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Arkansas Animal Science Department Report 2012

David L. Kreider
University of Arkansas, Fayetteville

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David L. Kreider, Editor

DIVISION OF AGRICULTURE
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Cover photo of cattle on pasture in Batesville, Ark. courtesy of University of Arkansas System Division of Agriculture.

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ARKANSAS ANIMAL SCIENCE DEPARTMENT REPORT 2012

Edited by

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INTRODUCTION

Welcome! This is the 15th edition of the *Arkansas Animal Science* publication. As always, thanks to the faculty, staff, and graduate students in the Department of Animal Science and to Dr. David Kreider who served as editor of this edition. *Arkansas Animal Science* continues to evolve as we strive to be a source of on-demand, unbiased, scientific-based knowledge both inside and outside the classroom. With all the new technologies today, 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. Last year we published for the first time an *Arkansas Animal Science–Research Highlights*. This allowed those interested to quickly read, in a few brief statements, the impact of our research and extension programs. A weblink to the entire report is included within each highlight.

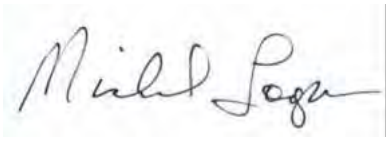
The research described in this report was conducted at the four main experiment stations used by the Department of Animal Science. These are the Arkansas Research and Extension Center at Fayetteville, the Southwest Research and Extension Center at Hope, the Southeast Research and Extension Center at 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. I 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)

TABLE OF CONTENTS

Beef Production and Management

Forages and Forage Management

Meats and Food Safety

Other Research

Demonstrations

Impact of different handling styles (good vs. aversive) on growth performance, behavior, and salivary cortisol concentrations in beef cattle

J. M. Bauer[1], E. B. Kegley[1], J. T. Richeson[2], D. L. Galloway[1], J. A. Hornsby[1], J. L. Reynolds[1], and J. G. Powell[1]

Story in Brief

The study objective was to determine effects of aggressive handling on growth performance, behavior, and salivary cortisol concentrations in beef calves. Crossbred calves (689 ± 10.3 lb; 24 steers and 30 heifers) from a single herd were stratified by gender, body weight, and initial chute score, then allocated randomly to 1 of 6 pens. Each pen was randomly assigned to 1 of 2 handling treatments (good vs. adverse) applied on days 7, 35, 63, and 91. The objective of the good treatment was to handle the calves quietly and gently to minimize stress. The objective of the adverse treatment was to move the calves rapidly and expose them to stressful stimuli. Body weight, exit velocity, and chute scores (based on 5 point subjective scale) were recorded and salivary samples for cortisol were collected (from the same 4 calves/pen) on days 0, 7, 35, 63, and 91. Pen scores (5 point subjective scale) were recorded on days 12, 42, and 87. Data were analyzed using a mixed model. Chute scores tended to be greater (more agitated) in the adverse treatment on day 7, but did not differ on subsequent days (treatment × day; $P = 0.06$). Salivary cortisol concentrations on day 63 were greater in cattle on the adverse treatment (treatment × day, $P = 0.001$). Body weight, exit velocity, and pen scores were not affected by treatment ($P \geq 0.24$). While differences were observed, cattle appeared to acclimate to short-term adverse handling, and it did not dramatically affect performance or behavior of beef cattle.

Introduction

Animal welfare has become an important current issue. People have been demanding and working for reform in livestock management worldwide. There have been citizen petitions and legislative bills calling for changes in the husbandry of animals in the livestock industry. Research in this area has become increasingly important.

Treatment of livestock is also a concern of producers, not only to comply with guidelines for animal welfare legislation but to increase productivity. More recently, the effect of animal handling on cattle behavior and the quality of product is a point of interest in research. In studies conducted by Hanna et al. (2006), Breuer et al. (1997), and Seabrook (1984), negative handling reduced milk yield in dairy cattle by 6% to 13%. Likewise, a study in Australia by Petherick et al. (2009) determined that adverse treatment negatively impacted live weight gain if the treatment was extreme enough.

This study was designed to determine the impact of different handling styles—good vs. adverse—on growth performance, behavior, and salivary cortisol concentrations in growing beef cattle.

Materials and Methods

Single source, crossbred Angus calves (n = 54; BW = 689 ± 10 lb; 24 steers and 30 heifers) from the University of Arkansas System Division of Agriculture cow/calf unit, were used. Calves had been previously weighed and handled numerous times since birth. Animals were penned in groups of 9 of mixed sex, housed on 6, 6-acre mixed grass pastures, and supplemented with dried distiller's grain (0.75% body weight per day basis). The amount of supplement was adjusted monthly based on recorded body weights. Water and a mineral supplement (Powell 4% Beef Mineral, Powell Feed and Milling Co. Inc, Green Forest, Ark.) were available ad libitum.

Initial Processing. The 92 d study began on February 16, 2011 (d -15). Cattle were weighed, and chute scores recorded, then stratified by gender, body weight, and chute score and allocated randomly to 1 of 6 pens. On d 0, all cattle were weighed and dewormed (Dectomax, Pfizer Animal Health, New York, N.Y.). Steers were implanted with Component TE-G (Ivy Animal Health, Inc., Overland Park, Kan.). Chute scores and exit velocity were recorded and an initial salivary sample was obtained. Cattle were sorted into assigned pens.

Treatments. Each pen was assigned randomly to 1 of 2 treatments (good or adverse handling). Calves in the good treatment groups were handled quietly with minimal stress and human interaction. This treatment involved moving calves from the pasture to the working facility as quietly as possible and with minimal prodding; a 15-min rest period where they were left alone in the holding pens, gentle handling through the chute, and a quiet environment inside the working facility. The goal of the adverse treatment groups was to work the calves in a manner that would maximize stress. This included moving the calves from the pasture to the holding pens as rapidly as possible, a 15-min period in the holding pens where they were exposed to extraneous noises and stimuli including an audible recording of distressed cattle noises and trains, slamming gates, ringing cow bells, banging on metal panels with rubber mallets, and prodding with livestock paddles. While being worked through the chute, cattle were exposed to loud talking and recorded sale barn noises, and cattle were aggressively prodded when they refused to move. These treatments were applied on d 7, 35, 63, and 91 of the study. Each treatment group was worked separately. Pens of cattle on the good treatment were worked first, returned to their pastures, and then the adverse treatment pens were brought to the working facility.

Measurements. On d 7, 35, 63, and 91 labor input was measured by recording 2 time intervals: the time it took to collect the calves from the pastures and the time it took to work calves through the chute. For the first factor, timing began when the handlers entered the pasture and stopped when the last calf exited the pasture, and for the second factor the amount of time between the pen of calves entering the working facility to the last calf exiting the restraining chute and re-entering the holding pen was recorded.

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Before entering the restraining chute each calf was weighed in a stanchion located behind the chute. While in the handling facility, a chute score was recorded to measure temperament. Each calf's chute score was recorded by 2 people independently and was based on a subjective 5 point scale (1 = calm, 2 = restless shifting, 3 = constant shifting with occasional shaking of weight box, 4 = continuous vigorous movement and shaking of weight box, and 5 = rearing, twisting, or violently struggling). Exit velocity was recorded when calves exited the restraining chute by using motion detecting equipment (Polaris Wireless Timer; FarmTek, Inc.; Wylie, Texas). Two laser barriers were placed 5 ft and 12 ft from the front of the chute. As the initial and secondary lasers were interrupted by an animal moving past them the equipment recorded the time it took each calf to traverse 7 ft.

To measure salivary cortisol concentrations, saliva samples from the same 4 pre-selected calves per pen were collected while cattle were in the restraining chute. Saliva samples were collected using a single-use synthetic sponge (2" × 0.5" × 0.5") held by a surgical clamp and inserted into the cheek. The sponge was then compressed within a syringe into a vial to collect approximately 2 mL of saliva sample. Samples were sealed and frozen at -10 °F until analysis. Saliva was analyzed for cortisol using a commercially available enzyme linked immunoassay kit as described in the manufacturer's instructions (Salimetrics, State College, Pa.).

On d 12, 42, and 87, subjective pen scores were recorded by an evaluator who scored the same 3 pre-selected calves per pen—selected randomly and marked by blue ear tags—upon initial approach in pasture and a second approach following a period of 5 min in the calves' presence. Pen scores were based on a 5 point scale (1 = unalarmed when approached, 2 = slightly alarmed and trots away, 3 = moderately alarmed and moves away quickly, 4 = very alarmed and runs off or charges, 5 = very excited and aggressive towards evaluator).

All data were analyzed using a mixed model of SAS (SAS Inst. Inc., Cary, N.C.). Fixed effects were treatment, sex, day when appropriate, and all interactions. Random effect was replication, and the subject was pen.

Results and Discussion

A tendency for a treatment × day interaction (Fig. 1, $P = 0.08$) was observed for time to gather cattle from the pasture. Good treatment groups tended to be faster ($P = 0.10$) on d 7 and slower ($P = 0.07$) on d 35. Times did not differ ($P \geq 0.31$) on d 63 and 91. There was a treatment × day interaction (Fig. 2, $P = 0.02$) for the time it took to work the cattle through the handling system, with the adverse groups faster on d 63, and good groups faster through the alley and chute on d 91. Inconsistencies in these results could be due to variable factors such as the calves distance from the gate, weather, or the work pace of handlers on a particular day. Although one of the stated goals for the adverse treatment was to move and work cattle faster, handling the cattle with that intent did not consistently produce that result.

Chute scores had a tendency for a treatment × day interaction (Fig. 3, $P = 0.06$). As expected, there was no difference between the treatment groups on d 0 since there was no treatment applied on that day, but on d 7, the first day of treatment application, the adverse groups had greater ($P = 0.001$) chute scores meaning those cattle appeared less content, presumably from being subjected to novel stress stimuli. However, on subsequent treatment days (d 35, 63, and 91) there again was no difference ($P \geq 0.31$) between the treatments suggesting the calves acclimated to the adverse stimuli. There was also an effect of sex ($P = 0.01$), with steers having greater chute scores than heifers.

Salivary cortisol concentrations were affected by a treatment × day interaction (Fig. 4, $P < 0.001$). Cortisol concentrations did not differ ($P = 0.85$) between treatments on d 0 before treatment was applied. Salivary cortisol concentrations did not differ ($P \geq 0.29$) on d 7, 35, and 91, but concentrations were greater ($P = 0.001$) in the adverse group on d 63, indicating this stress related hormone can be elevated in adversely handled cattle. There was also an effect of sex ($P = 0.02$), with heifers exhibiting greater salivary cortisol concentrations than steers. This was the opposite of what was observed with the subjective chute score, where steers appeared more agitated than heifers.

A treatment × sex interaction ($P = 0.01$, data not shown) was observed for pen scores. Heifers in the good treatment groups tended to exhibit a greater ($P = 0.16$) alarm response to the evaluator than heifers in the adverse handling groups; however, there was no difference ($P = 0.62$) in pen scores among the steers. This pattern was maintained in the 5 min evaluation as well (treatment × sex interaction, $P = 0.03$).

There were no treatment effects ($P \geq 0.24$) on either exit velocity (data not shown) or the final or any interim body weight and average daily gain (ADG). The final body weight of the cattle was 919 and 929 ± 15 lb and the ADG for the 92 d study was 2.48 and 2.56 ± 0.06 lb for the adverse vs. good treatments, respectively. The lack of treatment effect on performance is consistent with findings of Petherick et al. (2009) who observed that adverse handling (similar to ours) had only a temporary effect on liveweight gain. The concern among Petherick et al. (2009), as well as Hanna et al. (2006), was that handling methods used in their experiments were not extreme enough to produce the same results as previous studies. Based on the results for chute score, exit velocity, and cortisol concentrations, it appears the adverse handling in this study was not sufficient to produce responses as seen in previous studies. Results are also consistent with the findings of Burdick et al. (2009) that short-term exposure (acute stress) to elevated cortisol concentrations does not have an effect on health; whereas, a more prolonged exposure (chronic stress) would negatively impact productivity. Another concern among Hanna et al. (2006) and Petherick et al. (2009) was the predictability of the handlers' treatment. As the chute scores suggest, calves may have become acclimated to our adverse treatment. Similar patterns were observed in Petherick et al. (2009), indicating that the cattle began to anticipate the patterns of the adverse handling and thus the novelty and aversion to repeated stressful stimuli is reduced over time.

Implications

Results from the calf chute scores observed in this study suggest that cattle can become acclimated to repeated stressors. However, adverse treatment elevated cortisol concentrations, a physiological indicator of stress, in calves. The lack of a treatment effect on body weight suggests acute and repeated adverse treatment may not affect production in growing beef calves, consistent with previous findings that short-term exposure to adverse handling has little to no lasting effect on calves. Possibilities for further research would be to test the effects of long-term exposure and variation in the types of handler behavior on cattle production.

Acknowledgements

Research support was provided by The Dale Bumpers College of Agricultural, Food, and Life Science Undergraduate Research Program, the Honors College at the University of Arkansas, and the Division of Agriculture. Appreciation is expressed to Charles Rosenkrans for serving as a member of J. Bauer's Honors Committee.

Darren Bigner and Sterling Dewey are thanked for helping on work days, and the help of the Salimetrics technical support staff is gratefully acknowledged.

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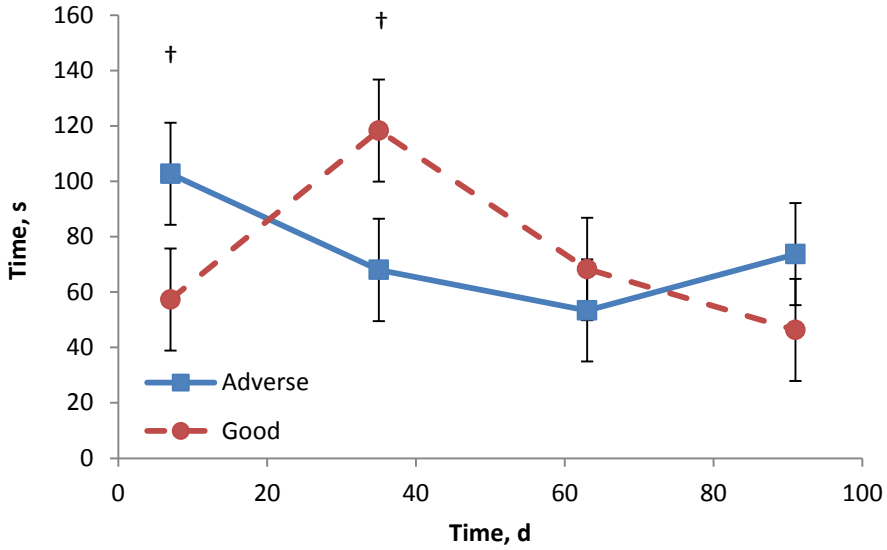

Fig. 1. Effect of handling style on time for handlers to collect the cattle and move them out of the pasture each work day. Effect of treatment × day (*P* = 0.08). Means within a day differ †*P* ≤ 0.10.

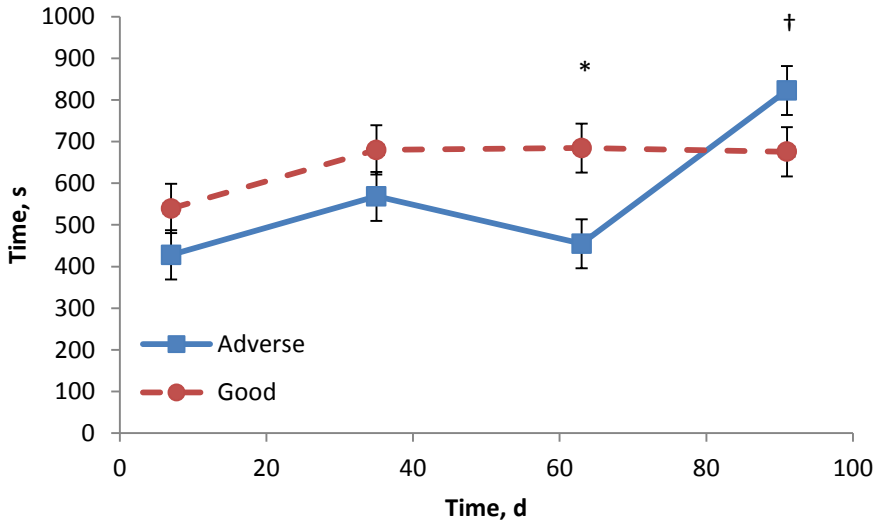

Fig. 2. Effect of handling style on time for cattle to be worked through the chute on each work day. Effect of day (*P* = 0.002), treatment × day (*P* = 0.02). Means within a day differ **P* ≤ 0.05, †*P* ≤ 0.10.

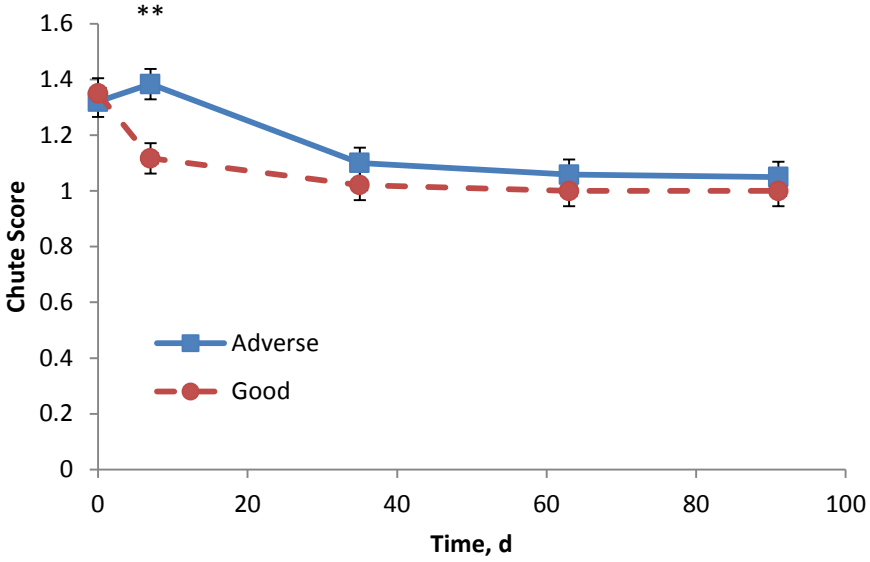

Fig. 3. Effect of handling style on subjective chute scores, 1 = calm to 5 = rearing, twisting, or violently struggling. Effects of day ($P < 0.001$), and treatment × day ($P = 0.06$). Means within a day differ **$P < 0.001$.

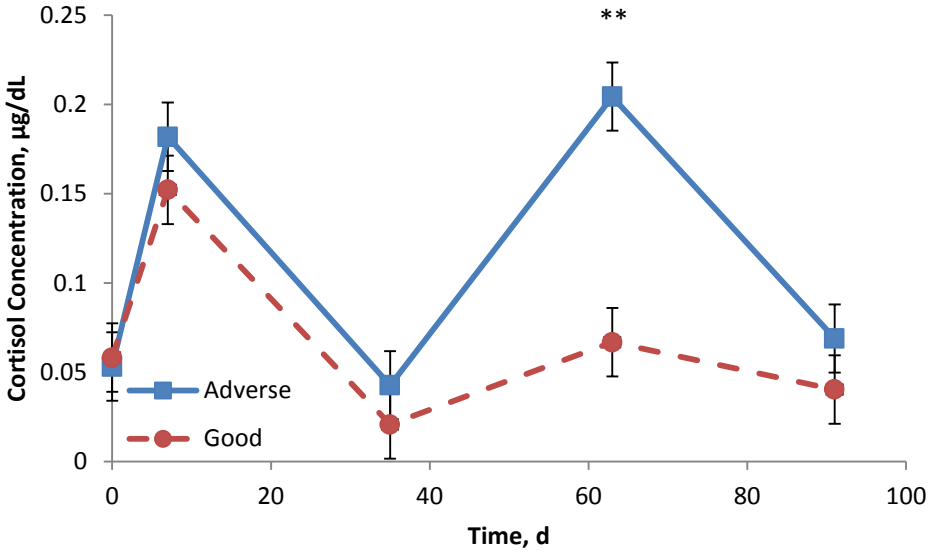

Fig. 4. Effect of handling style on salivary cortisol concentrations. Effects of treatment ($P = 0.09$), day ($P < 0.001$), and treatment × day ($P < 0.001$). Means within a day differ. **P < 0.001.

Effect of Rumensin® supplied via mineral or pressed protein block with or without Component TE-G with Tylan® implants on performance of steers grazing wheat pasture

Paul Beck[1], Thomas Hess[2], Don Hubbell[2], Brian Fieser[3], and Doug Hufstedler[4]

Story in Brief

This research was designed to evaluate the effect of Rumensin (Elanco Animal Health, Greenfield, Ind.) supplementation via mineral or pressed protein block with or without Component TE-G implants on performance of steers grazing wheat pasture in Arkansas over 2 years. Preconditioned steers (n = 360, BW = 525 ± 11.2 lb) grazed 15 four acre wheat fields in the fall (n = 60 steers each fall, stocking rate of 1 steer/acre) and 30 two acre wheat fields in the spring (n = 120 steers each spring, stocking rate of 2 steers/acre). Steers in each pasture were given free-choice access to non-medicated mineral (Control, MoorMan's WeatherMaster Range Minerals A 646AAA, ADM Alliance Nutrition, Inc., Quincy, Ill.), or were supplemented with Rumensin® (Elanco Animal Health, Greenfield, Ind.) via mineral containing 1,620 g monensin/ton (RMin, MoorMan's Grower Mineral RU-1620 590AR, ADM Alliance Nutrition, Inc.), or pressed-protein block containing 300 g/ton monensin (RBlock, MoorMan's Mintrate Blonde Block RU, ADM Alliance Nutrition, Inc.); and one-half of steers in each pasture were implanted with Component TE-G with Tylan (Elanco Animal Health). Animal performance data were analyzed as a randomized complete block design with a split-plot; using each season as the block, supplementation treatment as the whole plot, and implant as the split plot. Pasture was considered the experimental unit of the whole plot and steer the experimental unit of the split plot. Overall, Rumensin increased ($P \leq 0.03$) final body weight (BW) by 16 lb, total gain by 15 lb, and average daily gain (ADG) by 0.18 lb/day over non-medicated Control, but there was no difference ($P \geq 0.36$) due to type of supplement supplying Rumensin. Implanting steers increased ($P < 0.01$), final BW by 22 lb, total gain by 22 lb, and ADG by 0.32 lb/day over non-implanted steers. There was no interaction between Rumensin supplementation and implant ($P \geq 0.71$), indicating that the use of these technologies is additive. Steers that both received an implant and Rumensin gained 0.5 lb more per day and final BW was 39 lb greater than steers that did not receive Rumensin and were not implanted.

Introduction

Wheat pasture is high quality forage capable of producing gains in excess of 2 lb/day. Technologies such as growth promoting implants have been shown to increase gains of grazing calves by 0.18 to 0.25 lb/day (Brandt et al., 1995; Mader et al., 1994). Implants function by supplying very small amounts of compounds that act like naturally occurring hormones, thereby increasing muscle growth and reducing fat deposition. Because fat requires more energy to deposit than muscle, gains and feed efficiency are increased. Daily gain of growing cattle grazing wheat pasture has been increased by the addition of ionophores such as monensin (Rumensin®, Elanco Animal Health, Greenfield, Ind.) to supplements, and with the increased gains there was an increase in the economic value of supplementation programs. Horn et al. (1981) reported that daily gains of cattle grazing wheat pasture were increased by about 0.18 lb/day with the addition of monensin. There is skepticism by producers, consultants, and other livestock advisors as to whether giving calves both ionophores and implants will supply improvements in gain performance equal to each supplied individually. Therefore, this research was designed to evaluate the effect of Rumensin supplementation via mineral or pressed protein block with or without Component TE-G with Tylan implants on performance of steers grazing wheat pasture in Arkansas.

Experimental Procedures

Preconditioned steers (n = 360, BW = 525 ± 11.2 lb) grazed 15 four-acre wheat fields in the fall (n = 60 steers each fall, stocking rate of 1 steer/acre) and 30 two-acre wheat fields in the spring (n = 120 steers each spring, stocking rate of 2 steers/acre). Steers in each pasture were given free-choice access to non-medicated mineral (Control, MoorMan's WeatherMaster Range Minerals A, ADM Alliance Nutrition, Inc., Quincy, Ill.), or were supplemented with Rumensin® (Elanco Animal Health, Greenfield, Ind.) via mineral containing 1,620 g monensin/ton (RMin, MoorMan's Grower Mineral RU-1620, ADM Alliance Nutrition, Inc.), or pressed-protein block containing 300 g/ton monensin (RBlock, MoorMan's Mintrate Blonde Block RU, ADM Alliance Nutrition, Inc.). The guaranteed analysis of each mineral or block supplement are presented in Table 1. Supplements were offered free-choice in covered feeders designed for mineral supplementation (Ground Mineral Feeder, Sioux Steel Co., Sioux City, Iowa). Intake of the supplements was measured weekly. Targeted intake of the mineral supplements was 0.25 lb/steer/day and targeted intake of the pressed protein block was 0.3 to 1.3 lb/steer/day. One half of the steers in each pasture were implanted with 40 mg trenbolone acetate and 8 mg estradiol (Component TE-G with Tylan, Elanco Animal Health), remaining steers were not implanted.

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[2] University of Arkansas Livestock and Forestry Research Station, Batesville.
[3] ADM Alliance Nutrition, Quincy, Ill.
[4] Elanco Animal Health Beef Cattle-Stocker District, Guthrie, Okla.

This research took place on 60 acres of wheat pasture located at the University of Arkansas Livestock and Forestry Research Station near Batesville in northeast Arkansas (35°50' N, 91°48' W). The study site consisted of Peridge silt loam soil a deep well-drained upland soil with moderate fertility. Wheat was established the first week of September (90 lb wheat/acre) using either conventional tillage (six 4-acre pastures) or no-till (nine 4-acre pastures). The conventional tillage establishment protocol was to offset disk following removal of calves following grazeout the previous spring, chiseling twice, and light disking prior to planting, resulting in 4% residue cover at planting. No-till pastures were sprayed with Roundup Original Max® (Monsanto Co., St. Louis, Mo.) upon removal of calves following grazeout the previous spring, in mid-summer, and prior to planting and wheat planted directly into the stubble of previous crop, with >85% ground cover from residue. Supplementation treatments were factorialized across establishment methods so that each establishment method was equally represented. In the spring, pastures were divided into 2-acre tracts and supplementation treatments were re-randomized to the 30 resulting pastures by establishment method.

This study was analyzed as a randomized complete block design with a split-plot using the Mixed Procedure of SAS (SAS Inst., Cary, N.C.). Season was considered the block, supplementation treatment was the whole plot, and implant was in the split plot. Pasture was considered the experimental unit of the whole plot (supplementation treatment) and steer the experimental unit of the split plot (implant).

Results and Discussion

There was no significant interaction ($P \geq 0.71$) between supplement treatment and growth promoting implants, therefore the effects of supplement treatment and implant are presented separately in Tables 2 and 3, respectively. Daily gains were increased ($P < 0.01$) over Control by 0.15 to 0.21 lb/d, for RMin and RBlock, respectively. Total body weight (BW) gain was therefore increased ($P < 0.01$) by 15 lbs by both RMin and RBlock. Bodyweight at the end of the grazing season was increased ($P = 0.03$) from 714 lbs to 729 and 730 lbs for RMin and RBlock, respectively. The improved performance observed in this study is similar to gain increases reported by Horn et al. (1981) as well as very similar to the 2-year average improvement in performance (0.25 lb/d) reported by Fieser et al. (2007). Daily intake of the Control mineral was in excess of desired, averaging 0.36 ± 0.14 lb/calf. Intake of the RMin supplement was 0.23 ± 0.08 lb/calf, supplying 181 ± 73 mg Rumensin, while intake of RBlock averaged 0.73 ± 0.28 lb/calf each day, supplying 109 ± 41. The lack of performance differences ($P \leq 0.36$) between RMin and RBlock occurred even though monensin supplied by RBlock was less than what was supplied by RMin, this may indicate that this range of Rumensin dose is equally effective for calves grazing small-grain pasture or is an indication that the additional energy or protein supplied by RBlock may have had an influence on steer performance.

Implanting steers with Component TE-G increased ($P < 0.01$) average daily gain (ADG) by 0.32 lb/d and total BW gain by 22 lb/steer, which increased BW of steers at removal from pasture by 22 lb/head. This improvement in performance is greater than observed in other research (Brandt et al., 1995; Mader et al., 1994) and provides obvious economic incentives for stocker operators to utilize this technology. The lack of interactions between implantation and monensin supplementation indicates that the effects of these technologies are additive in nature. Steers that were not implanted and fed Control mineral supplement gained an average of 2.34 lb/d while steers supplied monensin (RMin and RBlock) and implanted gained an average of 2.84 lb/d. Thus, steers that both received implants and Rumensin gained 0.5 lb more per day and 44 lb more overall than steers that were not implanted nor received Rumensin increasing BW at removal from pasture by 39 lb, making it important for producers to consider supplying both implants and ionophores to grazing stocker calves.

Implications

These growth promoting technologies are cost effective ways to increase beef production by 21% without increasing feeding rates or pasture acreage. Utilizing ionophores and implants together for wheat pasture stocker cattle is calculated to decrease cost of gain by 25%. Implanting can increase net return per steer by $62. Rumensin supplementation can increase net return per steer by $26 when supplied by blocks or $52 when supplied by mineral, depending on supplement cost. Whereas profitability may be increased by $88 to $114/steer when both technologies are used.

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Table 1. Guaranteed analysis of supplements fed to steers grazing wheat pasture.

Item	Treatment[1]		
	Control	Rumensin Mineral	Rumensin Block
	----------As Fed Basis----------		
Ca, %			
Minimum	16.0	9.2	2.5
Maximum	19.2	11.0	3.5
P, % (Minimum)	8.0	6.0	1.5
NaCl, %			
Minimum	13.5	21.6	3.5
Maximum	16.2	25.9	4.5
Mg, % (Minimum)	2.5	0.3	-
K, % (Minimum)	0.2	0.8	1.2
Cu, ppm (Minimum)	1,100	1,120	-
Se, ppm (Minimum)	39	26.4	4.9
Zn, ppm (Minimum)	3,800	3,840	-
Vitamin A, IU (Minimum)	200,000	200,000	80,000
Crude protein, %	-	-	42.0
CP equivalent from NPN, %	-	-	12.0
Crude fat, %	-	-	0.5
Crude Fiber, %	-	-	7.0
Acid detergent fiber, %	-	-	10.0
Monensin, g per ton	-	1,620	300

[1] Steers in each pasture were given free-choice access to non-medicated mineral (**Control**, MoorMan's WeatherMaster Range Minerals A, ADM Alliance Nutrition, Inc., Quincy, Ill.), or were supplemented with Rumensin® via mineral containing 1,620 g Monensin/ton (**RMin**, MoorMan's Grower Mineral RU-1620, ADM Alliance Nutrition, Inc.), or pressed-protein block containing 300 g/ton Monensin (**RBlock**, MoorMan's Mintrate Blonde Block RU, ADM Alliance Nutrition, Inc.).

Table 2. Effect of Rumensin supplementation and supplement type on performance of steers grazing wheat pasture[1].

Item	Non-medicated Mineral	Rumensin Mineral	Rumensin Block	SE	Rumensin X Implant Interaction	Contrasts	
						Rumensin vs Non-medicated	Rumensin Mineral vs Block
Initial BW, lb	525	524	525	11.2	0.88	0.98	0.85
Final BW, lb	714	729	730	32.4	0.89	0.03	0.88
Gain, lb/steer	190	205	205	35.0	0.98	<0.01	0.98
ADG, lb/day	2.53	2.68	2.74	0.15	0.71	<0.01	0.36

[1] 60 steers (15 pastures) in falls of 2010 and 2011; and 120 steers (30 pastures) in springs of 2011 and 2012. BW = body weight; ADG = average daily gain.

Table 3. Effect of Component TE-G implant on performance of steers grazing wheat pasture[1].

Item	Control	Component TE-G	SE	P-value
Initial BW, lb	525	524	10.9	0.95
Final BW, lb	713	735	32.2	<0.01
Gain, lb/steer	189	211	35.0	<0.01
ADG, lb/day	2.49	2.81	0.14	<0.01

[1] 60 steers (15 pastures) in falls of 2010 and 2011; and 120 steers (30 pastures) in springs of 2011 and 2012, One half of the steers in each pasture were implanted with 40 mg trenbolone acetate and 8 mg estradiol (Component TE-G with Tylan, (Elanco Animal Health, Greefield, Ind.), remaining steers were not implanted. BW = body weight; ADG = average daily gain.

Single nucleotide polymorphisms of the follicle-stimulating hormone-β gene and effects on semen quality

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Story in Brief

Objectives of this study were to characterize single nucleotide polymorphisms in the promoter region of the bovine follicle stimulating hormone beta subunit gene, examine breed differences in polymorphisms, and determine effects of polymorphisms on semen quality. DNA samples were collected from 5 Angus, 13 Balancer, and 16 Brahman influenced bulls. Polymorphisms were identified by sequencing the promoter region revealing 17 polymorphisms and four insertion/deletions compared to the published sequence. Chi square indicated breed differences in frequency of occurrence for polymorphisms at base positions 169, 170, 171, 225, 353, 410, 411, 412, 485, 783, 1702, and in insertion/deletions at 413-414 ($P \leq 0.01$). In separate studies, semen collections were collected monthly from Brahman influenced bulls (June-August) and weekly from Angus and Balancer bulls (July-September). Computer assisted sperm analysis or stained smears were used to determine: % motile, progressive, rapid, and live sperm, path velocity, progressive velocity, track speed, lateral amplitude, beat frequency, % straightness, and % linearity. Additionally, minor:major axes of all sperm heads, average size of sperm heads, and % major, minor, and total abnormalities were measured. MIXED procedure for Angus and Balancer bulls indicated effects of week on the % linearity, average size of sperm heads, and % minor abnormalities ($P \leq 0.05$); effects of 485 on % motile, progressive, rapid, and live sperm, path velocity, progressive velocity, track speed, lateral amplitude, beat frequency, and average size of sperm heads ($P \leq 0.05$); effects of 1130 were observed on track speed, lateral amplitude, and % linearity ($P \leq 0.05$). For Brahman influenced bulls effects of polymorphism 783 were observed on % minor and total abnormalities ($P \leq 0.03$). Data from this study indicate breed differences in the frequency of polymorphisms in the promoter region of the follicle stimulating hormone beta subunit gene and that polymorphisms may be useful as markers related to semen quality.

Introduction

Follicle Stimulating Hormone (FSH) is a glycoprotein secreted by the anterior pituitary gland primarily in response to gonadotropin-releasing hormone (GnRH) and plays a role in the growth, development, and function of the ovaries and testes. The biological protein consists of an alpha (α) and beta (β) subunit; however the β subunit is responsible for the specific biological actions of FSH and its interactions with the FSH-receptor (Bernard, et al., 2010). Factors that influence the biological activity or pattern of secretion of FSH can alter development and function of the ovaries and testes and thereby affect fertility (Bernard, et al., 2010). In males, FSH promotes spermatogenesis, the process of sperm development, in the seminiferous tubules primarily via actions on Sertoli cells (Sairam and Krishnamurthy, 2001). Spermatogenesis is the developmental process in which spermatogonia are transformed into spermatozoa. Follicle stimulating hormone plays a critical role in the hormonal control of spermatogenesis by binding to FSH-receptors on Sertoli cells and stimulating primary spermatocytes to undergo the first meiotic division to form secondary spermatocytes (Senger, 2003). Since each Sertoli cell can only support a limited number of developing spermatozoa at a time, it is important we understand how polymorphisms of the FSHβ subunit gene can affect male fertility (Sairam and Krishnamurthy, 2001). Therefore, the objectives of this study were to characterize polymorphisms (SNP) in the promoter region of the bovine FSHβ gene and determine the relationship of polymorphisms of the FSHβ gene with measures of semen quality.

Materials and Methods

Animals and Treatments. The University of Arkansas Animal Care and Use Committee (Protocol # 11001) approved the animals and techniques used in this study. Five Angus (ANG) and 13 Balancer (BAL) bulls, mean age of 5.94 ± 1.47 years, from the University of Arkansas Beef Research Unit and 16 Brahman-influenced (BI) bulls, mean age of 15.13 ± 0.34 months, from the USDA-ARS Dale Bumpers Small Farms Research Center in Booneville, Ark. were used.

Semen samples were collected via electro-ejaculation weekly for ANG and BAL bulls from July through September, and monthly from June through August for BI bulls. Within five minutes of collection semen samples were evaluated using computer assisted sperm analysis (CASA) and stained smears were prepared using a nigrosin-eosin live dead stain to determine the following semen quality variables: percent motile (MOT), progressive (PROG), rapid (RAP), and live (LIVE) sperm, path velocity (VAP), progressive velocity (VSL), track speed (VCL), lateral amplitude (ALH), beat frequency (BCF), percent straightness (STR) and linearity (LIN). Additionally the minor:major axes of all sperm heads (ELONG), average size of sperm heads (AREA), and the percent major (MAJAB), minor (MINAB), and total abnormalities (TOTAB) were measured.

Polymerase Chain Reaction. Blood samples were collected from the animals listed above and genomic DNA extracted. Primers were designed using Primer3 (SourceForge.net®) to amplify three successive sections of the FSHβ gene promoter region via polymerase chain reaction (PCR). Agarose gel electrophoresis was used to ensure

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proper amplification region of PCR products. PCR products were purified and quantified according to manufacturer's instructions (Qiagen: QIAquick PCR Purification Kit; Qubit® 2.0 Fluorometer, Invitrogen, Eugene, Ore.). Purified PCR products were submitted to the University of Arkansas DNA Resource Center for sequencing using a ABI 3100 DNA Sequencer.

Identification of Polymorphisms. Polymorphisms were identified by comparing amplicons to the current FSHβ published sequence for *Bos taurus* (GenBank: M83753.1 GI:163063; NCBI) using ClustalW (European Bioinformatics Institute, Cambridge, U.K.) and electropherograms individually examined via Finch TV (Geospiza, Inc.; Seattle, Wash.).

Statistical Analysis. Chi square was used to examine breed differences in the frequency of occurrence of SNP identified in the 5 ANG, 13 BAL, and 16 BI bulls with JMP® Statistical Discovery Software (SAS Institute Inc., Cary, N.C.). The PROC MIXED procedure for repeated measures (SAS Institute Inc., Cary, N.C.) was used to determine: effects of week and SNP upon semen quality for the ANG and BAL bulls, and the effects of month and SNP upon semen quality for the BI bulls.

Results and Discussion

Transcriptional regulation is an important component in the control of gene expression. Initiation of transcription is the first rate limiting step in gene expression and is regulated by interactions of transcription factors with transcription factor binding sites in the promoter region (Dai, et al., 2009). Since transcription controls the production of FSH and its secretion, mutations of the regulatory region of a gene could result in either altered transcription factor binding sites or transcription initiation sites resulting in altered gene expression (Dai, et al., 2009). Therefore the purpose of this study was to: 1) identify polymorphisms (SNP) in the promoter region of the bovine FSHβ gene, and 2) determine the effects of polymorphisms of the FSHβ subunit gene upon measures of semen quality.

DNA samples were collected from 5 ANG, 13 BAL, and 16 BI bulls. Polymorphisms were identified by sequencing 3 sequential PCR products from the FSHβ promoter region. Seventeen SNP (A169G, G170T, T171C, C225G, C353A, G410T, G411T, T412A, G485A, A643G, G783A, A887G, C1130G, C1369T, C1376T, A1494T, C1702T) and 4 insertion/deletions (INDEL; CC413-414CAC, TC1063-1064TCC, TG1256-1257TCG, GA1703-1704GCA) were identified by comparing amplicons to the most recent FSHβ published sequence for *Bos taurus* (GenBank: M83753.1 GI:163063; NCBI). Naming of SNP occurred as follows: the letter before the base pair in which the SNP occurred represents the major allele, the letter following the base pair number represents the minor allele.

Breed Differences in the Frequency of Occurrence of SNP. Statistical analysis was performed using chi square to indicate breed differences in the frequency of occurrence of SNP. Of the polymorphisms identified, breed differences in the frequency of occurrence were observed for twelve SNP (A169G, G170T, T171C, C225G, C353A, G410T, G411T, T412A, CC413-414CAC, G485A, G783A, and C1702T; Table 1). The SNP occurring at bp 485 was a guanine (G) to adenine (A) substitution occurring in 40% of the ANG bulls, 84.62% of the BAL bulls, and 37.5% of the BI bulls ($P \leq 0.01$). Although SNP 170 was a G to thiamine (T) substitution and SNP 169 was a A to G substitution, both were homozygous substitutions occurring in 80% of the ANG bulls, 30.77% of the BAL bulls, and did not occur in any of the BI bulls ($P \leq 0.01$).

SNP C1702T, G783A, T412A, G411T, G410T, C353A, C225G, T171C, and INDEL CC413-414CAC were unique to the BI bulls in that they: 1) did not occur in any of the ANG and BAL bulls, and 2) there appears to be 100% linkage among these SNP with the exception of SNP G783A. SNP G783A only occurred in 31.25% of the BI bulls ($P = 0.01$) whereas the other SNP unique to the BI bulls occurred in 43.75% of that population ($P \leq 0.01$). When compared to the published sequence, INDEL CC413-414CAC appears to be an insertion of an A between two cytosine (C) bases occurring in 43.75% of the BI animals ($P \leq 0.01$).

Effects of SNP upon Semen Quality: *Angus and Balancer Bulls.* Two SNP appear to have affected semen quality for ANG and BAL bulls. Results indicate that SNP G485A affected the most semen quality variables (Table 2). Animals homozygous for the major allele (GG) had a decreased percentage of motile (MOT), progressive (PROG), rapid (RAP), and (LIVE) live sperm compared to heterozygous bulls ($P < 0.01$). Homozygous GG bulls also had a decreased path velocity (VAP), progressive velocity (VSL), track speed (VCL), and lateral amplitude (ALH), and an increased average size of sperm heads (AREA) and beat frequency (BCF) compared to heterozygous bulls ($P \leq 0.05$). Bulls homozygous for the major allele occurring at SNP C1130G (Table 3) experienced decreased track speed (VCL), lateral amplitude (ALH), and an increased percent linearity (LIN) compared to CG heterozygous bulls ($P \leq 0.05$). Sperm from CC homozygous bulls also had a strong tendency to move in a straighter pattern compared to heterozygous bulls (STR, $P = 0.06$).

Fewer total abnormalities (TOTAB) tended to occur in bulls homozygous for the major allele at SNP A169G and SNP G170T than for bulls homozygous for the minor allele (2.31 vs. 3.09, respectfully; $P = 0.06$). These bulls also tended to have fewer minor abnormalities (MINAB; 1.16 vs. 1.68, respectfully; $P = 0.07$).

Week by genotype interactions were observed for the percent linearity (LIN; Fig. 1), average size of sperm heads (AREA; Fig. 2), and the percent sperm with minor abnormalities (MINAB; Fig. 3) for SNP G485A ($P \leq 0.05$). Factors such as method of semen collection, environment, genetics, breed, animal health, prolonged periods of stress, and nutritional status of the animal have the potential to affect semen quality measurement (Foote, 1978). Therefore changes in any of these factors 61 days prior to semen collections could explain the genotype by week interactions observed for SNP G485A.

Brahman Influenced Bulls. Bulls homozygous for the major allele at SNP G783A (Table 4) had a decreased percentage of minor (MINAB) and total abnormalities (TOTAB) compared to heterozygous animals ($P = 0.03$). GG homozygous bulls also tended to have fewer sperm with major abnormalities (MAJAB, $P = 0.09$) and an increased beat frequency (BCF, $P = 0.07$) over heterozygous animals. Three genotypes were observed at SNP C1702T (Table 5). Heterozygous CT bulls tended to have an increased percentage of total abnormalities (TOTAB; $P = 0.06$), as well as, major (MAJAB) and minor abnormalities (MINAB; $P = 0.09$) compared to bulls homozygous for the major and minor allele.

In short, Angus and Balancer bulls heterozygous for the SNP identified in this study were associated with an increased percentage of live, motile, rapid, and progressively motile sperm. These bulls also exhibited increased path velocity, progressive velocity, track speed, and lateral amplitude, but a reduced sperm head area, beat frequency, and percent linearity and straightness. Heterozygous Brahman influenced bulls were also associated with increased track speed, path, and progressive velocity. Although most of the sperm quality measures evaluated appeared to be positively influenced by polymorphisms, Angus, Balancer, and Brahman influenced bulls heterozygous for the SNP identified appear to have an increased percentage of minor and total abnormalities when compared to bulls homozygous for the major allele.

Implications

Data from this study indicate breed differences in the frequency of occurrence of SNP in the FSHβ gene promoter region, and that SNP may be useful as markers related to semen quality.

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Table 1. Breed differences in frequency of occurrence of polymorphism in Angus, Balancer, and Brahman influenced bulls.

Base Pair Number	ANG (n=5)	BAL (n=13)	BI (n=16)	*P* Value
SNP A169G	4	4	0	< 0.01
SNP G170T	4	4	0	< 0.01
SNP T171	0	0	7	< 0.01
SNP C225G	0	0	7	< 0.01
SNP C353A	0	0	7	< 0.01
SNP G410T	0	0	7	< 0.01
SNP G411T	0	0	7	< 0.01
SNP T412A	0	0	7	< 0.01
INDEL CC413-414CAC	0	0	7	< 0.01
SNP G485A	2	11	6	< 0.01
SNP G783A	0	0	5	0.01
SNP C1702T	0	0	7	0.01

Chi square.

Table 2. Effects of SNP G485A on mean semen quality measurements for Angus and Balancer bulls.

	SNP G485A			
Variable	**GG**	**GA**	**SE**	***P* Value**
MOT	46.32	64.66	4.65	< 0.01
PROG	33.16	44.99	3.64	< 0.01
RAP	43.89	60.95	4.77	< 0.01
VAP	120.48	133.72	5.23	0.02
VSL	94.73	105.38	4.28	0.02
VCL	210.83	231.91	9.00	0.03
ALH	7.91	8.76	0.34	0.02
BCF	23.59	21.44	1.04	0.05
STR	78.71	78.76	1.15	0.97
LIN	48.33	48.32	1.26	1.00
ELONG	43.97	44.79	0.78	0.31
AREA	4.73	4.58	0.07	0.04
LIVE	66.40	76.22	3.08	< 0.01
TOTAB	2.63	2.69	0.46	0.91
MAJAB	1.11	1.36	0.28	0.38
MINAB	1.53	1.34	0.31	0.54

Least square means.

Table 3. Effects of SNP C1130G on mean semen quality measurements for Angus and Balancer bulls.

	SNP C1130G			
Variable	**CC**	**CG**	**SE**	***P* Value**
MOT	58.79	66.83	6.31	0.22
PROG	41.55	43.83	4.90	0.65
RAP	55.43	63.33	6.37	0.23
VAP	128.66	140.03	6.84	0.12
VSL	101.94	106.47	5.73	0.44
VCL	222.30	251.35	11.66	0.02
ALH	8.37	9.59	0.45	0.02
BCF	22.07	22.11	1.41	0.98
STR	79.16	76.06	1.51	0.06
LIN	48.81	45.17	1.68	0.05
ELONG	44.44	45.56	0.99	0.28
AREA	4.61	4.68	0.09	0.46
LIVE	73.53	75.56	4.29	0.64
TOTAB	2.75	2.32	0.59	0.48
MAJAB	1.32	1.02	0.36	0.41
MINAB	1.43	1.32	0.42	0.79

Least square means.

Table 4. Effects of SNP G783A on mean semen quality measurements for Brahman influenced bulls.

	SNP G783A			
Variable	**GG**	**GA**	**SE**	**P Value**
MOT	65.33	55.80	8.28	0.27
PROG	49.82	42.33	7.67	0.35
RAP	58.39	49.60	8.45	0.32
VAP	114.07	109.69	9.52	0.65
VSL	97.64	94.18	8.88	0.70
VCL	187.25	177.75	14.44	0.52
ALH	7.19	6.63	0.48	0.27
BCF	28.33	25.02	1.68	0.07
STR	84.15	78.80	3.83	0.18
LIN	54.03	51.73	3.27	0.49
ELONG	46.36	43.07	2.15	0.15
AREA	4.83	4.55	0.23	0.24
LIVE	77.48	73.00	4.97	0.38
MAJAB	10.09	14.80	2.57	0.09
MINAB	13.24	23.33	4.22	0.03
TOTAB	23.33	38.13	5.94	0.03

Least square means.

Table 5. Effects of SNP C1702T on mean semen quality measurements for Brahman influenced bulls.

	SNP C1702T				
Variable	CC	CT	TT	SE	P Value
MOT	65.11	55.50	78.67	16.80	0.31
PROG	49.30	42.00	64.00	15.71	0.35
RAP	57.81	49.72	71.67	17.27	0.40
VAP	113.68	111.49	111.07	19.89	0.97
VSL	97.24	95.33	97.87	18.65	0.98
VCL	186.94	182.23	172.77	29.52	0.86
ALH	7.24	6.81	6.27	0.97	0.48
BCF	28.41	25.09	30.47	3.44	0.12
STR	83.74	79.78	87.33	8.00	0.49
LIN	53.33	52.50	58.00	6.65	0.72
ELONG	46.00	43.89	48.00	4.57	0.53
AREA	4.86	4.58	4.73	0.49	0.53
LIVE	76.70	73.56	85.67	10.10	0.49
MAJAB	10.07[b]	14.78[a]	5.67[b]	5.26	0.09
MINAB	13.11[b]	22.89[a]	7.00[b]	8.70	0.09
TOTAB	23.19[d]	37.67[c]	12.67[d]	12.09	0.06

[ab]Least squares means tended to differ. *P* = 0.09.
[cd] *P* = 0.06.

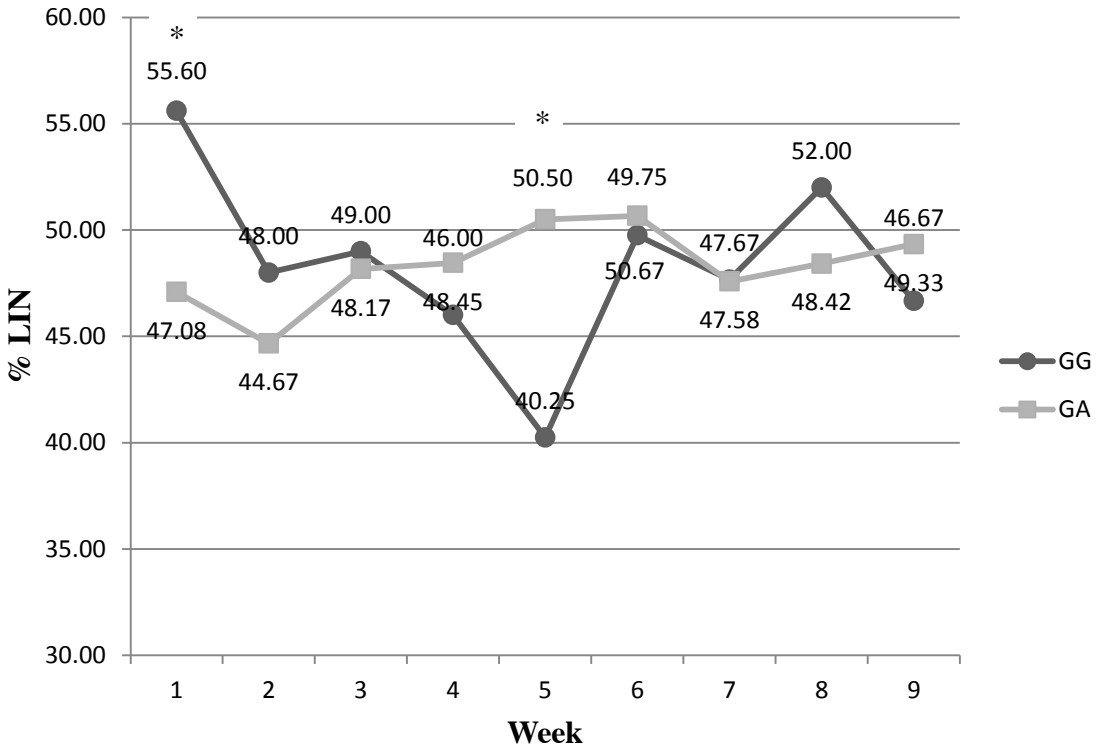

Fig. 1. Interaction between week and SNP G485A for LIN. * indicates P ≤ 0.05.

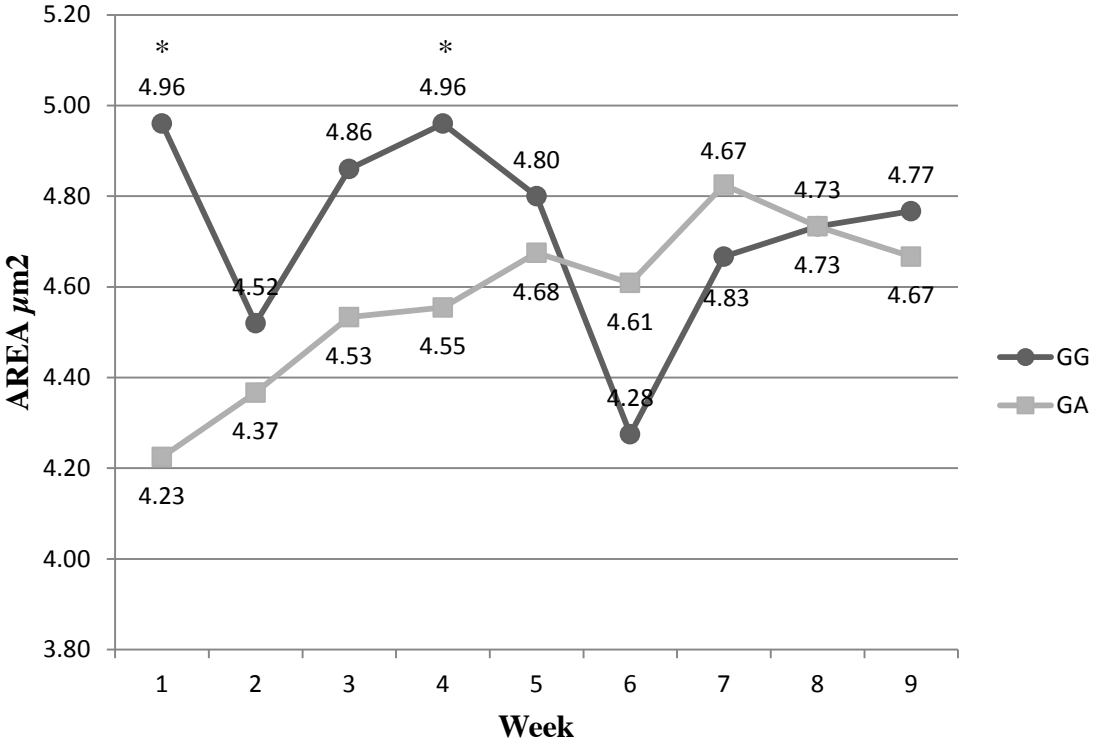

Fig. 2. Interaction between week and SNP G485A for AREA. * indicates P ≤ 0.05.

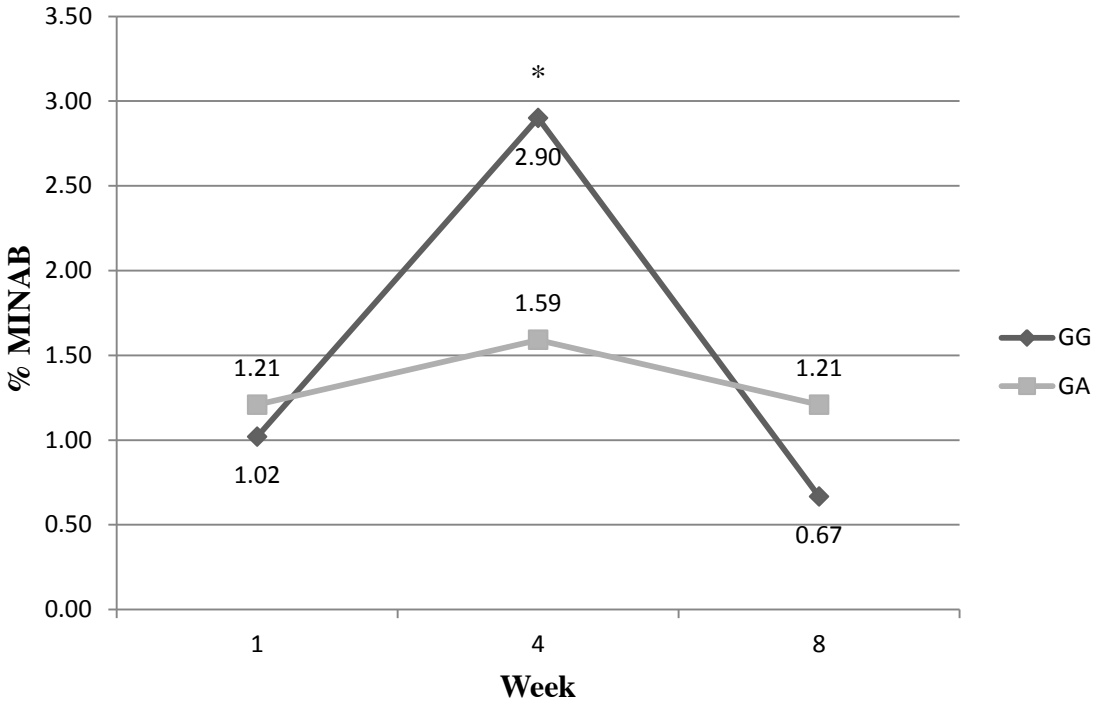

Fig. 3. Interaction between week and SNP G485A for MINAB. * indicates P ≤ 0.05.

Response of beef calves, not exposed to free choice mineral supplements, to an injectable trace mineral supplement

M.S. Gadberry[1] *and K. Simon*[1]

Story in Brief

The objective of this study was to examine growth and immune response of beef calves, not exposed to a free choice mineral supplement, to an injectable mineral formulation. Calves whose dams either did not receive or received trace mineral injection pre-breeding were randomly assigned within dam treatment to either a no-injectable mineral treatment or injectable mineral treatment. Calves assigned to the injectable mineral treatment were administered injectable mineral at 90 d of age processing and again at weaning. Neither dam treatment pre-breeding nor calf treatment affected weight gain from 90-d processing through a 46-d pre-conditioning period ($P \geq 0.27$). Type 1 bovine viral diarrhea virus neutralizing titer at weaning, 21 d post-weaning, or 46 d posting-weaning was not affected by pre-breeding dam treatment ($P \geq 0.59$) or calf treatment ($P \geq 0.44$). These results indicate injectable mineral does not improve growth or bovine viral diarrhea virus neutralizing titer for calves retained on-farm but not receiving a free choice mineral supplement.

Introduction

Commodity calves procured from livestock auctions and given injectable mineral have demonstrated increased growth and reduced illness (Richeson et al., 2009). This may be attributed to sub-optimal mineral supplementation pre-weaning and the effect minerals may have on calves being able to establish a good immune response to vaccination. Calves given an injectable mineral when exposed to a free choice mineral supplement pre-weaning, however, did not have a significant response to mineral injection (Kegley et al., 2011). The objective of this study was to examine growth and immune response of beef calves, not exposed to a free choice mineral supplement, to an injectable mineral formulation.

Materials and Methods

The study was conducted on a cooperator farm in Faulkner County, Ark. The 136-acre farm contained mixed warm-season grasses, over-seeded legumes, non-toxic fescue, and over-seeded winter annuals for fall and spring grazing. The soil analyzed 5.9 ± 0.5 pH, 69 ± 40 lb/ac P, and 264 ± 93 lb/ac K. Pastures were separated with electric fence and rotational grazing was implemented throughout the year. Hay was provided when grazeable forage was limiting and cows were supplemented with corn gluten feed pellets during lactation when hay analysis tested below cow nutritional requirements. With the exception of the mineral content of the corn gluten feed offered during lactation, no supplemental mineral was offered to the herd at any time throughout the year. Upon calf weaning, calves were supplemented with corn gluten feed at 0.3% body weight (BW).

On April 15, 2011, 35 beef cows and 7 heifers (combined weight 1035 ± 179 lbs) were assigned to either injectable mineral or no injectable mineral treatment. Treatments were randomly assigned to females after grouping for parity and previous calving date. Twenty-four of the 35 cows were nursing spring-born calves. All mature cows were commingled throughout the study (individual female was considered the experimental unit). The injectable mineral (Multimin 90, Multimin USA, Fort Collins, Colo.) contained 60 mg/mL zinc, 10 mg/mL manganese, 5 mg/mL selenium, and 15 mg/mL copper. The initial injection was given to cows on April 15, 2011. The dose was 0.5 mL/100 lb BW, and the injection was administered subcutaneous in the neck region, opposite the side vaccinations were administered. A placebo injection was not administered to control cows. The mineral product used in this study was donated by Multimin USA.

Calves nursing cows within each of the cow treatment groups were randomly assigned to either a no-injectable mineral treatment (n = 12) or an injectable mineral treatment (n = 12). Calves receiving the injectable mineral were treated at 90 d of age process (June 24, 2011) and weaning (October 28, 2011) using Multimin 90 at a dose of 1 mL/100 lb BW. Following weaning, calves were retained for a 46-d preconditioning period.

Calves were weighed and all bulls were castrated at approximately 90 d of age. Both steers and heifers were implanted (Ralgro, Intervet Inc., Merck Animal Health, Summit, N.J.) and Alpha 7 vaccine (Boehringer Ingelheim Vetmedica, St. Joseph, Mo.) administered. At weaning, steer calves were re-implanted, all calves were given a second dose of Alpha 7, and all calves were treated with an anthelmintic (Eprinex, Merial LTD., Duluth, Ga.) and vaccinated with Cattlemaster 4 VL5 (Pfizer Animal Health, New York, N.Y.). Calves were weighed and given a Cattlemaster booster on November 18 and final calf weighs were collected on December 13. Calves were co-mingled during all phases of management.

On Oct 28 (weaning), November 18 (re-vaccinating), and December 13 (end of 46-d retained ownership period), blood samples were collected and submitted to the Iowa State Veterinary Diagnostics Laboratory (Ames, Iowa) for type 1 bovine viral diarrhea (BVD) virus neutralizing (VN) titer determination.

Upon examining the proportion of male and female calves within dam and calf treatment, a greater percentage of male calves were allocated to the injectable mineral treatment compared to the no-injectable mineral treatment. Therefore, calf gender was included as a fixed covariate in the statistical analysis and least-square means were used to compare growth and serum BVD titers among treatments using SAS (SAS Institute Inc., Cary, N.C.) statistical software.

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Results and Discussion

Initial age and BW of calves at 90 d of age processing did not differ between dam treatments or calf treatments ($P \geq 0.27$, Table 1). Average daily gain (ADG) from 90 d processing to weaning did not differ between calves whose dams had received injectable mineral and calves whose dams had not received injectable mineral ($P = 0.27$). In addition, injectable mineral administered to the calves did not affect ADG from processing (90 d of age) to weaning ($P = 0.85$). Post-weaning weight gain to d 21 and d 46 was not affected by calves receiving injectable mineral ($P \geq 0.48$) or injectable mineral given to dams pre-breeding ($P \geq 0.31$). Neither dam mineral treatment pre-breeding nor calf mineral treatment at processing affected serum type 1 BVD VN titers ($P = 0.91$ and 0.45, respectively). The VN titer for type 1 BVD did not differ with dam treatment or calf treatment on d 21 post-weaning ($P = 0.59$ and 0.44, respectively) or d 46 post-weaning ($P = 0.79$ and 0.62, respectively).

Implications

Injectable mineral supplementation given to dams pre-breeding or their calves at 90 day of age processing followed by a second injection at weaning did not improve calf growth or bovine viral diarrhea titer response among retained calves that did not receive a free choice mineral supplement.

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Table 1. Performance of bovine calves whose dams received Multimin 90 injection pre-breeding and calves that received Multimin 90 injection at 90 d processing and a second injection at weaning

	Dam[2]				Calf[2]				
	No Injection	Multimin 90 Injection	Pooled SEM	P-value	No Injection	Multimin 90 Injection	Pooled SEM	P-value	Gender[1] P-value
N	13	11			12	12			
Percentage male	46	64			42	67			
90 d processing									
Age, d	89.1	95.4	8.0	0.59	88.3	96.2	8.1	0.50	0.26
BW, lb	253	277	15.4	0.27	264	266	15.6	0.96	0.45
Weaning									
BW, lb	467	515	18.1	0.08	494	488	18.0	0.81	0.08
ADG from 90 d, lb	1.70	1.84	0.08	0.27	1.78	1.76	0.08	0.85	0.02
WDA, lb	2.2	2.3	0.07	0.47	2.3	2.2	0.07	0.57	0.001
21 d revaccinate									
BW, lb	470	508	17.9	0.15	496	482	18.1	0.59	0.09
ADG from weaning, lb	0.18	-0.33	0.36	0.34	0.11	-0.26	0.36	0.48	0.80
46 d preconditioning									
BW, lb	518	558	17.2	0.12	541	534	17.2	0.79	0.03
ADG from weaning, lb	1.12	0.92	1.12	0.31	1.02	1.01	0.12	1.00	0.07
Serum BVD 1 VN[3]									
Weaning, d 0	8.3	8.4	0.73	0.91	8.7	7.9	0.74	0.45	0.27
Revaccinate, d 21	9.4	9.0	0.53	0.59	9.5	8.9	0.54	0.44	0.99
Final,d 46	9.4	9.2	0.43	0.79	9.5	9.1	0.42	0.62	0.73

[1]Gender included as a fixed covariate. Statistical model did not included interactive effects due to small sample size.

[2]Dams injected with Multimin 90 (0.5 ml/100 lb BW) prior to breeding. Calves injected with Multimin 90 (1 ml/100 lb BW) at 90 d post birth and a second injection at weaning.

[3]Log(2) Serum bovine viral diarrhea type 1 virus neutralizing titer.

BW = body weight; ADG = average daily gain.

Response of beef cows, not exposed to mineral supplements, to an injectable trace mineral supplement

M.S. Gadberry[1] and K. Simon[1]

Story in Brief

The objective of this study was to examine beef cow response to an injectable form of trace mineral supplementation in a production environment where forage and grazing was well managed but free choice mineral supplementation was not practiced. On April 15, 2011, 35 beef cows and 7 yearling heifers (average weight 1035 ± 179 lb) were assigned to either an injectable mineral or no-injectable mineral treatment. Female body weight (BW), body condition score (BCS), pregnancy rate, postpartum interval, and calculated number of days from bull exposure to breeding were determined over a 1-year cycle (pre-breeding 2011 to re-breeding 2012). Body weight did not differ ($P \geq 0.19$) between treatments at any point when compared among all females. Body condition score tended to be greater ($P = 0.10$) for injectable mineral at 90-d calf processing; however body condition score did not differ at weaning, calving or re-breeding. Pregnancy rate did not differ ($P = 0.36$) and was 90.4% for no-injectable mineral and 76.2% for injectable mineral. Retained injectable mineral cows that calved in the spring of 2011 had a similar ($P = 0.43$) postpartum interval compared to no-injectable mineral cows that calved in spring 2011. Calculated days from bull exposure to breeding did not differ among all retained females ($P = 0.89$). These results indicate injectable mineral may not increase pregnancy rate in beef cows not exposed to free choice mineral supplementation.

Introduction

In recent years, oral mineral supplement costs have increased, exceeding $20/50 lb bag. Most oral mineral supplements have an expected daily intake of 0.25 lb/cow. Therefore, at the current cost and typical consumption rate, the annual oral mineral supplementation cost of an individual cow could approach $40. As a result, cattle producers often question the cost-to-benefit value of free-choice, oral mineral supplementation. One reason for uncertainty is marginal trace mineral deficiencies typically cause subclinical production losses, and producers have a difficult time quantifying the benefits of mineral supplementation. In the absence of a mineral supplementation program, the average mineral composition of improved forages in Arkansas suggest that trace mineral deficiencies may be marginal and likely result in subclinical losses. Sodium is the most commonly deficient major mineral. There are few options to oral trace mineral supplements without purchasing a product that also includes several major minerals. Therefore, alternatives to a complete, oral mineral supplement, such as injectable trace mineral products, should be examined to determine if providing key trace mineral supplements at critical times of the production cycle will improve herd productivity. The objective of this study was to examine beef cow response to an injectable form of trace mineral supplementation in a production environment whereby forage and grazing is well managed but free choice mineral supplementation is not practiced.

Materials and Methods

The study was conducted on a cooperator farm in Faulkner County, Ark. The 136-acre farm contained mixed warm-season grasses, over-seeded legumes, non-toxic fescue, and over-seeded winter annuals for fall and spring grazing. The soil analyzed 5.9 ± 0.5 pH, 69 ± 40 lb/ac P, and 264 ± 93 lb/ac K. Pastures were separated with electric fence and rotational grazing was implemented throughout the year. Hay was provided when grazeable forage was limiting and cows were supplemented with corn gluten feed pellets during lactation when hay analysis tested below cow nutritional requirements. With the exception of the mineral content of the corn gluten feed offered during lactation, no supplemental mineral was offered to the herd at any time throughout the year.

On April 15, 2011, 35 beef cows and 7 heifers (combined weight 1035 ± 179 lbs) were assigned to either injectable mineral (ITM) or no injectable mineral (NITM) treatment. Treatments were randomly assigned to females after grouping for parity and previous calving date. All mature cows were commingled throughout the study (individual female was considered the experimental unit). The 7 heifers were managed separately through breeding then comingled with the mature cow herd. The injectable mineral (Multimin 90, Multimin USA, Fort Collins, Colo.) contained 60 mg/mL zinc, 10 mg/mL manganese, 5 mg/mL selenium, and 15 mg/mL copper and was provided by Multimin USA. The initial injection was administered subcutaneously in the neck region on April 15, 2011 at a dose of 0.5 mL/100 lb body weight (BW). The mineral injection was administered opposite the side vaccinations were administered. A placebo injection was not administered to control cows. Females administered ITM were given a second injection January 2012, prior to calving and a third injection April 2012, prior to re-breeding (beginning May 14, 2012).

The 7 heifers were exposed to a bull for 90 days beginning April 15, 2011 and mature cows were exposed to a bull for 90 days beginning May 14, 2011. All females were treated with Eprinex (Merial LTD., Duluth, Ga.) for internal parasites at the beginning of the study. Body weights and body condition scores (body condition score: BCS, 1 to 9 scale with 1 being emaciated and 9 being obese) were recorded at the start of the study, at 90-d calf processing (June 24), weaning (October 28), prior to calving (January 27, 2012), and prior to re-breeding (April 12, 2012). Blood samples were collected at weaning for pregnancy determination based on the BioPryn test (BioTracking, LLC, Moscow, Idaho). Non-pregnant cows and historically poor performing cows were culled in the fall of 2011. Prior to the 2012 calving season, cows were given Safeguard (Intervet Inc., Merck

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Animal Health, Summit, N.J.) and prior to the 2012 breeding season, cows were vaccinated with Cattlemaster 4-VL5 (Pfizer Animal Health, New York, N.Y.). Postpartum interval was calculated for 2011 spring calving cows and the number of days from bull exposure to breeding was calculated as: 2012 calving date – 283 – 1st date of bull exposure.

Age, BW, BCS, and postpartum interval were analyzed with R (www.r-project.org) using a generalized linear model. Pregnancy data and number of calves nursing at the start of the 2012 breeding season were analyzed with a chi-square test.

Results and Discussion

Table 1 contains the BW, BCS, and pregnancy test results for all females (defined as lactating females, breeding mature cows that calved the previous fall but exposed to the bull for spring calving, and developing heifers) and lactating females only (spring 2011 calving). Initial age, BW, and BCS did not differ between NITM and ITM prior to breeding. At 90-d calf processing, BW did not differ between treatments when compared among all females or lactating cows. Body condition score among all females tended to be greater ($P = 0.10$) for ITM compared to NITM at 90-d processing. When compared among lactating cows only at this time, BCS did not differ between treatments. At weaning, BW did not differ between treatments for all cows or lactating cows. Pregnancy rate did not differ ($P = 0.36$) between NITM and ITM.

Table 2 contains the BW, BCS, postpartum interval, calculated days from bull exposure to breeding and number of calves nursing cows retained in the herd as of April, 2012. The number of females in the herd after the 2011 fall culling was 29. The ITM treatment had 16 remaining females and the NITM treatment had 13 females remaining ($P = 0.50$). Body weight and BCS prior to breeding did not differ among treatments. Postpartum interval for cows that had calved in the spring of 2011 did not differ between ITM and NITM ($P = 0.43$). The calculated number of days from bull exposure to breeding in 2011 was 19.3 and 20.4 ± 5.7 (SEM) d and did not differ between treatments ($P = 0.89$). Body weight ($P = 0.99$) and body condition score ($P = 0.87$) prior to the 2012 breeding season did not differ between treatments, nor did the number of calves nursing ($P = 0.85$).

Implications

These results indicate that injectable mineral supplementation may not result in a sustained improvement in body weight, body condition or pregnancy rate for cows that do not receive a year-round free choice mineral supplement.

Table 1. Performance of bovine females receiving a trace mineral injection pre-breeding: pre-breeding to weaning.

	Treatment[1]			
	NITM	ITM	Pooled SEM	P-value
All females, n	21	21		
Lactating females, n	18	16		
Pre-breeding[2]				
Age, yr	4.7	5.0	0.69	0.73
BW, lb	1038	1033	40.1	0.92
BCS[3]	5.4	5.4	0.12	0.97
90-d calf processing[2]				
All females BW, lb	1138	1163	43.4	0.69
All females BCS	5.3	6.0	0.26	0.10
Lactating females BW, lb	1138	1144	43.5	0.93
Lactating females BCS	5.3	5.8	0.25	0.19
Calf weaning[2]				
Lactating females BW, lb	1184	1229	0.41	0.43
Lactating females BCS				
Lactating females pregnant, %	94	80		0.22
All females BW, lb	1157	1192	38.6	0.52
All females BCS				
All females pregnant, %	90.4	76.2		0.36

[1]NITM (no trace mineral injection) and ITM (trace mineral injection: Multimin 90 injection at 0.5mL/100 lb body weight (BW) 30 prior to breeding).
[2]Responses to treatment were determined at pre-breeding, trial initiation (April 15), at 90-d calf processing (June 24), and at weaning (October 28).
[3]BCS (body condition score scale from 1 to 9 whereby 1 = emaciated and 9 = obese).

Table 2. Performance of bovine females receiving a trace mineral injection pre-breeding: 2012 pre-calving to re-breeding.

	Treatment[1]			
	NITM	ITM	Pooled SEM	P-value
Retained, n	13	16		0.50
Pre-calving[2]				
BW, lb	1212	1188	24.5	0.73
BCS[3]	5.3	5.4	0.16	0.80
Postpartum interval, d	367	357	8.9	0.43
Exposure to breeding[4], d	19.3	20.4	5.7	0.89
Pre-breeding[2]				
BW, lb	1130	1130	55.4	0.99
BCS[3]	5.4	5.5	0.25	0.87
Calves nursing, n	12	14		0.71

[1]NITM (no trace mineral injection) and ITM (trace mineral injection: Multimin 90 injection at 0.5mL/100 lb body weight (BW) 30 prior to breeding).
[2]Responses to treatment were determined at pre-calving (January 27, 2012) and re-breeding (April 12, 2012).
[3]BCS (body condition score scale from 1 to 9 whereby 1 = emaciated and 9 = obese).
[4]Calculated as calving date – 283 – 1st date of bull exposure.

Relationship between horn fly population, month, breed type and temperament in beef cows[1]

A. R. Mays[2], M. A. Brown[3], and C. F. Rosenkrans, Jr.[2]

Story in Brief

Objectives of this study were to determine if horn fly populations and cow temperament were associated with month and breed type. Horn fly counts were collected over a six month period from fifty-one beef cows (1362 ± 140 lb body weight). Horn fly populations were affected by month of collection ($P < 0.001$) and breed type ($P = 0.02$). Cow weight was positively correlated (r = 0.25; P < 0.001) with horn fly populations for all breed types and exit velocity of Bonsmara (r = 0.37; $P < 0.05$), Charolais (r = 0.27; $P < 0.05$), and Romosinuano-sired cows (r = 0.70; $P < 0.002$). Cow weight and horn fly population was positively correlated with chute score (r= 0.46; $P < 0.01$ and r = 0.53; $P < 0.003$) in Gelbvieh-sired cows. Determination of factors affecting horn fly populations may serve as alternative methods of control on horn fly populations for producers.

Introduction

Horn flies (*Haematobia irritans*) are considered a major blood sucking pest in the cattle industry with economic losses in the United States estimated to be $876 million (Kunz et al., 1991). Stress caused by horn fly bites can lead to decreased gain and negative temperament and behavior. Development of resistance to antiparasitic treatments is forcing producers to seek alternative methods of horn fly population control. Environmental conditions and breed type are two factors associated with horn fly populations in beef cattle (Oyarzún et al., 2008 and Steelman et al., 1991). Therefore, the objective of this study was to investigate if horn fly populations and cow temperament were associated with month of collection and breed type.

Materials and Methods

Fifty-one crossbred beef cows (1362 ± 140 lb) were maintained on native rangeland pastures located at the United States Department of Agriculture (USDA) Grazinglands Research Laboratory in El Reno, Oklahoma. Breed types consisted of Bonsmara (BonsX; n = 5), Charolais (CharX; n = 10), Gelbvieh (GelvX; n = 5), Hereford (HerfX; n = 10) and Romosinuano-sired cows (RomoX; n = 7), as well as Brangus (Bran; n = 14). All experimental procedures were reviewed and accepted by the ARS Animal Care and Use Committee and the Committee for Institutional Animal Care and Use at the University of Arkansas.

Horn fly populations were counted for individual cow once a month beginning in May and ending in October. The procedures used for obtaining fly counts were similar to those previously utilized at the University of Arkansas, Fayetteville. A trained observer counted individual horn flies present on each cow when the total population was <25 flies, however, if the horn fly population was >25 flies the horn flies were counted in groups of 5. More detail on horn fly population counts can be found in Steelman et al., 1991. Horn flies were treated monthly after population counts were collected using Co-Ral (organophosphate) to ensure the overall health and well-being of the animals were maintained based on horn fly populations exceeding threshold numbers.

Temperament data consisted of exit velocity (EV) and chute scores (CS), and was collected monthly beginning in May and ending in October. Exit velocity was recorded to determine the rate at which cows exited the squeeze chute and traversed 6 ft. Cows were assigned a CS based on a 1-4 scale (1 = calm no movement; 2 = restless shifting; 3 = squirming continuous shaking of the squeeze chute and 4 = rearing, twisting, continuous violent struggle), which was adopted from previously described procedures (Grandin, 1993).

Data were analyzed utilizing analysis of variance (ANOVA), with breed and month as the main effects and month of horn fly counts treated as a repeated measure. Pearson correlations were calculated to assess the relationship between type and horn fly populations, cow weight, EV and CS.

Results and Discussion

Dependent variables were not affected ($P < 0.10$) by an interaction between breed type and month of collection; therefore, main affects will be presented. Horn fly populations were affected by month of collection ($P < 0.001$; Fig. 1). In August horn fly populations were highest (524 ± 42 flies), followed by October (410 ± 40 flies), September (366 ± 40 flies) and July (343 ± 40 flies; $P < 0.001$). Previous research conducted in Arkansas found similar results, which are likely due to the correlation between horn fly development and environmental temperatures.

Horn fly populations were significantly affected by breed type ($P = 0.02$; Fig. 2). Horn fly populations were significantly lower in RomoX and CharX cows (273 ± 43 and 296 ± 36 flies; $P = 0.02$) when compared to HerfX cows (438 ± 34 flies), with BonsX, Bran, and GelvX cows considered similar to the other breeds (299 ± 49, 349 ± 29 and 309 ± 49 flies). Previous research determined breed type be an important factor in relation to horn fly populations.

Cow weight was positively correlated (r = 0.25; $P < 0.001$) with horn fly populations for all breed types. Cow weight was positively correlated with EV in BonsX (r = 0.37; $P < 0.05$), CharX (r = 0.27; $P < 0.05$), and RomoX cows (r = 0.70; $P < 0.002$), indicating that increased cow weight led to increased time to traverse 6 ft. Gelbvieh-sired cows had a positive correlation between cow weight and CS

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(r = 0.46; $P < 0.01$) and cow weight and horn flies (r = 0.53; $P < 0.003$), indicating the heavier the cow and the greater the horn fly population, the greater the CS. Pearson correlations regarding cow weight found that increased weight was correlated with increased horn fly populations among all breed types and increased EV among certain breeds.

Implications

Our results indicate horn fly numbers are associated with month of horn fly population counts and breed type. Continued research utilizing more breed types and different geographic locations is needed to elaborate on the current findings. However based on these findings, selection of specific breed types with reduced horn fly populations may be useful. The relationship of horn flies on temperament data is being analyzed further to determine the exact effects of horn fly populations associated with temperament measurement techniques such as exit velocity and chute score.

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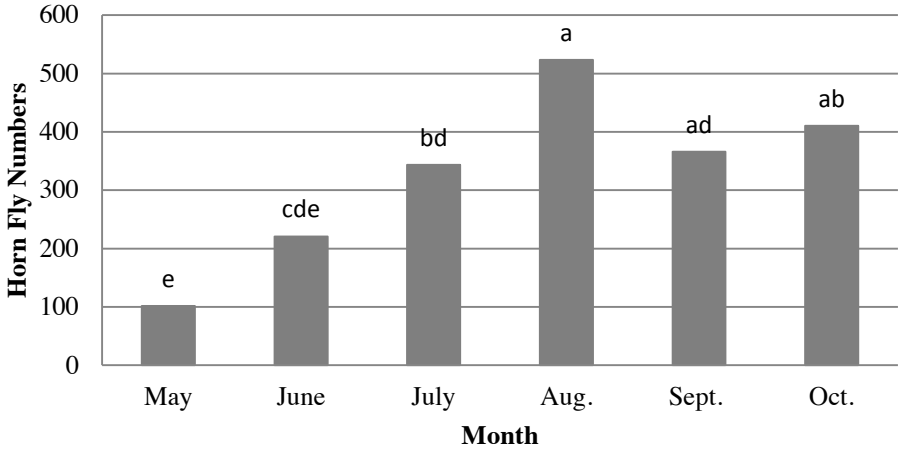

Fig. 1. Effect of month on horn fly population. Bars with different letters are significantly different ($P < 0.001$).

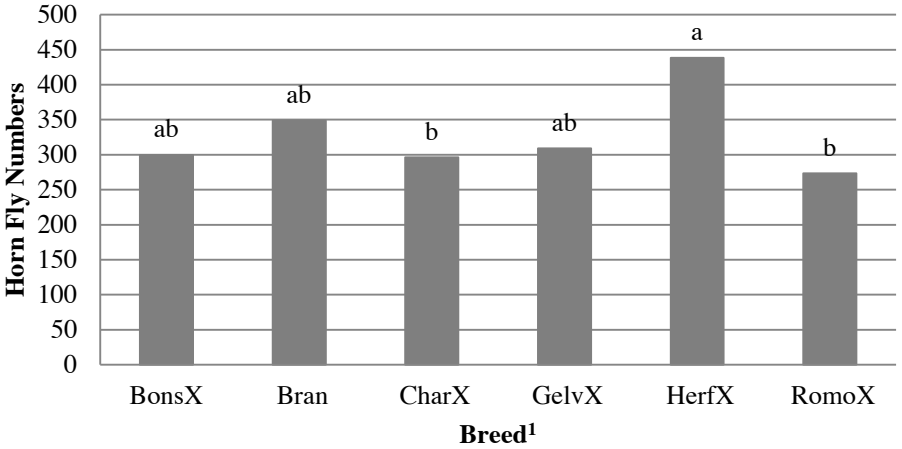

Fig. 2. Incidence of horn flies on various beef cow breed types. Bars with different letters are significantly different ($P = 0.02$).
[1] BonsX = Bonsmara-sired cows; Bran = Brangus; CharX = Charolais-sired cows; GelvX = Gelbvieh-sired cows; HerfX = Hereford-sired cows; RomoX = Romosinuano-sired cows.

Performance by fall-born calves weaned in the morning or evening using either fenceline or traditional weaning methods

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Story in Brief

Using different weaning methods in conjunction with time of day calves are weaned may reduce the negative effects of separation from their dams and improve performance. The objective of our study was to determine the effects of time of day and weaning method on behavior and performance of fall-born calves. Crossbred fall-born calves (n = 94) were stratified by body weight, age, sex, and age of their dam and allocated randomly to 1 of 8 groups 2 weeks prior to weaning. The groups were assigned to treatments consisting of: 1) Fenceline AM (FenAM; 2 replications); 2) Fenceline PM (FenPM; 2 replications); 3) Traditional AM (TradAM; 2 replications); 4) Traditional PM (TradPM; 2 replications). Calves vocalized more ($P \leq 0.05$) from AM compared with PM and from traditional compared with fenceline weaned. Average daily gain and calf gain (14 days) was greater ($P < 0.05$) from PM compared with AM and average daily gain and calf gain tended ($P = 0.08$) to be greater from fenceline compared with traditional weaning. Therefore, weaning fall-born calves in the evening may be more desirable, resulting in fewer calves vocalizing and greater calf weight gains. Fenceline weaning may improve some performance measurements.

Introduction

Weaning is one of the most important phases in cattle production. Traditionally, this is done by abruptly separating calves from their dams. However, fenceline weaning has gained popularity in recent years over traditional methods because calves vocalize less, spend more time eating (Stookey et al., 1997), and have increased average daily gain (ADG) (Price et al., 2003). Another factor that may impact weaning performance is time of day calves are weaned. In pigs, weaning in the evening increased feed intake by 5% and ADG by 6% over 28 days compared with morning weaning (Ogunbameru et al., 1992). Therefore, our objective was to determine the effects of weaning method (fenceline vs. traditional) and time of day (AM vs. PM) on behavior and performance of fall-born calves.

Materials and Methods

This study was conducted at the University of Arkansas Livestock and Forestry Research Station near Batesville, Ark. Crossbred fall-born calves (n = 94) were stratified by body weight, age, sex, and age of their dam and allocated randomly to 1 of 8 groups approximately 2 weeks (April 9, 2012) prior to weaning. The groups were assigned to treatments consisting of: 1) Fenceline AM (FenAM; 2 replications); 2) Fenceline PM (FenPM; 2 replications); 3) Traditional AM (TradAM; 2 replications); and 4) Traditional PM (TradPM; 2 replications). On April 23, 2012 all calves assigned to PM treatments were gathered at 1730 h, separated from their dams, weighed, and either placed in 4-acre paddocks adjacent to their dams or in 1-acre drylots away from their dams for 14 d. On April 24, 2012 all calves assigned to AM treatments were gathered at 0730 h and handled the same as the PM treatment groups. During the weaning period, all groups had ad libitum access to water, commercially available trace mineral salt, and were offered 2 lb/hd/d of dried distiller's grains; and in addition the traditional weaned groups were offered medium quality hay. At approximately 12, 24, 48, and 72 h after weaning, each group was evaluated for 10 minutes for vocalization and either walking rapidly, running, standing, or lying down. After the 14-d weaning period, calves were gathered and reweighed at 0730 and 1730 h for AM and PM, respectively.

Statistical Analysis. Behavioral measurements were treated as repeated measures and analyzed using PROC MIXED of SAS (SAS Institute, Inc., Cary, N.C.) where each group of animals in a specific pen/pasture was considered the experimental unit. Sampling time was considered the repeated measurement. Three pre-planned orthogonal contrasts were used to compare 1) mean of AM with the mean of PM, 2) mean of fenceline with the mean of traditional, and 3) interaction of weaning time and method. When a treatment × sampling time interaction was detected ($P \leq 0.05$), mean separations were performed using a Fisher's protected *t*-test. All data are reported as percentages of the total observations per treatment using least squares means.

Calf performance measurements were analyzed using PROC MIXED of SAS. The group of animals in a specific pen/pasture was considered the experimental unit and the same contrast statements were used as with behavioral measurements. Treatment means are reported as least squares means. Differences referred to as tendencies are those having a P-value between 0.05 and 0.10.

Results and Discussion

The percentage of calves walking rapidly, standing, or lying down did not differ ($P \geq 0.23$) across treatments (Table 1). A sampling time effect was detected ($P = 0.05$) for percentage of calves vocalizing, but interactions of treatment and sampling time were not detected ($P = 0.11$). Therefore, these data were pooled across sampling time. Percentage of calves vocalizing were greater ($P = 0.01$) from

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AM compared with PM (67% vs. 42% average, respectively) and from traditional compared with fenceline (62% vs. 46% average, respectively) weaning. A treatment × sampling time tendency ($P = 0.07$) was detected for percentage of calves running. When analyzed within sampling time, percentage of calves running did not differ ($P > 0.10$) across treatments at 48 and 72 h. However, percentage of calves running tended ($P \leq 0.10$) to be greater than the other treatments from TradPM at 12 h and from TradAM at 24 h.

Calf weights at weaning and end of the weaning period did not differ ($P \geq 0.46$) across treatments (Table 2). Calf gain and ADG during the 14-d weaning period were greater ($P < 0.05$) from PM compared with AM and tended ($P \leq 0.10$) to be greater from fenceline compared with traditional weaning. Daily gains by calves in this study were comparable across treatments with that previously reported by fall-born calves either fenceline or drylot weaned (Price et al., 2003).

Implications

Based on these results, fenceline weaning may result in fewer calves vocalizing during the weaning period than traditional weaning. Weaning calves in the late afternoon may reduce the number of calves vocalizing and may increase calf gains over the weaning period, which may benefit producers that sell calves to a cash market shortly after weaning.

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Table 1. Behavioral observations by fall-born calves weaned in the morning (AM) or evening (PM) using either fenceline or traditional weaning methods.

	Treatments[a]					Contrasts[b]		
Item[c]	FenAM	FenPM	TradAM	TradPM	SEM[d]	AM vs. PM	Fen vs. Trad	Interaction
Vocalizations, %[e]	54	39	80	45	5.7	0.01	0.05	0.13
Walking rapidly, %	4	1	34	13	14.6	0.46	0.23	0.60
Running, %	5	2	33	9	7.4	0.15	0.08	0.24
12	0^{h}	0^{h}	9^{gh}	37^{g}	13.4	–	–	–
24	0^{h}	0^{h}	75^{f}	0^{h}	13.4	–	–	–
48	0^{h}	8^{gh}	31^{gh}	0^{h}	13.4	–	–	–
72	21^{gh}	0^{h}	17^{gh}	0^{h}	13.4	–	–	–
Standing, %	100	95	98	96	2.8	0.26	0.86	0.61
Lying down, %	7	11	3	10	4.1	0.24	0.56	0.72

[a] FenAM = fenceline AM, FenPM = fenceline PM, TradAM = traditional AM, and TradPM = traditional PM.
[b] P-values for orthogonal contrasts comparing the mean of AM with the mean of PM, mean of fenceline with mean of traditional, or interaction of weaning time and method.
[c] Behavior variables were recorded for 10 minutes per group at 12, 24, 48, and 72 hours after weaning was initiated.
[d] SEM = Pooled standard error of the mean.
[e] Sampling time effect was detected ($P = 0.05$).
[fgh] Means in a row without a common superscript tended to differ ($P \leq 0.10$).

Table 2. Performance by fall-born calves weaned in the morning (AM) or evening (PM) using either fenceline or traditional weaning methods.

	Treatments[a]					Contrasts[b]		
Item	FenAM	FenPM	TradAM	TradPM	SEM[c]	AM vs. PM	Fen vs. Trad	Interaction
Calf weights, lb								
at weaning	478	482	500	490	21.2	0.86	0.46	0.81
at end weight[d]	504	524	512	522	22.5	0.54	0.90	0.83
Average daily gain, lb	2.0	3.1	0.9	2.3	0.42	0.04	0.08	0.73
Calf gain, lb	28	43	13	32	5.8	0.04	0.08	0.73

[a] FenAM = fenceline AM, FenPM = fenceline PM, TradAM = traditional AM, and TradPM = traditional PM.
[b] P-values for orthogonal contrasts comparing the mean of AM with the mean of PM, mean of fenceline with mean of traditional, or interaction of weaning time and method.
[c] SEM = Pooled standard error of the mean.
[d] End weight was the weight measured 14 d after weaning was initiated.

Genetic parameter estimates for susceptibility/resistance to Infectious Bovine Keratoconjunctivitis (IBK) in Angus calves

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Story in Brief

Pre-weaning records in Angus calves (n = 843) were used to obtain genetic parameter estimates for susceptibility/resistance to Infectious Bovine Keratoconjunctivitis (IBK). The single trait analysis, genetic, environmental, and phenotypic variances for IBK were 0.0778, 0.09099, and 0.09877, respectively. Estimates of heritability and environmental variance were 0.08 ± 0.074 and 0.92 ± 0.074, respectively. From the two trait analysis, genetic, environmental and phenotypic variation of IBK with birth weight were 0.27 ± 0.39, -0.03 ± 0.10, and 0.02 ± 0.03, respectively. The environmental and phenotypic correlations of IBK with weaning weight were -0.29 ± 0.10 and 0.05 ± 0.03, respectively. In these data, the heritability of IBK was low; however, because of the small sample size additional data may be required to further explain the inheritance of resistance/susceptibility in calves to IBK.

Introduction

Infectious Bovine Keratoconjunctivitis (IBK) is a serious eye disease of cattle. Infectious Bovine Keratoconjunctivitis impacts cattle of all ages, with its greatest impact being reduced performance of calves during the preweaning period (Snowder et al., 2005). Greater than 29% of cattle operations surveyed by the National Animal Health Monitoring System (NAHMS) in 1998 reported IBK as an economically important disease. Infectious Bovine Keratoconjunctivitis affects 10 million calves in the U. S. noting an estimated economic loss of more than $150 million (Richey, 2003). Incidence of IBK has been observed to be greater for bull calves (Ward and Nielson, 1979) and greater for *Bos taurus* genetic types relative to *Bos indicus* genetic types (Snowder, 2005). Infectious Bovine Keratoconjunctivitis impacts marketability of breeding cattle, particularly bulls, because they utilize their sense of vision to detect females in estrus (Geary and Reeves, 1992). Increased IBK incidence has been associated with reduced eyelid pigmentation. Published estimates of genetic parameters involving incidence of IBK are limited. Snowder et al. (2005) reported an overall direct heritability of 0.22 ± 0.02, while direct heritability was 0.25 ± 0.04 for the Angus breed. Due to the evident issues related to IBK, and because of the extensive cow-calf production in the southern U.S., coupled with the popularity of the Angus breed, a genetic study of resistance/susceptibility of Angus calves at weaning could aid in establishing selection programs for this trait. The objective of this study was to determine genetic parameter estimates for resistance/susceptibility to IBK.

Materials and Methods

Calves were born in the spring and fall at three Arkansas locations in 2009 and 2010 under procedures of objective 1a, Southern Regional Project, S1045. All calves were sired by purebred Angus bulls registered with the American Angus Association, one of which was Bon View New Design 878, the in common sire among locations. At weaning, incidence of IBK was determined using a subjective scoring system where 0 = no evidence of IBK in either eye, and 1 = evidence of IBK in one or both eyes. Scarring occurred in 19.6% of calves. Heritability, genetic, environmental, and phenotypic correlations were determined using variance component obtained with a single and two-trait animal model and MTDFREML. Fixed effects of contemporary group generated by birth year, season of birth, location and sex were included in the mixed model procedures. Age of dam and age of calf at weaning were included as covariates. Standard errors for the phenotypic correlations were estimated using residuals from the mixed model analysis.

Results and Discussion

Infectious Bovine Keratoconjunctivitis occurred in 5% of fall born calves while 30% of spring born calves had evidence of clinical IBK. Non-Infectious Bovine Keratoconjunctivitis calves had greater ($P < 0.05$) adjusted mean weaning weight than calves with IBK (268 ± 2.1 vs. 250 ± 3.9 Kg). Single trait analysis, genetic, environmental, and phenotypic variances for IBK were 0.0778, 0.09099, and 0.09877, respectively. Estimates of heritability and environmental variance were 0.08 ± 0.074 and 0.92 ± 0.074, respectively. From the two trait analysis, genetic, environmental and phenotypic variation of IBK with birth weight were 0.27 ± 0.39, -0.03 ± 0.10, and 0.02 ± 0.03, respectively. The environmental and phenotypic correlations of IBK with weaning weight were -0.29 ± 0.10 and 0.05 ± 0.03, respectively.

Implications

Phenotypic variation in the incidence of IBK presents the producers the possibility of artificial selection for IBK resistant animals leading to several advantages including recovery of the annual cost of treating this malady and reduced antibiotic dependency. Furthermore, producers could recover losses due to poor animal performance. As a result, producers would see economic benefits, while public safety concerns regarding the environment and food would be reduced.

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Influence of maternal environment during conception and late-gestation, and heifer fescue cultivar during development on growth and fertility of crossbred beef heifers

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Story in Brief

The objective was to determine effects of maternal forage type grazed during conception, cow body condition during conception and late-gestation, and heifer fescue cultivar grazed during development on heifer growth and fertility. Brahman-influenced cows (body condition score (BCS) = 5.6 ± 1.3) were assigned to graze common bermudagrass or toxic endophyte-infected tall fescue for a 60-d breeding season during 2 y. Cow body condition was assessed at d 0, 30, and 60 of breeding season. Cows were managed to achieve marginal (BCS = 4.2 ± 0.8) or good (BCS = 6.3 ± 0.8) body condition during the last trimester of pregnancy. At 9 to 10 mo of age, Angus-sired (1/4 to 3/8 Brahman) heifers (n = 80) from cows managed for marginal or good BCS were weighed and randomly assigned to replicated pastures of toxic or non-toxic endophyte-infected tall fescue for 190 d. Heifer body weight and growth data were collected at initiation of grazing (9 to 10 mo), yearling (11 to 12 mo), and prebreeding (13 to 14 mo). Heifer antral follicle count was determined by ultrasound at yearling and prebreeding. Maternal conception body condition × conception forage × late-gestation body condition × heifer fescue grazed during development 4-way interaction affected ($P < 0.0001$) heifer average daily gain (ADG), pelvic height, antral follicle count, and estrous activity. Heifers from cows in good body condition during conception had more antral follicles than heifers from cows in marginal body condition. Heifer pregnancy rate was influenced ($P < 0.05$) by cow body condition during last trimester × fescue cultivar grazed during heifer development (90.3%, 69.6%, 50.0%, 43.8 % pregnant: heifers grazing non-toxic fescue from cows in good body condition during the last trimester of pregnancy, heifers grazing non-toxic fescue from cows in marginal body condition during the last trimester of pregnancy, heifers grazing toxic fescue from cows in good body condition during the last trimester of pregnancy, and heifers grazing toxic fescue from cows in marginal body condition during the last trimester of pregnancy, respectively). Ensuring adequate body condition of cows during conception and late-gestation will increase pelvic growth, antral follicles, and pregnancy rates of heifer offspring.

Introduction

Maternal nutrition during conception and late-gestation could impact postnatal growth, development, and subsequent reproductive performance of offspring (Funston et al., 2010). Nutrient restricted cows often lose body condition (BC) during pregnancy and post-partum, which might impact offspring growth (Funston et al., 2010). Cattle grazing toxic endophyte-infected tall fescue (E+) can suffer fescue toxicosis which is characterized by elevated body temperature, reduced feed intake, and decreased average daily gain (ADG) (Paterson et al., 1995). Watson et al. (2004) demonstrated BCS of cows grazing E+ was lower, cow ADG was reduced, and cows birthed lighter calves. Heifers grazing E+ have suppressed growth which could be detrimental to reproductive processes including reduced pregnancy rates (Paterson et al., 1995). Fewer antral follicles could impact fertility as antral follicle count has been positively associated with fertility in cattle (Burns et al., 2005).

Research has demonstrated cattle grazing non-toxic endophyte-infected tall fescue do not exhibit signs of fescue toxicosis, indicating improved performance (Watson et al., 2004). Our objective was to determine effects of maternal forage type grazed during conception, cow body condition during conception and late-gestation, and heifer fescue cultivar during development on subsequent growth and fertility of beef heifers.

Materials and Methods

The committee for animal welfare at the USDA-ARS, Dale Bumpers Small Farms Research Center, Booneville, Arkansas, and the University of Arkansas Institutional Animal Care and Use Committee (No. 040200) approved animal procedures used in this study.

Cow Forage Management. To investigate the effects of maternal body condition and breeding season forage type grazed on beef heifer growth and fertility during development, Brahman-influenced cows [n = 80; BCS = 5.6 ± 1.3] were assigned, during a 60-d breeding season, to graze (1 cow/ 0.7 ha) either common bermudagrass [CB; *Cynodon dactylon* (L.) Pers.; n = 3 (16 ha) pastures/yr] or toxic tall fescue [E+; *Lolium arundinaceum* (Schreb.) Darbysh.; n = 3 (16 ha) pastures/yr].

Pastures of both forage types were established >20 yr and E+ pastures were >85% endophyte-infected. Pastures were characterized three times (d 0, 30, and 60) during the breeding season to determine forage mass, nutritive value, and concentration of ergovaline. Nutritional analysis of forage was carried out on random samples (20 samples/16 ha) collected at each sampling date. Dried forage samples were ground to pass through a 0.85-mm screen and analyzed for CP by rapid combustion (AOAC, 1990; Elementar Americas Inc., Mt. Laurel, N.J.) and NDF using Ankom Technology (Macedon, N.Y.) by the Agricultural Diagnostic Service Laboratory, University of Arkansas, Fayetteville. High-performance liquid chromatography was utilized to determine ergovaline on pooled samples taken from E+ pastures (20 samples/16 ha of pasture) cut into 5.1-cm pieces, and was stored at -4 °C until determination.

Cow Management. Cow body condition (where 1 = emaciated to 9 = obese; Watson et al., 2004) was assessed at d 0 (ConBC = cow BC during conception), 30, and 60 of the breeding season. From May 8 to July 11 (yr 1) and from May 8 to July 9 (yr 2), cows were exposed to Angus bulls (1 bull/ 20 cows) previously evaluated for breeding

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soundness. All cows grazed CB for 145 d following the 60-d breeding season, after which all cows were managed to achieve marginal (BCS = 4.2 ± 0.8) or good (BCS = 6.3 ± 0.8) BC (LateBC = cow BC during last trimester of pregnancy), and grazed stockpiled and spring-growth E+ at a stocking rate of 1 cow/ 0.3 ha (marginal BC; body weight (BW) = 1017 ± 178 lb; BCS = 4.2 ± 0.8) or 1 cow/ 0.8 ha (good BC; BW = 1287 ± 97 lb; BCS = 6.3 ± 0.8) for 150 d during the last trimester of pregnancy. Calving dates of cows ranged from February 11 to April 9 (yr 1) and from February 2 to April 9 (yr 2).

Heifer Forage Management. In two subsequent years, forty Angus-sired (1/4 to 3/8 Brahman) heifers (n = 80) were weighed (initial body weight = 528 ± 65.3 lb) at weaning and managed as a single herd on CB pasture (39.5 acres) for 64 ± 2 d until the start of the grazing study. At the initiation of the study, heifers (9 to 10 mo of age) were blocked by weight and randomly assigned (4 head/ pasture) to graze replicated pastures (2.5 acres) of E+ or non-toxic (Novel) endophyte-infected tall fescue (Hfes = heifer fescue cultivar grazed during development). Pastures consisted of three Kentucky-31 endophyte-infected tall fescue (E+), four HiMag (Novel) tall fescue with strain 4 endophyte (HiMag4) and four (three pastures in yr 2; Novel) Jesup tall fescue with the AR542 endophyte strain (MaxQ™).

Heifer Weight and Growth Measurements. Heifer weaning weight (7 to 8 mo), post-weaning weight (9 to 10 mo), yearling weight (10 to 11 mo), and prebreeding weight (13 to 14 mo) were collected. In addition to body weight, and chute exit velocity (EV; s/m), heifer growth measurement data were collected including: hip height (HH), hip width (HW), pelvic height (PH), pelvic width (PW), and pelvic area (PA). Exit velocity (rate at which the heifers exited the squeeze chute and traversed 1.83 m) was measured using two infrared sensors (FarmTek, Inc., North Wiley, Texas) and recorded as time (s)/distance (m). Hip height was measured using a sliding caliper. (Altitude Stick, NASCO, Fort Atkinson, Wis.). Hip width was measured by firmly closing a clamp over the edge of the hook bones and measuring the distance between the clamp ends. Measurements for PH and PW were obtained per rectum using a Rice Pelvimeter (Lane Manufacturing, Denver, Colo.). Pelvic height was determined as the vertical distance between the pubis symphysis and the sacral vertebrae. Pelvic width was determined as the horizontal distance between the shafts of the ilia. Pelvic area was calculated as the product of PH × PW.

Heifer Reproductive Traits. Antral follicle count (AFC; total number of follicles >3 mm in diameter on both ovaries, Burns et al., 2005) of heifers was determined by an individual technician using real-time ultrasonography (Aloka 500 V®; Corometrics, Wallingford, Conn., equipped with a 7.5-MHz transducer) twice each at yearling (d 73 ± 2 and 85 ± 2) and prebreeding (d 175 ± 2 and d 187). Heifers received 100 mg GnRH (Cystorelin, Merial LLC, Duluth, Ga.) on d 187 along with a controlled internal drug-releasing device (CIDR; Pfizer Animal Health, New York, N.Y.) for 7 d prior to 60 d breeding season, then administered PGF2α at removal of CIDR. On d 194, all heifers were fitted with a radio-telemetry (HeatWatch, Cow Chips LLC, Denver, Colo.) transmitter. All heifers were removed from fescue pastures and maintained as a single herd on CB pasture (16 ha) with three Angus bulls, previously evaluated for breeding soundness, during the 60 d breeding season until pregnancy diagnosis 60 d post-breeding season. Estrous activities were recorded for each heifer during the first 30 d of breeding season and included date and time of the onset of estrus, number of mounts received, duration (h) of estrus, as well as the quiescent (no estrus activity between two individual mounts) period between. Heifer pregnancy was determined by ultrasound 60 d after bull removal.

Statistical Analyses. Heifer growth measurements were analyzed by ANOVA within a split-plot design. Growth measurements, estrous behavior and AFC were analyzed using the mixed procedure of SAS (SAS Institute, Cary, N.C.) with pasture within year as the experimental unit, heifer age as a covariate, and heifer within year as a repeated measure for testing the main effects of maternal forage grazed during conception (ConFor = cow forage grazed during conception; E+ or CB), conception BC (ConBC), BC during the last trimester of pregnancy (LateBC), and heifer fescue cultivar grazed during development (Hfes = heifer fescue grazed during development; E+ or Novel) on heifer BW, growth measurements (HH, HW, PH, PW, and PA), and estrous behavior (mounts, duration, and quiescence). Percentage of heifers exhibiting estrus during the first 30 d of the breeding season and pregnancy rate were analyzed by chi-squared analysis of SAS.

Results and Discussion

Heifer Body Weight and Average Daily Gain. Heifer BW was ($P < 0.0001$) influenced by age of heifer. Interaction between ConBC × ConFor × LateBC × Hfes affected ($P < 0.0001$) heifer BW. Overall ADG of heifers was influenced ($P = 0.03$) by age. Heifer ADG was ($P < 0.0001$) affected by ConBC × ConFor × LateBC × Hfes 4-way interaction (Fig. 1). Overall ADG ($P < 0.0001$) was greater for heifers developed on Novel (1.2 ± 0.02 lb) compared to heifers grazing E+ (0.8 ± 0.02 lb) during development.

Heifer Growth Characteristics. Heifer HH was not ($P = 0.18$) affected by heifer age. Age of heifer influenced ($P = 0.003$) heifer HW. Hip height and HW increased ($P < 0.0001$) over time but were not altered by the interaction between ConBC × ConFor × LateBC × Hfes. Heifer PH ($P = 0.89$) and PA ($P = 0.12$) were not influenced by age of heifer. Heifer PH increased ($P < 0.0001$) over time. Interaction between ConBC × ConFor × LateBC × Hfes affected ($P < 0.0001$) heifer PH. Pelvic height of E+ heifers (37.0 ± 1.9 in) from cows in marginal-ConBC, grazing E+ ConFor, and in good-LateBC was greater compared to PH of E+ heifers from cows in marginal-ConBC, grazing CB ConFor, and in good-LateBC (26.1 ± 1.5 in). Heifer PW was influenced ($P = 0.001$) by age of heifer. Heifer PW and PA increased ($P < 0.0001$) over time but were not altered by the interaction between ConBC × ConFor × LateBC × Hfes.

Chute exit velocity was not ($P = 0.67$) influenced by age of heifer. Yearling heifer EV was ($P = 0.002$) affected by the ConBC × ConFor × Hfes 3-way interaction. Heifers developed on E+ (23.4 ± 11.9 s/m) from cows in good-ConBC, grazing CB during conception exited the chute the fastest compared to E+ heifers from cows in marginal-ConBC, grazing CB during conception (105.3 ± 13.6 s/m). Post-weaning and prebreeding heifer EV was not affected by the ConBC ($P = 0.32$), ConFor ($P = 0.86$), or Hfes ($P = 0.78$).

Heifer Reproductive Traits. Antral follicle count was influenced by scan date ($P < 0.0001$). Age of heifer did not ($P = 0.54$) affect yearling heifer AFC. Prebreeding heifer AFC was ($P = 0.05$) influenced by age of heifer. Yearling heifer AFC was ($P < 0.0001$) affected by the ConBC × ConFor × LateBC × Hfes interaction (Table 1). Novel heifers (25.1 ± 0.8 follicles) from cows in good-ConBC, grazing CB ConFor and in marginal-LateBC had the most antral follicles at yearling compared to E+ heifers from cows in marginal-ConBC, grazing CB ConFor and in good-LateBC (3.8 ± 2.4 follicles). Prebreeding heifer AFC was ($P = 0.002$) affected by the ConBC × ConFor × LateBC × Hfes interaction (Table 1). Heifers developed on E+ (24.4 ± 1.3 follicles) from cows in good-ConBC, grazing E+ ConFor and in good-LateBC had the most antral follicles at prebreeding compared to Novel heifers from cows in good-ConBC, grazing E+ ConFor and in marginal-LateBC (12.8 ± 1.3 follicles).

Interaction between ConFor and Hfes affected (P = 0.02) the percentage of Novel heifers exhibiting estrus as recorded by HeatWatch during the first 30 d of the breeding season (Table 2). More heifers exhibited estrus that were developed on Novel from cows grazing E+ during conception (83%) compared to Novel heifers from cows grazing CB during conception (54%). Interaction between ConBC and Hfes did not ($P > 0.13$) affect the percentage of E+ heifers exhibiting estrous. Interaction between LateBC and Hfes did not ($P > 0.74$) affect the percentage of Novel or E+ heifers exhibiting estrus. Number of mounts during estrous was ($P < 0.0001$) affected by the ConBC × ConFor × LateBC × Hfes interaction (Table 3). Novel heifers (24.9 ± 1.7 mounts) from cows in good-ConBC, grazing E+ ConFor and in marginal-LateBC had the most mounts during estrous compared to E+ heifers from cows in good-ConBC, grazing E+ ConFor and in marginal-LateBC (7.6 ± 2.0 mounts). Duration of estrus was ($P = 0.02$) affected by the ConBC × ConFor × LateBC × Hfes interaction (Table 3). Novel heifers (9.0 ± 0.6 hr) from cows in marginal-ConBC, grazing CB ConFor and in good-LateBC had the longest duration of estrus compared to E+ heifers from cows in marginal-ConBC, grazing E+ ConFor and in good-LateBC (2.6 ± 1.4 hr). Heifer quiescence was ($P < 0.0001$) affected by the ConBC × ConFor × LateBC × Hfes interaction (Table 3). Heifers developed on E+ from cows in marginal-ConBC, grazing E+ ConFor and in good-LateBC had the shortest (16.8 ± 8.4 min) quiescence, while E+ heifers from cows in good-ConBC, grazing CB ConFor and in marginal-LateBC had the longest (62.4 ± 4.2 min) quiescence.

Interaction between ConBC and Hfes did not ($P > 0.43$) affect the percentage of heifers pregnant. Interaction between ConFor and Hfes did not ($P > 0.20$) affect the percentage of heifers pregnant. Interaction between ConBC × ConFor × LateBC × Hfes is presented in Table 4. Heifer pregnancy rate was influenced by LateBC × Hfes (90.3, 69.6, 50.0, 43.8 % pregnant: Novel heifers from cows in good-LateBC, Novel heifers from cows in marginal-LateBC, E+ heifers from cows in good-LateBC, and E+ heifers from cows in marginal-LateBC, respectively).

The quality of maternal nutrition during conception and late-gestation could impact postnatal growth, development, and subsequent reproductive performance of offspring (Funston et al., 2010). Since the discovery of toxins in tall fescue, research has focused on production implications of livestock grazing E+ and the effects of this endophyte infected grass on different aspects of animal agriculture. Overall ADG of heifers developed on Novel was greater compared to heifers developed E+, which is consistent with previous research (Watson et al., 2004). Heifers developed on E+ from cows in marginal-BC during conception and from cows in marginal-BC during late gestation had significantly lower ADG. The double insult to these heifers of being born from thin cows and then developed on E+ most likely contributed to their reduced ADG.

Maternal environment has an important role in the regulation of and or variation in ovarian reserve as measured by AFC, as AFC has been positively associated with fertility in cattle (Burns et al., 2005). Maternal nutrient restriction during conception and the first trimester of pregnancy (the period encompassing the peak in fetal oocyte numbers) resulted in 60% lower AFC in heifers born to the nutrient restricted cows (Mossa et al., 2009). Mean AFC in the current study was lower in heifers from cows in marginal-BC during conception compared to heifers from cows in good-BC during conception. Our data are consistent with previous research in that cattle consuming E+ forage have reduced pregnancy rates (Paterson et al., 1995).

Heifers developed on toxic tall fescue reduced heifer growth compared to heifers grazing Novel fescue during development. Growth of E+ heifers was further decreased by a marginal-maternal-BC during conception and maternal nutrient restriction during late-gestation. Variations in maternal environment negatively impacted ovarian reserve, specifically noted by the significant effect of cow body condition during conception on AFC of heifer offspring. Overall pregnancy rates were lower for heifers developed on E+ compared to Novel, further impacted by maternal BC during conception and late-gestation.

Implications

Utilizing growth indicators such as skeletal body measurements in combination with non-toxic endophyte-infected tall fescue for development of replacement beef heifers can result in increased ADG. Ensuring cows are in adequate body condition during conception and late-gestation can result in increased pelvic growth, more antral follicles, and improved pregnancy rates of heifer offspring.

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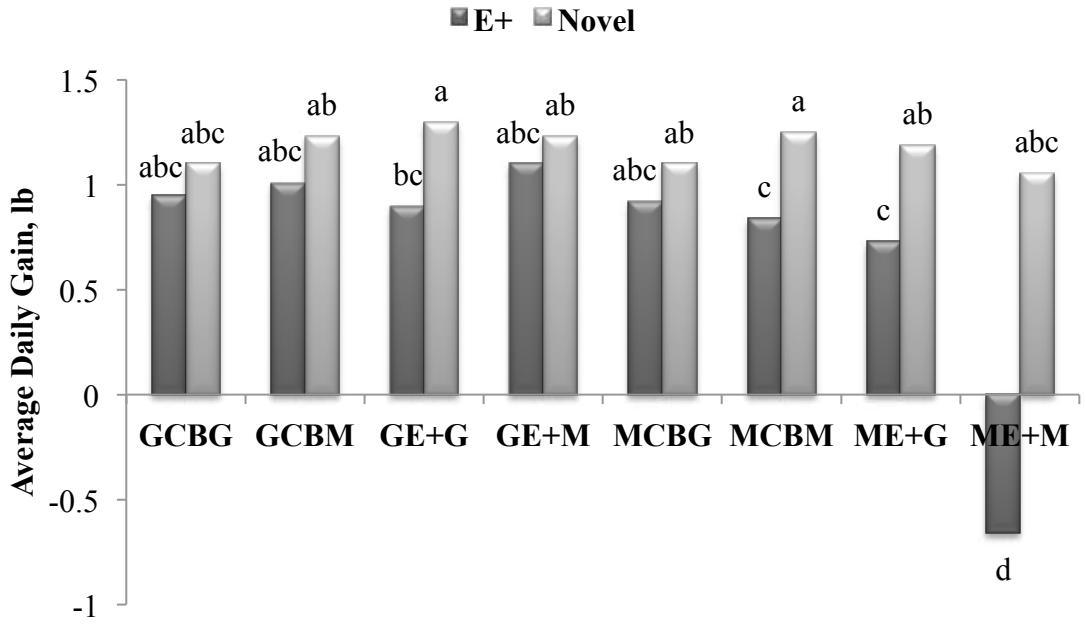

Fig. 1. Heifer average daily gain (ADG) during development effected by the interaction between conception body condition (BC) of cow (ConBC) x cow forage grazed during conception (ConFor) x cow body condition during late gestation (LateBC) x heifer fescue cultivar grazed during development. (*P* < 0.0001; SEM = 0.08 lb). ConBC = Good (BCS = 6.3 ± 0.8) or Marginal (BCS = 4.2 ± 0.8); Confor = common bermudagrass (CB) or toxic endophyte-infected tall fescue grazed (E+); LateBC = Good or Marginal; Heifer fescue cultivar = toxic (E+) or non-toxic (Novel) endophyte-infected tall fescue. Abbreviations: GCBG = good-ConBC, CB, good-LateBC; GCBM = good-ConBC, CB, marginal-LateBC; GE+G = good-ConBC, E+, good-LateBC; GE+M = good-ConBC, E+, marginal-LateBC; MCBG = marginal-ConBC, CB, good-LateBC; MCBM = marginal-ConBC, CB, marginal-LateBC; ME+G = marginal-ConBC, E+, good-LateBC; ME+M = marginal-ConBC, E+, marginal-LateBC.

Table 1. Heifer yearling and prebreeding antral follicle count (AFC) affected by the interaction between maternal conception body condition x maternal forage grazed during conception x maternal body condition during late gestation x heifer fescue grazed during development.

	Marginal-ConBC[1]								Good-ConBC[1]							
	CB[2]				E+[2]				CB				E+			
	Good-LateBC[3]		Marginal-LateBC[3]		Good-LateBC		Marginal-LateBC		Good-LateBC		Marginal-LateBC		Good-LateBC		Marginal-LateBC	
AFC[5]	E+[4]	Novel[4]	E+	Novel	E+	Novel	E+	Novel	E+	Novel	E+	Novel	E+	Novel	E+	Novel
Yr[5]	4^{d}	18^{c}	24^{ab}	19^{bc}	18^{bc}	19^{bc}	22^{ab}	18^{c}	25^{ab}	22^{ab}	25^{ab}	25^{ab}	23^{ab}	25^{ab}	20^{abc}	16^{c}
PBr[5]	N/A	15^{yz}	20^{xy}	17^{yz}	19^{xy}	16^{yz}	18^{yz}	16^{yz}	20^{xy}	18^{yz}	20^{yz}	19^{xy}	24^{x}	18^{yz}	14^{yz}	13^{z}

[1]ConBC = Cow body condition [BC; good (BCS = 6.3 ± 0.8) or marginal (BCS = 4.2 ± 0.8)] during conception.
[2]Cow conception forage type grazed [common bermudagrass (CB) or toxic endophyte-infected tall fescue (E+)].
[3]LateBC = Cow BC during last trimester of gestation.
[4]Heifer fescue cultivar grazed during development [E+ or non-toxic endophyte-infected tall fescue (Novel)].
[5]AFC = antral follicle count of heifers at yearling (Yr) and prebreeding (PBr).
[a,b,c,d]Means without common superscripts differ within day (Yr), between columns and rows ($P < 0.05$; pooled SE = 1.2).
[x,y,z] Means without common superscripts differ within day (PBr), between columns and rows ($P < 0.05$; pooled SE = 1.2).

Table 2. Percentage of crossbred beef heifers exhibiting estrous affected by the interaction between maternal conception body condition x maternal forage grazed during conception x maternal body condition during late gestation x heifer fescue cultivar grazed during development.

	Good-ConBC[1]							
	CB[2]				E+[2]			
	Good-LateBC[3]		Marginal-LateBC[3]		Good-LateBC[3]		Marginal-LateBC[3]	
	E+[4]	Novel[4]	E+	Novel	E+	Novel	E+	Novel
Estrus, % n =	66.7 (2/3)	100^{a} (8/8)	80.0 (4/5)	100^{a} (9/9)	100 (3/3)	90.0^{a} (9/10)	100 (2/2)	100^{a} (3/3)

	Marginal-ConBC[1]							
	CB[2]				E+[2]			
	Good-LateBC[3]		Marginal-LateBC[3]		Good-LateBC[3]		Marginal-LateBC[3]	
	E+	Novel	E+	Novel	E+	Novel	E+	Novel
Estrus, % n =	N/A	100^{a} (5/5)	80.0 (4/5)	100^{a} (5/5)	50.0 (1/2)	83.3^{a} (5/6)	0.0 (0/2)	50.0^{b} (2/4)

[1]ConBC = cow body condition [BC; good (BCS = 6.3 ± 0.8) or marginal (BCS = 4.2 ± 0.8)] during conception.
[2]Cow forage type grazed during conception [common bermudagrass (CB) or toxic endophyte-infected tall fescue (E+)].
[3]LateBC = cow BC during last trimester of pregnancy.
[4]Heifer fescue cultivar grazed during development [E+ or non-toxic endophyte-infected tall fescue (Novel)].
[a,b]Means without common superscripts tended ($P = 0.08$) to differ .
Means without superscripts did not differ ($P > 0.10$).

Table 3. Estrous activity of crossbred beef heifers affected by the maternal conception body condition x maternal forage grazed during conception x maternal body condition during late gestation x heifer fescue cultivar grazed during development interaction.

	Marginal-ConBC[1]								Good-ConBC[1]							
	CB[2]				E+[2]				CB				E+			
	Good-LateBC[3]		Marginal-LateBC[3]		Good-LateBC		Marginal-LateBC		Good-LateBC		Marginal-LateBC		Good-LateBC		Marginal-LateBC	
Item	E+[4]	Novel[4]	E+	Novel	E+	Novel	E+	Novel	E+	Novel	E+	Novel	E+	Novel	E+	Novel
Mnts	N/A	17^{b}	11^{bc}	12^{bc}	10^{bc}	15^{bc}	N/A	10^{bc}	16^{bc}	24^{a}	15^{bc}	21^{a}	9^{c}	14^{bc}	8^{c}	25^{a}
Dur, h	N/A	9^{j}	6^{j}	7^{j}	3^{k}	6^{j}	N/A	6^{j}	7^{j}	8^{j}	8^{j}	9^{j}	7^{j}	5^{k}	3^{k}	7^{j}
Quies, m	N/A	34^{yz}	37^{yz}	34^{yz}	17^{z}	29^{yz}	N/A	59^{x}	27^{yz}	22^{z}	60^{x}	32^{yz}	52^{xy}	26^{yz}	28^{yz}	20^{yz}

[1]ConBC = Cow body condition [BC; good (BCS = 6.3 ± 0.8) or marginal (BCS = 4.2 ± 0.8)] during conception.
[2]Cow conception forage type grazed [common bermudagrass (CB) or toxic endophyte-infected tall fescue (E+)].
[3]LateBC = Cow BC during last trimester of gestation.
[4]Heifer fescue cultivar grazed during development [E+ or non-toxic endophyte-infected tall fescue (Novel)].
[5]AFC = antral follicle count of heifers at yearling (Yr) and prebreeding (PBr).
[a,b,c,d]Means without common superscripts differ between columns and rows ($P < 0.05$; pooled SE = 1.6).
[j, k]Means without common superscripts differ between columns and rows ($P < 0.05$; pooled SE = 1.0).
[x,y,z] Means without common superscripts differ between columns and rows ($P < 0.05$; pooled SE = 4.6).

Table 4. Pregnancy rate of crossbred beef heifers affected by the maternal conception body condition x maternal forage grazed during conception x maternal body condition during late gestation x heifer fescue cultivar grazed during development interaction.

	Good-ConBC[1]							
	CB[2]				E+[2]			
	Good-LateBC[3]		Marginal-LateBC[3]		Good-LateBC[3]		Marginal-LateBC[3]	
	E+[4]	Novel[4]	E+	Novel	E+	Novel	E+	Novel
Pg, % n =	33.3 (1/3)	88.9 (8/9)	40.0 (2/5)	44.4 (4/9)	33.3 (1/3)	90.9 (10/11)	50.0 (1/2)	100 (4/4)

	Marginal-ConBC[1]							
	CB[2]				E+[2]			
	Good-LateBC[3]		Marginal-LateBC[3]		Good-LateBC[3]		Marginal-LateBC[3]	
	E+	Novel	E+	Novel	E+	Novel	E+	Novel
Pg, % n =	100 (1/1)	100 (5/5)	57.0 (4/7)	80.0 (4/5)	66.7 (2/3)	83.3 (5/6)	0.0 (0/2)	80.0 (4/5)

[1]ConBC = cow body condition [BC; good (BCS = 6.3 ± 0.8) or marginal (BCS = 4.2 ± 0.8)] during conception.
[2]Cow forage type grazed during conception [common bermudagrass (CB) or toxic endophyte-infected tall fescue (E+)].
[3]LateBC = cow BC during last trimester of pregnancy.
[4]Heifer fescue cultivar grazed during development [E+ or non-toxic endophyte-infected tall fescue (Novel)].

Season of collection and heat shock protein 70 haplotype influence semen quality characteristics of Holstein bulls

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Story in Brief

The objective was to determine season of collection and heat shock protein 70 haplotype effects on Holstein semen characteristics. Bulls (n = 26) were collected by artificial vagina, and ejaculates (n = 8964) evaluated for volume, total sperm, sperm concentration, motility, and potential number of breeding units calculated. Following extension and cryopreservation, post-thaw motility was evaluated visually. Bulls were haplotyped based on a heat shock protein 70 promoter sequence ("No SNP" = no SNP or the sequence does not differ from the published sequence; "Deletion" = cytosine deletion at nucleotide base 895; and "Yes SNP" = SNP other than Deletion). Distribution of Holstein bulls by heat shock protein 70 haplotype were 14, 8, and 4 for Deletion, No SNP, and Yes SNP haplotypes, respectively. Collection age (mature >2 yr; and young <2 yr) affected ejaculate volume, total sperm, sperm concentration, number of potential breeding units, and post-thaw motility. Interaction ($P < 0.0001$) between season of collection and haplotype affected mature bull ejaculate volume and total sperm. Interaction between season and haplotype on number of potential breeding units revealed mature Deletion bulls produced more ($P = 0.0002$) breeding units during spring (469 ± 40 units) and winter (474 ± 40 units) whereas Yes SNP bulls produced more units during summer (380 ± 72 units). Season by haplotype interaction affected young bull ejaculate volume ($P = 0.04$) and post-thaw motility ($P = 0.01$). Young Yes SNP bulls collected during winter had lower post-thaw motility (35 ± 5%) compared to Deletion (49 ± 1%) and No SNP (52 ± 2%). Haplotypes of heat shock protein 70 were associated with seasonal differences in semen characteristics. Incorporating heat shock protein 70 haplotypes into marker-assisted management could be beneficial in selecting breeding stock with consistent traits associated with successful reproduction.

Introduction

Environmental, management, and genetic factors were reported to impact semen quality particularly during stressful conditions (Mathevon et al., 1998). Stress alters cellular function in various tissues and heat shock protein 70 (Hsp70), a molecular chaperone, provides protection at the cellular level. Heat shock protein 70, located in reproductive tissues, has critical roles in events including spermatogenesis (Kamaruddin et al., 2004). Expression of Hsp70 was reported during developmental stages of male gametes (Kamaruddin et al., 2004). Seasonal effects can impact semen production resulting in decreased semen quality due to variations of semen characteristics such as ejaculate volume and sperm concentration (Mathevon et al., 1998).

Single nucleotide polymorphisms occurring in the Hsp70 promoter region may impact stress tolerance, and haplotypes of Hsp70 were related to cow fertility and heat tolerance (Rosenkrans et al., 2010; Basirico et al., 2011). Limited information is available investigating the effect of Hsp70 haplotype on bull semen quality. Therefore, our objective was to determine season of collection and Hsp70 haplotype effects on Holstein bull semen characteristics.

Materials and Methods

Test Animals. Data were obtained for Holstein bulls (n = 26) housed at Genex Cooperative, Inc. (a subsidiary of Cooperative Resources International) Artificial Insemination centers (Shawano, Wis.; Tiffon, Ohio; and Ithaca, N.Y.). Bulls categorized as young bulls (<2 yr of age) and mature bulls (>2 yr of age) were stimulated and ejaculates were collected by artificial vagina. Ejaculates (n = 8964) were evaluated for volume, sperm concentration, and motility. Proprietary laboratory software was utilized to record all evaluation data and calculate extension volume and number of potential breeding units. Ejaculates were treated with antibiotics in accordance with Certified Semen Service protocols (http://www.naab-css.org/about_css/CSSMinReqJan2011.rev.htm), and extended in milk-based extender before being packaged into straws and cryopreserved. Post-thaw motility was assessed visually using a light microscope with phase optics.

DNA Sequencing and Haplotypes. Genomic DNA was extracted from semen using the DNeasy® kit (QIAGEN Inc., Valencia, Calif.) and diluted to 20 ng/ µL. Bulls were haplotyped based on a 539-base sequence spanning a conserved region within the Hsp70 gene promoter (GenBank accession number M98823; base positions 749-1288) was amplified by polymerase chain reaction (PCR). Primers for Hsp70 promoter sequencing were [forward (5'-GCCAGGAAACCAGAGACAGA-3') and reverse (5'-CCTACGC AGGAGTAGGTGGT-3')]. 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 extension step consisted of 10 min at 68 °C. In each PCR, Approximately 100 ng genomic DNA, 10 pM forward primer, 10 pM of reverse primer and 43 µL of Platinum PCR Supermix (Invitrogen, Carlsbad, Calif.) for a total volume of 50 µL was used. A 2 µL sample of the amplicons were visualized via electrophoresis in 1.2% agarose gels containing 5 µL ethidium bromide in 1.0X Tris/Boric Acid/EDTA.

Amplication products were purified using QIAquick PCR purification kit (QIAGEN Inc., Valencia, Calif.). A Hoefer DyNA Quant 200 fluorometer (Amersham Biosciences Copr., Piscataway, N.J.) was utilized for DNA quantification following purification.

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Purified PCR products were sequenced at the University of Arkansas DNA Core Lab using ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif.). Both forward and reverse sequences were compared using the web-based software package ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html; European Bioinformatics Institute, Cambridge, U.K.). Homozygous and heterozygous alleles were identified by assessing sequence chromatograms using BioEdit.

Eleven Hsp70 SNP sites within the promoter region were evaluated and bulls were haplotyped based on the Hsp70 promoter reference sequence as described by Rosenkrans et al. (2010): No SNP = no SNP or sequence does not differ from the NCBI published sequence; Deletion = cytosine deletion at nucleotide base 895; Yes SNP = a SNP other than Deletion or sequence differs from the NCBI published sequence.

Statistical Analyses. Data were analyzed by ANOVA using mixed procedure of SAS (SAS Institute, Inc., Cary, N.C.). Main effects were season of collection, Hsp70 haplotype, and bull age group (mature or young). Based upon bull location, seasons were categorized as spring (April, May, June), summer (July, August, and September), fall (October and November), and winter (December, January, February, and March). For mature bulls, there were no Yes SNP haplotype bulls collected during the spring. Haplotype was used as a random component and season of collection as a fixed affect. The dependent variables of ejaculate volume, sperm concentration, total sperm, potential breeding units, initial (pre-freezing) motility, and post-thaw motility were evaluated.

Results and Discussion

Haplotype Identification and Distribution. Distribution of Holstein bulls by heat shock protein 70 haplotype were 14, 8, and 4 for Deletion, No SNP, and Yes SNP haplotypes, respectively. Six polymorphisms were identified in the 539-base segment of the bovine Hsp70 promoter region (Table 1). Of the six SNP, one deletion [C895D (cytosine deleted and not replaced with a nucleotide base; represented as D)]; two transitions [A1096G (adenine replaced with guanine)] and [T1204C (tyrosine replaced with cytosine)]; and three transversions [A1125C (adenine replaced with cytosine)], [G1128T (guanine replaced with tyrosine)], and [C1154G (cytosine replaced with guanine)] were identified. Alleles were noted as homozygous minor, heterozygous, or homozygous major and minor allele frequencies were calculated. The most prevalent SNP identified were T1204C (55.8%) and G1128T (52%; Table 1).

Ejaculate Volume. Interaction between season of collection and Hsp70 haplotype for mature Holstein bull ejaculate volume revealed Deletion bulls produced greater ($P < 0.0001$) ejaculate volume during spring and winter (Table 2). Young Yes SNP and No SNP bull ejaculate volume was greater ($P = 0.04$) compared to Deletion during fall (Table 2).

Sperm Concentration. Interaction between season of collection and Hsp70 haplotype did not differ for mature ($P = 0.33$) or young ($P = 0.95$) bull sperm concentration. Sperm concentration was not affected by haplotype for mature ($P = 0.65$) or young ($P = 0.66$) bulls. Season of collection affected sperm concentration of mature ($P < 0.002$) bulls. Mature bulls collected during fall (1215 ± 57 mil sperm/mL) had the lowest sperm concentration compared to spring (1286 ± 57 mil sperm/mL), summer (1262 ± 57 mil sperm/mL), and winter (1280± 57 mil sperm/mL). Young bull sperm concentration was affected ($P < 0.0001$) by season where more total sperm was produced during fall (1239 ± 110 mil sperm/mL) and winter (1226 ± 106 mil sperm/mL) compared to spring (1049 ± 108 mil sperm/mL) and summer (1039 ± 108 mil sperm/mL).

Total Sperm per Ejaculate. Interaction between season and haplotype affected total sperm per ejaculate of mature bulls (Table 3). Interaction ($P = 0.54$) between season and haplotype did not affect young bull total sperm. Total sperm of young bulls was not affected ($P = 0.84$) by haplotype. Season of collected affected ($P = 0.002$) total sperm of young bulls. Young bulls collected during fall (4.4 ± 0.6 bil sperm/ ejaculate) and winter (4.2 ± 0.6 bil sperm/ejaculate) produced more total sperm compared to during spring (3.5 ± 0.6 bil sperm/ejaculate) and summer (3.5 ± 0.6 bil sperm/ejaculate).

Number of Potential Breeding Units. Total number of potential breeding units was affected ($P = 0.0002$) by interaction between season and haplotype (Table 4). Interaction ($P = 0.64$) between season and haplotype did not affect number of potential breeding units of young bulls. Haplotype did not affect ($P = 0.81$) number of breeding units of young bulls. Season of collection affected ($P = 0.04$) number of potential breeding units of young bulls. Young bulls collected during winter (187 ± 27 units) produced more breeding units compared to fall (171 ± 28 units), summer (161 ± 27 units), and spring (152 ± 27 units).

Initial and Post-Thaw Motility. Initial motility of mature bulls was not affected ($P = 0.20$) by interaction between season and haplotype. Mature bull haplotype tended ($P = 0.07$) to affect initial motility. Mature Yes SNP bull initial motility (67 ± 2%) was lower than No SNP (71 ± 1%) and Deletion (72 ± 0.9%). Season of collection affected ($P < 0.0001$) initial motility of mature bulls. Mature bulls collected during spring (70 ± 0.6%) and summer (70 ± 0.6%) had greater initial motility compared to mature bulls collected during fall (69 ± 0.6%) and winter (69 ± 0.6%).Young bull initial motility was not affected ($P = 0.42$) by interaction between season and haplotype. Haplotype of young bulls did not affect (P = 0.31) initial motility. Season of collection tended ($P = 0.07$) to influence initial motility of young bulls. Young bull initial motility was lower during fall (67 ± 1%) compared to spring (68.8 ± 5%), winter (68 ± 1%), and summer (70 ± 1%).

Interaction ($P = 0.62$) between season and haplotype did not affect post-thaw motility of mature bulls. Post-thaw motility of mature bulls was not affected ($P = 0.18$) by haplotype. Season of collection affected ($P = 0.005$) mature bull post-thaw motility. Mature bulls collected during summer (53 ± 0.6%), fall (54 ± 0.6%) and winter (53 ± 0.6%) had greater post-thaw motility compared to mature bulls collected during spring (52 ± 0.6%). Young bull post-thaw motility was affected ($P = 0.01$) by interaction between season and haplotype where young Yes SNP bulls had the lowest post-thaw motility during the winter (Fig. 1).

Seasonal influences such as elevated temperature are related to variation in specific bull semen characteristics including ejaculate volume, sperm concentration, and motility (Mathevon et al., 1998). Semen characteristics can differ between young and mature bulls, and seasonal effects were shown to negatively affect semen quality of young bulls more than mature bulls (Mathevon et al., 1998).

Basirico et al. (2011) related haplotypes of Hsp70 to heat tolerance of dairy cows and beef cow fertility was affected by Hsp70 haplotypes (Rosenkrans et al., 2010). The seasonal and age-related effects on semen characteristics (Mathevon et al., 1998) and expression of Hsp70 during spermatogenesis (Kamaruddin et al., 2004) support our hypothesis that Hsp70 haplotypes affect sperm characteristics of Holstein bulls.

Shrum et al. (2010) demonstrated total sperm and motility were related to Hsp70 haplotype in young Brahman-influenced bulls collected during the summer. Shrum et al. (2010) reported Deletion bulls tended to have lower total sperm compared to No SNP and Yes

SNP during the summer. Total sperm of young Holstein bulls in our study did not differ by haplotype, but was influenced by season of collection. In another study, young No SNP Brahman-influenced bull motility tended to be greater than Yes SNP and Deletion (Shrum et al., 2010). Seasonal differences in initial motility were observed in the current study, but Hsp70 haplotype did not affect initial motility.

The results of this study are the first to report season of collection by Hsp70 haplotype influence semen characteristics of Holstein bulls. Haplotypes of Hsp70 are associated with seasonal differences in semen characteristics including ejaculate volume, sperm concentration, and motility which could impact fertility.

Implications

Total number of breeding units produced per ejaculate has production implications including increased progeny, increased revenue, and genetic progress. Identifying bulls with greater ejaculate volume, sperm concentration, and motility by season of collection and adjusting collection schedule could influence number of potential breeding units produced. Incorporating Hsp70 haplotypes into marker-assisted management could be beneficial in selecting breeding stock with consistent traits that are associated with successful reproduction.

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Table 1. The distribution of single nucleotide polymorphisms (SNP) of the bovine heat shock protein 70 promoter in Holstein bulls.

	Genotype Distribution[2]			
Polymorphism[1]	Homo	Hetero	homo	MAF[3]
C895D	12	7	7	40.4
A1096G	21	3	2	13.5
A1125C	12	4	10	46.2
G1128T	9	7	10	52.0
C1154G	21	3	2	13.5
T1204C	8	7	11	55.8

[1] Single nucleotide polymorphism occurred at the indicated number. Primary allele is indicated by the first letter and minor allele is indicated by the letter following the digits (D represents deletion of cytosine).
[2] The number of Holstein bulls homozygous for the primary allele (Homo), heterozygous (Hetero), and homozygous for the minor allele (homo).
[3] Minor allele frequency expressed as a percentage.

Table 2. The interaction between season of collection and heat shock protein 70 haplotype by bull age group (mature and young) for Holstein bull ejaculate volume (mL).

	Season of Collection			
Haplotype[1]	Spring	Summer	Fall	Winter
Mature[2] bulls				
Deletion	7.1 ± 0.6^{a}	6.7 ± 0.6^{b}	6.7 ± 0.6^{b}	7.1 ± 0.6^{a}
No SNP	6.5 ± 0.7^{b}	6.6 ± 0.7^{b}	6.4 ± 0.7^{b}	6.3 ± 0.7^{bc}
Yes SNP	N/A	4.9 ± 1.0^{c}	4.4 ± 1.0^{c}	5.0 ± 1.0^{c}
Young[3] bulls				
Deletion	3.2 ± 0.3^{b}	3.3 ± 0.3^{ab}	3.2 ± 0.3^{b}	3.5 ± 0.3^{a}
No SNP	3.5 ± 0.5^{a}	3.7 ± 0.5^{a}	3.4 ± 0.5^{a}	3.3 ± 0.5^{ab}
Yes SNP	2.4 ± 1.0^{b}	4.3 ± 0.9^{a}	4.1 ± 1.0^{a}	3.0 ± 1.0^{b}

[1]Haplotypes were Deletion = cytosine deletion at nucleotide base 895; No SNP = no single nucleotide polymorphism (SNP); Yes SNP = SNP other than a deletion.
[2]Mature = bulls > 2 yr of age; [3]Young = bulls < 2 yr of age.
[a, b, c] Means with different letters differ between rows and columns, within bull age group ($P < 0.05$).

Table 3. The interaction between season of collection and heat shock protein70 haplotype on total sperm (billion sperm/ejaculate) of mature (>2 yr of age) Holstein bulls.

	Season of Collection			
Haplotype[1]	Spring	Summer	Fall	Winter
Mature[2] bulls				
Deletion	9.5 ± 0.7[a]	8.5 ± 0.7[a]	8.2 ± 0.7[a]	9.4 ± 0.7[a]
No SNP	8.1 ± 0.9[a]	8.0 ± 0.9[ab]	7.6 ± 0.9[b]	7.7 ± 0.9[ab]
Yes SNP	N/A	7.0 ± 1.3[b]	5.5 ± 1.3[b]	6.5 ± 1.2[b]

[1]Haplotypes were Deletion = cytosine deletion at nucleotide base 895; No SNP = no single nucleotide polymorphism (SNP); Yes SNP = SNP other than a deletion.
[a, b] Means with different letters differ between rows and columns, within bull age group ($P < 0.05$).

Table 4. The interaction between season of collection and heat shock protein 70 haplotype on total number of potential breeding units (straws) of mature (>2 yr of age) Holstein bulls.

	Season of Collection			
Haplotype[1]	Spring	Summer	Fall	Winter
Mature bulls				
Deletion	469 ± 40[b]	432 ± 40[c]	406 ± 40[c]	474 ± 40[a]
No SNP	421 ± 47[c]	389 ± 47[c]	384 ± 47[c]	394 ± 47[c]
Yes SNP	N/A	380 ± 72[c]	253 ± 70[d]	326 ± 68[c]

[1]Haplotypes were Deletion = cytosine deletion at nucleotide base 895; No SNP = no single nucleotide polymorphism (SNP); Yes SNP = SNP other than a deletion.
[a, b, c] Means with different letters differ between rows and columns, within bull age group ($P < 0.05$).

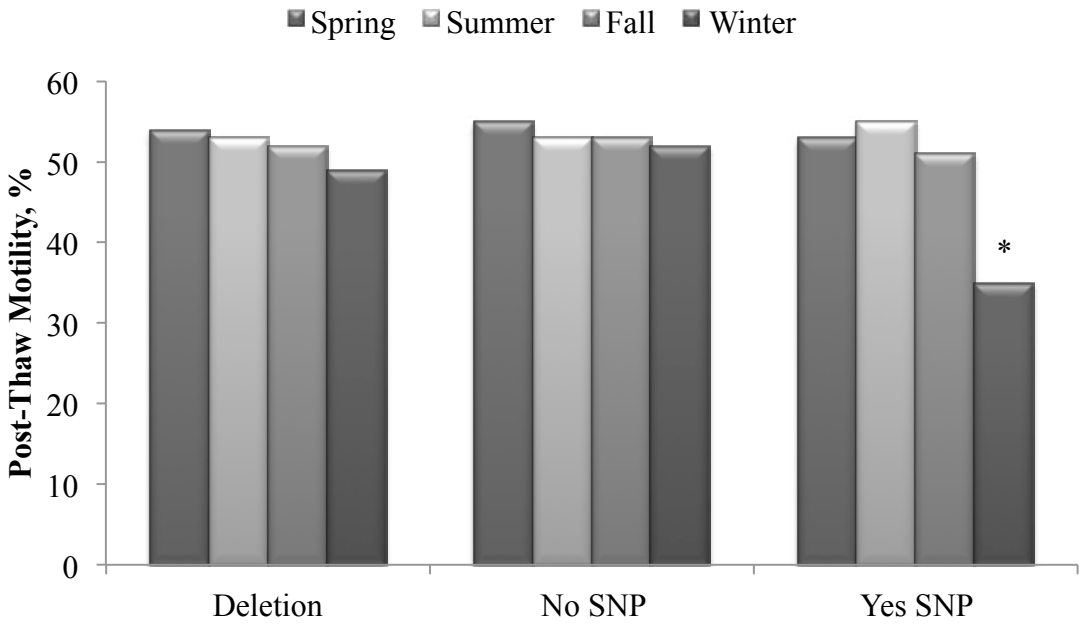

Fig. 1. The interaction between season of collection and heat shock protein 70 haplotype for post-thaw motility (%) of young Holstein bulls. Haplotypes were Deletion = cytosine deletion at nucleotide base 895; No SNP = no single nucleotide polymorphism (SNP); Yes SNP = SNP other than a deletion. (*$P < 0.05$; SEM = 2%).

Response to a modified-live virus respiratory vaccine in young calves versus a traditional preconditioning vaccination regimen at weaning

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Story in Brief

Crossbred beef calves were used to determine effects on the health, performance, and immune response of different pentavalent [bovine herpesvirus-1, bovine viral diarrhea virus (BVDV) types 1 and 2, parainfluenza-3 virus, and bovine respiratory syncytial virus] modified-live virus (MLV) vaccine regimens administered initially at either 62 or 188 d of age. The early vaccination treatment (EV) group was administered a pentavalent MLV respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid [Pyramid® 5 + Presponse® SQ, Boehringer Ingelheim Vetmedica, Inc. (BIVI), St. Joseph, Mo.] and a multivalent clostridial bacterin-toxoid (Alpha 7®, BIVI) on d 0 (average calf age = 62 ± 17 d). The traditional vaccination treatment (TV) group received the same respiratory vaccine and a multivalent clostridial bacterin-toxoid (Caliber® 7, BIVI) on d 126 (average calf age = 188 ± 17 d). Both treatment groups were revaccinated with the MLV respiratory and clostridial vaccines on d 147 (weaning). No calves required treatment for bovine respiratory disease during the study, which ended after an 84-d post-weaning period. Interim and overall gain performance were similar ($P \geq 0.84$) between vaccine treatments throughout the study. On d 0, serum BVDV type 1a antibody titers were present, but did not differ ($P = 0.50$) between vaccine treatments. However, BVDV antibody titers were greater for EV compared to TV on d 21, 126, and 147. There was a treatment × day interaction ($P = 0.05$) for the CD25 expression index of CD8+ cells; EV calves had a greater expression index than TV calves on d 126. Differences in BVDV type 1a titer concentrations and CD25 expression indices suggest that calves develop both humoral and cell-mediated immunity when vaccinated at 62 d of age. Furthermore, growth performance or health was not affected by vaccine regimen, which supports early vaccination as a cost-effective alternative to traditional calfhood vaccine regimens.

Introduction

Bovine respiratory disease (BRD) is thought to be the most expensive cattle disease in the U.S. Economic losses are a result of not only increased antibiotic treatment and death loss, but also reduced growth performance and carcass value (Schneider et al., 2009). Preconditioning reduces BRD morbidity because this comprehensive management practice mitigates predisposing disease factors by reducing physiological stress, and improving immunity through appropriate timing of vaccination before marketing and disease challenges occur (Smith, 2010).

Typically, preconditioning guidelines recommend vaccinating calves at or near weaning in consideration of the historical belief that maternal antibodies present from colostrum transfer interfere with the immune response to vaccination. However, although maternal antibodies received passively in colostrum may interfere with the antibody response to vaccination, cell-mediated immunity during this time is clearly activated (Platt et al., 2009). If efficacy of vaccination in immature beef calves in the presence of maternal antibody is established, it affords new opportunity to administer an initial modified live virus vaccine during the management event known as "branding" (calf age approximately 60 d) and to modify existing vaccination guidelines within preconditioning programs.

Our objective was to determine the effects of administering initial vaccines to calves at approximately 60 d of age versus a traditional preconditioning vaccination regimen administered near weaning age.

Materials and Methods

This study was conducted from May 2010 until July 2011 at the University of Arkansas Division of Agriculture Experiment Station located in Savoy and the University of Arkansas Division of Agriculture Livestock and Forestry Research and Extension Station located near Batesville. A total of 253 Angus-cross beef calves from 3 herds (58 in a spring-calving herd and 102 in a fall-calving herd at the Savoy unit and 93 in a spring-calving herd at the Batesville unit) were stratified by date of birth, gender (heifers or steers castrated at birth), calf body weight (BW), and cow BW, body condition score (BCS) and parity, then assigned randomly to 1 of 2 treatment groups. Calves assigned to the early vaccination treatment (EV) were administered a pentavalent [bovine herpesvirus-1 (BHV-1), BVDV type1a and type 2a, parainfluenza-3 virus (PI-3V), and bovine respiratory syncytial virus (BRSV)] MLV respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ, Boehringer Ingelheim Vetmedica, Inc. [BIVI], St. Joseph, Mo.) and a multivalent clostridial bacterin-toxoid (Alpha 7®, BIVI) on d 0 of the study when the mean age of calves was 62 ± 17 d. Calves assigned to the traditional vaccination treatment (TV) were administered the same pentavalent modified-live virus respiratory vaccine and a multivalent clostridial bacterin-toxoid (Caliber® 7, BIVI) on d 126 of the study (21 d prior to weaning; mean age = 188 ± 17 d). Both vaccine treatment groups were revaccinated with the same pentavalent modified-live virus respiratory vaccine and received a multivalent clostridial bacterin-toxoid (Caliber® 7, BIVI) booster on d 147 (d of weaning; mean

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age = 209 ± 17 d). Careful consideration of Beef Quality Assurance guidelines and manufacturer recommendations were followed with regard to vaccine handling and administration. All cows were vaccinated with a multivalent MLV vaccine containing BHV-1, BVDV type1a and type 2a, PI-3V, BRSV and five serovars of leptospira (Pyramid® 10, BIVI) and administered a pour-on anthelmintic (Cydectin, BIVI) on d 0 (branding). Cows and calves were separated into pasture groups based on calf treatment and grazed on replicated pastures until weaning (2 to 4 pasture replications/treatment within each herd).

All cows and calves were weighed 7 d prior to the initiation of the experiment to determine appropriate allocation to treatments. At this time, all calves assigned to the study were ear notched and tested for BVDV-persistent infection using the antigen capture-ELISA procedure at a commercial laboratory (Cattle Stats, LLC, Oklahoma City, Okla.). Calves were weighed individually at the initiation of the experiment (d 0; branding), d 21, 126 (21 d prior to weaning), 147 (weaning), and every 28 d throughout the backgrounding phase until the study ended on d 231. In addition to the appropriate vaccinations at weaning, a pour-on anthelmintic (Cydectin, BIVI) was administered to calves at this time.

During weaning at the Savoy location, pairs were split and calves were transported to the Savoy Stocker Cattle Unit and penned according to treatment, sex, and BW resulting in 4 pens/treatment. The feed management approach was designed to provide cattle with ad libitum hay and supplemental ration intended to achieve approximately 1.5 lb average daily gain (ADG) day over the 28-d weaning period. After this 28-d period, treatments were commingled and calves were moved to mixed-grass pastures and maintained for the duration of the study. During weaning at Batesville, calves were weaned as groups maintained by treatment and offered bermudagrass hay for 3 d in large holding pens. They were then moved to non-toxic novel endophyte-infected tall fescue pastures for the remainder of the study. Equal representation of vaccination treatments was maintained within each pasture. Grain supplementation (0.5% of average BW) was used to maintain calf growth as forage quality declined. Health was monitored daily for all calves throughout the cow-calf and backgrounding phases of the study.

Blood samples, used to determine serum antibody concentrations for BVDV, were collected via jugular venipuncture from 16 randomly selected calves (8 steers and 8 heifers) in each treatment group within each of the 3 herds. An additional 4 steers/treatment from the fall-calving herd at Savoy were bled so that titers would be known for all calves that were also sampled for the cell-mediated immune response assay, resulting in BVDV antibody concentrations being analyzed for a total of 104 calves. Serum samples from d 0, 21, 126, 147, 175, and 231 were shipped to the Iowa State University Veterinary Diagnostic Laboratory for determination of serum neutralizing antibody titer concentration against the homologous BVDV type 1a strain contained in the pentavalent MLV respiratory vaccine (Singer strain).

Blood samples from 33 steer calves in the fall-calving herd at Savoy were obtained between 24 and 48 h of birth to verify that steers being used for the cell-mediated immune assay had received maternal antibodies from colostrum. The 20 steers selected to be sampled for the cell-mediated immune assay had BVDV antibody titers ≥ 1024, indicating sufficient colostrum transfer occurred. To measure cell-mediated immune responses, jugular blood was collected on d 0 (branding), 7, 21, 42, 126, 147 (weaning), and 189 from a subset of 10 steers from each vaccination timing regimen to be utilized for flow cytometry analysis of T-cell populations and in vitro IFN-γ expression analyses according to procedures described previously (Platt et al., 2006, 2008).

Results were analyzed using the mixed procedure of SAS (SAS Institute, Inc., Cary, N.C.). The statistical model for BW and ADG included treatment, gender, and the treatment × gender interaction as fixed effects, and herd as a random effect. Antibody titer data were $\log_2$-transformed and analyzed as repeated measures with calf as the subject. The model included treatment, day, and the treatment × day interaction as fixed effects and herd as a random effect. The covariance model structure used was SP(POW). Flow cytometry and IFN-γ data were also analyzed as repeated measures. The model included treatment, day, and the treatment × day interaction.

Results and Discussion

No calves were found to be persistently infected with BVDV, nor were any calves treated for BRD at either location pre- or post-weaning. At no time during the experiment did BW (Table 1) differ ($P \geq 0.51$) between vaccination timing treatments. Similarly, neither pre-weaning nor post-weaning ADG, nor the combined ADG for the entire 231 d of the study were impacted ($P \geq 0.84$) by the timing of vaccination. Therefore, growth performance of calves was not negatively impacted by MLV vaccination at 62 d of age.

Bovine viral diarrhea virus type 1a antibody concentrations (Fig. 1) were affected by the timing of vaccination (treatment × day interaction; $P < 0.001$). Calves on both treatments had equivalent BVDV titers on d 0 (pre-vaccination), which can be attributed to maternal antibody transfer in the calves. The BVDV titers increased for EV from d 0 to 21, but did not for calves that had not been vaccinated (TV). Furthermore, EV calves continued to have increased BVDV titers on d 126, whereas BVDV titers declined for TV calves. The lowest serum anti-BVDV antibody concentrations for either vaccination treatment during the entire study were observed for the TV calves on d 126. Calves on the TV treatment were initially vaccinated on d 126 and BVDV antibody concentrations increased on d 147; however, at weaning (d 147) EV calves still exhibited greater ($P \leq 0.01$) BVDV titer concentrations even though TV had been vaccinated more recently. During the 84-d backgrounding period, all calves exhibited high serum BVDV antibody titers and vaccination treatment differences were not evident ($P \geq 0.07$).

There was a treatment × day interaction ($P = 0.05$) for the CD25 expression index (EI) for CD8+ cells (Fig. 2). Isolated CD8+ cells from EV calves had a greater CD25 EI than isolated CD8+ cells from TV calves on d 126. There was a tendency for a treatment × day interaction ($P = 0.08$) for the CD25+ EI, again cells from EV calves tended to have a greater CD25 EI than cells from TV calves on d 126 (data not shown). This increase in various CD25 EI parameters on d 126 (immediately before initial vaccination of the TV calves) is suggestive of stimulation of the cell-mediated immune response for EV. Platt and coworkers (2009) observed an increase in CD25 EI for vaccinated cattle, which corresponded with protection from subsequent challenge with BVDV. There was no effect of vaccination regimen ($P = 0.66$) or a treatment × day interaction ($P = 0.22$) on the CD25 EI for γδ-TCR+ cells (Table 2) in the current study. Although numerical increases were observed for EV, there were no statistical differences (treatment, $P = 0.23$; treatment × day, $P = 0.31$) in IFN-γ concentrations of supernatant after *in vitro* culture of PBMC for 4 d with BVDV (Fig. 3). Nevertheless, increases observed for some cell-mediated immune parameters in EV calves would suggest that MLV respiratory vaccine administered in the presence of maternal BVDV antibody stimulated the cell-mediated immune response.

Implications

Growth performance and health did not differ due to vaccination timing regimen for calves that remained on their origin ranch through an 84-day backgrounding period after weaning. Differences in serum bovine viral diarrhea virus type 1a antibody concentrations indicate that calves vaccinated at branding developed an antibody response despite maternal antibodies being present. Calves vaccinated early in life had greater bovine viral diarrhea virus type 1a titers during the pre-weaning phase, and, during the backgrounding phase, were equivalent to calves vaccinated in a traditional preconditioning regimen. Furthermore, vaccination with MLV respiratory vaccine during the presence of maternal antibodies stimulated the cell-mediated immune response.

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Table 1. Effect of vaccination timing regimen on growth performance of beef calves (least squares means ± SE; n = 253).

	Treatment		
Item	EV[a]	TV[b]	*P*-value
Body weight, lb			
Day 0	191 (± 4.3)	187 (± 4.3)	0.51
Day 21	222 (± 4.8)	224 (± 4.8)	0.68
Day 126	391 (± 7.5)	387 (± 7.6)	0.69
Day 147 (weaning)	424 (± 7.2)	422 (± 7.3)	0.80
Day 175	469 (± 7.3)	468 (± 7.4)	0.95
Day 203	481 (± 7.4)	477 (± 7.5)	0.72
Day 231	524 (± 7.6)	519 (± 7.7)	0.64
Average daily gain, lb			
Day 0 to147	1.59 (± 0.033)	1.60 (± 0.033)	0.84
Day 147 to 231	1.18 (± 0.038)	1.17 (± 0.039)	0.88
Day 0 to 231	1.44 (± 0.025)	1.44 (± 0.025)	0.97

[a]Early vaccination calves (EV) received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7™) on d 0 (62 d of age). Calves revaccinated on d 147 (weaning) with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster.

[b]Traditional vaccination calves (TV) received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on d 126 (188 d of age). Both vaccine treatment groups were revaccinated on d 147 (weaning) with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster.

Table 2. Effect of vaccination timing regimen on peripheral blood mononuclear cell populations of beef calves after being cultured in vitro with bovine viral diarrhea virus for 4 d (least squares means ± SE; n = 10/treatment sampled on d 0, 7, 21, 42, 126, and 189).

	Treatment		*P*-value	
Item	EV[a]	TV[b]	Treatment	Treatment × Day
CD25, EI[c]	2.4 (± 0.66)	1.8 (± 0.068)	0.56	0.08
γδ-TCRCD25, EI	3.1 (±1.4)	2.2 (± 1.4)	0.66	0.22
CD8+CD25, EI	1.5 (± 0.37)	1.1 (± 0.38)	0.47	0.05

[a] Early vaccination calves (EV) received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7™) on d 0 (62 d of age). Calves revaccinated on d 147 (weaning) with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster.

[b] Traditional vaccination calves (TV) received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on d 126 (188 d of age). Both vaccine treatment groups were revaccinated on d 147 (weaning) with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster.

[c] Expression index calculated by dividing the product (percentage CD25+ × mean fluorescent intensity of particular cell subset) from BVDV-stimulated cells by the product from mock antigen-stimulated cells of the same cell subset from the same animal.

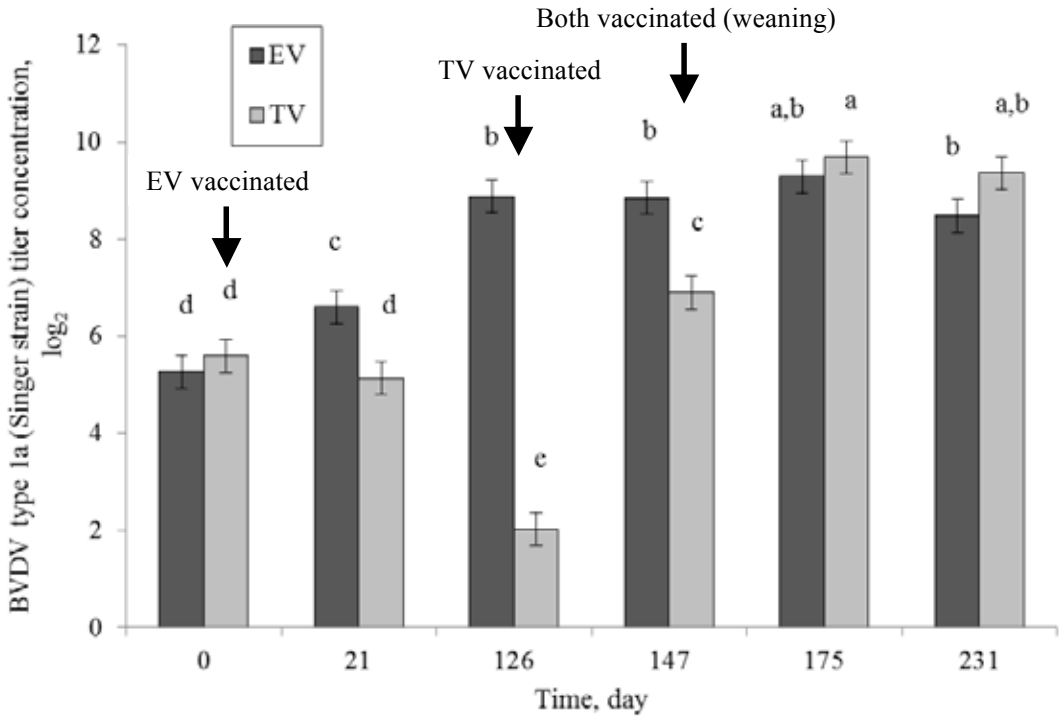

Fig. 1. Effect of vaccination timing regimen on serum bovine viral diarrhea virus type 1a antibody titers of beef calves (n = 52/treatment). Effect of treatment, *P* < 0.001; day, *P* < 0.001; treatment × day interaction, *P* < 0.001. EV = Early vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7™) on d 0 (62 d of age). TV = Traditional vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on d 126 (188 d of age). Both vaccine treatment groups were revaccinated with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster at weaning (d 147). Bars with different superscripts are significantly different (*P* < 0.05).

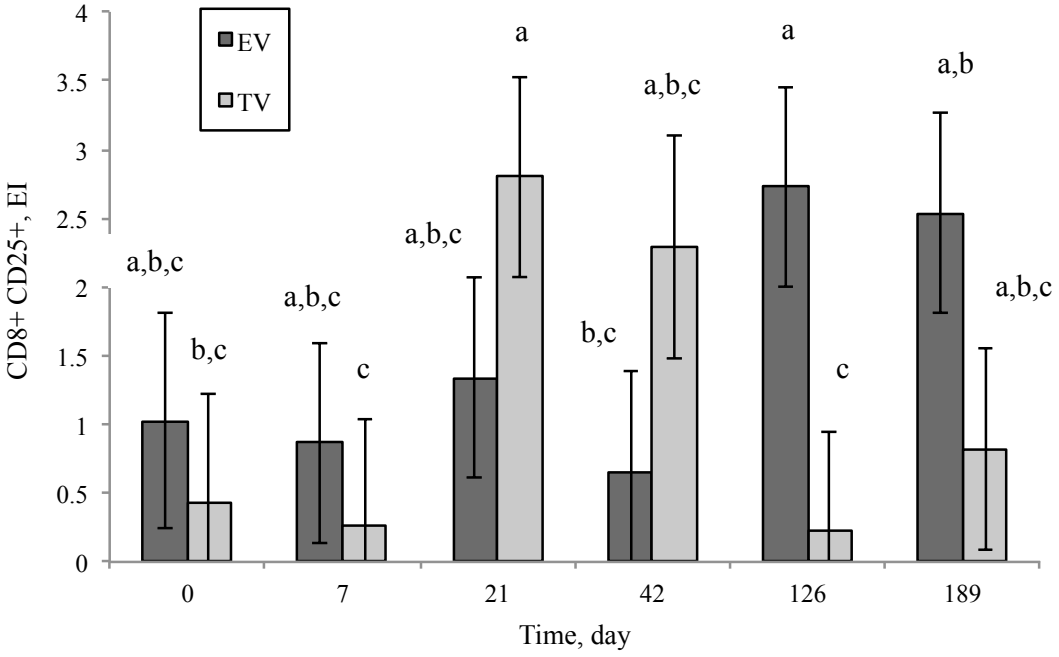

Fig. 2. Effect of vaccination timing regimen on CD8+ CD25 expression index (EI) of isolated peripheral blood mononuclear cells after 4 d of incubation with either bovine viral diarrhea virus or a mock antigen (n = 10/treatment). Treatment, *P* = 0.47; Day, *P* = 0.24; Treatment × day interaction, *P* = 0.05. EV = Early vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7™) on d 0 (62 d of age). TV = Traditional vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on d 126 (188 d of age). Both vaccine treatment groups were revaccinated with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster at weaning (d 147). Bars with different superscripts are significantly different (*P* < 0.05). Expression index (EI) calculated by dividing the product (percentage CD25+ × mean fluorescent intensity of CD8+CD25+ cells) from BVDV-stimulated cells by the product from mock antigen-stimulated cells from the same animal.

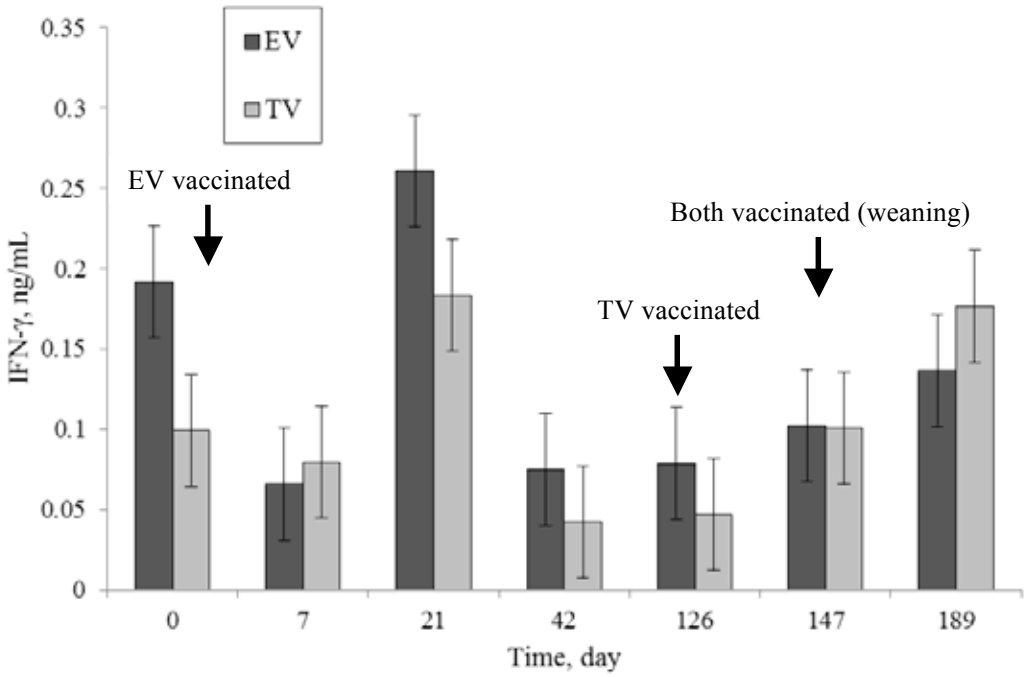

Fig. 3. Effect of vaccination timing regimen on interferon-gamma concentrations of supernatant of peripheral blood mononuclear cells cultured in vitro with bovine viral diarrhea virus for 4 d (n = 10/treatment). Treatment, *P* = 0.23; Day, *P* < 0.001; Treatment × day interaction, *P* = 0.31. EV = Early vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7™) on d 0 (62 d of age). TV = Traditional vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on d 126 (188 d of age). Both vaccine treatment groups were revaccinated with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* bacterin-leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster at weaning (d 147).

Comparison of a 5- or 14-day CIDR-based estrous synchronization protocols with sorted semen in beef heifers

J.G. Powell[1], T.D. Lester[1], J.L. Reynolds[1], A.J. Davis[1], J.A. Hornsby[1], and R.W. Rorie[1]

Story in Brief

This study compared the effectiveness of a 5-d versus a 14-d controlled internal drug-release device (CIDR) protocol when utilizing sorted semen during artificial insemination (AI) in beef heifers. A secondary objective was to determine effect of insemination timing on pregnancy rates when using sorted semen. Angus-cross beef heifers (n = 66) were randomly and equally distributed into two treatment groups based on pubertal status, age, body condition score and body weight. Treatment 1 heifers received a CIDR from d 0 to 5, gonadotropin-releasing hormone (GnRH) on d 0 and prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) on d 5. Treatment 2 heifers received a CIDR from d 0 to 14, GnRH on d 16, and $PGF_{2\alpha}$ on d 23. At the time of $PGF_{2\alpha}$ dosing, electronic mount detectors were placed on all heifers to monitor for estrus. Estrous response to synchronization for heifers in Treatment 1 and Treatment 2 was similar ($P = 0.62$) at 51.5% and 57.6%. A higher percentage (P = 0.03) of prepubertal heifers were induced to cycle by Treatment 2 compared to Treatment 1 (6/12, 50% vs. 1/11, 9.1%). The mean interval from $PGF_{2\alpha}$ to estrus, length of estrus and mounts during estrus were similar ($P > 0.14$) between Treatment 1 and Treatment 2. The sorted semen AI pregnancy rates for Treatment 1 and Treatment 2 also were similar ($P = 0.49$) at 41.2% and 52.6% respectively. Across treatments, AI pregnancy rates were similar ($P = 0.42$) at 30%, 56.3%, and 50% for heifers inseminated at 14 to 18 h, 19 to 23 h and 24 to 28 h after onset of estrus, respectively. The overall, seasonal pregnancy rate for heifers in Treatment 1 and Treatment 2 were similar ($P = 0.64$) at 93.9% and 90.9%, respectively. Based on estrous response, mean interval to estrus following $PGF_{2\alpha}$, AI conception rate and final pregnancy rates, 5-d and 14-d CIDR protocols were equally effective methods for synchronizing beef heifers in this study. The 14-d CIDR treatment did induce puberty in more heifers than the 5-d treatment. Additional studies are needed to determine if there is an advantage in pregnancy rate by delaying insemination until 19 h or longer after estrus onset when using sorted semen.

Introduction

Estrous synchronization has the potential to increase the proportion of beef heifers bred early in the breeding season and stimulate puberty in prepubertal heifers (Hall et al., 1997). Recent studies have shown that both 5-day and 14-day progesterone protocols are effective methods to synchronize estrus for artificial insemination (AI) in beef heifers (Bridges and Lake, 2011; Busch et al., 2007; Leitman et al., 2008; Leitman et al., 2009). Sorted semen is more costly than unsorted, indicating a need to maximize AI pregnancy rates. The objective of this study was to compare the effectiveness of a 5-day compared to a 14-day progesterone protocol when utilizing sorted semen during AI in beef heifers. A secondary objective was to determine any effect of timing of insemination on pregnancy rates when using sorted semen.

Material and Methods

Description of Animals and Treatments. Nulliparous Angus-cross beef heifers were randomly and equally distributed into two treatment groups based on pubertal status, age (1.37 yr ± 0.5), body condition score (BCS; 5.5 ± 0.08) and body weight (BW; 803 lb ± 23.8). Treatment 1 (TRT1) heifers (n = 33) received a controlled internal drug-release device (Fig. 1; CIDR; Eazi-Breed CIDR®, 1.38 g progesterone, Pfizer Animal Health, New York, N.Y.) followed by a 100 mcg dose of gonadotropin-releasing hormone (GnRH; Factrel®, Pfizer Animal Health; i.m.) on d 0 (November 14, 2011). The CIDR was removed on d 5, followed by a single 500 mcg dose of cloprostenol ($PGF_{2\alpha}$; Estrumate®, Merck Animal Health, Summit, N.J.; i.m.). Treatment 2 (TRT2) heifers (n = 33) received a CIDR on d 0, with removal of the CIDR on d 14. The TRT2 heifers then received GnRH dosing on d 16 followed by a 500 mcg dose of $PGF_{2\alpha}$ on d 23. At the time of $PGF_{2\alpha}$ dosing, electronic mount detectors (HeatWatch®, CowChips, LLC, Manalapan, N.J.) were placed on all heifers for monitoring of estrus behavior. Throughout the study, all animals were maintained and cared for in compliance with the University of Arkansas Animal Care and Use Committee Protocol.

Breeding Season and Pregnancy Diagnosis. One experienced technician performed AI at 14 to 28 h after onset of estrus with X-chromosome sorted semen. Following the end of the initial AI period, heifers were monitored for an additional 21 days for return to estrus. Heifers exhibiting a return to estrus were inseminated with non-sorted semen. At 28 d after the initial insemination, all heifers were then exposed to fertile bulls for approximately 30 d. Transrectal ultrasonography (Aloka 500V with 5-MHz transducer, Aloka Inc., Wallington, Conn.) was used to determine pregnancy status of heifers at 45 to 60 d after initial AI and again 30 d after removal of bulls. Fetal crown-rump length was used to determine if pregnancies resulted from AI or subsequent breeding.

Data and Statistical Analysis. Data were analyzed using statistical software (SAS, version 9.1, SAS Institute, Inc., Cary, N.C.). The AI pregnancy rate was defined as the number of heifers that were determined to be pregnant to AI service divided by the number of heifers exhibiting estrus within the 96-h period following PG dosing and insemination. Overall pregnancy rate was defined as the percentage of all heifers per treatment that were pregnant at the end of the breeding season. Non-parametric data were evaluated using the Chi-Square analysis, and all other data were evaluated by analysis of the variance. Initial models for reproductive responses contained fixed effects of treatment, BCS, BW, age, sire, pubertal status and their

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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, pubertal status, age, and AI sire had no effect on either AI pregnancy rate or overall pregnancy rate ($P \geq 0.17$). Results for heifer estrus response, hours to estrus following $PGF_{2\alpha}$, length of estrus, mounts during estrus and pregnancy rates are presented in Table 1. In the current study, a higher percentage ($P = 0.03$) of prepubertal heifers were induced to cycle by TRT2 compared to TRT1 (6/12, 50% vs. 1/11, 9.1%). These results are supported by a previous reported study (Hall et al., 1997) indicating progestin assisted prepubertal beef heifers in the attainment of puberty.

The present study indicated that estrus response to synchronization was similar ($P = 0.62$) for TRT1 and TRT2 at 51.5% and 57.6%, respectively. Our lab previously reported (Powell et al., 2011) that the same 14-d CIDR synchronization protocol utilized in the current study, resulted in a 75% estrus response. However, a previous study (Bridges and Lake, 2011) comparing a 5-d to a 14-d CIDR protocol in beef heifers undergoing timed artificial insemination (TAI) reported that heifers treated with a 5-d CIDR protocol tended ($P = 0.07$) to exhibit a lowered estrus response compared to heifers treated with a 14-d CIDR protocol at 53.5% to 63.5%, respectively. While our results for the 5-d CIDR protocol was similar to the Bridges and Lake study, our result for the 14-d CIDR protocol was less than both of the aforementioned studies.

The mean interval from $PGF_{2\alpha}$ to estrus (54.5 vs. 47.5 h) was similar ($P = 0.14$) between TRT1 and TRT2, respectively. These results were similar to data previously reported (Wilson et al., 2007) that indicated heifers treated with a 5-d CIDR protocol exhibited a mean interval of 51 h. However, previous studies determined intervals for heifers treated with a 14-d CIDR protocols were slightly higher than the interval noted in the current study. Leitman et al. (2008) reported a mean interval of 52 hours from $PGF_{2\alpha}$ to estrus in heifers on a 14-d CIDR protocol. In addition, Busch et al. (2007) and Leitman et al. (2009) reported mean intervals for heifers treated with a 14-d CIDR protocol as 65 h and 68 h, respectively.

In the current study, length of estrus (11.4 vs. 10.8 h) and mounts during estrus (44.2 vs. 40.3) were similar ($P \geq 0.69$) between TRT1 and TRT2, respectively. Furthermore, AI pregnancy rates were similar ($P = 0.49$) for TRT1 and TRT2 when using sorted semen (41.2% and 52.6% respectively). Additionally, Bridges and Lake (2011) did not find a treatment difference in AI pregnancy rates when comparing a 5-d to a 14-d CIDR protocol in beef heifers even though TAI and unsorted semen were utilized.

Across treatments, AI pregnancy rates were similar ($P = 0.42$) at 30%, 56.3%, and 50% for heifers inseminated at 14 to 18, 19 to 23 and 24 to 28 h after onset of estrus, respectively. Although no difference was noted, the numerical improvement in AI pregnancy at 19 hours and beyond warrants additional data collection to determine if delaying insemination until 19 h or more after the onset of estrus will improve pregnancy rates when utilizing sorted semen in beef heifers.

The pregnancy rate for 15 heifers returning to estrus and inseminated with unsorted semen was 80%. Furthermore, overall breeding season pregnancy rate for heifers in TRT1 and TRT2 were similar ($P = 0.72$) at 93.9% and 91.9%, respectively.

Current recommendations indicate that two separate doses of $PGF_{2\alpha}$ be administered at a 12-hour interval when utilizing a 5-d CIDR protocol in beef heifers. In the current study, heifers treated with the 5-d CIDR protocol received a single injection of $PGF_{2\alpha}$ following CIDR removal and an acceptable estrus response and AI result occurred. Our study utilized cloprostenol, which has a longer half-life in circulation, and thus might be more effective as a single dose than other prostaglandin products. Administering only one injection may be more favorable and acceptable to the beef industry by decreasing labor and treatment costs required to conduct the 5-d CIDR protocol.

Implications

In this study, the estrus response and subsequent pregnancy rate in beef heifers was similar for synchronization protocols utilizing either a 5-d or 14-d CIDR protocol. The 14-d progestin treatment was more effective in inducing cyclicity in prepubertal heifers. Additional data is needed to determine if delaying insemination until 19 h or later would increase pregnancy rates when utilizing sorted semen. Furthermore, results indicate that a single injection of $PGF_{2\alpha}$ was sufficient to induce luteal regression and consequently result in acceptable AI pregnancy rates when utilizing sorted semen in a 5-d CIDR protocol for beef heifers.

Acknowledgements

The authors thank Pfizer Animal Health (New York, N.Y.) for supplying pharmaceutical products for the experiment as well as Genex Cooperative, Inc (Shawano, Wis.) and Jac's Ranch (Bentonville, Ark.) for supplying bull semen utilized in the study. The authors also express appreciation to 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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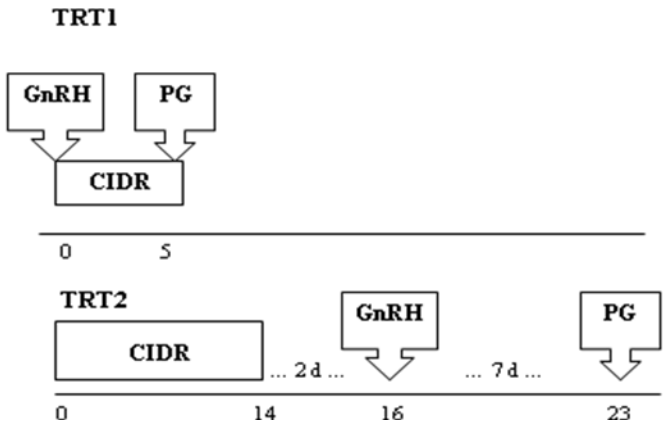

Fig. 1. Treatment schedule for heifers assigned to TRT1 and TRT2.

Table 1. Effect of 5-d and 14-d CIDR protocol on estrous response and pregnancy rates.

	Estrous synchronization treatment *		
	TRT 1	**TRT 2**	**P value**
Estrus response	17/33 (51.5%)	19/33 (57.6%)	0.62
Prepubertal estrus response	1/11 (9.1%)	6/12 (50%)	0.03
PG to estrus, hours	54.5 ± 3.1	47.5 ± 3.4	0.14
Length of estrus, hours	11.4 ± 1.1	10.8 ± 1.2	0.69
Mounts during estrus	44.2 ± 7.3	40.3 ± 7.8	0.72
Sorted semen pregnancy rate	7/17 (41.2%)	10/19 (52.6%)	0.49
Overall pregnancy rate	31/33 (93.9%)	30/33 (90.9%)	0.64

*Heifers in TRT 1 and 2 received 5-d and 14-d CIDR as progesterone sources, respectively.

Estrous response for progestin-based estrous synchronization protocols and subsequent pregnancy rates when using X-chromosome sorted semen in postpartum beef cows

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Story in Brief

Estrous response and subsequent artificial insemination pregnancy rate were evaluated after synchronization of beef cows with a modified 14-d progesterone protocol. Also evaluated was the effect of timing of insemination on pregnancy rate, when using sorted semen. Angus-cross beef cows were randomly and equally distributed into two treatments groups based on cyclicity, parity, weight, body condition and days postpartum. Treatment 1 cows received a controlled internal drug-release device (CIDR) progesterone insert on d 0. The CIDR was removed on d 14, followed by treatment with gonadorelin (GnRH) on d 16, and prostaglandin $F_{2\alpha}$ (PGF) on d 23. Treatment 2 cows received the same synchronization treatment, except an additional dose of PGF was given on d 7 of CIDR treatment. Cows in both treatments were observed over a 96 h period for estrus and inseminated with X-chromosome sorted semen 10 to 24 h after onset of estrus. A week later, all cows were exposed to fertile bulls for 56 days. Ultrasonography was used to determine pregnancy status of cows ~45 d after insemination and again 31 d after bull removal. The percentage of cows exhibiting estrus did not differ ($P = 0.78$) at 62.9% and 60.4% for treatments 1 and 2, respectively. Artificial insemination pregnancy rates were similar ($P = 0.40$) at 58.8% and 68.8% for treatments 1 and 2, respectively. Across treatments, AI pregnancy rate tended to be higher ($P = 0.07$) for cows inseminated 15 to 19 h after onset of estrus (73.7%) compared to those inseminated 10 to 14 h (60%) or 20 to 24 h (38.5%) after estrus onset. Beef cows are routinely inseminated about 10 to 14 h after the onset of estrus. Results suggest that when using sorted semen, delaying insemination until approximately16 to 18 h after onset of estrus may improve subsequent pregnancy rate.

Introduction

Producers can pre-determine the gender of calves born by artificial insemination (AI) with sorted semen. However, sorted semen is more costly and often results in lower pregnancy rates than that achieved with unsorted semen. Therefore, it is important to use estrous synchronization protocols that maximize expression of estrus and AI pregnancy rates when utilizing sorted semen. Our laboratory has observed good synchrony and AI pregnancy rates for cows synchronized with 14-d controlled internal drug release (CIDR) progesterone treatment, followed by gonadorelin (GnRH) on d 16 and prostaglandin $F_{2\alpha}$ (PGF) on d 23. In absence of luteal progesterone, a 14-d CIDR treatment results in development of a persistent follicle. Thus, our protocol might be improved by the addition of PGF on d 7 of the CIDR treatment, to insure endogenous progesterone is low and that a persistent follicle will develop, and respond to GnRH given on d 16. This study evaluated the estrous response and subsequent AI pregnancy after synchronization of beef cows with a 14-d CIDR protocol, where an additional PGF treatment was given on d 7 or progestin treatment. A secondary objective was to evaluate the effect of time of insemination after onset of estrus on pregnancy rate when using sorted semen.

Materials and Methods

Angus-cross multiparous (n = 72) and primiparous (n = 35) lactating beef cows at one location were randomly and equally distributed into two treatments groups based on cyclicity, parity, weight, body condition and days postpartum (Table 1). Treatment 1 (TRT1) cows received a CIDR progesterone insert (Eazi-Breed CIDR) on d 0. The CIDR was removed on d 14, followed by treatment with 100 µg of GnRH (Factrel) on d 16, and 25 mg of PGF (Lutalyse) on d 23. Treatment 2 (TRT2) cows received the same synchronization treatment, except an additional 25 mg dose of PGF was given on d 7 of the CIDR treatment. A mount detection patch (Estrotect) was placed on all cows at the time of PGF on day 23. Cows were observed for estrus for 96 h after PGF, and those exhibiting estrus were inseminated with X-chromosome sorted semen 10 to 24 h after onset of estrus. A week after the estrus detection period, all cows were exposed to fertile bulls for 56 days. Transrectal ultrasonography was used to determine pregnancy status of cows at ~45 d of gestation and again 31 days after bull removal. Fetal crown-rump length was used to determine if pregnancies resulted from artificial insemination or subsequent matings. Non-parametric data were evaluated using the Chi-Square analysis, while all other data were evaluated by analysis of the variance.

Results and Discussion

Previously, we have reported (Powell et al., 2011) a synchronization protocol consisting of 14-d CIDR treatment, followed by GnRH on d 16 and PGF on d 23 resulting in 80% or more of treated cows in estrus within a 3 to 4 d period. The protocol was based on the assumption that the long-term CIDR treatment would result in development of a large persistent follicle capable of ovulating in response to GnRH given a couple days after CIDR removal. However, if a cow has a functional corpus luteum during the CIDR treatment period, the additional progesterone from the corpus luteum prevents a persistent follicle from developing and the GnRH treatment will be ineffective. Injection of PGF treatment on d 7 of the CIDR treatment should regress any corpus luteum present and insure a persistent follicle develops that in turn, will respond to GnRH. Thus, this study was conducted to determine if such a PGF treatment would improve the estrous response to the synchronization protocol.

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In the present study, the estrous response to synchronization was similar ($P = 0.78$) at 62.9 and 60.4% for TRT1 and TRT2, respectively (Table 2). The low estrous response, and lack of response to the PGF treatment, might have been due to less than 60% of the cows being cyclic at the start of synchronization treatment (Table 1). Only cows that were already cyclic and had a functional corpus luteum at the start of synchronization could have responded to the PGF given on d 7 of the CIDR treatment. Therefore, the PGF treatment would have been ineffective on at least 40% of the cows. Across treatments, the estrus response to synchronization was greater ($P = 0.02$) for multiparous than primiparous cows (69.4% vs. 45.7%, respectively; Table 3). At the start of the study 48.5% of primiparous cows and 37.5% of multiparous cows assigned across treatments were not cyclic. The higher estrous response compared to the percentage of cyclic cows indicates the 14-d CDIR treatment helped initiate cyclicity in some of the cows. Previous studies have shown that progesterone treatment can stimulate cows to cycle sooner after calving than would occur naturally.

Synchronization treatment had no effect ($P = 0.62$; Table 2) on the interval from treatment to estrus, but the interval tended to be less ($P = 0.09$) for multiparous vs. primiparous cows (Table 3). Within treatments, AI pregnancy rates were similar ($P = 0.40$) at 58.8% and 68.8% for TRT 1 and 2, respectively. Across treatments, AI pregnancy rate tended to be higher ($P = 0.07$) for cows inseminated 15 to 19 h after onset of estrus (73.7%) than those inseminated 10 to 14 h (60%) or 20 to 24 h (38.5%) after estrus onset (Fig. 1). We have previously reported that with unsorted semen, pregnancy rates are similar for insemination of beef cows at times ranging from 7 to 25 h after the onset of estrus (Rorie et al., 2002). Therefore, timing of insemination in relation to estrus appears to be more critical when using sorted versus unsorted semen. These results suggest that delaying insemination until ~16 to 18 h after onset of estrus will improve pregnancy rates when using sorted semen. Seasonal pregnancy rates were similar ($P = 0.40$) at 72.2% for TRT1 and 79.3% for TRT2 (Table 2). When compared across treatments (Table 3), multiparous cows had a higher ($P = 0.01$) pregnancy rate (83.3%) than primiparous cows (60.0%). This difference was likely due to the lower body condition in the primiparous versus multiparous cows (mean of 4.7 vs. 5.5, respectively) at the start of the study.

Implications

There was no advantage in either estrous response or subsequent AI pregnancy rate with the addition of PGF treatment on d 7 of a synchronization protocol consisting of CIDR for 14 d, GnRH on d 16 and PG on d 23. When using sorted semen, delaying the time of insemination in relation to onset of estrus may improve subsequent pregnancy rate, and merits further study.

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Table 1. Distribution of beef cows across synchronization treatments.

Parameter	TRT1 (Control)	TRT2 (D7 PG)	P value
Weight (lbs)	1210.2 ± 25.2	1194.4 ± 25.4	0.66
Body condition (BCS)	5.27 ± 0.14	5.25 ± 0.14	0.94
Post partum interval (d)	55.4 ± 2.5	57.2 ± 2.5	0.62
Cows cyclic at synchronization	32/54 (59.3%)	31/53 (58.5%)	0.94

Table 2. Effect of synchronization treatment on estrus response and pregnancy rate.

Parameter	TRT1 (Control)	TRT2 (D7 PG)	P value
Estrus response	34/54 (62.9%)	32/53 (60.4%)	0.78
Interval, PGF to estrus (h)	55.6 ± 1.8	55.9 ± 1.9	0.90
AI pregnancy rate	20/34 (58.8%)	22/32 (68.8%)	0.40
Season pregnancy rate	39/54 (72.2%)	42/53 (79.3%)	0.40

Table 3. Comparison of multiparous versus primiparous cows across treatments.

Parameter	Multiparous cows	Primiparous cows	P value
Estrus response	50/72 (69.4%)	16/35 (45.7%)	0.02
Interval, PGF to estrus (h)	54.5 ± 1.5	59.6 ± 2.6	0.09
AI pregnancy rate	32/50 (64.0%)	10/16 (62.5%)	0.91
Season pregnancy rate	60/72 (83.3%)	21/35 (60.0%)	0.01

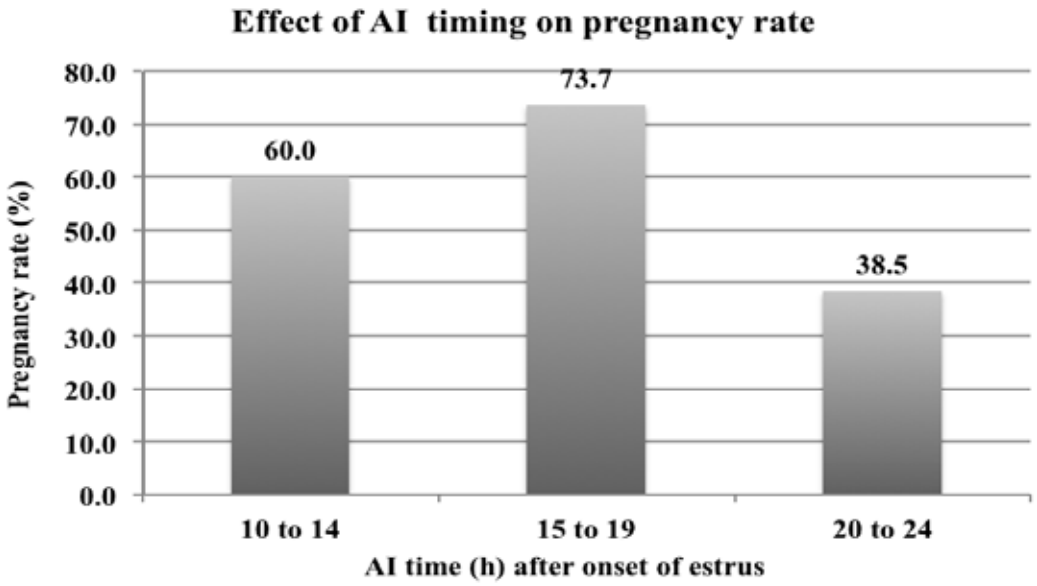

Fig. 1. Effect of timing of insemination after estrus on AI pregnancy rate.

Post-weaning sera isoenzymes of LDH and G6PDH and subsequent carcass traits in finished beef cattle

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Story in Brief

Our objective was to determine if serum lactate dehydrogenase (LDH) activity and (or) serum isoenzymes of LDH and glucose 6-phosphate dehydrogenase (G6PDH) of feeder calves could be used as an indicator of subsequent carcass traits. Samples were collected from crossbred calves (n = 181) prior to finishing in a feedlot (approximately 150 d). Individual isoenzymes were classified as high (H; when value was > 1 SD above mean), medium (M; when value was within 1 SD of mean), low (L; when value was < 1 SD below mean), or without (W; no band present). Carcass weight increased ($P < 0.05$) as the density of isoenzymes L1 and G1 increased. Carcass weight was inversely related ($P < 0.05$) to isoenzyme L3 and total LDH isoenzymes.

Introduction

In recent years, there has been an increased interest in selecting animals which produce products that meet consumer demands (i.e., tender lean with juiciness and flavor). Understanding fundamental mechanisms associated with the major metabolic processes, especially lipogenesis, is required to meet those selection goals (Smith and Smith, 1995). Lipogenesis requires reducing equivalents (NADPH, NADH, FADH, etc). Approximately half of the reducing equivalents produced in the cell arise from glucose 6-phosphate dehydrogenase (G6PDH) activity, a rate-limiting enzyme of the pentose phosphate pathway (Smith, 1983). The activity of G6PDH is high in adipose tissue, low in skeletal muscle, and highly related to lipogenesis (Belk et al., 1993). Lactate dehydrogenase (LDH) is another enzyme that supplies NADH for metabolic reactions and a very important enzyme in gluconeogenesis and lipogenesis (Whitehurst et al., 1981). The LDH activity of cattle varies considerably between animals and type of tissue sampled (Kaneko, 1989). Our objectives were to: characterize the relationships between the presence of isoenzymes of LDH and G6PDH in the sera of feeder cattle, and determine if the proportion of any one isoenzyme in the sera of feeder calves prior to the finishing phase could be used as an indicator of carcass traits at slaughter.

Material and Methods

Animals. Crossbred calves (n = 181; 7 to 15 mo of age) were finished for approximately 150 d under typical commercial feedlot conditions. Calf feeding and handling were consistent with established humane procedures. All calves were slaughtered at commercial packing plants on dates determined by the feedlot manager in consultation with the owner of the cattle. At slaughter, hot carcass weight (CARCWT) was determined. Following a 24 hr chill period, longissimus muscle area (LMA) was determined using a standard grid method and back fat (BF) thickness measured between the 12th and 13th ribs. Marbling scores (MS) were visually estimated by trained personnel as a USDA quality grade was assigned to each carcass. The relationship between numerical MS and USDA quality grades were as follows: Prime (700 < MS < 999), Choice (400 < MS < 699), Select (200 < MS < 399), and No-Rating (MS ≤ 199). As calves entered the feedlot, blood samples were collected via jugular venipuncture. Blood was collected using individual syringes fitted with 18 gauge hypodermic needles. Immediately after collection, blood was placed into ice-cold borosilicate glass tubes where it was allowed to clot for 24 hr at 5 °C. Serum was harvested by centrifugation of blood at 700 × g for 25 min, and stored frozen (-20 °C) until enzyme analysis.

LDH Activity. Total serum LDH activity (TOTAL, IU/mL) for each animal was determined using a commercial kit (Sigma Chemicals, Saint Louis, Mo.). That colorimetric assay is based on the enzymatic conversion of lactate to pyruvate.

LDH Isoenzymes. Isoenzymes of LDH from serum samples were separated by polyacrylamide gel electrophoresis (PAGE, Rosenkrans et al., 2000). Serum (15 uL) was diluted to 100 L with ddH_2O and 15 L sample buffer added to the mixture. Isoenzyme separation was performed using a mini gel format with electrophoresis conducted in an ice bath at a constant voltage of 75 volts and a total running time of about 1.5 hr. The running gels consisted of acrylamide/bis acrylamide (7.5%/2.6%) covered by a stacking gel of 4% acrylamide/2.6% bis acrylamide. The running buffer (pH 8.3) consisted of Tris HCl (0.01 M) and glycine (0.383 M). Staining solution was prepared approximately 30 min before the staining procedure. All gels were stained at 39 °C for approximately 30 min. Staining solution for LDH consisted of lithium lactate (0.057 M), NAD (1.9 mM), phenazine methosulphate (0.33 mM), nitro blue tetrazolium (0.49 mM), and tris HCl (50 mM). Five proteins were identified as having LDH activity.

G6PDH Isoenzymes. Serum proteins with G6PDH activity were separated as described above for LDH isoenzymes in non denaturing polyacrylamide running gels (9.5% acrylamide/2.6% bisacrylamide) with 4% acrylamide/2.6% bis acrylamide stacking gels. The staining solution used was that described by Belk et al. (1993). Four proteins with G6PDH activity were detected.

Following the staining process, gels were scanned by an enhanced laser densitometer (Ultroscan XL laser densitometer; LKB, Sweden) to quantify the isoenzymes. Area percentage of each protein peak represented the expression of the corresponding isoenzyme. Therefore, for each calf, adding the area percentage of the isoenzymes summed to 100 percent each for LDH and G6PDH.

Isoenzyme Designation. Isoenzyem proteins with LDH activity, were labeled L1 (homozygous heart subunit), which moved fastest towards the anode, followed by L2, L3, L4, and L5 (homozygous muscle subunit), which was the slowest in electrophoresis. Percentage

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of LDH heart subunit was calculated based on the molecular composition of each isoenzyme (heart LDH % = L1 % + 0.75 (L2 %) + 0.50 (L3 %) + 0.25 (L4 %)). The four isoenzyme proteins representing bands with G6PDH activity, were labeled G1 (most anodic), G2, G3, and G4 (most cathodic).

To aid in the determination of differences in isoenzyme activity by carcasses grading Choice, each isoenzyme was classified into four arbitrary LDH and G6PDH isoenzyme classification: High, Medium, Low, and None.

Statistical Analysis. All statistical analyses were performed using procedures of SAS (SAS Institute, Inc., Cary, N.C.). Pearson correlation coefficients among isoenzymes and carcass traits were determined. In addition, partial correlation coefficients were obtained with the MANOVA option of PROC GLM with carcass weight in the model. Those correlations were calculated to adjust for differences in carcass weight. The proportion of carcasses grading USDA Choice (the seven carcasses grading Prime were grouped with Choice) among isoenzyme classifications were examined by Chi-square analysis.

Stepwise regression and partial least-squares analyses were used to examine relationships among carcass traits and isoenzymes. Ordinary least-squares regression seeks linear functions of the predictors that explain as much variation in the response as possible and has the goal of minimizing sample response prediction error. Partial least-squares (PLS) have the additional goal of accounting for variation in the predictors and should provide better prediction for new observations when the predictors are highly correlated. Principal components regression (PCR) was used and the randomization-based model comparison test of van der Voet (1994) was used to test models with different numbers of extracted factors against the model that minimized the predicted residual sum of squares (PRESS). Regression coefficients were computed with the number of factors found to be important. These coefficients, along with those obtained from stepwise regression, are reported.

The Variable Importance for Projection (VIP) of Wold (1994) was calculated and predictors with small values of VIP (<0.8) were deleted and analyses rerun with remaining predictors and the number of factors determined by the PRESS statistic. These coefficients, along with those obtained from stepwise regression, are reported.

Results and Discussion

One of our objectives was to determine if the proportion of any one isoenzyme in the sera of feeder calves prior to the finishing phase could be used as an indicator of carcass traits at slaughter. Ultimately, our goal was to determine if isoenzyme profiles could be used prior to the finishing phase to identify those calves that partition fat disproportionately toward intramuscular fat based on MS. We found one LDH (L4) and two G6PDH (G1 and G4) isoenzymes that appeared to have met that interest. Categorizing the isoenzyme resulted in groups of animals in which back fat thickness was not affected by classification, but MS and percentage of animals grading USDA Choice were affected by isoenzyme classification. The other isoenzymes that increased MS and percentage of animals grading USDA Choice also increased BF; therefore, increasing overall fat deposition and not intramuscular fat.

Lipogenesis is subject to several possible limitations, including the supply of acetyl CoA, NADPH, NADH, and the regulation of synthesis from the precursors (Vernon, 1986). Glucose 6-phosphate dehydrogenase (G6PDH), a rate limiting enzyme of the pentose cycle, contributes 30% to 50% of reducing equivalents (NADPH) for lipogenesis as well as other metabolic processes (Smith, 1983). The remainder is thought to be provided by NADP-malate dehydrogenase system (Smith and Prior, 1981). In ruminant adipose tissue, other than glucose, lactate is the important precursor for lipid synthesis (Hanson and Ballard, 1967; Whitehurst et al., 1978). However, glucose has an effect on stimulating the incorporation of lactate into fatty acids (Whitehurst et al., 1981). This effect may be due to NADPH generation via the pentose cycle.

Utilization of lactate as a precursor of fatty acid synthesis requires lactate dehydrogenase to convert lactate to pyruvate and the generation of reducing equivalents (NADH). This scenario has been confirmed by studies with the hydrogen acceptor, methylphenazonium methosulphate, which indicated that in bovine adipose tissue there is a paucity of cytosolic NADH (Smith, 1983). The low NADH concentration would limit the conversion of oxaloacetate, formed via ATP-citrate lyase, to malate which would restrict the production of NADPH via NADP-malate dehydrogenase under those conditions (Prior et al., 1981). Leaving G6PDH as the primary enzyme responsible for the production of NADPH required for lipogenesis.

This mechanism is supported by the fact that measured activities of G6PDH in bovine adipose tissue far exceed reducing equivalent requirements for fat synthesis (Whitehurst et al., 1978). Thus, the interaction and relative activities of G6PDH and LDH could be a significant factor in regulation of lipogenesis by regulating the cellular ratio of NADH to NADPH. The biological and coordinated interaction between G6PDH and LDH is supported by our data, where LDH isoenzymes are significantly correlated with G6PDH isoenzymes in bovine sera. Our assumption is that in healthy calves the isoenzymic distribution in sera is representative of the isoenzyme profile for the whole body.

In addition to lipogenesis, LDH has been shown to be correlated with muscle characteristics in ruminants (Vernon, 1986), and greater weight gain for cattle with more muscle type LDH (Jurie et al., 1995). Our results support those findings in part. We found that the presence of isoenzyme L5 (homozygous muscle isoform) was not related to LMA, but as isoenzyme L4 increased LMA increased and MS decreased. It is not known why the heterozygous L4 (A3B) was more closely related to LMA than the homozygous L5 (A4). Our data set consisted of genetically diverse calves that were representative of those available for finishing in the cattle industry. The correlation between BF and MS varies from low positive (Brethour, 2000) to high positive (Lamb et al., 1990) with our results intermediate (r = 0.54). One can conclude that due to the inconsistency in the relationship between BF and MS that some type of selection assistance could be helpful for marbling. Currently, EPDs have proven to be an effective means of selecting for animals that will marble but not deposit additional back fat (Gwartney et al., 1996). However, if cattle of unknown genetic background are purchased EPDs are not useful. Trained technicians have developed ultrasound techniques that can be used as a tool for sorting cattle into outcome groups (Brethour, 2000). We conclude that the use of isoenzyme analysis also could be used to sort animals of unknown genetic background into outcome groups. In addition, based on the moderate heritability coefficient for LDH activity in serum ($h^2 > 0.35$) of calves, we believe that LDH might be useful as a selection tool for marbling in herds without EPD information.

Implications

Our results indicate that the isoenzymes of lactate dehydrogenase and glucose-6-phosphate dehydrogenase in feeder calf serum are related to carcass characteristics of cattle. Those isoenzyme patterns were determined as calves entered a finishing phase; therefore, enzyme

activities may be useful in assigning cattle of unknown genetic background into slaughter outcome groups. As genome mapping matures into proteonomics, our data suggests that isoenzymes may be very useful in determining the functionality of various genes and gene products.

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Breed group effects for chute exit velocity as an indicator trait for temperament in weaned calves

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Story in Brief

The objective of this study was to determine breed group differences in chute exit velocity (CEV) in weaned calves (n = 3176). Data were collected from 2004 through 2008 under a regional project with the following states contributing data: Florida, Louisiana, and Mississippi. Chute exit velocity was the time (seconds) required for a calf to traverse 6 feet when released from the working chute and calculated as (velocity = distance (f)/time (s)). Angus (A), Braford (BF), Brangus (BN), Brahman (B), Charolais (C), Hereford (H), Romosinuano (R), Commercial (X) and 2-and 3-way crosses of these groups were included. Data were analyzed by location with analysis of variance. At Brooksville, Fla., mean chute exit velocity did not differ among breed types involving A, B, and R breeds. At Marianna, Fla., breed group affected mean chute exit velocity; A was similar to CA, CBN, BN and ABN; B was similar to ABN, AB, BN, CBN, and CA. At Baton Rouge, La., mean chute exit velocity was affected ($P < 0.01$) and B had greater ($P < 0.05$) mean chute exit velocity when compared to A and BF; whereas, BF had lesser ($P < 0.05$) mean chute exit velocity than A and B. Breed types (A and BN) did not differ in mean CEV at Iberia, La. At Raymond, Miss., mean chute exit velocity was affected ($P = 0.03$) by breed type, but AX (lowest value) did not differ from AB, BNX, and A. The HX (highest value) was similar to BNX, AB and A for mean chute exit velocity. Breed type-differences for chute exit velocity may have potential for among breed selection for temperament in weaned calves.

Introduction

The Southern region of the U.S. produces 42% of the nation's beef cattle. Genetic selection for beef cattle breeds to match environmental conditions for productivity may lead to behavior and temperament challenges. Handler safety and animal welfare may be at risk due to temperament. Animals with calm temperaments show reduced stress and improved welfare, health, meat quality, and fertility (Grandin, 1997, 2003; Cooke, et al., 2009). Conversely, animals with excitable temperaments may increase production costs through increased bruising by running into equipment during handling and resulting loss of yield. Research has shown temperament differences among breeds, among individuals within breeds, and those differences are heritable (Stricklin, et al., 1980). Methods to assess temperament include pen score, chute score, and chute exit velocity. Exit velocity is an objective measure and has been shown to be correlated with cortisol response, suggesting increased stress, and is repeatable over time (Curley, et al., 2006). The goal of this study was to determine breed group differences in chute exit velocity in weaned calves.

Materials and Methods

Data (n = 3176) were collected from 2004 through 2007 in Florida, Louisiana, and Mississippi. Chute exit velocity (CEV) was calculated by recording the time (seconds) required for the calf to cross 6 feet when released from the working chute. Infrared sensors were used to remotely trigger the start and stop of timing. Velocity was calculated by distance (feet)/time (s). Breeds analyzed were Angus (A), Braford (BF), Brangus (BN), Brahman (B), Charolais (C), Hereford (H), Romosinuano (R), Commercial (X), and 2- and 3-way crosses of the above breeds. Not all breeds were represented at all locations. Data were analyzed by location by ANOVA, with the model terms for an overall mean, year, breed, calf gender where applicable, calf age, dam age, and error. Calf age and dam age were covariates. Means were separated using PDIFF of SAS (SAS Institute, Inc., Cary N.C.) with significance set at $P \leq 0.05$.

Results and Discussion

Chute exit velocity did not differ among location or year for calf gender. The number of observations by year, location, and gender are provided in Table 1. Cattle at Brooksville, Fla. did not differ ($P = 0.42$) in CEV by breeds or crossbreeds (Table 2). Breed differences among Marianna, Fla., cattle approached significance ($P < 0.10$) for CEV measures, with A similar to CA, CBN, BN and ABN; B was similar to ABN, AB, BN, CBN, and CA (Table 3). At Baton Rouge, La., mean CEV was affected by breed ($P < 0.01$) and B had greater ($P < 0.05$) mean CEV compared to A and BF, whereas BF had lesser ($P < 0.05$) mean CEV than A and B (Table 4). The CEV at Iberia, La., did not differ by breed groups (Table 5). At Raymond, Miss., (Table 6) mean CEV was affected ($P = 0.03$) by breed type, but AX (lowest value) did not differ ($P > 0.05$) from AB, BNX, and A. The HX (highest value) was similar ($P > 0.05$) to BNX, AB and A for mean CEV.

Implications

Previous studies have shown that selection for temperament can improve profitability, handler safety, and animal welfare. Chute exit velocity is an objective, repeatable tool to assess animal temperament. Breed type-differences for chute exit velocity has potential for among breed selection for temperament in weaned calves.

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Table 1. Number of observations by year, location, and gender.

Location	Sex	2004	2005	2006	2007	n
Fla.,	Bulls	12	16	16	26	70
Brooksville	Steers	243	125	159	162	689
	Heifers	223	150	191	198	762
Fla.,	Bulls		26	19	5	50
Marianna	Steers		95	163	61	319
	Heifers		109	163	77	339
La., Central	Heifers	23	41	29	29	122
La., Iberia	Heifers	61	77	48	20	206
Miss.	Bulls	2	2	115	2	134
	Steers	74	80	-	84	238
	Heifers	91	98	81	105	375
Totals		729	832	974	769	3304

Table 2. Chute exit velocity at Brooksville, Fla.

Breed	n	CEV*	SE
3-way X	581	1.92	± 0.02
AB-BA (Angus/Brahman x Brahman/Angus)	182	2.12	± 0.03
Angus (A)	207	1.97	± 0.03
AR-RA (Angus/Romo x Romo/Angus)	257	1.94	± 0.03
RB-BR (Romo/Brahman x Brahman/Romo)	383	1.92	± 0.02
RR (Romosinuono)	352	1.97	±0.02
Total	1962		

*Chute exit velocity.

Table 3. Chute exit velocity at Marianna, Fla.

Breed	n	CEV*	SE
Angus (A)	58	2.73[b]	± 0.12
Angus x Brangus (ABN)	30	3.07[ab]	± 0.17
Angus x Brahman (AB)	198	3.01[a]	± 0.07
Brangus (BN)	192	2.98[ab]	± 0.07
Charolais x Angus (CA)	27	2.83[ab]	± 0.18
Charolais x Brangus (CBN)	26	2.86[ab]	± 0.18
Brahman (B)	57	3.23[a]	± 0.12
Total	588		

*Chute exit velocity.
[ab]Within a column, means without a common superscript differ ($P = 0.08$).

Table 4. Chute exit velocity at Baton Rouge, La. (Central). Heifers only.

Breed	n	CEV*	SE
Brahman (B)	32	5.32[a]	± 0.39
Angus (A)	51	3.27[b]	± 0.21
Braford (BF)	39	2.61[c]	± 0.22
Total	122		

*Chute exit velocity.
[abc]Within a column, means without a common superscript differ ($P < 0.05$).

Table 5. Chute exit velocity at Iberia, La.

Breed	n	CEV*	SE
Angus (A)	136	3.08	± 0.07
Brangus (BN)	112	2.96	± 0.09
Total	278		

*Chute exit velocity.

Table 6. Chute exit velocity at Raymond, Miss.

Breed	n	CEV*	SE
Angus (A)	54	2.38[ab]	± 0.14
Angus x Brahman (AB)	308	2.22[ab]	± 0.06
Angus X (AX)	394	2.09[b]	± 0.06
Brahman X (BNX)	139	2.33[ab]	± 0.98
Polled Hereford X (HPX)	75	2.43[a]	± 0.13
Total	970		

*Chute exit velocity.
[ab]Within a column, means without a common superscript differ (P = 0.03).

Heat shock protein 70 gene polymorphisms to horn fly infestation

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Story in Brief

The relationship of single nucleotide polymorphisms (mutations) within the heat shock protein 70 (Hsp 70) gene and horn fly infestation was investigated utilizing unrelated Angus, Brahman, and reciprocal-cross breed types (n = 68). During the study, subjects were maintained on either endophyte infected tall fescue (E+) or common bermudagrass (BG). Cattle were observed for a twenty-one week cycle in which horn fly counts were noted individually when ≤25, or in groups of 5 when numbers exceeded 25. Counts were taken from a distance of 5 to 10 m. Single nucleotide polymorphisms identified included G2033C of the coding sequence in tandem with C895D and G1128T of the promoter region. Animals with homozygous guanine (GG) genotype of base 2033 generally had fewer flies compared to those with the guanine to cytosine base substitution (GC). Furthermore, the homozygous cytosine deletion (DD) at position 895 and homozygous thymine (TT) of base 1128 displayed fewer fly counts in comparison to other respective base position haplotypes. A forage by date interaction ($P < 0.0001$) occurred during each sequencing set with cattle grazing endophyte infected tall fescue generally displaying fewer flies.

Introduction

Haematobia irritans (Linnaeus), introduced from Europe in the late 19th century, is one of the most economically important ectoparasites of cattle (Koehler et al., 2005). Economic impacts to the industry from the horn fly totaled in excess of $730 million in 1981 (Drummond, 1987) and $876 million in 1991 (Kunz et al., 1991). Current estimates presume losses ranging from $700 million to $1 billion with additional expenditure of $60 million (Cupp et al., 1998) regarding pesticide application and usage.

At this time, control focuses on use of insecticide impregnated ear tags incorporating pyrethroid and organophosphates due to ease of obtainment. However, horn flies are now showing cross resistance within the continental U.S. Thus, chemical application is no longer a cure-all solution and should begin only when average counts reach an economical threshold of 200 per head for beef cattle or 100 per head for dairy. As a result, individual and breed susceptibility must be taken into account.

Furthermore, forage type fluctuates in growth, and nutritional value influencing physical productivity of cattle (Brown et al., 1993) providing another variable of interest. Thus, the objectives were to determine relationships between Hsp 70 single nucleotide polymorphisms, forage, and date to horn fly infestation.

Materials and Methods

Unrelated Angus (n = 20), Brahman (n = 17), Angus-Brahman (n=18) and Brahman-Angus (n = 13) crossbred cattle located in Booneville, Ark., were used as experimental models. Subjects during the trial grazed either endophyte-infected tall fescue (E+) or common bermudagrass (BG). Over the 21 wk trial period, May to October, trained observers obtained individual horn fly counts by walking around the subjects at a distance of 5 to 10 m. Numbers were recorded individually when <25 flies were present and in groups of 5 when ≥25.

Subsequently, cattle were genotyped for the G2033C single nucleotide polymorphism of heat shock protein 70 coding sequence, along with C895D and G1128T SNPs of the promoter region. Resulting data was analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, N.C.). Fixed effects were generated as genotype, forage, date, forage × date, and genotype × date. Random effect for each trial was designated as breed, while repeated measure was date.

Results and Discussion

In this study, results revealed 2 genotypes of the G2033C SNP: homozygous guanine (GG) and heterozygous guanine-cytosine (GC). The G2033C homozygous guanine haplotype, with a tendency of significance ($P = 0.069$), displayed fewer flies as seen within Fig. 1.

The C895D SNP promoter region disclosed three genotypes: homozygous cytosine (CC), heterozygous cytosine deletion (CD), and homozygous for the cytosine deletion (DD). Cattle homozygous for the cytosine deletion at base position 895 displayed fewer flies than other haplotypes of this base position (Fig. 2). The effect of base position 895 was a tendency effect ($P = 0.067$).

Three genotypes of G1128T promoter region G1128T ($P = 0.049$) were seen: homozygous guanine (GG), heterozygous guanine to thymine (GT), and homozygous thymine (TT). The heterozygous GT haplotype displayed higher fly counts over the duration of the trial (Fig. 3). In contrast, both homozygous haplotypes demonstrated similar counts, except within the month of August.

Forage by date interaction was noted ($P < 0.0001$) with cows grazing E+ typically displaying fewer flies. However, August shows the opposite for all SNPs. At this time, we are working under the hypothesis that increased endophyte concentrations and temperatures lead to reduced resistance to the horn fly regardless of genotype during this month.

Implications

Different Hsp 70 genotypes, forage, and month were associated with fewer horn fly numbers. Use of genotype in combination with forage type could be useful in identifying production systems to reduce horn fly infestation without the need for pesticide application.

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Further research is needed to conclude if Hsp 70 genotypes alone provide a superior genetic marker for horn fly resistant cattle.

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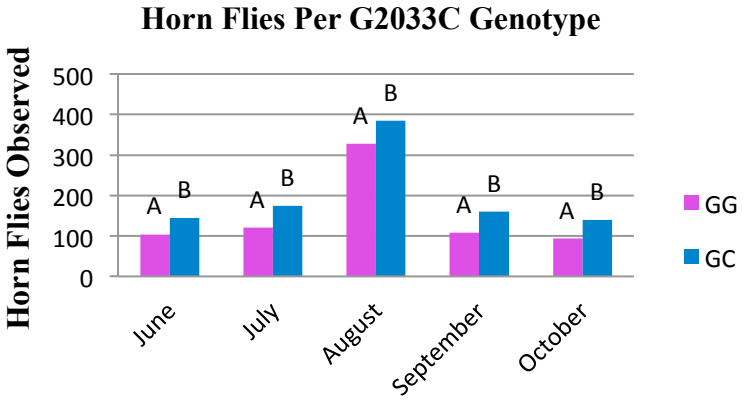

Fig. 1. The effect of infestation × haplotype interaction for heat shock protein 70 gene SNP of G2033C. Bars without a common superscript differ ($P < 0.05$) within month.

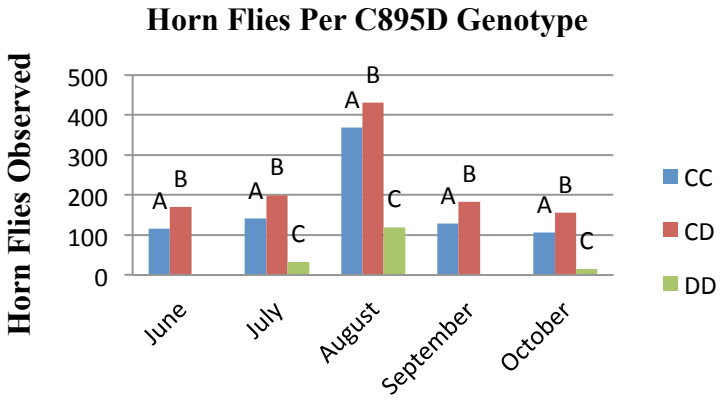

Fig. 2. The effect of infestation × haplotype interaction for heat shock protein 70 gene SNP of C895D of the promoter region. Bars without a common superscript differ ($P < 0.05$) within month. Note no observations were made for the DD haplotype in June or September.

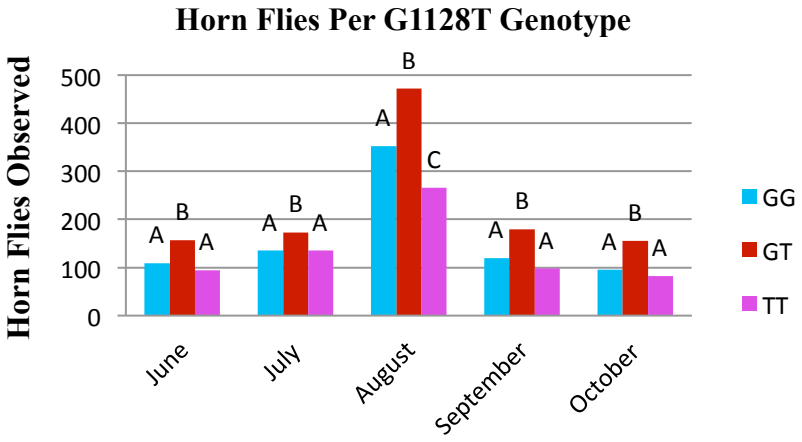

Fig. 3. The effect of infestation × haplotype interaction for heat shock protein 70 gene SNP of G1128T of the promoter region. Bars without a common superscript differ ($P < 0.05$) within month.

Performance by yearling Katahdin ewes grazing tall fescue pastures using continuous or rotational grazing schemes—1 year summary

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Story in Brief

Rotational grazing schemes have become increasingly popular in livestock production systems. However, they have not been well documented for Katahdin hair sheep. Our objectives were to compare performance, parasite load, and reproductive measurements by yearling Katahdin ewes grazing endophyte-infected tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh; E+] in late spring through summer using either continuous or rotational grazing schemes. Beginning May 5, 2011, yearling Katahdin ewes (n = 25; 123.7 ± 2.74 lb initial body weight; 3.5 ± 0.3 initial body condition score) were stratified by body weight and allocated randomly to one of five 1-acre tall fescue pastures representing two treatments: 1) continuous (C; two replications) or 2) four-cell rotation (4R; three replications). At the initiation of the breeding season (May 19, 2011), one ram was placed with each group of ewes for 40 d. Body weight at beginning and end of breeding, final body weight, total body weight gain, and average daily gain did not differ ($P \geq 0.62$) across treatments. At breeding, end of breeding, and final body condition scores and FAMACHA© scores did not differ ($P \geq 0.14$) across treatments. Lambing rates were greater ($P \leq 0.01$) from four-cell rotation compared with continuous. Number of lambs born/ewe exposed did not differ ($P = 0.16$) across treatments. Therefore, rotationally grazing endophyte-infected tall fescue pastures in late spring through summer may not improve yearling Katahdin ewe performance and parasite load, but may increase lambing rates.

Introduction

Rotational grazing schemes have become increasingly popular in livestock production systems. These grazing schemes may increase carrying capacity and may help maintain forages in a vegetative state, but may not increase cattle performance when compared with continuous grazing methods (Sharrow and Krueger, 1979; Ball et al., 2007). However, little research has been done to examine the effects of rotational compared with continuous grazing in sheep, especially using Katahdin hair sheep on endophyte-infected tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh; E+]. Therefore, the objective of this study was to compare performance, parasite load, and reproductive measurements by yearling Katahdin ewes grazing E+ pastures in late spring through summer using either continuous or rotational grazing schemes.

Materials and Methods

This study was conducted at Lincoln University Carver Farm in Jefferson City, Mo. Twenty-five yearling Katahdin ewes (123.7 ± 2.7 lb initial body weight (BW); 3.5 ± 0.3 initial body condition score (BCS)) were stratified by body weight and allocated randomly on May 5, 2011 to one of five 1-acre E+ pastures at a stocking rate of five ewes/acre. Pastures were assigned randomly to one of two treatments, consisting of 1) continuous grazing (C; 2 replications) or 2) four-cell rotational grazing (4R; 3 replications). Beginning May 19, 2011, one ram that passed a breeding soundness exam was placed in each pasture for a 40-d breeding season and controlled internal drug-release (CIDR) inserts were removed from ewes. The CIDR inserts were administered fourteen days prior to ram introduction. Immediately following CIDR removal, each ewe was administered 400 IU of PG600 and returned back to their respective pastures. Body weight, BCS (1-5 scale; 1 = emaciated; 5 = obese; Russel et al., 1969), and FAMACHA© scores (1-5 scale; 1 = healthy; 5 = severely anemic; Bath et al., 2001) were determined at the start of breeding, end of breeding, and at end of the study. Reproductive measurements consisted of lambing rate and number of lambs born/ewe exposed.

Ewe performance and number of lambs born/ewe exposed were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, N.C.) with pasture or group of animals as the experimental unit. Lambing rates were analyzed by Chi-square using PROC FREQ of SAS. Treatment means are reported as least squares means.

Results and Discussion

Start breeding, end breeding, and final BW, BCS, and FAMACHA© scores did not differ ($P \geq 0.14$) across treatments for the duration of the study (Table 1). Average daily gain and total gain did not differ ($P \geq 0.71$) across treatments, which agrees with previous research in Romney sheep (Sharrow and Krueger, 1979). FAMACHA© scores averaged a score of one for the duration of the project, thus ewes were considered to be healthy and not burdened by parasite loads.

Lambing rates were greater ($P = 0.01$) from 4R compared with C (93% vs. 50%, respectively). However, number of lambs born/ewe exposed did not differ ($P = 0.16$) between C and 4R, although numerically 4R had on average one lamb more compared to C. Therefore, rotational grazing hair sheep in late-spring through summer may not improve yearling ewe performance but may increase lambing rates.

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Implications

Based on these results, producers using a continuous grazing system with endophyte infected tall fescue pastures may increase lambing rates by switching to a rotational grazing system, thus allowing for more lambs to be marketed.

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Table 1. Performance by yearling Katahdin ewes grazing endophyte-infected tall fescue pastures using either a continuous or four-cell rotation grazing scheme.

	Treatment[a]			
Item	**C**	**4R**	**SEM[b]**	***P*-Value**
Body Weight, lb				
at breeding	126	124	2.8	0.62
end of breeding	133	133	3.3	0.97
end of study	142	141	3.3	0.90
Body condition score				
at breeding	4	3	0.2	0.14
end of breeding	4	3	0.2	0.22
end of study	3	3	0.1	0.87
FAMACHA©				
at breeding	1	1	0.2	0.30
end of breeding	1	1	0.1	0.27
end of study	1	1	0.2	0.69
Average daily gain, lb	0.2	0.2	0.020	0.72
Total gain, lb	17.6	18.3	2.20	0.71
Lambing rate, %[c]	50	93	–	0.01
Number of lambs/ewe exposed	0.8	1.9	0.43	0.16

[a]C = Continous; 4R = four-cell rotation.
[b]SEM = Pooled standard error of the mean.
[c]Analyzed using Chi-square procedure of SAS.

Replacing synthetic nitrogen with clovers or alfalfa in bermudagrass pastures for growing calves

Paul Beck[1], Thomas Hess[2], and Don Hubbell[2]

Story in Brief

The objective of this research was to determine the impact of alfalfa or clover additions to bermudagrass pastures on performance of growing calves and pasture carrying capacity compared to commercial N. In October 2008, bermudagrass (*Cynodon dactylon* [L.] Pers.) pastures (n = 8; 2 acre) were interseeded with 12 lb red clover/acre (Trifolium pratense, cv. Morningstar, Cal/West Seeds, Woodland, CA) and 3 lb/acre ladino white clover (Trifolium repens, cv. Regal Graze, Cal/West Seeds) or with 25 lb alfalfa/acre (Medicago sativa, cv. PGI 459, Producers Choice, Woodland, Calif.). Due to stand losses in alfalfa pastures, the 4 established alfalfa pastures were destroyed in fall 2009 and alternate pastures were established to another alfalfa variety selected for grazing under conditions in the southern U.S. (cv. Rebel, Producers Choice, Woodland, Calif.). The 12 bermudagrass pastures received 0, 50, or 100 lb N/acre as ammonium nitrate. Over 3 years, growing beef steers (n = 431, body weight = 542 ± 73 lb) grazed pastures in this put and take experiment in which each year four steers were selected for each pasture to measure animal performance and additional steers were added or removed in order to maintain similar forage allowance among pastures. Grazing was initiated in mid April or May in alfalfa and clover pastures each year and in May in fertilized bermudagrass pastures. Data were analyzed as a completely randomized design with the mixed procedure of SAS. Single df contrasts were used to determine the linear N fertilization rate effect, the effects of alfalfa and clover vs. each N fertilization rate, and the alfalfa vs. clover comparison. Stand counts of initial alfalfa pastures decreased from 34% in May 2009 to 15% in October 2009, but alfalfa stands in replacement pastures remained between 45% and 55% through 2010 and 2011. Clover pastures maintained stands of 38% to 55% over the 3 year period. Daily gains increase linearly ($P < 0.01$) with increasing N rate. Daily gains of clover and alfalfa pastures did not differ ($P = 0.64$) from 50 lb N rate, but were less ($P < 0.01$) than 100 lb N rate. Gains of clover and alfalfa steers did not differ ($P = 0.59$). Body weight gain/acre increased linearly ($P < 0.01$) with increasing N rate.The legumes produced more ($P < 0.01$) bodyweight gain/acre and grazing-days/acre than all N fertilizer rates. Grazing-d/acre of alfalfa was greater ($P = 0.05$) than clover, yet bodyweight gain/acre did not differ ($P = 0.54$).

Introduction

Increasing costs of production are causing beef producers to look at alternative production systems and production practices (Rouquette and Smith, 2010). From 2002 to 2008, the cost of synthetic N has increased by over 300%, contributing to elevated costs of production and cost of bodyweight gain (Rouquette and Smith, 2010). The inexpensive N fertilizers of the 1950s and 1960s led to replacement of grass-clover pasture combinations with more productive N fertilization of grass pastures (Rouquette and Smith, 2010). With increasing N fertilizer cost, economic conditions are present for a shift back to legume inclusion in pasture systems. Although estimates vary depending on a multitude of conditions, clovers contribute from 20 to over 200 lb N/acre to pastures (Knight, 1970), but direct transfer of N from legumes to grasses growing in the same season is extremely low (Morris et al., 1990). The objective of this research was to determine the effects of white and red clover or alfalfa additions to bermudagrass pastures on steer performance in relation to a range of N fertilization rates.

Materials and Methods

This research took place on 40 acres of bermudagrass pasture located at the University of Arkansas Livestock and Forestry Research Station near Batesville in northeast Arkansas (35°50' N, 91°48' W). The study site consisted of Peridge silt loam soil which is a deep well-drained upland soil with moderate fertility. In October 2008, bermudagrass (*Cynodon dactylon* [L.] Pers.) pastures (n = 8; 2 acre) were interseeded with cool-season legumes. Four pastures were seeded with 12 lb red clover/acre (Trifolium pratense, cv. Morningstar, Cal/West Seeds, Woodland, Calif.) and 3 lb ladino white clover/acre (Trifolium repens, cv. Regal Graze, Cal/West Seeds). Four pastures were seeded with 25 lb alfalfa/acre (Medicago sativa, cv. PGI 459, Producers Choice, Woodland, Calif.). Due to stand losses in alfalfa pastures, the 4 established alfalfa pastures were destroyed in fall 2009, and alternate bermudagrass pastures were established to another variety selected for grazing under conditions in the southern United States (cv. Rebel, Producers Choice, Woodland, Calif.). The remaining 12 bermudagrass pastures received 0, 50, or 100 lb N/acre as ammonium nitrate (n = 4 pastures per N rate) in split applications (one-half of total N per application) in May and July each summer.

Over 3 years, growing beef steers (n = 431, bodyweight = 542 ± 73 lb) grazed pastures in this put-and-take experiment in which each year four steers were selected for each pasture to measure animal performance and additional steers were added or removed in order to maintain similar forage allowance among pastures. Steer bodyweight gain/acre were calculated based on daily gains of 4 tester animals in each pasture and the total number of grazing days of grazers and testers per acre. Grazing was initiated in all pastures on May 29, 2009 in year 1 and on May 25 in year 2. In year 3, alfalfa pastures were ready to graze on April 14 and clover pastures were ready to graze by April 29, while grazing of bermudagrass pastures was initiated on May 11, 2011. Stand counts of the initial alfalfa pastures decreased from 34% in May, 2009 to 15% in October 2009, but alfalfa stands in replacement pastures were 46% at the end of grazing in 2010

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and 55% at the end of grazing in 2011. Clover pastures had stand densities of 38%, 43%, and 55% at the end of grazing in 2009, 2010, and 2011, respectively.

This study was analyzed as a randomized complete block design with a split-plot using the Mixed Procedure of SAS (SAS Institute, Cary, N.C.). Year was considered a random effect. Pasture was considered the experimental unit. Single df contrasts were used to determine the linear N fertilization rate effect, the effects of alfalfa and clover vs. each N fertilization rate, and the alfalfa vs. clover comparison.

Results and Discussion

The performance of steers grazing bermudagrass pastures with N fertilization or interseeded with clovers or alfalfa is presented in Table 1. As N fertilization rate increased, average daily gain (ADG) of steers increased in a linear fashion ($P < 0.01$), increasing from 1.35 lb/d for steers grazing unfertilized pastures to 1.80 lb/d for steers on the pastures fertilized at the 100 lb/acre rate. Daily gains of steers grazing alfalfa and clover pastures did not differ ($P \geq 0.59$) from each other or the 50 lb N/acre rate averaging 1.59 lb/d, but were greater ($P < 0.01$) than ADG of steers on unfertilized pastures. Steers grazing pastures fertilized with 100 lb N/acre gained 0.21 lb/d more ($P < 0.01$) than steers grazing legume pastures. Bodyweight upon removal from pastures was, as observed with ADG, increased linearly with increasing N fertilization ($P < 0.01$). Bodyweight when steers were removed from pastures did not differ ($P \geq 0.12$) between alfalfa and clover pastures or between legume pastures and 50N or 100N fertilization rates.

As would be expected, total bodyweight gain per acre was increased ($P < 0.01$) linearly with increasing N fertilization rates, but surprisingly there was no change ($P = 0.42$) in grazing-day per acre with increasing N fertilization. The lack of increase in grazing days may be due to drought conditions that have been encountered during 2-years of the 3-year study. Due to slightly longer grazing season and greater stocking rate during the early grazing season, alfalfa and clover pastures had greater ($P < 0.01$) grazing-days/acre than all N fertilization rates. Bodyweight gain/acre was thence greater ($P < 0.01$) for legume pastures compared with all N fertilization rates. Steer grazing-days/acre were greater ($P = 0.05$) for alfalfa than clover pastures yet bodyweight gain per acre did not differ ($P = 0.55$).

Using regression analysis on the bodyweight gain per acre observed at the varying N fertilization rates indicated that for each lb of N fertilizer, bodyweight gains were increased by 1.25 lb/acre. Alfalfa pastures produced the equivalent gain/acre of 185 lb N/acre and clovers produced the equivalent of 168 lb N/acre. Although outside of the range of data the N response curve was determined, the N equivalent observed with legume indicates that legumes can effectively replace high rates of N fertilizer.

Implications

Nitrogen fertilizer can effectively be replaced by either clovers or alfalfa in bermudagrass pastures for growing beef steers. Alfalfa variety selection is an important factor to consider when utilizing this legume for grazing cattle. Varieties of alfalfa selected under similar management and growing conditions would be expected to persist longer than varieties selected for hay production in other environments. Species of clovers must be selected to match both the site and growing season of main use and no one species of clover would be expected to produce through the entire grazing season.

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Table 1. Effect of alfalfa or white and red clovers interseeded into bermudagrass pastures compared with N fertilization on performance of growing beef steers.

	N fertilization Rate						Contrasts				
							Linear N	Legume vs.			Alfalfa vs. Clover
Item	0	50	100	Alfalfa	Clover	SE		0N	50N	100N	
Bodyweight, lb											
Initial	535	540	542	544	549	36.3	0.55	0.25	0.53	0.66	0.65
Final	665	689	715	708	713	27.8	<0.01	<0.01	0.12	0.73	0.73
Average daily gain, lb/d	1.35	1.55	1.80	1.57	1.61	0.14	<0.01	<0.01	0.64	<0.01	0.59
Grazing-days/acre	225	243	238	335	304	18.5	0.42	<0.01	<0.01	<0.01	0.05
Gain/acre, lb	293	370	415	529	509	37.7	<0.01	<0.01	<0.01	<0.01	0.55

Digestibility by lambs offered alfalfa hay treated with a buffered propionic acid hay preservative and baled at different concentrations of moisture[1]

K. Coffey[2], W. Coblentz[3], A. Young[2], M.G. Bertram[4], and R. Carter[2]

Story in Brief

Eighteen crossbred wether lambs (76.1 ± 8.18 lb initial body weight (BW)) were used for a 2-period digestion study to evaluate the effect of hay preservative concentration (0%, 0.56%, or 0.98% buffered propionic acid[5]) and hay moisture concentration at baling (19.6%, 23.8%, or 27.4% moisture) on digestibility of alfalfa hay. Lambs were stratified by weight and allocated randomly such that two lambs each period were offered one of the nine treatment combinations. Alfalfa hay was chopped (1.5"), and then offered to lambs for 10 days of adaptation at 2.2% of BW on an as-fed basis in equal feedings at 0800 and 1700 h. Total feces were collected twice daily over a 7-d period to determine fecal output beginning on day 11. Lambs were allocated randomly to a different treatment in the second period. Digestibility was greatest ($P < 0.05$) from lambs offered hay baled at 19.6% moisture and treated with 0.56% propionic acid, and those offered hay baled at 23.8% moisture and treated with 0.98% propionic acid. The lowest ($P < 0.05$) digestibilities were from hay baled at 23.8% moisture without preservative and those baled at 27.4% moisture with either concentration of preservative. Therefore, propionic acid was able to enhance digestibility of hay baled at a moderately excessive moisture concentration (23.8%) but not at excessive moisture (27.4%).

Introduction

Unfavorable weather conditions during the hay making process often force producers to make a choice between allowing their hay to be rained on, or baling their hay at excessive moisture. Mold and other microbial growth in hay baled at excessive moisture can result in reduced hay quality, digestibility, and acceptability by animals. Hay preservatives such as buffered propionic acid products are often used to reduce mold growth in hay that is baled at excessive moisture, thereby reducing some of the risk associated with hay making. However, the window of opportunity at which propionic acid preservatives are effective in reducing mold growth and forage quality, and the effective application rate of propionic acid at greater moisture concentrations needs further definition. The objective of this study was to determine the digestibility of alfalfa hay baled in large square bales at different moisture concentrations and treated with different levels of a buffered propionic acid product as a hay preservative.

Materials and Methods

Alfalfa hay was grown and baled at the University of Wisconsin Marshfield Agricultural Research Station, Marshfield, Wis. Hay was packaged in 3 × 3 × 6-ft. large square bales at 19.6%, 23.8%, or 27.4% moisture. At baling, approximately one third of the bales within each moisture concentration were not treated, or were treated with either 0.56% or 0.98% propionic acid. Bales were stored inside a barn through the winter and then shipped to Fayetteville, Ark. in the spring. Hay was then stacked under a shed with a metal roof to prevent weather effects on the bales. Prior to feeding, two representative bales from each moisture and propionic acid treatment combination were selected. The center 1/3 of each bale was chopped using a commercial bedding shreader to an approximately 3" particle length. The two bales within each moisture concentration and acid treatment combination were chopped into the same stack, mixed and stored in plastic bags until feeding.

Eighteen wether lambs (76.1 ± 8.18 lb initial body weight (BW)) were obtained from one local producer and were crosses between black-faced (Suffolk/Hampshire) and Gulf Coast Native breeding. The crossbred lambs were produced by breeding ewes with a greater percentage of Gulf Coast Native to a black-faced ram and those ewes having a greater percentage of black-faced genetics to a Gulf Coast Native ram. Upon arrival at the University of Arkansas facilities, the lambs were weighed, dewormed with levamisole hydrochloride (Prohibit® Soluble Drench Powder Anthelmintic, Agri Laboratories, St. Joseph, Mo. 64503), then placed randomly in individual pens (3.5 × 5 ft) with expanded metal floors. The pens were located inside of an enclosed metal building equipped with ventilation fans. Each pen was equipped with a metal feeder and an automatic nipple waterer, but water was also provided for ad libitum consumption via rubber water pails placed inside of each pen.

During each period, lambs were weighed, stratified by BW, and then allocated randomly such that 2 lambs each were offered 1 of the 9 treatment combinations. Lambs were offered alfalfa hay for 10 days of adaptation at 2.2% of BW on an as fed basis in equal feedings at 0800 and 1700 h. Samples of hay offered daily and any hay refused were dried at 122 °F to determine dry matter. Lambs were fitted with fecal collection bags and total feces were collected twice daily, weighed, and dried at 122 °F to determine fecal output over a 7-d period beginning on day 11. At the end of period 1, lambs were removed from their pens, weighed, offered the low-moisture control alfalfa hay as a group for 8 d, and allowed access to a pasture with

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information, and does not imply either recommendation or endorsement by the U.S. Department of Agriculture.

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[4] Superintendent, University of Wisconsin Marshfield Agricultural Research Station, Marshfield, Wis.

[5] FRESH CUT Plus; Kemin AgriFoods North America, Inc.; Des Moines, Iowa.

bermudagrass and broadleaf weeds. Lambs were then weighed and allocated randomly to 1 of the 9 treatments for a second period but were not offered the same treatment as in period 1. All procedures were the same for the second period as described for period 1.

Chemical composition is reported on the specific bales offered to lambs in the digestion study. Since the results reflect only the chemical composition from two bales that were mixed together for the purposes of feeding, statistical analyses were not conducted on forage quality measurements. Statistical analyses on digestibility data were conducted using PROC MIXED of SAS (SAS Institute, Inc., Cary, N.C.) for a completely randomized design. Treatment was considered a fixed effect and period was considered a random effect.

Results and Discussion

Chemical composition of the specific bales offered to lambs is shown in Table 1. As mentioned previously, statistical analyses were not conducted since the values in Table 1 represent those from the two large square bales that were offered to 4 different lambs over the course of the digestion study. Therefore, discussion of these trends should be viewed with caution since there is no measure of variability. Concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin increased with increasing moisture concentration at baling, but were reduced within the elevated moisture concentrations when propionic acid was applied. These changes would be expected as soluble carbohydrates are metabolized to a greater extent in hay baled at elevated moisture concentrations, and propionic acid treatment generally reduces the metabolism of soluble carbohydrates. When soluble carbohydrates are metabolized, concentrations of other components are elevated as a result of loss of these carbohydrates. Concentrations of crude protein (CP) did not appear to follow a particular trend. The proportion of total CP that is bound in the ADF fraction is used to represent heat damage in forages baled at excessive moisture concentrations. Without acid treatment, concentrations of ADF-bound CP were elevated in hay baled at excessive moisture concentrations (>20% moisture). Within hay baled at excessive moisture (23.8% and 27.4% moisture at baling) propionic acid appeared to reduce heat damage, particularly in hay baled at 23.8% moisture as reflected in lower concentrations of acid detergent insoluble crude protein (ADICP).

The moisture concentration × propionic acid application rate interaction was detected ($P < 0.05$) for digestibility of dry matter (DM). Digestibility of DM was greatest ($P < 0.05$) from lambs offered hay baled at 19.6% moisture and treated with 0.56 % propionic acid, and those offered hay baled at 23.8% moisture and treated with 0.98% propionic acid. The lowest ($P < 0.05$) digestibilities of DM were from hay baled at 23.8% moisture without preservative and those baled at 27.4% moisture with either concentration of preservative. The remaining treatment combinations were intermediate in digestibility. Based on this information, it appears that propionic acid treatment was effective in maintaining digestibility when the alfalfa hay was baled at 23.8% moisture, but was not able to maintain digestibility when baled at greater moisture concentrations.

Implications

Baling hay at excessive moisture concentrations is necessary sometimes in order to avoid adverse weather conditions causing even greater damage to the hay. In instances where hay is baled at moderately excessive moisture concentrations, treatment of the hay with propionic acid may help reduce chemical reactions in the hay that reduce overall digestibility. However, at excessive moisture concentrations at baling, up to 1% propionic acid may not be effective in reducing those negative chemical reactions.

Table 1. Nutrient composition of alfalfa hay that was untreated or treated with different levels of propionic acid and baled at different concentrations of moisture[1].

Moisture at baling		**19.6**			**23.8**			**27.4**	
Rate of propionic acid	**0**	**0.56**	**0.98**	**0**	**0.56**	**0.98**	**0**	**0.56**	**0.98**
Neutral detergent fiber, %	58.82	56.34	58.45	61.14	57.69	56.29	63.31	62.72	60.58
Acid detergent fiber, %	41.74	39.14	43.55	42.73	38.03	37.74	45.13	44.96	43.65
Acid-detergent lignin, %	7.33	6.44	7.41	7.54	5.82	6.10	7.86	8.04	7.60
Crude protein, %	18.61	17.43	18.77	18.81	17.60	18.24	17.70	18.93	18.70
Acid-detergent insoluble crude protein, % of dry matter	2.34	2.14	2.47	3.08	1.66	1.99	2.45	2.60	2.37
Acid-detergent insoluble crude protein, % of crude protein	12.59	12.31	13.15	16.37	9.43	10.88	13.83	13.72	12.68

[1]Values represent those from only two large-square bales per treatment. Therefore, statistical analyses were not conducted.

Table 2. Intake and digestibility by lambs offered alfalfa hay baled at different moisture concentrations and treated with different rates of propionic acid as a hay preservative.

Moisture at balling		**19.6**			**23.8**			**27.4**		
Rate of propionic acid preservative	**0**	**0.56**	**0.98**	**0**	**0.56**	**0.98**	**0**	**0.56**	**0.98**	**SEM**
Wt 1, lb	72.5	73.5	74.5	76.0	73.8	75.8	73.8	75.3	73.0	4.42
Dry matter intake, % of body weight	2.05	2.03	2.00	1.94	2.01	1.99	2.00	1.91	1.92	0.358
Dry matter digestion, %[1]	54.6^{B}	56.6^{A}	54.4^{B}	52.4^{C}	54.2^{B}	57.6^{A}	53.0^{B}	52.4^{C}	51.8^{C}	0.601

[1]Means without a common letter differ ($P < 0.05$).

Diurnal variation in fecal concentrations of indigestible-acid detergent fiber, acid-detergent insoluble ash, and alkaline-peroxide lignin from cattle fed bermudagrass hays of varying quality

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Story in Brief

The effect of time of fecal sampling on the accuracy of indigestible acid detergent fiber, acid detergent insoluble ash, and alkaline-peroxide lignin for the prediction of fecal output in cattle was evaluated. Eight cannulated cows (1307 ± 78.0 lbs) were allocated randomly to 4 bermudagrass hay diets having a wide range of crude protein concentrations (7.9 to 16.4% dry matter (DM)), with 2 replicates/diet for 3 periods (n = 24). Cows were individually fed their respective hay at a total of 2% DM of body weight (BW) in equal feedings at 0800 and 1700 h for a 10-d adaptation period, followed by a 5-d total fecal collection. Fecal grab samples were taken each day during the fecal collection period at 0600, 1200, 1800, and 2400 h either directly from the rectum or from fresh feces, and were composited by cow and time across the 5 d of total collection. Duplicate samples of each hay, ort, and fecal sample were incubated for 144 h in the rumen of 2 cows for each period (n = 6 cows), followed by analysis of acid detergent fiber to obtain adjusted indigestible acid detergent fiber. Forage, ort, and fecal samples were analyzed for concentrations of alkaline peroxide lignin and acid detergent insoluble ash. Time of sampling affected the concentration of adjusted indigestible acid detergent fiber ($P < 0.01$), while acid detergent insoluble ash and alkaline-peroxide lignin concentrations in fecal samples were similar ($P \geq 0.24$) across sampling times and to those determined based on total collection. Estimates of dry matter digestibility and fecal output by a representative sample from total fecal collection and those from all grab sampling times were not ($P \geq 0.85$) different from actual dry matter digestibility and fecal output values as determined by total collection irrespective of internal markers. Therefore, there is little variation in concentrations of acid-detergent insoluble ash and alkaline-peroxide lignin in daily fecal excretion, and 2 daily fecal samplings can be used in the prediction of dry matter digestibility and fecal output.

Introduction

Due to the expense and difficulty of testing a large numbers of forage samples using in vivo techniques for measuring dry matter intake (DMI), fecal output (FO), and dry matter digestibility (DMD), indirect methods using external and internal markers can be applied (Owens and Hanson, 1992) to save labor expenses and avoid total collection of feces. Several studies have reported variation within 1 d in fecal concentration of external markers (Titgemeyer, 1997), but few studies have evaluated diurnal fecal concentration pattern of internal markers (Momont et al., 1994; Sampaio et al., 2011). Information is needed on the variation of internal markers during a 24-h period to determine whether or not sampling time affects marker recovery. Therefore, the objective of this study was to evaluate the effect of time of fecal sampling on the accuracy of indigestible acid detergent fiber (IADF), acid detergent insoluble ash (ADIA), and alkaline peroxide lignin (APL) to predict fecal output (FO) and dry matter digestibility (DMD) in cattle fed varying quality of bermudagrass hays.

Materials and Methods

Eight ruminally cannulated cows (1307 ± 78.0 lbs) were allocated randomly to 4 bermudagrass hay diets having a wide range of crude protein concentrations (7.9% to 16.4% of DM), with 2 replicates/diet for 3 periods (n = 24). Cows were individually fed their respective hay at a total of 2% of BW in equal feedings at 0800 and 1700 h for a 10-d adaptation period followed by a 5-d total fecal collection (TC) period in 9.8 × 14.1-ft pens fitted with rubber mats.

Fecal Grab Sample Collection and Preparation for In Situ Analysis. Fecal grab samples (approximately 0.66 lbs for each sample) were taken 4 times daily (0600, 1200, 1800, and 2400 h) directly from the rectum of each cow or from freshly excreted feces, and samples were oven-dried at 50 °C for DM determination and further chemical analysis. Fecal grab samples were composited by cow and time of sampling within period. Fecal grab samples were ground to pass a 2-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, N.J.). Dacron bags (3.9 by 7.87-inches; 53 ± 10-µm pore size; ANKOM Technology Corp., Fairport, N.Y., USA) were filled with 0.18 oz. fecal grab samples and closed with rubber bands. Duplicate bags (n = 192) were prepared for each fecal grab sample.

In Situ Experiment for Analyzing IADF. Fecal grab samples were analyzed for rumen undegradable dry matter (RUDM), indigestible neutral detergent fiber (INDF), and IADF by in situ incubation followed by neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) extraction. Duplicate fecal grab samples from the same period were incubated in two cows (n = 6) for 144 h. Cows were offered a total of 2% BW of a bermudagrass hay-based diet in equal meals at 0800 and 1600 h and had ad libitum access to water and mineral salt (4 oz/cow per day). Diet fed during in situ incubation period consisted of bermudagrass hay (CP = 10.8% DM) fed at 1.7% BW, supplemented with a concentrate mix (CP = 21.0% DM) fed at 0.3% BW. The in situ procedure was modified in that bags were not soaked in tepid water prior incubation because of the long incubation time (144 h). Individual bags were placed in 1.2 × 1.6-ft mesh bags and inserted into the ventral rumen immediately prior to feeding on d 10 of the adaptation period. After 144 h of incubation, the Dacron bags

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were removed from the rumen and were subjected to a hand washing (rinsing) with cold-water until the water was clear (approximately 10 times) to prevent any loss of sample due to washing machine use. All rinsed bags were dried to a constant weight at 122 °F and allowed to equilibrate to ambient temperature and residual DM remaining was recorded before proceeding to a sequential analysis of NDF and ADF.

Chemical Analysis of $IADF_a$, APL, and ADIA in Fecal Grab Samples. The IADF on the residual DM from the in situ was analyzed by using the batch procedure of ANKOM Technol. Corp. (Fairport, N.Y.), where 0.018 ± 0.00035 oz of each residual DM were put in filter bags (#F57; ANKOM Technol. Corp.) and sequentially analyzed for NDF and ADF. Adjusted IADF ($IADF_a$) was obtained by multiplying the initial fecal grab sample weight incubated by the corresponding correction factor (CF) obtained from samples of total fecal collection to obtain the initial fecal DM corrected for DM loss. Furthermore, fecal grab samples were ground to pass 1-mm screen Willey mill, and analyzed for ADIA using the ANKOM procedure (ANKOM Technol. Corp., Fairport, N.Y.), where 0.018 ± 0.00035 oz of fecal sample were analyzed for ADF, and the remaining ADF residue was ashed in a muffle furnace at 932 °F for 8 h. Alkaline peroxide lignin analysis was performed by placing 0.018 ± 0.00035 oz of fecal matter in filter bags, incubated in alkaline hydroxide peroxide (AHP; pH = 11.5) solution for 24 h, and subsequently rinsed to neutral pH with hot distilled water. The AHP residue was then analyzed for ADF and acid detergent lignin (ADL) to quantify APL concentrations in fecal grab samples.

Calculation of DMD and FO Using Fecal Grab Samples. The estimated DMD by fecal grab samples taken at different times was calculated as:

$$DMD = 100 \times \left(1 - \frac{M_{fd}}{M_{ftime}}\right) \quad \text{Eq. (1)}$$

where M_{fd} is the concentration of marker in consumed hay, and M_{ftime} is the marker concentration in each fecal grab sample at a particular sampling time.

Estimate of FO by fecal grab sample taken at different times was calculated according to the following expression:

$$FO = DMI \times \frac{M_{fd}}{M_{ftime}} \quad \text{Eq. (2)}$$

The 4 sampling times resulted in 15 different combinations of times to compare to in vivo data and a representative sample from TC. The values were then compared to determine diurnal variation in marker concentration, as well as to determine how close the concentrations of marker in the grab samples were to those obtained by TC and which time, or combination of times, of sampling could provide the marker concentrations closest to those from TC to predict DMD and FO.

Statistical Analysis. Data for marker concentration in grab samples were analyzed as a replicated 4 × 4 Latin-square design, with one period missing using PROC GLM of SAS. Effects of cow, diet, and period were included in the model and significances among treatments were noted at $P < 0.05$. Data for DMD and FO estimates from grab sampling were also analyzed separately for each marker for the prediction of actual DMD and FO using PROC GLM of SAS. Effect of diet, time, and diet by time interaction were include in the model to compare digestibility and FO estimates using different sampling times with the in vivo value of DMD and FO.

Results and Discussion

Marker Concentration in Feces by Sampling Time. The concentration of adjusted $IADF_a$ was affected by sampling time ($P < 0.01$; Table 1) and diet ($P = 0.02$). Time of fecal grab-sampling seemed to overestimate the concentration of $IADF_a$ in feces in comparison with the marker concentration derived from TC. There were no ($P \geq 0.62$) diet by time of sampling interactions for all three markers. The concentrations of ADIA and APL were not ($P \geq 0.24$) affected by sampling time, but diet affected ($P < 0.01$) ADIA and APL concentrations.

Fecal Output Estimation and Digestibility by Sampling Time. Estimates of FO for the three internal markers ($IADF_a$, ADIA, APL) were similar ($P = 0.36$) to the value obtained by in vivo procedure (Table 2). Estimates of DMD determined from a combination of 4 fecal grab samples per/d differed ($P < 0.01$) by markers, even though their estimates were similar to the in vivo value. There was a diet × marker interaction ($P = 0.01$) on DMD but not ($P = 0.86$) for FO. Alkaline peroxide lignin (APL) overestimated the DMD of low quality bermudagrass (502 vs. 56.9; Table 3), whereas $IADF_{a1234}$ underestimated the DMD of low quality bermudagrass (502 vs. 392). Estimates of DMD and FO (Table 4) by $IADF_a$, ADIA, and APL at the 4 fecal sampling times were similar to the in vivo values ($P \geq 0.85$).

Implications

Estimates of digestibility and fecal output by fecal sampling times were similar to actual values irrespective of internal markers. There was little variation in concentrations of acid detergent insoluble ash and alkaline-peroxide lignin in daily fecal excretion and 2 daily fecal samplings can be used in the prediction of digestibility and fecal output.

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Table 1. Mean fecal concentrations (% dry matter, DM) of different internal markers ($IADF_a$, ADIA, APL) derived from different sampling times compared with fecal concentrations of these markers with values from total collection (TC).

Marker[b]	Time of sampling				TC[c]	SEM[d]	*P*-value[a]		
	0600	1200	1800	2400			D	T	D × T
$IADF_a$	36.3[ef]	37.2[e]	36.1[ef]	35.0[fg]	34.5[g]	0.45	0.02	<0.01	0.98
ADIA	5.9	5.8	6.1	5.8	5.8	0.15	<0.01	0.46	0.62
APL	5.5	5.9	5.8	5.8	5.6	0.13	<0.01	0.24	0.94

[a]D= diet; T= time; and D × T= diet by time interaction.
[b]$IADF_a$, adjusted indigestible acid detergent fiber; ADIA, acid detergent insoluble ash; and APL, alkaline peroxide lignin.
[c]TC, total fecal collection.
[d]SEM, standard error of the mean.
[efg]Means with different superscripts in the same row differ, $P < 0.05$.

Table 2. Comparison of in vivo dry matter digestibility (DMD, % DM) and fecal output (FO, lb/d) with estimates obtained by different internal markers using four fecal grab sampling per day.

Item[c]	Marker[a]			TC[d]	SEM[e]	*P*-value[b]		
	$IADF_{1234}$	$ADIA_{1234}$	APL_{1234}			D	M	D × M
DMD (%)	51.6[g]	56.1[f]	57.1[f]	53.9[fg]	1.27	<0.01	<0.01	<0.01
FO (lb/d)	9.61	8.78	8.65	9.24	0.433	0.10	0.36	0.86

[a]$IADF_a$, adjusted indigestible acid detergent fiber using sample from four grab sampling per day (0600, 1200, 1800, and 2400); ADIA, acid detergent insoluble ash using four grab samples per day (0600, 1200, 1800, and 2400); and APL, alkaline peroxide lignin using four grab samples per day (0600, 1200, 1800, and 2400).
[b]D = diet effect; M = marker effect; and D × M = diet by marker interaction.
[c]DMD, dry matter digestibility; FO, fecal output.
[d]TC, total fecal collection technique.
[e]SEM, standards error of the mean.
[fg]Means with different superscripts in the same row differ at $P < 0.05$.

Table 3. Estimates of dry matter digestibility (DMD, % DM) by diet by marker interaction ($P < 0.001$) using four fecal grab samples per day.

Marker within time[b]	Treatments[a]				SEM[c]	Effect
	L	ML	MH	H		
TC	50.2[e]	55.2[de]	54.5[de]	56.2[de]	2.30	D × M
$ADIA_{1234}$	53.8[de]	54.0[de]	56.8[d]	60.0[d]		
APL_{1234}	56.9[d]	56.6[d]	56.4[de]	58.5[d]		
$IADF_{1234}$	39.2[f]	55.5[de]	55.1[de]	56.8[d]		

[a]L, low crude protein (CP = 7.9 % DM); ML, medium low crude protein (CP = 11.0 % DM); MH, medium high crude protein (CP = 13.0 % DM); H, high quality diet (CP = 16.4 % DM).
[b]$IADF_a$, adjusted indigestible acid detergent fiber using four sampling (0600, 1200, 1800, and 2400); ADIA, acid detergent fiber using four sampling (0600, 1200, 1800, and 2400) ; and APL, alkaline peroxide lignin using four sampling (0600, 1200, 1800, and 2400).
[c]SEM, standard error of the mean.
[def]Means with different superscripts within row and column differ at $P < 0.05$.

Table 4. Comparison of the actual in vivo values of digestibility (% DM) and fecal output (lb/d) and their estimates determined by adjusted indigestible acid detergent fiber ($IADF_a$), acid detergent fiber (ADIA), alkaline peroxide lignin (APL) using samples from different sampling time and their combinations.

Item[b]		Time of sampling[a]					SEM[d]	*P*-value
		0600	1200	1800	2400	TC[c]		
$IADF_a$								
	DMD	51.6	53.0	51.8	50.0	53.9	1.20	0.85
	FO	9.59	9.35	9.59	9.97	9.24	0.473	0.99
ADIA								
	DMD	55.7	55.4	56.6	55.1	53.9	1.36	0.97
	FO	8.87	8.95	8.65	8.95	9.24	0.400	0.96
APL								
	DMD	55.0	57.6	57.4	56.1	53.9	1.42	0.89
	FO	9.02	8.58	8.62	8.78	9.24	0.433	0.99

[a]1, sampled at 0600; 2, sampled at 1200; 3, sampled at 1800, 4, sampled at 2400, and TC, total fecal collection.
[b]DMD, dry matter digestibility calculated using different markers at different sampling time; FO, fecal output calculated using different markers at different sampling time.
[c]TC, total fecal collection.
[d]SEM, standard error of the mean.

In situ evaluation of internal markers for predicting digestibility and fecal output in cattle fed various bermudagrass hays

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Story in Brief

The potential of in situ indigestible neutral detergent fiber, indigestible acid detergent fiber, and rumen undegradable dry matter for predicting dry matter digestibility and fecal output by cattle offered bermudagrass hay of varying qualities was evaluated. Eight cannulated cows (1,307 ± 78.0 lbs) were allocated randomly to 4 bermudagrass hay diets categorized by their low, medium low, medium high, and high crude protein concentrations (7.9%, 11.0%, 13.0%, and 16.4% crude protein, respectively). Diets were offered in 3 periods to provide 2 replicates per diet and period (n = 24). Cows were individually offered their respective hay at a total of 2% of body weight (BW) in equal feedings at 0800 and 1700 h for a 10-d adaptation and a 5-d total fecal collection in each period. Duplicate samples of each of the hay, ort, and fecal samples from each period were incubated in Dacron bags for 144 h in the rumen of two cows, followed by a sequential analysis of neutral detergent fiber and acid detergent fiber. Recovery of rumen undegradable dry matter, indigestible neutral detergent fiber, and indigestible acid detergent fiber and their respective adjusted values were expressed as the ratio of the quantity of marker excreted in the feces per unit of marker consumed. Diet affected dry matter intake ($P = 0.01$) but did not affect fecal output ($P = 0.16$) and apparent dry matter digestibility ($P = 0.23$). All recovery rates were incomplete and differed by marker ($P < 0.01$).There was no diet by marker interaction ($P > 0.25$). Estimates of dry matter digestibility were underestimated ($P < 0.01$) while fecal output estimates were overestimated ($P < 0.01$) for all in situ markers. However, when an adjustment was applied, rumen undegradable dry matter, indigestible neutral detergent fiber, and indigestible acid detergent fiber recovery rates increased to 90.0%, 89.0%, and 92.4%, respectively. Standardized adjustment can be applied to obtain acceptable recovery of in situ markers.

Introduction

In forage-based ruminant feeding, knowledge of nutritive value of the basal diet is crucial to decide whether or not supplements are needed to meet the animal's energy and other nutrient requirements. One way of estimating energy values of feed is to conduct an in vivo digestion study and to determine organic matter digestibility (OMD) which is theoretically equal to total digestible nutrients (TDN) or digestible energy (DE) (Lofgreen, 1953), but these types of studies are very labor-intensive.

Internal markers offer the opportunity to link indigestible or nearly indigestible feed components to the overall feed digestibility and value. However, the variability of internal markers in predicting digestibility and fecal output across different types of forages (Sunvold and Cochran, 1991) requires a validation of marker recovery on a specified diet before its application in research. Therefore, our objective was to evaluate the potential of in situ indigestible neutral detergent fiber (INDF), indigestible acid-detergent fiber (IADF), and rumen undegradable dry matter (RUDM) as internal markers in predicting apparent dry matter digestibility (DMD) and fecal output (FO) of bermudagrass hay of varying qualities fed to cattle.

Materials and Methods

Treatments and Experimental Design for In Vivo Digestion. Eight ruminally cannulated cows (n = 8, BW = 1,307 ± 78.0 lbs) were allocated to four diet treatments of bermudagrass hay varying in nutritional quality: 1) low [crude protein (CP) = 7.9% DM]; 2) medium low (CP = 11.0% DM); 3) medium high (13.0% DM); and 4) high (CP = 16.4% DM). The experimental design used was a replicated 4 × 4 Latin Square with one period missing. Pairs of cows were allocated randomly to one of the four different diet treatments for each of the three periods. This resulted in 24 (n = 4 × 3 × 2 = 24) total in vivo observations. Each period consisted of a 10-d adaptation period and a 5-d of data collection period, and there were 14 d rest scheduled between two consecutive periods.

Hay Acquisition. Bermudagrass hay used in this study was harvested at three different locations: Batesville (3 bales), Watershed Research and Education Center (WREC, Fayetteville) (5 bales), and Monticello (4 bales) to represent a wide range in quality and maturity. The bales were large round bales weighing between 802 to 1102 lbs with average bale dimensions of 3.9 × 4.9- ft. Bales were grouped based on CP concentration, irrespective of location, and one bale from each treatment (total of 12) was fed to 2 cows during each period. A total of 12 large round bales were used for the 45-d feeding of the 3 periods.

Feeding and Sample Collection. Cows were offered two meals a day (0800 and 1700 h) in equal amounts to achieve an intake of approximately 2% BW per day per cow. This feeding level was chosen to minimize refusal. Cattle were housed in 9.8 × 14.1-ft individual pens equipped with rubber mats. Water was provided for ad libitum consumption via rubber water tanks. Feed sampling began on d 9, orts on d 10, and feces on d 11. Samples of forage offered were taken at each feeding sequence and a daily composite sample was placed in paper bags, weighed immediately, and dried in a forced-air oven at 122 °F until no further weight loss was detected for DM determination and subsequent chemical analysis. Samples of orts (refusals) were collected each morning before feeding (0700 h) in paper bags weighed and dried in a forced-air oven at 50 °C until no further weight loss was detected. Total feces from each cow were collected throughout the day by scraping them directly from the pen rubber mats and stored temporarily in plastic-lined trash cans. Total feces per cow were weighed, mixed in a commercial concrete mixer (Mixer Model 043206 Type A, Monarch Industries Inc., Canada),

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and a representative fecal sample (approximately 0.66 lbs of fresh feces) from the individual total daily fecal excretion was taken and placed in paper/aluminum plates, and dried in a forced-air oven at 50 °C for subsequent analysis of chemical composition and marker concentration. Total fecal output of each cow was corrected to include the dry weight of the 20 fecal grab samples taken per period. Cows were moved from their pens and allowed to graze for 14 d between each period to exercise and reduce the carryover effects of the previous hay treatment.

Sample Preparation for In Situ Procedure. Samples of the bermudagrass hay offered, ort, and feces excreted from the total collection (in vivo) experiment were analyzed for RUDM, INDF, and IADF by in situ incubation followed by neutral detergent fiber (NDF) and acid detergent fiber (ADF) extraction. Samples of hay offered, orts, and feces excreted were ground to pass a 0.079-inch screen of a Wiley mill (Thomas Scientific, Swedesboro, N.J.). Dacron bags (3.9 by 7.9-inches; 53 ± 10-µm pore size; ANKOM Technology Corp., Fairport, N.Y.) were filled with 0.18 oz of ground forage, ort, or feces and closed with rubber bands. Duplicate bags (n = 108) were prepared for each hay, ort, and feces samples. In total, there were 24 samples of hay offered, 48 samples of feces, and 36 samples of orts (3 cows in period 2 and 3 cows in period 3 did not have orts).

In Situ Analysis. Duplicate samples of hay, orts, feces from the same period were incubated in two cows (n = 6) for 144 h (6 d). During the incubation, cows were offered a total of 2% BW of a bermudagrass hay-based diet in equal meals at 0800 and 1600 h and had ad libitum access to water and mineral salt (4 oz/cow per day).

The in situ procedure was modified in a way that bags were not soaked in tepid water prior to incubation because of the long incubation time (144 h). Individual bags were placed in 14.2 × 19.7-inches mesh bags and inserted into the ventral rumen immediately prior to feeding on d 10 of the adaptation period. After 144 h of incubation, the Dacron bags were removed from the rumen and were subjected to a hand washing with cold water until the water was clear (approximately 10 times). All rinsed bags were dried to a constant weight at 122 °F and allowed to equilibrate to ambient temperature prior to weighing.

Dry Matter Loss Analysis and Adjustment of Concentrations of Markers. After initial evaluation of RUDM, INDF, and IADF, recovery rates were adjusted based on the proportion of each marker that washed out of the sample bags that were not incubated in the rumen, but at the same time were subjected to washing procedures similar to those used for the bags incubated in the rumen. The correction (adjustment) factor (CF) was calculated as the ratio of DM remaining after washing to the initial sample weight on a DM basis. The initial DM incubated for in situ RUDM, INDF, and IADF evaluation was then multiplied by CF to obtain the initial DM corrected for differential DM loss of forage, ort, and feces.

Chemical Analysis of Forages, Orts, Feces and Internal Markers. Forage samples were analyzed for DM, total ash, OM, total N; NDF, ADF, and ADL in forage, ort, and feces were analyzed sequentially. The same method was used to analyze INDF and IADF on the residual DM from the in situ procedure by placing 0.018 ± 0.00035 oz in filter bags and analyzing these sequentially for NDF and ADF. Hemicelluloses were estimated from the values obtained in sequential analyses of NDF and ADF and was calculated as the difference between NDF and ADF.

Recovery Rate, Digestibility, and Fecal Output Calculation. The concentration of marker in consumed forage (Mfd) was expressed as follows:

$$M_{fd} = \frac{[(M_{of} \times Q_{of}) - (M_{or} \times Q_{or})]}{DMI} \quad \text{Eq. (1)}$$

where M_{of} is the concentration of marker in hay offered; Q_{of} is the amount of hay offered; M_{or} is the concentration of marker in orts; Q_{or} is the amount of orts refused (Q_{or}), and DMI is the actual DMI.

The recovery of RUDM, adjusted RUDM ($RUDM_a$), INDF, adjusted INDF ($INDF_a$), IADF, and adjusted IADF ($IADF_a$) were expressed as the ratio of the quantity of marker excreted in the feces per unit of marker consumed according to the following relationship:

$$R\ (recovery) = \frac{M_{fc} \times FO}{[(M_{of} \times Q_{of}) - (M_{or} \times Q_{or})]} \quad \text{Eq. (2)}$$

where FO is the fecal DM excretion; M_{fd} is the marker concentration in consumed feed; M_{fc} is the marker concentration in feces.

Apparent dry matter digestibility (DMD) was calculated by the following formula:

$$DMD = \frac{100 \times (DMI - FO)}{DMI} \quad \text{Eq. (3)}$$

The estimate of dry matter digestibility (DMD) using internal markers was given by the following expression:

$$DMD = 100 \times \left(1 - \frac{M_{fd}}{M_{fc}}\right) \quad \text{Eq. (4)}$$

Estimate of fecal output (FO) using internal markers was given by the following expression:

$$FO = DMI \times \frac{M_{fd}}{M_{fc}} \quad \text{Eq. (5)}$$

Statistical Analysis. Data for intake, digestibility and DM loss were analyzed as a replicated 4 × 4 Latin-Square design with one period missing using PROC GLM of SAS. Effects of cow, diet and period were included in the model. Cow was considered as the experimental unit for the diet effect and differences were considered significant at $P < 0.05$. Data of internal marker recovery, estimates of apparent DMD and FO were analyzed for prediction of digestibility and fecal output using PROC GLM of SAS, where diet, marker, and diet by marker interaction were included in the model.

Results and Discussion

Data for DMI, apparent DMD, and FO are presented in Table 1. Forage intake was affected by diet ($P = 0.02$), while apparent DMD ($P = 0.23$) and FO ($P = 0.16$) were not affected by diet. Forage intake increased as forage quality increased while apparent DMD and FO only numerically increased.

The recovery rates per marker before and after adjustment are presented in Table 2. The average recovery rates were affected by marker ($P < 0.01$) and diet ($P < 0.01$). However, there was no diet by marker interaction ($P = 0.25$). Although there has been a significant improvement (80.0% vs. 90.5%, $P < 0.05$, respectively for unadjusted vs. adjusted) of marker recovery after correcting for difference in DM loss among forage, ort, and feces during in situ procedure, none of the adjusted markers ($RUDM_a$, $INDF_a$, and $IADF_a$) presented a satisfactory recovery close to 100%. The highest recovery rate (92.4%) was found on $IADF_a$ and differed from 100% ($P < 0.012$).

Estimates of DMD and FO were affected by type of marker (Table 3, $P = 0.01$). The entire in situ markers predicted poorly actual DMD ($P < 0.001$) and FO ($P < 0.012$). Diet affected the prediction of actual DMD and FO ($P < 0.01$). However, there was no diet by marker interaction for DMD and FO ($P \geq 0.87$). In general, DMD was underestimated while FO was overestimated because of incomplete recovery of these in situ markers in feces. However, there was an improvement in prediction of actual DMD and FO by adjusted internal markers ($RUDM_a$, $INDF_a$, and $IADF_a$). The adjusted IADF ($IADF_a$) was the closest in predicting DMD (53.9% vs. 49.2% DM; CV = 9%) and FO (4.2 vs. 4.6; CV = 8.6%).

Implications

None of the in situ internal markers accurately predicted apparent digestibility and fecal output. A standardized adjustment to DM loss due to difference in particle size among forage, ort, and feces during in situ process may provide acceptable recovery that allows the prediction of digestibility and fecal output.

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Table 1. Dry matter intake (DMI), fecal output (FO), and dry matter digestibility (DMD) of bermudagrass hay fed to cattle for estimating internal marker recovery based on total collection (TC).

Item	Treatments[a]				SEM[b]	*P*-value
	L	ML	MH	H		
DMI (lbs/d)	17.0[d]	19.8[cd]	22.4[c]	21.6[c]	0.93	0.01
FO (lbs/d, on DM basis)	8.4	9.0	10.3	9.5	0.56	0.16
DMD (% DM)	51.0	54.4	53.9	56.1	1.64	0.23

[a]L, low quality hay (CP = 7.9% DM); ML, medium low quality hay (CP = 11.0% DM); MH, medium high quality hay (CP = 13.0% DM); and H, high quality hay (CP = 16.4% DM).
[b]SEM, standard error of the mean.
[cd]Means with different superscripts in the same row differ at $P < 0.05$.

Table 2. Recovery (%) of internal markers in feces from cattle fed bermudagrass hays varying in quality. Values are given for markers pre- and post-correction for particle loss during the analytical procedures.

Item[b]	Treatments[a]				Average	SEM[c]	*P*-value[d]		
	L	ML	MH	H			D	M	D × M
IADF	69.9	84.4	87.7	88.8	82.7[f]	1.55	0.01	0.01	0.25
$IADF_a$	79.7	96.0	97.5	96.4	92.4[e]				
INDF	68.2	77.5	85.1	83.3	78.5[f]				
$INDF_a$	78.7	89.4	94.8	92.7	88.9[e]				
RUDM	69.5	78.1	85.2	82.7	78.9[f]				
$RUDM_a$	81.0	90.8	95.8	92.9	90.1[e]				

[a]L, low quality hay (CP = 79% DM); ML, medium low quality hay (CP = 11.0% DM); MH, medium high quality hay (CP = 13.0% DM); and H, high quality hay (CP = 16.4% DM).
[b]RUDM, rumen undegradable dry matter; $RUDM_a$, rumen undegradable dry matter adjusted; INDF, indigestible neutral detergent fiber; $INDF_a$, indigestible neutral detergent fiber adjusted; IADF, indigestible acid detergent fiber; $IADF_a$, indigestible acid detergent fiber.
[c]SEM, standard error of the mean.
[d]D, diet; M, marker; and D × M, diet by marker interaction.
[ef]Means with different superscripts within column differ at $P < 0.05$.

Table 3. Dry matter digestibility (DMD, % DM) and fecal output (FO, lbs/d) derived from total collection (TC) compared with estimates using various internal markers.

Item[b]	Method[a]							SEM[c]	*P*-value
	TC	RUDM	$RUDM_a$	INDF	$INDF_a$	IADF	$IADF_a$		
DMD	53.9[d]	40.5[f]	48.3[e]	39.6[f]	47.5[e]	41.6[f]	49.2[e]	1.04	<0.001
FO	9.2[f]	12.0[d]	10.5[e]	12.0[d]	10.4[e]	11.5[d]	10.1[e]	0.23	<0.001

[a]TC, total fecal collection; RUDM, rumen undegradable dry matter ; $RUDM_a$, rumen undegradable dry matter adjusted; INDF, indigestible neutral detergent fiber; $INDF_a$, indigestible neutral detergent fiber adjusted ; IADF, indigestible acid detergent fiber; $IADF_a$, indigestible acid detergent fiber.
[b]DMD, dry matter digestibility; FO, fecal output.
[c]SED, standard error of the mean.
[def]Means with different superscripts within row differ at $P < 0.05$.

Intake and digestibility, of bermudagrass hay by lactating beef cows offered corn or hominy feed as supplements

Z. Madzonga[1], A. Young[1], K. Coffey[1], D. Philipp[1] and E. Kegley[1]

Story in Brief

Hominy feed, a co-product of dry corn milling, has been evaluated to a limited extent in feedlot and dairy rations, but has not been evaluated as a supplemental energy source for lactating beef cows. The objective of this study was to determine the effect of level of hominy feed supplementation on intake and digestibility of medium quality bermudagrass hay. Five ruminally cannulated lactating beef cows (1314 ± 30.6 lb. initial body weight (BW)) were used in an experiment with a 5 × 5 Latin square design. Treatments were low hominy (0.25% of BW), medium hominy (0.50% of BW), low corn (0.25% of BW), medium corn (0.50% of BW) and no supplement. Supplements were offered at 0800 daily, and hay was offered to maintain 10% refusal. Fresh water was offered for ad libitum consumption. Fecal samples were collected twice daily to estimate fecal output using acid-detergent insoluble ash as an internal marker. Hay dry matter intake was not affected ($P = 0.35$) by supplementation. Total dry matter intake (% of BW) was greater ($P < 0.05$) for medium corn and hominy compared with low hominy and no supplement. Dry matter digestibility did not differ ($P = 0.25$) among treatments but medium corn and hominy had greater ($P < 0.05$) digestible dry matter intake compared with no supplement and low corn. Hay fraction B (potentially degradable DM) was greater ($P < 0.05$) for low hominy and medium corn compared with medium hominy. Therefore, either ground corn or hominy feed can be used as supplemental feedstuffs at these levels for lactating beef cows offered bermudagrass hay to increase digestible dry matter intake without negatively affecting hay intake.

Introduction

The use of corn co-products has increased as livestock feeds in recent years as a result of abundant availability from the milling, alcohol and ethanol industries, and the comparatively lower retail prices of co-products. Corn co-products that are available today include corn gluten feed, corn gluten meal, corn bran, distiller's grain with solubles, and hominy feed. These co-products differ from the original corn grain by having greater concentrations of fiber and lower concentrations of starch. Because of the greater concentrations of fiber, which is highly digestible generally, these co-products can be used effectively in ruminant diets as a source of digestible energy. Hominy feed has received little attention, and limited research has been published about its potential use as a supplemental energy source for cows offered lower-quality forages compared with other corn co-products. Therefore, the objective of this study was to determine the effect of hominy feed supplementation on intake, digestibility, in situ dry matter disappearance, and passage rate of bermudagrass hay in lactating beef cows.

Materials and Methods

Animal Management. Five multiparous, lactating, ruminally-cannulated fall-calving beef cows (BW = 1314 ± 30.6 lb) were used in a study with a 5 × 5 Latin Square Design to compare 5 dietary treatments during 5 experimental periods. Cows were housed individually in 20 × 20 ft pens with wood chips for bedding in an enclosed facility that allowed air circulation. Each period consisted of a 10-d dietary adaptation period followed by a 6-d sample collection period.

Treatments. Cows were offered a bermudagrass hay basal diet (Table 1) along with either no supplemental concentrate (CONT) or with supplements of hominy offered at 0.25 (LH) or 0.50% of BW (MH), or ground corn offered at 0.25 (LC) or 0.50% of BW (MC) on an as-fed basis. Supplements were offered at 0800 daily. The hay was taken from large-round bales and offered as long hay in 2 feedings at approximately 0830 and 1630 to maintain a minimum of 10% refusal. Water was supplied ad libitum and a commercial mineral supplement[2] (~4 oz; Purina Wind and Rain All Season 4, Purina Mills, Gray Summit, Mo.) was offered to each cow including the CONT cow at 0800 daily.

Dry Matter Digestion. Fecal samples were collected from each cow between 0800 and 0830, and again between 1630 and 1700 for 5 d beginning on d 11 and dried to constant weight. Acid-detergent insoluble ash was used as an internal marker to determine DM digestibility.

Passage rate (K_p) was estimated using total ruminal evacuation. On d 16 of each experimental period, total ruminal evacuations were carried out immediately preceding the morning feeding (0730) and at 6 h after feeding. Representative samples of ruminal contents were dried to constant weight. Acid-detergent insoluble ash was used as an internal marker to determine ruminal fill and passage rate.

Ruminal In Situ Dry Matter Disappearance. Hay DM disappearance in the rumen was determined using the nylon bag procedure. On d 10 through 15 of each period, duplicate bags of hay and supplement were placed in mesh lingerie bags and inserted in reverse order under the ruminal mat in the ventral rumen. Hay samples were incubated for 124, 100, 76, 52, 24, 16, 12, 8, and 4 h. All bags were removed simultaneously on d 15 at 2100, placed in cold tap water to rinse off particles adhering to them and to inhibit any further microbial activity, rinsed 10 times in a top-loading washing machine, then dried.

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[2] Purina Wind and Rain All Season 4 Mineral contained CP not less than 5%, crude fat not less than 3%, crude fiber not more than 2%, Ca min 5%; Ca max, 5%; P min 4%; Mg min 1%; K min 3%; Zn min 2,100 ppm; Mn min 1,650 ppm; Cu min 730 ppm; Co min 75 ppm; I min 68 ppm; Se min13 ppm; Vitamin A min 176,000 IU/lb; Vitamin D min 44,000 IU/lb; Vitamin E min 220 IU/lb.

Statistical Analysis. Dry matter intake, digestibility, and passage rate data were analyzed using mixed-models procedures of SAS (SAS Institute, Inc., Cary, N.C.) for a 5 × 5 Latin Square design. Treatment was considered a fixed effect and period and animal were considered random effects. In the event of significant treatment effects ($P < 0.05$) or tendencies ($0.05 < P < 0.10$), means were separated using the least-significant difference test (PDIFF option) at the respective *P*-value.

The proportion of DM remaining in the in situ bags at each incubation time were fit to a non-linear statistical model (Mertens and Loften, 1980) using PROC NLIN of SAS (SAS Institute, Inc., Cary, N.C.). The fraction that was degraded at a measurable rate (fraction B), the disappearance lag time, the rate of DM disappearance (K_d), and the undegradable fraction (fraction C) were derived directly from the model whereas the immediately-soluble fraction (fraction A) was calculated as 100 – B – C. Effective ruminal degradation was determined as $A + [B \times (K_d/(K_d + K_p))]$. Data derived from the non-linear model were analyzed using mixed-models procedures of SAS (SAS Institute, Inc., Cary, N.C.) as described previously.

Results and Discussion

Data for DM intake, digestibility, ruminal fill, and passage rate are presented in Table 2. Hay DM intake (lb/d and % of BW) did not differ ($P \geq 0.15$) across treatments, indicating that the types and levels of supplements used in this study were not having a negative effect on forage intake. Total DM intake was greater ($P < 0.05$) for MC and MH compared with CONT, LC and LH when measured as lb/d, and was greater ($P < 0.05$) from MC and MH compared with CONT and LH when expresses as a percentage of cow BW.

Dry matter digestibility was not different ($P = 0.25$) across treatments. However, digestible DM intake (lb/d) was greater ($P < 0.05$) for cows offered MC and MH compared with those offered the other treatments. When expressed as a percentage of cow BW, digestible DM intake was greater ($P < 0.05$) for cows offered MH compared with those offered CONT, LC, and LH. Digestible DM intake (% of BW) for cows offered MC tended to ($P = 0.07$) be lower than that from cows offered MH, but did not differ ($P = 0.27$) from that for cows offered LC. Cows offered MC consumed more ($P < 0.05$) digestible DM (% of BW) than cows offered CONT or LH. Dry matter fill, passage rate, and ruminal retention time did not differ ($P \geq 0.31$) across treatments.

Based on previous studies (Sanson, 1993), 0.5% of BW as supplemental corn appears to be the point where forage intake begins to be reduced. When this occurs, we are replacing corn for hay instead of adding corn to the hay diet, and therefore are not getting the full benefit of the supplementation. Total DM intake in this study was 13.3% greater by cows offered MC and MH compared with those offered no supplement. Cows offered LC and LH only increased their total DMI numerically by 5.5% and 3.5% respectively compared with those offered no supplement. These increases in total DM intake resulted in 15.7% and 21.3% increases in digestible DM intake from cows offered MC and MH, respectfully, compared with those offered CONT.

Overall, in situ data (Table 3) were consistent with digestibility data in that no differences were observed among treatments for rate of DM disappearance, or effective ruminal DM disappearance, as was the case for total tract digestibility and passage rate (Table 2). However, cows offered LH had greater potential disappearance of forage DM and a lower undegradable fraction compared with those offered MH, LC, and CONT. These differences could not be readily explained by differences in DM intake or total tract digestibility. Numerically, the lowest potentially-degradable fraction and the greatest undegradable fraction were from cows offered MH, which also had the greatest DMD and digestible DMI.

Implications

Lactating beef cows require additional energy in instances where forage quality is limiting, but supplementation with corn is facing growing competition from domestic use. Based on our research, either corn or hominy feed, a co-product from dry corn milling, can be fed at levels up to 0.50% of body weight to lactating beef cows consuming medium-quality bermudagrass hay without negatively affecting intake or digestibility. This resulted in greater intake of digestible dry matter, which would improve cow energy balance. Therefore, hominy feed can be used as an alternative to corn as an energy supplement with similar results on potential animal performance indicators.

Literature Cited

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Table 1. Chemical composition of bermudagrass hay and supplements offered to lactating, ruminally-cannulated cows (dry matter basis).

Item	Bermudagrass hay	Corn	Hominy feed
Crude protein	10.3	12.3	11.3
Neutral detergent fiber	70.7	16.0	27.5
Acid detergent fiber	32.4	4.3	7.0
Ash	8.0	2.5	3.0
Ether extract	1.0	4.0	7.0

Table 2. Intake, digestibility, and ruminal dry matter (DM) fill by lactating, ruminally-cannulated beef cows offered medium quality bermudagrass hay and supplemented with either corn or hominy.

	Treatments[a]						
Item	**CONT**	**LC**	**LH**	**MC**	**MH**	**SEM**	**Effects[b]**
DM intake, lb/d							
Hay	31.5	30.5	29.6	30.0	29.8	1.64	ns
Supplement	0.0c	2.8b	3.0b	5.9a	6.0a	0.26	S
Total DMI	31.5b	33.3b	32.7b	35.8a	35.8a	1.72	S
DM intake, % BW							
Hay	2.4	2.4	2.3	2.3	2.3	0.15	ns
Total	2.4c	2.6ab	2.5bc	2.7a	2.7a	0.15	S
DM digestibility[c], %	53.0	54.7	53.0	54.4	56.3	2.10	ns
Digest. DM intake[c], lb/d	16.7c	18.3b	17.3bc	19.5a	20.1a	1.14	S
Digest. DM intake[c], % BW	1.27d	1.44b	1.34c	1.47ab	1.54a	0.107	S
DM fill, lb,[d]	30.0	28.9	28.2	31.8	31.3	1.43	ns
DM fill, % BW	2.3	2.3	2.2	2.4	2.4	0.14	ns
Passage rate, h^{-1}	0.037	0.039	0.040	0.035	0.036	0.0033	ns
Retention time, h	27.6	26.2	25.3	28.8	29.6	2.61	ns

Means within a row without a common letter differ ($P < 0.05$).
[a] CONT, control (no supplement); LC, low corn; LH, low hominy; MC, medium corn; MH, medium hominy.
[b] S, supplement effect ($P < 0.05$); ns, no statistical difference.
[c] Digestibility as determined using actual DMI and fecal output determined using acid-detergent insoluble ash as an internal marker.
[d] DM fill represents the average of the ruminal fill measured by total ruminal evacuation immediately prior to feeding and 6 h after feeding.

Table 3. In situ dry matter (DM) disappearance in lactating, ruminally-cannulated beef cows offered medium quality bermudagrass hay and supplemented with different levels of corn or hominy.

Item[b]	**CONT**	**LC**	**LH**	**MC**	**MH**	**SEM**	**Effects[c]**
Water-soluble fraction, %	22.0	22.0	22.9	21.7	21.9	0.16	ns
Potentially-degradable fraction, %	47.8[bc]	48.4[bc]	50.1[a]	49.2[ab]	47.5[c]	1.51	S
Undegradable fraction, %	30.3[ab]	30.3[ab]	28.0[c]	29.1[bc]	30.6[a]	0.46	S
Rate of disappearance (K_d), h^{-1}	0.034	0.032	0.028	0.030	0.032	0.0020	ns
Lag time, h	1.2	0.91	2.3	1.8	1.8	0.39	ns
Effective ruminal degradation of DM, %	44.8	43.8	42.4	44.2	44.7	1.49	ns

Means within a row without a common letter differ ($P < 0.05$).
[a] CONT, control (no supplement); LC, low corn; LH, low hominy; MC, medium corn; MH, medium hominy.
[b] A, immediately soluble fraction; B, degradable fraction; C, undegraded fraction; K_d, degradation rate; ED, effective ruminal DM disappearance.
[c] S, supplement effect ($P < 0.05$); ns, no statistical difference.

Ruminal fermentation of bermudagrass hay by lactating beef cows offered corn or hominy feed as supplements

Z. Madzonga[1], A. Young[1], K. Coffey[1], E. Kegley[1] and D. Philipp[1]

Story in Brief

A number of co-products from corn processing are available today. Hominy feed, a co-product of dry corn milling, has been evaluated to a limited extent in feedlot and dairy rations, but has not been evaluated as a supplemental energy source for lactating beef cows. The objective of this study was to determine the effect of level of corn or hominy feed supplementation on ruminal fermentation characteristics of medium quality bermudagrass hay. Five ruminally cannulated lactating beef cows (1314 ± 30.6 lb. initial body weight, BW) were used in an experiment with a 5 × 5 Latin Square design. Treatments were low hominy (0.25% of BW), medium hominy (0.50% of BW), low corn (0.25% of BW), medium corn (0.50% of BW) and no supplement. The cows were housed individually, and supplements were offered at 0800 daily. Hay and fresh water were offered for ad libitum consumption, and a mineral supplement was offered daily. Five consecutive 16-d periods were used, with 13 d for diet adaptation. Ruminal fluid was sampled on d 14 of each period immediately prior to supplement feeding and at 1, 3, 5, 7, 9, 11, and 13 h after supplement feeding to measure pH and for analysis of concentrations of volatile fatty acids and rumen ammonia-N. Mean ruminal pH tended ($P = 0.07$) to be greater for no supplement and low corn compared with low hominy. Ruminal ammonia-N and total volatile fatty acid concentrations were not affected ($P \geq 0.77$) by supplements. The sampling time × treatment interaction affected ($P < 0.05$) concentrations of acetate, propionate and the acetate:propionate ratio. Generally, the acetate:propionate ratio was greatest from no supplement and lowest from medium corn and hominy. Therefore, hominy feed and corn had similar effect on ruminal fermentation by lactating beef cows offered bermudagrass hay.

Introduction

The nutrient demands required to maintain lactation in beef cows is greater than the potential nutrient consumption from poor- to medium-quality hay. Providing supplements containing starch, such as corn, to meet these nutrient deficiencies may cause potential negative effects to occur in the rumen. Co-products from various corn processing industries have increasingly been incorporated as energy supplements in forage based diets in lieu of corn because of their increased availability and because of their lower starch and greater digestible fiber concentrations. Hominy feed has received little attention, and limited research has been published about its potential use as a supplemental energy source for cows offered lower-quality forages compared with the other corn co-products. Therefore the objective of this study was to determine the effect of hominy feed supplementation on ruminal fermentation of bermudagrass hay-based diets in lactating beef cows.

Materials and Methods

Animal Management. Five multiparous, lactating, ruminally-cannulated Gelbvieh × Angus crossbred fall-calving beef cows (BW = 1314 ± 30.6 lb) were used in a study with a 5 × 5 Latin Square design to compare 5 dietary treatments during 5 experimental periods. The cows were approximately 45 d post-calving at the initiation of the study. Cows were housed individually in 20 × 20 ft pens with wood chips for bedding in an enclosed facility that allowed air circulation. Each period consisted of a 13-d dietary adaptation period prior to rumen fluid collection on d 14.

Cows were offered a bermudagrass hay basal diet (Table 1) along with either no supplemental concentrate (CONT) or with supplements of hominy offered at 0.25 (LH) or 0.50% of BW (MH), or ground corn offered at 0.25 (LC) or 0.50% of BW (MC) on an as-fed basis at 0800 daily. The hay taken from large-round bales was offered as long hay in 2 feedings at approximately 0830 and 1630 to maintain a minimum of 10% refusal. Water was supplied ad libitum and approximately 4 oz of a commercial mineral supplement[2] (~110 g; Purina Wind and Rain All Season 4 , Purina Mills, Gray Summit, Mo.) was offered to each cow including the CONT cow at 0800 daily.

Ruminal fluid was sampled on d 14 of each period immediately prior to supplement feeding and at 1, 3, 5, 7, 9, 11, and 13 h after the morning supplement feeding. Ruminal pH was measured and recorded immediately, and ruminal fluid samples were frozen with specific preservatives for later volatile fatty acid (VFA) and ammonia-N analyses.

Volatile fatty acids were analyzed using automated gas chromatography. Ammonia-N concentrations were determined using the phenol-hypochlorite procedure (Broderick and Kang, 1980) by spectroscopy.

Statistical Analysis. Ruminal pH, VFA, and ammonia-N data were analyzed using mixed-model procedures of SAS (SAS Institute, Inc., Cary, N.C.) for a repeated-measures experiment with a 5 × 5 Latin Square design. Treatment was considered a fixed effect, period and animal were considered random effects, and sampling time was considered a repeated measurement. Effects of treatment × sampling time and cow (treatment × period) were included in the statistical model. In the event of significant treatment effects ($P < 0.05$) or tendencies ($0.05 < P < 0.10$), means were separated using the least-significant difference test (PDIFF option) at the respective *P*-value. In the event of a significant treatment × sampling time interaction ($P < 0.05$), treatment means were compared within sampling time only using the least-significant difference test.

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

[2] Purina Wind and Rain All Season 4 Mineral contained CP not less than 5%, crude fat not less than 3%, crude fiber not more than 2%, Ca min 5%; Ca max, 5%; P min 4%; Mg min 1%; K min 3%; Zn min 2,100 ppm; Mn min 1,650 ppm; Cu min 730 ppm; Co min 75 ppm; I min 68 ppm; Se min13 ppm; Vitamin A min 176,000 IU/lb; Vitamin D min 44,000 IU/lb; Vitamin E min 220 IU/lb.

Results and Discussion

Ruminal pH was affected ($P < 0.05$) by sampling time and tended ($P < 0.10$) to be affected by supplementation and the supplementation by time interaction (Table 2). Ruminal pH tended ($P < 0.10$) to be greater for cows offered LC compared with those offered LH, MC, and MH. Greater pH would be expected from cows offered CONT, LC, or LH because of lower concentrations of readily fermentable carbohydrates in those diets. The suppressed pH in cows offered LH compared with those offered CONT and LC cannot be explained based on other research. We suspect that the digestible fiber content and residual starch in the hominy may have been fermented by both fibrolytic and amylolytic bacteria to yield low pH relative to that yielded by fermentation of corn.

There were no effects of supplementation ($P = 0.94$) on ruminal ammonia-N concentrations), but there was a sampling time effect ($P < 0.05$). Ruminal ammonia concentrations were greatest ($P < 0.05$) at 1 h followed by that at 3 h after supplement feeding (data not shown) compared with the remainder of the sampling times. These elevated ammonia-N concentrations at 1 and 3 h post-feeding were likely due to the rapid degradation of soluble protein from the bermudagrass hay. Ruminal ammonia concentrations were low through the sampling times, but the level was still within and/or above the range required (2 to 5 mg/dL) by microbes for microbial protein synthesis (Satter and Slyter, 1974).

Concentrations of total VFA were not affected ($P = 0.77$) by supplement treatments, but varied ($P < 0.05$) across sampling times as well. Concentrations of total VFA were greater ($P < 0.05$) at 13 h after feeding the supplements compared with immediately prior to supplement feeding, or 1, 3, or 9 h after feeding supplements (data not shown) indicating delayed fermentation throughout the day. The sampling time × treatment interaction affected ($P < 0.05$) proportions of acetate, propionate, and the acetate:propionate ratio (Figs. 1, 2, and 3, respectively). Supplementation affected ($P < 0.05$) concentrations of isobutyrate and tended ($P < 0.10$) to affect proportions of isovalerate (Table 2). Concentrations of isobutyrate were greater ($P < 0.05$) from cows offered MC compared with those offered the other treatments, whereas concentrations of isovalerate tended ($P < 0.10$) to be greater from cows offered MC, LC and CONT compared with those offered LH.

In general, ruminal acetate concentrations were greater from cows offered CONT compared with those offered supplements, particularly when the supplements were offered at 0.5% of BW (Fig. 1). When offered at 0.5% of BW, no differences were observed ($P \geq 0.36$) in concentrations of acetate between cows offered corn and hominy feed at any of the sampling times. Concentrations of acetate were greater ($P < 0.05$) from cows offered LC compared with those offered LH at 9 h after feeding, but not ($P \geq 0.05$) at the other sampling times. Greater concentrations of ruminal acetate are generally associated with the fermentation of fiber.

Concentrations of propionate generally followed an opposite trend as observed with concentrations of acetate (Fig. 2). Propionate concentrations were numerically the lowest throughout all sampling times from cows offered CONT and LC, and the greatest concentrations were generally from cows offered MC or MH. Propionate concentrations from cows offered LH were intermediate between those offered MC and MH and those offered LC and CONT. These results were expected as greater concentrations of propionate are generally associated with fermentation of starch. Propionate is also used more efficiently for energy by the host animal.

Because of the negative relationship between acetate and propionate mentioned previously, the acetate:propionate ratios closely mirrored those of acetate concentrations. The acetate:propionate ratio was greatest for cows offered CONT and LC compared with those offered MC and MH at 3 h and continuing for the rest of the time periods (Fig. 3). Cows offered CONT also had greater acetate:propionate ratios than LH, MC, and MH at 1 h after supplement feeding. The dietary effects on the acetate:propionate ratios in this study were most likely due to the acetate produced during degradation of hay in CONT and the low impact LC had on propionate production, combined with the propionate production from fermentation of starch and readily fermentable fiber in MH and MC.

Implications

Ruminal pH changes observed in this study were not sufficient to have major effects on fiber digestion. Although no differences in total volatile fatty acid concentrations were observed, propionate concentrations and the acetate:propionate ratios were more favorable throughout much of the day from cows offered MC and MH. This should result in greater dietary energy recovery and greater overall energy balance in cows offered the increased levels of supplements (0.5% of body weight). Only minimal differences were observed between corn and hominy feed when offered at the same levels, indicating that cost can drive purchasing decisions without other production considerations.

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Table 1. Chemical composition of bermudagrass hay and supplements offered to lactating, ruminally-cannulated cows (dry matter basis).

Item	Bermudagrass hay	Corn	Hominy feed
Crude protein	10.3	12.3	11.3
Neutral detergent fiber	70.7	16.0	27.5
Acid detergent fiber	32.4	4.3	7.0
Ash	8.0	2.5	3.0
Ether extract	1.0	4.0	7.0

Table 2. Ruminal fluid characteristics of lactating, ruminally-cannulated beef cows offered medium quality bermudagrass hay supplemented with either corn or hominy.

	Treatments[a]						
Item	**CONT**	**LC**	**LH**	**MC**	**MH**	**SEM**	**Effects[b]**
pH	6.3ab	6.4a	6.1c	6.2bc	6.2bc	0.14	s, T, s × t
Ammonia-N, mg/dL	4.7	4.4	4.8	4.3	4.4	0.78	T
Total volatile fatty acids, mM	106.4	99.4	107.3	108.2	105.4	5.36	T
	----------% of total VFA----------						
Acetate	70.6	69.6	68.3	67.4	67.8	0.50	S, T, S × T
Propionate	17.1	17.5	18.1	19.1	19.2	0.41	S, T, S × T
Acetate:propionate	4.2	4.0	3.8	3.5	3.6	0.11	S, T, S × T
Butyrate	10.2	10.6	11.2	11.0	10.7	0.32	T
Isobutyrate	0.65b	0.68b	0.67b	0.73a	0.69b	0.02	S, T
Valerate	0.91	0.90	0.91	0.94	0.95	0.39	T
Isovalerate	0.76a	0.77a	0.70b	0.86a	0.74ab	0.05	s, T

Means within a row without a common letter tended to differ ($P < 0.10$).

[a] CONT, control (no supplement); LC, low corn; LH, low hominy; MC, medium corn; MH, medium hominy.

[b] S and s, supplement effect ($P < 0.05$ and 0.1, respectively); T, sampling time effect; S × T and s × t, sampling time × treatment interaction ($P < 0.05$ and 0.1, respectively); ns, no supplement effect.

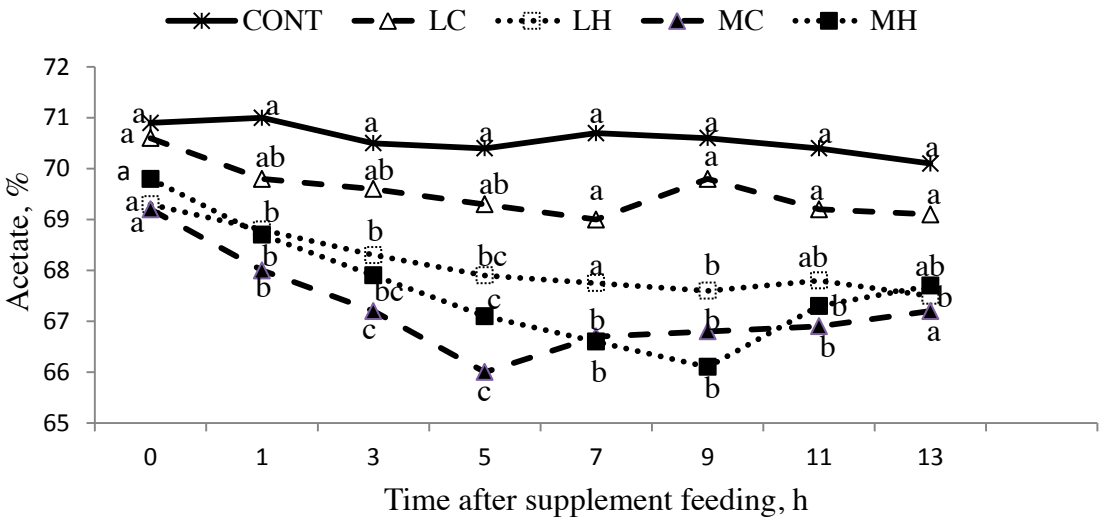

Fig. 1. Ruminal % acetate in lactating, ruminally-cannulated beef cows offered medium-quality bermudagrass hay supplemented with either corn or hominy. Sampling time × treatment effect ($P < 0.05$). Means with different letters differ within a time. SE = 0.50.

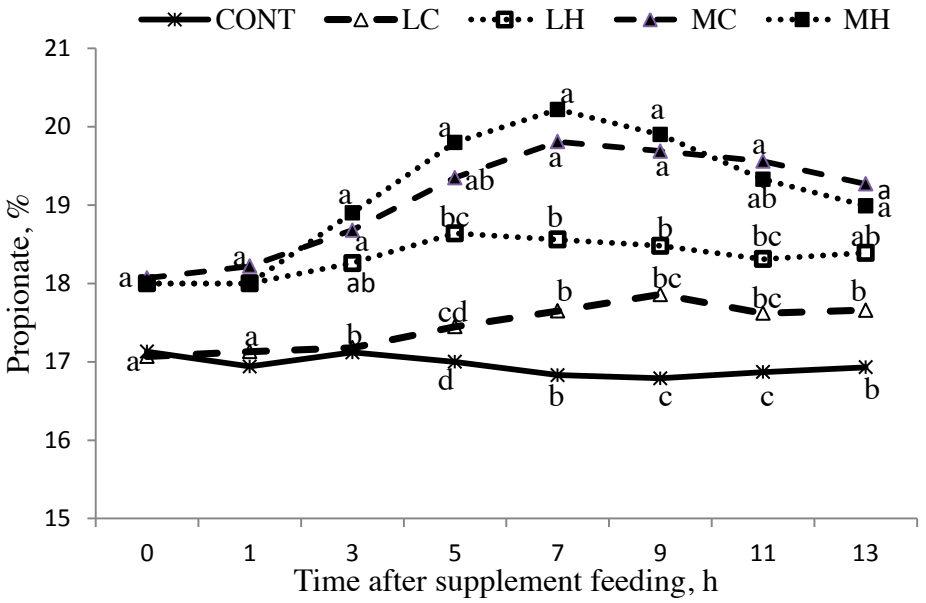

Fig. 2. Ruminal % propionate in lactating, ruminally-cannulated beef cows offered medium-quality bermudagrass hay supplemented with either corn or hominy. Sampling time × treatment effect (*P* < 0.05). Means with different letters differ within a time. SE = 0.42.

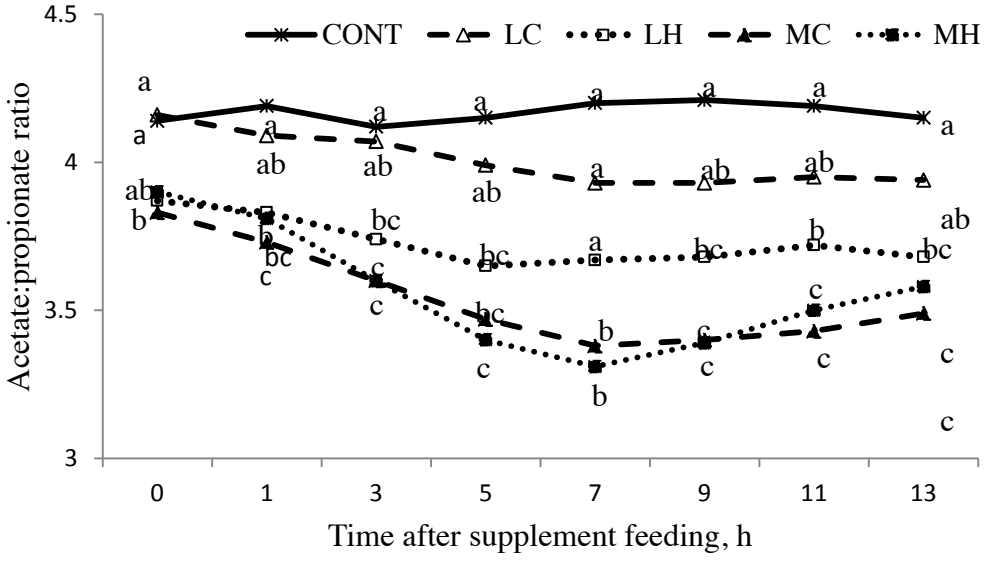

Fig. 3. Ruminal acetate:propionate ratio in lactating, ruminally-cannulated cows beef cows offered medium-quality bermudagrass supplemented with either corn or hominy. Sampling time × treatment (*P* < 0.05). Means with different letters differ within a time. SE = 0.11.

Impact of lauric arginate alone or followed by other antimicrobials as decontamination interventions on ground beef instrumental color properties

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Story in Brief

In this study, inoculated (*Escherichia coli* and *Salmonella* 10^5 CFU/ml) beef trimmings (1.6 kg/ treatment/replicate) were spray treated (~0.1 ml/g) with lauric arginate (LA; 5%) alone or followed by 0.4% cetylpirinidium chloride (LAC), 4% sodium metasilicate (LAN), 10% trisodium phosphate (LAT), 0.02% peracetic acid (LAP) or sterile water (LAW). Then un-inoculated untreated (CON) and inoculated untreated (INCON) control trimmings as well as spray treated trimmings were individually ground two times and 200 g of individual samples were placed on plastic foam trays and over wrapped with polyvinyl chloride film. The experiment was repeated 3 times. The ground beef packages were stored under simulated retail conditions (4 °C) until sampled on day 0, 1, 2, and 3 of display for CIE L*, a* and b* measurements (n = 3/sample; Hunter lab mini scan; illuminant A/10° observer). The LA, LAN, LAT and LAW treated ground beef had similar ($P > 0.05$) lightness (L*) to CON. Additionally, ground beef processed from LA alone or followed by water had more ($P < 0.05$) redness (a*) value compared to all the other treatments and control samples. However, LAC, LAN, LAP and LAT treated samples maintained a similar ($P > 0.05$) redness to CON and INCON samples. Comparison of treatments indicate that use of lauric arginate alone or accompanied by water, sodium metasilicate, trisodium phosphate, or peroxyacetic acid when used as potential pre-grinding interventions may not cause adverse effects on ground beef color. Additionally, lauric arginate alone and followed by water surpassed other treatments in enhancing redness in ground beef.

Introduction

Although the United States Department of Agriculture has approved many antimicrobial agents that can be used in meat decontamination, some antimicrobial agents pose a negative impact on quality characteristics of the final meat product. Consumers often associate the bright redness of the meat product with freshness and discriminate the color-changed meat products. Thus any negative impacts on meat color and other quality attributes lead to economic losses in the meat industry. Lauric arginate is a novel antimicrobial compound that can be used as a bactericidal agent against wide range of pathogenic bacteria on fresh beef surfaces. Many studies have provided scientific evidence to conclude the efficacy of peroxyacetic acid (Quilo et al., 2009) cetylpyridinium chloride (CPC; Cutter et al., 2000) and trisodium phosphate (TSP; Pohlman et al., 2002) for improving beef product safety. An added benefit in application of CPC and TSP antimicrobial interventions, as reported by Pohlman et al. (2002) and Jimenez-Villarreal et al. (2003), is that these agents may enhance redness (a*) and oxymyoglobin stability (630 nm/580 nm) without affecting the odor characteristics in ground beef. The application of lauric arginate alone or followed by other antimicrobials as decontamination interventions in ground beef production systems is still under-investigated and very little or no information is available on its impact on ground beef color properties. Therefore, the objective of this study was to evaluate ground beef instrumental color properties when lauric arginate alone or followed by water, cetylpirinidium chloride sodium metasilicate, trisodium phosphate, or peracetic acid were applied to decontaminate beef trimmings prior to grinding.

Materials and Methods

Inoculation Process. Beef trimmings (40 kg) were inoculated with a cocktail mixture (4 °C) of *E.coli* O157:H7, O26, O103, O111, O121, O45, and O145 (EC) and *Salmonella Typhimurium* DT 104 and *Salmonella* Newport MDR-AmpC (S) at 10^5 CFU/g by following the procedures explained by Pohlman et al. (2002). Following inoculation, the trimmings were left overnight at 4 °C for further bacterial attachment.

Antimicrobial Treatment Applications. Beef trimmings (1.6 kg/ treatment/replicate), arranged in stainless steel trays, were subjected to conventional spray (~0.1 ml/g) applications of 5% (v/v) Lauric arginate (LA; CytoGuard®, A & B Ingredients, Fair Field, N.J.) alone or followed by 0.4% (v/v) cetylpirinidium chloride (LAC; Cecure®, Safe Foods Cooperation, Little Rock, Ark.), 4% (w/v) sodium metasilicate (LAN; PQ Corporation, Valley Forge, Pa.), 0.02% (v/v) peracetic acid (LAP; Blitz®, FMC Corporation, Philadelphia, Pa.), 10% (w/v) trisodium phosphate (LAT; ICL performance products, St. Louis, Mo.) or sterile water (LAW). Subsequent to spray applications on both sides (3 replications/treatment), the treated trimmings were allowed to drip for 3 min prior to and after assigned second antimicrobial applications (3 replicates/treatment).

Sample Processing. Untreated un-inoculated (CON) and inoculated untreated (INCON) control beef trimmings along with all the treated beef trimmings were individually ground twice ground using an American Eagle grinder (Model AEG-12N, Food Machinery Inc. Chicago, Ill.) with a 3.2 mm chopping plate. Next, 200 g of individual ground beef samples processed from each treatment and control were placed on plastic foam trays and over wrapped with polyvinyl chloride film (O_2 transmission rate = 14,000 cc/mm^2/24 h/1 atm;

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Koch Supplies, Inc., Kansas City, Mo.). The packages were stored under simulated retail conditions (4 °C, under 1,630 lux of deluxe warm white fluorescent lighting; Phillips Inc., Somerset, N.J.) until further evaluation.

Instrumental Color Analysis. On days 0, 1, 2, and 3 of simulated retail display, the samples were evaluated for CIE L* (lightness), a* (redness), and b* (yellowness) using a Hunter-Lab MiniScan XE Spectrocolorimeter, Model 4500L (Hunter Associates Laboratory, Reston, W. Va.). All the values were determined from the mean of three measurements of each sample using Illuminant A/10° observer. The hue angle (arctan (b*/a*), saturation index $((a^{*2}+ b^{*2}))^{0.5}$, and reflectance ratio (630/580 nm) of these samples were also determined.

Analysis of Data. The L*, a*, b*, hue angle, saturation index, and reflectance ratio were analyzed for the main effects of antimicrobial treatment, days of display and treatment by day of display interaction using the GLM procedure of SAS (SAS Institute, Inc., Cary, N.C.). Least squares means were generated for all variables and were separated using the PDIFF option of SAS.

Results and Discussion

Table 1 shows the effect of antimicrobial treatment on lightness (L*), redness (a*), yellowness (b*) and saturation index (which represents the chroma or total color of a sample) of processed ground beef. The results indicated that the treatment × day interaction was not significant ($P > 0.05$) for yellowness (b*) and saturation index. Ground beef from LA, LAN, LAT and LAW treatments had similar lightness ($P > 0.05$) compared to CON. The lightness of LAC-treated ground beef was significantly higher ($P < 0.05$) compared to all the other treatments. The LA and LAW treatments were more ($P < 0.05$) red (a*) compared to all the other treatments and control samples. However, there was no significant difference ($P > 0.05$) in redness among LAC, LAN, LAP, LAT and CON samples. All treated samples had a yellowness (b*) similar ($P > 0.05$) to CON except the LAC treatment. Further, all treatments, excluding LAP, had a similar saturation index compared to CON and INCON. Both L* and b* intensities significantly decreased ($P < 0.05$) from day 0 through 3 of display (Table 2). In addition, the LA treated ground beef maintained a similar ($P > 0.05$) hue angle (which represents a change from red color to yellow) and reflectance ratio (630 nm/580 nm) compared to INCON and CON samples on day 0 through 3 of display (Table 3). The ground beef from LAP and LAT also maintained a similar hue angle compared to CON and INCON samples on day 1 through 3 and day 2 and 3 of display, respectively. Findings from this study indicate that use of lauric arginate alone or accompanied by water, sodium metasilicate, trisodium phosphate, or peroxyacetic acid may provide successful pre-grinding decontamination interventions without adverse effects on ground beef color properties. Nevertheless, lauric arginate alone and followed by water were superior to other treatments in enhancing redness in ground beef.

Implications

The use of lauric arginate alone or along with water, sodium metasilicate, trisodium phosphate, or peroxyacetic acid may extend ground beef shelf life. This research provides beef processors new antimicrobial intervention options which generally have no effects on desirable meat color. However, further studies to evaluate the impact of lauric arginate alone or accompanied by other antimicrobials on ground beef instrumental color and sensory color under uninoculated state are recommended.

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Table 1. Effect of antimicrobial treatment on least squares mean of ground beef lightness (L*), redness (a*), yellowness (b*) and saturation Index[1].

Treatment[2]	L*	a*	b*	Saturation Index
INCON	55.84^{b}	14.27^{a}	18.35^{ab}	23.45^{a}
CON	49.35^{a}	15.49^{a}	17.41^{a}	23.53^{a}
LA	51.08^{a}	17.05^{b}	18.64^{ab}	25.49^{ab}
LAC	62.50^{c}	13.23^{a}	19.42^{b}	23.75^{a}
LAN	53.82^{ab}	14.24^{a}	18.29^{ab}	23.40^{a}
LAP	55.65^{b}	15.68^{a}	19.02^{ab}	24.89^{ab}
LAT	52.86^{a}	15.91^{a}	18.97^{ab}	24.93^{ab}
LAW	51.11^{a}	18.67^{b}	18.82^{ab}	26.64^{b}

[1] Saturation index = $((a^{*2}+ b^{*2}))^{0.5}$.

[2] Treatment: INCON = untreated inoculated control, CON = untreated un-inoculated control, LA = 5% lauric arginate, LAC = 5% LA followed by 0.5% cetylpyridinium chloride, LAN = 5% LA followed by 4% sodium metasilicate, LAP = 5% LA followed by 0.02% peracetic acid, LAT = 5% LA followed by 10% trisodium phosphate, LAW = 5% LA followed by water.

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

Table 2. Effect of day of display on least squares mean of ground beef lightness (L*), redness (a*), yellowness (b*) and saturation Index[1].

Day of display	L*	a*	b*	Saturation Index
0	57.83^{c}	16.68^{b}	19.42^{b}	25.64^{b}
1	54.97^{b}	16.57^{b}	18.76^{b}	25.15^{b}
2	53.88^{b}	19.81^{c}	18.56^{ab}	27.21^{b}
3	49.44^{a}	9.21^{a}	17.70^{a}	20.04^{a}

[1] Saturation index = $((a^{*2}+ b^{*2}))^{0.5}$.

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

Table 3. Effect of antimicrobial treatment and day of display interaction on least squares mean of ground beef hue angle and reflectance ratio.

Instrumental color Property	Treatment[1]	Days of display			
		0	1	2	3
Hue Angle[2]	INCON	55.23^{b}	48.59^{a}	44.14^{a}	63.67^{b}
	CON	52.16^{b}	45.10^{a}	39.45^{a}	60.83^{b}
	LA	50.11^{ab}	43.66^{a}	39.53^{a}	61.13^{b}
	LAC	49.61^{a}	57.93^{c}	49.46^{b}	69.37^{c}
	LAN	45.38^{a}	54.76^{c}	47.30^{ab}	64.84^{bc}
	LAP	48.42^{a}	49.12^{a}	43.66^{a}	64.18^{bc}
	LAT	47.49^{a}	50.46^{b}	43.14^{a}	62.14^{b}
	LAW	47.93^{a}	43.02^{a}	39.46^{a}	54.14^{a}
Reflectance Ratio[3]	INCON	0.41^{a}	0.52^{b}	0.60^{ab}	0.29
	CON	0.47^{a}	0.56^{b}	0.66^{bc}	0.32
	LA	0.52^{ab}	0.63^{b}	0.71^{bc}	0.30
	LAC	0.48^{a}	0.39^{a}	0.55^{a}	0.30
	LAN	0.63^{b}	0.39^{a}	0.50^{a}	0.33
	LAP	0.52^{ab}	0.51^{ab}	0.64^{bc}	0.32
	LAT	0.58^{b}	0.48^{a}	0.63^{b}	0.41
	LAW	0.53^{ab}	0.67^{b}	0.77^{c}	0.29

[1] Treatment: INCON = untreated inoculated control, CON = untreated un-inoculated control, LA = 5% lauric arginate, LAC = 5% LA followed by 0.5% cetylpyridinium chloride, LAN = 5% LA followed by 4% sodium metasilicate, LAP = 5% LA followed by 0.02% peracetic acid, LAT = 5% LA followed by 10% trisodium phosphate, LAW = 5% LA followed by water.

[2] Hue angle - ($\tan^{-1}(b^*/a^*)$.

[3] Reflectance Ratio - (630/580 nm).

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

Novel decontamination approaches for beef trimmings using lauric arginate to reduce O157:H7 and non-O157:H7 shiga toxin producing *E. coli* and *Salmonella* in ground beef

P. N. Dias-Morse[1], F. W. Pohlman[1], J. A. McDaniel[2], R. D. Guidry[1], C. L. Coffman[1], and T. L. Devine[1]

Story in Brief

This study evaluated novel lauric arginate (LA; 5%) alone or followed by 0.4% cetylpirinidium chloride (LAC), 4% sodium metasilicate (LAN), 0.02% peracetic acid (LAP), 10% trisodium phosphate (LAT) or sterile water (LAW) as a pre-grinding treatment on beef trimmings to reduce ground beef microbial populations. Beef trimmings (40 kg) were inoculated with a 10^5 CFU/ml cocktail containing *E. coli* O157:H7 and six non-O157:H7 Shiga Toxin-producing (STEC) sero groups (EC) and *Salmonella* Typhimurium DT 104 and *Salmonella* Newport MDR-AmpC (S). Subsequently, inoculated beef trimmings (1.6 kg/treatment/replicate) were spray treated (~0.1 ml/g) with 5% LA alone or followed by other assigned treatments. The experiment was repeated 3 times. All treated trimmings and inoculated un-treated control (INCON) were ground twice and 200 g of individual samples were overwrap-packaged and stored under simulated retail conditions (4 °C) until sampled on day 0, 1, 2, and 3 of display for microbiological analysis. All the treatments showed significantly lower ($P < 0.05$) coliform (CO), EC, and S counts compared to the inoculated control from day 1 to 3 of display. Ground beef from LAC, LAN, LAT, and LAP treatments surpassed other treatments in controlling *Salmonella* population with >1 log reductions on day 0 through 3 of display. The results suggest that application of LA as a single or multiple chemical hurdle approach with selected antimicrobials on beef trimmings may provide successful decontamination intervention to enhance microbial quality of consequent ground beef.

Introduction

Presence of pathogens including shiga toxin producing *Escherichia coli* (STEC) in meat products can cause serious consumer illnesses. The recent large number of ground beef recalls due to the presence of possible pathogenic bacteria indicates that the practice of intervention techniques in processing and handling of ground beef products is inadequate. Therefore, there is a greater need for new and advanced decontamination interventions to enhance microbial safety of ground beef products. According to Pohlman et al. (2002) application of antimicrobial decontamination techniques on beef trimmings resulted in efficient reduction of pathogenic bacteria populations in ground beef. Although *Escherichia coli* O157:H7 is the most commonly known shiga toxin producing *E. coli* (STEC) responsible for food borne outbreaks in the U.S., six non-O157: H7 STEC serogroups (O26, O45, O103, O111, O121, and O145) are gaining public health concern as they have the potential to cause human illnesses (Fratamico et al., 2011; Kasper et al., 2010). Since ground beef processing involves mixing and grinding of meat from various animals, developing and implementing antimicrobial interventions in the production line would help to control any STEC organisms if they were ever present. Numerous chemical interventions have been studied with *E. coli* O157:H7 as the target pathogen. However there is limited information on novel and existing chemical interventions on non-O157:H7 STEC. Therefore, there is a great need for validation of novel and commonly practiced antimicrobial interventions to control *E. coli* O157:H7 and non-O157H7. The overall objective of this research was to utilize antimicrobial properties of lauric arginate alone or followed by cetylpirinidium chloride (LAC), sodium metasilicate (LAN), peracetic acid (LAP), trisodium phosphate (LAT) or sterile water (LAW), on beef trimmings to control ground beef *E. coli* O157:H7, non-O157H7 and *Salmonella* populations.

Materials and Methods

Inoculation Process. Beef trimmings (40 kg) were inoculated with a cocktail mixture (4 °C) of *E.coli* O157:H7, O26, O103, O111, O121, O45, and O145 (EC) and *Salmonella* Typhimurium DT 104 and *Salmonella* Newport MDR-AmpC (S) at 10^5 CFU/g by following the procedures explained by Pohlman et al. (2002). After leaving overnight at 4 °C for further bacterial attachment, the inoculated beef trimmings (1.6 kg/treatment/replicate) were arranged in stainless steel trays.

Antimicrobial Treatment Applications. Each side of the beef trimmings (1.6 kg/treatment/replicate) were treated with conventional spray (~0.1 ml/g) applications of 5% (v/v) Lauric arginate (LA; CytoGuard®, A & B Ingredients, Fair Field, N.J.) alone or followed by 0.4% (v/v) cetylpirinidium chloride (LAC; Cecure®, Safe Foods Cooperation, Little Rock, Ark.), 4% (w/v) sodium metasilicate (LAN; PQ Corporation, Valley Forage, Pa.), 0.02% (v/v) peracetic acid (LAP; Blitz®, FMC Corporation, Philadelphia, Pa.), 10% (w/v) trisodium phosphate (LAT; ICL performance products, St Louis, Mo.) or sterile water (LAW). The LA- treated samples were allowed to drip for 3 min prior to and after assigned second antimicrobial applications (3 replicates/treatment).

Sample Processing. All treated and untreated inoculated (INCON) beef trimmings were ground twice and 200 g of individual samples were placed on plastic foam trays and over wrapped with polyvinyl chloride film (O_2 transmission rate = 14,000 cc/mm^2/24 h/1 atm; Koch Supplies, Inc., Kansas City, Mo.). The packages were stored under simulated retail conditions (4 °C, under 1,630 lux of deluxe warm white fluorescent lighting; Phillips Inc., Somerset, N.J.).

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[2] Cargill Value Added Meats, Springdale, Ark.

Microbiological Analysis. On day 0, 1, 2, and 3 of simulated retail display, *Salmonella* (S) counts on *Salmonella* shigella agar (DIFCO Laboratories, Detroit, Mich.), aerobic plate count (APC), and *E. coli* (EC)/coliform (CO) counts on Petrifilm® (3M Corporation, St. Paul, Minn.) were determined for ground beef from all treatments. The 25 g samples of ground beef from each antimicrobial treatment were placed in sterile whirlpack bags (Nasco, Ft Atkinson, Wis.) separately and 225 ml of 0.1% buffered peptone water was added and samples were homogenized for 2 min in a stomacher (Model 400 Lab Stomacher; Seward, London, U.K.). Subsequently, serial 10-fold dilutions were made and spread plating was done in duplicates. The EC, APC and S counts were read after 48 h, whereas coliform plates were read after 24 h. All the counts were recorded as colony forming units per gram (CFU/g).

Analysis of Data. The bacterial values were transformed to log values and then analyzed for the main effects of antimicrobial treatment, days of display and treatment by day of display interaction using the GLM procedure of SAS (SAS Institute, Inc., Cary, N.C.). Least square means were generated for all variables and were separated using the PDIFF option of SAS.

Results and Discussion

Table 1 shows the antimicrobial treatment by days of display interaction for CO, EC, S and APC counts, respectively. All the treatments effectively lowered ($P < 0.05$) CO, EC, and S counts compared to the inoculated control from day 0 to 3 of display. Ground beef from LAP, LAW and LAC showed a greater reduction ($P < 0.05$) of coliform counts compared to LA and LAT treatments on day 0 while LAN-treated ground beef reported the lowest coliform count ($P < 0.05$) compared to all the treatments on day 2 of display. All the treatments encountered >1 log reduction of EC counts in ground beef on days 0 and 1, and afterward, >2 log reduction on day 2 of display. Similarly, all treatments controlled *Salmonella* populations with >1 log reductions on day 0 through 3 of display compared with INCON. On days 2 and 3 of display, all the treatments showed >1 log APC count reduction while LAC and LAN showed >2 log reduction. Our results are in agreement with studies conducted by Pohlman et al. (2002) which recognized successful application of 0.5% cetylpirinidium chloride (CPC) and 10% trisodium phosphate (TSP) on beef trimmings to reduce similar bacterial counts of ground beef under retail display. However, these studies were focused on *E. coli* O157:H7 as the target pathogen. Therefore, overall, it can be concluded that tested chemical interventions in this study were effective against *E. coli* O157:H7 as well as non-O157:H7 and multi-drug-resistant *Salmonella* in ground beef and can be adopted in meat decontamination interventions in ground beef production systems. However, further investigations on the impact of these antimicrobials on quality characteristics of ground beef are recommended.

Implications

The results suggested that applications of lauric arginate alone or followed by cetylpirinidium chloride, sodium metasillicate, peracetic acid, trisodium phosphate, or sterile water on contaminated beef trimmings can result in >1 log or in some days up to >2 log reduction of *E. coli* O157:H7, non-O157H7 and *Salmonella* populations in ground beef. Hence, lauric arginate alone or along with other interventions may function as successful candidates in ground beef decontamination interventions, especially in reducing the pathogens of recent concern.

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Table 1. Effect of antimicrobial treatment by day of display interaction on least squares mean log CFU*/g coliform, *Escherichia coli*, *Salmonella* and aerobic plate counts of ground beef.

		Days of display			
	Treatment**	0	1	2	3
Coliform	INCON	6.09^{c}	6.03^{c}	6.22^{c}	7.98^{c}
	LA	4.71^{b}	4.52^{ab}	4.97^{b}	6.99^{b}
	LAC	4.47^{a}	4.39^{a}	4.76^{b}	5.84^{a}
	LAN	4.58^{ab}	4.62^{b}	4.45^{a}	5.89^{a}
	LAP	4.39^{a}	4.44^{a}	5.00^{b}	6.07^{b}
	LAT	4.63^{b}	4.74^{b}	4.97^{b}	6.03^{ab}
	LAW	4.48^{a}	4.74^{b}	4.83^{b}	6.90^{b}
Escherichia coli					
	INCON	6.10^{c}	6.05^{c}	7.24^{c}	8.00^{c}
	LA	4.72^{b}	4.72^{b}	5.00^{b}	7.02^{b}
	LAC	4.66^{ab}	4.40^{a}	4.80^{ab}	5.91^{a}
	LAN	4.61^{ab}	4.63^{b}	4.57^{a}	5.95^{a}
	LAP	4.42^{a}	4.41^{a}	4.99^{b}	6.11^{a}
	LAT	4.78^{b}	4.78^{b}	4.98^{b}	6.08^{a}
	LAW	4.46^{a}	4.52^{ab}	4.89^{b}	6.92^{b}
Salmonella					
	INCON	4.41^{d}	5.28^{e}	5.28^{e}	6.09^{d}
	LA	2.44^{a}	3.95^{d}	3.93^{d}	4.36^{c}
	LAC	2.53^{ab}	2.97^{b}	3.04^{b}	3.39^{a}
	LAN	2.78^{b}	2.98^{b}	2.78^{a}	3.71^{b}
	LAP	2.39^{a}	2.89^{ab}	2.98^{b}	3.76^{b}
	LAT	2.66^{b}	2.77^{a}	2.88^{ab}	3.40^{a}
	LAW	2.36^{a}	3.27^{c}	3.32^{c}	4.36^{c}
Aerobic plate count	INCON	6.56^{d}	6.88^{d}	7.66^{e}	8.08^{c}
	LA	4.77^{ab}	4.87^{b}	6.00^{c}	7.06^{b}
	LAC	4.82^{b}	4.82^{b}	5.59^{b}	6.06^{a}
	LAN	5.65^{cd}	4.65^{a}	4.62^{a}	6.02^{a}
	LAP	4.61^{a}	4.61^{a}	5.98^{c}	6.14^{a}
	LAT	5.35^{c}	4.99^{c}	6.14^{d}	6.28^{a}
	LAW	6.28^{d}	5.28^{c}	5.65^{b}	7.00^{b}

*Colony forming units.

**Treatments: INCON = untreated inoculated control, LA = 5% lauric arginate, LAC = 5% LA followed by 0.5% ctylpyridinium chloride, LAN = 5% LA followed by 4%, sodium metasilicate, LAP = 5% LA followed by 0.02% peracetic acid, LAT = 5% LA followed by 10% trisodium phosphate, LAW = 5% LA followed by sterile water.

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

Effect of octanoic acid on color characteristics of ground beef applied using conventional and electrostatic spray

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Story in Brief

Effectiveness of octanoic acid applied to beef trimmings at three different levels (0.5%, 1.5% and 3.0%) using electrostatic and conventional sprayers and its effect on instrumental color characteristics of ground beef through simulated retail display was examined. Beef trimmings were inoculated with a *Salmonella* and *E. coli* species cocktail mixture 10^5/ml colony forming unit or cfu/g; (2.5 kg/treatment/rep) and spray treated with three levels of octanoic acid with conventional and electrostatic spray. Trimmings were ground twice and were placed on plastic styrofoam trays with absorbent pads and overwrapped with polyvinyl chloride film and sampled on days 0, 1, 2, 3 and 7 for instrumental color characteristics. Results of this study showed that ground beef treated with octanoic acid and trisodium phosphate had no significant difference ($P > 0.05$) on lightness (L^*) values compared to inoculated control on 0, 2, and 3 day of display. Whereas, on day 1 and 7 water treated ground beef were lighter (L^*, $P < 0.05$) compared to inoculated control. Redness (a^*) values were significant ($P < 0.05$) for ground beef treated with trisodium phosphate and 0.5% octanoic acid on 7 day of display. Beef trimmings sprayed with trisodium phosphate, water and 3.0% octanoic acid using conventional sprayer were lighter ($P < 0.05$) in comparison to the inoculated control whereas, remaining treatments did not differ ($P > 0.05$) in lightness values compared to inoculated control. Electrostatic spray had no significant effect ($P < 0.05$) on ground beef color (L^*, $P > 0.05$) compared to the inoculated control. There was no significant difference ($P > 0.05$) in the redness (a^*) and yellowness (b*) of the ground beef with both application methods. Trisodium phosphate treated ground beef was more intensely yellow ($P < 0.05$) compared to the control. Electrostatic sprayed ground beef showed all the levels of octanoic acid treatments were also similar ($P > 0.05$) in hue angle compared to inoculated control. Trisodium phosphate treated ground beef maintained a larger ($P < 0.05$) hue angle. A significant difference ($P > 0.05$) in saturation index occurred between conventional sprayed ground beef for all the treatments compared to inoculated control; whereas, electrostatic spray showed no significant difference among treatments.

Introduction

Escherichia coli O157:H7 and *Salmonella* are food borne pathogens that have resulted in outbreaks of illnesses in the U.S. Although *E.coli* O157:H7 is the most commonly known shiga toxin-producing *E.coli* (STEC), six non-O157:H7 STEC serogroups (O26, O45, O103, O111, O121, and O145) are gaining public health concern as they have the potential to cause human illnesses (Fratamico et al., 2011). Decontaminating beef trimmings using antimicrobial treatments prior to grinding has shown to be effective in reducing the amount of pathogenic bacteria in final ground beef (Pohlman et al., 2002). Octanoic acid (OA) is a food-grade chemical that has been approved by the Food Safety and Inspection Service (FSIS, 2011) for use as an antimicrobial agent in ready-to-eat meat and poultry products up to 400 ppm by weight of the final product and is generally considered as safe by the U.S. Food and Drug Administration (GRAS 21 CFR 184.1025). Therefore, the purpose of this study was to investigate the effectiveness of OA on beef trimmings applied using an electrostatic and conventional sprayer and its effect on instrumental color characteristics.

Materials and Methods

Inoculation Process. A bacterial cocktail containing 10^5 log CFU *E. coli* O157:H7, O26, O103, O111, O121, O45 and O145 and *Salmonella* Typhimurium DT 104, (ST), *S. Newport* were prepared from frozen (-80 °C) pure cultures according to the procedures described by Pohlman et al. (2002). To make the cocktail, 0.1 mL of each strain of *E. coli* and *S.* Typhimurium were inoculated into 10 ml aliquots of Brain Heart Infusion agar (BHI; Difco Laboratories Becton Dickinson and Company, Sparks Md.). The inoculated tubes were incubated at 37 °C for 18 hours non-shaking. Following incubation, the tubes were centrifuged (3500 g for 20 minutes at 37 °C) (Beckman GS-6 series, Fullerton, Calif.). The liquid supernatant was discarded and the bacterial pellets were resuspended with buffered peptone water (BPW; Difco Laboratories, Becton Dickinson and Company, Sparks Md.). Finally, the bacterial cocktail (10^5 CFU) was stored in a 4 °C cooler until use. Beef trimmings (2.5 kg/trt/rep) were inoculated with bacterial cocktail and placed at 4 °C for 12 to 14 hours for microbial attachment.

Antimicrobial Treatment Preparation. Three levels of OA (0.5%, 1.5% and 3.0 %) were prepared using appropriate amounts of water. Beef trimmings were spray treated (3 ml/sec/60 psi) using an electrostatic spray system (ESS; Electrostatic Spraying Systems, Inc. Watkinsville, Ga.) with either: (1) 10% TSP (Trisodium phosphate anhydrous (FG), ICL performance products, St. Louis, Mo.), (2) 0.5 % OA, (3) 1.5 % OA or (4) 3.0 % OA. The control treatments included in this experiment were: treated inoculated beef trimmings (InCon), and inoculated beef trimmings treated with water (InW). The treated and untreated beef trimmings were ground twice (American Eagle Model: AEG-12N , 12-1/8 chopper plate Food machinery Inc., Chicago, Ill.) and 200 g of individual samples were

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placed on plastic foam trays with absorbent pads and overwrapped with polyvinyl chloride film (O_2 transmission rate = 14,000cc/mm^2/24h/1atm; Koch Supplies, Inc. Kansas City, Mo.) and stored under retail display condition at 4 °C under 1,630 lx of deluxe warm white fluorescent lighting (Phillips Inc., Somerset, N.J.). Sampling was done for instrumental color characteristics including (lightness (L^*), redness (a^*), yellowness (b^*), hue angle, saturation index and spectral reflectance for day 0, 1, 2, 3, and 7.

Statistical Analysis. Least squares means for significant interactions and main effect were identified using LSMEANS statement and separated using the probability of difference procedure (PDIFF) of SAS. The experimental design includes 6 different treatments applied using two methods of spraying and 5 display days (0, 1, 2, 3 and 7).The analysis model included the main effects of antimicrobial treatment, spray method, day of display, and interaction effects were spray × treatment, spray × day, treatment × day and spray × treatment × day interactions.

Results and Discussion

Effect of Antimicrobial Treatment by Day of Display Interaction on Instrumental Color Characteristics. The effect of antimicrobial treatment by day of display interaction across 7 days under simulated retail display on lightness (L^*), redness (a^*), yellowness *(b)**, hue angle, saturation index and the proportion of oxymyoglobin to metmyoglobin (630/580 nm) are summarized in Table 1.

Ground beef treated with OA and TSP had no significant difference ($P > 0.05$) on CIE L^* values compared to InCon on 0, 2, and 3 day of display. On day 1 and 7, water treated ground beef was different ($P < 0.05$) from inoculated control (InCon) and ground beef were lighter (L^*, $P < 0.05$) compared to InCon. There was a day of display by antimicrobial treatment interaction effect on CIE a^* values ($P < 0.05$) for ground beef treated with TSP and 0.5% OA on day 7 of display. On day 0 of display, the 1.5% OA treated ground beef were less ($P < 0.05$) red compared to the InCon and contained less ($P < 0.05$) oxymyoglobin (630 compared with 580 nm). However, on day 1, 2 and 3 of display there was no difference ($P > 0.05$) for ground beef treated with different levels of OA for a^* or 630 and 580 nm values compared with InCon. On day 7 of display, ground beef treated with TSP and 0.5% OA treatment remained red ($P < 0.05$) in color and contained more ($P < 0.05$) oxymyoglobin than all other treatments whereas, no difference ($P > 0.05$) in redness intensity were found in any of the treatments and oxymyoglobin was similar ($P > 0.05$) in all treatments. In our study, 10% TSP retained high oxymyoglobin proportion compared to other treatments through 7 days of display. Pohlman et al. (2002) reported that ground beef previously inoculated and treated with TSP was redder (a^*) and had higher oxymyoglobin contents when compared to inoculated control (InCon). Inoculated Water (InW) treated ground beef was only different ($P < 0.05$) on day 0, 2, 3 and 7 of display for b^* (yellowness) values compared to the control, however, OA treated ground beef was similar ($P > 0.05$) for b^* values compared to the control through 0, 1, 2 and 3 day of display. Significant differences ($P > 0.05$) were observed for OA treatments compared to the control for CIE b^* values for day 7 of display.

The hue angle is calculated using a^* and b^* values to determine the hue color of a sample. The effect of treatments over days of display did not affect hue angle measurements of ground beef. Ground beef treated with water, TSP and three levels of OA were similar ($P > 0.05$) in hue (hue angle) compared to InCon through 7 day of display, while TSP treated ground beef tended to have higher hue angle, but was only slightly higher ($P < 0.05$) on day 3 and 7 of display. Similar findings have been reported for hue angle values for antimicrobials treated patties.

Saturation index is determined by intensity of their corresponding a^* values and b^* values. Because of lower a^* and b^* values, ground beef treated with TSP and different levels of OA were less ($P > 0.05$) vivid in color (saturation index) by day 0, 1 and 3 of display compared to InCon. On day 2 and 7, TSP treated ground beef had higher values indicating more vividness in color compared to InCon. Estimated oxymyoglobin proportions (630/580 nm) were higher ($P < 0.05$) for 0.5% OA when compared to control on day 2 of retail display. On day 0, 1 and 3 of display, all treatments had similar ($P > 0.05$) oxymyoglobin values when compared to InCon. On day 7 of display the TSP and OA had decrease in oxymyoglobin proportion value. The treatments with low oxymyoglobin also yielded low a^* values among all treatments. Similar observations were reported by Quilo et al. (2009) for ground beef treated with different antimicrobials.

Effect of Antimicrobial Treatments by Spray Application Method. Effect of antimicrobial treatment by spray application method for instrumental color measures are shown in Tables 2a and 2b. Beef trimmings sprayed with TSP, water and 3.0% OA using conventional sprayer, were lighter ($P < 0.05$) in comparison to the InCon whereas, remaining treatments did not differ ($P > 0.05$) in CIE L^* values compared to InCon. Electrostatic spray had no effect on ground beef color (L^* $P > 0.05$) except for water when compared to InCon. There was no difference ($P > 0.05$) in the redness (a^*) of the ground beef with both application methods. Trisoduim phosphate treated ground beef was more intense yellow color ($P < 0.05$) compared to the control; whereas, there was no difference ($P > 0.05$) between any treatments and the control for CIE b^* values. This is in agreement with Pohlman et al. (2002) findings which reported that ground beef treated with 10% trisodium phosphate exhibited lower b^* values in display. Ground beef sprayed with conventional sprayer for 0.5%, 1.5% and 3.0% OA had similar ($P > 0.05$) hue angle whereas, TSP and water had larger ($P < 0.05$) hue angle. Electrostatic sprayed ground beef showed OA treatment at all levels; treatments were also similar ($P > 0.05$) in hue angle compared to InCon; and TSP treated ground beef maintained larger ($P < 0.05$) hue angle. Difference ($P > 0.05$) in saturation index occurred between conventional sprayed ground beef for all the treatments compared to InCon whereas, electrostatic spray showed no significant difference ($P < 0.05$) among treatment. There was no difference ($P > 0.05$) in oxymyoglobin proportion values for ground beef sprayed with conventional and electrostatic sprayer. The pH of electrostatically applied TSP on the ground beef was different ($P < 0.05$) from the InCon; whereas, conventionally applied TSP did not vary ($P > 0.05$). Difference was observed ($P < 0.05$) with OA treatments when applied using conventional sprayer.

Implications

Water treated ground beef was lighter (L^*) compared to inoculated control on day 1 and 7 of display; whereas, redness of the ground beef was more with trisodium phosphate and 0.5% octanoic acid on day 7 of display. With regard to application methods, beef trimmings sprayed with trisodium phosphate, water and 3.0% octanoic acid using conventional sprayer were lighter whereas, electrostatic spray had no effect on ground beef color in comparison to the inoculated control.

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Table 1. Antimicrobial treatment[a] by days of display interaction effect on the least-squares means (±SE) for CIE *L[b], *a**[c], *b**[d], hue angle[e], saturation index[f] and 630/580 nm[g] value of ground beef.**

Attribute	Treatment	Day of display				
		0	1	2	3	7
CIE *L**	InCon	52.66	48.32^{bc}	46.67	46.52	41.84^{c}
	InW	53.76	54.36^{a}	52.08	50.56	50.21^{ab}
	TSP	48.90	54.02^{ab}	51.73	49.37	50.70^{a}
	0.5 OA	49.13	47.81^{c}	47.33	44.74	45.86^{bc}
	1.5 OA	46.64	47.62^{c}	46.05	45.22	46.35^{abc}
	3.0 OA	48.74	51.03^{abc}	49.94	46.44	47.09^{ab}
	SE	1.31	1.05	1.57	1.20	0.84
CIE *a**	InCon	12.54^{b}	9.09	7.32	8.85	8.28^{a}
	InW	13.55^{ab}	9.07	7.47	9.30	7.55^{ab}
	TSP	15.20^{ab}	8.28	8.23	7.29	5.92^{b}
	0.5 OA	13.76^{ab}	8.58	8.67	8.15	5.88^{b}
	1.5 OA	15.65^{a}	8.37	8.13	8.00	6.56^{ab}
	3.0 OA	14.02^{ab}	10.09	7.14	8.66	6.82^{ab}
	SE	0.52	0.64	0.37	0.41	0.34
CIE *b**	InCon	16.57^{b}	15.41	13.80^{b}	12.99^{b}	11.64^{b}
	InW	19.26^{a}	16.48	16.23^{a}	17.42^{a}	14.89^{a}
	TSP	18.39^{ab}	16.58	16.70^{ab}	16.61^{a}	14.95^{a}
	0.5 OA	17.56^{ab}	15.15	15.46^{ab}	14.83^{ab}	13.98^{a}
	1.5 OA	18.13^{ab}	14.85	14.82^{ab}	15.43^{ab}	13.92^{a}
	3.0 OA	18.30^{ab}	16.44	14.88^{ab}	15.82^{ab}	14.45^{a}
	SE	0.45	0.41	0.49	0.56	0.36
Hue angle	InCon	57.52^{a}	59.44	62.13	55.73^{b}	54.51^{b}
	InW	57.52^{a}	61.05	65.47	61.82^{ab}	59.42^{a}
	TSP	57.52^{a}	64.02	63.49	65.95^{a}	60.10^{a}
	0.5 OA	57.52^{a}	60.81	60.75	61.23^{ab}	60.51^{a}
	1.5 OA	57.52^{a}	60.84	61.52	62.31^{ab}	59.66^{a}
	3.0 OA	55.48^{b}	59.39	64.23	60.95^{ab}	57.42^{ab}
	SE	0.00	1.61	1.13	1.59	0.57
Saturation index	InCon	24.03	17.89	15.64^{b}	15.73	14.29^{b}
	InW	24.03	18.84	17.89^{ab}	19.76	20.42^{a}
	TSP	24.03	18.58	18.65^{a}	18.25	19.21^{a}
	0.5 OA	24.03	17.47	17.74^{ab}	16.97	19.16^{a}
	1.5 OA	24.03	17.09	16.96^{ab}	19.87	20.13^{a}
	3.0 OA	24.56	19.37	16.52^{ab}	18.06	19.93^{a}
	SE	0.17	0.58	0.52	1.10	0.34
630/580 nm	InCon	2.33	1.87	1.15^{c}	2.42	2.72^{a}
	InW	2.51	1.57	1.46^{bc}	2.27	2.15^{b}
	TSP	2.79	1.64	1.79^{ab}	2.25	2.10^{b}
	0.5 OA	2.68	1.50	2.01^{a}	2.32	2.04^{b}
	1.5 OA	3.28	1.66	1.67^{abc}	2.17	2.28^{b}
	3.0 OA	2.74	1.81	1.41^{bc}	2.20	2.30^{ab}
	SE	0.19	0.10	0.09	0.09	0.07

[a] InCon, control; InW, water; TSP, trisodium phosphate; 0.50 OA, 0.5% Octanoic acid; 1.50 OA, 1.5% Octanoic acid; 3.0OA, 3.0% of Octanoic acid.
[b] *L**: 0 = black and 100 = white.
[c] *a**: -60 = green and +60 = red.
[d] *b**: -60 = blue and +60=yellow.
[e] Calculated as $\tan^{-1}(b^*/a^*)$.
[f] Calculated as $(a^{*2} + b^{*2})^{0.5}$.
[g] Calculated as the ratio 630 nm/580 nm reflectance.
Least- squares means within an attribute and a column bearing different letters (a-c) are different ($P < 0.05$).

Table 2a. Antimicrobial treatment[a] by application method[b] interaction effect on the least-squares means (±SE) for CIE *L[c], *a**[d], *b**[e], hue angle[f], saturation index[g], 630 nm/580 nm[h] and pH value of ground beef.**

Attribute	CIE *L**		CIE *a**		CIE *b**	
	Spray method		Spray method		Spray method	
Treatment	CS	ES	CS	ES	CS	ES
InCon	47.20^{c}	47.20^{b}	9.22^{a}	9.22^{a}	14.08^{e}	14.08^{e}
InW	50.88^{ab}	53.51^{a}	8.94^{a}	9.84^{a}	16.46^{bcd}	17.25^{abc}
TSP	53.49^{a}	48.39^{b}	9.14^{a}	8.83^{a}	18.40^{a}	14.88^{de}
0.5 OA	47.4^{bc}	46.49^{b}	8.69^{a}	9.33^{a}	15.88^{cde}	14.91^{de}
1.5 OA	46.31^{c}	46.44^{b}	9.01^{a}	9.68^{a}	15.08^{de}	15.77^{cde}
3.0 OA	49.64^{ab}	47.65^{b}	10.32^{a}	8.37^{a}	17.82^{ab}	14.14^{e}
SE	0.77	0.77	0.29	0.29	0.29	0.29

[a] InCon, control; InW, water; TSP, trisodium phosphate; 0.50 OA, 0.5% octanoic acid; 1.50 OA, 1.5% Octanoic acid; 3.0OA, 3.0% of Octanoic acid.
[b]Conventional spray; Electrostatic spray.
[c] *L**: 0 = black and 100 = white.
[d] *a**:-60 = green and +60 = red.
[e] *b**:-60 = blue and +60 + yellow.
[f]Calculated as $\tan^{-1}(b^*/a^*)$.
[g]Calculated as $(a^{*2}+b^{*2})^{0.5}$.
[h]Calculated as the ratio 630 nm/580 nm reflectance.
Least- squares means within an attribute and a column bearing different letters (a-e) are different ($P < 0.05$).

Table 2b. Antimicrobial treatment[a] by application method[b] interaction effect on the least-squares means (±SE) for CIE *L[c], *a**[d], *b**[e], hue angle[f], saturation index[g], 630 nm/580 nm[h] and pH value of ground beef.**

Attribute	Hue angle		Saturation index		630/580 nm		pH	
	Spray method		Spray method		Spray method		Spray method	
Treatment	CS	ES	CS	ES	CS	ES	CS	ES
Incon	57.86^{b}	57.86^{b}	17.51^{de}	17.51^{de}	2.09^{a}	2.09^{a}	6.41^{cd}	6.36^{d}
InW	58.76^{cd}	60.91^{ab}	20.52^{ab}	19.84^{abcd}	1.94^{a}	2.04^{a}	6.28^{d}	6.32^{d}
TSP	62.71^{a}	61.70^{ab}	22.04^{a}	17.44^{de}	2.14^{a}	2.08^{a}	6.71^{abc}	6.71^{abc}
0.5OA	59.82^{ab}	59.82^{ab}	20.35^{abc}	17.79^{cde}	2.13^{a}	2.09^{a}	6.83^{a}	6.37^{d}
1.5OA	59.41^{ab}	59.84^{bc}	20.69^{ab}	18.53^{bcde}	2.26^{a}	2.17^{a}	6.92^{a}	6.33^{d}
3.0OA	57.93^{ab}	61.05^{ab}	22.26^{a}	17.10^{e}	2.19^{a}	1.99^{a}	6.76^{ab}	6.46^{bcd}
SE	0.52	0.52	0.40	0.40	0.07	0.07	0.04	0.04

[a] InCon, control; InW, water; TSP, trisodium phosphate; 0.50 OA, 0.5% octanoic acid; 1.50 OA, 1.5% Octanoic acid; 3.0OA, 3.0% of Octanoic acid.
[b]Conventional spray; Electrostatic spray.
[c] *L**: 0 = black and 100 = white.
[d] *a**:-60 = green and + 60 = red.
[e] *b**:-60 = blue and + 60 + yellow.
[f]Calculated as $\tan^{-1}(b^*/a^*)$.
[g]Calculated as $(a^{*2}+b^{*2})^{0.5}$.
[h]Calculated as the ratio 630 nm/580 nm reflectance.
Least- squares means within an attribute and a column bearing different letters (a-e) are different ($P < 0.05$).

Incorporation of lean finely textured beef improved select quality characteristics of ground beef patties

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Story in Brief

Lean finely textured beef (LFTB), commonly used with ground beef to yield a lower fat, lower cost product, is created from fatty trimmings (greater than 80% fat), centrifuged to remove the fat, leaving a product that is 94% to 97% lean. The objective of this study was to determine the effects of LFTB on fresh and cooked quality characteristics of ground beef patties. Ground beef was formulated into 6 treatments in a 2 × 3 factorial of 82% or 93% lean and 0%, 10%, or 20% LFTB. Batches of each treatment combination (n = 5) were ground through a 3/8-in plate and formed into 1/3-lb patties, then overwrapped with a PVC film and placed in simulated retail display for 5 d. Instrumental color was measured daily, whereas lipid oxidation was measured on patties after 0, 1, 2, and 4 d of display. Additional patties were cooked to 160 °F before measuring internal instrumental color, cooked lipid oxidation, cooking loss, and Lee-Kramer shear force. The pH of fresh patties increased ($P < 0.05$) with increasing fat levels and LFTB percentage. Regardless of lean percentage, increasing LFTB in fresh patties resulted in lighter, redder, and less yellow patties ($P < 0.05$) throughout display. Even though lipid oxidation was similar among fresh patties on d 0 of display, oxidation values were lower ($P < 0.05$) with increasing LFTB on d 1, 2, and 4 of display. Cooking loss was lowest ($P < 0.05$) in 20% LFTB patties, whereas shear force declined ($P < 0.05$) with increasing fat levels and increased LFTB; however, LFTB inclusion percentage did not ($P > 0.07$) affect the internal color or oxidation of cooked patties. Overall, LFTB incorporation up to 20% improved the shelf-life of fresh ground beef patties and the tenderness of cooked patties, with no detrimental effects on cooked color.

Introduction

Lean Finely Textured Beef (LFTB) is a commonly used beef product made from high fat trimmings and is between 94% and 97% lean beef. When included in ground beef, it usually comprises 10-15% of the total product. By allowing around 10-lb more lean beef to be recovered from each carcass, LFTB is a way of turning meat and fat excluded from other cuts into a diverse and profitable product. Due to the formation of ammonium hydroxide and subsequent increase in pH during processing, the number of potential pathogens can also be reduced, improving food safety.

The LFTB process begins with the recovery of lean from fatty trim (greater than 80% fat) removed from the carcass during fabrication. It is difficult and economically infeasible to remove the lean from fatty trim by hand. Trim pieces are ground and heated to around 100 °F and the lean is separated using centrifugal force. In the case of Beef Products Incorporated (BPI), the lean beef is treated with a small amount of ammonia gas which combines with moisture in the beef to create ammonium hydroxide. This treatment raises the pH of the beef to reduce potential pathogens. The final step in the process is to rapidly freeze and cut the LFTB into large blocks or small pieces for shipping.

Although LFTB has been widely used in its current form for around twenty years, little research could be found concerning its effects on the quality characteristics of fresh and cooked ground beef. Therefore, the objective of this study was to determine the effects of LFTB on the fresh pH and color stability, as well as cooking loss, cooked color, shear force, and lipid oxidation of ground beef patties.

Materials and Methods

Denuded knuckles (97% lean) and beef trimmings with a 50:50 lean to fat ratio were ground once in a commercial mixer/grinder through a 5/8-in plate, and mixed appropriately to formulate 25-lb batches of either 82% or 93% lean ground beef. Additionally, lean finely textured beef (LFTB) was incorporated at 0%, 10%, and 20%, replacing the appropriate amount of knuckles (6 treatments with 5 batches/treatment). Batches were ground through a 3/8-in plate and 1/3 lb patties formed using a commercial patty forming machine. Patties for cooked Thiobarbituric Acid Reactive Substances (TBARS), cooked color, and Lee-Kramer evaluations were vacuum-packaged and stored frozen.

For display color, patties were packaged in pairs on foam trays and covered with PVC film. They were placed in simulated retail display (34 °F) and randomly shuffled daily to account for any differences within the display case. Measurements of lightness, redness, and yellowness (L*, a*, and b*, respectively) were taken after 0, 1, 2, 3, and 4 d of display. Three scans were taken from each package using a Hunter MiniScanXE (Hunter Associates Laboratory, Inc, Reston, Va.) with a 1- in aperture and illuminant A light source.

Patties for fresh TBARS, a measure of lipid oxidation, were aerobically packaged in individual foam trays and stored in simulated retail display. They were removed from display and frozen after 0, 1, 2, and 4 d display. Patties were thawed before TBARS were assayed.

To determine cooked TBARS, patties were thawed overnight at 34 °F and cooked to 160 °F on countertop electric griddles turning every 2 min, monitored with a handheld thermometer. They were allowed to cool to room temperature then refrozen for storage. Patties were thawed and TBARS assayed as described previously.

Patties for Lee-Kramer shear force were stored, thawed, and cooked similarly to cooked TBARS patties. A 2.4 × 2.4-inch square was cut from the center of each cooked patty, and shear force was measured using an Instron Universal Testing Machine (Instron Worldwide Headquarters, Norwood, Mass.) with a 200-kg load cell and 6 blade Lee-Kramer shear attachment.

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To measure pH, a 1-g sample was removed from each Lee-Kramer patty prior to cooking. This sample was homogenized with 10-mL of distilled water and pH measured using a pH meter.

Patties for cooked color were stored, thawed, and cooked as described previously. Immediately after cooking, these patties were placed in plastic bags and immersed in an ice bath to halt post cooking temperature changes. Each patty was sliced in half parallel to the surface exposing the internal surface, and lightness, redness, and yellowness (L*, a*, and b*, respectively) was measured using a Hunter MiniScanXE with a 1-in aperture and illuminant A light source. These patties were also weighed and measured before and after cooking to calculate cooking loss.

Data were analyzed as a 2 × 3 factorial design with fat level and LFTB level as main effects. The experimental unit was a batch of ground beef with a repeated measure of day for color display analysis. Data were analyzed using the Mixed Models Procedure in SAS (SAS Institute Inc., Cary, N.C.) with least square means separated when $P < 0.05$.

Results and Discussion

In fresh ground beef patties, pH values were greater ($P < 0.0001$) with increasing LFTB inclusion (Table 1). Patties formulated with 82% lean were also shown to have greater pH values ($P = 0.017$). The greater pH of patties containing LFTB was expected due to the presence of ammonium hydroxide, a processing aid known to increase pH values.

Fresh color measurements were affected ($P < 0.05$) by LFTB inclusion (Table 1). Lightness values (L*) increased ($P < 0.0001$) as LFTB increased and were greater ($P < 0.0001$) in 82% lean patties. Patties with 20% LFTB were shown to have greater ($P = 0.055$) a* values (indicating a redder patty) than 0% LFTB patties with 10% LFTB were intermediate. Values for b* (yellowness) decreased ($P = 0.009$) with LFTB inclusion meaning patties were less yellow. These results of an increase in lightness and redness with a decrease in yellowness could be accredited to the greater pH which tends to protect color.

Values for fresh TBARS decreased ($P < 0.0001$) with increasing LFTB indicating these patties were less oxidized (Table 1). Fresh TBARS were not shown to be affected ($P > 0.05$) by fat level. However, fresh TBARS were affected ($P = 0.012$) by an LFTB inclusions × display duration interaction (Fig. 1). The TBARS were similar ($P > 0.05$) across treatments on the day of patty formation and increased (indicating greater lipid oxidation; $P < 0.05$) with each day of display for all patties. This was expected as patties will continue to oxidize throughout display. Patties containing 10% LFTB had lower TBARS values ($P < 0.0001$) than 0% LFTB patties at d 1 and 4, whereas 20% LFTB patties were lower ($P < 0.0001$) than both 0% and 10% LFTB patties on each day of display after day 0. These results indicate that patties containing LFTB oxidize at a slower rate and would have a longer shelf life than patties without LFTB.

No difference in cooking loss was seen between patties with 0% and 10% LFTB inclusion but was lower ($P = 0.01$) in 20% LFTB patties (Table 2). Cooking loss was also lower ($P < 0.0001$) in 93% than 82% lean patties. No differences ($P > 0.05$) were found in cooked TBARS between 0% LFTB patties and those with LFTB inclusion (Table 2). However, 93% lean patties had lower ($P < 0.0001$) overall TBARS values than 82% lean, meaning they were less oxidized.

No differences ($P > 0.05$) were found in internal cooked color of ground beef patties (Table 2). Inclusion of LFTB did not affect ($P > 0.05$) lightness, redness, or yellowness (L*, a*, or b*). Furthermore, no differences in internal cooked color ($P > 0.05$) were seen between 82% and 93% lean patties. The result of LFTB having no effect on cooked color was unexpected due to the association between the higher pH seen in dark cutters and persistent pinking in cooked beef. Given the results of this study, it does not appear that pH increase due to ammonium hydroxide inclusion has the same effect on persistent pinking.

Lee-Kramer shear force values decreased ($P < 0.0001$) in cooked patties with each increase in LFTB inclusion (Table 2). Since these values are related to texture or tenderness, lower values would be indicative of more tender patties and higher values with tougher patties. Patties of 82% lean also yielded lower ($P < 0.0001$) shear force values than those with 93% lean.

Implications

The results of this study indicate that the inclusion of lean finely textured beef up to 20% could lend many positive quality characteristics to both fresh and cooked ground beef patties. Decreased lipid oxidation along with improved fresh color could result in a product with greater shelf life and more appealing color to consumers. With no negative effects on cooked color or cooking loss and the potential for increased tenderness, lean finely textured beef inclusion is a viable way to produce a desirable product while ensuring more complete utilization of beef carcasses.

Table 1. Quality characteristics of fresh ground beef patties as affected by lean finely textured beef inclusion and lean composition.

	Lean finely textured beef, %				Lean composition, %		
	0	10	20	SE	82	93	SE
pH	5.84[c]	6.11[b]	6.49[a]	0.028	6.19[a]	6.10[b]	0.024
Lightness (L*)[1]	45.66[c]	46.74[b]	47.71[a]	0.208	49.06[a]	44.34[b]	0.170
Redness (a*)[1]	19.23[b]	19.78[ab]	20.12[a]	0.258	19.95	19.47	0.211
Yellowness (b*)[1]	17.60[a]	17.17[b]	16.94[b]	0.150	17.32	17.16	0.123
TBARS[2]	1.47[a]	1.25[b]	1.05[c]	0.031	1.28	1.23	0.025

[1] L* values measure darkness to lightness (greater L* values indicate a lighter color); a* values are a measure of redness (greater a* values indicate a redder color); and b* values are a measure of yellowness (greater b* values indicate a more yellow color).
[2] 3-thiobarbituric acid reactive substances (mg of maldenaldehyde/kg of tissue).
Within a row and main effect, least squares means lacking a common superscript letter differ ($P < 0.05$).

Table 2. Quality characteristics of cooked ground beef patties as affected by lean finely textured beef inclusion and lean composition.

	Lean finely textured beef, %				Lean composition, %		
	0	10	20	SE	82	93	SE
Cooking Loss (%)	27.90[a]	27.50[a]	24.60[b]	0.008	29.47[a]	23.87[b]	0.006
TBARS[1]	0.87	0.91	0.88	0.046	1.05[a]	0.72[b]	0.038
Lightness (L*)[2]	56.14	56.23	55.18	0.772	56.53	55.17	0.629
Redness (a*)[2]	15.56	17.06	17.63	0.739	16.19	17.31	0.603
Yellowness (b*)[2]	18.18	18.78	19.02	0.395	18.41	18.91	0.323
Lee-Kramer shear[3]	176.07[a]	162.05[b]	134.81[c]	3.483	143.56[b]	171.72[a]	2.850

[1] 3-thiobarbituric acid reactive substances (mg of maldenaldehyde/kg of tissue).
[2] L* values measure darkness to lightness (greater L* values indicate a lighter color); a* values are a measure of redness (greater a* values indicate a redder color); and b* values are a measure of yellowness (greater b* values indicate a more yellow color).
[3] Greater values indicate tougher patties.
Within a row and main effect, least squares means lacking a common superscript letter differ ($P < 0.05$).

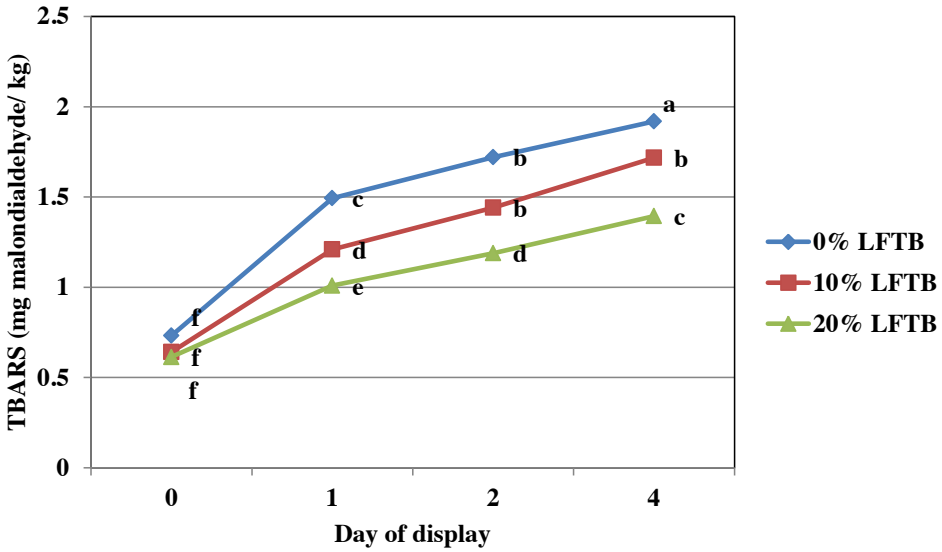

Fig. 1. Interactive effects of day of display and percentage of lean finely textured beef (LFTB) on Thiobarbituric Acid Reactive Substances (TBARS; a measure of oxidation) in fresh ground beef patties. Mean values with different letters differ ($P < 0.05$).

Impact of a whole yeast product on sow, litter, and nursery performance

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Story in Brief

Three groups of gestating gilts and sows (n = 98) were used to determine the effects of Pichia guilliermondii (Pg), a whole yeast product, on dam and litter performance. Within 24 hours of breeding, gilts and sows were assigned to one of three dietary treatments consisting of a control diet, or control supplemented with either 0.1% or 0.2% CitriStim. Dietary treatments were maintained through lactation. At approximately 21 days of age, pigs from the first two farrowing groups were individually weighed and allotted to either a control diet, or a diet containing CitriStim in a 3 (0, 0.1, or 0.2% Pg) × 2 (with or without Pg) factorial arrangement (336 pigs in Exp. 1 and 288 pigs in Exp. 2). Pigs were provided ad libitum access to feed and water during phase 1 (P1; 7 d), phase 2 (P2; 14 d) and phase 3 (P3; 14 d) in both studies. More pigs were born alive to sows fed Pg compared to controls. Additionally, number weaned and preweaning mortality were greater, while the percentage of piglets weighing less than 2.0 lb at birth was reduced in sows provided 0.1% or 0.2% Pg. There were no differences in gestation BW gain, farrowing (d 110 to 48 h post-farrowing) or lactation BW loss (d 110 to weaning), individual birth and weaning weight, or number of mummies or stillborns ($P > 0.05$). Inclusion of Pg in sow diets increased average daily gain (ADG) during nursery P1-2 and BW at the end of P2 in Exp. 1 ($P < 0.05$). Additionally in Exp. 1, an interaction between sow and nursery treatment was observed in ADG for P1, in ADFI for P1, P1-2, P3, and the overall nursery period, G:F for P2, P1-2, and the overall nursery period, and BW at the end of P1. In Exp. 2, a linear increase ($P < 0.05$) was observed in ADG, ADFI and G:F for P1, P2, P1-2, and the overall nursery period as the level of Pg fed to sows increased. Additionally, BW at the end of P2 and P3 increased linearly with increasing Pg in gestation sow diets. In conclusion, CitriStim in sow and nursery diets improved litter and nursery performance, and this improvement may be additive.

Introduction

Yeast products have been shown to enhance swine performance as both a probiotic (living, viable microorganism, such as active dry yeast) or prebiotic (nondigestible food ingredient, such as yeast cell culture). One component of the yeast cell wall that is believed to provide beneficial effects is mannan oligosaccharide. Several theories have been proposed as to how these benefits are conveyed, including direct binding of pathogenic bacteria in the intestinal lumen, thereby flushing them out, as well as immunomodulation.

The effect of yeast products vary. Veum et al. (1995) observed no performance differences when sows were provided a yeast culture product from day 60 of gestation through lactation. However, Shen et al. (2011) fed a commercial yeast cell culture product from breeding through lactation and reported an increase in litter wean weight and piglet average daily gain. Additionally, improvements in number born alive and weaned, as well as body weight at birth, and d 14 and 28 of lactation were reported in pigs from sows fed another yeast product from 4 weeks prior, until 4 weeks following farrowing (Czech et al., 2010). A beneficial effect of yeast on growth performance in nursery pigs has also been demonstrated (Davis et al., 2004).

CitriStim (ADM Alliance Nutrition) is a whole yeast [*Pichia guilliermondii* (Pg)] coproduct of citric acid extraction, containing the whole yeast cell and its components. Several unpublished studies have demonstrated the potential benefit of Pg in swine. Therefore the objective of this research was to determine the effects of Pg on dam and litter performance.

Materials and Methods

Sow and Litter. A total of 98 GPK 35 gilts and sows were allotted to 1 of 3 dietary treatments based on parity, and body weight at breeding. The 3 dietary treatments (Table 1) were a gestation control diet (SC), or the control diet supplemented with 0.1% (S1), or 0.2% Pg (S2). Gilts and sows were housed in individual gestation stalls throughout gestation and provided approximately 5 lb of feed per day and free access to water throughout the gestation period. On day 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, sows were fed ad libitum, maintaining gestation treatments through the lactation period. Both gestation and lactation diets were formulated to meet or exceed National Research Council (NRC) requirements for gestating and lactating sows, respectively. Individual body weight was recorded for gilts and sows at breeding, 110 d of gestation, 48 hours post farrowing, and at weaning, which occurred approximately 21 days post farrowing. At farrowing, and again at weaning, the total number of live piglets was counted and individual body weight was recorded. Additionally, the number of stillborns and mummies were recorded. Individual daily feed intake of each sow was also recorded during lactation.

Nursery. Pigs from the first 2 breeding groups of gilts and sows were used to determine whether Pg fed to sows during gestation and lactation impacted growth performance of their progeny. The study was designed in a 3 (SC, S1, or S2) × 2 (nursery pigs with [NPg] or without [NC] Pg) factorial arrangement in an randomized complete block design. For Exp. 1, a total of 336 weaned pigs (12.8 lb average BW) were individually weighed and grouped into weight blocks with stratification based on sex and litter and assigned to pens (7 pigs/pen, 8 replicates/treatment). Pigs were phase fed either a control diet (NC) or control with Pg (NPg) inclusion at 0.2%, 0.1%, and 0.1% in phase 1 (7 d), phase 2 (14 d), and phase 3 (14 d), respectively. In Exp. 2, a total of 288 weaned pigs (14.1 lb average BW) were individually weighed and grouped into weight blocks with stratification based on sex and litter and assigned to pens (6 pigs/pen, 8 replicates/treatment). Pigs were phase fed, as in Exp. 1, either the NC or NPg (0.2% Pg in all phases) diet. Diets were formulated to meet or exceed NRC requirements (Table 2). Weaning occurred at approximately 21

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d of age and feed and water were provided *ad libitum* for both studies. Pigs were housed in a conventional nursery facility in elevated, wire floored pens.

Pigs were individually weighed at weaning and the end of each phase. Pen feed intake was also determined at the end of each phase. These measurements were used to calculate average daily gain, average daily feed intake, and gain to feed ratio.

Statistical Analysis. The PROC STEPWISE procedure of SAS was used to determine significant independent variables in the sow and litter study. Then, the PROC MIXED procedure of SAS was performed using relevant independent variables. Replicate and parity were included as random variables. For nursery Exp. 1 and 2, performance data was analyzed as a randomized complete block design using the General Linear Model function of SAS with pen as the experimental unit.

Results and Discussion

Sow and Litter. There were no significant differences observed in gestation weight gain, total weight loss after farrowing, overall average daily feed intake, birth weight, number of stillborn or mummies, weaning weight or average daily gain. However, there was a tendency (sow quadratic, $P = 0.07$) for increased average daily feed intake during week 3 in S1 gilts and sows, and a tendency (sow quadratic, $P = 0.11$) for decreased lactation body weight loss in the same group (Table 3).

Sows supplemented with Pg had approximately 1 pig per litter more than those receiving the control diet (Fig. 1), however average piglet birth weight did not differ. Thus, pig size was consistent though litter size increased in sows provided diets supplemented with Pg. Additionally, sows provided Pg had a lower percentage of pigs weighing less than 2.0 lbs at birth (Fig. 2). Sows provided Pg during gestation and lactation had a small, but significant increase in the number of pigs weaned (Fig. 3). It should be noted that, similar to the increase in number born alive, there was no difference among groups in average weaning weight. However, there was a small increase in preweaning mortality for sows provided Pg (Fig. 4).

In summary, inclusion of Pg in gestation diets increased the number born alive, and decreased the percentage of pigs born weighing less than 2.0 lbs. Providing Pg in gestation and lactation diets increased the number weaned, but also increased percent preweaning mortality.

In conclusion, the inclusion of the whole yeast product Pg in sow gestation and lactation diets may have a beneficial effect on sow reproductive, and litter performance.

Nursery. Exp. 1. There was an increase in average daily gain in pigs from sows fed Pg in the overall phase 1-2 period when compared to those from sows that were fed the control diet (Fig. 5). Nursery pigs from sows provided with Pg during gestation and lactation had increased feed efficiency during phase 2, the overall phase 1-2 period, and the overall nursery period compared to those provided the control diet (Fig. 5). Body weight of pigs from S2 were heavier than those from either S1 or SC at the end of phase 1, and pigs from sows fed Pg (S1 and S2) being just over 1 lb heavier than pigs from SC at the end of phase 2 (Fig. 6).

No differences were observed between any level of Pg inclusion in sow diets for pigs receiving NC for phase 1 average daily gain, however there was a significant increase in average daily gain in NPg pigs as level of Pg inclusion increased in sow diets (Fig. 7). A similar pattern for average daily feed intake was observed during phase 1 (Fig. 7). During phase 2 there is a tendency for a decrease in average daily feed intake in pigs fed NC as the level of Pg increased in sow diets, while average daily feed intake for NPg for all sow treatments ($P = 0.06$). During the overall phase 1-2 period, phase 3, and the overall nursery period (Fig. 8) there was an increase in average daily feed intake in NPg pigs as the level of Pg increased in sow diets, and a decrease as level of Pg increased in sow diets for NC pigs.

During phase 2, phase 1-2 overall, the overall nursery period (Fig. 8) G:F increased in NC as the level of Pg inclusion increased in sow diets, with no change in NPg regardless of sow treatment.

Finally, body weight of NPg pigs increased as the level of Pg inclusion increased in gilt and sow gestation and lactation diets.

Nursery Exp. 2. In phase 1, as well as the overall nursery period, average daily gain was greater in S2 than SC of S1. During phase 2, and the phase 1-2 overall period, pigs from S2 had the highest average daily gain, followed by S1 and SC was lowest (Fig. 9).

Average daily feed intake was highest in pigs from S2, intermediate in S1, and lowest in pigs from SC during phase 1 and the overall nursery period. During phase 2 and the phase 1 and 2 period combined, pigs from sows provided Pg had a higher average daily feed intake than those from control fed sows (Fig. 10).

Feed efficiency was greater in pigs from S2 during phase 1, phase 2, and the combined phase 1 and 2 period. For the overall nursery period, feed efficiency was greater in pigs from S2 than those from SC, with S1 being intermediate (Fig. 11).

Interestingly, pigs from S2 began the study lighter than SC, with S1 being intermediate. However, by the end of phase 2, pigs from S2 were the heaviest, and maintained that position through the end of the study, being approximately 4 lb heavier than SC at the completion of the study (Fig. 12).

In summary, weaned pigs from sows provided Pg had increased average daily gain, feed efficiency, and body weight. The effect of Pg inclusion may be additive when fed to nursery pigs as observed in the improvement in average daily gain as well as feed intake in NPg as the level of Pg increased in sow diets. Finally, Pg inclusion in sow diets was beneficial to efficiency in pigs receiving control nursery diets.

Implications

Supplementation of standard gestation and lactation diets with Pg may be beneficial to sow reproductive performance, as well as to the performance of the litter. Additionally, continued inclusion of the whole yeast product, Pg, in nursery diets improved weaned pig performance, and this improvement may be additive.

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Table 1. Composition (as-fed) of gestation and lactation diets[1].

Ingredients (%)	Gestation	Lactation
Corn	54.52	54.81
Soybean meal, 48%	9.50	28.00
DDGS	30.00	10.00
Fat (Yellow Grease)	1.00	2.00
Dicalcium phosphate	1.875	2.40
Limestone	1.175	0.75
Salt	0.45	0.50
L-Lysine	0.15	0.175
L-Threonine	0.00	0.04
Other	1.33	1.33
TOTAL	100.00	100.00
Calculated composition[2]		
ME (Mcal/lb)	1.50	1.51
CP (%)	17.07	20.67
SID Lysine (%)	0.652	1.065
Available P (%)	0.544	0.547
Ca (%)	0.952	0.956
SID M+C:Lys	81	56
SID Thr:Lys	73	64
SID Trp:Lys	19	19
SID Ile:Lys	80	69
SID Val:Lys	100	78

[1]Control diets for gestation and lactation. For the 0.1% Pg and 0.2% Pg diets, 2 lb/ton and 4 lb/ton Pg, respectively, was added at the expense of corn.

[2]ME = metabolizable energy; CP = crude protein; SID = standard ileal digestible.

Table 2. Composition (as-fed) of nursery diets[1]

Ingredients (%)	Phase 1	Phase 2	Phase 3
Corn	40.66	50.34	56.70
Soybean meal, 48%	19.50	31.50	24.50
DDGS	—	5.00	15.00
Oat Groats	10.00	—	—
Poultry Fat	1.50	1.00	—
L-Lysine	0.30	0.27	0.30
DL-Methionine	0.17	0.11	0.03
L-Threonine	0.11	0.08	0.07
L-Tryptophan	0.01	—	—
Whey	16.45	8.00	—
Plasma protein	4.00	—	—
Fish meal	4.00	—	—
Other	3.30	3.70	3.40
TOTAL	100.00	100.00	100.00
Calculated composition[2]			
ME (kcal/kg)	1.52	1.50	1.50
CP (%)	22.18	21.80	20.64
SID Lysine (%)	1.43	1.26	1.10
Available P (%)	0.52	0.40	0.40
Ca (%)	0.89	0.84	0.85
SID M+C:Lys	58	58	58
SID Thr:Lys	62	62	63
SID Trp:Lys	17	18	17
SID Ile:Lys	56	64	65
SID Val:Lys	66	70	74

[1]Control diets for nursery. Exp. 1 - Pg added at 0.2 (4 lb/ton), 0.1 (2 lb/ton), and 0.1% in Phase 1, 2, and 3, respectively; Exp. 2 - Pg added at 0.2% in all phases at the expense of corn.
[2]ME = metabolizable energy; CP = crude protein; SID = standard ileal digestible.

Table 3. Effect of supplementation of *Pichia guilliermondii* on sow reproductive performance.

	Treatment[1]			P Value		
Variable[2]	SC	S1	S2	TRT	Linear Contrast	Quadratic Contrast
Sow Gestation BW Gain, lb	113.01 ± 5.58	118.65 ± 5.53	115.74 ± 5.56	0.4171	0.5175	0.2435
Sow Farrowing BW Loss, lb	-25.49 ± 5.58	-31.13 ± 5.53	-28.24 ± 5.56	0.4171	0.5175	0.2435
Sow Lactation BW Loss, lb	-27.56 ± 10.19	-17.39 ± 10.12	-22.16 ± 10.16	0.1700	0.3160	0.1074
Sow Total BW Loss, lb	-52.58 ± 6.44	-51.46 ± 6.35	-49.67 ± 6.35	0.8811	0.6201	0.9500
Week 1 Lactation ADFI, lb	10.23 ± 0.44	9.94 ± 0.44	10.21 ± 0.44	0.7429	0.9518	0.4429
Week 2 Lactation ADFI, lb	15.43 ± 0.33	15.15 ± 0.33	15.34 ± 0.31	0.8023	0.8383	0.5250
Week 3 Lactation ADFI, lb	15.85 ± 0.33	16.60 ± 0.31	16.01 ± 0.31	0.1911	0.7355	0.0743
Overall Lactation ADFI, lb	13.60 ± 0.86	14.09 ± 0.86	13.76 ± 0.86	0.6770	0.7811	0.3989
Total Birth Weight, lb	39.09 ± 1.21	37.41 ± 1.17	38.40 ± 1.17	0.5679	0.6681	0.3240
Average Birth Weight, lb	2.98 ± 0.02	2.95 ± 0.02	2.95 ± 0.02	0.7916	0.5229	0.7965
Number of Stillborn	0.86 ± 0.22	0.82 ± 0.21	1.17 ± .021	0.3837	0.2773	0.4178
Number of Mummies	0.44 ± 0.11	0.22 ± 0.11	0.39 ± 0.11	0.3199	0.7321	0.1397
Total Weaning Weight, lb	139.20 ± 3.64	136.57 ± 3.55	135.91 ± 3.53	0.7480	0.4675	0.7998
Average Weaning Weight, lb	13.29 ± 0.35	13.03 ± 0.33	12.85 ± 0.33	0.6246	0.3350	0.9041
Piglet Average Daily Gain, lb/d	0.489 ± 0.003	0.489 ± 0.003	0.484 ± 0.003	0.3762	0.2454	0.4502
Percent weaned ≤ 7.0 lb	3.60 ± 1.33	5.11 ± 1.31	6.28 ± 1.31	0.3704	0.1607	0.9149

[1]SC = Control diet; S1 = Control diet supplemented with 0.1% Pg; S2 = Control diet supplemented with 0.2% Pg.
[2]BW = body weight; ADFI = average daily feed intake.

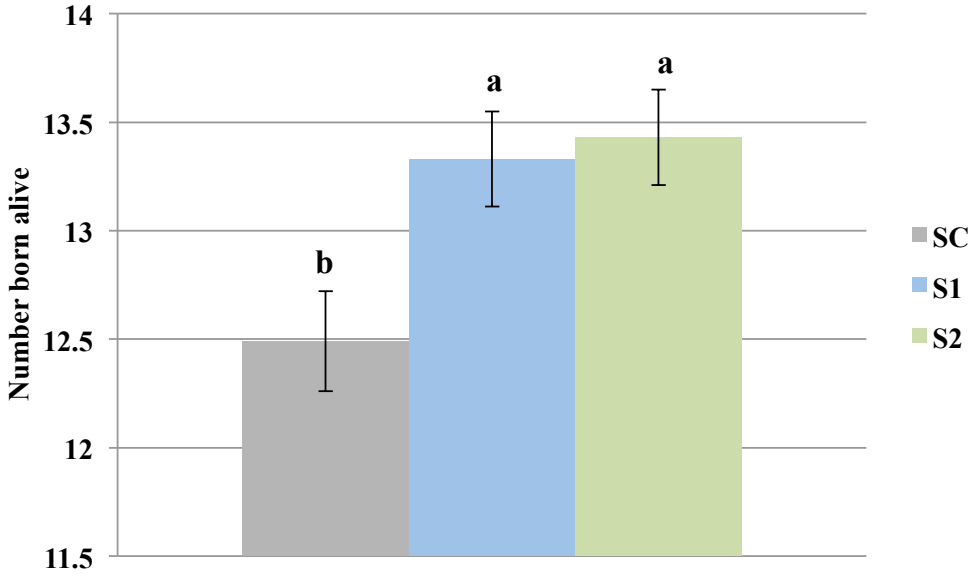

Fig. 1. Number born alive (*P* = 0.004). SC – Control; S1 – Control + 0.1% Pg; S2 – Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

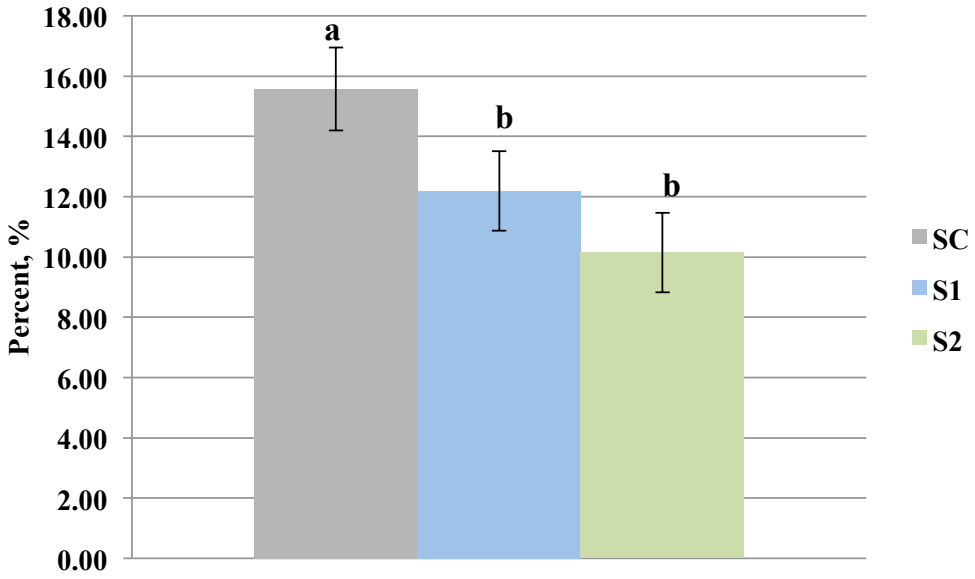

Fig. 2. Percentage of pigs born alive weighing less than, or equal to, 2 pounds (*P* = 0.02). SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

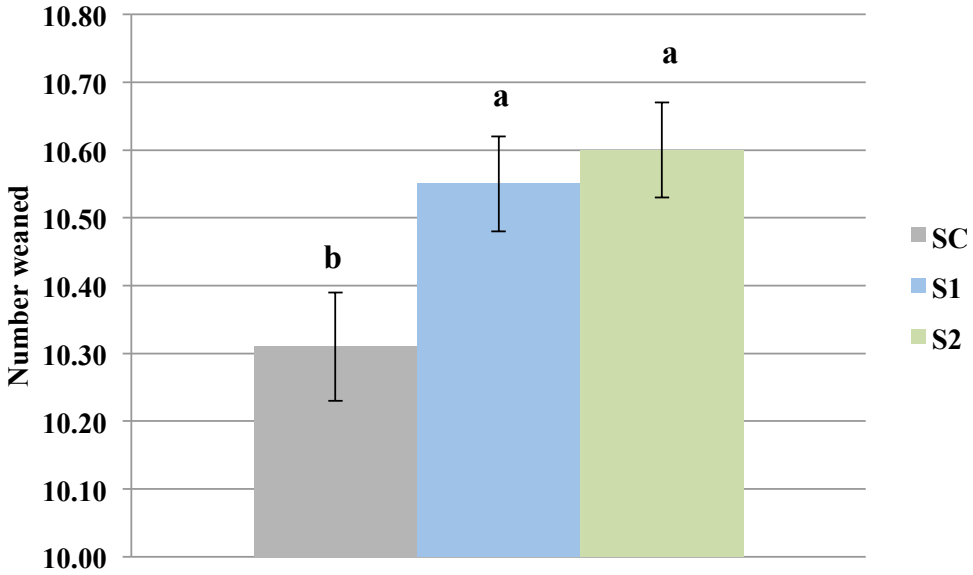

Fig. 3. Number weaned (*P* = 0.02). SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

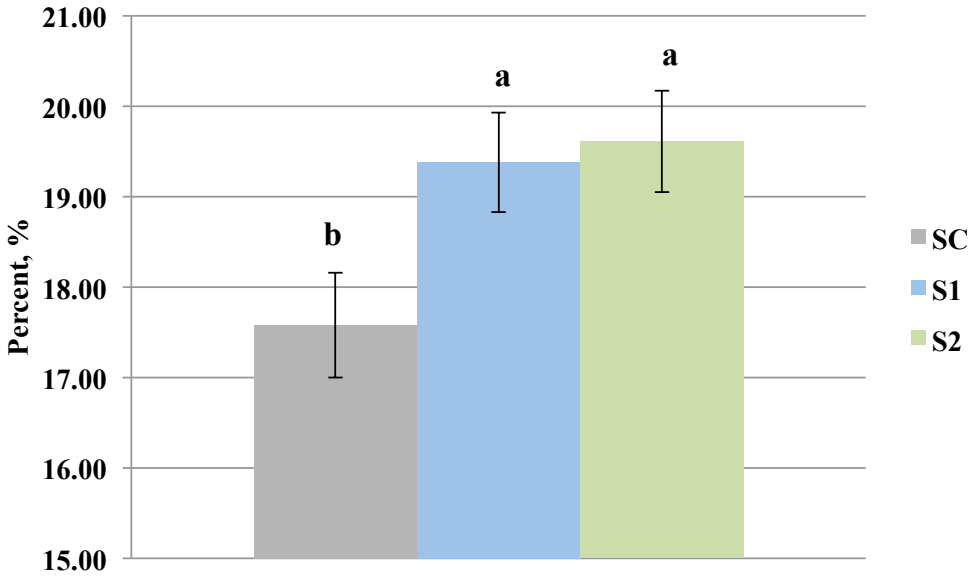

Fig. 4. Percentage preweaning mortality (*P* = 0.03). SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

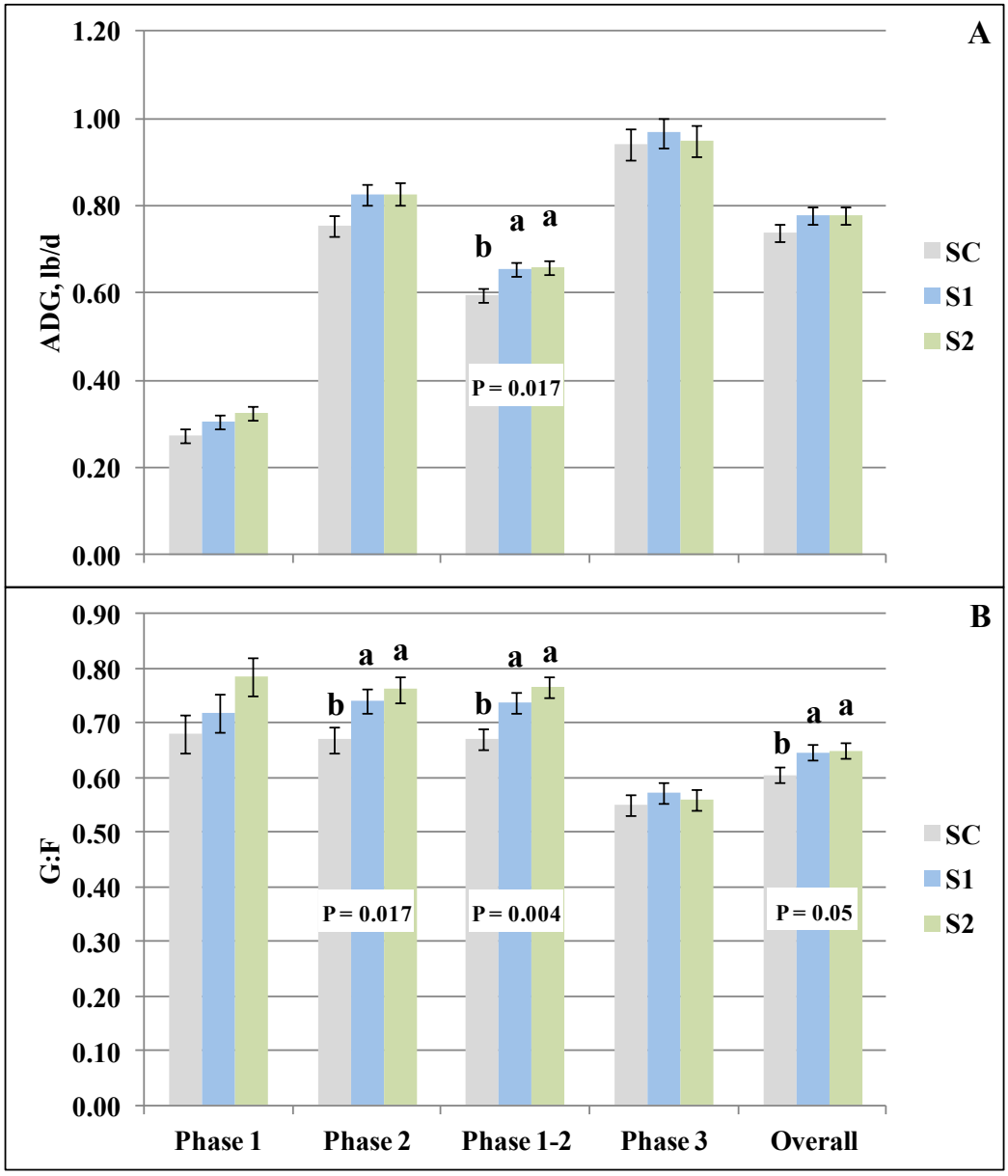

Fig. 5. Exp. 1 average daily gain (panel A) and feed efficiency (panel B). SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

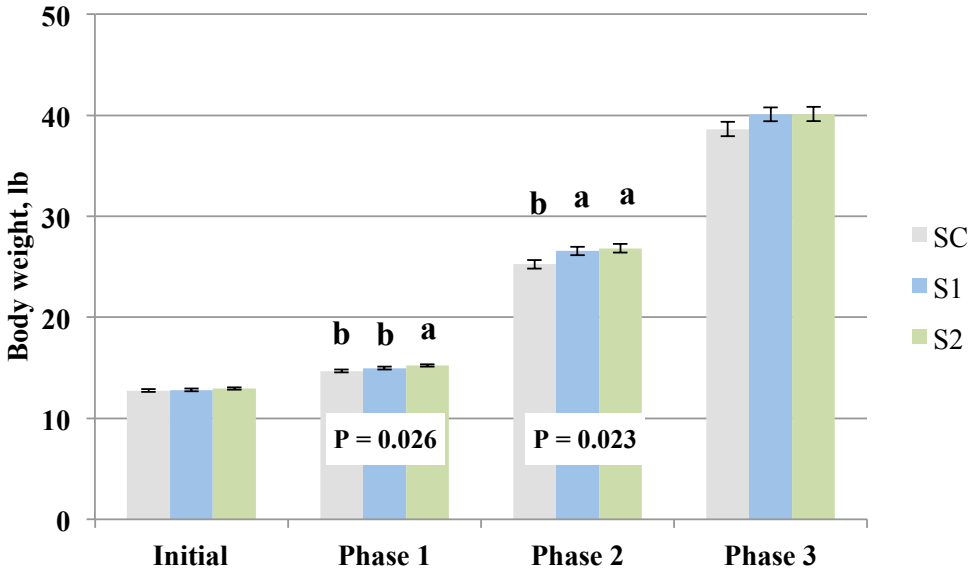

Fig. 6. Exp. 1 body weight. SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

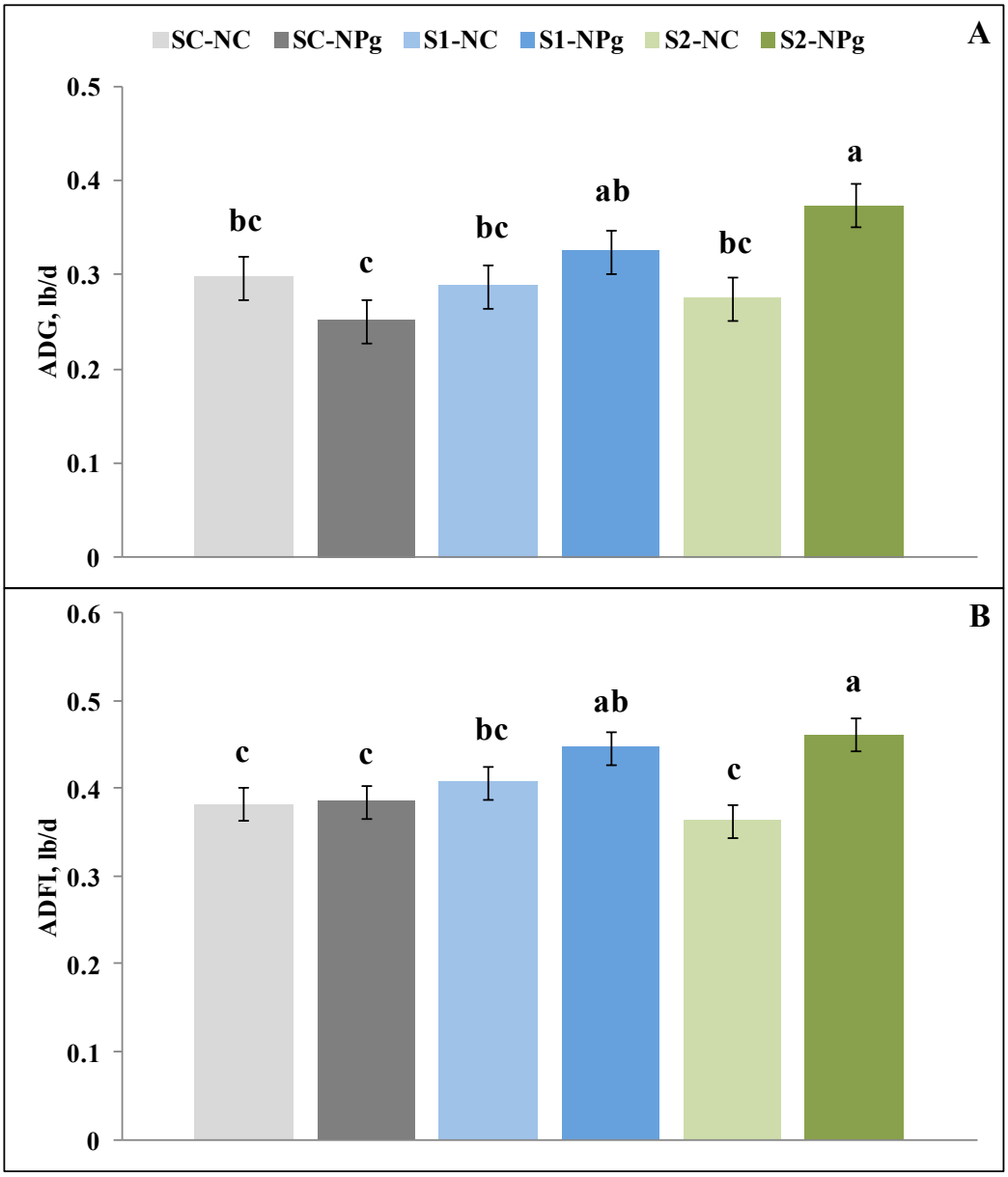

Fig. 7. Exp. 1 Phase 1 average daily gain [panel A (sow treatment x nursery, *P* = 0.01)] and average daily feed intake [panel B (sow treatment x nursery, *P* = 0.05)] . SC = Sow gestation/lactation control; S1 = Sow gestation/lactation control + 0.1% Pg; S2 = Sow gestation/lactation control + 0.2% Pg; NC = Nursery control; NPg = Nursery control + Pg. Bars with different letters differ (*P* < 0.05).

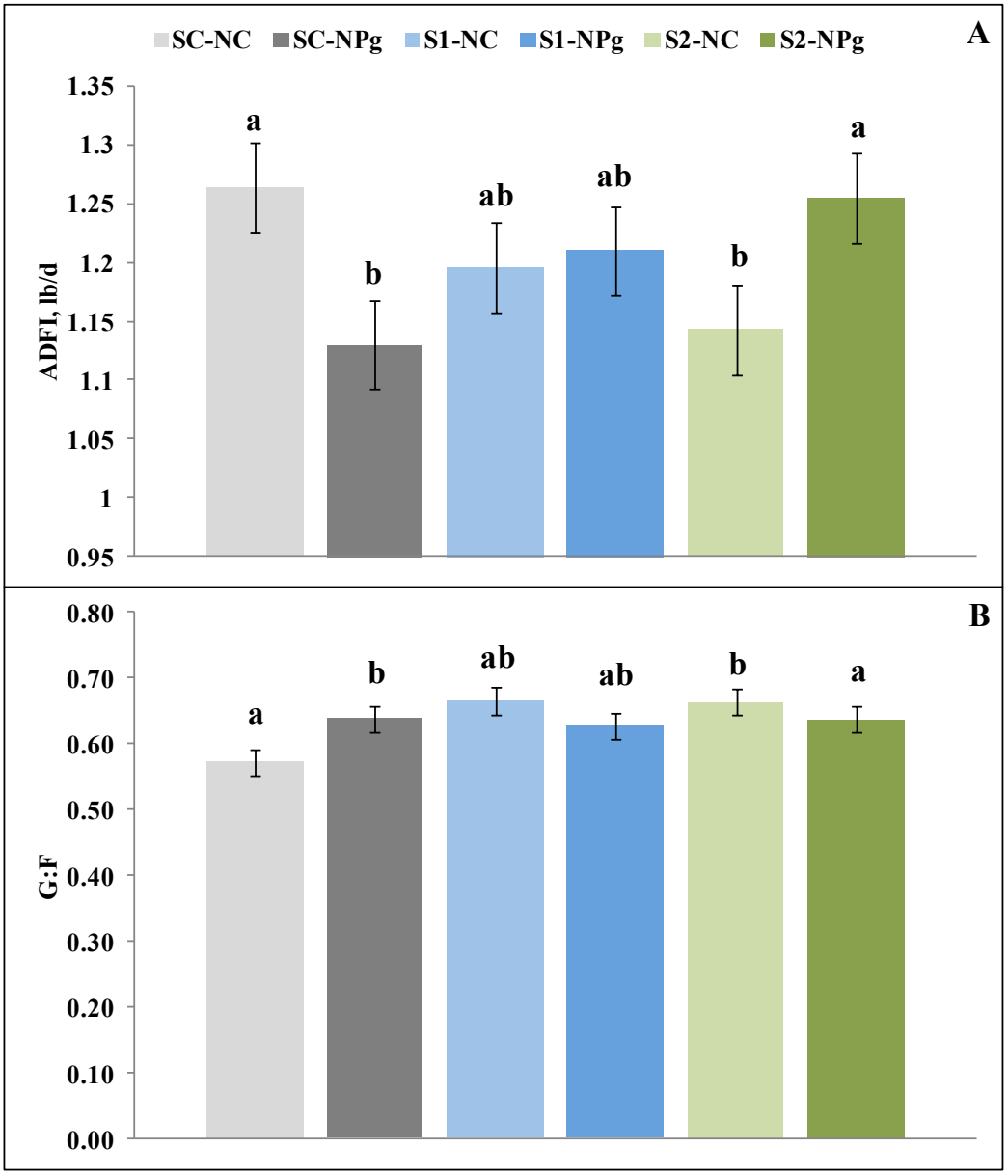

Fig. 8. Exp. 1 Overall average daily feed intake [panel A (sow treatment x nursery, *P* = 0.01)] and feed efficiency [panel B(sow treatment x nursery, *P* = 0.02)]. SC = Sow gestation/lactation control; S1 = Sow gestation/lactation control + 0.1% Pg; S2 = Sow gestation/lactation control + 0.2% Pg; NC = Nursery control; NPg = Nursery control + Pg. Bars with different letters differ (*P* < 0.05).

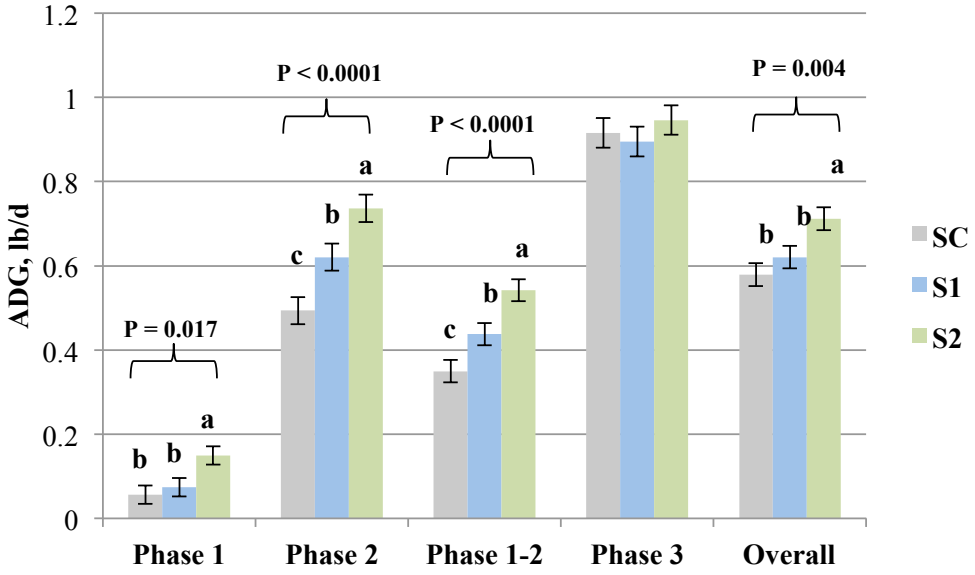

Fig. 9. Exp. 2 average daily gain. SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

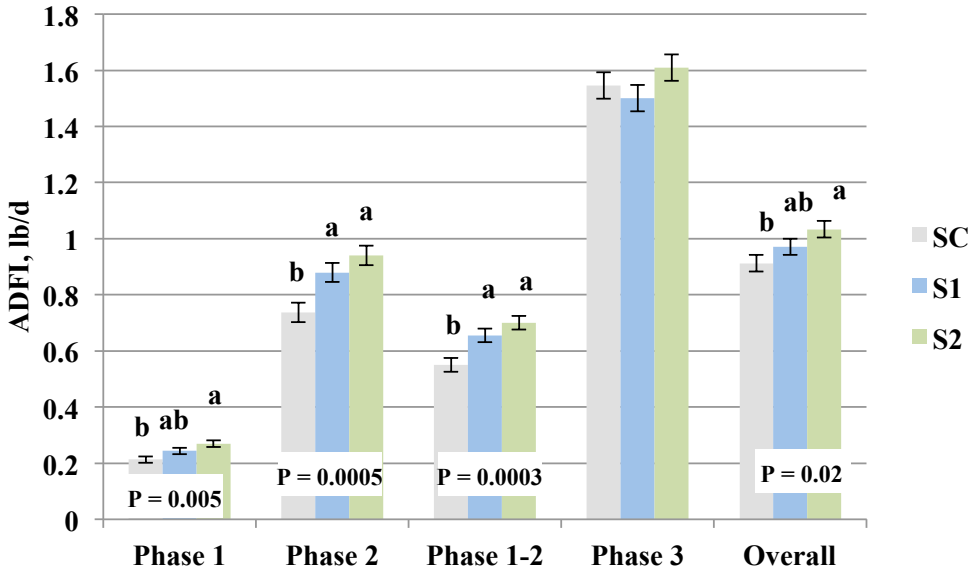

Fig. 10. Exp. 2 average daily feed intake. SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

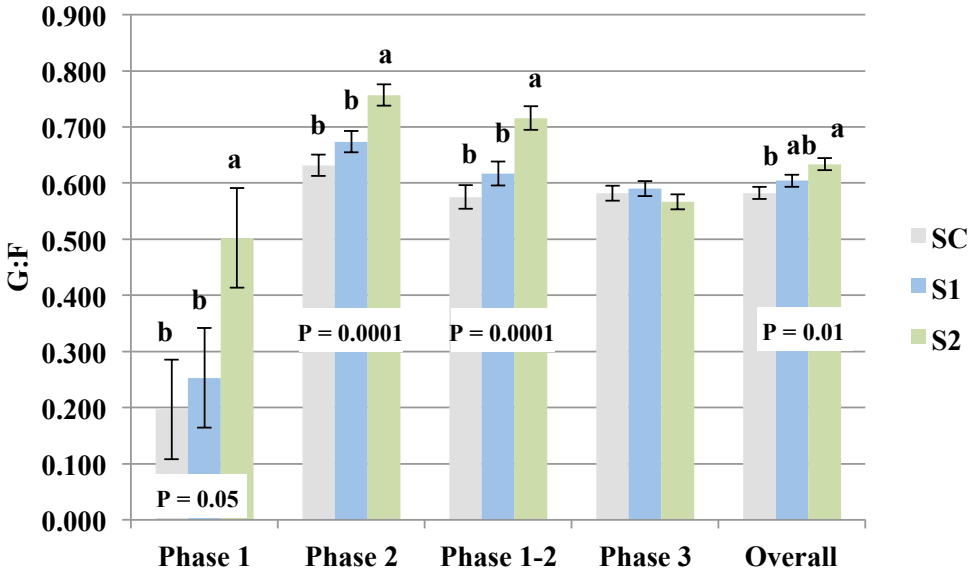

Fig. 11. Exp. 2 feed efficiency. SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

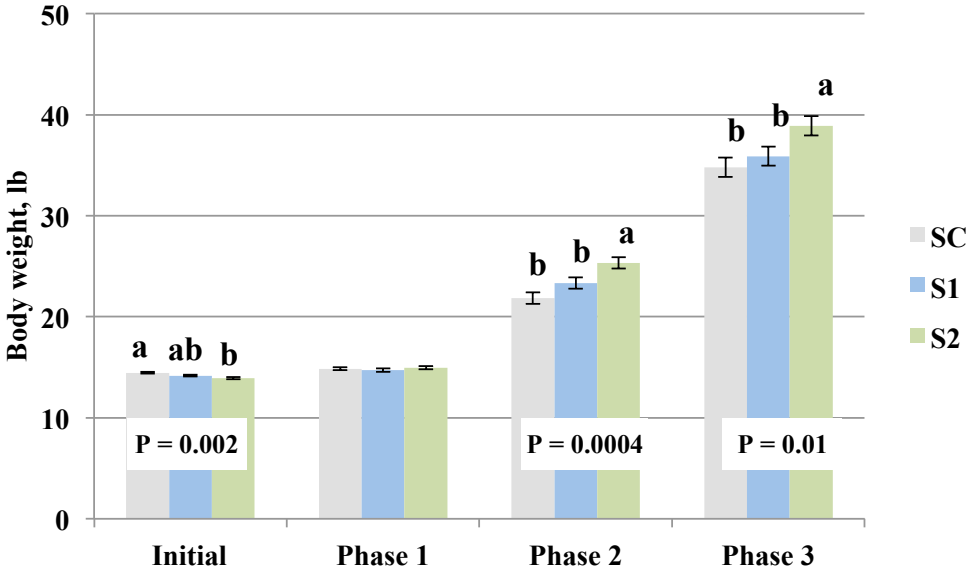

Fig. 12. Exp. 2 body weight. SC = Control; S1 = Control + 0.1% Pg; S2 = Control + 0.2% Pg. Bars with different letters differ (*P* < 0.05).

300-Day Grazing Demonstration—Cattle Management Report Year Four

T. R. Troxel[1], J. A. Jennings[1], M. S. Gadberry[1], K. Simon[1], J.G. Powell[1], D. S. Hubbell, III[2] and J.D. Tucker[2]

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) feed hay ≤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 2011-2012 was 500 and 485 lb for the steers and heifers, respectively. Sixty-two percent of the calves were medium frame and 38% were scored large-framed, and all calves were scored a muscle score 1. The calves (n = 39) were weaned on May 2, 2012 and the average weaning weight and value was 532 lbs and $878, respectively, with a total weaning value of $34,242. The overall cow efficiency was 45% (calf adjusted 205 d wt/cow wt at weaning). The cow efficiency goal of 50% was not reached this year and the herd fell 18 lb short of the 550 lb weaning weight goal. Over a 55-day grazing period, the calves gained 1.60 lbs average daily gain (ADG) resulting in an average weight and value of 620 lbs and $908 ($1.46/lb), respectively. As a result of grazing an extra 55 days, the calves returned $30/calf above their value at weaning. Continued emphasis on improved management and selection of high quality bulls and selective culling, can potentially increase production.

Introduction

Livestock producers continue to suffer from increased input costs. As a result, producers are challenged to determine what management adjustments are necessary for their operation to reduce costs. 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 of these decisions, producers may face 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) achieve 60 d or less of hay feeding, 4) maintain a 90% net calf-crop and 5) wean calves at an average weight of 550 pounds.

Materials and Methods

The cow herd was comprised of 38 Balancer mature cows with an August 30 to November 1 calving season and a November 29 to January 27 breeding season. In the fall of 2010, 2 Hereford bulls were leased and tested for breeding soundness and trichomoniasis prior to the breeding season.

On April 11, 2012 calves from the August 30 to November 1, 2011 calving season were administered an 8-way clostridial vaccine (Covexin 8; Merck Animal Health, Summit, N.J.) and a killed vaccine containing respiratory viruses, *leptospirosis*, and *vibriosis* (Virashield 6 + VL5; Novartis Animal Health, Greensboro, N.C.). In addition, cows and calves were dewormed with Cydectin Pour-on (Boehringer Ingelheim Vetmedica Inc. St. Joseph, Mo.). Blood samples were collected from the cows to determine pregnancy rate for the 2011-2012 breeding season (Veterinary Diagnostic Laboratory, Arkansas Livestock & Poultry Commission, Little Rock). Body weight of cows and calves were determined for the cow herd performance program.

On May 2, 2012 calves were administered a Virashield 6 + VL5 and Covexin 8 booster and all steers were implanted with a Ralgro (Intervet/ Schering-Plough Animal Health, Millsboro, Del.). In addition, all cows and calves were dewormed with Cydectin Pour-on. At processing, a certified livestock market reporter determined the selling value of the calves. Following processing, the herd was returned to pasture for fenceline weaning. Fenceline weaning was accomplished by placing calves in a high quality pasture containing endophyte infected tall fescue and white clover, with dams placed in an adjacent pasture. Following weaning, calves were retained for a 55-day post-weaning grazing period. Due to excessive dry conditions, calves were sold on June 26.

Results and Discussion

Adjusted 205-day weaning weights, mature cow body condition score (BCS) and pregnancy rates are reported in Table 1. Test results showed the pregnancy rate was 97% (37 of the 38 cows). The average 205-d adjusted weaning weights were 500 and 485 lb for the steers (n = 20) and heifers (n = 19), respectively. Sixty-two percent of the calves were medium frame and 38% were large framed, and all calves were scored a muscle score 1. The adjusted 205-day weaning weights increased from the first year of the demonstration. Overall cow efficiency was 45% (calf adjusted 205 d wt/cow wt at weaning). The cow efficiency goal of 50% was not reached this year. Given the cow size of the females (1,107 lb), the cow efficiency goal may be more ambitious than is realistically possible. Looking at the four year results (Table 1), there has been a steady increase in weaning weights over time. Using high quality sires and culling low performing dams along with proving high quality forage may have contributed to this improvement.

One of the management strategies that were implemented is a strict culling program. All open females or females that lost a calf for any reason were culled and replaced with a female of similar stage of production. A cow that lost a calf after calving was replaced with another cow/calf pair and a female that was determined to be open following pregnancy test was replaced with a bred female. Over the four years, 17 of the 38 (45%) original cows were culled for various reasons or died. Animals that were added to the herd as replacements were culled at about the same rate (43%). Failure to breed (38.5% of

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cull cows) and bad feet/fescue foot (34.2% of cull cows) were the two most common reasons for culling (Table 2).

Weaning weights and post weaning performance results are summarized in Table 3. Calves were weaned on May 2, 2012. Steers and heifers weighed 549 and 515 lb, respectively, for an overall average of 532 lb. Steers and heifers were valued at $1.70 ($933/hd) and $1.60/lb ($824/hd), respectively. Different Hereford bulls were used for the 2011-2012 calf crop compared to the 2010-2011 calf crop. For the past four years, calves were weaned in early to mid-May and grazed until late June (2010-2011 and 2011-2012) or mid-July (2008-2009 and 2009-2010).

While grazing primarily Bermudagrass pastures with no rainfall from May 2 to June 26, the calves gain was 1.60 ADG. Because of the lack of rainfall and subsequent effects on forage growth, the calves were sold on June 26. Overall the calves weighed 620 lbs and were sold for $908/hd (Table 3) with a post weaning total value of $35,397. Retaining ownership for 55 d returned $1,155 or $30 per calf. Although retained ownership was profitable in yr 4 it wasn't as profitable as previous yrs (3.4% vs. 12.2%, 14.3% and 8.6%, for yr 4, 1, 2, and 3, respectively). The primary reason for the reduced return was a drop in selling price in late June.

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.

Literature Cited

Troxel, T. R., J. A. Jennings, M. S. Gadberry, B. L. Barham, K. Simon, J. Powell and D. S. Hubbell, III. 2009. 300 day grazing demonstration. Arkansas Animal Science Department Report. Arkansas Agricultural Experiment Station. Research Series 574.

Table 1. 205-day adjusted weights and cow performance summary for the 300-day grazing demonstration.

	Year			
	2008-2009	2009-2010	2010-2011	2011-2012
Steer weaning weight, lbs	446	480	535	500
Heifer weaning weight, lbs	416	448	476	485
Overall weaning weight, lbs	437	464	507	493
Cow Efficiency, %	43%	45%	50%	45%
Cow Body Condition Score[a,b]	5.0	5.8	5.5	6.0
Cow Weight, lbs[b]	1016	1039	1042	1107
Pregnancy Rate, %	84%	97%	86%	97%

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

[b] Body condition scores and cow weights were obtained at the time calves 205-day weights were collected (April 11, 2012).

Table 2. Reasons for culling cows in the 300-day grazing demonstration.

Reason	Percentage[a]
Bad Feet/Fescue Foot	34.2%
Open	38.5%
Calf Death Loss	15.4%
Cow Death Loss	12.8%

[a]Percentage of cows culled.

Table 3. Four year post-weaning results summary for the 300-day grazing demonstration.

Year	Weaning Weight	Average Weaning Value	Total Weaning Value	Post Weaning Weight	Average Daily Gain	Post Weaning Average Value	Post Weaning Total Value	Value Difference
2008-2009	471lbs	$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
2011-2012	532 lbs	$878	$34,242	620 lbs	1.60	$908	$35,397	$1,155

300-Day Grazing Demonstration—Year 4 Financial Report

T. R. Troxel[1], J. A. Jennings[1], M. S. Gadberry[1], K. Simon[1], J.G. Powell[1], D. S. Hubbell, III[2] and J.D. Tucker[2]

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, 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 (AU), 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 (BW) 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 AI cost, breeding soundness examinations, replacement heifer or cow purchase, fertilizer, lime, purchased hay, herbicide, and miscellaneous. The number of mature cows grazed remained the same (n = 38) except for yr 3 but the number of AU increased (38 to 40.7 from 1 to 4, respectively). The mature cow calf-crop percentage increased steadily from yr 1 to yr 4. Most expenses remained rather constant over the 4-yr period except fly control and fertilizer cost increased. The total expense for yr 4 ($634/AU) was 10% greater than the average expense for yr 1 and 2 ($575/AU). Twenty-one steers (623 lbs), 20 heifers (576 lbs) and 10 cows (1,165 lbs) were sold in yr 4. The total gross income was $1,077/AU. The herd sold 36,253 lbs of beef (857 lb/AU) at an average price of $1.26/lb. Herd breakeven for yr 4 was $0.74/lb. Income over specified cost/AU was 121% greater in yr 4 compared to yr 1 by applying research based management practices.

Introduction

Livestock producers continue to suffer from increased input costs. Producers are challenged to determine what management adjustments are necessary for their operation. To survive economically, some producers chose to not make purchases (i.e. fertilizer), reduce livestock numbers, cut expenses at the risk of reducing livestock performance, or employ 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) achieve hay feeding of 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 and animal units of mature cows. 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 a 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 for yr 1, 2, 3 and 4 is summarized in Table 1. As in yr 1 and 2, there were 38 mature cows in the yr 4 demonstration herd. The yr 4 mature cow AUs slightly increased compared to yr 1and 2 due to increased cow weights in yr 4 (1,107 lb) compared to yr 1 (1,016 lb) and 2 (1,039 lb).

Production information is summarized in Table 2. Mature cow calf-crop percentage steadily increased from yr 1 to 4 (84% to 92%). Out of all the mature cows exposed to a bull, four mature cows were sold, replaced and did not wean a calf. One cow had twins, improving the calf crop percentage. The resulting calf crop percentage was 92% (35/38). Improved mature cow calf-crop percentage may have been due to a result of culling non-productive cows and improving forage quality and quantity. The four cows sold were replaced with cows with calves resulting in 39 calves at weaning. The cost of the replacement cows ($1,300 per hd) was charged to the demonstration.

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The expenses for yr 1, 2, 3 and 4 are summarized in Table 3. Many of the expense amounts remained rather constant over the 4-yr period. A few expense items slightly decreased in yr 4 compared to yr 1 and 2. These included salt and mineral, veterinarian costs, lime and herbicide. Fertilizer and fly control expense increased almost $100 and $8.02/AU, respectively, (compared to the average of yr 1 and 2) to $166.62 ad $13.08/AU, respectively, in yr 4. The reason for the increased in fertilizer cost was the application of potash. The total expense for yr 4 ($634) was 10% greater than the average total expense for yr 1 and 2 ($575/AU).

Twenty-one steers weighing 623 lbs sold for $151/cwt. ($19,755), 20 heifers weighing 576 lb sold for $141/cwt. ($16,243) and 10 cows weighing 1,165 lbs for $81.40/cwt. ($9,483) were sold. The herd sold 36,253 lbs of beef (857 lb/AU) at an average price of $1.26/lb in yr 4 (Table 4). Because of the increased in selling price, yr 4 recorded the highest income/AU, however, because of higher specified cost/AU the income over specified cost/AU was second to yr 3.

The average selling price increased $0.42/lb (50%) from yr 1 to 4. 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 4 budget. After adjusting the yr 4 selling price to $0.84/lb, the income per AU was $721, which was $78 less than yr 1. Approximately the same amount of pounds of beef was sold than in yr 1 and 4 (36,156 lbs vs. 36,253 lbs, respectively). The income over specified cost/AU for the adjusted yr 4 budget was $87/AU, which was $113/AU less than yr 1. Expenses per AU were higher in yr 4 ($633.68.00/AU) compared to yr 1 ($598.85/AU), therefore, higher production cost was the primary reason for the reduction in income over specified cost/AU for the adjusted yr 4 budget compared to the actual yr 1 budget.

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.

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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 4	Year 1	Year 2	Year 3	Year 4
Mature cows	38	38	31	38	38.0	38.0	33.1	40.7
H1[b]	0	0	5	0	0	0	4.5	0
H2[c]	0	0	10	0	0	0	11.3	0
Total	38	38	46	38	38.0	38.0	48.9	40.7

[a] Animal unit is equal to the metabolizable energy (ME) requirement for 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. Four year production information summary for the 300-day grazing demonstration.

Production item	Year 1	Year 2	Year 3	Year 4
Mature cows:				
Calf-crop percentage[a]	84% (32/38)	95% (37/38)	100% (31/31)	92% (35/38)
Culling percentage[b]	16% (6/38)	13% (5/38)	10% (3/31)	11% (4/38)
Death loss percentage[c]	3% (1/38)	3% (1/38)	3% (1/31)	0% (0/31)
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.
[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, 2 and 4.
[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.

Table 3. Four year expense per animal unit[a] summary for the 300-day grazing demonstration.

Expense item	Year 1	Year 2	Year 3	Year 4
Salt and mineral	$30.81	$27.75	$23.94	$26.70
Supplemental feed	$0.00	$0.00	$0.00	$0.00
Vet. medicine	$23.42	$21.20	$23.36	$17.97
Growth implants	$1.58	$2.36	$0.95	$0.71
Fly control	$5.12	$5.00	$0.00	$13.08
Sale commission	$36.47	$34.03	$37.02	$39.07
Hauling	$7.63	$14.34	$3.88	$10.87
Pregnancy test	$2.69	$2.72	$2.55	$2.69
Bull lease	$15.79	$15.79	$16.23	$14.18
Fertility testing bulls	$4.08	$2.11	$1.43	$3.78
Replacement cows	$328.95	$251.32	$175.80	$297.72
Fertilizer	$69.74	$108.79	$32.50	$166.62
Lime	$17.68	$0.00	$0.00	$0.00
Purchased hay	$15.79	$46.74	$21.46	$36.86
Herbicide	$6.97	$11.12	$3.46	$0.00
Miscellaneous	$32.13	$7.07	$28.41	$3.42
Total expenses	$598.85	$550.34	$371.00	$633.68

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

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

Item	Year 1	Year 2	Year 3	Year 4
Total lbs sold	36,156	30,325	35,467	36,253
Average price per lb received	$0.84	$0.93	$1.16	$1.26
Income/AU[a]	$799	$745	$844	$1,077
Income over specified cost/AU[b]	$200.41	$195.11	$472.68	$442.93
Herd breakeven[c]	$0.63	$0.69	$0.51	$0.74

[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 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.

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