issues affecting consumer health and triggering product recalls involve microbial and particularly bacterial pathogen contamination (Sofos, 2008).

Meat decontamination techniques are beneficial in reducing or eliminating bacteria that may be human pathogens, as well as those that may cause meat spoilage (Huffman, 2002). When beef carcasses are fabricated into retail cuts, any microbial contamination present on carcasses will be introduced to newly exposed surfaces (Emswiler, et al., 1976). The use of antimicrobials in meat decontamination continues to advance; however, the effectiveness of these antimicrobials must be tested and implemented in order to have maximum performance without impacting quality characteristics.

The objectives of this study were to evaluate the effectiveness of antimicrobial interventions on reducing *Escherichia coli* and *Salmonella* Typhimurium of pre-inoculated beef *biceps femoris* muscles.

Material and Methods

Bacterial Preparation and Inoculation. *Escherichia coli* (ATCC #11775) and a nalidixic acid resistant strain of *Salmonella* Typhimurium (ATCC #1769NR) inocula were prepared from frozen (-80 °C) stock cultures. Frozen cultures of *E. coli* and *Salmonella* Typhimurium were thawed, and 0.1 ml of *E. coli* suspension was inoculated into 40 separate 40 ml aliquots of BHI, and 0.1 ml of *Salmonella* Typhimurium suspension were inoculated into 40 separate 40 ml aliquots of BHI with nalidixic acid. Following 18 h of incubation at 37 °C, bacteria were then harvested by centrifugation ($3500 \times g$ for 20 min at 37 °C), and re-suspended with 40 ml of 0.1% buffered peptone water (BPW) and pooled together (1600 ml of *E. coli* and 1600 ml of Typhimurium) to make a bacterial cocktail. The bacterial cocktail (3200 ml; 10^7 CFU/ml *E. coli* and 10^7 CFU/ml Typhimurium) was cooled to 4 °C and then combined. Beef *biceps femoris* muscles (n = 12) were cut into three subsections (n = 36 subsections), and brush inoculated with the bacterial cocktail and placed in a sterile bag (n = 5 subsections/sterile bag) and stored in a 4 °C cooler for 12 to 14 h to allow for further microbial attachment.

Antimicrobial Treatment Application and Sample Processing. The antimicrobial treatments of 3% KL (UltraLac KL-60, Hawkins Inc., Minneapolis, Minn.), 4% NMS (Metso Pentabead® 20, PQ Corporation, Valley Forge, Pa., USA), 0.5% CPC (Cecure®, Safe Foods Cooperation, Little Rock, Ark.), 10% (w/v) TSP (Trisodium phosphate anhydrous (FG), ICL performance products, St. Louis, Mo.), were prepared by mixing appropriate amounts with water. Inoculated untreated samples (IN) and uninoculated untreated samples (C) were used as control treatments of the experiment. The C was not used for microbial analysis, but it was used for instrumental color characteristics. Inoculated subsections (n = 5 subsections per treatment) (subsections were approximately 10.2 cm by 27.9 cm) were spray (SureSpray™ Sprayer Deluxe, applied 50 ml on 44 square inch area in 10 sec) treated with either: (1) water; (2) 3% KL; (3) 4% NMS; (4) 0.5% CPC; (5) 10% TSP; (6) IN; or (7) C. Each subsection was cut into three individual steaks (n = 105) allowing fifteen steaks per treatment per display day. Steaks were placed on styrofoam trays with absorbent pads and overwrapped with polyvinyl chloride film (O^2 transmission rate = 14, 000 cc/mm^2/24h/1 atm). The steaks were stored at 4 °C under 1630 lx of deluxe warm white fluorescent lighting.

Microbial Sampling. On days 0, 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. The 2.5 g samples were placed in sterile whirl pack bags

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separately and 22.5 ml of 0.1% buffered peptone water was added. The samples were then homogenized for 2 minutes at normal speed in a stomacher. 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) Petrifilm ® plates (3M Corporation, St. Paul, Minn.). The ST counts were done on *Salmonella Shigella* agar containing nalidixic acid. Plates were then incubated at 37 °C in an aerobic incubation chamber. The EC, APC, and ST counts were read after 48 h, whereas CO plates were read at 24 h. All counts were recorded as colony forming units per g (CFU/g).

Statistical Analysis. The experiment was replicated three times. A randomized, complete block, six by five factorial design, was analyzed using the GLM procedure of SAS. Bacterial counts were transformed to log values. Treatments were blocked by replicate then analyzed for main effects of antimicrobial treatment, day of display, and treatment by day of display interaction. For variables confounded by interactions, interaction least squares means were generated and separated using the PDIFF option of SAS. Least-squares means for all other variables were generated and separated using the PDIFF option of SAS.

Results and Discussion

The effect of antimicrobial treatment at the sub-primal level by day of display interaction on microbial populations of *biceps femoris* beef steaks is summarized in Tables 1, 2, 3, and 4. The KL treatment reduced (~1 log; $P < 0.05$) CO, EC and APC compared with IN on day 0 of display. The water (W) and CPC treatments also showed a significantly ($P < 0.05$) lower count in CO and EC compared to the inoculated control on day 0 of display. On day 1 of display, NMS significantly ($P < 0.05$) reduced CO and EC counts. The NMS treatment had a greater ($P < 0.05$) reduction in CO and EC on day 1 versus CPC, IN, KL, TSP, and water. Compared with IN, the CPC and TSP treatments significantly ($P < 0.05$) reduced CO and EC counts on day 2 of display, and furthermore, the water treatment also significantly ($P < 0.05$) reduced EC counts on beef steaks on day 2 of display. On day 2 of display, the water and CPC treatments significantly ($P < 0.05$) reduced APC counts. Both CPC and NMS treatments significantly ($P < 0.05$) reduced EC, APC and CO counts on day 3 of display; and furthermore, water treatment also significantly ($P < 0.05$) reduced CO counts on day 3 of display. On day 5 of display there was no significant difference ($P > 0.05$) for CO counts of beef steaks. *E. coli* and APC counts on day 5 and 7 of display were similar ($P > 0.05$) between all treatments. On day 0 of display, there was no significant difference ($P > 0.05$) among treatments for ST counts of beef steaks. On day 2 of display ST counts were significantly reduced ($P < 0.05$) by water and TSP compared to IN, CPC, KL, and NMS (3.90 and 4.00, compared to 4.96, 4.42, 4.92, and 4.53, respectively). Both NMS and water treatments significantly ($P < 0.05$) reduced ST counts on day 3 of display when compared to IN, CPC, KL, and TSP (2.13 and 3.02 compared to 4.07, 3.30, 3.91, and 3.74, respectively). On day 5 of display, the CPC, NMS, TSP, and KL treatments significantly ($P < 0.05$) reduced ST counts; and furthermore, on day 7 of display, CPC significantly ($P < 0.05$) reduced ST counts on beef steaks compared with IN.

Implications

With an increase in food borne pathogens, it is imperative to study the use of antimicrobial treatments and their effects on microbial characteristics. There is little research displaying the effects of treating sub-primals with antimicrobials to determine effectiveness of reducing microbial load. Both CPC and NMS reduced EC through 3 days of display. NMS reduced ST through 3 days of display. Spray treating *biceps femoris* muscles at the sub-primal level with certain antimicrobials may reduce microorganisms on steaks through 3 days of display.

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Table 1. Effect of antimicrobial treatment at the sub-primal level by day of display interaction on least squares means (± SE) log CFU*/g *E. coli* (EC) counts of *biceps femoris* beef steaks.

	Days of Display					
Treatment†	**0**	**1**	**2**	**3**	**5**	**7**
IN	8.27 ± 0.30^{d}	5.26 ± 0.30^{b}	5.95 ± 0.30^{bc}	6.34 ± 0.30^{bc}	6.91 ± 0.30	8.26 ± 0.30
W	7.40 ± 0.30^{abc}	4.78 ± 0.30^{b}	4.88 ± 0.30^{a}	5.51 ± 0.30^{ab}	7.03 ± 0.30	8.27 ± 0.30
CPC	7.01 ± 0.30^{ab}	4.69 ± 0.30^{b}	4.80 ± 0.30^{a}	4.96 ± 0.30^{a}	6.98 ± 0.30	8.33 ± 0.30
KL	6.96 ± 0.30^{a}	4.75 ± 0.30^{b}	6.48 ± 0.30^{c}	6.37 ± 0.30^{c}	7.34 ± 0.30	8.21 ± 0.30
NMS	7.82 ± 0.30^{bcd}	3.70 ± 0.30^{a}	5.48 ± 0.30^{ab}	5.05 ± 0.30^{a}	6.94 ± 0.30	8.09 ± 0.30
TSP	7.93 ± 0.30^{cd}	5.14 ± 0.30^{b}	5.00 ± 0.30^{a}	6.95 ± 0.30^{c}	7.19 ± 0.30	8.16 ± 0.30

* 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-d] Least squares means within a column with different superscripts are different ($P < 0.05$).

Table 2. Effect of antimicrobial treatment at the sub – primal level by day of display interaction on least squares means (± SE) log CFU*/g *Salmonella* (ST) counts of *biceps femoris* beef steaks.

	Days of Display					
Treatment†	**0**	**1**	**2**	**3**	**5**	**7**
IN	6.48 ± 0.31	4.44 ± 0.31^{ab}	4.96 ± 0.31^{b}	4.07 ± 0.31^{c}	5.71 ± 0.31^{c}	4.84 ± 0.31^{b}
W	6.30 ± 0.31	3.68 ± 0.31^{a}	3.90 ± 0.31^{a}	3.02 ± 0.31^{b}	4.98 ± 0.31^{bc}	4.53 ± 0.31^{ab}
CPC	5.93 ± 0.31	3.99 ± 0.31^{ab}	4.42 ± 0.31^{ab}	3.30 ± 0.31^{bc}	4.30 ± 0.31^{ab}	3.91 ± 0.31^{a}
KL	6.17 ± 0.31	4.68 ± 0.31^{b}	4.92 ± 0.31^{b}	3.91 ± 0.31^{c}	3.97 ± 0.31^{a}	4.07 ± 0.31^{ab}
NMS	6.01 ± 0.31	4.52 ± 0.31^{ab}	4.53 ± 0.31^{ab}	2.13 ± 0.31^{a}	4.51 ± 0.31^{ab}	4.60 ± 0.31^{ab}
TSP	5.71 ± 0.31	4.45 ± 0.31^{ab}	4.00 ± 0.31^{a}	3.74 ± 0.31^{bc}	4.23 ± 0.31^{ab}	4.63 ± 0.31^{ab}

* 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 treatment at the sub-primal level by day of display interaction on least squares means (± SE) log CFU*/g coliform (CO) counts of *biceps femoris* beef steaks.

	Days of Display					
Treatment†	**0**	**1**	**2**	**3**	**5**	**7**
IN	8.26 ± 0.30^{c}	5.24 ± 0.30^{b}	6.02 ± 0.30^{bc}	6.44 ± 0.30^{b}	6.95 ± 0.30	8.23 ± 0.30^{ab}
W	7.36 ± 0.30^{ab}	4.76 ± 0.30^{b}	5.36 ± 0.30^{ab}	5.55 ± 0.30^{a}	6.95 ± 0.30	8.26 ± 0.30^{ab}
CPC	7.33 ± 0.30^{ab}	4.63 ± 0.30^{b}	4.79 ± 0.30^{a}	5.04 ± 0.30^{a}	7.08 ± 0.30	8.33 ± 0.30^{b}
KL	6.95 ± 0.30^{a}	4.94 ± 0.30^{b}	6.29 ± 0.30^{c}	6.43 ± 0.30^{b}	7.33 ± 0.30	8.21 ± 0.30^{ab}
NMS	8.13 ± 0.30^{bc}	3.67 ± 0.30^{a}	5.39 ± 0.30^{ab}	5.18 ± 0.30^{a}	6.94 ± 0.30	8.09 ± 0.30^{a}
TSP	7.93 ± 0.30^{bc}	5.09 ± 0.30^{b}	4.92 ± 0.30^{a}	7.00 ± 0.30^{b}	7.13 ± 0.30	8.14 ± 0.30^{ab}

* 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 treatment at the sub-primal level by day of display interaction on least squares means (± SE) log CFU*/g aerobic plate counts (APC) of *biceps femoris* beef steaks.

	Days of Display					
Treatment†	**0**	**1**	**2**	**3**	**5**	**7**
IN	8.40 ± 0.33^{b}	4.94 ± 0.33^{a}	6.56 ± 0.33^{b}	6.57 ± 0.33^{bc}	7.28 ± 0.33	8.52 ± 0.33
W	7.63 ± 0.33^{ab}	4.71 ± 0.33^{a}	5.24 ± 0.33^{a}	5.71 ± 0.33^{ab}	7.17 ± 0.33	8.34 ± 0.33
CPC	7.68 ± 0.33^{ab}	4.63 ± 0.33^{a}	5.00 ± 0.33^{a}	5.07 ± 0.33^{a}	7.07 ± 0.33	8.34 ± 0.33
KL	7.41 ± 0.33^{a}	5.53 ± 0.33^{ab}	6.61 ± 0.33^{b}	6.51 ± 0.33^{bc}	7.33 ± 0.33	8.24 ± 0.33
NMS	8.31 ± 0.33^{ab}	4.82 ± 0.33^{a}	6.60 ± 0.33^{b}	5.15 ± 0.33^{a}	7.00 ± 0.33	8.27 ± 0.33
TSP	8.31 ± 0.33^{ab}	5.99 ± 0.33^{b}	6.19 ± 0.33^{b}	7.11 ± 0.33^{c}	7.16 ± 0.33	8.35 ± 0.33

* 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 ($P < 0.05$).

The effects of hydrochloric/citric acid mixture, cetylpyridinium chloride, trisodium phosphate, or sodium metasilicate as antimicrobial sequential treatment combinations on microbiological properties of beef *Longissimus lumborum* muscle

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Story in Brief

The impact of antimicrobial sequential treatment combinations on microbiological properties of beef loin eye (*Longissimus lumborum*) muscles treated at the sub-primal and steak level was evaluated. Beef muscles (n = 12) were further cut into three subsections (n = 36 total subsections) and brush inoculated with *Escherichia coli* (ATCC # 11775) and *Salmonella* Typhimurium (ATCC # 1769NR) at 10^7 CFU/ml. Each inoculated subsection was then treated with either: (1) 20% hydrochloric/citric acid mixture followed by 0.4% cetylpyridinium chloride; (2) 20% hydrochloric/citric acid mixture followed by 10% trisodium phosphate; (3) 20% hydrochloric/citric acid mixture followed by 4% sodium metasilicate; (4) 0.4% cetylpyridinium chloride followed by 10% trisodium phosphate; (5) 0.4% cetylpyridinium chloride followed by 4% sodium metasilicate or (6) inoculated untreated control. Three steaks were obtained from each sub-section allowing fifteen steaks per treatment per day of display. Individual steaks were treated again with the same treatment combinations allotted at the sub-primal level for a multiple intervention application. The experiment was carried out in three replicates. Subsequently, individually over-wrapped packages of steaks were removed from simulated retail display (4 °C) on day 1, 2, 3, 5, and 7 for microbial enumeration. On day 2 of display, coliform, Escherichia coli and aerobic plate counts were significantly ($P < 0.05$) reduced by 0.4% cetylpyridinium chloride followed by 10% trisodium phosphate. Coliform, *Escherichia coli* and aerobic plate counts were significantly ($P < 0.05$) reduced by 20% hydrochloric/citric acid mixture followed by 0.4% cetylpyridinium chloride on day 3 of display. Furthermore, *Salmonella* Typhimurium counts were significantly ($P < 0.05$) reduced by 20% hydrochloric/citric acid mixture followed by 0.4% cetylpyridinium chloride on days 2, 3, and 5 of display. Findings from this study indicate that use of hydrochloric/citric acid blend along with trisodium phosphate or sodium metasilicate may be used as potential multiple decontamination interventions at sub-primal and steak level to enhance beef product safety.

Introduction

The pathogenic contamination of beef products has prompted consumer fear and global concern, threatened trade and economic profit and stimulated ideas in developing new process and control measures (Edwards and Fung, 2006). Viral pathogens will continue to be of concern at food service, and bacterial pathogens such as *Escherichia coli* O157:H7, *Salmonella*, and *Campylobacter* will continue to affect safety of raw meat. Decontamination techniques are targeted to reduce or eliminate bacteria that are human pathogens as well as those that may cause meat spoilage (Sofos, 2008; Huffman, 2002).

Recently, research has been conducted to determine the use of single antimicrobial interventions, and furthermore, the use of multiple antimicrobial interventions. The order of application of antimicrobial treatments is an important factor when using multiple intervention technology (Pohlman et al., 2002). Using the principles of the hurdle technology, Leistner and Gould (2002) found that initial microbial load could be substantially reduced as a result of decontamination procedures, which are then more easily inhibited by following decontamination procedures (Huffman, 2002).

The United States Department of Agriculture, Federal Food Safety and Inspection Service (USDA, FSIS, 2007) recently suggested that antimicrobial interventions should be applied at the sub-primal level. Therefore, this study is an attempt to evaluate hydrochloric/citric acid blend, cetylpyrinidium chloride, trisodium phosphate, and sodium metasilicate treatment combinations at the sub-primal and steak levels to enhance microbial properties of beef products.

Material and Methods

Bacterial Preparation and Inoculation. An inoculum cocktail (10^7 CFU/ml) containing *Escherichia coli* (ATCC #11775) and a nalidixic acid resistant strain of *Typhimurium* (ATCC #1769NR) was prepared from frozen (-80 °C) stock cultures as described by Pohlman et al. (2002). Beef *Longissimus lumborum* muscles (n = 12) were cut into three subsections (10.2 cm × 27.9 cm; n = 36 subsections), and brush inoculated with the bacterial cocktail. Then they were placed in sterile bags (n = 5 subsections/sterile bag) and stored in a 4 °C cooler for 12 to 14 hr to allow for further microbial attachment.

Antimicrobial Treatment Application and Sample Processing. The antimicrobial treatments of 20% CIT (Citrilow™, Safe Foods Cooperation, Little Rock, Ark.), 4% NMS (Metso Pentabead® 20, PQ Corporation, Valley Forge, Pa.), 0.4% CPC (Cecure ®, Safe Foods Cooperation, Little Rock, Ark.), and 10% (w/v) TSP (Trisodium phosphate anhydrous (Food Grade), ICL performance products, St. Louis, Mo.), was prepared by mixing appropriate amounts of municipal purified water. Inoculated subsections (n = 5 subsections per treatment) were immersed in the first treatment for 10 s allowed to drip for 2 min, and then immersed in the second treatment for 10 s and allowed to drip for 2 min. Treatments included (1) 20% CIT followed by 0.4% CPC (CIT/CPC); (2) 20% CIT followed by 10% TSP (CIT/TSP); (3) 20% CIT followed by 4% NMS (CIT/NMS); (4) 0.4% CPC followed by 10% TSP (CPC/TSP); (5) 0.4% CPC followed by 4% NMS (CPC/NMS); and (6) untreated inoculated control (IN). Following primary treatment application, each subsection was cut into three individual steaks (n = 105) allowing fifteen steaks per treatment per display day. The individual steaks were subjected to the

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same immersion procedures with the same treatment combinations allotted at the sub-primal level for a multiple intervention application. All treated and untreated steaks were placed on styrofoam trays with absorbent pads and overwrapped with polyvinyl chloride film (O_2 transmission rate = 14, 000 cc/mm^2/24 h/1 atm; Koch Supplies, Inc., Kansas City, Mo.). The steaks were stored at 4° C under 1630 lx of deluxe warm white fluorescent lighting (Phillips Inc., Somerset, N.J., USA).

Microbial Sampling. On days 1, 2, 3, 5, and 7 of simulated retail display, the microbial enumeration for steaks from each treatment was carried out by aseptically removing 2.5 g from the surface using a sterile scalpel and forceps. 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. The microbial plating procedure was carried out as explained in Pohlman et al. (2002).

Analysis of Data. The experimental design was a randomized complete block, (six by five factorial design), and replicated three times. Bacterial counts were transformed to log values and then analyzed for the main effects of antimicrobial treatment, day of display, and treatment by day of display interaction using the GLM procedure of SAS (SAS Inst., Inc., Cary, N.C.; Version 9.1). Least squares means were generated for all variables and were separated using the PDIFF option of SAS.

Results and Discussion

On day 2 of display, the CPC/TSP treatment significantly ($P < 0.05$) reduced CO, EC and APC counts compared to IN. Furthermore, CPC/TSP treatment achieved >1 log reduction of CO on day 2 of display. In addition, on day 2 of display, the CIT/CPC treatment significantly ($P < 0.05$) reduced EC counts of beef steaks. The CIT/CPC treatment showed 2 log reductions ($P < 0.05$) on CO, EC and APC and close to 1 log reduction in ST counts on day 3. The ST counts on days 2, 3, and 5 were significantly reduced ($P < 0.05$) by the CIT/CPC treatment. Pohlman et al. (2002) reported that acetic acid followed by CPC, a chlorine dioxide followed by CPC, and a CPC followed by TSP pre-grinding treatments were effective in reducing *Salmonella Typhimurium* in ground beef. Our results are comparable to these findings, and the CPC treatment combination outperformed the other treatment combinations in reducing CO, EC, APC and ST counts.

Implications

The use of antimicrobial sequential treatment combinations at the sub-primal and steaks levels may reduce the number microorganisms on steaks through 3 days of display. However, careful selection of treatment combination is important to achieve maximum reduction of bacteria in ground beef.

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Table 1. Effect of antimicrobial sequential treatment combinations at the sub-primal and steak levels by day of display interaction on least squares means (±SE) log CFU*/g *E. coli* (EC) counts of *Longissimus lumborum* beef steaks.

	Days of Display				
Treatment†	**1**	**2**	**3**	**5**	**7**
IN	6.41 ± 0.41^{ab}	7.84 ± 0.41^{cd}	8.04 ± 0.41^{b}	8.84 ± 0.41	9.08 ± 0.41
CIT/CPC	5.77 ± 0.41^{ab}	5.50 ± 0.41^{a}	5.73 ± 0.41^{a}	8.62 ± 0.41	8.97 ± 0.41
CIT/TSP	5.30 ± 0.41^{a}	8.54 ± 0.41^{d}	9.14 ± 0.41^{b}	9.10 ± 0.41	9.23 ± 0.41
CIT/NMS	5.60 ± 0.41^{ab}	6.87 ± 0.41^{bc}	8.67 ± 0.41^{b}	8.85 ± 0.41	8.85 ± 0.41
CPC/TSP	5.56 ± 0.41^{ab}	6.02 ± 0.41^{ab}	8.86 ± 0.41^{b}	9.11 ± 0.41	9.20 ± 0.41
CPC/NMS	6.53 ± 0.41^{b}	7.72 ± 0.41^{cd}	8.68 ± 0.41^{b}	8.89 ± 0.41	8.97 ± 0.41

* Colony forming units.

† Treatment: IN = untreated inoculated control, CIT/CPC = 20% hydrochloric/citric acid mixture followed by 0.4% cetylpyridinium chloride, CIT/TSP = 20% hydrochloric/citric acid mixture followed by 10% trisodium phosphate, CIT/NMS = 20% hydrochloric/citric acid mixture followed by 4% sodium metasilicate, CPC/TSP = 0.4% cetylpyridinium chloride followed by 10% trisodium phosphate, CPC/NMS = 0.4% cetylpyridinium chloride followed by 4% sodium metasilicate.

[a-d] Least squares means within a column with different superscripts are different ($P < 0.05$).

Table 2. Effect of antimicrobial sequential treatment combinations at the sub-primal and steak levels by day of display interaction on least squares means (±SE) log CFU*/g *Salmonella* (ST) counts of *Longissimus lumborum* beef steaks.

	Days of Display				
Treatment†	**1**	**2**	**3**	**5**	**7**
IN	4.50 ± 0.31	4.81 ± 0.31^{bc}	5.38 ± 0.31^{b}	4.46 ± 0.31^{b}	5.30 ± 0.31
CIT/CPC	4.09 ± 0.31	3.69 ± 0.31^{a}	4.42 ± 0.31^{a}	3.49 ± 0.31^{a}	5.21 ± 0.31
CIT/TSP	3.81 ± 0.31	5.20 ± 0.31^{c}	6.05 ± 0.31^{bc}	4.51 ± 0.31^{b}	4.95 ± 0.31
CIT/NMS	4.13 ± 0.31	4.00 ± 0.31^{ab}	5.66 ± 0.31^{bc}	4.53 ± 0.31^{b}	4.52 ± 0.31
CPC/TSP	4.25 ± 0.31	4.48 ±0.31^{abc}	6.29 ± 0.31^{c}	4.41 ± 0.31^{b}	5.05 ± 0.31
CPC/NMS	3.95 ± 0.31	4.61 ± 0.31^{bc}	6.25 ± 0.31^{bc}	4.52 ± 0.31^{b}	4.87 ± 0.31

* Colony forming units.

† Treatment: IN = untreated inoculated control, CIT/CPC = 20% hydrochloric/citric acid mixture followed by 0.4% cetylpyridinium chloride, CIT/TSP = 20% hydrochloric/citric acid mixture followed by 10% trisodium phosphate, CIT/NMS = 20% hydrochloric/citric acid mixture followed by 4% sodium metasilicate, CPC/TSP = 0.4% cetylpyridinium chloride followed by 10% trisodium phosphate, CPC/NMS = 0.4% cetylpyridinium chloride followed by 4% sodium metasilicate.

[a-c] Least squares means within a column with different superscripts are different ($P < 0.05$).

Table 3. Effect of antimicrobial sequential treatment combinations at the sub-primal and steak levels by day of display interaction on least squares means (±SE) log CFU*/g coliform (CO) counts of *Longissimus lumborum* beef steaks.

	Days of Display				
Treatment†	**1**	**2**	**3**	**5**	**7**
IN	6.22 ± 0.40^{ab}	8.12 ± 0.40^{bc}	8.05 ± 0.40^{b}	8.88 ± 0.40	9.21 ± 0.40
CIT/CPC	6.08 ± 0.40^{ab}	7.13 ±0.40^{abc}	6.03 ± 0.40^{a}	8.69 ± 0.40	8.97 ± 0.40
CIT/TSP	5.48 ± 0.40^{a}	8.25 ± 0.40^{c}	9.18 ± 0.40^{b}	9.34 ± 0.40	9.33 ± 0.40
CIT/NMS	5.84 ± 0.40^{ab}	7.02 ± 0.40^{ab}	8.70 ± 0.40^{b}	8.99 ± 0.40	9.07 ± 0.40
CPC/TSP	5.86 ± 0.40^{ab}	6.42 ± 0.40^{a}	8.89 ± 0.40^{b}	9.34 ± 0.40	9.34 ± 0.40
CPC/NMS	6.62 ± 0.40^{b}	7.90 ± 0.40^{bc}	8.69 ± 0.40^{b}	9.04 ± 0.40	9.21 ± 0.40

* Colony forming units.

† Treatment: IN = untreated inoculated control, CIT/CPC = 20% hydrochloric/citric acid mixture followed by 0.4% cetylpyridinium chloride, CIT/TSP = 20% hydrochloric/citric acid mixture followed by 10% trisodium phosphate, CIT/NMS = 20% hydrochloric/citric acid mixture followed by 4% sodium metasilicate, CPC/TSP = 0.4% cetylpyridinium chloride followed by 10% trisodium phosphate, CPC/NMS = 0.4% cetylpyridinium chloride followed by 4% sodium metasilicate.

[a-c] Least squares means within a column with different superscripts are different ($P < 0.05$).

Table 4. Effect of antimicrobial sequential treatment combinations at the sub-primal and steak levels by day of display interaction on least squares means (±SE) log CFU*/g aerobic plate counts (APC) counts of *Longissimus lumborum* beef steaks.

	Days of Display				
Treatment†	**1**	**2**	**3**	**5**	**7**
IN	6.20 ± 0.40^{ab}	8.10 ± 0.40^{bc}	8.02 ± 0.40^{b}	8.90 ± 0.40	9.18 ± 0.40
CIT/CPC	6.07 ± 0.40^{ab}	7.20 ±0.40^{abc}	6.02 ± 0.40^{a}	8.67 ± 0.40	8.97 ± 0.40
CIT/TSP	5.53 ± 0.40^{a}	8.24 ± 0.40^{c}	9.18 ± 0.40^{c}	9.33 ± 0.40	9.34 ± 0.40
CIT/NMS	5.85 ± 0.40^{ab}	7.01 ± 0.40^{ab}	8.78 ± 0.40^{bc}	9.07 ± 0.40	9.08 ± 0.40
CPC/TSP	5.89 ± 0.40^{ab}	6.45 ± 0.40^{a}	8.97 ± 0.40^{bc}	9.33 ± 0.40	9.34 ± 0.40
CPC/NMS	6.68 ± 0.40^{b}	7.92 ± 0.40^{bc}	8.85 ± 0.40^{bc}	9.10 ± 0.40	9.10 ± 0.40

* Colony forming units.

† Treatment: IN = untreated inoculated control, CIT/CPC = 20% hydrochloric/citric acid mixture followed by 0.4% cetylpyridinium chloride, CIT/TSP = 20% hydrochloric/citric acid mixture followed by 10% trisodium phosphate, CIT/NMS = 20% hydrochloric/citric acid mixture followed by 4% sodium metasilicate, CPC/TSP = 0.4% cetylpyridinium chloride followed by 10% trisodium phosphate, CPC/NMS = 0.4% cetylpyridinium chloride followed by 4% sodium metasilicate.

[a-c] Least squares means within a column with different superscripts are different ($P < 0.05$).

The antimicrobial efficacies of novel organic acids as single antimicrobial intervention for the control of *Escherichia coli* O157:H7 in inoculated beef trimmings

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Story in Brief

The microbial safety of raw ground beef remains challenged by the potential for emergence of new pathogens and re-emergence of known pathogens. *E. coli* O157:H7 is known to have an inherent ability to evolve, mutate, and adapt to several stressors including antimicrobial treatments and allowing them to survive. The objective of this study was to determine the antimicrobial efficacies of peroxyacetic and other novel food grade organic acids on reduction of *E. coli* O157:H7 on beef trimmings. Beef trimmings inoculated with *E. coli* O157:H7 (10^5 CFU/g) were dipped for 15 s in solutions of novel organic acids [fumaric acid, malic acid, citric acid, gluconic acid, levulinic acid, pyruvic acid, caproic acid, caprylic acid, capric acid, and peroxyacetic acid]. Following antimicrobial treatment, beef trimming samples were processed and spread plated on aerobic plate count, *E. coli*/coliform Petrifilm® plates. Among all treatments, caprylic acid was most effective in reducing more than 4.78, 4.73, and 2.48-logs of *E. coli*, coliform, and of aerobic plate counts, respectively. Results of microbial assays revealed that 3% of pyruvic acid yielded 2.48, 0.31, and 0.2-logswhile peroxyacetic acid, citric acid, levulinic acid, and capric acid resulted in ~0.3 to 0.5 log reduction of *E. coli*, coliform, and aerobic plate counts as compared to the control. Treatment of beef trimmings with 3% of caprylic acid, fumaric acid, and malic acid were the most effective antimicrobial treatments in reducing *E.coli* O157:H7 population. The results of this study suggest that the application of novel organic acids may have practical application for killing food-borne pathogens on beef trimmings destined for ground beef production.

Introduction

Ground beef is characterized as a commodity of high potential risk of food-borne illness. Since ground beef is sourced from different cattle and locations, further grinding and mixing operations during ground beef production could potentially contaminate the uncontaminated product. In the past decades, *E. coli* O157:H7 has emerged as a high profile food-borne pathogen, and frequent ground beef product recalls due to *E. coli* O157:H7 contamination continue to be a serious concern to the U.S. meat industry and consumers. Direct antimicrobial application to meat as a decontamination technique has been shown to be effective in reducing pathogenic bacteria populations in the final product (Dorsa et al., 1998). Recent studies have suggested that decontamination of beef trimmings destined for ground beef production prior to grinding is effective in reducing bacterial counts in ground beef (Pohlman et al., 2002; Stivarius, et al., 2002). This study was designed to evaluate the effectiveness of antimicrobial interventions on reducing *Escherichia coli* O157:H7 (EC), coliform (CO), and aerobic plate count (APC) of pre-inoculated beef trimmings. Therefore, our objective was to determine the antimicrobial efficacies of different concentrations of peroxyactic acid (PAA) and other novel food grade organic acids [fumaric acid (FA), malic acid (MA), citric acid (CA), gluconic acid (GA), levulinic acid (LA), pyruvic acid (PY), caprioc acid (CR); caprylic acid (CL); capric acid (CP)] on reduction of *E. coli* O157:H7 on beef trimmings.

Material and Methods

Inoculation Preparation and Inoculation. *Escherichia coli* O157:H7 (EC) inoculum was prepared from frozen (-80 °C) stock cultures. Frozen cultures of EC were thawed, and 0.1 ml of EC suspension was inoculated into 40 ml aliquots of Brain Heart Infusion broth (BHI). Following 18 h of incubation at 37 °C, bacteria were then harvested by centrifugation (3500 × g for 20 min at 25 °C) (Beckman GS-6 series, Fullerton, Ca.), and re-suspended with 40 ml of 0.1% buffered peptone water (BPW) (Difco Laboratories, Becton Dickinson and Company, Sparks, Md.). The bacterial suspension (log 10^5CFU/ml EC) was cooled to 4 °C and then mixed. Beef trimmings were inoculated with the bacterial suspension and placed in a sterile bag and placed in a 4 °C cooler for 12 to 14 hr to allow for further microbial attachment.

Antimicrobial Treatment Application and Sample Processing. A model system was developed to mimic a commercial dip treatment system for beef trimmings. Beef trimmings inoculated with *E. coli* O157:H7 (10^5 CFU/g; 25 g/treatment) were processed with antimicrobial treatments by dipping for 15 s in 100 mL solution of peroxyacetic acid (PAA; 0.02%) and 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc acid (CR), caprylic acid (CL), capric acid (CP), and peroxyacetic acid (PAA)]. Following antimicrobial treatment, beef trimming samples were processed and spread plated using serial 10-fold dilution in duplicates on aerobic plate count (APC), *E. coli* (EC) / coliform (CO) Petrifilm® plates. Plated samples were incubated at 37 °C for up to 48h in an aerobic incubation chamber. Inoculated beef trimmings treated with and without water (IN; IN+W) and un-inoculated (UN) samples served as controls.

Microbial Sampling. The microbial enumeration was carried out by homogenizing 25 g beef trim in a sterile whirl pack bag (Nasco, Ft Atkinson, Wis.) in 225 ml of 0.1% buffered peptone for 2 minutes in a stomacher (Model 400 Lab Stomacher; Seward, London, UK)

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Subsequently, serial 10-fold dilutions were made and spread plating was done in duplicates on aerobic plate counts (APC), and *E. coli* (EC)/coliform (CO) counts of Petrifilm® (3M Corporation, St. Paul, Minn.). Plates were then incubated at 37 °C in an aerobic incubation chamber (VWR Model 5015 and Model 3015 incubators, VWR Scientific, Cornelius, Ore.). The EC and APC counts were read after 48 h, whereas CO plates were read at 24 h. All counts were recorded as colony forming units per gram (CFU/g).

Statistical Analysis. The bacterial values were transformed to log values and then analyzed for the main effects of antimicrobial treatment using the PROC Mixed procedure of SAS (SAS Inst., Inc., Cary, N.C.). Least-squares means for protected F-tests ($P < 0.05$) were separated by using least significant differences (LSD; $P < 0.05$). The experiment was replicated two times.

Results and Discussion

The effect of antimicrobial treatment with PAA and other novel organic acids [FA, MA, CA, GA, LA, PY, CR, CL, CP, and PAA)] on *E. coli* O157:H7 population of beef trimmings is shown in Figs. 1, 2, and 3. Treatment of beef trimmings prior to grinding with MA, FA, PY, and CL reduced ($P < 0.05$) survival of CO (Fig. 1). Among all antimicrobials MA, FA, and CL were most effective in reducing ($P < 0.05$) EC counts (Fig. 2) while PY yielded only 0.3, and 0.2-log reduction ($P<0.05$) of EC, and APC counts as compared to the control (IN+W) (Figs. 2 and 3). Among all antimicrobial treatments, CL and FA were the most effective treatments in reducing CO and EC populations within 15 s (Figs. 1 and 2). Caprylic acid reduced ($P < 0.05$) 4.78, 4.73, and 2.48-logs and FA reduced 3.23, 3.07, and 2.16-logs of EC, CO, and APC respectively (Figs. 1, 2, and 3). Among other treatments, 3% MA resulted in 2.23, 2.25, and 0.2-log reduction ($P < 0.05$) of EC, CO, and APC counts as compared to the control (IN+W). Dorsa et al., (1998) reported similar findings that antimicrobial treatment with 2% lactic acid on beef trimmings was not effective on reducing mesophilic aerobic bacteria through 7 days of storage.

Treatment of beef trimmings with PAA, CA, LA, and CP resulted in ~0.3 to 0.5 log reduction of EC and CO and APC. The highest concentration (3%) of novel organic acid (GA) tested in this study yielded no reduction in EC, CO, and APC counts compared to the control. Results suggest that the deleterious effects exhibited by the organic acids and PAA were consistent with other studies (King et al., 2005; Pohlman et al., 2002ab). These data suggest that CL negatively affects EC and CO enumeration efficacy. The results of this study indicated that CL, PA, and MA were the most effective antimicrobial treatments in reducing *E.coli* O157:H7 population on beef trimmings. The results of the antimicrobial application on beef trims were as expected for potent antimicrobial agents: PAA and other novel organic acids exhibited deleterious effects on survival of EC, CO, and APC.

Implications

This research provides a practical and cost-effective, novel decontamination technology for beef processors that can be immediately implemented for commercial application of antimicrobial interventions during ground beef production. The results from this research validate the antimicrobial efficacies of peroxyacetic acid and/or novel organic acids and their application on beef trimmings and/or ground beef to substantially reduce *E.coli* O157:H7 contamination.

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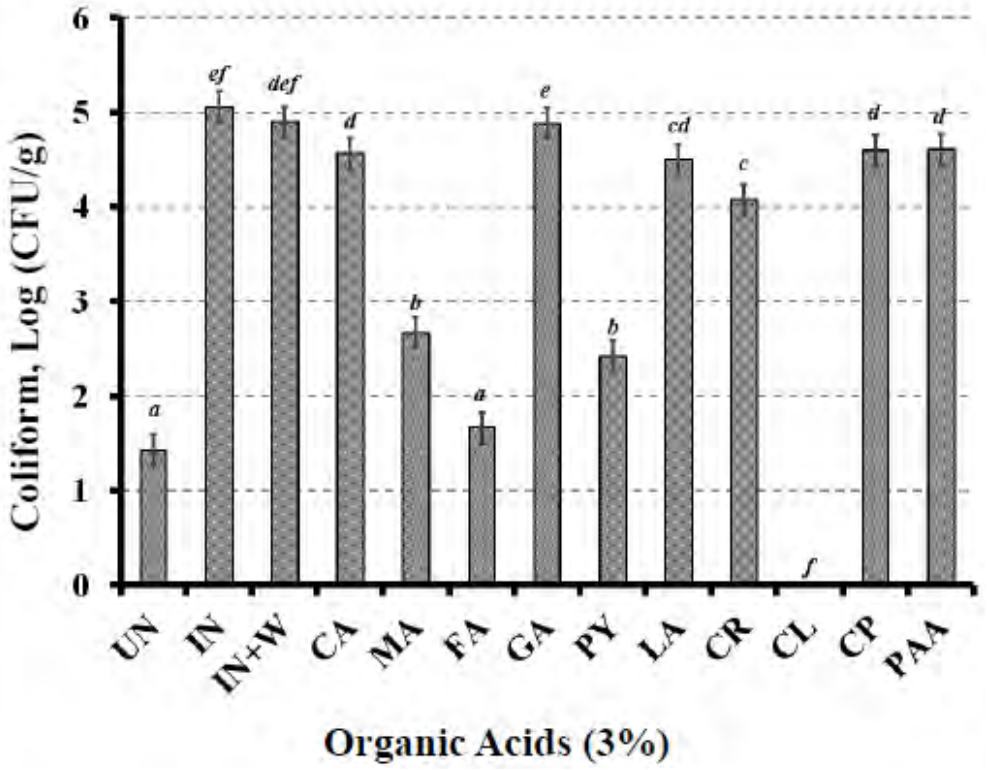

Fig. 1. Effects of 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc acid (CO), caprylic acid (CL), and capric acid (CP)] and peroxyacetic acid (200 ppm)on log CFU/g of *coliform* (CO) counts of inoculated beef trimming.

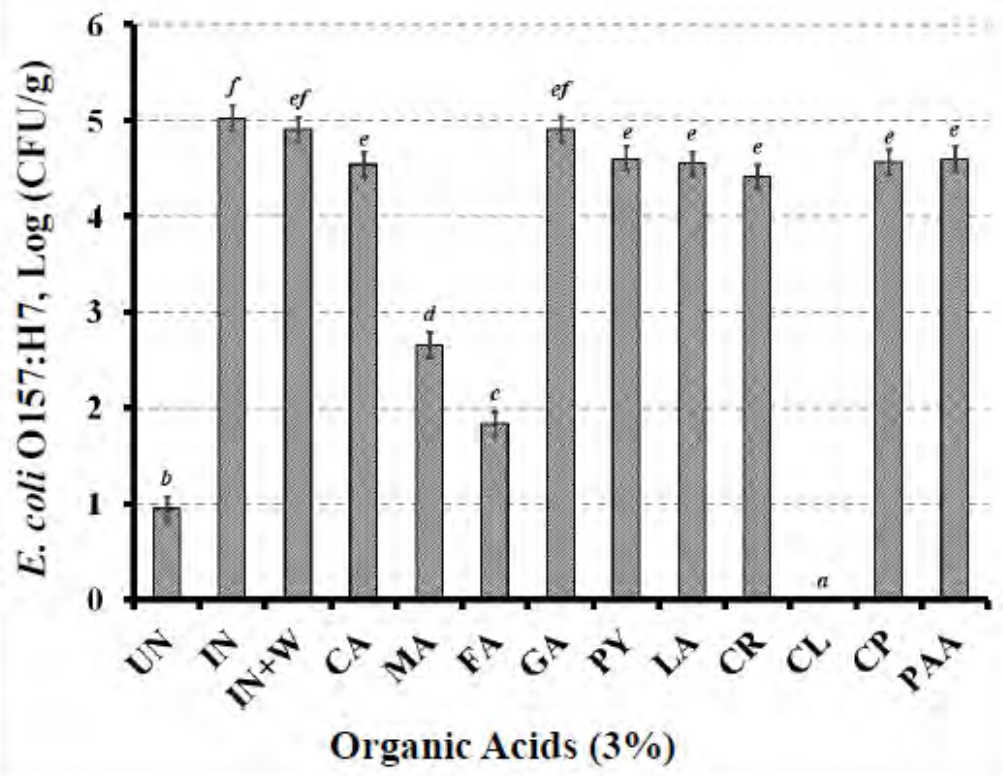

Fig. 2. Effects of 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc acid (CO), caprylic acid (CL), and capric acid (CP)] and peroxyacetic acid (200 ppm) on log CFU/g of *E. coli* O157:H7 (EC) counts of inoculated beef trimming.

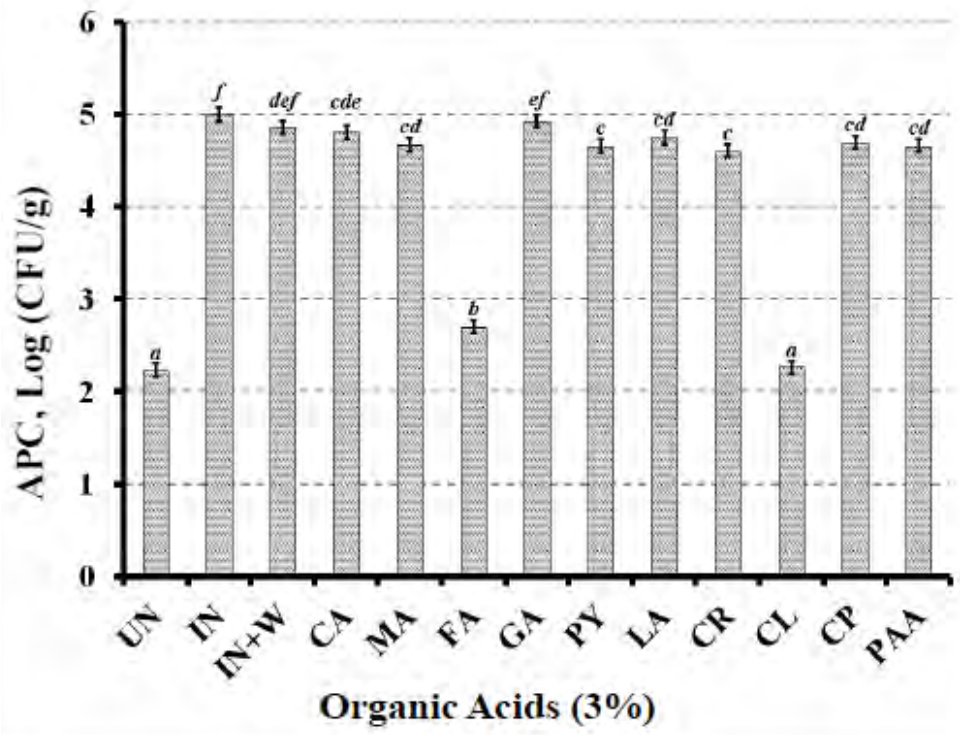

Fig. 3. Effects of 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc acid (CO), caprylic acid (CL), and capric acid (CP)] and peroxyacetic acid (200 ppm) on log CFU/g of Aerobic Plate counts (APC) of inoculated beef trimming.

The effects of peroxyacetic acid, octanoic acid, malic acid, and potassium lactate in reducing *E. coli*, *Salmonella*, *Coliform*, and aerobic plate counts in inoculated beef trimmings

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Story in Brief

In spite of decades of research, ground beef meat remains a major source of food-borne pathogens and consequent safety recalls. Decontamination of beef trimming destined for ground beef production is considered one of the most important steps for controlling food-borne pathogenic bacteria during or at the end of the production line-chain. The objective of this study was to evaluate the impact of antimicrobial agents peroxyacetic acid, malic acid, octanoic acid, and potassium lactate followed by 10% trisodium phosphate with ultra-chilled CO_2 shower to beef trimmings on microbial counts of *Escherichia coli*, coliform, aerobic plate count, and *Salmonella* Typhimurium, of the produced ground beef. Beef trimmings were inoculated by dipping into a bacterial cocktail containing log 10^7 CFU/mL of *Escherichia coli* (ATCC #25922) and *Salmonella* Typhimurium (ATCC #333310NR). After grinding, 200 g of the ground beef were overwrapped with polyvinyl chloride film and displayed under retail conditions for 1, 2, 3, 5, and 7 days. Treatment of beef trimmings prior to grinding with peroxyacetic acid, malic acid, and octanoic acid reduced ($P < 0.05$) up to 2 logs of *Escherichia coli*, coliform, *Salmonella* Typhimurium, and aerobic plate count throughout the days of retail display. The results of this study indicated that octanoic acid, as a novel microbiological intervention strategy tended to be the most effective antimicrobial agent currently approved for use in reducing total bacterial populations on beef carcass tissue and beef trimmings. Similar reductions were observed on total bacterial populations on peroxyacetic acid and malic acid treated beef trimmings. Therefore, treatment of beef trimmings before grinding with peroxyacetic acid, octanoic acid, malic acid, and potassium lactate may improve ground beef safety and enhance shelf-life.

Introduction

Ground beef is a focus of concern for producers and consumers and is characterized as a commodity of highest potential risk of food borne illness. Grinding and mixing operations during ground beef production creates a high potential for cross-contamination. In fact, ground beef is the leading source of EC infections in the United States (CDC 2006).In the United States every year, there are an estimated 76 million cases of food-borne illnesses, resulting in $5-17 billion in economic and productivity losses annually (Edwards and Fung, 2006).The Center for Disease Control estimates 1.4 million cases of food poisoning due to *Salmonella* each year. In spite of decades of research, ground beef remains a major source of food-borne pathogens and consequent safety recalls.

A crucial need exists for United States beef processors to implement decontamination technologies of beef trimmings intended for production of ground beef to eliminate the incidents of food-borne pathogens and consequent safety recalls (USDA-FSIS, 2007). Direct antimicrobial application to meat as a decontamination technique has been shown to be effective in reducing pathogenic bacteria populations in the final product (Pohlman et al., 2002a). However, the challenge of direct antimicrobial application is that active substances can be neutralized upon contact or diffuse rapidly from the surface into the food mass. Studies have suggested that decontamination of beef trimmings destined for ground beef production and prior to grinding is effective in reducing bacterial counts in ground beef (Pohlman et al., 2002b; Stivarius, et al., 2002). The objective of this study was to evaluate the effectiveness of antimicrobial interventions on pre-inoculated beef trimming with *Escherichia coli* and *Salmonella* Typhimurium.

Material and Methods

Inoculation Preparation and Inoculation. *Escherichia coli* (EC;ATCC #25922) and a nalidixic acid resistant strain of *Salmonella* Typhimurium (ST;ATCC #333310NR) inoculums were prepared from frozen (-80 °C) stock cultures. Frozen cultures of EC and ST were thawed, and 0.1 ml of EC suspension was inoculated into 60 separate 40 ml aliquots of Brain Heart Infusion broth (BHI), and 0.1 ml of ST suspension was inoculated into 60 separate 40 ml aliquots of BHI with nalidixic acid. Following 18 h of incubation at 37 °C, bacteria were harvested by centrifugation (3500 × g for 20 min at 25 °C) (Beckman GS-6 series, Fullerton, Ca.), re-suspended with 40 ml of 0.1% buffered peptone water (BPW) (Difco Laboratories, Becton Dickinson and Company, Sparks, Md.) and pooled together to make a bacterial cocktail. The bacterial cocktail (log 10^7 CFU/ml EC and log 10^7 CFU/ml ST) was cooled to 4 °C and then mixed. Beef trimmings were inoculated with the bacterial cocktail and placed in a sterile bag and placed in a 4 °C cooler for 12 to 14 h to allow for further microbial attachment.

Antimicrobial Treatment Application and Sample Processing. The antimicrobial treatments of 0.02% peroxyacetic acid (PAA), 0.04% octanoic acid (OA), 2% mailc acid (MA), and 2% potassium lactate (KL) followed by 10% trisodium phosphate (TSP) was prepared by mixing appropriate amounts of water. Inoculated beef trimmings were treated with either: (1) 0.02%PAA; (2) 0.04%OA; (3) 2% MA; or(4) 2% KL. Each antimicrobial treated sample was then subsequently mixed with 10% (weight/volume) TSP and chilled with liquid CO_2. For the antimicrobial treatments, 5.4 kg of beef trimmings (80%lean and 20% fat) were placed into a CO_2 mixer (Model 814, Food Processing Equipment Co., Springdale, Ark.) with 1L of the selected antimicrobial solution and mixed with the

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meat for 3 min and chilled with CO_2 for 30 s. After the antimicrobial application and chilling steps were completed, the beef trimmings were ground twice using a Hobart grinder (Model 310, Hobart Inc., Troy Ohio) with a3.2-mm plate. After grinding, 200 g of the ground beef were placed on foam trays with absorbent pads, overwrapped with polyvinyl chloride film (oxygen transmission rate of 14,000 cc/mm^2/24 h/1 atm; Koch Supplies, Inc., Kansas City, Mo.) and stored under simulated retail conditions (4 °C; deluxe warm white fluorescent lighting; 1600 lx; Phillips Inc., Somerset, N.J.) for 7 d. Experiments were repeated three times for antimicrobial treatments. Styrofoam trays containing ground beef for each treatment were packaged to allow for independent use for microbial evaluation and instrumental color sampling. Treated ground beef pH was also sampled immediately after grinding by homogenizing a 1.8 g portion of ground beef in 18 ml of distilled water and evaluated using an Orion Model420A pH meter with a ROSS electrode (Model 8165BN,Orion Research, Inc., Beverly, Mass.).

Microbial Sampling. On days 1, 2, 3, 5, and 7 of simulated retail display, the microbial enumeration for each ground beef sample was carried out by aseptically removing 25 g from the surface then placing in a sterile whirl pack bags (Nasco, Ft. Atkinson, Wis.) along with 225 ml of 0.1% buffered peptone water, and homogenized for 2 minutes (Model 400 Lab Stomacher; Seward, London, UK). Subsequently, serial 10-fold dilutions were made and spread plating was done in duplicates on aerobic plate count (APC), and EC/CO counts of Petrifilm® (3M Corporation, St. Paul, Minn.). *Salmonella* Typhimurium counts were performed on *Salmonella Shigella* agar (Difco Laboratories, Becton Dickinson and Company, Sparks, Md.) containing nalidixic acid. Plates were then incubated at 37 °C in an aerobic incubation chamber (VWR Model 5015 and Model 3015 incubators, VWR Scientific, Cornelius, Ore.). The EC, APC, and ST counts were read after 48 h, whereas CO plates were read at 24 h. All counts were recorded as colony forming units per gram (CFU/g).

Statistical Analysis. The bacterial values were transformed to log values then analyzed for the main effects of antimicrobial treatment, day of display and treatment by day of display interaction using the PROC Mixed procedure of SAS (SAS Inst., Inc., Cary, N.C.). Least squares means for protected F-tests ($P < 0.05$) were separated by using least significant differences (LSD; $P < 0.05$).The experiment was replicated three times.

Results and Discussion

The antimicrobial treatment of beef trimmings and its effect on reduction of microbial populations (CO, EC, ST, and APC) is summarized in Tables 1, 2, 3, and 4, respectively. The bactericidal effect of novel organic acids PAA, MA, and OA evaluated in this study were effective in reducing the survival of EC, CO, ST, and APC during the retail display. Among all organic acids evaluated in this study, 0.04% OA exhibited greater microbial inactivation and reduced survival ($P < 0.05$) of EC, CO, ST, and APC throughout retail display. The highest concentration of KL (2%) tested in this study yielded approximately1-2 log reduction of EC on d 1, 2, and 7 and approximately 1 log of CO and APC on d 1 of the display as compared to control. However, treatment of beef trimmings with KL exhibited approximately 2 log reductions in ST through days 1 to 3 and approximately 1 log on days 5 and 7 of the retail display. In a similar study, Pohlman et al. (2009) found that 4% sodium metasilicate (NMS) treatment showed >1 log reduction on EC, CO and APC counts on inoculated ground beef.

The PAA treatment reduced ($P < 0.05$) CO, EC, and ST counts through days 1, 2, 3, and 5 of display (Table 1, 2, and 4).King et al. (2005) evaluated PAA as a sanitizer for meat contact surfaces and reported that PAA was effective in reducing the bacterial load, but total elimination of *E. coli* O157:H7 was not achieved. In the present study, MA achieved lower reductions in bacterial populations than did PAA and OA or EC, CO, and APC (Tables 1, 2, and 3).Dorsa et al. (1998) observed that antimicrobial treatment of 2% lactic acid on beef trimmings did not effectively reduce mesophilic aerobic bacteria through 7 days of storage.

Nevertheless, all antimicrobial treatments achieved approximately 1.4 log CFU/g reduction ($P < 0.05$) for APC on day 1. The results of the antimicrobial application on beef trimmings were as expected for potent antimicrobial agents: OA had the most deleterious effect on survival of EC, CO, ST, and APC and its antimicrobial effect was synergistically enhanced by sequential treatment of beef trim with 10% TSP throughout the simulated retail display.

Implications

The results of this study indicated that peroxyacetic acid, octanoic acid, and malic acid, were effective in reducing total bacterial populations on beef trimmings. Furthermore, peroxyacetic acid, octanoic acid, and malic acid in general reduced *Escherichia coli,* coliform, and *Salmonella* Typhimurium populations. Therefore, treatment of beef trimmings before grinding with peroxyacetic acid, octanoic acid, malic acid, and potassium lactate may improve the safety and shelf-life of the ground beef.

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Table 1. Effect of antimicrobial treatment by day of display interaction on least squares means (± SE) log CFU*/g *E. coli* (EC) counts of bulk ground beef.

	Days of display				
Treatment**	**1**	**2**	**3**	**5**	**7**
IN	6.38 ± 0.19^{b}	7.40 ± 0.19^{d}	6.50 ± 0.19^{c}	6.65 ± 0.19^{c}	7.26 ± 0.19^{d}
IN+CO_2	6.42 ± 0.19^{b}	6.52 ± 0.19^{c}	7.29 ± 0.19^{d}	6.37 ± 0.19^{c}	7.21 ± 0.19^{d}
PAA	5.43 ± 0.19^{a}	5.38 ± 0.19^{a}	5.44 ± 0.19^{b}	5.53 ± 0.19^{b}	7.44 ± 0.19^{d}
MA	5.60 ± 0.19^{a}	6.01 ± 0.19^{b}	5.52 ± 0.19^{b}	6.20 ± 0.19^{c}	6.06 ± 0.19^{b}
OA	5.34 ± 0.19^{a}	5.35 ± 0.19^{a}	4.40 ± 0.19^{a}	4.37 ± 0.19^{a}	4.36 ± 0.19^{a}
KL	5.42 ± 0.19^{a}	6.28 ± 0.19^{bc}	6.47 ± 0.19^{c}	6.67 ± 0.19^{c}	6.91 ± 0.19^{c}

* Colony forming units.
** Treatment: IN = untreated inoculated control, IN+CO_2 = untreated inoculated control treated and chilled with CO_2, PAA = 0.02% peroxyacetic acid, MA = 2% malic acid, OA = 0.04% octanoic acid, KL = 2% potassium lactate.
[a-d] Least squares means within a column with different superscripts are different ($P < 0.05$).
All treatments were chilled with CO_2 for 30 s.

Table 2. Effect of antimicrobial treatment by day of display interaction on least squares means (± SE) log CFU*/g *coliform* (CO) counts of bulk ground beef.

	Days of display				
Treatment**	**1**	**2**	**3**	**5**	**7**
IN	6.59 ± 0.21^{bc}	6.75 ± 0.21^{b}	6.68 ± 0.21^{cd}	6.94 ± 0.21^{c}	7.47 ± 0.21^{c}
IN+CO_2	6.78 ± 0.21^{c}	6.81 ± 0.21^{b}	6.92 ± 0.21^{d}	7.75 ± 0.21^{d}	7.64 ± 0.21^{c}
PAA	5.77 ± 0.21^{a}	5.69 ± 0.21^{a}	5.55 ± 0.21^{a}	5.52 ± 0.21^{b}	7.58 ± 0.21^{c}
MA	6.15 ± 0.21^{b}	5.84 ± 0.21^{a}	6.38 ± 0.21^{bc}	7.40 ± 0.21^{d}	6.60 ± 0.21^{b}
OA	5.61 ± 0.21^{a}	5.69 ± 0.21^{a}	5.44 ± 0.21^{a}	4.49 ± 0.21^{a}	4.67 ± 0.21^{a}
KL	5.61 ± 0.21^{a}	5.89 ± 0.21^{a}	6.33 ± 0.21^{b}	6.70 ± 0.21^{c}	7.43 ± 0.21^{c}

* Colony forming units.
** Treatment: IN = untreated inoculated control, IN+CO_2 = untreated inoculated control treated and chilled with CO_2, PAA = 0.02% peroxyacetic acid, MA = 2% malic acid, OA = 0.04% octanoic acid, KL = 2% potassium lactate.
[a-d] Least squares means within a column with different superscripts are different ($P < 0.05$).
All treatments were chilled with CO_2 for 30 s.

Table 3. Effect of antimicrobial treatment by day of display interaction on least squares means (± SE) log CFU*/g *aerobic plate counts* (APC) of bulk ground beef.

	Days of display				
Treatment**	**1**	**2**	**3**	**5**	**7**
IN	7.21 ± 0.20^{c}	7.66 ± 0.20^{b}	7.61 ± 0.20^{c}	7.01 ± 0.20^{b}	7.82 ± 0.20^{b}
IN+CO_2	6.98 ± 0.20^{bc}	7.75 ± 0.20^{b}	7.68 ± 0.20^{c}	8.13 ± 0.20^{c}	8.23 ± 0.20^{b}
PAA	5.51 ± 0.20^{a}	7.86 ± 0.20^{b}	7.24 ± 0.20^{bc}	7.94 ± 0.20^{c}	7.87 ± 0.20^{b}
MA	6.43 ± 0.20^{b}	7.57 ± 0.20^{b}	7.80 ± 0.20^{c}	7.91 ± 0.20^{c}	7.91 ± 0.20^{b}
OA	5.72 ± 0.20^{a}	5.62 ± 0.20^{a}	4.87 ± 0.20^{a}	4.82 ± 0.20^{a}	7.27 ± 0.20^{a}
KL	5.76 ± 0.20^{a}	7.93 ± 0.20^{b}	7.52 ± 0.20^{b}	8.07 ± 0.20^{c}	8.11 ± 0.20^{b}

* Colony forming units.
** Treatment: IN = untreated inoculated control, IN+CO_2 = untreated inoculated control treated and chilled with CO_2, PAA = 0.02% peroxyacetic acid, MA = 2% malic acid, OA = 0.04% octanoic acid, KL = 2% potassium lactate.
$^{a-c}$ Least squares means within a column with different superscripts are different ($P < 0.05$).
All treatments were chilled with CO_2 for 30 s.

Table 4. Effect of antimicrobial treatment by day of display interaction on least squares means (± SE) log CFU*/g S*almonella* (SM) counts of bulk ground beef.

	Days of display				
Treatment**	**1**	**2**	**3**	**5**	**7**
IN	5.54 ± 0.11^{d}	5.61 ± 0.11^{c}	5.59 ± 0.11^{d}	5.63 ± 0.11^{d}	5.76 ± 0.11^{c}
IN+CO_2	5.50 ± 0.11^{d}	5.62 ± 0.11^{c}	5.53 ± 0.11^{d}	5.61 ± 0.11^{d}	5.83 ± 0.11^{c}
PAA	3.35 ± 0.11^{a}	3.35 ± 0.11^{a}	3.39 ± 0.11^{a}	4.14 ± 0.11^{b}	4.38 ± 0.11^{b}
MA	4.40 ± 0.11^{c}	4.67 ± 0.11^{b}	4.52 ± 0.11^{c}	4.56 ± 0.11^{c}	4.85 ± 0.11^{b}
OA	3.42 ± 0.11^{ab}	3.85 ± 0.11^{b}	3.38 ± 0.11^{a}	3.40 ± 0.11^{a}	3.47 ± 0.13^{a}
KL	3.70 ± 0.11^{b}	3.45 ± 0.11^{a}	3.73 ± 0.11^{b}	4.44 ± 0.11^{c}	4.72 ± 0.11^{b}

* Colony forming units
** Treatment: IN = untreated inoculated control, IN+CO_2 = untreated inoculated control treated and chilled with CO_2, PAA = 0.02% peroxyacetic acid, MA = 2% malic acid, OA = 0.04% octanoic acid, KL = 2% potassium lactate
$^{a-d}$ Least squares means within a column with different superscripts are different ($P < 0.05$)
All treatments were chilled with CO_2 for 30 s.

The impact of peroxyacetic acid, octanoic acid, malic acid, and potassium lactate as antimicrobial interventions on instrumental color characteristics of beef trimmings

Anand Mohan[1], F. W. Pohlman[1], J. A. McDaniel[1]

Story in Brief

The impact of multiple antimicrobial interventions on ground beef instrumental color characteristics was evaluated. Beef trimmings were inoculated with *Escherichia coli* and *Salmonella* at 10^7 CFU / mL and treated with either: (1) 2% malic acid; (2) 2% potassium lactate; (3) 0.02% peroxyacetic acid; 0.04% octanoic acid; (5) inoculated untreated control; and (6) uninoculated untreated control. Following initial antimicrobial treatments, trimmings were further treated with 10% trisodium phosphate and rapidly chilled with carbon dioxide (CO_2) snow for 30 seconds. Subsequently, treated and untreated inoculated control trimmings were then ground and packaged on styrofoam trays with absorbent pads, overwrapped with polyvinyl chloride film and sampled for instrumental color characteristics [lightness (L^*), redness (a^*), yellowness (b^*), hue angle, saturation index (intensity of redness), 630/580 reflectance ratio for oxymyoglobin proportion, and a/b ratio on days 1, 2, 3, 5, and 7 of retail display. CO_2 treatment did not affect ($P < 0.05$) instrumental a^*-value of untreated un-inoculated control + CO_2 as compared to untreated un-inoculated control. There was no difference ($P > 0.05$) in a^*-values among different antimicrobial treatments of beef trimmings except octanoic acid compared to control samples. When comparing controls (both chilled vs. un-chilled) there were no difference ($P > 0.05$) for L^*, b^*, and saturation index; however, as compared inoculated untreated control, inoculated untreated control+CO_2 were tended to be darker during retail display. The antimicrobial treated samples exhibited similar color performance throughout display. Therefore, results of this study indicate that these treatments applied to beef trimmings generally produced ground beef of similar or improved color as an untreated control throughout display.

Introduction

Ground beef has been a focus of concern for producers and consumers due to the high potential risk for food borne illness. Ground beef is produced from trimmings and sourced from different cattle and locations. Within the meat industry, new and emerging pathogens continue to provide an obstacle for producers and consumers concerning meat safety. Dorsa et al. (1997) found that the use of hot water or organic acid interventions in the processing of beef carcasses were effective in reducing bacteria, but had detrimental effect on quality characteristics. Pohlman et al., (2009) found that potassium lactate (KL) and sodium metasilicate (NMS) treatments maintained oxymyoglobin content and redness of color through simulated retail display.

Currently available decontamination treatments and methods are somewhat effective in reducing microbial populations; however, most of these available techniques/methods render the detrimental effects on treated products quality attributes. Consumers demand not only safe meat products but also products that are high in quality regarding color, taste and appearance. Direct antimicrobial application to meat as a decontamination technique has been shown to be effective in reducing pathogenic bacteria populations in the final product. However, the challenge of direct antimicrobial application is that active substances can be neutralized upon contact or diffuse rapidly from the surface into the food mass. Furthermore, most antimicrobial agents impact meat color adversely depending upon the type and amount of antimicrobial applied. Maintaining a bright red color of meat is a delicate interaction of applied antimicrobial and myoglobin chemistry. The objectives of this study were to evaluate the impact of antimicrobial treatment on instrumental color characteristics of ground beef produced from treated beef trimmings.

Materials and Methods

Antimicrobial Treatment Application and Sample Processing. The antimicrobial treatments of 0.02% peroxyacetic acid (PAA), 0.04% octanoic acid (OA), 2% malic acid (MA), 2% potassium lactate (UltraLac KL -78, Hawkins Inc., Minneapolis, Minn.) (KL), and 10% (w/v) trisodium phosphate (TSP; Trisodium phosphate anhydrous (FG), ICL performance products, St. Louis, Mo.), was prepared by mixing appropriate amounts of municipal purified water. Inoculated samples treated with and without CO_2 (IN; IN+CO_2) and un-inoculated treated with and without CO_2 (C; C+CO_2) treatments were run as control treatments of the experiment. The un-inoculated samples (C and C+CO_2) were only used for instrumental color characteristics measurements and not for microbial analysis. Inoculated beef trims were treated with either: (1) 0.02% PAA; (2) 0.04% OA; (3) 2% MA; and (4) 2% KL. Each antimicrobial treated sample was then subsequently mixed with 10% (w/v) TSP and chilled with liquid CO_2. For the antimicrobial treatments, 5.4 kg of beef trimmings (80% lean and 20% fat) were placed into a CO_2 mixer (Model 814, Food Processing Equipment Co., Springdale, Ark.) with 1 L of the selected antimicrobial solution and mixed with the meat for 3 min and chilled with CO_2 for 30 s. After the antimicrobial application step and chilling were completed, the beef trimmings were ground twice using a Hobart grinder (Model 310, Hobart Inc., Troy Ohio) with a 3.2-mm plate. After grinding, 200 g of the ground beef were placed on foam trays with absorbent pads and over wrapped with polyvinyl chloride film (oxygen transmission rate of 14,000 cc/mm^2/24 h/1 atm; Koch Supplies, Inc., Kansas City, Mo.) was used as an over wrap and stored under simulated retail conditions (4 °C; deluxe warm white fluorescent lighting; 1600 lx; Phillips Inc., Somerset, N.J.) for 7 days. Styrofoam trays containing ground beef for each treatment were packaged to allow for independent use for microbial

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evaluation and instrumental color sampling. Treated ground beef pH was also sampled immediately after grinding by homogenizing a 1.8 g portion of ground beef in 18 ml of distilled water and evaluated using an Orion Model420A pH meter with a ROSS electrode (Model 8165BN,Orion Research, Inc., Beverly, Mass.).

Instrumental Color. On days 1, 2, 3, 5, and 7 of simulated retail display, instrumental color of the ground beef were measured using a Hunter-Lab Miniscan XE Spectrocolorimeter (Model 45/-L, Hunter Associates Laboratory, Inc., Reston, W.Va.). The samples were evaluated using illuminant A/10° observer for the Commission *Internationale de l'Eclairage* (CIE; L^*, a^*, and b^*) color values. In the visible spectrum from 580 to 630 nm reflectance measurements were also taken to estimate the proportion of oxymyoglobin content. In addition, hue angle [$(\tan^{-1}(b^*/a^*)$] and the saturation index [$(a^{*2}+b^{*2})^{0.5}$], were also calculated according AMSA color guidelines (AMSA, 1991). Three measurements were taken of each sample and averaged for statistical analysis.

Statistical Analysis. The experimental design was a randomized complete block with 3 replications with a 6 × 5 factorial arrangement of treatments consisting of 6 different treatment applications and 5 display days (1, 2, 3, 5, and 7). The model included the main effects of antimicrobial treatment, day of display, and treatment by day interactions. The Proc Mixed procedure of SAS (2003) was used to perform type-3 tests of fixed effects. Least squares means for protected F-tests ($P < 0.05$) were separated by using least significant differences (LSD; $P < 0.05$). The experiment was replicated three times.

Results and Discussion

Effect of Antimicrobial Treatment on Instrumental Color Characteristics. Impact of antimicrobial treatments on instrumental color characteristics of the ground beef produced from treated beef trimmings is summarized in Table 1. There were main effects of day of display ($P > 0.05$) for L^*, a^*, b^*, 630 nm /580 nm, a/b reflectance ratio, hue angle, and saturation index values. Ground beef produced from the beef trimmings treated with antimicrobials PA, MA, OA, and KL was less red ($P > 0.05$) and contained lower a/b ratio as the display day advanced from day 1 through day 7. However, all antimicrobial treatments affected ($P > 0.05$) ground beef oxymyoglobin redox form (630 nm/580 nm ratio). The ground beef produced from beef trimmings treated with all antimicrobial treatments (PA, MA, OA, and KL) had higher ($P > 0.05$) 630 nm / 580 nm ratio on days 1 and 2 compared to days 3, 5, and 7 of the retail display. In general, numerical values for 630 nm / 580 nm ratio decreased with increase in the day of display indicating dissipation of oxymyoglobin redox form with advancement of days of display from day 1 through 7. There was a similar trend observed for a^*-values and hue angle. Saturation index values indicate intensity of redness of the ground beef during day of retail of display. Saturation index values were significantly high ($P > 0.05$) on day 1 and had same values ($P > 0.05$) for day 2 and 3 of display. Day 7 of display exhibited lowest ($P > 0.05$) value of saturation index indicating significant discoloration of the ground beef.

Pohlman et al (2002) found that ground beef treated with 10% TSP and 0.5% CPC were more ($P < 0.05$) red (a^*). Jimenez-Villarreal et al. (2003) found that ground beef patties treated with 0.5% CPC were lighter (L^*, $P < 0.05$) in color compared to the control, the 10% TSP treatment and the control did not differ ($P > 0.05$) in L^* value, and the 10% TSP treatment was found to be more ($P < 0.05$) red (a^*) in color than the control, whereas 0.5% CPC was similar ($P > 0.05$) in redness (a^*) to the control. Consumers associate redness to freshness, and discriminate against discolored meat, thus the redness of meat is an important factor (Hood and Riordan 1973; Morrisey et al., 1994). Antimicrobial treatments affected ($P > 0.05$) ground beef lightness (L^*) values and the treated ground tended to be more darker ($P > 0.05$) than control on days 2 through 7 of display, . The antimicrobial treatments reduced yellowness (b^*-values) and exhibited similar trend as L^* values during the display.

Effect of Duration of Display, Pooled Across Antimicrobial Treatments on Instrumental Color Characteristics. The effect of duration of display, pooled across antimicrobial treatments of the ground beef produced from beef trimmings treated with antimicrobials on instrumental color characteristics is summarized in Table 2. Individual effect of treatments were detected for L^*, a^*, b^*, and hue angle, saturation index values, 630 nm/580 nm, and a/b reflectance ratio. The ground beef redness became less red (a^*), and less yellow (b^*) for antimicrobial treatments PA, MA, OA, and KL. The hue angle values were same ($P > 0.05$) for UN, UN+CO_2, and OA treatment while IN, IN+CO2, PA, MA, and KL had the same ($P > 0.05$) hue angle values. Antimicrobial treatments applied to beef trimmings affected ($P > 0.05$) ground beef oxymyoglobin content (630 nm /580 nm) and reduced a/b ratio that represents loss of red color through retail display.

Implications

The results of this study suggest that application of antimicrobials such as PA, OA, MA, and KL in decontamination of beef trimmings will produce ground beef of similar or improved color as an untreated one. Antimicrobial treatments to beef trimmings could affect ground beef oxymyoglobin content through retail display. Subsequent application of 10% TSP followed with antimicrobial treatment (PA, OA, MA, and KL) may stabilize ground beef color stability during retail display.

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Table 1. Least squares means (± SE) for the main effects of day of display on *L, *a**, *b**, hue angle, saturation index, 630/580and a/b ratio of ground beef during simulated retail display.**

Color Parameters	Day of display				
	1	2	3	5	7
*L**	39.56 ± 0.82[b]	37.97 ± 0.82[ab]	37.26 ± 0.82[ab]	37.11 ± 0.82[a]	37.37 ± 0.82[ab]
*a**	23.35 ± 0.65[d]	19.70 ± 0.65[c]	18.14 ± 0.65[bc]	17.08 ± 0.65[b]	10.08 ± 0.65[a]
*b**	18.15 ± 0.38[c]	15.72 ± 0.38[b]	15.63 ± 0.38[b]	15.10 ± 0.38[b]	12.85 ± 0.38[a]
Hue angle	37.91 ± 0.93[a]	38.68 ± 0.93[ab]	41.17 ± 0.93[bc]	42.28 ± 0.93[c]	52.02 ± 0.93[d]
Saturation index	29.59 ± 0.68[d]	25.21 ± 0.68[c]	24.00 ± 0.68[bc]	22.89 ± 0.68[b]	16.43 ± 0.68[a]
630/580 ratio†	2.13 ± 0.68[d]	2.16 ± 0.68[c]	2.00 ± 0.68[bc]	2.89 ± 0.68[b]	1.43 ± 0.68[a]
a/b ratio††	4.19 ± 0.17[d]	3.98 ± 0.17[d]	3.46 ± 0.17[c]	2.96 ± 0.17[b]	1.06 ± 0.17[a]

[a-d]Least squares means within a row with different superscripts are different ($P < 0.05$).
†630/580 ratio = 1 = brown to 5 = bright purplish red.
††a/b ratio = 1 = brown to 5 = bright purplish red.
[a-d] Least squares means within a row with different superscripts are different ($P < 0.05$).

Table 2. Least squares means (± SE) for the main effects of duration of display on *L, *a**, *b**, hue angle, and saturation index and 630/580 and a/b ratio of ground beef during simulated retail display.**

	Treatment*							
	UN	**UN+CO_2**	**IN**	**IN+CO_2**	**PA**	**MA**	**OA**	**KL**
*L**	52.0 ± 0.9^{c}	52.5 ± 0.9^{c}	49.2 ± 0.9^{b}	46.2 ± 0.9^{a}	47.7 ± 0.9^{ab}	48.7 ± 0.9^{b}	48.3 ± 0.9^{ab}	46.0 ± 0.9^{a}
*a**	17.5 ± 0.9^{b}	17.4 ± 0.9^{b}	19.1 ± 0.9^{c}	16.8 ± 0.9^{b}	16.6 ± 0.9^{b}	16.7 ± 0.9^{a}	14.8 ± 0.9^{a}	16.3 ± 0.9^{b}
*b**	17.4 ± 0.5^{c}	16.8 ± 0.5^{bc}	16.1 ± 0.5^{b}	15.1 ± 0.5^{b}	14.7 ± 0.5^{ab}	14.4 ± 0.5^{ab}	14.3 ± 0.5^{ab}	14.0 ± 0.5^{a}
S-Index	24.9 ± 1.0^{c}	24.4 ± 1.0^{c}	25.2 ± 1.0^{c}	22.8 ± 1.0^{bc}	22.2 ± 1.0^{ab}	22.1 ± 1.0^{ab}	20.7 ± 1.0^{a}	21.6 ± 1.0^{ab}
630/580†	2.1 ± 0.09^{a}	2.1 ± 0.09^{a}	2.3 ± 0.09^{b}	2.3 ± 0.09^{b}	2.5 ± 0.09^{d}	2.5 ± 0.09^{d}	2.4 ± 0.09^{c}	2.5 ± 0.09^{d}
a/b ratio§	1.0 ± 0.04^{a}	1.0 ± 0.04^{a}	1.2 ± 0.04^{a}	1.1 ± 0.04^{a}	1.1 ± 0.04^{a}	1.2 ± 0.04^{a}	1.0 ± 0.04^{a}	1.2 ± 0.04^{a}
Hue angle	45.5 ± 1.4^{b}	44.5 ± 1.4^{bc}	41.2 ± 1.4^{a}	42.0 ± 1.4^{ac}	41.6 ± 1.4^{a}	40.8 ± 1.4^{a}	44.9 ± 1.4^{b}	40.4 ± 1.4^{a}

*Treatment: UN = untreated un-inoculated control not chilled with CO_2, UN+CO_2 = untreated un-inoculated control chilled with CO_2, IN = untreated inoculated control, IN+CO_2 = untreated inoculated control treated with chilled with CO_2, PA = 0.02% ppm peroxyacetic acid, MA = 2% malic acid, OA = 0.04%octanoic acid, KL = 2% potassium lactate.

†630/580 ratio = 1 = brown to 5 = bright purplish red.

§a/b ratio = 1 = brown to 5 = bright purplish red.

[a-d] Least squares means within a row with different superscripts are different ($P < 0.05$).

Inactivation of *E. coli* cells at low dose rates of gamma radiation

Anand Mohan[1], F. W. Pohlman[1], M. C. Hunt[1]

Story in Brief

This study was conducted to evaluate the effect of low dosage rates of gamma irradiation on actively growing cells of *Escherichia coli*. A mathematical model was developed for calculating the minimum dose required for complete elimination of actively growing bacteria in nutrient rich medium. Following gamma-ray irradiation of culture flasks, microbiological analyses were performed to enumerate surviving organisms after exposure to 0.05, 0.1, 0.15, and 0.2 kGy of gamma irradiation. Results indicate that low-dose gamma irradiation reduced all microorganisms. The irradiation dose (kGy) required to reduce a known number of microorganisms by 90% or by 1-log reduction), calculated from linear regression analysis had the highest irradiation dose of 0.24 kGy. *E. coli* cells showed quantitative stress responses in terms of growth and survival depending on the dose rates of energy deposition, i.e. dose-rate of radiation. Based on these results, a dose of 0.20 kGy should be sufficient to achieve a 5-log reduction of a known number of microorganisms by inactivation of *E. coli* as recommended by the National Advisory Committee for Microbiological Criteria for Foods.

Introduction

Gamma radiation is one of the commonly employed methods employed for the destruction of microbial cells. The killing effect of radiation in microorganisms is generally expressed by the decimal reduction dose or D_{10} value (Thornley, 1963). The D_{10} value is the reciprocal of the slope of the exponential part of a survival curve. This value may also be obtained from the following equation:

$$D_{10} = \frac{RadiationDose}{\log_{10}(X_0 - X)}$$

Where X_o is the initial number of organisms, and X is the number of organisms surviving the radiation dose.

The effect of dose rate on the survival of rapidly renewing mammalian cells exposed to gamma irradiation has been studied (Mayer et al., 2005). However, no systematic study is reported on the effect of low dose-rates on actively growing bacterial cells. This study was designed evaluate the effect of low dose rates of gamma irradiation on actively growing cells of *Escherichia coli* (ATCC # 35218) and a mathematical model was developed for calculating the minimum dose rate required for complete elimination of an actively growing bacterium in nutrient rich medium.

Materials and Methods

Inoculum Preparation and Gamma Irradiation. The ATCC # 35218 strain of *E. coli* was used and grown either in Luria-Bertani (LB) broth or on LB agar as required. One ml aliquot (10^6CFU/ml) of an overnight culture of *E. coli* was suspended in 10 ml LB broth contained in 100 ml capacity conical flasks. The final concentration of cells in the flasks at the beginning of the experiment was ~10^5CFU/ml. Irradiation of the culture flask was started by bringing up the irradiation source rack out of the water pool. Flasks were withdrawn at fixed intervals to estimate the surviving population of the bacterium. The number of survivors was determined by the standard viable cell count on LB agar plates after incubation for 24 h at 37 °C. Irradiation was carried out in a prototype Food Package Irradiator (FPI; Atomic Energy of Canada Ltd). Six positions at different distance from the source (cobalt-60) rack of the FPI were identified and at each position the dose-rate delivered was determined using the equation, $D = h \times (S/d^2)$, where D, h, S, and d represent the dose of gamma-ray irradiation (D), irradiation time (h), dose of the source (S), and the distance from the source (d) to sample tube, respectively. Figure 2 (B) shows the six positions around the source rack and the corresponding dose rates where the flasks were kept for irradiation. The dose rate at the six positions varied from 0.05 to 0.20 kGy/h. Cobalt 60 (half-life 5.2 years) was the gamma-ray source used in this experiment. The actual D_{10} value for the *E. coli* cells was determined in a small (2 L) experimental irradiator with a fairly high dose rate (Gamma Cell 220, Atomic Energy of Canada Ltd., dose rate 21 Gy/min).

Mathematical Modeling. A mathematical model has been generated to explain the results. The model synthesizes equations of microbial growth in a rich nutrient medium and inactivation of microbes by gamma irradiation. The resulting equation can predict the requirements of dose rate for killing a microbe.

Quantitative relationship of bacterial growth is given by the following equation:

$$\frac{dX}{dt} = \mu X \qquad \text{Eq. (1)}$$

Where X denotes the cell number, and μ is the instantaneous growth rate given by generation time tg($\mu = \ln 2/tg$).

Equation (1) expressing the exponential growth phase can be modified to include the trend toward the stationary phase, which occurs at the high population densities. A second term is therefore added to the equation that expresses the maximum population attainable (X_m) for the organism under the environmental conditions specified (Bazni, 1983). The equation (1) then assumes a form often called the logistics equation (2) as shown below:

$$\frac{dX}{dt} = \mu X - \frac{\mu}{X_m X^2} \qquad \text{Eq. (2)}$$

If we assume the gamma radiation inactivation of a microbe follows the usual exponential decay with the decay constant λ, then the population of the microbe will be governed by the equation

$$\frac{dX}{dt} = (\mu - \lambda)X - \frac{\mu}{X_m X^2} \qquad \text{Eq. (3)}$$

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The solution of Eq. (3) can be obtained from

$$X_{(t)} - X_1 = C\left[X_{(t)} - X_2\right]e^{-\mu\frac{t(X_1 - X_2)}{Xm}}$$

Eq. (4)

Where C is the constant of integration to be obtained from the initial condition, and $X_1$and X_2 are the two roots of the quadratic equation

$$(\mu - \lambda)X - \frac{\mu X^2}{X_m} = 0$$

Eq. (5)

The roots have the value of .X_1 = 0, and X_2 = (μ-λ) X_m/μ. Substituting these values in equation (iv) one can then write the solution of Eq. (3) as:

$$X(t) = \frac{C(\mu - \lambda)X_m / \mu e^{(\mu - \lambda)t}}{Ce^{(\mu - \lambda)t} - 1} = 0$$

Eq. (6)

If X_o denotes the initial cell density at time $t = 0$, then Eq. (6) can be used to obtain constant C.

$$C = \frac{X_o}{X_o - (\mu - \lambda / \mu)X_m}$$

Eq. (7)

The instantaneous growth rate μ is related to the generation time t_g and the decay constant λ to the D_{10} value as follows

$$\mu = 0.693 / t_g$$

Eq. (8)

$$\lambda = 2.3026\varphi / D_{10}$$

Eq. (9)

Here φ denotes the incident radiation dose rate (dose/unit time). Given the two constants μ and λ Eq. (6) completely determines the time evolution of the microbial cells. For example in Fig. 2(A), cell population as a function of incident dose-rate during a 24-h exposure time has been plotted. The dotted line in this figure has been obtained from Eq. (6), while the solid lines represent the experimental data points [Fig. 2(A)]. As one can see from the figure, the calculated values show good agreement with the experimental values.

Equation (5) can be used to estimate the minimum dose required for complete elimination of an actively growing bacterium. For this we note that the decay of cell number $X_{(t)}$ is essentially controlled by the exponent (μ-λ). The condition for $X_{(t)}$ to vanish for sufficiently large t is

$$\mu - \lambda < 0$$

Eq. (10)

Substituting for μ and λ, we finally obtain the required condition as

$$\varphi > 0.3D_{10} / t_g$$

Eq. (11)

From the experimentally known value φ for the complete elimination of the microbe, one can estimate the D_{10} value or the generation time of the microbe, if one of the two is known. For example for the experimental value of φ = 1.2 and D_{10} = 0.25 kGy/h, the doubling time of the *E. coli* cells in this experiment will be 20.1 min (given by Eq. (10)). The derived experimental value of the critical dose rate required to inactivate an actively growing bacterium and those derived from the model show a good agreement.

Results and Discussion

The D_{10} value (0.24 kGy/h) was calculated using survival curve for *E. coli* cells suspended in 0.9% saline exposed to gamma radiation at a high dose rate (21 Gy/min). Figure 1 shows the exposure of *E. coli* cells to gamma radiation reduced cell survival ($P < 0.05$) and in some cases reduced it below the limits of detection (Figs. 1C and 1D). The irradiation dose-rate of 0.20 kGy/h yielded no survivors after 24 h of exposure in all positions except position 1A (Figs. 1A, B, C, D, E, and F). Positions 3 and 4 inactivated more than 4.5 logs ($P < 0.05$) of *E. coli* cells after 8 h of exposure with 0.20 kGy/h irradiation dose-rate (Figs. 1C and 1D).Increasing irradiation dose-rates from 0.05 kGy/h to 0.15 kGy/h rendered elimination of more than 2.0 logarithm reduction of *E. coli* cells (Figs. 1E and 1F), and further exposure to 0.20 kGy dose-rate reduced the cells survival below the limits of detection for the experiment ($P < 0.05$). Wang and others (2004) studied the inactivation of two strains of *E. coli* O157: H7 by ionizing radiation in apple cider and tryptic soy broth and reported D_{10}-values within the same magnitude (0.25 to 0.29 kGy). Buchanan and others (1998) reported similar D_{10}-values for *E. coli* O157:H7 in apple juice and found that increasing the solids in the juice increased the D_{10}-value. Results from this study strongly suggest that in a nutrient rich medium capable of supporting growth and multiplication of bacterial cells, destruction of the microbial population by gamma irradiation is a function of dose-rate.

Implications

The results indicate that low dose-rates of gamma irradiation affected *E. coli* population at a dose-rate of 0.20 kGy, which is sufficient to achieve the recommended 5-log inactivation of other species of *E. coli*. Better understanding of the mechanisms involved in bacterial resistance to radiation exposure need to be explored.

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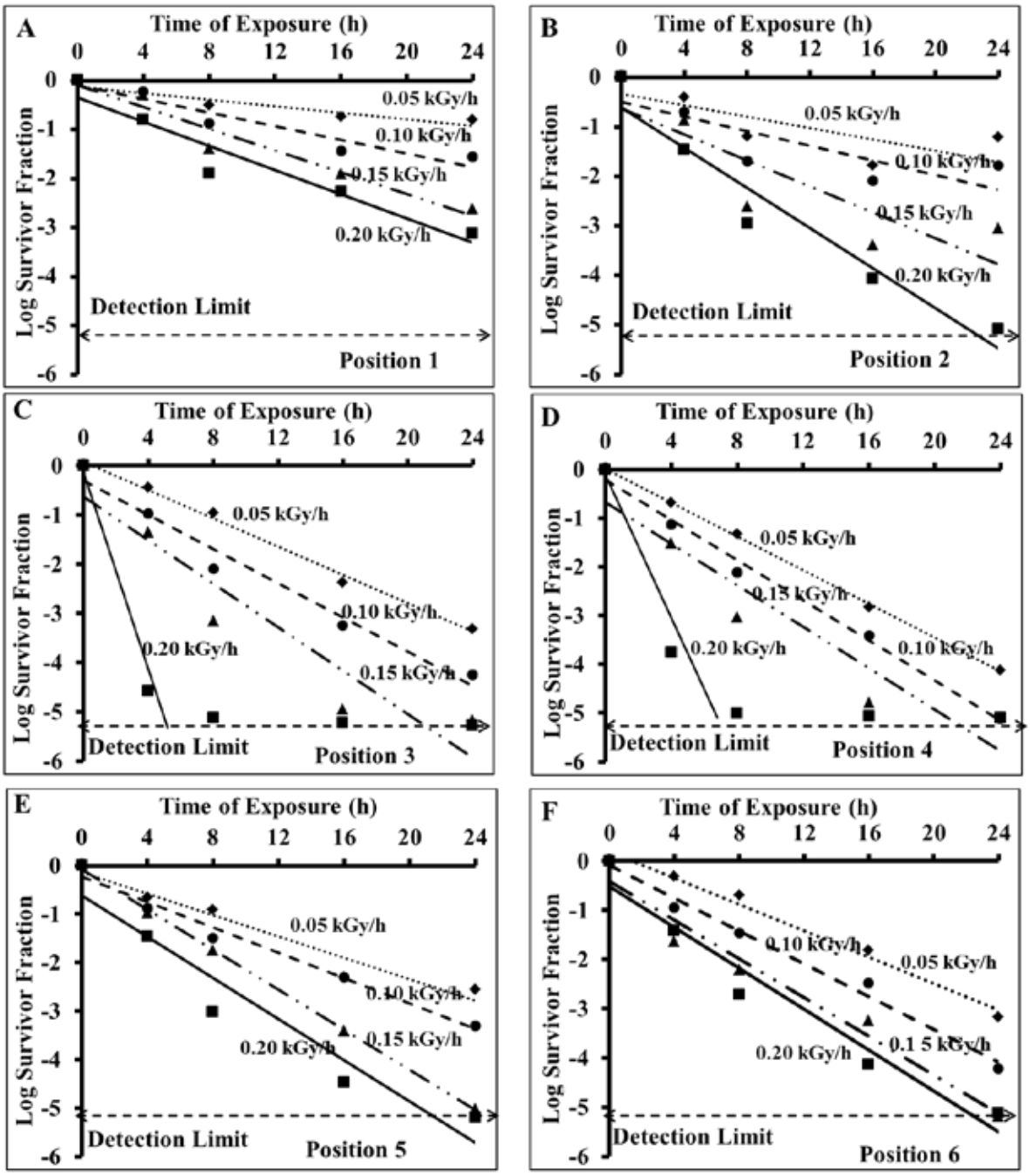

Fig. 1. Inactivation of *E. coli* cells after 4, 8, 12, 16, 20 or 24 hours of exposure to at low dose-rates of 0.05, 0.10, 0.15, and 0.20 kGy/h of gamma irradiation at positions 1, 2, 3, 4, 5, and 6.

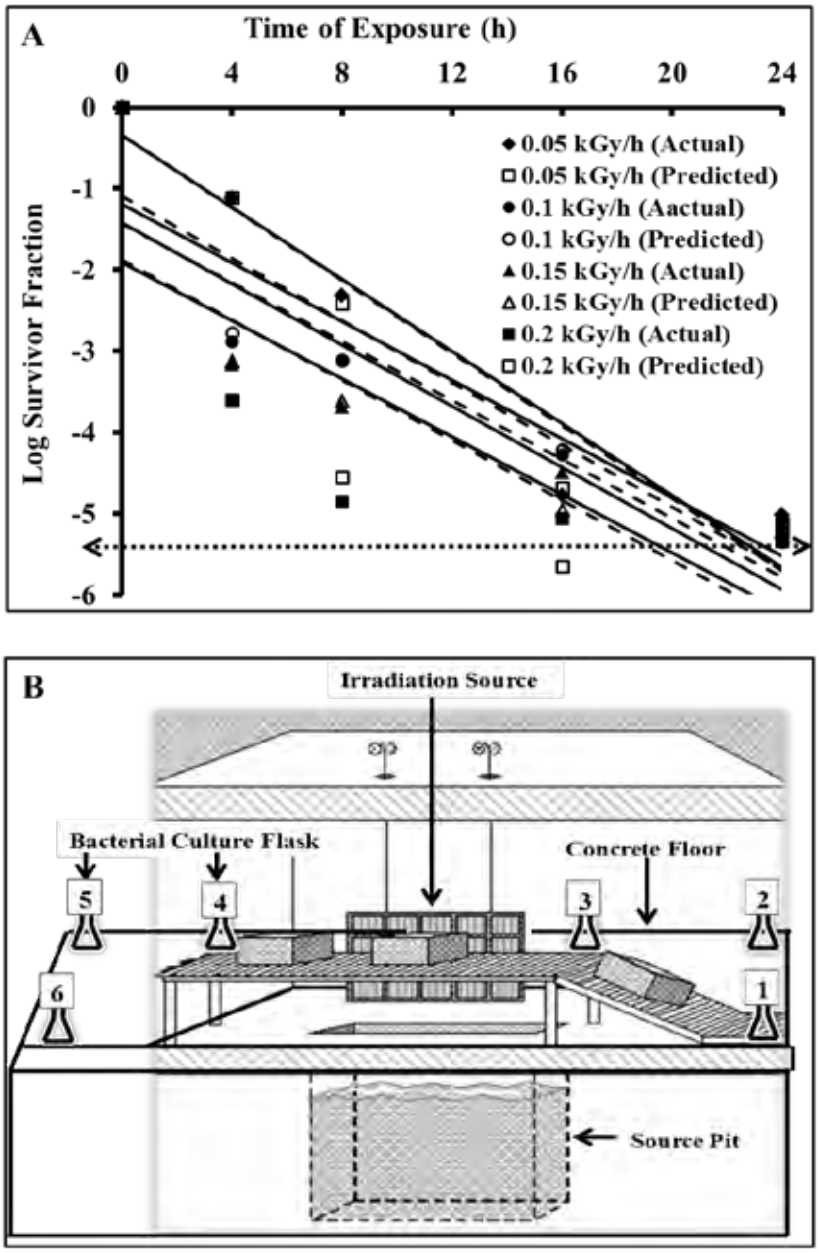

Fig. 2. (A). Depiction of the actual and predicted inactivation curve of the *E. coli* cells after 4, 8, 12, 16, 20 or 24 hours of exposure to low dose-rates of 0.05, 0.10, 0.15, and 0.20 kGy/h of gamma irradiation at position 4; (B) diagrammatic representation of irradiation exposure position of bacterial culture flask inside the irradiation chamber.

300-Day Grazing Demonstration – Cattle management report for the third year

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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 hay feeding to ≤60 d per year, 4) maintain a 90% net calf-crop and 5) wean at an average weight of 550 pounds. The average adjusted 205-d weaning weight for 2010-2011 was 535 and 476 lb for steers and heifers, respectively. 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 50% (calf adjusted 205 d wt/ cow wt at weaning). The goal for weaning 50% of the cow's body weight was reached this year, but the herd still fell short of the 550 lb weaning weight goal. Given the cow size of the females that make up the herd, the 550 lb average 205-day weaning weight goal may be more ambitious than is realistically possible since that would mean cows would need to wean more than 50% of their body weight. Calves were weaned on May 18, 2011 and average weaning weight and value was 603 lbs and $801, respectively, with a total weaning value of $31,248. Over a 45 day grazing period calves gained 1.46 lbs average daily gain (ADG) resulting in an average weight and value of 665 lbs and $870, respectively. As a result of grazing an extra 45 days, calves return an extra $2,685, $68/calf or $64/day. With continued emphasis of improved management, selection of high quality bulls and selective culling, increases in production and calf income may be possible.

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), reduce 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 hay feeding to 60 d or less, 4) maintain 90% net calf-crop and 5) wean calves at an average weight of 550 pounds.

Materials and Methods

The cow herd (45 head total) was comprised of Balancer females (30 head), Angus 2-yr-old (first calf) heifers (10 head), and Balancer x Hereford yearling heifers (5 head) with a September 1 to November 1 calving season and a November 21 to January 2 breeding season. In the fall of 2009, 2 Hereford bulls were leased and fertility-tested prior to the breeding season. The five head of yearling heifers were synchronized using a two-shot Lutalyse (Pfizer Animal Health, Madison, N.J.) procedure and AI-bred to a low birth weight Angus bull on standing heat once, before turning out with Hereford bulls.

On April 11, 2011 calves from the 2010 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 2011 blood samples were collected from cows to determine pregnancy rate for the 2009-2010 breeding season (SEK Genetics, Inc., Galesburg, Kan.). Cows and calves were weighed and the weights were determined for the cow herd performance program.

On May 18, 2011 calves were administered a Virashield 6 + VL5 booster and all steers and all heifers not retained as replacements 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 fence-line weaning. Fence-line weaning was accomplished by placing calves in a high quality pasture containing novel endophyte tall fescue and red clover with their dams were placed in an adjacent pasture.

Results

Blood test results showed an overall pregnancy rate of 86% (Table 1); 37 of the 38 cows (97%), 5 out of 7 first calf heifers (91%) and 2 out of 5 yearling heifers (40%) were determined to be pregnant. Pregnancy rates for the mature cows and first calf heifers are acceptable and within expectations, however, the pregnancy rates for the yearling heifers are well below expected values. It is unclear why these heifers failed to breed as they were in good body condition and were above their target body weights prior to the breeding season.

The average adjusted 205-d weaning weight was 535 and 476 lb for the steers and heifers, respectively (Table 1). The average frame score was 4.1 for steers and 4.3 for 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. Calf weaning weights are higher than in the first year of the demonstration and suggest the importance of sire selection on the overall production level of a cowherd. The overall cow efficiency was 50% (calf adjusted 205 d wt/ cow wt at weaning). The goal for weaning 50% of the cow's body weight was reached this year but the herd still fell short of the 550 lb weaning weight goal. Given the cow size of the females that make up the herd, the 550 lb average 205 day weaning weight goal may be more ambitious than is realistically possible. Looking at the three year results (Table 1), there has been a steady increase in weaning weights over time. Using high quality sires and culling low

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performing dams along with proving high quality forage are likely keys to this improvement.

One of the management strategies that has been implemented in this herd is a strict culling program. All open females or females that lose a calf for any reason are culled and replaced with a female of similar stage of production. A cow that loses a calf after calving is replaced with another cow/calf pair and a female that is determined to be open following a pregnancy test is replaced with a bred female. Over the three years, 19 of the 38 (50%) original cows have been culled for various reasons or have died. Animals that have been added to the herd as replacements to the original cows unfortunately have been replaced at an even higher rate (62%). Failure to breed is the most common reason for replacement (Table 2).

The post weaning results are summarized in Table 3. For the past three years, calves were weaned in mid-May and grazed until late June (2010–2011) or mid-July (2008-2009 and 2009-2010). For year 3 (2010–2011) calves were weaned on May 18, 2011 and grazed until June 29, 2011 (45 days) at which time they were sold. The average weaning weight and value was 603 lbs and $801, respectively, with a total weaning value of $31,248. Over a 45 day grazing period, the calves average daily gain (ADG) was 1.46 lbs resulting in an average weight and value of 665 lbs and $870, respectively. As a result of grazing an extra 45 days, calves return an extra $2,685, $68/calf or $64/day. For the past three years, not selling at weaning but grazing fall born calves for an extra 45 to 60 days was a cost effective practice. Although not statically analyzed, calf performance appeared to have improved over the three year demonstration.

Implications

Livestock producers are faced with greater input costs and volatile markets. The 300-day grazing demonstration is a discovery farm to show the integration of science-based management practices. No attempt was made to compare different management practices or systems. The demonstration gives producers an opportunity to observe the effects of management changes before adopting recommended practices. Cow-calf production efficiencies can be improved to reduce cost and increase the opportunity for success.

Acknowledgements

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Table 1. Three-year weaning results summary for the 300-Day Grazing Demonstration.

	Year		
	2008-2009	**2009-2010**	**2010-2011**
Steer WWT, lbs	446	480	535
Heifer WWT, lbs	416	448	476
Overall WWT, lbs	437	464	507
Cow Efficiency, %	43%	45%	50%
Cow Body Condition Score at Weaning[a]	5	5.8	5.5
Cow Weight at Weaning, lbs	1016	1039	1042
Pregnancy Rate, %	84%	97%	86%

[a]Body condition scores was based on a 1 to 9 scale where 1 = an emaciated animal, 5 = a moderate animal, and 9 = a very fat animal.

Table 2. Reasons for cow replacement summary for the 300-Day Grazing Demonstration.

Reason	**Percentage of total replacements**
Bad Feet/Fescue Foot	15.8%
Open	55.3%
Calf Death Loss	21.0%
Cow Death Loss	7.9%

Table 3. Three year post-weaning results summary for the 300-Day Grazing Demonstration.

Year	Weaning Weight	Ave Weaning Value	Total Weaning Value	Post Weaning Weight	ADG	Post Wean Ave Value	Post Wean Total Value	Value Difference
2008-2009	471 lbs	$518	$19,704	576 lbs	1.81	$581	$22,112	$2,408
2009-2010	562 lbs	$637	$24,206	634 lbs	1.01	$728	$27,664	$3,458
2010-2011	603 lbs	$801	$31,248	665 lbs	1.46	$870	$33,933	$2,685

ADG = average daily gain.

300 Day Grazing Demonstration
Forage management practices - 2008 through 2010

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Story in Brief

Staff at the Livestock and Forestry Research Station at Batesville, Ark. and the Animal Science faculty began a project in 2008 to apply research based management practices to demonstrate 300 days of grazing. The forage production goals of the program were to 1) enhance the utilization of forages, 2) demonstrate efficient and targeted fertilizer use, 3) extend the grazing season to 300 days or more and to reduce hay needs to 60 days or less, and 4) plan high quality forage for a fall calving cow herd and for calves retained after spring weaning. Initial pasture composition was 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 spring, summer, fall, and winter to match the fall-calving herd. By using forward planning, rotational grazing, fenceline weaning, strip grazing, stockpiled forages, and overseeding clover, grazing seasons exceeding 300 days were achieved for three consecutive years.

Introduction

Extending the grazing season has a strong positive impact on profitability of cattle operations. Most producers obtain adequate forage yield for their livestock, but management is focused on the added expense of producing hay for a long hay feeding season instead of producing more lower-cost grazing days. The 300 Day Grazing Program was developed to demonstrate how livestock producers may be able to better manage costs. The concept was to plan forage production practices at least one season ahead to provide grazing options under changing conditions. The challenge for this project was to develop a 300 day grazing plan, starting with only tall fescue and bermudagrass, for a fall-calving herd and calves retained after weaning. Primary management practices included targeted fertilization and rotational grazing to extend the grazing period during the growing season. Stockpiled forages were used during winter after forage growth ceased for the year. The forage production goals were to 1) enhance the utilization of forages, 2) demonstrate efficient and targeted fertilizer use, 3) extend the grazing season to 300 days or more and reduce hay needs to 60 days or less, and 4) plan high quality forage for a fall calving cow herd and for calves retained after spring weaning.

Materials and Methods

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 days of grazing. Forage management practices were implemented to provide quality forage to a fall-calving cow herd (calving season of September 1–November 1) of predominately Brangus × Balancer females (38 cows) with a November 21 to January 2 breeding season. Pastures in 2008 consisted of 40 acres of common bermudagrass divided into four 10-acre paddocks (Bm1, Bm2, Bm3 and Bm4), and 90 acres of cool-season forages comprised primarily of tall fescue divided into four 22.5 acre pastures (Fld9, Fld10, Fld11, and Fld12). Red clover, white clover, and alfalfa were added to the system in 2009 and 2010 (Fig. 1). In 2010, cool-season forage pastures included 33 acres of KY-31 endophyte infected toxic (E+) fescue (Fld10 and Fld11), 12 acres of Ky-31 fescue/white clover, 22.5 acres of Novel endophyte (NE+) nontoxic fescue/red clover (Fld 12), and 18.5 acres of mixed grass of which about 5 acres was NE+ fescue (Fld 9) and 4 acres of alfalfa. Cool-season pastures were managed for spring and fall grazing and for winter stockpiled forage. The bermudagrass pastures were managed for grazing from June through October. All pastures were soil tested in 2008. Soil pH was >6.0 and soil P was >100 lb/acre for all pastures. Soil K was high (>300 lb/acre) in bermuda pastures, but was medium for fescue pastures (200 lb/acre). Nitrogen was applied at 50 lb/a to bermudagrass in summer on 10-acre paddocks as needed. Nitrogen was applied to Fld 10 and Fld 11 in early September each year for stockpiled forage. Fertilizer N was not applied to fescue in spring except to one fescue pasture in spring 2009. Potash fertilizer was only applied to fescue pastures where clover was overseeded. Each of the pastures contained ponds or water tanks for livestock water. All pastures were fenced with electric fences and could be subdivided as necessary for rotational grazing management. The overall stocking rate was 2.7 acres/AU. An AU (animal unit) was defined based on metabolizable energy requirements for a 1000-lb cow as described by Gadberry and Troxel (1999). Forage management practices implemented from 2008 thru 2010 are shown in Table 1. Primary practices were strip-grazing of stockpiled forages for winter, rotational grazing through spring, summer, and fall, fenceline weaning, and overseeding clover in fescue pastures.

Results and Discussion

By using a seasonal forage plan, grazing seasons exceeding 300 days were achieved for each of the three years since the demonstration began. Grazing seasons were 347, 311, and 326 days for 2008/09, 2009/10, and 2010/11, respectively. Hay feeding periods were 18, 54, and 39 days for the same respective years. Rainfall distribution was excellent through summer of 2008 and 2009 resulting in very good forage growth. In 2009, total annual rainfall reached the highest on record for central Arkansas. But, growing conditions in summer and fall of 2010 were poor. In central Arkansas, 117 days reached temperatures over 90 °F and fall rainfall was extremely low across the state resulting in very poor forage growth. Many producers across the state started feeding hay in September and October. Yet producers

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enrolled in the 300 Day Grazing Whole Farm demonstrations did not feed hay until late December or early January. Their stocking rates ranged from 2.1 to 2.5 acres/AU. On the Livestock and Forestry Research Station, hay feeding did not begin until February 8. The stocking rate was 2.7 acres/AU. This would be considered a moderate stocking rate, but is similar to the 300 day Grazing Whole Farm demonstrations. The moderate stocking rate provided the management flexibility for a 300 day grazing season. High stocking rates in cow-calf systems (<2 acres/AU) may increase numbers of calves initially, but often lead to overgrazing, inadequate seasonal forage availability, higher hay and feed costs, and ultimately lower overall system profitability. These results emphasize the importance of following a grazing and forage management plan in both good and poor conditions.

Forage management practices were planned at least one season or more in advance for spring, summer, fall, and winter each year. This strategy provided options if growing conditions were different than expected. Management practices such as fertilization, forage canopy and weed control, and other items should be in place before forage growth begins each season for a producer to have maximum effect on forage growth. Once a growing season begins, the field conditions in place will largely dictate forage production. For example, heavy infestation of winter annual weeds or fescue on bermudagrass pastures will delay bermuda greenup and reduce early summer forage growth. But controlling early season winter weeds and fescue will improve early growth of the bermuda forage. Also, grazing or clipping forage residue from fescue fields in late summer along with early September fertilization will greatly improve quality and fall growth for stockpiled fescue.

Forages were managed through each season with the cattle production cycle in mind. Spring forage was managed to support cows in late lactation and growing calves. Red clover/NE+ fescue pasture was managed to provide high quality forage from mid-May through early summer to support growth of weaned calves. Bermudagrass was managed to support dry cows through summer. Fescue and clover were used to support cows during fall calving and into the early breeding season. Stockpiled fescue was managed to support cows during winter.

Soil tests were used to guide fertilizer application when needed. Spring growth of fescue pastures that were fertilized the prior fall for stockpiling tends to be more vigorous than unfertilized pastures (personal observation). Therefore, N fertilization was very limited on spring fescue pastures to avoid producing excess forage that could not be utilized. Legumes improve forage quality and help offset negative effects of fescue toxicity. During the winter of 2009/2010 and 2010/2011 several cows were affected with fescue foot resulting from grazing the stockpiled toxic KY-31 fescue pastures. Symptoms of severe lameness of the rear feet were observed following onset of bitterly cold weather in January. To help reduce future fescue toxicity problems and to reduce N fertilizer requirement, a 6-acre block of Ky-31 fescue in each of pastures Fld 10 and Fld 11 was overseeded with white clover (Var: Patriot) in February 2010. In April 2010, alfalfa was planted in 4 acres of Fld 9 to provide some additional high quality forage for weaning and breeding periods. By April 2010, legumes had been seeded into 43% of the fescue pasture area. More white clover was overseeded in February 2011 which increased the legume acreage to 62% of the fescue pasture area. Broadleaf weed infestation in the cool-season pastures was minimal and was easily controlled with the grazing animals.

Implications

Growing conditions over the past three years have ranged from extremely good to extremely poor at the Livestock and Forestry Research Station, yet grazing seasons exceeding 300 days were achieved each year. The project started with a very simple forage base of bermudagrass and fescue and later included clover and alfalfa. Key forage management practices included targeted fertilizer planning, seasonal forage planning, rotational grazing, strip-grazing, fenceline weaning, leader/follower grazing, and stockpiling forage for winter. This integrated approach on a "discovery farm" in a controlled setting suggests that a well-planned forage management strategy works in both good and poor growing conditions.

Acknowledgements

The authors acknowledge the Livestock and Forestry Research Station for supporting this project.

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Table 1. Forage management practices used in the Livestock and Forestry Research Station 300 Day Grazing Demonstration

	2008 Forage Management Practices (project began July 1, 2008)
Summer	Applied 50 lb/a N to 20 acres of bermuda
	Rotationally grazed cows on bermuda until October
Fall	Grazed Fld 10 and Fld 11 to prepare for stockpiled fescue for winter grazing
	Fertilized Fld 10 with 50 lbs/a N in September for stockpiling
	Strip grazed Fld 12 in Oct/Nov to prepare for overseeding red clover
	Drilled red clover in October in Fld 12 at 10 lbs/a (Var: Redland Max)
Winter	Began grazing stockpiled fescue Nov. 25
	Hay feeding began on March 8 and lasted 18 days
	2009 Forage Management Practices
Spring	Fertilized Fld 10 with 34 lbs/a N for early spring grazing
	Sprayed glyphosate on bermuda stubble in February for weed control
	Rotationally grazed fescue pastures through spring
	Fenceline weaned calves onto Fld 12 (Novel endophyte fescue/red clover) in May
Summer	Fertilized 10 acres of bermuda with 50 lbs/a N in early June
	Grazed cows and calves in leader/follower system across red clover then bermuda until calves were sold in July
	Cut accumulated forage on Fld 10 and Fld 11for hay to prepare for stockpiling fescue
Fall	Applied 50 lbs/a N in September to Fld 10 and Fld 11 for stockpiling fescue
	Grazed cows on bermuda until early November
	Grazed Fld 12 NE+ fescue/red clover through November prior to breeding
Winter	Started grazing stockpiled fescue in early December
	Limited hay feeding began in January and began continuously in early March
	Hay was fed until April 8 for a total of 54 days
	2010 Forage Management Practices
Spring	No fertilizer was applied to fescue pastures in spring 2010
	Burned bermuda stubble in February and sprayed in early March with glyphosate for controlling fescue and winter annual weeds
	Overseeded white clover in 6-acre blocks in each of Fld 10 and Fld 11 (Var: Patriot)
	Planted alfalfa in a 4-acre block of Fld 9 (Var: MagnaGraze and Magnum 601
	Rotationally grazed fescue through spring
	Fenceline weaned calves onto Fld 12 (Novel endophyte fescue/red clover) in May
Summer	Fertilized 10 acres of bermuda with 50 lbs/a N in early June, July, and late July
	Grazed cows and calves in leader/follower system across red clover then bermuda until calves were sold in July
	Grazed Fld 10 and Fld 11in August to prepare for stockpiling fescue
Fall	Applied 50 lbs/a N to Fld 10 and Fld 11 in September for stockpiling fescue
	Grazed cows on bermuda until early November
	Grazed alfalfa, NE+ fescue/red clover, and Ky-31/white clover through Dec. 26
Winter	Started grazing stockpiled fescue December 27
	Hay feeding began on February 8 and lasted 35 days

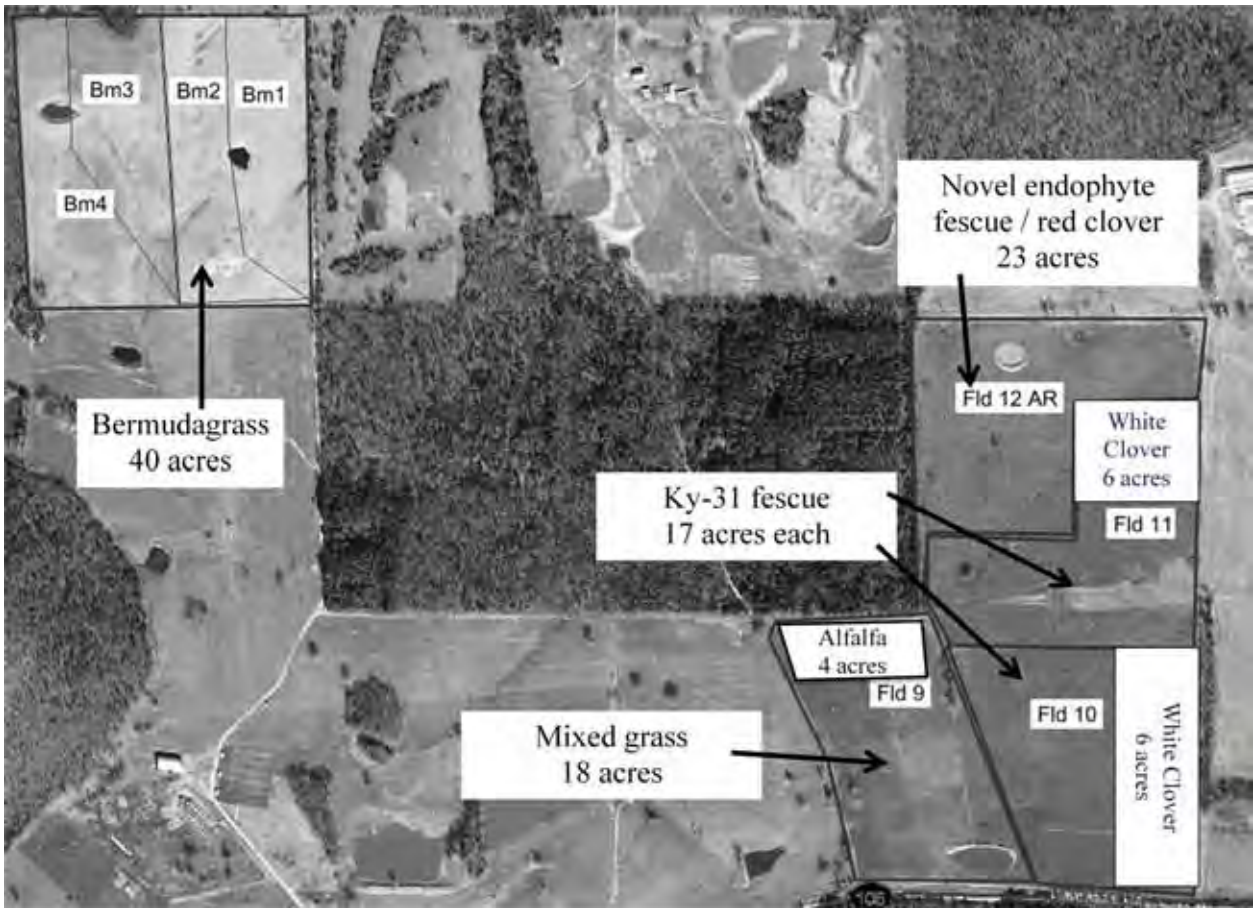

Fig. 1. 2010 map of the pastures involved in the 300 day grazing demonstration at the Livestock and Forestry Research Station. Pastures contain bermudagrass (Bm1, Bm2, Bm3 and Bm4) and fescue, red and white clover, and alfalfa (Fld9, Fld10, Fld11 and Fld12).

300-Day Grazing Demonstration – Year 3 financial report

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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) limit hay feeding to ≤60 d, 4) maintain a 90% net calf-crop and 5) wean an average weight of 550 pounds. An enterprise budget was used to summarize herd inventory, number of animal units, production information, income and expenses. Production information included calf-crop percentage, culling percentage, replacement rate and death loss. Income summary included the number of head sold, average body weight per head, and average price per lb sold. The specified expenses included: salt and mineral, supplemental feed, veterinarian costs, growth implants, fly control, sale commission, hauling, pregnancy testing, bull or artificial insemination (AI) cost, breeding soundness examinations, replacement heifer or cow purchase, fertilizer, lime, purchased hay, herbicide, and miscellaneous. The number of cattle grazed increased 18% and the number of animal units increased 26% from yr 1 to 3. Mature cow calf-crop percentage was 84% in yr 1 and 100% in yr 3. Most expenses remained rather constant over the 3-yr period except for replacement cow costs and fertilizer cost which decreased. The total expenses for yr 3 were $371/animal unit while average total expenses for yr 1 and yr 2 was $575/animal unit. Twenty-one steers (622 lbs), 18 heifers (582 lbs), 3 cows (1,047 lbs), 3 weaned heifers that have not conceived (915 lbs) and 4 heifers that were pregnant or nursing their first calf but not pregnant with a second calf (898 lbs) were sold in yr 3. The total gross income was $789/animal unit. The herd sold 33,024 lbs of beef (675 lb/animal unit) at an average price of $1.17/lb. Herd breakeven for yr 1 and 3 was $0.63 and $0.45/lb, respectively. Research based management practices were demonstrated.

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) limit hay feeding to 60 d or less, 4) maintain 90% net calf-crop and 5) wean an average weight of 550 pounds.

Materials and Methods

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).

An enterprise budget was used to summarize herd inventory, number of animal units (AU), production information, income, and expenses. An animal's animal unit equivalence was determined from its metabolizable energy (ME) requirement in Mcal/lb. For example, since a 1,000 lb non-lactating cow was equal to 1 AU (ME = 17.3 Mcal/lb), then a 1,100 lb cow (ME = 18.5 Mcal/lb) was equal to 1.07 AU (18.5 ÷ 17.3 = 1.07 AU; Gadberry, 2010). Production performance and costs were determined on a fiscal year of July 1 to June 30. The herd inventory reflected the number of animals as of July 1. It included the number of mature cows, weaned heifers that have not conceived (H1), heifers that are pregnant or nursing their first calf but not pregnant with a second calf (H2) and the number of AUs for each classification. Production information included calf-crop percentage, culling percentage, replacement rate and death loss. 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 body weight (BW) per head, and average price per lb sold. Included in the income section were calculated values for total lb sold, total gross income, average selling price, total lb sold per AU, and income per AU.

The specified expenses included: salt and mineral, supplemental feed, veterinarian costs, growth implants, fly control, sale commission (including insurance, yardage and check off programs), hauling, pregnancy testing, bull cost or AI, breeding soundness examinations, replacement heifer or cow purchase, fertilizer, lime, purchased hay, herbicide, and miscellaneous (ear-tags for calves, posts, poly-wire, 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.

Results and Discussion

The herd composition and number of AU grazed for yr 1, 2 and 3 is summarized in Table 1. For the first 2 yr of the demonstration, cow numbers and AU totals remained constant. There were no H1s or H2s in the herd for yr 1 and 2. In yr 3, the number of cattle grazed increased 18% (38 to 46 head,) which increased the number of AU by 29% (38.0 to 48.9 AU). The number of AU increased at a higher percentage due to an increase in mature cow BW resulting in a higher metabolizable energy requirement and the addition of 10 H2s which had a higher metabolizable energy requirement than a 1,000 lb non-lactating mature cow.

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Production information is summarized in Table 2. The mature cow calf-crop percentage steadily increased from yr 1 to 3 (84% to 100%). In yr 3 only one mature cow did not wean a calf but a cow had twins resulting in a 100% calf crop. Improved mature cow calf crop percentage was a result of culling non-productive cows and improving forage quality and quantity. The pregnancy rate for the H1 was low (40%). The reason for the low pregnancy rate for H1s was not clear. Puberty is a function of weight and age. It was believed the H1s developed properly and reached their respective target weight by breeding time. The H1s were artificially inseminated and then placed with a bull for breeding. The breeding season for the H1s was 30 days longer than the mature cow breeding season, giving the heifers numerous opportunities to conceive. All non-pregnant H1s were culled.

Of the 10 H2s that began yr 3, 2 died and 1 was sold. One H2 died before the breeding season. Of the 7 remaining H2's, 5 conceived with their second calf. Three H2s were culled at weaning due to severe fescue foot. Of the 10 H2s purchased, 4 remained with the herd.

The expenses for yr 1, 2 and 3 are summarized in Tables 3. Many of the expense amounts remained rather constant over the 3-yr period. A few expense items decreased in yr 3 compared to yr 1 and 2. These included replacement cow and fertilizer expenses. The replacement cow expense decreased because fewer replacements were purchased. In addition to replacing 2 mature cows, 5 bred H2s were added in yr 3. The purchase price for an H2 was $1,000.

Fertilizer expense was reduced from almost $90/AU (average of yr 1 and 2) to $32.50/AU in yr 3. The incorporation of clovers (red and white) was very important in providing nitrogen fixation to the soil, reducing the need for commercial fertilizer. Miscellaneous expense increased in yr 3 compared to yr 1 and 2. Structures were constructed to provide shade for the cattle in several pastures. The expenses for the structures were placed into the miscellaneous category. The total expense for yr 3 ($371) was 35% less than the average total expense for yr 1 and 2 ($575/AU).

Twenty-one steers weighing 689 lbs sold for $135/cwt. ($19,533) and 18 heifers weighing 640 lbs sold for $125/cwt. ($14,400). Three cows (1,047 lbs for $69.50/cwt. ($2,183)), 3 H1s (915 lbs for $85.30/cwt. ($2,341)) and 4 H2s (898 lbs for $78.40/cwt. ($2,815)) were culled in yr 3. The total gross income for yr 3 was $844/AU (Table 4). The herd sold 35,467 lbs of beef (725 lb/AU) at an average price of $1.16/lb in yr 3.

The average selling price increased $0.32/lb (38%) from yr 1 to 3. In order to remove the impact of the market from the budget, the average price received from yr 1 ($0.84/lb) was incorporated into the yr 3 budget. After adjusting the yr 3 selling price to $0.84/lb, the gross income per AU was $612, which was $187 less than yr 1. In yr 1, more pounds of beef were sold than in yr 3 (36,156 lbs vs. 35,467 lbs) which resulted in the lower gross income. The income over specified cost/AU for the adjusted yr 3 budget was $241/AU, which was $41/AU higher than yr 1. Reduced production cost in yr 3 ($371/AU) over yr 1($596.85/AU) was the reason why the income over specified cost improved. This improvement in production efficiency was also supported by herd breakeven. Herd breakeven for yr 1 and 3 was $0.63 and $0.51/lb, respectively. Production efficiency was improved by applying research based management practices.

Implications

Livestock producers are faced with greater input costs and volatile markets. Developing environmentally and financially sustainable systems to improve forage utilization thus reducing dependency on hay, fertilizer and supplemental feed will improve opportunities for success. The 300 day grazing demonstration is a discovery farm to show the integration of science based management practices. No attempt was made to compare different management practices or systems. The demonstration gives producers an opportunity to observe the effects of management changes before adopting recommended practices. Cow-calf and forage production efficiencies can be improved to reduce cost and increase the opportunity for success.

Acknowledgements

The authors acknowledge the Livestock and Forestry Research Station for supporting this project.

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Table 1. The herd composition and number of animal units in the 300 day grazing herd.

	Number of head			Number of animal units[a]		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Mature cows	38	38	31	38.0	38.0	33.1
H1[b]	0	0	5	0	0	4.5
H2[c]	0	0	10	0	0	11.3
Total	38	38	46	38.0	38.0	48.9

[a] One animal unit is equivalent to a 1,000 lb non-lactating cow.
[b] H1 = weaned heifers that have not conceived.
[c] H2 = heifers that are pregnant or nursing their first calf but are not pregnant with a second calf.

Table 2. Three year production information summary for the 300 day grazing demonstration.

Production item:	Year 1	Year 2	Year 3
Mature cows:			
Calf-crop percentage[a]	84% (32/38)	95% (37/38)	100% (31/31)
Culling percentage[b]	16% (6/38)	13% (5/38)	10% (3/31)
Death loss percentage[c]	3% (1/38)	3% (1/38)	3% (1/31)
H1[d, e]:			
Pregnancy rate[f]			40% (2/5)
Culling percentage[b]			60% (3/5)
Death loss[c]			0
H2 [d, g]:			
Calf-crop percentage[a]			56% (5/9)
Culling percentage[b]			20% (2/10)
Death loss percentage[c]			20% (2/10)

[a] number of calves weaned from mature cows or H2s divided by the number of mature cows or H2, respectively, exposed to the bull.
[b] number of mature cows, H1 or H2 culled divided by the total number of mature cows, H1 or H2, respectively.
[c] number of mature cows, H1 or H2 that died divided by the total number of mature cows, H1 or H2, respectively.
[d] There were no H1s and H2s in the herd for yr 1 and 2.
[e] H1s are weaned heifers that have not conceived.
[f] number of pregnant H1 (pregnant with first calf) divided by the number of H1s exposed to the bull.
[g] H2s are heifers that are pregnant or nursing their first calf but not pregnant with a second calf.

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Table 3. Three year expense summary for the 300-day grazing demonstration. Expenses are expressed on an animal unit basis.

Expense item:	Year 1	Year 2	Year 3
Salt and mineral	$30.81	$27.75	$23.94
Supplemental feed	$0.00	$0.00	$0.00
Vet. Medicine	$23.42	$21.20	$23.36
Growth implants	$1.58	$2.36	$0.95
Fly control	$5.12	$5.00	$0.00
Sale commission	$36.47	$34.03	$37.02
Hauling	$7.63	$14.34	$3.88
Pregnancy test	$2.69	$2.72	$2.55
Bull lease	$15.79	$15.79	$16.23
Fertility testing bulls	$4.08	$2.11	$1.43
Replacement cows	$328.95	$251.32	$175.80
Fertilizer	$69.74	$108.79	$32.50
Lime	$17.68	$0.00	$0.00
Purchased hay	$15.79	$46.74	$21.46
Herbicide	$6.97	$11.12	$3.46
Miscellaneous	$32.13	$7.07	$28.41
Total expenses	$598.85	$550.34	$371.00

Table 4. Three year production information and income summary for the 300 day grazing demonstration.

Item:	Year 1	Year 2	Year 3
Total lbs sold	36,156	30,325	35,467
Average price per lb received	$0.84	$0.93	$1.16
Income/AU[a]	$799	$745	$844
Income over specified cost/AU[b]	$200.41	$195.11	$472.68
Herd breakeven[c]	$0.63	$0.69	$0.51

[a] Animal unit is equal to the metabolizable energy (ME) requirement for a 1,000 lb non-lactating cow.

[b] 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 artificial insemination (AI), breeding soundness examinations, replacement heifer or cow purchase, fertilizer, lime, purchased hay, herbicide, and miscellaneous.

[c] Total specified cost divided by lb of beef sold.

Growth performance by heifers grazing annual ryegrass pastures fertilized with nitrogen or overseeded with crimson, ladino, or both crimson and ladino clovers during spring: Three-year summary

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Story in Brief

Interest in substituting legumes in place of nitrogen 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 nitrogen or overseeded with legumes. Gelbvieh × Angus crossbred heifers [n = 120; 581.3 ± 100.57 lb initial body weight (BW)] were assigned to 1 of 8, 5-acre pastures in the spring of each of the three years of study between 2009 and 2011. All pastures were overseeded with 'Marshall' annual ryegrass, and were not seeded with any clover (commericial nitrogen applied) or overseeded with 'Dixie' crimson, 'Osceola' ladino, or a combination of crimson and ladino clover (CL). Heifers were turned out between January to March and grazed pastures until early to mid-May. Total body weight gain and average daily gain was not different ($P > 0.05$) among treatments; however, number of grazing days was higher for nitrogen treated pastures versus pastures that contained legumes ($P < 0.01$). It appears that with an equal number of grazing days, clovers may not be able to totally eliminate the need for fertilizer, but they may 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 USDA-Economic Research Service (USDA, 2011) stated that ammonia prices paid by farmers increased from $227 per ton to $521 per ton between 2000 and 2006. The increased cost of conventional fertilizers encourages producers to look at lower cost alternatives. Legumes have the ability to fix atmospheric N in conjunction with a symbiotic relationship with Rhizobia bacteria and therefore reducing the 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.

Materials and Methods

Gelbvieh × Angus crossbred spring-born heifers [n = 120; 581.3 ± 100.57 lb initial body weight (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, AR. 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. Each year of the study, a different set of heifers were used. The stocking rate was 1 cow/acre.

The experimental pastures consisted of common bermudagrass (*Cynodon dactylon*) that was sod-seeded mid-September by broadcasting with 30 lb/acre (actual seeding rate) of annual ryegrass (*Lolium multiflorum* cv. Marshall) after a light disking. 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. Soil fertility was adjusted if needed according to soil test recommendations.

On November 3, 2008, control treatments received 300 lbs/acre of 19-19-19 (57 lbs/acre actual N). The following day, November 4, 2008, legume treatments received 200 lbs/acre of 0-23-30. February 23, 2009, control treatments received 150 lbs/acre of 34-0-0 (51 lbs/acre actual N) as spring application. The same day, legumes also received N at the amount of 60 lbs/acre of 34-0-0 (20 lbs/acre actual N). It was recognized at this point that without early-spring fertilization of legume treatments, the grazing starting date in these pastures and therefore grazing days among treatments would likely differ to an extent which would make treatment comparisons unrealistic. The fertilizer quantities applied were considered large enough to initiate ryegrass biomass production yet small enough to not limit N fixation rates substantially.

This fertilizer practice was continued during the second and third years of the experiment. On November 9, 2009, control treatments received 300 lbs/acre of 19-19-19 (57 lbs/acre actual N). Legume treatments received 300 lbs/acre of 6-24-24 (18 lbs/acre actual N) the same day. On March 4, 2010, control treatments received 180 lbs/acre ammonium nitrate (61 lbs/acre actual N), and legume treatments received 100 lbs/acre ammonium nitrate (34 lbs/acre actual N). A quantity of 1.5 tons/acre of lime was also applied in 2009. For the third year of study, legume plots were fertilized with 300 lbs/ac 6-24-24 (18 lbs/acre actual N) and the control paddocks were fertilized with 300 lbs/acre 19-19-19 (57 lbs/acre actual N) November 10, 2010.

During the first year of the study, cattle were stocked on control pastures January 23, 2009. However, legume/ryegrass treatment pastures were not stocked until March 6, 2009 due to a lack of available forage. Heifers remained on their respective pastures until May 11, 2009. Remaining forage biomass was cut for hay on May 27, 2009. For

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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.

the second year of study, the randomization structure of treatments remained. Heifers were again rotated between cells and weighed at the same intervals used in the first year. Heifers were stocked on their respective pastures on March 15, 2010 when forage biomass was great enough to begin grazing. In 2010, grazing days were possibly affected across all pastures due to heavy damage by grazing wildlife early in 2010. Cattle remained on their respective pastures until May 12, 2010. The third year of study was managed in the same manner as the first and second. During 2011, cattle were stocked February 17 and were removed May 3.

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, ending BW, total BW gain, and average daily gain (ADG) did not differ among treatments ($P > 0.1$; Table 1). However, grazing days were significantly higher ($P < 0.01$) for control pastures than pastures that contained legumes. This is primarily due to a delay in grazing during the first year (2009) when growth in legume pastures lagged behind. The fertilizer regime was therefore changed during following years to reduce these differences. Thus far, it appears that legumes may offset fertilizer costs, but this may be difficult during spring grazing. The time available for legume growth is relatively short between broadcasting in Sep/Oct and start of grazing early in the year. Thus, the amount of N fixed will be small, too. A positive result from this study is that ADG was not different across treatments ($P > 0.05$). However, it is possible that these numbers are more related to annual ryegrass which also has a relatively high nutritive value. Conversely, ADG did not change during the summer months of 2009 and 2010 (data no shown) when cattle grazed primarily bermudagrass. A detailed economic analysis is currently underway to determine if clover seed and planting costs would offset or even cost less than the incurred costs in N fertilizer.

Implications

Producers may use legumes and reduced amounts of commercial N in a pasture system to obtain similar animal production in comparison to pastures without legumes fertilized with traditional N rates. However, increased management requirements with respect to legume establishment and persistence need to be taken into consideration when a long term substantial reduction of synthetic N fertilizer is the goal.

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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 2009-2011.

	Nitrogen	Crimson	Ladino	Crimson + Ladino	SEM
Initial wt., lb	565	586	584	589	53.3
End wt., lb	780	768	761	768	43.7
Total study gain, lb	215	182	178	179	18.2
Avg. grazing days/acre	79[a]	66[b]	66[b]	66[b]	5.8
ADG, lbs/day	2.7	2.8	2.7	2.7	0.36

[ab]Within a row, means that do not have a common superscript letter differ, $P < 0.05$.
ADG = average daily gain.

What is the probability for a hay sample to contain a dangerously high nitrate concentration?

M.S. Gadberry[1], J.A. Jennings[1], and P.A. Beck[2]

Story in Brief

The University of Arkansas provides a feedstuff analysis service for Arkansas livestock producers. Test options include routine analysis consisting of dry matter, crude protein, acid detergent fiber, and neutral detergent fiber, plus major minerals, minor minerals, and nitrate. These tests are independently available; therefore, a routine analysis may be requested without a nitrate analysis. Grass hays that analyze high in crude protein may be indicative of high nitrate. The objective of this study was to examine the relationship of nitrate-nitrogen with routine forage test components to establish a prediction equation for the probability of a hay sample testing dangerously high for nitrates. The University of Arkansas, feedstuff analysis database was queried for hay samples (n = 17,765). Of the 17,765 hay samples, 6,420 (36.1%) were tested for nitrate-nitrogen and were included in the probability analysis. Observations that measured >1,400 ppm nitrate-nitrogen were considered dangerously high nitrate. Crude protein was positively correlated with nitrate-nitrogen ($r = 0.42$). When grass hays and legume hays were examined, legume hays exhibited the lowest probability of containing a dangerously high concentration of nitrate and should not require testing. The probability of observing a dangerously high nitrate concentration exceeded 33% for nitrate accumulating hays (johnsongrass, sorghum, and sorghum-sudangrass) contained greater than 12% crude protein. Analysis of this data set indicates that the probability that a hay sample contains a dangerously high concentration of nitrate can be estimated from forage type and crude protein. This information could be presented with forage test results when non-legume hays test high in crude protein and a nitrate test was not requested.

Introduction

Each fall, winter supplementation meetings are offered across the state through local county Cooperative Extension offices to educate cattle producers about forage testing, how to interpret a forage test, and how to choose a supplement using forage test results. Cattle producers participating in this program submit hay samples for analysis which includes dry matter (DM), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF), as well as, total digestible nutrients (TDN) estimation. In 2010, several samples contained high CP. This pattern was alarming since some samples were predominately johnsongrass which is known to be a nitrate accumulating plant. When surveyed at the meeting, these instances shared a similar story of N fertilization followed by limited rainfall between fertilization and harvest. Based on these observations, a question was presented, "Could forage type and routine analysis results be used to identify samples with a higher probability of containing a dangerously high nitrate concentration?"

The objective of this study was to use a database of hay samples analyzed at the University of Arkansas Agriculture Diagnostics Laboratory to examine the relationship of nitrate-N with routine forage test components to establish a prediction equation for the probability of a hay sample testing dangerously high for nitrates.

Materials and Methods

The University of Arkansas provides a feedstuff testing service available through county Cooperative Extension offices. Samples are submitted to the Agriculture Diagnostics Laboratory (Fayetteville, Ark.) for routine (DM, CP, ADF, and NDF), major minerals, minor minerals, or nitrate analysis. The laboratory is a National Forage Testing Association (Avoca, Neb.) certified lab and routine analysis is performed by Near Infrared Spectroscopy (FOSS NIRSystems, Laurel, Md.) and wet chemistry according to Association of Analytical Chemists procedures. Nitrate is determined using a colorimetric procedure.

The University of Arkansas, feedstuff analysis database was queried for hay samples (n = 17,765). Of the 17,765 hay samples, 6,420 (36.1%) were tested for nitrate-N and were included in the analysis. Correlation coefficients for pairwise complete observations of DM, CP, ADF, NDF, and nitrate-N were calculated. This was used to determine which constituents would be modeled and to minimize the number of independent covariates in a prediction model that would be correlated. A new binary variable was created based on nitrate-N to categorize the sample as containing a dangerously high concentration of nitrate. Observations that tested >1,400 ppm nitrate-N were given a reference value of 1; otherwise, the reference value was 0. Nitrate-N concentrations exceeding 1,400 ppm may result in poor appetite, reduced growth rate, abortions, and decreased milk production. Death can occur when concentrations exceed 2,100 ppm.

Forage specie was re-coded into a forage type variable. Forage type was categorized as legume (LEG), warm-season grass (WSG), cool-season grass (CSG), mixed grass (MIX), or nitrate accumulating grass (NIT). A few observations were identified as native grasses or weeds and were omitted from the dataset. Once the forage type and independent forage analysis covariates to be modeled were chosen, model estimates were determined by fitting the data to a generalized linear model with a binomial distribution using R statistical software (http://www.r-project.org). Pairwise comparisons were made for forage type parameter estimates adjusting for Tukey's honestly significant difference using the multcomp package for R.

Results and Discussion

Table 1 contains the forage species allocated to each of the forage types modeled. Bermudagrass was the primary specie categorized as WSG, fescue was the primary specie categorized as CSG, MIX consisted of all samples that were originally coded as mixed grasses which represent forages harvested from heterogeneous WSG and CSG meadows. The NIT category of hays consisted of 64% sorghum-sudangrass, 25% johnsongrass, and 11% sorghum. The

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CP, ADF, NDF, and nitrate-N content means and standard deviations are summarized by forage type and presented in Table 2. Nitrate-N averaged 798, 996, 569, 836, and 1,313 ppm for WSG, CSG, LEG, MIX, and NIT forage types, respectively. Nitrate-N concentration was highly variable. Table 3 presents a summary of the correlations among DM, CP, ADF, NDF, and nitrate-N for all hay samples. Of the feed analysis variables, CP had the greatest correlation with nitrate-N (r = 0.42). The second most correlated variable with nitrate-N was ADF (r = -0.151); however, ADF expressed a greater correlation with CP (r = -0.541). As a result, CP was chosen as the only forage analysis component to model the probability of observing a dangerously high nitrate concentration.

Table 4 contains the model parameter estimates and their standard errors for the log-odds of observing a dangerously high (>1,400 ppm nitrate-N) nitrate concentration. Based upon forage type and CP concentration, the log-odds of the sample containing a dangerous concentration of nitrate can be determined. For interpretative purpose, the log-odds can be converted to a probability

$$P = \frac{1}{1 + e^{-(x+0.349(CP))}}$$

where x = -7.507, -7.086, -11.411, -7.363, and -5.189 for WSG, CSG, LEG, MIX, and NIT, respectively. A two variable sensitivity table for the lower and upper 95% confidence limits of the probability of observing a dangerous nitrate concentration is presented in Table 5. Legumes exhibit the lowest probability of containing a dangerously high concentration of nitrate and do not require testing. Hay meadows that contain a moderate to high percentage of legume in the specie composition mix will likely not be fertilized with high rates of N. The log-odds parameter estimate was greater for NIT compared to all other forage type categories ($P < 0.001$). The probability of observing a dangerously high nitrate concentration exceeded 33% with NIT hays testing greater than 12% CP. The log-odds estimate for WSG and CSG were similar ($P = 0.11$), and MIX did not differ from either WSG ($P = 0.87$) or CSG ($P = 0.61$). At 18% CP, 20% WSG, 24% CSG and 20% MIX grasses will likely have a dangerously high nitrate concentration.

Similarities in log-odd estimates for grass forages categorized as WSG, CSG, and MIX suggests a reduced model that excludes LEG and separates forage types into two categories, nitrate accumulators and non-nitrate accumulators, could be sufficient. A reduced model would yield the following probability equation

$$P = \frac{1}{1 + e^{-(x+0.341(CP))}}$$

where x = -5.091 for NIT grasses and -7.293 for all other grasses.

Implications

In conclusion, the probability of a hay to contain a dangerously high concentration of nitrate can be estimated from forage type and CP. This information could be presented with forage test results when non-legume hays test high in CP and a nitrate test was not requested. Since the average CP of NIT forages was 11% and being 95% confident that 12% CP NIT forages will have a 21 to 33% probability of containing dangerously high concentrations of nitrate, county Extension agents should recommend a nitrate test any time hay from these forage species are being submitted for the routine analysis.

Table 1. Forage species within forage type category for all hay samples.

	Forage type[a]				
Forage specie	WSG (n)	CSG (n)	LEG (n)	MIX (n)	NIT (n)
Alfalfa			414		
Alfalfa-grass mix			80		
Clover			34		
Legume-grass mix			291		
Bahiagrass	384				
Bermudagrass	7,132				
Dallisgrass	50				
Mixed grasses				5,773	
Fescue		1,535			
Oats		28			
Orchardgrass		271			
Rye		70			
Ryegrass		385			
Wheat		128			
Johnsongrass					297
Sorghum					133
Sorghum-sudangrass					760
Total	7,566	2,417	819	5,773	1,190

[a]WSG, warm season grass; CSG, cool season grass; LEG, legume; MIX, mixed grass; and NIT, nitrate accumulating grasses.

Table 2. Summary of forage analysis results by forage type.

	Forage type[a]				
	WSG	**CSG**	**LEG**	**MIX**	**NIT**
CP, %	13.0 ± 3.7	11.6 ± 3.3	16.7 ± 4.9	11.5 ± 3.0	11.1 ± 3.6
ADF, %	33.0 ± 4.2	37.7 ± 5.0	33.7 ± 6.6	37.8 ± 4.6	38.1 ± 6.1
NDF, %	71.1 ± 5.2	65.6 ± 6.1	51.4 ± 12.0	66.6 ± 5.7	65.0 ± 5.5
Nitrate-N, ppm	798 ± 1538	996 ± 1383	569 ± 430	836 ± 1142	1313 ± 1538

[a]WSG, warm season grass; CSG, cool season grass; LEG, legume; MIX, mixed grass; and NIT, nitrate accumulating grasses.

Table 3. Correlations among the pairwise, complete hay analysis observations.

	DM	**CP**	**ADF**	**NDF**	**Nitrate-N**
DM	1.000	-0.079	-0.019	0.028	-0.090
CP		1.000	-0.541	-0.285	0.412
ADF			1.000	0.186	-0.151
NDF				1.000	-0.086
Nitrate-N					1.000

Table 4. Summary of the model parameters, parameter estimates, standard error and probabilities.

Parameter[a]	**Estimate**	**Standard error**	**Pr (> |z|)**
Intercept	-7.086	0.305	< 0.001
CP	0.349	0.018	< 0.001
LEG	-4.326	1.039	<0.001
MIX	-0.278	0.204	0.173
NIT	1.897	0.224	<0.001
WSG	-0.421	0.179	0.019

[a]WSG, warm season grass; CSG, cool season grass; LEG, legume; MIX, mixed grass; and NIT, nitrate accumulating grasses.

Table 5. Two-variable sensitivity table showing the 95% confidence limits for the predicted probability of observing nitrate-N at a dangerously high concentration.

	Forage type[a] 95% confidence limits[b]									
Forage CP	WSG		CSG		LEG[c]		MIX		NIT	
	lower (%)	upper (%)	lower (%)	upper (%)	lower (%)	upper (%)	lower (%)	upper (%)	lower (%)	upper (%)
10%	1.3	2.3	1.8	3.6	0.0	0.1	1.4	2.6	11.3	19.6
12%	2.7	4.3	3.6	6.9	0.0	0.2	3.0	5.1	21.0	32.9
14%	5.6	7.9	7.2	12.8	0.0	0.4	5.9	9.6	35.1	50.1
16%	11.1	14.5	13.6	22.9	0.0	0.9	11.3	17.7	52.0	67.6
18%	20.0	25.5	24.0	38.0	0.0	1.8	20.1	30.7	68.3	81.6
20%	32.9	41.5	38.6	56.3	0.0	3.5	33.1	48.2	80.9	90.6

[a]WSG, warm season grass; CSG, cool season grass; LEG, legume; MIX, mixed grass; and NIT, nitrate accumulating grasses.
[b]Lower and upper 95% confidence limits calculated as $\hat{p} \pm 1.96 \times (standard\ error\ of\ prediction)$.
[c]Negative lower confidence limits were suppressed to zero.

Effect of sodium hydroxide treatment of selected mature forages

E. Hatungimana[1], D. W. Kellogg[1], K. S. Anschutz[1], and A. H. Brown, Jr.[1]

Story in Brief

Low digestibility of roughage limits ruminant performance. The objective of this experiment was to evaluate in vitro degradability of selected mature forages including bermudagrass, crabgrass, tall fescue, eastern gamagrass, switchgrass, johnsongrass, and sweet sorghum when treated with sodium hydroxide. Samples of forages were harvested late in the growing season during 2008, and dry matter yield was calculated from field replicates. Samples were pre-treated with 0, 2, 4, and 6% sodium hydroxide for 24 h. Then, in vitro dry matter disappearance was determined in duplicate. The concentrations of crude protein, neutral detergent fiber, and acid detergent fiber varied among forages. Compared to no pretreatment, vitro dry matter disappearance was improved with sodium hydroxide. With 4% sodium hydroxide treatment means were 61.1% for sweet sorghum, 44.3% for crabgrass, 52.4% for bermudagrass, 46.8% for eastern gammagrass, 48.4% for johnsongrass, 47.0% for tall fescue and 45.5% for switchgrass. Treatment with sodium hydroxide was more effective in forages with greater neutral detergent fiber.

Introduction

Cellulose and hemicellulose constitute the major fibrous components of grass. In low-quality forages carbohydrates are associated with lignin and other compounds that make the fiber less available to microorganisms in the intestinal tract, decrease the rate and extent of digestibility and reduce animal performance (Darcy and Belyea, 1980). Even high-quality forages have greater concentrations of fiber at maturity compared to earlier in the growth phase. The fiber of mature plants is slowly and incompletely digested by microorganisms and limits energy intake by ruminant animals.

Switchgrass has been proposed as a cash crop for biomass that could be used to produce ethanol. Lignification of cellulosic biomass is also a problem for research on ethanol production from plant materials such as switchgrass because more dry matter (DM) would be available at maturity (Schroeder, 1996). Chemical treatment has been used to break bonds between lignin and hemicelluloses or cellulose, and sodium hydroxide (NaOH) has efficiently improved digestibility of fibrous roughages (Klopfenstein, 1979).

The objective of this experiment was to pre-treat with NaOH to determine the effects on in vitro digestibility of selected mature forages.

Materials and Methods

Selected mature forage samples of bermudagrass (*Cynodon dactylon* (L.) Pers.), crabgrass (*Digitaria ciliaris*), eastern gamagrass (*Trypsacum dactyloides* (L.) L.), johnsongrass (*Sorghum halepens* (L.) Pers.), sweet sorghum (*Sorghum bicolor* (L.) Moench), tall fescue (*Lolium arundinaceum* Schreb.), and switchgrass (*Panicum virgatum* L.) were harvested late in the 2008 growing season at the University of Arkansas Experiment Station in Fayetteville. Fields were divided into 4 quadrants and a ¼-m^2 frame was used to sample randomly or, when appropriate, plants in 1-meter of row was selected randomly. The height of plants was measured and the area was clipped to provide 3 replicates in each quadrant. Samples were weighed and dried under forced air at 50 °C to determine field DM composition. Samples were composited over field replications before laboratory analyses.

Pretreatment was conducted in 50-mL test tubes, in triplicate, using different concentrations of NaOH (0, 2, 4 and 6%) by adding 2 mL of distilled water or diluted NaOH to 0.25 g of each forage subsample. Treated material was held at room temperature for 24 h to allow reaction.

Ruminal fluid was collected from a rumen-fistulated heifer maintained on alfalfa hay and a corn-based supplement with ad libitum access to water. After dilution with the buffer-nutrient solution, 30 mL of ruminal fluid was added to each tube of the pretreated samples. Duplicate tubes were placed in a 39 °C water bath for different incubation times (0, 3, 6, 12, 24, 48 and 72 h). After each time of incubation, tubes were removed from the water bath and placed in a freezer. At a later time, materials were thawed and filtered with filter paper. The residue was retained on the filter paper and was sealed to be dried in an oven at 100 °C for 8 h after which samples were weighed to determine the extent of degradability (IVDMD).

Means and standard deviations were calculated for the field data and nutritive values of the forages. For the IVDMD experiment, a 7 × 4 × 7 factorial arrangement was used for 7 forages types, 4 NaOH concentrations, and 7 in vitro incubation times. Data were analyzed using GLM procedures of SAS version 9.1 (SAS Institute Inc., Cary, N.C.) with the 3-way interaction used as error term. Both 2-way interactions involving NaOH were significant ($P < 0.01$) suggesting re-running the analysis by NaOH concentrations. Then a 7 × 7 factorial was used for each NaOH concentration with the 2-way interaction of forage by time being the error term.

Results and Discussion

The results of plant height, DM content, forage yield and nutritive value (composition of crude protein (CP), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignan (ADL), and fat) are presented in Table 1. The height of plants ranged from 47.0 cm for bermudagrass to 343.5 cm for sweet sorghum. Forages were all harvested near the end of the growing season. Data for DM content of fall fescue was lost, so it was not possible to calculate DM yield. It would appear that the tall growing plants (sweet sorghum and switchgrass) provided the most DM yield and would be more attractive to harvest for biomass conversion into sugars. However, the composition of the plants may impact that decision.

Table 1 shows that CP content ranged from 4.9% in switchgrass to 13.5% in bermudagrass. Eastern gamagrass and switchgrass had the greatest NDF percentage, although all forages tested were above

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70% NDF except sweet sorghum. The plant composition of ADF ranged from 26.1% in sweet sorghum to 43.1% in switchgrass. Acid detergent lignin ranged from 2.3% in sweet sorghum to 5.5% in eastern gamagrass. In this experiment, sweet sorghum had the least percentage of NDF, ADF and ADL. Other forage species used in this experiment had a greater fiber content, and this predicts low digestibility of those forage species without pretreatment. Switchgrass had the greatest concentrations of NDF and ADF. The energy content of forages ranged between 4441 calories/g for switchgrass and 3779 calories/g for crabgrass.

Table 2 shows that the IVDMD of forages depended on the species ($P < 0.01$), different time of incubation ($P < 0.01$), and concentration of NaOH treatment used ($P < 0.01$). Significant interactions were observed between forages and NaOH treatment ($P < 0.01$) and between NaOH treatment and incubation time ($P < 0.01$). An interaction between forages and incubation time was not detected ($P > 0.10$). The IVDMD of all forages increased ($P < 0.01$) with longer incubation time.

There was an interaction between forage and concentration of NaOH treatment. Treatment of forages with 2 and 4% NaOH (Table 3) increased IVDMD for all forages, but treatment with 6% NaOH decreased IVDMD of some forages, probably due to the increased alkalinity (pH = 7.6 to 7.8) after samples were treated with 6% NaOH. Although the solubilization of hemicelluloses should have been greater with 6% NaOH treatment, the accumulation of fermentation end products should have also impaired microbial attachment in the first hours of incubation and reduced digestibility (Arisoy, 1998). Bermudagrass, switchgrass, tall fescue, and eastern gamagrass responded better to the 4% NaOH treatment than other forages (Table 3). Johnsongrass and sweet sorghum responded better to the 2% NaOH better than other forages. Crabgrass responded better to 6% NaOH treatment. Differences in response may have been due to the chemical composition of the forage species.

In conclusion, NaOH treatment of forages improved IVDMD of forages. Treatment with 4% NaOH offered improvement of IVDMD compared to other concentrations of NaOH used in this experiment for most of forages species. Forages with greater fiber content were improved most, in terms of percentage units of increased IVDMD over untreated controls. Despite the improved digestibility with NaOH treatment, the elevated pH was a problem, especially in samples treated with 6% NaOH. Further investigations are needed to use high concentrations of NaOH without compromising the pH environment of rumen microbes. Emphasis should be also placed on techniques of removing end products from fermentation to allow microbial attachment and enhance microbial activity.

Implications

Treatment of forages with sodium hydroxide is effective in increasing invitro digestibility and could be used to increase the digestibility or low quality forages; however, the high pH created by higher concentrations of sodium hydroxide may adversely affect the pH and environment of the rumen.

Acknowledgements

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Table 1. Average height, field dry matter (DM) percentage, DM yield, and nutrient composition of selected mature forages[1].

Item	Height, cm	Field DM, %	Yield, kg/ha	CP, %	Ash, %	NDF, %	ADF, %	ADL, %	Fat, %	Gross Energy, calories/g
Bermudagrass										
Mean	47.0	29.0	3909	13.5	9.3	74.9	34.0	3.4	2.2	4231
SD	6.0	2.0	1149	1.0	1.0	1.8	0.4	0.3		
Crabgrass										
Mean	95.0	33.6	7250	8.9	7.7	72.5	39.3	4.8	1.5	3779
SD	8.0	2.6	2400	0.7	0.7	1.1	0.7	0.4		
Tall fescue[2]										
Mean	122.7	-----	------	9.2	8.1	70.7	40.6	4.5	2.1	4103
SD	8.9	-----	------	2.9	1.9	2.2	1.5	0.6		
Eastern gamagrass										
Mean	164.9	36.2	3160	8.6	7.5	77.6	40.6	5.5	1.5	3928
SD	15.1	2.3	914	3.0	0.2	1.6	2.5	3.5		
Johnsongrass										
Mean	183.9	34.6	11264	8.1	7.1	72.9	41.9	4.0	1.6	4108
SD	43.3	0.35	1516	0.9	0.3	1.2	0.8	0.2		
Sweet sorghum										
Mean	343.5	31.6	21603	5.7	4.4	47.2	26.1	2.3	2.2	3972
SD	26.5	1.05	2496	1.0	0.1	3.8	1.4	0.4		
Switchgrass[3]										
Mean	-----	45.50	18180	4.9	3.8	77.2	43.1	4.6	1.9	4441

[1]CP = Crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, GE = gross energy.
[2]Data for DM of fescue was lost.
[3]Switchgrass samples were provided by Dr. Charles West (Crop, Soil and Environmental Sciences Department, Fayetteville), and SD values were not available.

Table 2. Effect of forage type, incubation time, NaOH treatment and their interactions on in vitro dry matter disappearance (IVDMD)[1].

	NaOH concentration					*P* -value					
	0%	2%	4%	6%	SE	Forage	Time	NaOH	NaOH × Time	Forage × NaOH	Forage × Time
Forages[2]											
BG	35.0	49.1	52.4	48.0	0.4	< 0.01	0.05	< 0.01	< 0.01	< 0.01	>0.1
EGG	28.7	40.9	46.8	47.3	0.4	< 0.01	0.2	< 0.01	< 0.01	< 0.01	>0.1
JG	34.9	46.1	48.4	39.3	0.3	< 0.01	0.002	< 0.01	< 0.06	< 0.01	>0.1
CB	34.5	49.4	44.3	43.8	0.3	< 0.01	<0.01	< 0.01	< 0.01	< 0.01	>0.1
TF	34.3	44.7	47.0	40.6	0.3	< 0.01	0.04	< 0.01	< 0.01	< 0.01	>0.1
SS	54.4	63.7	61.1	59.6	0.2	< 0.01	<0.01	< 0.01	< 0.01	< 0.01	>0.1
SG	30.1	42.4	45.5	44.2	0.1	< 0.01	0.08	< 0.01	0.002	< 0.01	>0.1

[1] Mean digestibility of forages at different times of incubation (0, 3, 6, 12, 24, 48, and 72 h) per NaOH concentrations (0, 2, 4, and 6%).
[2] BG = bermudagrass, EGG = eastern gamagrass, JG = johnsongrass, CB = crabgrass, TF = tall fescue, SS = sweet sorghum, SG = switchgrass.

Table 3. Effect of forage type and NaOH treatment on in vitro dry matter disappearance of forages[2].

	NaOH concentration			
	0%	**2%**	**4%**	**6%**
Forages[2]				
BG	35.0[b]	49.2[b]	52.4[b]	48.0[b]
EGG	28.7[c]	40.9[c]	46.8[cd]	47.3[bc]
JG	34.9[b]	46.1[bc]	48.4[c]	39.3[e]
CB	34.5[b]	49.4[b]	44.3[e]	43.8[cd]
TF	34.3[b]	44.7[c]	47.6[cd]	40.6[de]
SS	54.4[a]	63.7[a]	61.2[a]	59.6[a]
SG	30.1[c]	42.4[c]	45.5[de]	44.7[bcd]
SE	0.7	0.5	0.3	0.4

[a, b, c, d, e] Means in the same column with different superscripts differ ($P < 0.01$).

[1]Mean digestibility of forages at different times of incubation (0, 3, 6, 12, 24, 48, and 72 h) per NaOH concentrations.

[2]BG = bermudagrass, EGG = eastern gamagrass, JG = johnsongrass, CB = crabgrass, TF = tall fescue, SS = sweet sorghum, SG = switchgrass.

Effect of endophyte infected tall fescue seed extract on cytochrome P450 system

A. S. Moubarak[1], Z. B. Johnson[1] and C. F. Rosenkrans, Jr.[1]

Story in Brief

Endophyte infected tall fescue is the base diet for nearly all beef cattle in the southern U.S. It has been linked to a variety of toxicological conditions due to the presence of large numbers of ergot alkaloids. This study was designed to study the effects of endophyte infected tall fescue seed extract and selected ergot alkaloids on the detoxification pathway by cytochrome P450 enzyme system. Tests were performed using the P450-Glo cytochrome P450 enzyme activity kit (Promega, Wis.), according to the manufacturer's manual. Endophyte infected tall fescue seed was extracted with 50/50 methanol/ 25 mM ammonium carbonate, cleaned and concentrated on Strata-X reversed phase column (Phenomenex). The extracts were evaluated on an HPLC, and then tested using a serial dilution method. Commercially available ergonovine (EN) and ergocryptine (EC) were tested individually using 0 to 44 nM concentrations. Seed extract of endophyte infected tall fescue produced a significant ($P < 0.05$) dose dependent inhibition of cytochrome P450 enzyme activity similar to that produced by the commercially available ergot alkaloids. Ergocryptine and ergonovine inhibited cytochrome P450 enzyme activity in a dose dependent manner ($P < 0.05$) with ergocryptine being most potent, followed by ergonovine (70% and 10% at 44 nM concentration). The similarity of the inhibition curves of seed extract to that of the commercially available ergot alkaloids suggest a related mode of action and that the use of such ergot alkaloids and cytochrome P450 assay is a good model to study the toxicity of tall fescue. Furthermore, it provides the foundation to identify the individual toxic components of purified endophyte infected tall fescue extract.

Introduction

Tall fescue grass is considered to be the primary diet for nearly all beef cattle in southern United States and has been linked to the incidence of fescue toxicosis syndrome due to the presence of ergot alkaloids. Cytochrome P450 (CYP) enzyme systems play a key role in the biotransformation of many endogenous and exogenous compounds including both toxins and drugs (Porter and Coon, 1991, Pollock, 1994). The CYP enzyme family consists of a large number of proteins with different substrate specificities and catalytic properties which are membrane-bound, mostly localized to the endoplasmic reticulum and in mitochondrial inner membranes. Ergot alkaloids found in tall fescue are a large family of chemical compounds with two major subfamilies. The tetracyclic ergoline nucleus group, such as lysergic acid ethylamide, ergonovine, and lysergic acid, and the ergopeptide alkaloids group which share the tetracyclic ergoline nucleus in addition to tricyclic amino acids such as ergotamine, ergocryptine, and bromocriptine. The metabolism of ergot alkaloids, such as bromocriptine, dihydroergotamine, and other structurally similar ergot derivatives is mediated mainly by CYP3A4 (Ball et al., 1992). Moochhala et al. (1989) reported that bromocriptine interferes with P450-dependent oxidative metabolism of xenobiotics. Later it was demonstrated that cytochrome P450 3A exhibits a particularly high affinity for ergopeptides. Ergot alkaloids have been shown to be metabolized by CYP3A; however, information on the effects of this alkaloids on CYP3A activates is very limited. The metabolism of ergot alkaloids has been linked to CYP3A; therefore, activation or inhibition of the induction process of such enzyme systems can have severe consequences on the metabolism of the ergot alkaloids. Witkamp et al. (1995) reported that tiamulin, a semi-synthetic antibiotic frequently used in agricultural animals, strongly inhibited the hydroxylation rate of testosterone at the 6 beta position via the formation of a cytochrome P450 3A4 metabolic intermediate complex in both microsomes and hepatocytes. Although we have reported previously (Moubarak et al. 2003) the lack of an induction effects of dihydroergotamine and ergonovine on rat CYP3A, this study was designed to examine the effects of seed extract of endophyte infected tall fescue and selected ergot alkaloids on the detoxification pathway by cytochrome P450 (CYP3A4) enzyme system in vitro.

Materials and Methods

All the chemicals and reagents used in these experiments were of the highest quality available and were purchased from Sigma Chemical Co. (St. Louis, Mo.) unless stated otherwise. Tests were performed using the P450-Glo CYP3A4 enzyme activity kit (Promega, Wis.), according to the manufacturer's manual. Briefly, 12.5 µl of test compound (seed extract or pure pushed ergot alkaloids) was added per well of a 96-well plate, followed by 12.5 µl of the 4 times concentrated CYP450 reaction mixture with and without CYP450 for the blank reactions. Then the plate was pre-incubated at 37 °C for 10 minutes. The reaction was initiated by adding 25 µl of the 2 times concentrated NADPH regeneration system and after 30 minutes incubation at 37 °C, 50 µl of reconstituted luciferin detection reagent was added. The plate was mixed for 10 seconds on an orbital shaker and incubated at room temperature for 20 minutes to stabilize the luminescent signal. And then the luminescence was recorded using Perkin Elmer 1420 Victor 3 V.

Endophyte infected tall fescue seed (E+) was extracted with 50/50 methanol/25 mM ammonium carbonate (10 ml/gm) for one hour, cleaned and concentrated on Strata-X reversed phase column (Phenomenex). The extracts were evaluated by a Millennium-32 Workstation HPLC system with auto-sample injector and a gradient programmer. The detection was accomplished using a Applied Biosystems 980 programmable fluorescence detector (excitation at 250 nm and emission at 370 nm long pass filter). Separation was conducted on a 3 × 3 CR C18 cartridge column using acetonitrile and 2.6 mM ammonium carbonate in 10% methanol gradient elution at 1 ml/min flow rate. The extracts were evaluated using 80% serial dilution method, and the commercially available ergonovine (EN) and ergocryptine (EC) were tested individually using 0 to 44 nM concentrations.

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Results and Discussion

Toxins found in seed extract of endophyte infected tall fescue (Fig. 1) influenced CYP3A4 enzyme activity in a similar inhibition action as that of commercially available ergot alkaloids (EN and EC). Seed extract of E+ produced a ($P < 0.05$) dose dependent inhibition (59 and 81% inhibition at 11 and 100% of extract concentrations respectively) of CYP3A4 enzyme activity. Non-infected tall fescue seed extract (E-) produced no significant effect on CYP3A4 activity due to the lack of the toxins (Fig. 3). Ergocryptine and EN inhibited CYP3A4 enzyme activity in a significant ($P < 0.05$) dose dependent manner (0.0 to 44.0 nM) with EC being most potent (70%), and EN the least (10%) at 44 nM concentrations (Fig. 2). The similarity of the inhibition curves and the chemical structure of toxins found in seed extract to that of the commercially available ergot alkaloids EC and EN suggest that the toxins found in E+ appears to target the same location on the CYP3A4 and follow the same mechanism of inhibition.

Implications

The connection between the effects of fescue toxins and specific effects of the ergot alkaloids, ergocryptine and ergonovine, on CYP enzyme systems has been established. Such association helps in understanding of the effects of E+ in general and lays the foundation to study the detailed effects of each single component of fescue toxins on liver detoxification mechanisms. Also the use of P450-Glo CYP3A4 enzyme activity kit appeared to be a good instrument to study and identify the toxic individual components of purified endophyte infected tall fescue extract.

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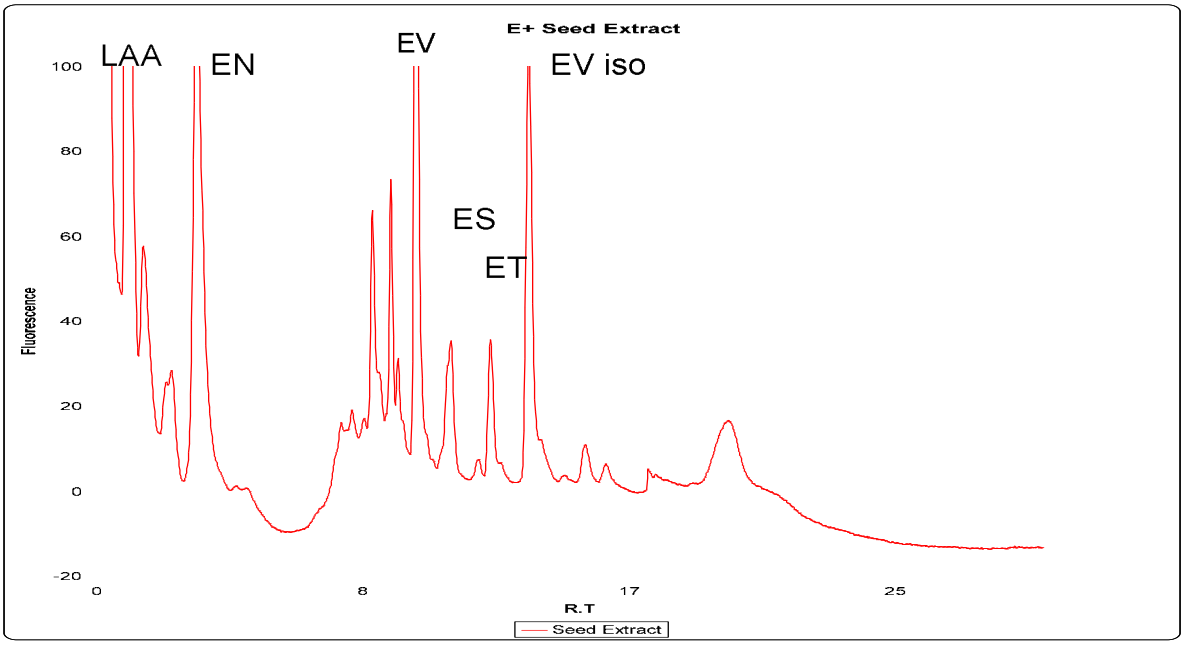

Fig. 1. High-performance (pressure) liquid chromatography chromatogram showing the typical profile of seed extract of endophyte infected tall fescue showing lysergic acid amide group (LAA), ergonovine (EN), ergovaline and the isomers (EV and EV iso), ergosine (ES) and ergotamine (ET).

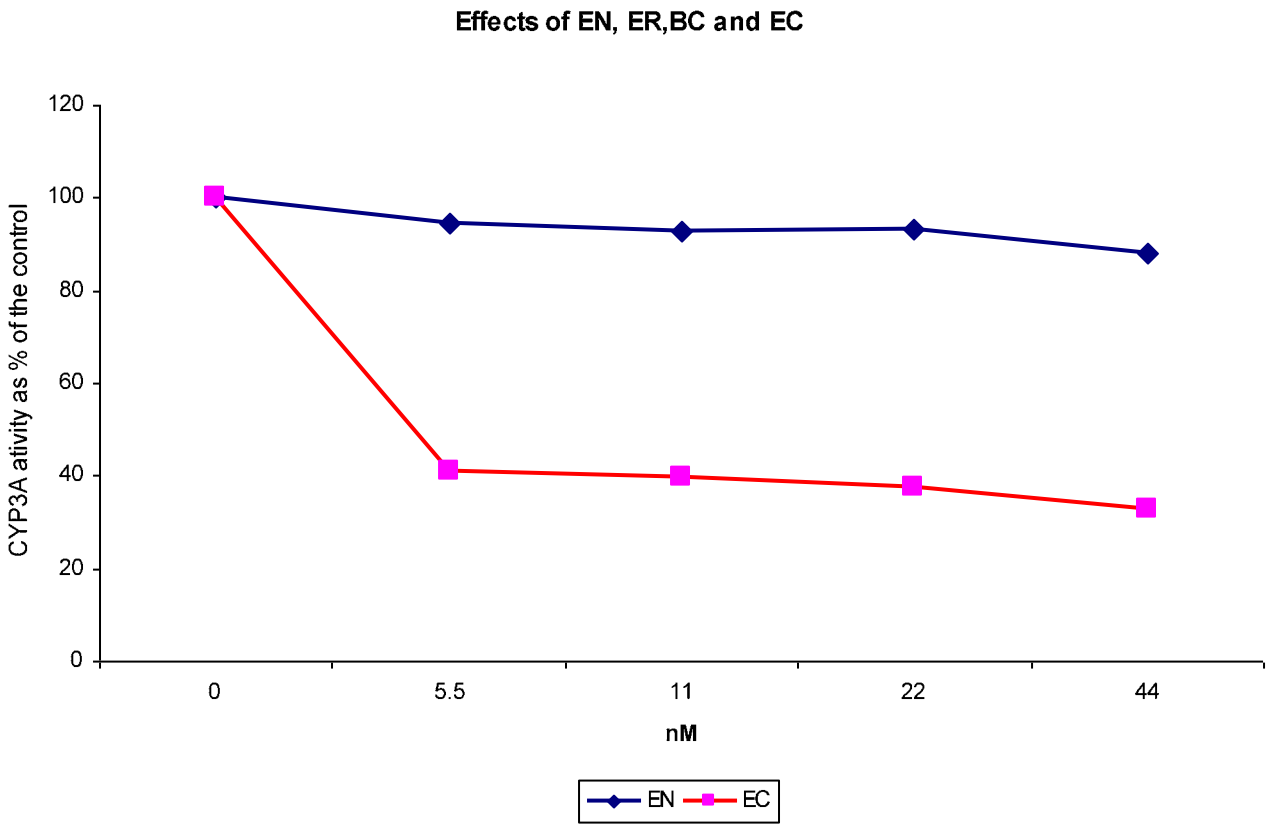

Fig. 2. Effects of increasing concentrations of ergonovine (EN) and ergocryptine (EC) on the in vitro activity of CYP3A4.

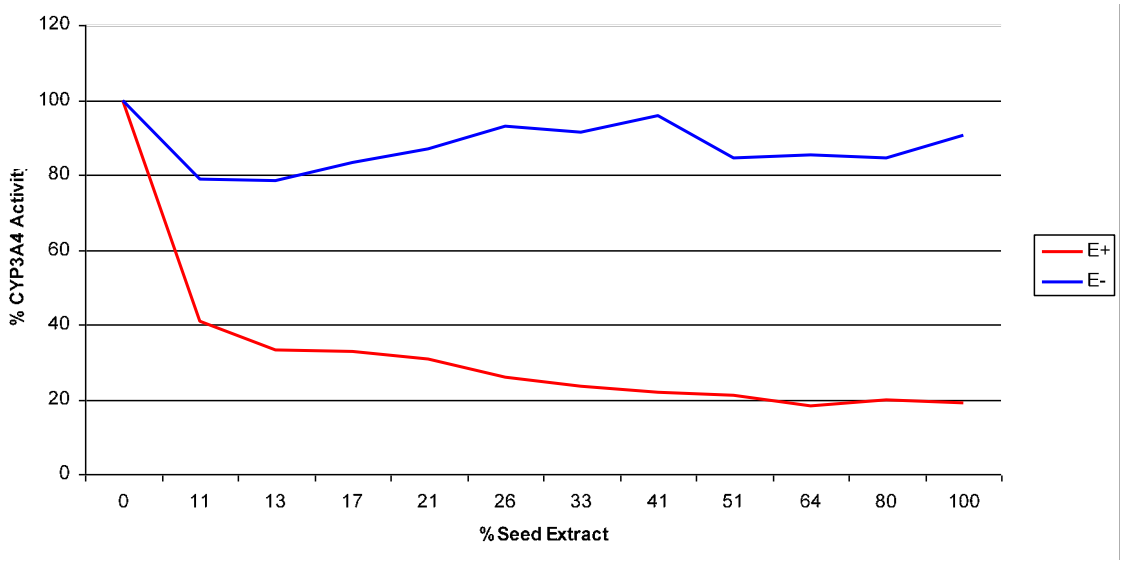

Fig. 3. Effects of increasing concentrations of seed extract of endophyte infected tall fescue on the in vitro activity of CYP3A4.

Nutritive value of pearl millet hay as affected by moisture concentration at time of baling

J. Kanani[1], D. Philipp[1], K.P. Coffey[1], A.N. Young[1], and R. Rhein[1]

Story in Brief

The objective of this study was to evaluate the effects of moisture at baling [15, 21, and 28% of dry matter (DM)] at baling on chemical composition and in situ disappearance of pearl millet in cannulated cows. Three acres of pearl millet were divided into three blocks and three subplots to assign moisture treatments of which a total of 27 round bales were obtained and stored for 71 d. Duplicate sample bags for in situ (10 × 20 cm) analysis were incubated in six ruminally cannulated cows for 0, 6, 12, 18, 24, 36, 48, 72, 96, and 120 h. Crude protein concentration was not affected ($P > 0.1$) by moisture or sampling depth, but increasing moisture tended ($P = 0.07$) to affect neutral detergent fiber, organic matter, and acid detergent fiber negatively. Increasing moisture increased ($P < 0.05$) neutral detergent insoluble nitrogen (40.8, 47.5, and 49.8% of total nitrogen), acid detergent insoluble nitrogen (7.0, 9.5, 22.8% of total nitrogen), and acid detergent lignin (3.0, 2.9, and 6.0% DM); however, hemicellulose decreased ($P < 0.05$) with increasing moisture (31, 31, and 25% DM). Increasing bale moisture also reduced the rate of dry matter disappearance (0.047, 0.043, 0.036/h; $P = 0.03$).

Introduction

Pearl millet [*Pennisetum americanum* (L.) Leeke] is an annual warm-season (C_4) grass that possesses a high yield potential especially during warm summer months when other traditionally used forage grasses become increasingly heat-stressed. In Arkansas, pearl millet could be an alternative feed resource to common bermudagrass which currently constitutes the backbone of the warm-season perennial grasses used for grazing and haying. However, pearl millet hay production is complicated by curing due to relatively thick stems which retain moisture longer than leaves. Therefore, baling pearl millet at higher than optimum moisture concentrations may be unavoidable due to prolonged drying times and frequent precipitation events during spring and summer in Northwest Arkansas. High moisture provides an environment which may promote the growth of unfavorable microorganisms in hay during storage (Turner et al., 2003). Presently, only limited data are available either on pearl millet hay production in Arkansas or on heat-damaged pearl millet hay and its effects on digestibility in cattle. Therefore, the objective of this research was to evaluate nutritive value and in situ digestibility of hay in cattle baled at different moisture concentrations.

Materials and Methods

Pearl millet was planted on July 6, 2008, at approximately 30lbs/acre with a 7-foot no-till drill (Tye Pasture Pleaser; AGCO Co., Duluth, Ga.) and 12-inch row spacing. On September 20, 2008 pearl millet was cut with a New Holland 1411 disc mower–conditioner (New Holland Agriculture, New Holland, Pa.). Forage was fluffed with a tedder device on September 20 to facilitate drying. The field was then sub-divided into three blocks and again into three plots (experimental units) to assigned moisture treatments. Cut forage was monitored for moisture concentrations by repeatedly placing grab samples into a microwave oven to determine dry matter (DM). Baling started whenever forage reached moisture concentrations of 28% (T3), 21% (T2), and 15% (T1), using a commercial hay round baler (Vermeer 604 XL, Pella, Iowa). Bale dimensions were approximately 47 inches wide with a diameter of 59 inches. From each treatment and replication, three bales were obtained (total of 27), labeled, weighed, and sampled to a depth of 18 inches with a Uni-forage core sampler (Star Quality Samplers, Edmonton, Alberta) to determine pre-storage moisture concentration. Bale openings were closed with plastic foil to minimize air intrusion during storage. The bales were then stacked individually on wooden pallets to prevent soil moisture intrusion.

Chemical Analysis of Forages. At the end of the storage period of 71 d in the open, bales were sampled at three locations within the bale. The flat sides of each bale were evenly marked into a center core, middle area, and outer layer (L1, L2, and L3, respectively). Sampling was performed to a depth of 18 inches from the outside using a core sampler described above. Two samples from each location were combined to represent one sample within the same treatment and block. Samples were transferred to the laboratory, freeze-dried, and ground to pass a 2-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, N.J.).

Forage samples for chemical analysis were re-ground to pass a 1-mm screen using the Wiley mill described above and analyzed for dry matter (DM), total ash (TA), organic matter (OM), crude protein (CP) or total N, neutral detergent insoluble nitrogen (NDIN), and acid detergent insoluble nitrogen (ADIN) according to the methods described by AOAC (2001) and were reported on the basis of total N. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were sequentially analyzed (ANKOM Technology, Fairport, N.Y.). Cellulose and hemicelluloses were estimated from the values obtained in sequential analysis of NDF, ADF, and ADL.

In Situ Experiment. Six ruminally cannulated crossbreed cows (BW = 1290 ± 83 lbs) were used to determine in situ disappearance characteristics. Animals were housed in individual pens (8 × 14 feet) with concrete floors and were allowed an adaptation period of 12 days prior to data collection. Pens were cleaned regularly, animals had ad libitum access to fresh water and were fed a total mixed ration of 2% of BW of bermudagrass and concentrate in equal feeding at 0830 and 1630 h. The adaptation diet of bermudagrass was necessary due to high nitrate concentrations in the pearl millet hay (mean of 1,722 ppm for five samples taken randomly).

For preparation of the in situ trial, bags (53 ± 10 μm pore size (Ankom Technology Corp., Fairport, N.Y.) were filled with 5 g of forage sample and closed with rubber bands. Duplicate bags were prepared for each sample combination period. In total, there were

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27 samples duplicated with 10 incubation periods (27 × 2 × 10 = 540 samples). Forage samples were ruminally incubated for 0, 6, 12, 18, 24, 36, 48, 72, 96, or 120 h.

Results and Discussion

Results for moisture effects on chemical composition are summarized in Table 1. Crude protein was not affected ($P = 0.91$) by moisture treatments, but other variables tended ($P < 0.1$) to respond to the different moisture levels at baling, including OM, NDF, ADF, and ash. Neural detergent insoluble nitrogen, ADIN, ADL, and hemicellulose were affected ($P < 0.05$) by moisture treatments. Elevated levels of NDIN and ADIN indicate heat damage resulting from higher moisture levels than the recommended 18% at the time of baling. Spontaneous heating occurs above a certain sustained temperature in bales with high moisture which supports microbial growth and enzymatic reactions and prolonged respiration that produces heat. With respect to producing pearl millet hay, it appears that even at recommended moisture levels of 18%, pockets of higher moisture concentrations may remain compared with forages that are easier to cure, such as bermudagrass.

With respect to DM disappearance, our data did not suggest any clear effect of moisture level on DM disappearance characteristics, although fraction C and K_d were affected ($P < 0.05$). Therefore, differences in hay nutritive value and hay digestibility of pearl millet baled between 15 and 28% moisture may not be as large as expected. Although ADIN was markedly higher under 28% moisture, effects of higher moisture on other parameters have been inconsistent. This may indicate that pearl millet hay baled at 15% may still contain pockets of moisture comparable to those that occur with higher moisture treatments.

Implications

This study indicated that pearl millet baled at moisture levels between 15 and 28% did not result in substantial differences regarding nutritive value or in situ digestibility; however, these results should be treated interpreted with caution because it is possible that pockets of high moisture concentration remained even in hay baled at the 15% moisture concentration. Care should be taken during the hay curing process to ensure all plant parts are equally dry for consistent hay quality.

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Table 1. Effects of moisture levels at baling on chemical composition (%) in pearl millet hay.

	Treatment[1]				
Item[2]	15	21	28	SEM[3]	P-value[4]
OM	85.0	84.6	82.3	0.74	0.07
NDF	63.6	63.8	60.2	0.89	0.07
ADF	32.7	33.2	35.2	0.59	0.08
HEM	30.4[a]	30.4[a]	25.0[b]	0.82	0.02
ASH	14.9	15.5	17.7	0.74	0.07
CP	11.6	11.6	12.0	0.66	0.91
NDIN	40.8[b]	47.5[a]	49.8[a]	1.90	0.01
ADIN	7.0[b]	9.5[b]	23.0[a]	0.1	0.01
ADL	3.0[b]	2.9[b]	6.1[a]	0.53	0.02

[1]Moisture at baling (15, 21, 28%).
[2]DM, dry matter; OM, organic matter; NDF, neutral detergent fiber;ADF, acid detergent fiber; HEM, hemicellulose; CP; crude protein; NDIN, neutral detergent insoluble nitrogen; ADIN, acid detergent insoluble nitrogen; ADL, acid detergent lignin.
[3]SEM, standard error of the means.
[4]Differences considered significant at $P < 0.05$.
Means within a row with different letter superscripts denote differences between these means.

Table 2. Effects of moisture levels at baling of pearl millet hay on situ disappearance in cows.

	Treatment[1]				
Item[2]	**15**	**21**	**28**	**SEM[3]**	**P-value**
A, g/kg	253.1	264.9	259.9	6.79	0.42
B, g/kg	537.3	527.3	552.1	7.64	0.12
C, g/kg	211.8	212.2	187.8	6.04	0.05
K_d/h	0.047	0.043	0.036	0.002	0.03
Lag time, h	2.64	2.51	2.34	0.761	0.88
ED, g/g	566.4	561.5	545.4	5.73	0.12

[1]Moisture at baling (15, 21, 28%).
[2]A, immediately degradable fraction; B, degradable fraction at measurable rate; C, indigestible fraction; K_d, rate of disappearance; Lag, discrete lag time; ED, Effective disappearance.
[3]SEM, standard error of the means.

Influence of dietary L-arginine supplementation to sows during late gestation on sow and litter performance during lactation

B. E. Bass[1], C. L. Bradley[1], Z. B. Johnson[1], R. D. Boyd[2], J. L. Usry[3], C. V. Maxwell[1], and J. W. Frank[1]

Story in Brief

Ninety-nine gilts and sows were used in a study to evaluate the effects of feeding an additional 1% L-arginine during the final 3 wk of gestation on sow and litter performance. From d 93 of gestation to farrowing, sows were provided 6.0 lb feed/d of either a control diet with no additional L-arginine, or a diet providing an additional 25 g/d arginine in two equal meals. On d 110, animals were weighed and moved into farrowing. A common lactation diet was provided upon farrowing. Sows were additionally weighed 48 h post-farrowing and at weaning. Late gestational body weight gain (d 93 to 110), farrowing (d 110 to 48 h post-farrowing) and lactation body weight loss (d 110 to weaning) were calculated. Sow and litter performance data, including number born alive, number weaned, individual birth and weaning weights, and total placenta weight were recorded. Plasma samples were collected on d 93 and 110 and IGF-1 (insulin-like growth factor 1), insulin, and blood urea nitrogen concentrations analyzed. There was a tendency for a greater late gestation body weight gain ($P = 0.06$) in sows receiving an additional 25 g/d arginine compared to the control diet (31.7 and 26.5 lbs respectively). Additionally, a tendency for a parity by treatment interaction was observed for late gestation body weight gain with parity 1 sows receiving an additional 25 g/d arginine gaining the most, and parity 0 sows receiving an additional 25 g/d arginine intermediate to all other treatments ($P = 0.1$). There were no differences between treatment groups observed in farrowing or lactation body weight loss, number born alive, number weaned, birth weight, weaning weight, or placenta weight ($P > 0.05$). Additionally, there were no differences between controls versus gilts and sows receiving an additional 25 g/d arginine plasma IGF-1, insulin, or blood urea nitrogen concentration at d 93 or 110 of gestation ($P > 0.05$). In conclusion, late gestational supplementation with L-arginine may be beneficial to sow body weight gain, independent of litter and placental weight, but had no affect on litter size or lactation performance.

Introduction

Through genetic selection the swine industry has improved not only average litter size, but also fetal growth rate potential in modern sows compared to past breeds. One consequence of this improvement is an apparent negative impact on uterine and umbilical blood flow per fetus, which could lead to disproportionate delivery of nutrients to the developing fetuses. One potential solution to these issues is the use of supplemental dietary L-arginine (L-Arg) in gestation diets of gilts and sows.

Arginine is important in metabolism as a substrate for protein synthesis and is a precursor of several important metabolites which are involved in embryogenesis and may enhance blood flow. Interestingly, arginine has been found in high concentrations in amniotic and allantoic fluids in early gestation.

In several previous studies, gestation diets were supplemented with L-Arg with varying outcomes dependent on the time during gestation supplementation occurred. Li and associates (2010) observed a reduction in uterine weight, total number of fetuses, number of corpora leutea, total fetal weight, and amniotic and amniotic fluid volume at d 25 of gestation when sows were provided an additional 0.8% L-Arg immediately following breeding to d 25 of gestation.

In contrast, gilts fed a gestation diet supplemented with 1.0% L-Arg from d 30 of gestation until parturition had more piglets born alive, an increased litter birth weight of piglets born alive, and decreased number of piglets born dead when compared to controls (Mateo et al., 2007). In another study, piglets of sows fed a lactation diet supplemented with an additional 1.0% L-Arg had increased body weight gain from d 0 to 7, and d 0 to 21, and were heavier at d 7, 14, and 21 of lactation compared to piglets of control fed sows (Mateo et al., 2008). Finally, supplementation of gestation diets from d 14 to 28 of gestation with an additional 25 g/d L-Arg resulted in an increase of approximately one pig per litter (Ramaekers et al., 2006) in one study, and an increase in total number, and weight of fetuses at d 75 of gestation (Berard and Bee, 2010) in another. Supplementation of L-Arg in early gestation appears to have a beneficial effect on litter performance during lactation, but it is unclear if there is an optimal time and duration of arginine supplementation during gestation. The objective of this study was to determine the effects of late gestation supplementation of 1.0% L-Arg to gestation diets on sow and litter performance during lactation.

Materials and Methods

Animals. Ninety-nine gilts and sows were allotted to one of two dietary treatments based on parity (0, 1, or 2+), as well as body weight at the initiation of the study on d 93 of gestation. Dietary treatments were a gestation control diet with no additional arginine, or the control diet with an additional 1% arginine (Table 1). In order to keep the diets isonitrogenous, alanine was added to the control diet. Alanine was chosen because it is nontoxic, is not a substrate for arginine synthesis and is catabolized by pigs. Gilts and sows were housed in individual gestation stalls throughout gestation and provided 5.0 lb/d of their respective diet and free access to water. From d 93 to d 110 of gestation, gilts and sows were provided 6.0 lb of their respective diets in two equal meals. On d 110 of gestation, gilts and sows were individually weighed and moved to the farrowing facility where they were housed in individual farrowing crates. Upon farrowing, a common lactation diet was fed. Individual body weight

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was recorded for gilts and sows at d 93 and 110 of gestation, 48 h post farrowing, and at weaning. At farrowing, and again at weaning, the total number of live piglets was counted and individual body weight was recorded. Additionally the number and total weight of stillborns and mummies were recorded. Total placenta weight was also recorded. Individual feed intake of each sow was recorded during lactation. Finally blood samples were obtained via jugular venapuncture on d 93 and d 110 of gestation and plasma concentrations of insulin-like growth factor-1, insulin, and urea nitrogen were determined.

Statistical Analysis. Data were analyzed using the PROC MIXED procedures of SAS with treatment as the fixed effect and parity as a covariate.

Results and Discussion

There were no differences in body weight loss following farrowing, or from farrowing to weaning between gilts and sows fed a control diet, or a diet supplemented with 1% L-Arginine (Table 2). Additionally, there were no differences in number born alive, number weaned, individual birth weight and weaning weight, number of stillborns and mummies, or total stillborn, mummy, or placenta weight (Table 2).

However, there was a tendency (P = 0.06) for increased late gestation body weight gain between d 93 and d 110 in gilts and sows fed the diet supplemented with arginine compared to those fed the control diet with arginine supplemented gilts and sows gaining approximately 4.4 lb more than controls (Fig. 1). There was also a tendency (P = 0.10) for a gestational treatment by parity interaction. We observed a greater increase in late gestation body weight gain in parity 0 and parity 1 gilts and sows fed the diet supplemented with 1% arginine from d 93 to d 110 of gestation than those of the control group (Fig. 2). Interestingly, there was no difference in late gestation body weight gain between control and arginine supplemented parity 2+ sows. The improvement observed may be due to an increased arginine requirement, which was at least partially met with supplementation, in parity 0 and 1 gilts and sows that are still growing.

Plasma concentrations of IGF-1, insulin, and urea nitrogen were similar between gilts and sows fed control or arginine supplemented late gestation diets on d 93 and 110 of gestation (Table 3).

In conclusion, supplementation of 1% dietary arginine to a Corn-soybean meal-distillers grains gestation diet for the final 17 d of gestation increased body weight gain in gilts and parity 1 sows. However, this increase in weight was independent of litter weight and had no affect on litter size at farrowing or subsequent lactation performance.

Implications

Supplementation of gestation diets with 1% arginine from day 93 to 110 of gestation may be beneficial, as improved body weight gain was observed during that period in parity 0 and 1 gilts and sows.

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Table 1. Composition of gestation and lactation diets (as-fed).

Ingredients, %	Control	+ Arginine	Lactation
Corn	52.65	52.65	62.54
SBM, 48%	9.00	9.00	21.25
DDGS	30.00	30.00	8.00
Fat (Darling, Yellow Grease)	1.00	1.00	2.75
Dicalcium phosphate	2.10	2.10	2.70
Limestone	1.15	1.15	0.70
Salt	0.45	0.45	0.40
L-lysine	0.15	0.15	0.20
L-threonine			0.03
K-Mg sulfate	0.65	0.65	0.50
Sow Add Pack[1]	0.25	0.25	0.25
Vitamin Premix[2]	0.25	0.25	0.25
Mineral Premix[3]	0.15	0.15	0.15
Ethoxiquin	0.03	0.03	0.03
Corn starch	0.00	1.05	0.00
L-alanine	2.05	0.00	0.00
L-arginine	0.00	1.00	0.00
Tylosin-40	0.12	0.12	0.00
BMD-50[4]	0.00	0.00	0.25
Total	100.0	100.0	100.0
Calculated composition			
NRC ME (Mcal/lb)	1.500	1.497	1.527
CP, %	18.7	18.7	17.6
Total Lysine, %	0.782	0.782	1.03
TID Lysine, %[5]	0.628	0.628	0.904
TID M+C:Lys, %	82.9	82.9	58.5
TID Thr:Lys, %	74.4	74.4	63.3
TID Trp:Lys, %	19.2	19.2	18.5
TID Ile:Lys, %	81.3	81.3	68.2
TID Val:Lys, %	101.2	101.2	78.6
TID Arg:Lys, %	119.5	276.4	108.0
Total P, %	0.829	0.829	0.883
Available P, %	0.582	0.582	0.580
Ca, %	0.987	0.987	0.978
Na, %	0.238	0.238	0.192

[1]The sow add pack provided the following per kg of complete diet: 22.05 IU of vitamin E, 551.15 mg of choline, 1.65 mg of folic acid, 4.96 mg of vitamin B_6, 0.22 mg of biotin, and 0.20 mg of chromium.

[2]The vitamin premix provided the following per kg of complete diet: 397.5 mg of Ca, 11,022.9 IU of vitamin A, 1,377.9 IU of vitamin D_3, 44.09 IU of vitamin E, 0.0386 mg vitamin B_{12}, 4.41 mg of menadione, 8.27 mg of riboflavin, 27.56 mg of D-pantothenic acid, and 49.6 mg of niacin.

[3]The mineral premix provided the following per kg of complete diet: 84 mg of Ca, 165 mg of Fe, 165 mg of Zn, 39.6 mg of Mn, 16.5 mg of Cu, 0.3 mg of I, and 0.3 mg of Se.

[4]Provided 250 g/ton bacitracin methylene disalicylate.

[5]TID = total ileal digestible.

Table 2. Sow and litter performance.

Trait	Control	Arginine
Farrowing BW loss, lb	-46.78 ± 4.21	-49.31 ± 4.14
Lactation BW loss, lb	-47.07 ± 4.01	-41.42 ± 4.10
Number born alive	13.59 ± 0.37	13.92 ± 0.37
Number weaned	10.65 ± 0.28	10.71 ± 0.28
Average piglet BW at birth, lb	3.11 ± 0.05	3.14 ± 0.05
Average piglet BW at weaning, lb	12.78 ± 0.28	12.99 ± 0.28
Number of stillborns	1.11 ± 0.22	1.05 ± 0.22
Number of mummies	0.52 ± 0.13	0.60 ± 0.13
Total BW of stillborns, lb	2.96 ± 0.62	2.85 ± 0.62
Total BW of mummies, lb	0.43 ± 0.17	0.47 ± 0.17
Placenta weight, lb	7.79 ± 0.36	8.15 ± 0.36

*$P > 0.05$.

Table 3. Concentration of plasma IGF-1, insulin, and urea nitrogen.

	Control	Arginine
IGF-1[1]	46.47 + 1.74	45.81 + 1.73
Insulin[2]	16.22 + 1.06	15.26 + 1.05
Urea nitrogen[3]	4.73 + 0.13	4.75 + 0.13

*$P > 0.05$.
[1]IGF-1 = Insulin-like growth factor 1, ng/ml.
[2]Insulin, µU/ml.
[3]Plasma urea nitrogen, mg/dl.

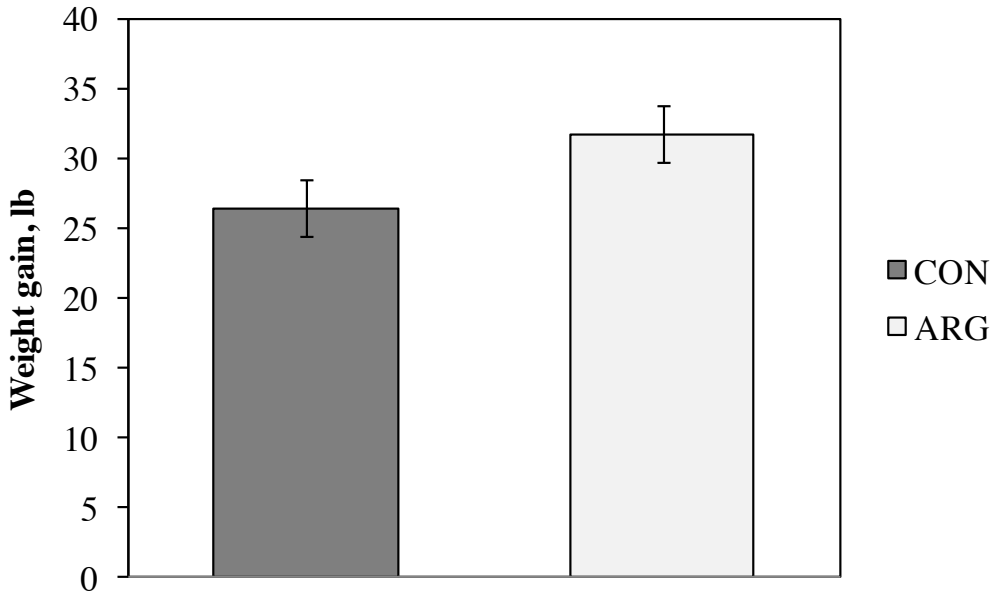

Fig. 1. Late gestation (d 93 to d 110) body weight gain ($P = 0.06$). CON = control, ARG = 1.0 % L-arginine.

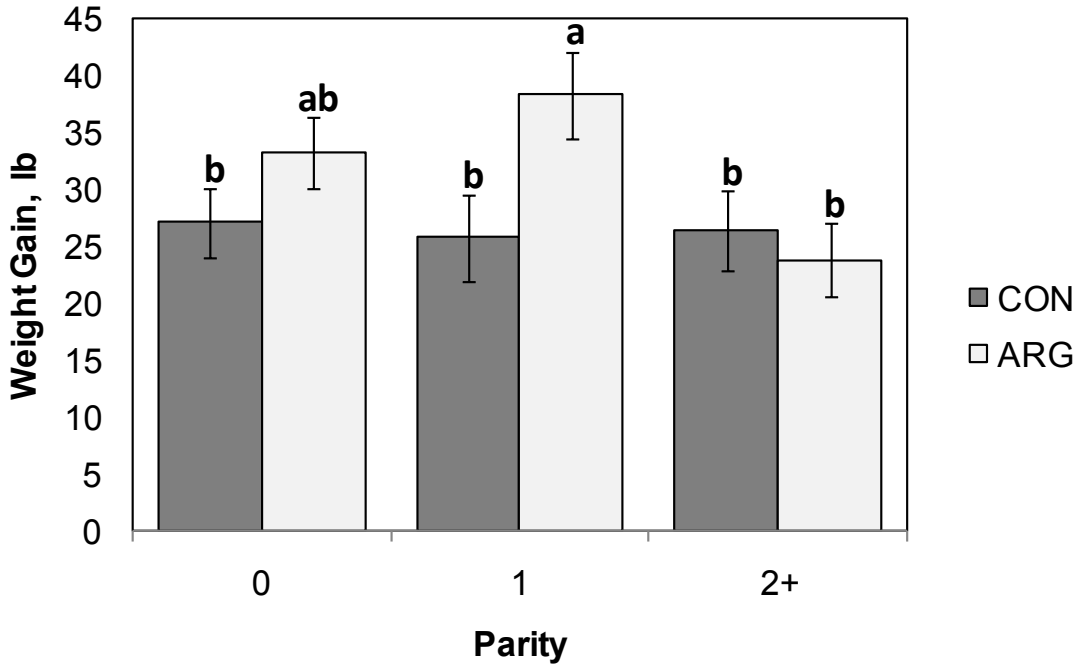

Fig. 2. Gestation treatment x sow parity interaction (*P* = 0.1) for late gestation body weight gain. [ab]Bars with different letters differ (*P* < 0.05). CON = control, ARG = 1.0 % L-arginine.

Relationships among lactate dehydrogenase activities, isoenzyme patterns, and hematological values in resting Thoroughbred and miniature horses

C. Bokina[1], A. R. Starnes[1], C. F. Rosenkrans, Jr.[1]

Story in Brief

Our objective was to determine the relationships among lactate dehydrogenase activities, isoenzyme patterns, and hematological values in Thoroughbred and Miniature horses. Samples were collected from Thoroughbred horses (n = 25) and Miniature horses (n = 34) at 0700-0800. Five lactate dehydrogenase (1-5) bands were evaluated utilizing digital densitometer to quantify isoenzymes. Total lactate dehydrogenase activity was determined using a modified colormetric kinetic assay. The pattern of lactate dehydrogenase isoenzyme distribution in the blood plasma was: 22% lactate dehydrogenase-1, 25% lactate dehydrogenase-2, 40% lactate dehydrogenase-3, 13% lactate dehydrogenase-4, and 1% lactate dehydrogenase-5. Hematological values were determined using Cell-Dyne 3500 hematological analyzer. Age was negatively correlated ($r \leq -0.34$; $p < 0.01$) with lactate and lactate dehydrogenase values. Lactate and lactate dehydrogenase activities were positively correlated ($r \geq 0.24$; $P \leq 0.10$) with white blood cells. Band lactate dehydrogenase-3 had a positive correlation with platelets ($r = 0.23$; $P = 0.08$) and white blood cells ($r = 0.37$; $P = 0.004$); whereas, it had a negative correlation to red blood cells ($r = -0.33$; $P = 0.01$), hemoglobin ($r = -0.51$; $P = 0.0001$), and hematocrit ($r = -0.53$; $P = 0.0001$). Age was related ($P < 0.05$) to monocytes, eosinophils, red blood cells (red blood cells), and weight, neutrophils, and lymphocytes. Breed was related ($P < 0.01$) to all lactate dehydrogenase activities, lactate, hemoglobin, hematocrit, height, and weight. Interactive effects of breed and gender affected ($P < 0.05$) red blood cells, hemoglobin, height, hematocrit, platelets, and weight. Additional research is warranted to determine the physiological and genetic mechanism related to lactate dehydrogenase effects on growth and immune function of horses.

Introduction

For over thirty years, horse owners have been interested in horse fitness and health status to meet the expectations of equine breeders and performance trainers. Most horse owners have relied on sound genetics and medicinal interventions to maintain animal health and performance. Ultimately an understanding of the physiological mechanisms and coordination of their body systems is necessary. Biochemical and physiological applications used to assess human fitness may be useful for performance assessment of physical conditioning in horses.

Lactate dehydrogenase (LDH) is one name for several proteins with the same enzymatic activity. There are five isoenzymes of LDH, which can be separated by electrophoresis. The five isoenzymes are tetramer proteins that consist of two 35-kDa subunits: A and B (Jungmann et al., 1998). Type A (or M) is primarily found in skeletal muscle and liver; whereas, type B (or H) is primarily located in the heart. Various combinations of these two subunits produce five possible isoenzymes in somatic cell types; LDH-1 (B4), LDH-2 (AB3), LDH-3 (A2B2), LDH-4 (A3B), and LDH-5 (A4) (Jungman et al., 1998; Goldberg, 1963). Our objective was to determine the relationships among LDH activities, isoenzyme patterns, and hematological values in Thoroughbred and Miniature horses.

Materials and Methods

Thoroughbred (n = 25) and Miniature (n = 34) horses from farms around Northwest Arkansas were utilized for this study per owner approval, and in agreement with the University of Arkansas Animal Care and Use Committee (protocol #04020). Miniature horses on visited farms had free access to pasture, whereas the Thoroughbred horses had access to pasture in early morning to late evening and were stalled for the remainder of evening. Upon arrival at each destination (0700 to 0800) each animal's weight, height, age, temperature, heart rate, and respiration rate were recorded. Blood (~20 mL) samples were collected via the jugular vein using 18 gauge needles and Vacutainer™ containing EDTA (BD, Franklin Lakes, N.J.). Immediately after collection, blood samples were placed on ice for approximately 1.5 h until centrifuged at 1,200 × g for 30 minutes. Following centrifugation, plasma was decanted and stored at -20 °C until assayed.

The Accutrend™ Lactate Analyzer (Roche Diagnostics, Alameda, Calif.) was used to determine lactate concentrations (mmol/L) in whole blood. Plasma lactate dehydrogenase activity (IU/mL), forward and reverse activity (LDHf and LDHr, respectively), was corrected for plasma protein (mg/ml). Both LDH activities and plasma proteins were determined using modified colormetric kinetic assay on a Spectra Max 250 (CV within 10%; Molecular Devices, Sunnyvale, Calif.). Forward reaction of LDH was determined using reagents β-nicotinamide adenine dinucleotide (0.0194 mmol/L; Sigma, St. Louis, Mo.) and pyruvic acid (16.2 mmol/L; Sigma). Reverse reaction of LDH was determined using reagents NAD^+, Free Acid (7mmol/L; Calbiochem, San Diego, CA) and L-lactic acid lithium salt (50 mmol/L; Tokyo Kasei Kogyo Co., Tokyo, Japan). Samples were analyzed at 340 nm three times, 30 seconds apart. Total protein concentrations were determined using Pierce commercial BCA Protein Assay Kit (#23225). Blood differential counts were determined using a Cell-Dyne 3500 hematology analyzer (Abbott Laboratories, Abbott Park, Ill.).

Isoenzymes of LDH from plasma samples were separated using a mini-format polyacrylamide gel electrophoresis (PAGE). Briefly, plasma protein (15 µg) was mixed with BioRad buffer (15 µl) and 10 µl of the mixed solution loaded into each well of the gel. Electrophoresis was conducted in an ice bath at a constant voltage of 110 volts and a total running time of about 1.5 h. Running gels consisted of acrylamide/bis acrylamide (7.5%/2.6%) covered by a stacking gel of 5% acrylamide/2.6% bis acrylamide. Running buffer (pH 8.9) consisted of 0.5 M Tris HCl, and glycine (0.383 M). All gels were stained at 37 °C for approximately 1 hr. Staining solution

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consisting of lithium lactate (6.1 mM), NAD (1.3 mM), phenazine methosulphate (0.09 mM), nitro blue tetrazolium (0.4 mM), and tris HCl (50 mM) was prepared approximately 30 min before staining. Following staining, five proteins were identified to have LDH activity and their relative amount was digitized using Kodak ID Image Analysis Software (Eastman Kodak Company, Rochester, N.Y.).

Data were analyzed using procedures of SAS (SAS inst. Inc., Cary, N.C.). Pearson correlation coefficients were determined among vital signs, blood values, and isoenzymes. Age was used as a covariant for ANOVA which contained main effects of breed, gender, and interaction of breed and gender. Dependent variables were: vital signs, blood profiles, LDH activity, and lactate.

Results and Discussion

Table 1 presents the distribution of gender by breed, and pregnancy status for Thoroughbred and Miniature horses. Table 2 presents the mean, standard deviation, minimum and maximum for each trait. Pearson correlation coefficients between age, heart rate, respiratory rate, and blood characteristics are presented in Table 3. Age was negatively correlated ($P < 0.01$) with lactate and all LDH values ($r \leq -0.34$; $P < 0.01$). All LDH activities and lactate were positively correlated ($r \geq 0.24$; $P \leq 0.10$) with WBC. Hemoglobin and hematocrit were negatively correlated ($r \leq -0.27$; $P < 0.05$) with LDH values. Heart rate had a positive correlation ($r = 0.23$; $P \leq 0.10$) with hemoglobin and hematocrit. Lymphocytes had a positive correlation with LDHr, LDHrp, and lactate ($r \geq 0.22$; $P \leq 0.10$). Neutrophils had a negative correlation with lactate ($r = -0.22$; $P \leq 0.10$). Monocytes had a negative correlation with LDHf, LDHr, and LDHrp ($r = -0.34$; $P < 0.01$), and lactate ($r = -0.27$; $P < 0.05$). Plasma LDH activity had a negative correlation with basophils, but positive correlation with platelets.

Table 4 presents means for LDH isoenzyme band intensities. Band LDH-3 had the greatest relative intensity and LDH-5 the least. Band LDH-5 was negatively correlated to eosinophils % ($r = -0.25$; $P = 0.06$). Band LDH 4 had a positive correlation to platlets ($r = 0.35$; $P = 0.06$), and WBC ($r = 0.32$; $P \leq 0.05$), and negative correlations with hemoglobin ($r = -0.36$; $P = 0.06$) and hematocrit ($r = -0.38$; $P = 0.003$). Band LDH-3 had a positive correlation with platelets ($r = 0.23$; $P = 0.08$) and WBC ($r = 0.37$; $P = 0.004$); whereas it had a negative correlation to RBC ($r = -0.33$; $P = 0.01$), hemoglobin ($r = -0.51$; $P = 0.0001$), and hematocrit ($r = -0.53$; $P = 0.0001$). Band LDH-2 had a positive correlation ($r = 0.23$; $P = 0.07$) to hemoglobin, and ($r = 0.25$; $P = 0.06$) to hematocrit. Band LDH-1 relative intensity showed a negative correlation with platelets ($r = -0.27$; $P = 0.04$), WBC ($r = -0.35$; $P = 0.006$), but was positively correlated to hemoglobin ($r = 0.41$; $P = 0.001$), and hematocrit ($r = 0.43$; $P = 0.0006$).

Effects of age, breed, and gender on LDH activity, blood values, and vital signs are presented in Table 5. Age was related ($P < 0.05$) to monocytes, eosinophils, RBC, weight, neutrophils, and lymphocytes. Breed was related ($P < 0.01$) to all LDH activities, lactate, hemoglobin, hematocrit, height, and weight. In addition, Thoroughbreds had more ($P < 0.05$) monocytes and basophils than Miniature horses; however, Miniature horses tended ($P \leq 0.10$) to have more WBC than Thoroughbreds. Males had a greater ($P < 0.05$) percentage of neutrophils and a lower ($P < 0.05$) percentage of lymphocytes than females. Interactive effects of breed and gender affected ($P < 0.05$) RBC, hemoglobin, height, hematocrit, platelets, and weight.

Proteins with LDH enzyme activity are distributed in all tissues and are involved with carbohydrate metabolism, and specifically LDH catalyzes the reversible oxidation and reduction between lactate and pyruvate. Pyruvate plays a pivotal role in cellular metabolism as a key substrate n regulation of glycolysis, gluconeogenesis, lipogenesis, and the TCA cycle.

The LDH-3 band had the greatest net intensity followed by band 2 and 1, with LDH band 4 and 5 having the least intensity. Our results indicate that LDH-4 and LDH-5 are not as predominant as the other three which is understandable since horses were measured at resting value. The fact that our LDH-3 band had the most intensity may relate to freezing of our samples. Another feasible explanation could be related to our sample population resting respiration rates. The isoenzyme LDH-3 is predominantly found in lung tissue, and when looking at Table 1, we see the respiration rate mean (17.8 breaths per minute (brpm) and maximum (24 brpm) of our population to be slightly higher than the normal. A further analysis of Table 5 shows the female Miniatures (17.6 brpm), male miniatures (18.4 brpm), and female Thoroughbreds (18.1 brpm) to all be slightly higher than the expected normal rate. Therefore, the slightly higher respiration rate might have caused an increase in LDH-3 isoenzyme activity.

A normal resting plasma lactate concentration is approximately 1.5 mmol/l in horses (Nappert and Johnson, 2001). Our findings indicate normal ranges for horses but the Miniature horses had a slightly higher range than Thoroughbreds. Reasons for this can be two-fold. First, the Miniatures utilized in this study were not accustomed to constant human interaction; therefore, anxiety may have been an influence. Second, the Miniature horses had free access to pasture and this may have lead to a higher lactate concentration than for the Thoroughbreds kept in stalls. This suggestion is similar to findings by Nogueira et al. (2002) which reported fillies on pasture had higher lactate concentrations than stabled horses. Many factors can influence the plasma activity of enzymes such as age, sex, exercise and training programs. In relation to sex, it has been demonstrated that elevated plasma muscle enzyme is more prevalent in mares than in male horses, suggesting a hormonal predisposition (Munoz et al., 2002).

There are two basic types of horses and many breeds have developed. Horses of Arabian ancestry are called "hot-blooded" and include Arab, Thoroughbred, Standardbred, and Quarterhorse breeds. "Cold-blooded" horses are the draft stock and ponies. Because ponies are considered a cold-blooded type of horse versus the hot-blooded Thoroughbreds or related breeds, it has been documented that miniature horses are similar to ponies, and donkeys; having a lower PCV, HGB, and RBC values, but have increased HCT, MCV, MCH, and MCHC values indicating a larger RBC size (Jeffcott, 1977). Our study is in agreement with this study, as our miniature horses had lower HGB and RBC compared to our Thoroughbreds, and our Thoroughbreds had higher hematocrit values. The same study showed RBC hematological values of Miniature horses having increased total lymphocytes, but lowered neutrophil values, causing the ratio of segmented neutrophils versus lymphocytes to be the reverse of reference values (1:1.5, rather than 1:1 or 1.5:1). Our study does not replicate this finding in that our Miniature versus Thoroughbred horses for the neutrophil and lymphocyte ratios showed no breed differences; however, age, gender, and pregnancy effects were significant.

In general, MCV increases in horses through the first 3 years of life, and smaller cells are considered normal in equine neonates. The larger cell volume of erythrocytes in aged horses may reflect an age-associated change in RBC maturation. Age interactions also were apparent in our study when correlated with our leukocytes. Other authors observed that horses greater than 8 years old had higher leukocyte counts; whereas some authors reported no effect of age on hematological parameters of Thoroughbreds.

Implications

The horse's vital signs, blood values, and lactate measurements can aid in a preliminary assessment of health which establishes a baseline reference for the individual animal. These individual reference ranges are important in understanding ailments and physiological processes for maintenance of health and training schedules. Further analysis would be required to ascertain how or if hormonal fluctuations played a role, and to determine the physiological and genetic mechanisms related to LDH effects on growth and immune function of horses.

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Table 1. Distribution of gender by breed, and pregnancy status.

	Thoroughbred		Miniature	
Gender	**N**	**Age**	**N**	**Age**
Colt	0	-	3	0.5
Gelding	16	11.2	0	-
Stallion	0	-	1	5
Filly	0	-	3	0.5
Non-gravid mare	7	10.1	9	1.4
Gravid Mare	2	8.0	18	7.1

N = number of horses.
Age = mean of horse age.

Table 2. Means of traits from fifty-nine horses.

Trait	Mean	SD	Minimum	Maximum
Protein,mg/ml	92.3	11.81	68.2	129.6
LDHf, IU/ml	281.2	105.43	118.9	624.5
LDHr, IU/ml	81.3	48.10	6.2	222.6
LDHfp,IU/mg protein	3.1	1.21	1.1	6.5
LDHrp,IU/mg protein	0.9	0.53	0.05	2.3
Lactate,mM/L	2.21	0.82	1.3	6.1
WBC,$10e^3/\mu L$	7.3	2.42	3.7	17.4
NEU,%	58.1	12.00	23.0	82.4
LYM,%	31.7	13.10	8.5	68.5
MONO,%	6.6	1.90	2.0	9.8
EOS,%	2.8	1.75	0.2	8.0
BAS,%	0.8	0.40	0.07	1.9
RBC,$(10e^6/\mu L)$	8.6	1.6	5.5	12.1
HGB,(g/dL)	13.05	2.00	9.7	18.7
HCT,%	37.4	6.12	27.0	55.0
PLT,(k/µL)	123.8	73.11	22.6	513.0
Height,(cm)	114.1	38.84	60.9	174.75
Weight,(kg)	268.3	194.6	37.7	659.1
Temperature,◦C	37.2	0.43	36.5	38.6
Respiration Rate, brpm	17.8	4.21	6.0	24.0
Heart Rate, bpm	40.81	9.00	15.0	60.0

LDHf = Lactate dehydrogenase forward activity; LDHr = Lactate dehydrogenase; reverse activity; LDHfp = Lactate dehydrogenase forward protein; LDHrp = Lactate dehydrogenase reverse protein; WBC = White blood cell, (x $10^3/\mu L$); NEU = Neutrophils, %; LYM = Lymphocytes, %; MONO = Monocytes, %; EOS = Eosinophils, %; BAS = Basophils, %; RBC = Red blood cells, (x $10^3/\mu L$); HGB = Hemoglobin, g/dL; HCT = Hematocrit, %; PLT = Platelet count, (x $10^3/\mu L$).

Table 3. Pearson correlation coefficients of age and blood chemistry profiles of Thoroughbred and Miniature horses.

Item	AGE	WBC	RBC	HGB	HCT	LYM	NEU	MONO	EOS	BAS	PLT
LDHf	-0.43**	0.24†	-0.19	-0.32*	-0.33*	0.18	-0.12	-0.34**	-0.08	-0.30*	0.28*
LDHfp	-0.45**	0.27*	-0.09	-0.27*	-0.27*	0.16	-0.09	-0.31	-0.13	-0.24†	0.37**
LDHr	-0.42**	0.26*	-0.19	-0.34**	-0.36**	0.24†	-0.18	-0.34**	-0.06	-0.31*	0.29*
LDHrp	-0.45**	0.33**	-0.14	-0.32*	-0.34**	0.22†	-0.16	-0.34**	-0.11	-0.31*	-0.45**
Lactate	-0.34**	0.61**	0.15	-0.14	-0.15	0.25†	-0.22†	-0.27*	0.31	-0.14	0.45**
Heart rate	-0.03	0.04	0.14	0.23†	0.23†	0.04	-0.07	0.004	0.10	0.31	0.14
Respiratory rate	0.11	0.11	-0.06	0.06	0.05	-0.10	0.05	-0.04	0.06	0.12	-0.01

** $P < 0.01$.

* $P < 0.05$.

† $P \leq 0.10$.

WBC = White blood cells, ($\times 10^3$ /µL); RBC = Red blood cells, ($\times 10^3$ /µL); HGB = Hemoglobin, g/dL; HCT = Hematocrit, %; LYM = Lymphocytes, %; NEU = Neutrophils, %; MONO = Monocytes, %; EOS = Eosinophils, %; BAS = Basophils, %; PLT = Platelet count, ($\times 10^3$ /µL); LDHf = Lactate dehydrogenase forward activity; LDHr = Lactate dehydrogenase reverse activity; LDHfp = Lactate dehydrogenase forward protein; LDHrp = Lactate dehydrogenase reverse protein.

Table 4. Separation of lactate dehydrogenase isoenzymes (LDH) in plasma collected from Thoroughbred and Miniature Horses.

Item	NI	SD	RI	SD
LDH band				
1	4832.4	2913.82	22	0.14
2	6759.6	4133.12	25	0.08
3	11675.2	7340.83	40	0.09
4	4135.1	3830.50	13	0.07
5	205.9	597.14	1	0.02

NI = Net Intensity; SD = Standard deviation from the mean; RI = Relative Intensity, %.

Table 5. Effects that age, breed, and gender had on LDH activity, blood values, and vital signs between Thoroughbred and Miniature Horses.

	Thoroughbreds		Miniatures		
Trait	**Female**	**Male**	**Female**	**Male**	**Effects**
Number	9.0	16.0	30.0	4.0	
LDHf	232.4 ± 28.8	206.8 ± 23.1	332.5 ± 16.3	303.5 ± 44.0	B^{**}
LDHr	44.2 ± 11.8	42.8 ± 9.5	110.2 ± 6.7	101.2 ± 17.9	B^{**}
LDHfp,IU/mg protein	2.5 ± 0.3	2.3 ± 0.3	3.6 ± 0.2	4.0 ± 0.5	B^{**}
LDHrp,IU/mg protein	0.5 ± 0.1	0.5 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	B^{**}
Lactate,mM/L	1.6 ± 0.2	1.7 ± 0.2	2.6 ± 0.1	2.6 ± 0.4	B^{**}
WBC,$10e^3/\mu L$	6.4 ± 0.8	6.3 ± 0.6	8.0 ± 0.4	7.8 ± 1.2	$B^{\dagger}$
NEU,%	50.7 ± 3.9	59.7 ± 3.1	58.4 ± 2.2	65.4 ± 6.0	A^{**},G^{*}
LYM,%	38.9 ± 4.1	28.7 ± 3.3	31.8 ± 2.3	26.1 ± 6.3	A^{**},G^{*}
MONO,%	7.2 ± 0.6	7.9 ± 0.5	5.8 ± 0.3	6.1 ± 0.9	A^{*},B^{*}
EOS,%	2.3 ± 0.6	2.7 ± 0.5	3.2 ± 0.3	1.7 ± 0.9	$A^{*},G^{\dagger}$
BAS,%	1.0 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	$A^{\dagger},B^{*}$
RBC,($10e^6/\mu L$)	10.2 ± 0.5	9.1 ± 0.4	7.8 ± 0.3	9.5 ± 0.7	$A^{*},B^{\dagger},BG^{**}$
HGB,(g/dL)	15.7 ± 0.5	13.5 ± 0.4	12.1 ± 0.3	12.8 ± 0.8	B^{**},BG^{**}
HCT,%	45.5 ± 1.6	39.1 ± 1.3	34.3 ± 0.9	36.4 ± 2.5	B^{**},BG^{*}
PLT,(k/μL)	104.4 ± 22.0	91.5 ± 17.6	133.9 ± 12.4	221.9 ± 33.6	$B^{**},G^{\dagger},BG^{*}$
Height,(cm)	153.3 ± 2.1	161.4 ± 1.7	83.1 ± 1.2	70.0 ± 3.3	B^{**},BG^{**}
Weight,(kg)	463.2 ± 13.6	486.0 ± 10.9	119.0 ± 7.7	83.0 ± 20.8	A^{*},B^{**},BG^{*}
Respiration Rate	17.2 ± 1.5	17.4 ± 1.1	18.3 ± 0.8	17.1 ± 2.2	NS
Heart Rate, bpm	43.7 ± 3.1	39.1 ± 2.5	41.3 ± 1.7	37.5 ± 4.7	NS

** $P < 0.01$.
* $P < 0.05$.
† $P \leq 0.10$.
A=Age; B=Breed; G=Gender; BG=Breed x Gender; NS= No Significance; LDHf = Lactate dehydrogenase forward activity; LDHr = Lactate dehydrogenase reverse activity; LDHfp = Lactate dehydrogenase forward protein; LDHrp = Lactate dehydrogenase reverse protein; WBC = White blood cells, ($\times 10^3$ /μL); NEU = Neutrophils, %; LYM = Lymphocytes, %; MONO = Monocytes, %; EOS = Eosinophils, %; BAS = Basophils, %; RBC = Red blood cells, ($\times 10^3$ /μL); HGB = Hemoglobin, g/dL; HCT = Hematocrit, %; PLT = Platelet count, ($\times 10^3$ /μL).

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